ALAGAPPA UNIVERSITY

[Accredited with 'A+' Grade by NAAC (CGPA:3.64) in the Third Cycle
and Graded as Category–I University by MHRD-UGC]

(A State University Established by the Government of Tamil Nadu)

KARAIKUDI – 630 003

Directorate of Distance Education

M.Sc. (Microbiology)

I - Semester

364 13

MICROBIAL PHYSIOLOGY

Author

Rahul Dev Ambedkar, *Assistant Professor, Sri Aurobindo College, Delhi*
Units (1-14)

"The copyright shall be vested with Alagappa University"

All rights reserved. No part of this publication which is material protected by this copyright notice may be reproduced or transmitted or utilized or stored in any form or by any means now known or hereinafter invented, electronic, digital or mechanical, including photocopying, scanning, recording or by any information storage or retrieval system, without prior written permission from the Alagappa University, Karaikudi, Tamil Nadu.

Information contained in this book has been published by VIKAS® Publishing House Pvt. Ltd. and has been obtained by its Authors from sources believed to be reliable and are correct to the best of their knowledge. However, the Alagappa University, Publisher and its Authors shall in no event be liable for any errors, omissions or damages arising out of use of this information and specifically disclaim any implied warranties or merchantability or fitness for any particular use.



Vikas® is the registered trademark of Vikas® Publishing House Pvt. Ltd.

VIKAS® PUBLISHING HOUSE PVT. LTD.
E-28, Sector-8, Noida - 201301 (UP)
Phone: 0120-4078900 • Fax: 0120-4078999
Regd. Office: 7361, Ravindra Mansion, Ram Nagar, New Delhi 110 055
• Website: www.vikaspublishing.com • Email: helpline@vikaspublishing.com

Work Order No. AU/DDE/DE1-238/Preparation and Printing of Course Materials/2018 Dated 30.08.2018 Copies - 500

SYLLABI-BOOK MAPPING TABLE

Microbial Physiology

Syllabi	Mapping in Book
BLOCK I: BACTERIAL GROWTH AND NUTRITION	
UNIT - 1: Growth of Bacteria - Phases of Growth. Growth Kinetics - Batch Culture, Continuous Culture and Synchronous Culture. Factors Affecting Growth - Nutrition, Aeration, Temperature and pH.	**Unit 1:** Bacteria : Phases of Growth and Growth Kinetics **(Pages 1-18)**
UNIT - 2: Nutritional Diversity in Microorganisms, Nutritional Types - Autotrophy, Heterotrophy, Chemotrophy, Phototrophy, Lithotophy and Organotropy. Nutrition - Essentiality of Major and Minor Elements.	**Unit 2:** Microbial Nutrition **(Pages 19-28)**
UNIT - 3: Chemotrophism and their Importance, Reverse Electron Transport, CO_2 Assimilation and Reductive Acetyl COA Pathway.	**Unit 3:** Chemotrophism: An Introduction **(Pages 29-39)**
UNIT - 4: Chemoheterotrophism - Acetogens, Methanogens, Methanogenenesis and its Importance. Physiology and Economic Importance of Methylotrophs.	**Unit 4:** Chemoheterotrophism **(Pages 40-54)**
BLOCK II: BACTERIAL PHOTOSYNTHESIS, NITROGEN METABOLISM AND STRESS RESPONSE	
UNIT - 5: Bacterial Photosynthesis - General Types of Microbial Photosynthesis, Oxygenic and Anoxygenic. Structure of Photosynthetic Pigments - Chlorophylls, Bacteriochlorophyll, Carotenoids and Phycobilins.	**Unit 5:** Bacterial Photosynthesis **(Pages 55-78)**
UNIT - 6: Photosynthetic Bacteria - Green Sulphur and Purple. Mechanism of Photosynthesis Non-Cyclic and Cyclic Electron Transport. Photo Phosphorylation.	**Unit 6:** Bacterial Photosynthesis: Mechanism **(Pages 79-89)**
UNIT - 7: Microbial Stress Responses - Osmotic Stress and Osmoregulation, Aerobic to Anaerobic Transitions, Oxidative Stress, pH Stress and Acid Tolerance, Thermal Stress and Heat Shock Response, Nutrient Stress and Starvation Stress.	**Unit 7:** Microbial Stress Responses **(Pages 90-108)**

BLOCK III: MICROBIAL METABOLISM

UNIT - 8: Nitrogen Metabolism - Nitrogen Cycle - Ammonification, Nitrification, Denitrification and Nitrogen Fixation. Nitrogenase Enzyme, Physiology of Nitrogen Fixation in Symbiotic and Free Living Bacteria.

Unit 8: Nitrogen Metabolism **(Pages 109-124)**

UNIT - 9: Aerobic Respiration - TCA Cycle - Intracellular Location and Reactions, Amphibolic Reactions. Glyoxalate Cycle.

Unit 9: Aerobic Respiration **(Pages 125-150)**

UNIT - 10: Mechanisms of Substrate - Level Phosphorylation. Respiratory Electron Transport in Mitochondria and Bacteria. Mechanism of Oxidative Phosphorylation.

Unit 10: Mechanism of Substrate **(Pages 151-165)**

UNIT - 11: Anaerobic Respirations - Sulphate, Nitrate, Carbonate Respirations and their Ecological Significance.

Unit 11: Anaerobic Respiration **(Pages 166-184)**

BLOCK IV: BACTERIAL TRANSPORT AND QUORUM SENSING

UNIT - 12: Bioenergetics - Entropy, Enthalpy, Electron Carriers, Artificial Electron Donars, Inhibitors, Uncouplers, Energy Bond and Phosphorylation.

Unit 12: Bioenergitics **(Pages 185-200)**

UNIT - 13: Transport Across Membrane - Diffusion, Osmosis, Active Transport and Group Translocation.

Unit 13: Transport Across Membrane **(Pages 201-210)**

UNIT - 14: Quorum Sensing - Mechanism and Signaling Molecules.

Unit 14: Quorum Sensing **(Pages 211-228)**

CONTENTS

INTRODUCTION

BLOCK I: BACTERIAL GROWTH AND NUTRITION

UNIT 1 BACTERIA : PHASES OF GROWTH AND GROWTH KINETICS 1-18

1.0 Introduction
1.1 Objectives
1.2 Bacteria : General Introduction
1.3 Growth of Bacteria
  1.3.1 Phases of Growth
1.4 Growth Kinetics – Batch Culture, Continous Culture and Synchronous Culture
  1.4.1 Microbial Growth Kinetics
1.5 Types of Microbial Culture
  1.5.1 Batch Fermentation
  1.5.2 Continuous Fermentation
  1.5.3 Synchronous Growth
1.6 Factors Affecting Becterial Growth
  1.6.1 Nutrients
  1.6.2 Temperature
  1.6.3 pH Levels
  1.6.4 Water
  1.6.5 Elements Present
  1.6.6 Ions and Salt
1.7 Answers to Check Your Progress Questions
1.8 Summary
1.9 Key Words
1.10 Self Assessment Questions and Exercises
1.11 Further Readings

UNIT 2 MICROBIAL NUTRITION 19-28

2.0 Introduction
2.1 Objectives
2.2 Nutritional Diversity in Microogranism

2.3 Nutritional Types of Microoganisms
2.4 Requirement for Nitrogen, Phosphorus and Sulfur
2.5 Growth Factor
2.6 Answers to Check Your Progress Questions
2.7 Summary
2.8 Key Words
2.9 Self Assessment Questions and Exercises
2.10 Further Readings

UNIT 3 CHEMOTROPHISM: AN INTRODUCTION **29-39**

3.0 Introduction
3.1 Objectives
3.2 Chemotrophism and its Importance
3.2.1 Maximum Yield Explains Chemotropism
3.3 Reverse Electron Flow
3.4 CO_2 Assimilation
3.5 Calvin Cycle
3.6 Answers to Check Your Progress Questions
3.7 Summary
3.8 Key Words
3.9 Self Assessment Questions and Exercises
3.10 Further Readings

UNIT 4 CHEMOHETEROTROPHISM **40-54**

4.0 Introduction
4.1 Objectives
4.2 Chemoheterotrophism
4.2.1 Acetogen
4.2.2 Methanogenesis
4.3 Phyiology and Econimic Importance of Methylotrophs
4.4 Answers to Check Your Progress Questions
4.5 Summary
4.6 Key Words
4.7 Self Assessment Questions and Exercises
4.8 Further Readings

BLOCK II: BACTERIAL PHOTOSYNTHESIS, NITROGEN METABOLISM AND STRESS RESPONSE

UNIT 5 BACTERIAL PHOTOSYNTHESIS **55-78**

5.0 Introduction
5.1 Objectives
5.2 Photosynthesis: General Introduction
5.3 Types of Bacterial Photosynthesis
5.4 Structure of Photosynthetic Pigments
5.5 Answers to Check Your Progress Questions
5.6 Summary
5.7 Key Words
5.8 Self Assessment Questions and Exercises
5.9 Further Readings

UNIT 6 BACTERIAL PHOTOSYNTHESIS: MECHANISM **79-89**

6.0 Introduction
6.1 Objectives
6.2 Photosynthetic Bacteria- Green Sulphur and Purple
  6.2.1 Green S Bacteria
  6.2.2 Purple S Bacteria
6.3 Photophosphorylation
6.4 Mechanism of Photosymthesis of Non-Cyclic and Cyclic Electron Transport
  6.4.1 Non-cyclic Electron Transport in Photosynthesis
  6.4.2 Cyclic Electron Transport in Photosynthesis
6.5 Answers to Check Your Progress Questions
6.6 Summary
6.7 Key Words
6.8 Self Assessment Questions and Exercises
6.9 Further Readings

UNIT 7 MICROBIAL STRESS RESPONSES **90-108**

7.0 Introduction
7.1 Objectives
7.2 Microbial Stress Response
  7.2.1 Osmotic Stress
  7.2.2 Osmoregulation

7.3 Aerobic to Anerobic Transitions
7.4 Oxidative Stress
7.4.1 Free Radicals
7.4.2 Antioxidants
7.4.3 Damaged Caused by Oxidative Stress
7.5 pH Stress and Acid Tolerance
7.6 Thermal Stress and Heat Shock Response
7.7 Nutrient Stress and Starvation Stress
7.8 Answers to Check Your Progress Questions
7.9 Summary
7.10 Key Words
7.11 Self Assessment Questions and Exercises
7.12 Further Readings

BLOCK III: MICROBIAL METABOLISM

UNIT 8 NITROGEN METABOLISM 109-124

8.0 Introduction
8.1 Objectives
8.2 Process of Nitrogen Metabolism
8.3 Nitrogen Cycle
8.3.1 Ammonification and Nitrification
8.4 Nitrogenase Enzyme
8.5 Physiology of Nitrogen Fixation in Symbiotic and Free Living Bacteria
8.6 Answers to Check Your Progress Questions
8.7 Summary
8.8 Key Words
8.9 Self Assessment Questions and Exercises
8.10 Further Readings

UNIT 9 AEROBIC RESPIRATION 125-150

9.0 Introduction
9.1 Objectives
9.2 Aerobic Respiration
9.2.1 Stage I of Respiration: Glycolysis (EMP-Pathway of Glycolytic Breakdown)
9.2.2 Stage II of Respiration: Pyruvate Oxidation or Conversion of Pyruvic Acid to Acetyle Coenzyme A
9.3.3 Stage III of Respiration: TCA Cycle or Krebs Cycle

9.3 Glyoxylate Cycle
9.3.1 Overview of the Glyoxylate Cycle
9.3.2 Steps Involved in Glyoxylate Cycle
9.3.3 Significance of Glyoxylate Cycle
9.2.4 Stage IV of Respiration: Terminal Oxidation
9.4 Answers to Check Your Progress Questions
9.5 Summary
9.6 Key Words
9.7 Self Assessment Questions and Answers
9.9 Further Readings

UNIT 10 MECHANISM OF SUBSTRATE **151-165**

10.0 Introduction
10.1 Objectives
10.2 Mechanisms of Substrate-Level Phosphorylation
10.3 Respiratory Electrons Transport in Mitochondria and Bacteria
10.4 Mechanism of Oxidative Phosphorylation
10.5 Answers to Check Your Progress Questions
10.6 Summary
10.7 Key Words
10.8 Self Assessment Questions and Exercises
10.9 Further Readings

UNIT 11 ANAEROBIC RESPIRATION **166-184**

11.0 Introduction
11.1 Objectives
11.2 Anaerobic Respiration
11.3 Common Types of Anaerobic Respiration
11.4 Nitrate Reduction and Denitrification
11.5 Sulflate and Sulphur Reduction
11.6 Carbonate (CO_2) Respiration
11.6.1 Acetogenesis
11.6.2 Methanogenesis
11.7 Answers to Check Your Progress Questions
11.8 Summary
11.9 Key Words
11.10 Self Assessment Questions and Exercises
11.11 Further Readings

BLOCK IV: BACTERIAL TRANSPORT AND QUORUM SENSING

UNIT 12 BIOENERGITICS **185-200**

12.0 Introduction
12.1 Objectives
12.2 Bioenergitics
12.3 Entropy
12.4 Enthalpy
12.5 Electron Carriers
12.6 Artificial Electron Donors
12.7 Inhibitors
  12.7.1 Uses of Inhibitors
12.8 Uncouplers
12.9 Phosphorylation
  12.9.1 Control of Phosphorylation
12.10 Bond Energy (E)
12.11 Answers to Check Your Progress Questions
12.12 Summary
12.13 Key Words
12.14 Self Assessment Questions and Exercises
12.15 Further Readings

UNIT 13 TRANSPORT ACROSS MEMBRANE **201-210**

13.0 Introduction
13.1 Objectives
13.2 Transport Across Membrane
13.3 Answers to Check Your Progress Questions
13.4 Summary
13.5 Key Words
13.6 Self Assessment Questions and Exercises
13.7 Further Readings

UNIT 14 QUORUM SENSING **211-228**

14.0 Introduction
14.1 Objectives
14.2 Quorum Sensing
14.3 Mechanism and Signalling Molecules
14.4 Answers to Check Your Progress Questions
14.5 Summary
14.6 Key Words
14.7 Self Assessment Questions and Exercises
14.8 Further Readings

INTRODUCTION

NOTES

Microbiology is that branch of biology which studies microorganisms and their effects on human beings and other living organisms. The study of microorganisms include the unicellular or cell-cluster microscopic organisms, such as eukaryotes which includes fungi and protists, and prokaryotes which includes bacteria and certain algae. Microbiology subdivided into divisions including physiology, bacteriology, virology, mycology, parasitology and others.

Microbial physiology is defined as the study of how microbial cell structures, growth and metabolism function in living organisms. It covers the study of viruses, bacteria, fungi and parasites. The study of microbial cell functions includes the study of microbial growth, microbial metabolism and microbial cell structure. Microbial physiology is important in the field of metabolic engineering and also functional genomics. Fundamentally, microbial physiology is an enormous discipline encompassing the study of thousands of different microorganisms.

This book, *Microbial Physiology*, is divided into four block, which is further divided into fourteen units which will help you understand basics of microbiology, bacterial growth and nutrition, growth of bacteria, phases of growth, growth kinetics, continuous culture and synchronous culture, nutritional diversity in microorganisms, nutritional types, chemotrophism, CO_2 assimilation and reductive acetyl CoA pathway, chemoheterotrophism, bacterial photosynthesis, structure of photosynthetic pigments, photosynthetic bacteria, mechanism of photosynthesis non-cyclic and cyclic electron transport, microbial stress responses, osmotic stress and osmoregulation, aerobic to anaerobic transitions, oxidative stress, pH stress and acid tolerance, thermal stress and heat shock response, nutrient stress and starvation stress, nitrogen metabolism, nitrogen cycle, nitrogenase enzyme, physiology of nitrogen fixation in symbiotic and free living bacteria, aerobic respiration, TCA cycle, Glyoxalate cycle, mechanisms of substrate, anaerobic respirations, bioenergetics, transport across membrane, quorum sensing mechanism and signaling molecules.

The book follows the self-instruction mode or the SIM format wherein each unit begins with an 'Introduction' to the topic followed by an outline of the 'Objectives'. The content is presented in a simple and structured form interspersed with 'Check Your Progress' questions and answers for better understanding. A list of 'Key Words' along with a 'Summary' and a set of 'Self Assessment Questions and Exercises' is provided at the end of the each unit for effective recapitulation.

NOTES

BLOCK - I
BACTERIAL GROWTH AND NUTRITION

UNIT 1 BACTERIA : PHASES OF GROWTH AND GROWTH KINETICS

Structure

1.0 Introduction
1.1 Objectives
1.2 Bacteria : General Introduction
1.3 Growth of Bacteria
  1.3.1 Phases of Growth
1.4 Growth Kinetics – Batch Culture, Continous Culture and Synchronous Culture
  1.4.1 Microbial Growth Kinetics
1.5 Types of Microbial Culture
  1.5.1 Batch Fermentation
  1.5.2 Continuous Fermentation
  1.5.3 Synchronous Growth
1.6 Factors Affecting Becterial Growth
  1.6.1 Nutrients
  1.6.2 Temperature
  1.6.3 pH Levels
  1.6.4 Water
  1.6.5 Elements Present
  1.6.6 Ions and Salt
1.7 Answers to Check Your Progress Questions
1.8 Summary
1.9 Key Words
1.10 Self Assessment Questions and Exercises
1.11 Further Readings

1.0 INTRODUCTION

Bacteria are microscopic single-celled organisms that thrive in diverse environments. They can live within soil, in the ocean and inside the human gut. Human's relationship with bacteria is complex. Sometimes they lend a helping hand, by curdling milk into yogurt, or helping with our digestion. At other times they are destructive, causing diseases like pneumonia and MRSA. Bacteria are among the most abundant life forms on Earth. They are found in every ecosystem and are vital for everyday life. For example, bacteria affect what people eat, drink, and breathe, and there

NOTES

are actually more bacterial cells within a person's body than mammalian cells. Because of the importance of bacteria, it is preferable to study particular species of bacteria in the laboratory. To do this, bacteria are grown under controlled conditions in pure culture, meaning that only one type of bacterium is under consideration. Bacteria grow quickly in pure culture, and cell numbers increase dramatically in a short period of time. By measuring the rate of cell population increase over time, a 'growth curve' to be developed. This is important when aiming to utilize or inoculate known numbers of the bacterial isolate, for example to enhance plant growth, increase bio-degradation of toxic organics, or produce antibiotics or other natural products at an industrial scale.

Bacteria reproduce via binary fusion, where one bacterial cell divides into two cells. The time that is required for the division of cell is known as the mean generation time, or doubling time, which is the time required for the number of cells to double.

In this unit, you will study about growth of bacteria- phases of growth, growth kinetics - batch culture, continuous culture and synchronous culture, factors affecting growth - nutrition, aeration, temperature and pH.

1.1 OBJECTIVES

After going through this unit you will be able to:

- Discuss growth of bacteria
- Analyse different phases of growth
- Explain growth kinetics - batch culture, continuous culture and synchronous culture
- Understand factors that affect the growth

1.2 BACTERIA : GENERAL INTRODUCTION

Bacteria are among the most abundant life forms on Earth. They are found in every ecosystem and are vital for everyday life. For example, bacteria affect what people eat, drink, and breathe, and there are actually more bacterial cells within a person's body than mammalian cells. Because of the importance of bacteria, it is preferable to study particular species of bacteria in the laboratory. To do this, bacteria are grown under controlled conditions in pure culture, meaning that only one type of bacterium is under consideration. Bacteria grow quickly in pure culture, and cell numbers increase dramatically in a short period of time. By measuring the rate of cell population increase over time, a 'growth curve' to be developed. This is important when aiming to utilize or inoculate known numbers of the bacterial isolate, for example to enhance plant growth, increase bio-degradation of toxic organics, or produce antibiotics or other natural products at an industrial scale.

Each cell division results in a doubling of the cell number. At low cell numbers, the increase is not very large; however, after a few generations, cell numbers increase explosively. After n divisions, there are 2n cells.

NOTES

1.3 GROWTH OF BACTERIA

To understand and define the growth of particular microorganisms, they are placed in a flask, where the nutrient supply and environmental conditions are controlled. If the liquid medium supplies all the nutrients required for growth and environmental parameters conducive to growth, the increase in numbers can be measured as a function of time to obtain a growth curve. Several distinct growth phases can be observed within a growth curve (Refer Figure 1.1).

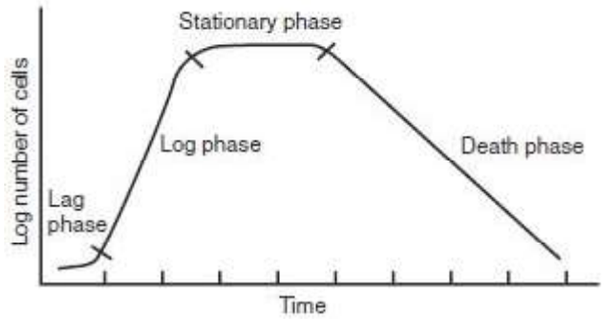


Fig. 1.1 *A Typical Growth Curve for a Bacterial Population*

1.3.1 Phases of Growth

There are four phases of growth named; lag phase, the exponential or log phase, the stationary phase, and the death phase, each of which are associated with specific physiological changes (Refer Table 1.1).

1. Lag Phase

When bacteria are inoculated into new fresh media, it does not divide immediately. Bacteria takes some time to adjust to the new environment. The time period in which bacteria is metabolically active but do not divide is called as lag phase. Lag phase is characterized by the period during which there is no increase in number of cell. Size of bacteria increase continuously so the bacteria have largest size at the end of lag phase. In this phase, microorganism tries to adopt in new environment. It is the phase of adjustment necessary for the synthesis of enzymes and co-enzymes for physiological activities. Time is required for adjustment in physical environment around each cell. Duration of lag phase varies according to conditions and species of bacteria. If the culture organism is taken from old culture, the duration will be longer but if the culture is fresh, duration is short. Similarly, if the culture media is different from the previous culture then duration is long because bacteria takes some more time to adjust in the new media. At the end of lag phase, bacteria become fully prepared for cell division.

NOTES

2. Log Phase or Exponential Phase

During this phase bacteria divides continuously at constant rate and the number of bacteria increase exponentially. In this phase all bacteria are in their rapid stage of cell division and show balanced growth. Due to rapid cell division, bacteria have smallest size in this phase. Bacterial population is nearly uniform in terms of their metabolic activities, chemical composition of cell and other physiological characteristics. Biochemical and physiological characteristics are commonly used for identification of bacteria are manifested during log phase of growth. Generation time of bacteria is usually determined during log phase. However, it is not same for all bacteria in culture. Generation time is shortest during log phase and is strongly dependent upon growth factors present in the medium. This phase lasts for several hour depending on the type of organism, conditions of growth and density of organism.

3. Stationary Phase

The bacteria growth reaches a state during which there is no net increase in bacterial population. This is called as stationary phase. In this phase a constant bacterial population is maintained by balance between cell division and cell death. In some bacteria, complete cessation of cell division occurs hence there is no net increase or decrease in number of bacteria. Stationary phase is induced by- increased bacterial cell density, depletion of nutrition in media and accumulation of toxic secondary metabolic wastes. Production of antibiotics such as Penicillin, streptomycin etc. and enzymes by certain bacteria occur during stationary phase of their growth. In endospore forming bacteria, sporulation occurs as the bacteria enter stationary phase.

4. Death Phase or Decline Phase

In this phase, number of bacteria decrease continuously and exponentially. During this phase, total count of bacteria may remain constant but the viable count decreases. It is just inverse of log phase. But the death rate is slower than growth rate. Death phase is brought about by various reasons, such as depletion of nutrition and accumulation of toxic wastes. Not all bacteria die at same rate, some die faster and some are more resistant and remain viable for longer time, for example in case of spore forming bacteria.

Table 1.1 *Phases of Growth*

Phase	Characteristics
Lag Phase	Slow growth or lack of growth due to physiological adaptation of cells to culture conditions or dilution of exoenzymes due to initial low cell densities.
Exponential or Log Phase	Optimal growth rates, during which cell numbers double at discrete time intervals known as the mean generation time.
Stationary Phase	Growth (cell division) and death of cells counterbalance each other resulting in no net increase in cell numbers. The reduced growth rate is usually due to a lack of nutrients and/or a buildup of toxic waste constituents.
Death Phase	Death rate exceeds growth rate resulting in a net loss of viable cells.

Check Your Progress

1. What is lag phase?
2. How is lag phase characterized ?
3. What happens in log phase?
4. How many phases of growth are there? Name them.
5. Define stationary phase.

NOTES

1.4 GROWTH KINETICS – BATCH CULTURE, CONTINOUS CULTURE AND SYNCHRONOUS CULTURE

Kinetics of growth refers to the rate at which the number of individual cells (or, more general, of active biomass) changes in a defined system. However, the area of microbial growth kinetics consists not just of the simple dynamic recording of biomass increase as a function of time in a culture but it is more the extraction of parameters that allow to quantitatively formulate general principles, to construct mathematical models that allow to describe and predict microbial growth processes and that provide a basis for further experimentation. Growth kinetics are therefore an indispensable tool not only in the applied fields of industrial and environmental biotechnology, but also in fundamental areas such as microbial genetics, physiology, and ecology, including competition, selection, and evolution.

1.4.1 Microbial Growth Kinetics

During the lag phase dX/dt and dS/dt are essentially zero. However as exponential growth phase begins it is possible to measure dX/dt and dS/dt values which are very useful for defining important microbial kinetic parameters. Using corresponding observations of dS/dt and dX/dt obtained just after the onset of exponential growth phase in Figure 2 we can compute the yield coefficient Y_{XS} and the specific growth rate μ as:

Yield Coefficient

$$Y_{XS} = \frac{dX}{dS} = \frac{\text{mass of new cells}}{\text{mass of substrate consumed}}, [\text{dimensionless}]$$

Specific Growth Rate

$$\mu = \frac{dX}{X_0 dt} = \frac{\text{mass of cells produced}}{\text{original mass of cells . time}}, \left[\frac{1}{\text{time}}\right]$$

The yield coefficient, commonly referred to as the substrate-to-biomass yield, is used to convert between cell growth rate dX/dt and substrate utilization

rate dS/dt. The yield coefficient and the specific growth rate used to develop three types of microbial growth kinetic relationships; Monod, first order, and zero order kinetics.

NOTES

Microbial Growth Kinetics describes about the growth pattern of a microbe in a fermenter. This factor has its own significance in determining the best batch timing for product recovery. A typical growth curve is explained on the basis of a typical batch type fermentation which includes:

- Lag Phase
- Logarithmic/Exponential Phase
- Static/Stationary Phase
- Death/Decline Phase

Lag Phase: Where we find no growth or very minimal growth in the fermenter. Reasons behind are inoculum inoculated into medium need to be adapted to the new environment.

Logarithmic/Exponential Phase: As inoculum got adapted to the environment, here we observe fast growth in population due to rapid division of adapted inoculum and with this phase we find the highest cellular growth rate and rapid utilization or uptake of nutrients or substrate.

Static/Stationary Phase: Here the growth in microbial population ceases and an equilibrium state exists among the number of cells dividing and number of cells dying. Reasons for growth cease are depletion of essential growth nutrients, accumulation of toxic by-products. Here the formation of non-growth associated products are observed.

Death/Decline Phase: The final stage of the microbe in its growth cycle. Here, the number of cells dying will be more than that of the number of cells dividing. Major reason behind the death of cells is exhaustion of all nutrients in the medium and accumulation of toxic products.

Check Your Progress

6. What is growth kinetics?
7. What happens in death phase?
8. What is the major reason behind the death of cells in death phase?

1.5 TYPES OF MICROBIAL CULTURE

Microbial culture processes can be carried out in different ways. There are three models of fermentation used in industrial applications: batch, continuous and fed batch fermentation.

NOTES

1.5.1 Batch Fermentation

A batch fermentation system is a closed system. At time t=0, the sterilized nutrient solution in the fermenter is inoculated with microorganisms and incubation is allowed to proceed at a suitable temperature and gaseous environment for a suitable period of time. In the course of the entire fermentation nothing is added, except oxygen (in case of aerobic microorganisms), an anti-foam agent, acid or base to control pH. The composition of the medium, the biomass concentration and the metabolite concentration generally change constantly as a result of metabolism of the cells. After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, six typical phases of growth are observed (Refer Figure 1.2).

Growth is a result of consumption of nutrients. The initial lag phase is a time of no apparent growth but actual biochemical analyses show metabolic turnover, indicating that cells are in the process of adapting to the environmental conditions and that new growth will eventually begin. There is then a transient acceleration phase as the inoculum begins to grow, which is quickly followed by an exponential phase. In the exponential phase, microbial growth proceeds at the maximum possible rate for that organism with nutrients in excess, ideal environmental parameters and growth inhibitors absent. However, in batch cultivation exponential growth is of limited duration and as nutrient conditions change, growth rate decreases, entering the deceleration phase, to be followed by the stationary phase, when overall growth can no longer be obtained owing to nutrient exhaustion. The final phase of the cycle is the death phase when growth rate has ceased. Most biotechnological batch processes are stopped before this stage because of decreasing metabolism and cell lysis. Typical microbial cultures in the laboratory (in a flask) are batch cultures.

Batch culture systems provide the following advantages and disadvantages:

Advantages

- Reduced risk of contamination or cell mutation as the growth period is short.
- Lower capital investment when compared to continuous processes for the same bioreactor volume.
- More flexibility with varying product/biological systems.
- Higher raw material conversion levels, resulting from a controlled growth period.

Disadvantages

- Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
- Increased focus on instrumentation due to frequent sterilization.
- Greater expense incurred in preparing several subcultures for inoculation.

NOTES

- Higher costs for labor and/or process control for this non-stationary procedure.
- Larger industrial hygiene risks due to potential contact with pathogenic microorganisms or toxins.

Common Applications for Batch Cultures

- Products that must be produced with minimal risk of contamination or organism mutation.
- Operations in which only small amounts of product are produced.
- Processes using one reactor to make various products.
- Processes in which batch or semi-continuous product separation is adequate.

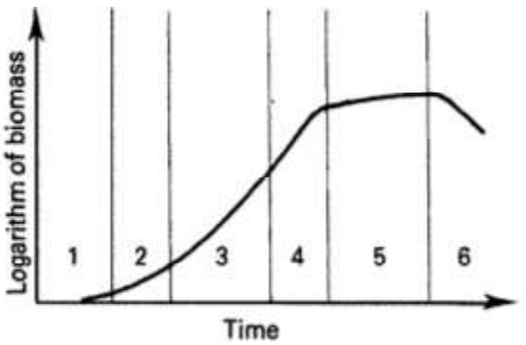


Fig. 1.2 *Growth Characteristics In A Batch Culture Of A Microorganism,*

In this figure the numbers denotes the different phases, i.e., 1 shows the Lag Phase, 2 shows the Transient Acceleration, 3 shows the Exponential Phase, 4 shows the Deceleration Phase, 5 shows the Stationary Phase, 6 shows the Death Phase

1.5.2 Continuous Fermentation

In continuous fermentation an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system. In a homogeneously mixed bioreactor, we can have a chemostat or a turbidostat. In the chemostat, in the steady state adjusting the concentration of one substrate controls cell growth. In the turbidostat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.

In the chemostat, constant chemical environment is maintained, while in a turbidostat constant cell concentration is maintained. In a chemostat the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from the reservoir. The volume of fluid in the growth chamber is maintained at a constant level by some sort of overflow

drain. Fresh medium is allowed to enter the growth chamber at a rate that limits the growth of the bacteria. The rate of addition of fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances and the accumulation of excess cells in the culture which are the parameters that initiate the stationary phase of the growth cycle.

A simple laboratory fermenter operating on a continuous cultivation basis is shown in Figure 1.3.

There are several major advantages and disadvantages of using continuous cultures as opposed to batch cultures, few of them are stated below

Advantages

- Continuous reactions offer increased opportunities for system investigation and analysis. As the variables remain unchanged, a benchmark can be determined for the process results, and then the effects of even minor changes to physical or chemical variables can be evaluated. By changing the growth-limiting nutrient, changes in cell composition and metabolic activity can be tracked. The constancy of the continuous process also provides a more accurate picture of kinetic constants, maintenance energy and true growth yields.
- Continuous culture provides a higher degree of control than a batch culture. Growth rates can be regulated and maintained for extended periods. By varying the dilution rate, biomass concentration can be controlled. Secondary metabolite production can be sustained simultaneously along with growth. In steady state continuous culture, mixed cultures can be maintained using chemostat cultures – unlike in a batch process where one organism usually outgrows another.
- Bioreactors operated as chemostats can be used to enhance selectivity for thermophiles, osmotolerant strains or mutant organisms with high growth rates. Also the medium composition can be optimized for biomass and product formation using a pulse- and shift method that injects nutrients directly into the chemostat. As changes are observed, the nutrient is added to the medium supply reservoir and a new steady state is established.
- Because of the steady state of continuous culture, the results are not only more reliable but also more consistent leading to a better quality product.
- It also results in higher productivity per unit volume, as time consuming tasks, such as cleaning and sterilization are unnecessary.
- The ability to automate the process makes it more cost-efficient and less sensitive to the impact of human error.

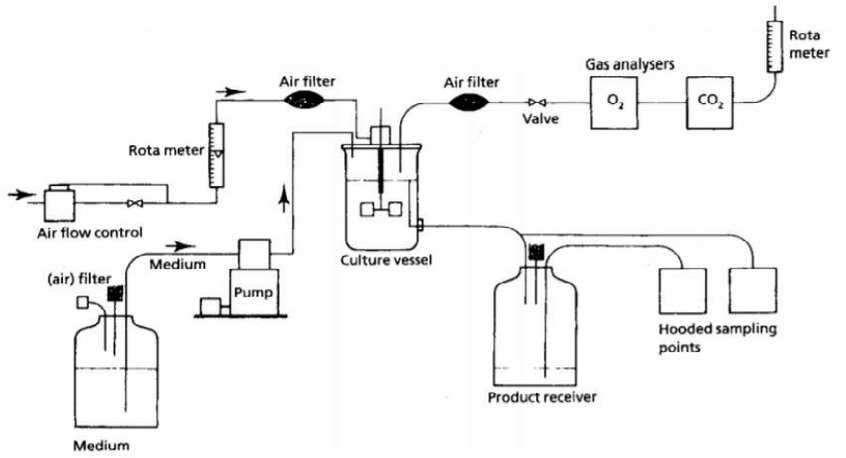


Fig. 1.3 *A Simple Laboratory Fermenter Operating on a Continuous Cultivation Basis.*

Disadvantages

- The control of the production of some non-growth related products is not easy. For this reason, the continuous process often requires feed-batch culturing and a continuous nutrient supply.
- Wall growth and cell aggregation can also cause wash-out or prevent optimum steady-state growth.
- The original product strain could be lost over time if a faster growing one overtakes it.
- The viscosity and heterogeneous nature of the mixture can also make it difficult to maintain filamentous organisms.
- Long growth periods not only increase the risk of contamination but also dictate that the bioreactor must be extremely reliable and consistent, incurring a potentially larger initial expenditure in higher quality equipment.

1.5.3 Synchronous Growth

Synchronous growth of a bacterial population is that during which all bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time. Synchronous growth helps studying particular stages or the cell division cycle and their interrelations.

In most of the bacterial cultures the stages of growth and cell division cycle are completely random and thus it becomes difficult to understand the properties during the course of division cycle using such cultures. To overcome this problem, the microbiologists have developed synchronous culture techniques to find synchronous growth of bacterial population.

Synchronous culture is that in which the growth is synchronous, i.e., all the bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time.

A synchronous culture can be obtained either by manipulating environmental conditions such as by repeatedly changing the temperature or by adding fresh nutrients to cultures as soon as they enter the stationary phase, or by physical separation of cells by centrifugation or filtration.

An excellent and most widely used method to obtain synchronous cultures is the Helmstetter-Cummings Technique (Refer Figure 1.4) in which an unsynchronized bacterial culture is filtered through cellulose nitrate membrane filter.

The loosely bound bacterial cells are washed from the filter, leaving some cells tightly associated with the filter. The filter is now inverted and fresh medium is allowed to flow through it.

New bacterial cells, that are produced by cell division and are not lightly associated with the filter, are washed into the effluent. Hence, all cells in the effluent are newly formed and are, therefore at the same stage of growth and division cycle. The effluent thus represents a synchronous culture.

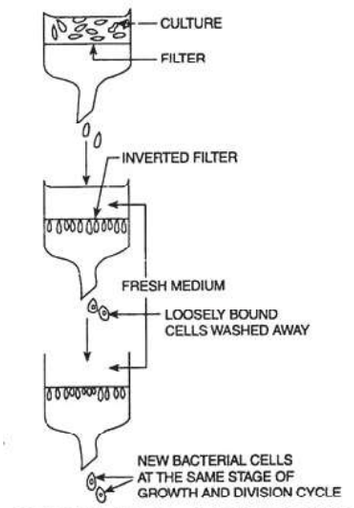


FIG. 19.4. Helmstetter-Cumming technique of obtaining synchronous cultures.

Fig 1.4 *The Helmstetter-Cummings Technique*

Check Your Progress

9. In how many ways microbial culture carried out?
10. Give two advantages of batch culture.
11. Give two disadvantages of batch culture.
12. What is the use of synchronous growth?

NOTES

1.6 FACTORS AFFECTING BECTERIAL GROWTH

Microorganisms are similar to more complex organisms in that they need a variety of materials from their environment to function and accomplish two primary goals—supply enough energy to manage their processes and extract building blocks to repair themselves or procreate. In addition to what they take in, microorganisms also thrive in particular environments. These environments vary as much as the organisms do themselves, and even the amount and distribution of elements in any particular environment can be very important. Scientists use this information to grow microorganisms in laboratories for experimentation.

1.6.1 Nutrients

All microorganisms need food. Different bacteria have different nutritional requirement. The food sources can vary, but the organisms primarily extract carbon and nitrogen from substances such as proteins, fats and carbohydrates. Some microorganisms seek out and absorb such particles. Others may perform chemical reactions with surrounding elements such as carbon dioxide to gain what they need, while still others can produce their own simple sugars through photosynthesis similar to plants. Nitrogen, which is used to synthesize proteins, can be taken from the surrounding atmosphere or from other organic matter.

If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate. With increase in concentration nutrition, growth rate of bacteria increases up to certain level and then growth rate remains constant irrespective of nutrition addition.

The relationship between substrate concentration (nutrition) and growth rate is shown in Figure 1.5.

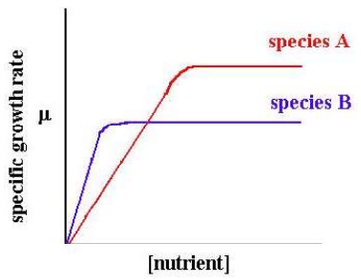


Fig. 1.5 *Nutrient vs. Growth Rate*

1.6.2 Temperature

In general, the higher the temperature, the more easily microorganisms can grow up to a certain point. Very high and very low temperatures both obstruct the enzyme processes microorganisms depend on to survive, but individual species of

microorganisms have grown to prefer different levels of temperature. Scientists usually divide them into three different groups: psychrophiles, mesophiles and thermophiles.

- Psychrophiles prefer temperatures from 0 to 5 degrees Celsius
- Mesophiles like it in the middle, 20-45 degrees Celsius
- Thermophiles like it hot, thriving in temperatures around or above 55 degrees

The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature. There is no growth below minimum and above maximum temperature. Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs. Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases. When temperature is increases continuously from its minimum, growth rate of bacteria increases because the rate of metabolic reaction increases with increase in temperature. At certain temperature the growth rate become maximum, this temperature is known as optimal temperature. On further increasing the temperature above optimal, growth rate decreases abruptly and completely cease with reaching maximum temperature.

The relationship between temperature and growth rate is shown in Figure 1.6 below.

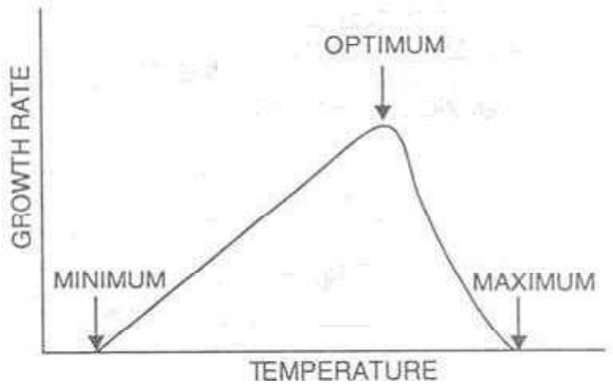


Fig. 1.6 *Temperature vs. Growth Rate*

1.6.3 pH Levels

Microorganisms also prefer a certain pH level in the substance or environment in which they grow,i.e., they prefer to have particular acidic qualities in their surroundings. Most microorganisms, including most human pathogens, are neutrophils, organisms that prefer a neutral pH level. Some like high pH levels, but most often, if conditions are too acidic, then the organism's enzymes break down.

NOTES

The relationship between pH and bacterial growth is given in Figure 1.7 below.

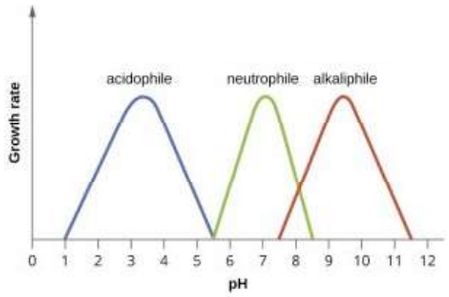


Fig. 1.7 *pH vs Growth Rate*

1.6.4 Water

The free flow of water is vital to microorganisms for their cells to exchange materials and for their metabolic processes. All microorganisms require some level of water, but a few can survive in low-moisture conditions by conserving all the water they find and by staying in a moisture-rich environment. As a general rule, though, the more moisture, the more microorganisms will be found there.

1.6.5 Elements Present

In addition to water, microorganisms usually require the presence of certain elements in the air- gases that they absorb to produce needed nutrients. Nitrogen is one necessary element, as is oxygen and carbon dioxide. Oxygen is required for aerobic respiration and obligate aerobic bacteria must require Oxygen for growth. For example, Mycobacterium, Bacillus. For obligate anaerobes Oxygen is harmful or sometime lethal. However facultative anaerobes can tolerate low concentration of Oxygen. Carbon-dioxide is needed for capnophilic bacteria. Such as Campylobacter, Helicobacter pylori.

1.6.6 Ions and Salt

All bacteria require metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++, Mn++, etc. to synthesize enzymes and proteins. Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt. There are some halophilic bacteria such as Archaebacteria that require high concentration of salt in media.

Check Your Progress

13. What are the factors that affect microbial growth?
14. What is minimum temperature?
15. What is maximum temperature
16. Why is free flow of water important to organisms?

1.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

NOTES

1. The time period in which bacteria is metabolically active but do not divide is called as lag phase.
2. Lag phase is characterized by the period during which there is no increase in number of cell.
3. During log phase bacteria divides continuously at constant rate and the number of bacteria increase exponentially.
4. There are four phases of growth named; lag phase, the exponential or log phase, the stationary phase, and the death phase, each of which are associated with specific physiological changes.
5. The bacteria growth reaches a state during which there is no net increase in bacterial population. This is called as stationary phase.
6. Kinetics of growth refers to the rate at which the number of individual cells (or, more general, of active biomass) changes in a defined system.
7. Death/Decline phase is the final stage of the microbe in its growth cycle. Here, the number of cells dying will be more than that of the number of cells dividing.
8. Major reason behind the death of cells in death phase is the exhaustion of all nutrients in the medium and accumulation of toxic products.
9. Microbial culture processes can be carried out in different ways. There are three models of fermentation used in industrial applications: batch, continuous and fed batch fermentation.
10. Two advantages of batch culture are -
    - Reduced risk of contamination or cell mutation as the growth period is short.
    - Lower capital investment when compared to continuous processes for the same bioreactor volume.
11. Two disadvantages of batch culture are -
    - Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
    - Increased focus on instrumentation due to frequent sterilization.
12. Synchronous growth helps studying particular stages or the cell division cycle and their interrelations.
13. The factors affecting microbial growth are- nutrients, temperature, pH levels, water, elements present, ions and salt.

NOTES

14. The lowest temperature that allows the growth is called minimum temperature.
15. The highest temperature that allows growth is called maximum temperature.
16. The free flow of water is vital to microorganisms for their cells to exchange materials and for their metabolic processes.

1.8 SUMMARY

- Bacteria are among the most abundant life forms on Earth.
- They are found in every ecosystem and are vital for everyday life.
- Bacteria affect what people eat, drink, and breathe, and there are actually more bacterial cells within a person's body than mammalian cells.
- Bacteria are grown under controlled conditions in pure culture, meaning that only one type of bacterium is under consideration.
- Bacteria grow quickly in pure culture, and cell numbers increase dramatically in a short period of time.
- By measuring the rate of cell population increase over time, a 'growth curve' to be developed.
- Bacterial reproduction occurs via binary fission, in which one bacterial cell divides and becomes two cells.
- The time needed for cell division is known as the mean generation time, or doubling time, which is the time needed for the number of cells to double.
- Each cell division results in a doubling of the cell number. At low cell numbers, the increase is not very large; however, after a few generations, cell numbers increase explosively. After n divisions, there are 2n cells.
- There are four phases of growth named; lag phase, the exponential or log phase, the stationary phase, and the death phase, each of which are associated with specific physiological changes.
- When bacteria are inoculated into new fresh media, it does not divide immediately.
- Bacteria takes some time to adjust to the new environment. The time period in which bacteria is metabolically active but do not divide is called as lag phase.
- Lag phase is characterized by the period during which there is no increase in number of cell.
- Size of bacteria increase continuously so the bacteria have largest size at the end of lag phase. In this phase, microorganism tries to adopt in new environment.
- During log phase bacteria divides continuously at constant rate and the number of bacteria increase exponentially.

- In log phase all bacteria are in their rapid stage of cell division and show balanced growth.
- Due to rapid cell division, bacteria have smallest size in this phase. Bacterial population is nearly uniform in terms of their metabolic activities, chemical composition of cell and other physiological characteristics.
- The bacteria growth reaches a state during which there is no net increase in bacterial population. This is called as stationary phase. In this phase a constant bacterial population is maintained by balance between cell division and cell death.
- Production of antibiotics such as Penicillin, streptomycin etc. and enzymes by certain bacteria occur during stationary phase of their growth. In endospore forming bacteria, sporulation occurs as the bacteria enter stationary phase.
- Growth is a result of consumption of nutrients. The initial lag phase is a time of no apparent growth but actual biochemical analyses show metabolic turnover, indicating that cells are in the process of adapting to the environmental conditions and that new growth will eventually begin.
- Synchronous growth of a bacterial population is that during which all bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time.
- In most of the bacterial cultures the stages of growth and cell division cycle are completely random and thus it becomes difficult to understand the properties during the course of division cycle using such cultures.
- All microorganisms need food. Different bacteria have different nutritional requirement. The food sources can vary, but the organisms primarily extract carbon and nitrogen from substances such as proteins, fats and carbohydrates. Some microorganisms seek out and absorb such particles.
- Microorganisms also prefer a certain pH level in the substance or environment in which they grow—that is, they prefer to have particular acidic qualities in their surroundings.

1.9 KEY WORDS

- **Lag phase:** The time period in which bacteria is metabolically active but do not divide.
- **Log phase or exponential phase:** The time period in which bacteria divides continuously at constant rate and the number of bacteria increase exponentially.
- **Stationary phase:** The time period when bacteria growth reaches a state during which there is no net increase in bacterial population.

NOTES

- **Death phase or decline phase:** In this phase, number of bacteria decrease continuously and exponentially.
- **Minimum temperature:** The lowest temperature that allows the growth.
- **Maximum temperature:** The highest temperature that allows growth.

1.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Give a brief introduction about bacteria.
2. What is log phase?
3. Distinguish between stationary phase and death phase.
4. What is batch fermentation?
5. Explain in brief about synchronous growth.
6. Name the factors that effects bacterial growth and explain them in brief.

Long-Answer Questions

1. Explain growth of bacteria in detail.
2. Discuss about growth kinetics in detail.
3. Explain about synchronous culture.
4. Discuss about the factors affecting bacterial growth in detail.
5. Discuss about different types of microbial culture.

1.11 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 2 MICROBIAL NUTRITION

NOTES

Structure

2.0 Introduction
2.1 Objectives
2.2 Nutritional Diversity in Microogranism
2.3 Nutritional Types of Microoganisms
2.4 Requirement for Nitrogen, Phosphorus and Sulfur
2.5 Growth Factor
2.6 Answers to Check Your Progress Questions
2.7 Summary
2.8 Key Words
2.9 Self Assessment Questions and Exercises
2.10 Further Readings

2.0 INTRODUCTION

Nutrients are materials that are acquired from the environment and are used for growth and metabolism. Microorganisms or microbes vary significantly in terms of the source, chemical form, and amount of essential elements they need. Some examples of these essential nutrients are carbon, oxygen, hydrogen, phosphorus, and sulfur.There are two categories of essential nutrients: macro-nutrients which are needed in large amounts and micro-nutrients which are needed in trace or small amounts. Macro-nutrients usually help maintain the cell structure and metabolism. Micro-nutrients help enzyme function and maintain protein structure. Organic nutrients contain some combination of carbon and hydrogen atoms. Inorganic nutrients are elements or simply molecules that are made of elements other than carbon and hydrogen.

There are two sources of energy available to microorganisms, and based on this they are of two types, i.e., phototrophs in which energy for growth is derived from sunlight and chemotrophs in which energy for growth is derived from the oxidation of either organic and inorganic chemical compounds.

In this unit, you will study about nutritional diversity in microorganisms, nutritional types - autotrophy, heterotrophy, chemotrophy, phototrophy, lithotrophy and organotrophy, nutrition essentiality of major and minor elements.

2.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand nutritional diversity in microorganisms
- Explain about the nutritional types

- Discuss learn about autotrophy, heterotrophy, chemotrophy, phototrophy, lithotrophy and organotrophy
- Analyse the nutrition essentiality of major and minor elements

NOTES

2.2 NUTRITIONAL DIVERSITY IN MICROOGRANISM

To obtain energy and construct new cellular components, organisms, must have a supply of raw materials or nutrients. Nutrients – are substances used in biosynthesis and energy production.

Nutrient Requirements

Microbial cell composition shows that 95% of cell dry weight is made up of a few major elements: Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.

Macronutrients or Macro Elements

These are required by microorganisms in relatively large amounts. Carbon, oxygen, hydrogen nitrogen, sulfurs and phosphorous are components of carbohydrates, lipids, proteins and nucleic acids. The remaining four macro elements (K, Ca, Mg and Fe) exist in the cell as cations.

- K^+ - Is required for the activity by a number of enzymes, including those involved in protein synthesis.
- $Ca2^+$ - Contributes to the heat resistance of bacterial endospores. 15% of spore contains dipicolinic acid and calcium.
- $Mg2^+$ - Serves as a cofactor for many enzymes, complexes with ATP and stabilizes ribosomes and cell membranes.
- $Fe2^+$ and $Fe2^+$ - Part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

Micronutrients or Trace Elements

These are manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.

- Zn^{2+} - Is present at the active site of some enzymes but is also involved in the association of regulatory and catalytic sub-units in *E. coli* aspartate carbomoyltransferase.
- Mn^{2+} - Aids many enzymes catalyzing the transfer of phosphate groups.
- Mo^{2+} - Required for nitrogen fixation.
- Co^{2+} - Is a component of Vitamin B12.

NOTES

Besides macro and micro nutrients, some microorganisms may have particular requirements that reflect the special nature of their morphology or environment. Diatoms need silicic acid to construct their beautiful cell walls of silica. Bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ion. Microorganisms require a balanced mixture of all the above nutrients for proper growth.

Requirements For Carbon, Hydrogen and Oxygen

- Carbon is needed for the skeleton or backbone of all organic molecules and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms. One important carbon source that does not supply hydrogen or energy is CO_2 . Autotrophs – can use CO_2 as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source. Some autotrophs oxidize inorganic molecules and derive energy from electron transfer. Heterotrophs – are organisms that use reduced pre-formed organic molecules as carbon sources. For example, Glycolytic pathway produces carbon skeleton for use in biosynthesis and also releases energy as ATP and NADH. *Actinomycetes* will degrade amyl alcohol, paraffin and even rubber. *Burkholderia cepacia* can use over 100 different carbon compounds. Some microorganisms can metabolize even relatively indigestible human-made substances such as pesticides. Indigestible molecules can be oxidized and degraded in the presence of a growth promoting nutrient that is metabolized at the same time, a process called Co-metabolism. The products of this breakdown can then be used as nutrients by other microorganisms.

Check Your Progress

1. What are the nutrient found in the cell?
2. Name some of the micro-nutrients.
3. Name some of the macro-nutrients.
4. Why is carbon essential as a nutrient?

2.3 NUTRITIONAL TYPES OF MICROOGANISMS

In addition to carbon, hydrogen and oxygen all organisms require sources of energy and electrons for growth.

Carbon sources

- **Autotrophs** - CO_2 sole or principal biosynthetic carbon source
- **Heterotrophs -** Reduced, preformed organic molecules from other organisms.

NOTES

Energy sources

- **Phototrophs** – Use light as their energy source.
- **Chemotrophs** – Obtain energy from the oxidation of chemical compounds (either organic or in organic)

Electron sources

- **Lithotrophs** – Use reduced inorganic substances as their electron source.
- **Organotrophs** – Extract electrons from organic compounds.

Four major nutritional classes based on their primary sources of carbon, energy and electrons are:

- Photolithotrophic Autotrophs or Photoautotrophs or Photolithoautotrophs
- Source of energy – Light energy
- Source of electrons – Inorganic hydrogen/ electron
- Carbon source - CO_2
- Example: Algae, purple and green sulfur bacteria and cyanobacteria.
- Photoorganotrophic Heterotrophy or Photoorganoheterotrophs
- Source of energy – Light energy
- Source of electrons – Organic hydrogen/ electron
- Carbon source – Organic carbon sources (CO_2 may also be used)
- Example: Purple and green non-sulfur bacteria (common inhabitants of lakes and streams)
- Chemolithotrophic autotrophs or chemolithoautotrophs
- Source of energy – Chemical energy source (inorganic)
- Source of electrons – Inorganic hydrogen/ electron donor
- Carbon source - CO_2
- Example: Sulfur-oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron-oxidizing bacteria.
- Chemoorganotrophic Heterotrophs or Chemoorganoheterotrophs
- Source of energy – Chemical energy source (organic)
- Source of electrons – Inorganic hydrogen/ electron donor
- Carbon source – Organic carbon source
- Example: Protozoan, fungi, most non-photosynthetic bacteria (including most pathogens)

The most common nutritional types are photolithoautotrophs and chemoorganoheterotrophs. Bacteria *Beggiatoa* rely on inorganic energy sources

and organic (or sometimes CO_2) carbon sources. These microbes are sometimes called mixotrophic because they combine chemolithoautotrophic and heterotrophic metabolic processes.

NOTES

Check Your Progress

5. Which organisms uses carbon as a source?
6. Which organisms uses energy as a source?
7. Which organisms uses electron as a source?
8. Name four major nutritional classes based on their primary sources of carbon, energy and electrons.

2.4 REQUIREMENT FOR NITROGEN, PHOSPHORUS AND SULFUR

Nitrogen is needed for the synthesis of amino acids, purines, pyramidines, some carbohydrates and lipids, enzyme cofactors and other substances. Most phototrophs and many non-photosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction. A variety of bacteria like many Cyanobacteria and Rhizobium can reduce and assimilate atmospheric nitrogen using the nitrogenase systems. Phosphorous is present in nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell components. All microorganisms use inorganic phosphate as their phosphorous source and incorporate it directly. *E. coli* can use both organic and inorganic phosphate. Organophosphates such as hexose 6- phosphate can be taken up directly by transport proteins. Other organophosphates are often hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate which is then transported across the plasma membrane. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel. Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates biotin and thiamine. Most of them use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; a few require a reduced form of sulfur such as cysteine.

2.5 GROWTH FACTOR

Many microorganisms have the enzymes and pathways necessary to synthesize all cell components. Many lack one or more enzymes and hence require organic compounds because they are essential cell components or precursors of such components and cannot be synthesized by the organisms are called – growth factors.

NOTES

There are three major classes of growth factors

- Amino acids – needed for protein synthesis.
- Purines and Pyramidines – for nucleic acid synthesis
- Vitamins – small organic molecules that usually make up all or part of enzyme cofactors, and only very small amounts sustain growth.

Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth response assays for a variety of substances. The observation that many microorganisms can synthesize large quantities of vitamins has led to their use in industry. Several water-soluble and fat-soluble vitamins are produced using industrial fermentations.

Riboflavin – *Clostridium, Candida, Ashbya, Eremothecium*

- Coenzyme A – *Brevibacterium*
- Vitamin B12 – *Streptomyces, Propionibacterium, Pseudomonas*
- Vitamin C – *Gluconobacter, Erwinia, Corynebacterium*
- â- Carotene – *Dunaliella*
- Vitamin D – *Saccharomyces*

Check Your Progress

9. What is the use of nitrogen?
10. What does most of the phototrophs and many non-photosynthetic microorganisms do?
11. Where is phosphorous present?
12. Which phosphate does *E. coli* use?
13. What happens when inorganic phosphate is outside the bacterium?

2.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Microbial cell composition shows that 95% of cell dry weight is made up of a few major elements: Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.
2. Some of the micro-nutrients are manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.
3. Some of the macro-nutrients are carbon, oxygen, hydrogen nitrogen, sulfurs and phosphorous are components of carbohydrates, lipids, proteins and

nucleic acids. The remaining four macro elements (K, Ca, Mg and Fe) exist in the cell as cations.

4. Carbon is needed for the skeleton or backbone of all organic molecules and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms.
5. Organisms that uses carbon as a source are autotrophs as CO_2 sole or principal biosynthetic carbon source and heterotrophs as educed, preformed organic molecules from other organisms.
6. Organisms that uses energy as a source are phototrophs use light as their energy source and chemotrophs obtain energy from the oxidation of chemical compounds (either organic or in organic)
7. Organisms that uses electron as a source are lithotrophs that uses reduced inorganic substances as their electron source and organotrophs use extract electrons from organic compounds.
8. Four major nutritional classes based on their primary sources of carbon, energy and electrons are -
   - Photolithotrophic autotrophs or photoautotrophs or photolithoautotrophs
   - Photoorganotrophic heterotrophy or photoorganoheterotrophs
   - Chemolithotrophic autotrophs or chemolithoautotrophs
   - Chemoorganotrophic heterotrophs or chemoorganoheterotrophs.
9. Nitrogen is needed for the synthesis of amino acids, purines, pyramidines, some carbohydrates and lipids, enzyme cofactors and other substances.
10. Most phototrophs and many non-photosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction.
11. Phosphorous is present in nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell components.
12. *E. coli* can use both organic and inorganic phosphate.
13. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel.

2.7 SUMMARY

- Nutrients are materials that are acquired from the environment and are used for growth and metabolism.
- Microorganisms or microbes vary significantly in terms of the source, chemical form, and amount of essential elements they need. Some examples of these essential nutrients are carbon, oxygen, hydrogen, phosphorus, and sulfur.

NOTES

- There are two categories of essential nutrients: macro-nutrients which are needed in large amounts and micro-nutrients which are needed in trace or small amounts.
- Macro-nutrients usually help maintain the cell structure and metabolism.
- Micro-nutrients help enzyme function and maintain protein structure.
- Organic nutrients contain some combination of carbon and hydrogen atoms.
- Inorganic nutrients are elements or simply molecules that are made of elements other than carbon and hydrogen.
- There are two sources of energy available to microorganisms, and based on this they are of two types, i.e., phototrophs in which energy for growth is derived from sunlight and chemotrophs in which energy for growth is derived from the oxidation of either organic and inorganic chemical compounds.
- To obtain energy and construct new cellular components, organisms, must have a supply of raw materials or nutrients. Nutrients – are substances used in biosynthesis and energy production.
- Microbial cell composition shows that 95% of cell dry weight is made up of a few major elements: Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.
- Macronutrients or macro elements are required by microorganisms in relatively large amounts. Carbon, oxygen, hydrogen nitrogen, sulfurs and phosphorous are components of carbohydrates, lipids, proteins and nucleic acids. The remaining four macro elements (K, Ca, Mg and Fe) exist in the cell as cations.
- Micronutrients or trace elements are manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.
- Besides macro and micro nutrients, some microorganisms may have particular requirements that reflect the special nature of their morphology or environment.
- Diatoms need silicic acid to construct their beautiful cell walls of silica. Bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ion.
- Microorganisms require a balanced mixture of all the above nutrients for proper growth.
- Carbon is needed for the skeleton or backbone of all organic molecules and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms.
- One important carbon source that does not supply hydrogen or energy is CO_2 .

- Autotrophs – can use CO_2 as their sole or principal source of carbon.
- Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source.
- Some autotrophs oxidize inorganic molecules and derive energy from electron transfer.
- Heterotrophs – are organisms that use reduced pre-formed organic molecules as carbon sources.
- *Actinomycetes* will degrade amyl alcohol, paraffin and even rubber. *Burkholderia cepacia* can use over 100 different carbon compounds.
- Some microorganisms can metabolize even relatively indigestible human-made substances such as pesticides.
- Indigestible molecules can be oxidized and degraded in the presence of a growth promoting nutrient that is metabolized at the same time, a process called Co-metabolism.

2.8 KEY WORDS

- **Phototrophs:** Phototrophs are the organisms that uses energy for growth is derived from sunlight.
- **Chemotrophs:** Chemotrophs are the organisms that uses energy for growth is derived from the oxidation of either organic and inorganic chemical compounds.
- **Nutrients:** Nutrients are materials that are acquired from the environment and are used for growth and metabolism. Microorganisms or microbes vary significantly in terms of the source, chemical form, and amount of essential elements they need.
- **Heterotrophs:** A heterotroph is an organism that cannot manufacture its own food by carbon fixation and therefore derives its intake of nutrition from other sources of organic carbon,
- **Lithotrophs**: Lithotrophs are a diverse group of organisms using inorganic substrate (usually of mineral origin) to obtain reducing equivalents for use in biosynthesis (e.g., carbon dioxide fixation) or energy conservation (i.e., ATP production) via aerobic or anaerobic respiration.
- **Organotrophs**: An organotroph is an organism that obtains hydrogen or electrons from organic substrates. This term is used in microbiology to classify and describe organisms based on how they obtain electrons for their respiration processes.

NOTES

2.9 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Why are nutrients essential for microorganisms?
2. What are micro-nutrients?
3. What are macro-nutrients?
4. Why is carbon, hydrogen and oxygen required by microorganisms?
5. Which organisms uses carbon as a source?
6. What is growth factor?

Long-Answer Questions

1. Write about nutritional diversity in microorganisms.
2. Write a note on all the major micro-nutrients stating their use.
3. Elaborate about all the macro-nutrients and also their functions.
4. Explain about the essentiality of nutrients for the microorganisms and also the nutritional types of microorganisms.
5. Discuss about the growth factor in detail.
6. Elaborate about the need for nitrogen, phosphorous and sulphur.

2.10 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 3 CHEMOTROPHISM: AN INTRODUCTION

NOTES

Structure

3.0 Introduction
3.1 Objectives
3.2 Chemotrophism and its Importance
3.2.1 Maximum Yield Explains Chemotropism
3.3 Reverse Electron Flow
3.4 CO_2 Assimilation
3.5 Calvin Cycle
3.6 Answers to Check Your Progress Questions
3.7 Summary
3.8 Key Words
3.9 Self Assessment Questions and Exercises
3.10 Further Readings

3.0 INTRODUCTION

Chemotropism is the growth of organisms or parts of an organism, including individual cells such as bacteria and plants, navigated by chemical stimulus from outside of the organism or organisms part. The response of the organism or organism part is termed 'positive' if the growth is towards the stimulus or 'negative' if the growth is away from the stimulus. An example of chemotropic movement can be seen during the growth of the pollen tube, where growth is always towards the ovules. It can be also written that conversion of flower into fruit is the example of chemotropism. Fertilization of flowers by pollen is achieved because the ovary releases chemicals that produce a positive chemotropic response from the developing pollen tube. An example of positive and negative chemotropism is shown by a plant's roots; the roots grow towards useful mineralsdisplaying positive chemotropism, and grow away from harmful acids displaying negative chemotropism. Another example of chemotropic movement of includes the growth of individual neuronal cell axons in response to extra cellular signals, which guide the developing axon to innervate the correct target tissue. Also, the addition of atmospheric nitrogen, also called nitrogen fixation, is an example of chemotropism.

According to botanists, plants can display both negative and positive chemotropisms. Studies have shown that some plants tend to turn away from poor-quality soil and face better growing conditions instead, suggesting a form of chemical sensing in the root and stem system.Because of chemotropism, some plant roots develop an increased sensitivity to potential attackers such as fungi or bacteria.

NOTES

In this unit, you will study about chemotropism and its importance, reverse electron transport, CO_2 assimilation and reductive Acetyl-CoA pathway.

3.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand about chemotrophism and their importance
- Explain reverse electron transport
- Discuss CO_2 assimilation
- Know about reductive Acetyl-CoA pathway

3.2 CHEMOTROPHISM AND ITS IMPORTANCE

In plant biology, any type of 'tropism' is a plant's physical response to some sort of external stimuli. The response is typically movement of some sort.

Chemotropism is a form of tropism whereby the plant's organism grows in response to a chemical stimulus. According to botanists, plants can display both negative and positive chemotropisms.

Studies have shown that some plants tend to turn away from poor-quality soil and face better growing conditions instead, suggesting a form of chemical sensing in the root and stem system.

3.2.1 Maximum Yield Explains Chemotropism

Because of chemotropism, some plant roots develop an increased sensitivity to potential attackers such as fungi or bacteria. Similarly, these plants have a heightened awareness of nutrient sources and automatically proliferate in areas with a high concentration of iron. Additionally, some studies have shown that pollen can also respond to chemical stimulation, whereby the pollen tube starts to learn towards external stimuli.

According to botanists, plants also move in the following main ways:

- Hydrotropism (growth or developmental response to water)
- Thermometer (response dependent upon temperature)
- Phototropism (movement toward light)
- Gravitropism and geotropism (movement relative to a gravitational field, or toward the center of the Earth)
- Thigmotropism (plant growth in response to physical contact)

NOTES

Check Your Progress

1. What is chemotropism?
2. What type of chemotropism plant show?
3. What is hydrotropism?
4. Define thigmotropism.

3.3 REVERSE ELECTRON FLOW

Reverse electron flow (also known as reverse electron transport) is a mechanism in microbial metabolism. Chemolithotrophs using an electron donor with a higher redox potential than NAD(P)+/NAD(P)H, such as nitrite or sulfur compounds, must use energy to reduce NAD(P)+. This energy is supplied by consuming proton motive force to drive electrons in a reverse direction through an electron transport chain and is thus the reverse process as forward electron transport. In some cases, the energy consumed in reverse electron transport is five times greater than energy gained from the forward process. Autotrophs can use this process to supply reducing power for inorganic carbon fixation.

3.4 CO_2 ASSIMILATION

Although most microorganisms can fix or assimilate carbon dioxide (CO_2), only autotrophic ones use CO_2 as their sole or principal carbon source.

The reduction or assimilation of CO_2 takes place at the expense of much energy. Usually autotrophic microorganisms obtain the required energy by trapping light during photosynthesis (photoautotrophs), but some derive it from the oxidation of reduced inorganic electron donors (chemoautotrophs).

Microorganisms can fix CO_2 or convert this inorganic molecule to organic carbon and assimilate it in certain major ways, which are the Calvin cycle (also called Calvin-Benson cycle, or reductive pentose phosphate pathway), the reductive tricarboxylic acid cycle (also called reductive TCA cycle, or reverse citric acid cycle), the hydroxypropionate cycle or Acetyl-CoA pathway.

Carbon-dioxide (CO_2) is incorporated by almost all microbial autotrophs using Calvin cycle, a special metabolic pathway. Although this cycle in most photosynthetic microorganisms, it is absent in archaea (archaebacteria), some obligately anaerobic bacteria, and some microaerophilic bacteria. These microorganisms usually use rest of the two above mentioned pathways.

NOTES

The reductive tricaboxylic cycle is used by some archaea (archaebacteria), for example, Thermoproteus, Sulfolobous and by bacteria such as Chlorobium, a green sulphur bacterium. Chloroflexus, a green non-sulphur photoautotroph uses the unique pathway of hydroxypropionate.

Check Your Progress

5. What is reverse electron flow?
6. How do autotrophs use reverse electron flow?
7. What do autotrophs use as carbon source?
8. How does reduction of CO_2 occur?
9. How do autotrophs obtain energy?

3.5 CALVIN CYCLE

Phototropic microorganisms (microalgae, cyanobacteria, purple and green bacteria), like plants, assimilate CO_2 to produce carbohydrate principally through Calvin cycle (Refer Figure 3.1). The latter is named for its discover Melvin Calvin and is also popular by the names Calvin-Benson cycle or reductive pentose phosphate cycle.

The Calvin cycle requires NAD(P)H and ATP and two key enzymes, ribulose-1, 5-biphosphate carboxylase (ribulose bisphosphate carboxylase) and phosphoribulokinase. To understand the Calvin-cycle easily, it can be divided into three phases:

- Carboxylation
- Reduction
- Regeneration

1. The Carboxylation Phase

During this phase of CO_2 fixation, the enzyme ribulose-1, 5-biphosphate carboxylase or ribulose bisphosphate carboxylase (in short form called RUBISCO) catalyzes the incorporation of CO_2 to ribulose-1, 5-biphosphate (RuBP) to generate two molecules of 3-phosphoglyceric acid (PGA).

2. The Reduction Phase

3-phosphoglyceric acid (PGA) is reduced to glyceraldehyde 3-phosphate with the involvement of two enzymes. Phosphoglycerate kinase enzyme reduces 3-phosphoglyceric acid into 1, 3-biphosphate glyceric acid which is then reduced to glyceraldehyde 3-phosphate by enzyme glyceraldehyde 3-phosphate dehydrogenase.

NOTES

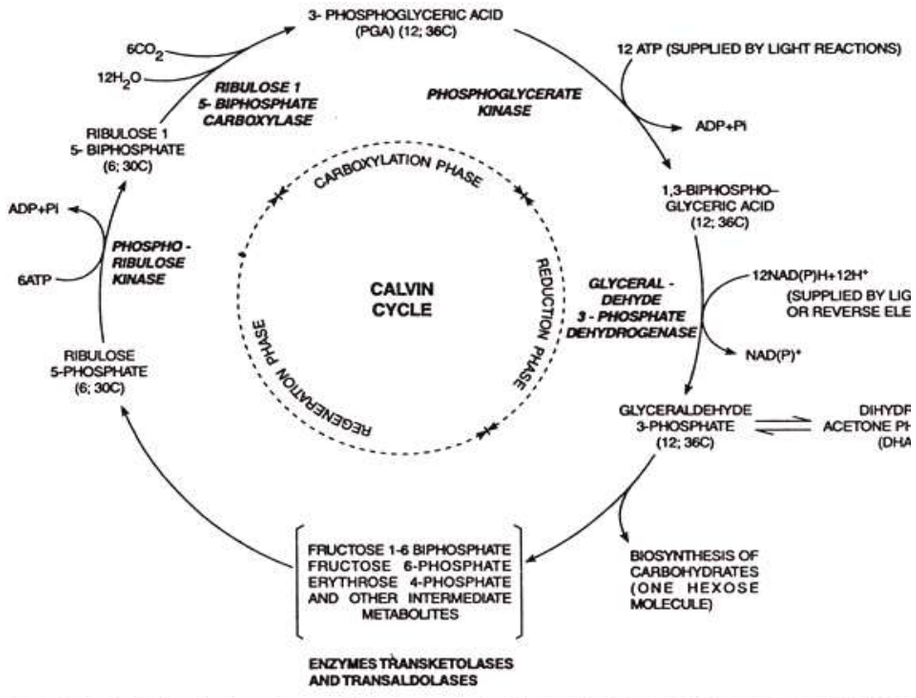


Fig. 3.1 *The Calvin Cycle*

3. The Regeneration Phase

During this phase the ribulose-1, 5-biphosphate (RuBP) is regenerated and carbohydrates such as fructose and glucose are produced. Glyceraldehyde 3-phosphate is converted to dihydroxyacetone phosphate (DHAP); this conversion is reversible.

Most of these two,i.e., glyceraldehyde 3-phosphate and DHAP are used to regenerate ribulose-1, 5-biphosphate via various intermediate steps involving transketolase and transaldolase reactions, the remaining ones are used in the biosynthesis of carbohydrates.

The stoichiometry of the Calvin cycle can be represented in few words as 12 NADPH and 18ATP are required to synthesize 1 hexose molecule (glucose) from 6 molecules of CO_2

The overall equation can be summarized as under:

$6CO_2 + 18ATP + 12NADPH + 12H+ + 12H_2O \rightarrow 1$ Hexose + 18ADP+ 18Pi + 12NADP+

Reductive or Reverse Tricarboxylic Acid Cycle (Reduced TCA Cycle)

The reductive tricarboxylic acid cycle (reduced TCA cycle, reduced Krebs cycle, or reduced citric acid cycle), also called reverse TCA cycle is used as alternative

NOTES

mechanism of fixation by phototrophic green sulphur bacteria (for example, Chlorobium) and by some non-phototrophic archaebacteria (Thermoproteus, Sulfolobus and Aquifex).

In this cycle, the CO_2 fixation takes place by a reversal of steps in the tricarboxylic acid cycle (a major pathway of respiration by which pyruvate is completely oxidised to CO_2). In Chlorobium, there are two ferredoxin-linked enzymes that catalyse the reductive fixation of CO_2into intermediates of the tricarboxylic acid cycle.

The two ferredoxin-linked reactions involve the carboxylation of succinyl-CoA to á-ketoglutarate and the carboxylation of Acetyl-CoA to pyruvate (Refer Figure 3.2).

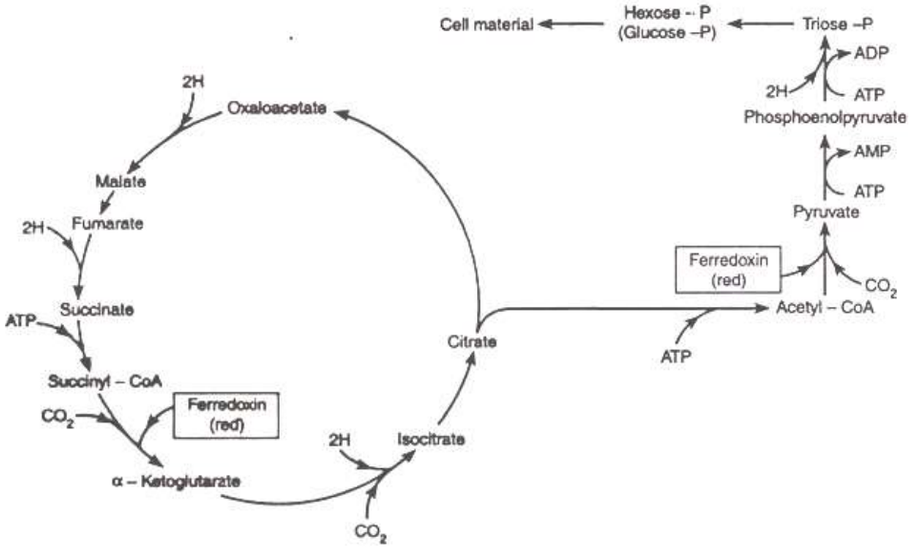


Fig. 3.2 *The Reductive Tricarboxylic Acid Cycle*

The reductive tricarboxylic acid cycle starts from oxaloacetate and each complete turn of the cycle results in three molecules of CO_2 being incorporated and pyruvate as the product.

All reactions of the cycle are catalysed by enzymes of normal tricarboxylic acid cycle, but they work in reverse. One exception is citrate lyase, an ATP-dependent enzyme that cleaves citrate into Acetyl-CoA and oxaloacetate in green sulphur bacteria.

Citrate lyase replaces citrate synthetase that produces citrate from oxaloacetate and Acetyl-CoA in the normal TCA cycle. However, the Acetyl-CoA of reduced TCA cycle produces pyruvate, which is converted to phosphoenolpyruvate that then results in triose-phosphate. The triose-phosphate converts into hexose- phosphate (glucose-phosphate), which is utilized in cell material.

NOTES

Hydroxypropionate Pathway

Hydroxypropionate pathway (Refer Figure 3.3) is also a mechanism of autotrophic CO_2 fixation unique to green non-sulphur bacteria (Chloroflexus). Chloroflexus, an anoxygenic photoautotroph, uses either H_2 or H_2S as electron donors.

In hydroxypropionate pathway, two molecules of CO_2 are reduced to glyoxylate. Acetyl-CoA is carboxylated to yield methylmalnonyl-CoA. This intermediate is rearranged to yield Acetyl-CoA and glyoxylate. The latter is converted to cell material.

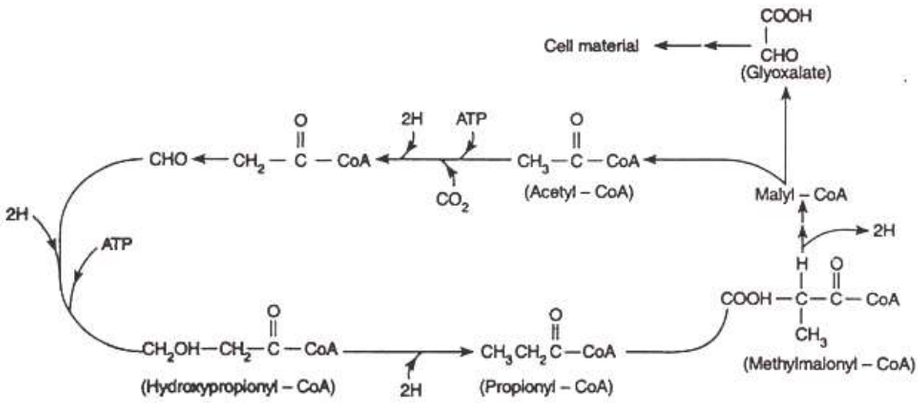


Fig. 3.3 *The Hydroxypropionate Pathway*

Hydroxypropionate pathway has so far been confirmed only in Chloroflexus and appears to be of evolutionary significance. Chloroflexus is a "hybrid" photoautotroph in the sense that its photosynthetic mechanism shows features characteristic of both purple sulphur bacteria and green sulphur bacteria. Bacteriochlorophyll a located in the cytoplasmic membrane of cells of Chloroflexus is arranged to form a photosynthetic reaction center structurally similar to those of purple bacteria.

Chloroflexus, on the other hand, contains bacteriochlorophyll c and chlorosomes (oblong bacteriochlorophyll-rich bodies bound by a thin, non-unit membrane lying attached to the cytoplasmic membrane in the periphery of the cell) like green sulphur bacteria.

It has thus been proposed that modern Chloroflexus may be a vestige of a very early phototrophic ancestor that perhaps first evolved a photosynthetic reaction center and then received chlorosome-specific genes by lateral transfer.

Reductive Acetyl CoA Pathway

The conversion of carbon dioxide (CO_2) into organic material drives the global carbon cycle and is a prerequisite for all life forms that evolved on earth. The

NOTES

biological conversion of CO_2 into reduced compounds needs the extraction of energy from the environment. Plants and many microbes use the reductive pentose phosphate cycle, also known as Calvin-Benson cycle, which is the predominating way of CO_2 fixation. Enzymes of the reductive pentose phosphate cycle are oxygen-tolerant, which is likely responsible for their wide distribution. The reductive Acetyl-CoA pathway, also called Wood-Ljungdahl pathway, is a way along which CO_2 enters the microbial carbon cycle under anaerobic conditions. Among the six different known pathways of autotrophic carbon fixation, the reductive Acetyl-CoA pathway is most likely the most ancient and evolved long before the oxygenation of the atmosphere. This pathway was likely present in the last universal common ancestor. In contrast to CO_2 fixation along the reductive pentose phosphate cycle, the reductive Acetyl-CoA pathway is restricted to strictly anoxic conditions. It was likely because of the anoxic conditions found in the early history of life on earth that metalloenzymes with complex metal clusters could evolve to catalyse the respective reactions. The reliance on these oxygen-sensitive metalloenzymes is the likely reason why prokaryotes employing the reductive Acetyl-CoA pathway are restricted to anoxic niches. The reductive Acetyl-CoA pathway is found in phylogenetically diverse microbes, including anaerobic acetogenic, sulfidogenic, methanogenic and hydrogenogenic bacteria, and archaea. Accordingly, the pathway operates in diverse anaerobic habitats from the gastrointestinal tract of animals and insects to the exotic environment of hot springs. The reductive Acetyl-CoA pathway has a unique appeal to mechanistic biochemists and structural biologists, due to the unusual enzymes with unprecedented cofactors and unique reactions found in the pathway. Two of the central catalysts of the pathway contain Ni, Fe and S ions in a unique assembly that resembles Ni, Fe, S-containing minerals with related catalytic capabilities.

Check Your Progress

10. How many phases does Calvin- cycle have?
11. What happens in carboxylation phase?
12. What is reduction phase?
13. What is hydroxypropionate pathway?

3.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Chemotropism is a form of tropism whereby the plant's organism grows in response to a chemical stimulus.
2. According to botanists, plants can display both negative and positive chemotropisms.

NOTES

3. Hydrotropism is the growth or developmental response to water.
4. Thigmotropism is plant growth in response to physical contact.
5. Reverse electron flow also known as reverse electron transport is a mechanism in microbial metabolism.
6. Autotrophs can use this process to supply reducing power for inorganic carbon fixation.
7. Although most microorganisms can fix or assimilate carbon dioxide (CO_2), only autotrophic ones use CO_2 as their sole or principal carbon source.
8. The reduction or assimilation of CO_2 takes place at the expense of much energy.
9. Usually autotrophic microorganisms obtain the required energy by trapping light during photosynthesis (photoautotrophs), but some derive it from the oxidation of reduced inorganic electron donors (chemoautotrophs).
10. Calvin-cycle easily, is divided into three phases:
    - Carboxylation
    - Reduction
    - Regeneration
11. During carboxylation phase CO_2 fixation, the enzyme ribulose-1, 5-biphosphate carboxylase or ribulose bisphosphate carboxylase (in short form called RUBISCO) catalyzes the incorporation of CO_2 to ribulose-1, 5-biphosphate (RuBP) to generate two molecules of 3-phosphoglyceric acid (PGA).
12. During reduction phase 3-phosphoglyceric acid (PGA) is reduced to glyceraldehyde 3-phosphate with the involvement of two enzymes. Phosphoglycerate kinase enzyme reduces 3-phosphoglyceric acid into 1, 3-biphosphate glyceric acid which is then reduced to glyceraldehyde 3-phosphate by enzyme glyceraldehyde 3-phosphate dehydrogenase.
13. Hydroxypropionate pathway is also a mechanism of autotrophic CO_2 fixation unique to green non-sulphur bacteria (Chloroflexus). Chloroflexus, an anoxygenic photoautotroph, uses either H_2 or H_2S as electron donors.

3.7 SUMMARY

- Chemotropism is a form of tropism whereby the plant's organism grows in response to a chemical stimulus.
- According to botanists, plants can display both negative and positive chemotropisms.
- Studies have shown that some plants tend to turn away from poor-quality soil and face better growing conditions instead, suggesting a form of chemical sensing in the root and stem system.

NOTES

- Because of chemotropism, some plant roots develop an increased sensitivity to potential attackers such as fungi or bacteria.
- Reverse electron flow (also known as reverse electron transport) is a mechanism in microbial metabolism.
- Chemolithotrophs using an electron donor with a higher redox potential than NAD(P)+/NAD(P)H, such as nitrite or sulfur compounds, must use energy to reduce NAD(P)+.
- Although most microorganisms can fix or assimilate carbon dioxide (CO_2), only autotrophic ones use CO_2 as their sole or principal carbon source.
- The reduction or assimilation of CO_2 takes place at the expense of much energy.
- Usually autotrophic microorganisms obtain the required energy by trapping light during photosynthesis (photoautotrophs), but some derive it from the oxidation of reduced inorganic electron donors (chemoautotrophs).
- The Calvin cycle requires NAD(P)H and ATP and two key enzymes, ribulose-1, 5-biphosphate carboxylase (ribulose bisphosphate carboxylase) and phosphoribulokinase.
- The reductive tricarboxylic acid cycle , also called reverse TCA cycle is used as alternative mechanism of fixation by phototrophic green sulphur bacteria and by some non-phototrophic archaebacteria .
- Hydroxypropionate pathway is also a mechanism of autotrophic CO_2 fixation unique to green non-sulphur bacteria (Chloroflexus).
- Chloroflexus, an anoxygenic photoautotroph, uses either H_2 or H_2S as electron donors.
- In hydroxypropionate pathway, two molecules of CO_2 are reduced to glyoxylate. Acetyl-CoA is carboxylated to yield methylmalnonyl-CoA. This intermediate is rearranged to yield Acetyl-CoA and glyoxylate. The latter is converted to cell material.

3.8 KEY WORDS

- **Chemotropism :** It is a form of tropism whereby the plant's organism grows in response to a chemical stimulus.
- **Thermometer:** It is the response dependent upon temperature.
- **Phototropism:** It is the movement toward light.
- **Gravitropism and geotropism:** It is the movement relative to a gravitational field, or toward the center of the Earth.
- **Thigmotropism:** It is plant growth in response to physical contact.
- **Hydrotropism:** It is growth or developmental response to water.

3.9 SELF ASSESSMENT QUESTIONS AND ANSWERS

NOTES

Short-Answer Questions

1. Define the terms-
   (i) Phototropism
   (ii) Gravitropism
   (iii) Thigmotropism
2. Write a short note on reverse electron flow.
3. What is CO_2 assimilation?
4. Write in brief about Calvin cycle.
5. What is reductive Acetyl-CoA pathway?

Long-Answer Questions

1. Define chemotropism and their importance.
2. Write about Calvin-cycle and its phases.
3. Explain reduced TCA cycle.
4. Write about reductive Acetyl-CoA pathway.
5. Explain about hydroxypropionate pathway.

3.10 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 4 CHEMOHETEROTROPHISM

NOTES

Structure

4.0 Introduction
4.1 Objectives
4.2 Chemoheterotrophism
  4.2.1 Acetogen
  4.2.2 Methanogenesis
4.3 Phyiology and Econimic Importance of Methylotrophs
4.4 Answers to Check Your Progress Questions
4.5 Summary
4.6 Key Words
4.7 Self Assessment Questions and Exercises
4.8 Further Readings

4.0 INTRODUCTION

Chemoheterotroph is the term for an organism which derives its energy from chemicals, and needs to consume other organisms in order to live. That means you: your body gets its energy from food, and you must consume other organisms such as plants and animals in order to survive. All organisms must solve two problems in order to survive: they must be able to obtain energy, and they must be able to obtain cellular 'building materials' such as proteins, lipids, and carbohydrates. Most organisms that consume organic material use it for both energy and building materials. Animals and fungi both, for example, obtain energy by breaking down our food, and also get the building materials for our own cells from the food we eat.

A few rare organisms, however, use inorganic chemicals for energy, and yet cannot make their own building materials. These organisms – almost always bacteria – require both inorganic chemical energy sources, and other organisms whose organic materials they can consume, in order to survive. All animals are chemoheterotrophs. So are fungi – although fungi may look like plants, they don't perform photosynthesis, but rather derive their energy by breaking down organic material in soil. Many bacteria are also chemoheterotrophs, including many bacteria that live inside the human body, many infectious bacteria, and some sulfur bacteria.

In this unit, you will study about chemoheterotrophs, acetogens, methanogens, methanogenesis and its importance, physiological and economic importance of methylotrophs.

4.1 OBJECTIVES

NOTES

After going through this unit, you will be able to:

- Understand about chemoheterotrophism and its related terms
- Discuss about physiology and economic importance of methylotrophs
- Define acetogens, methanogens
- Know about methanogenesis and its importance

4.2 CHEMOHETEROTROPHISM

Chemoheterotrophs, unlike chemoautotrophs, are unable to synthesize their own organic molecules. Instead, these organisms must ingest preformed carbon molecules, such as carbohydrates and lipids, synthesized by other organisms. They do, however, still obtain energy from the oxidation of inorganic molecules like the chemoautotrophs. Chemoheterotrophs are only able to thrive in environments that are capable of sustaining other forms of life due to their dependence on these organisms for carbon sources. Chemoheterotrophs are the most abundant type of chemotrophic organisms and include most bacteria, fungi and protozoa.

These bacteria obtain both carbon and energy from organic compounds, such as carbohydrates, lipids and proteins. The carbon source as well as the source of energy are mostly the same for these bacteria. Most of the bacteria are chemo- heterotrophs. Figure 4.1 shows the flow chart explaining about sources of carbon and energy.

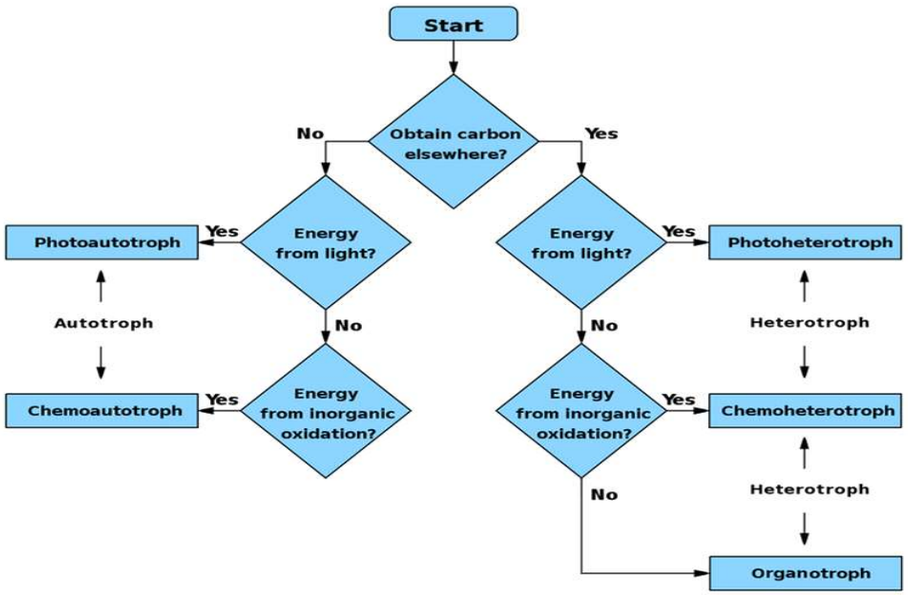


Fig. 4.1 *Flow Chart Showing Sources of Carbon and Energy*

Glucose or Monosaccharide [(CH_2O) n] + $O_2 \rightarrow CO_2 + H_2O$ + Energy

Chemoheterotrophs may belong to one of the three main categories that differ in how they obtain their organic nutrients.

NOTES

These are:

- **Parasitic:** These bacteria obtain their food from living hosts on which these grow. Parasites which cause diseases are known as pathogens, for example *Clostridium*, *Mycobacterium*, etc.
- **Saprophytic:** These bacteria obtain their food from dead and organic remains like fruits, vegetables, leaves, meat, faeces, corpses and other non-living products. The anaerobic breakdown of carbohydrates is fermentation while that of proteins is called putrefaction, for example putrefying bacteria like *Bacillus mycoides*, *B. Ramosus*, etc.
- **Symbiotic:** These bacteria live in close association with organs of other organisms (higher plants and animals) in such a way that both the concerned organism receives mutual benefit from this association. This is called symbiosis, for example *Rhizobium leguminosarum* in the root nodules of the leguminous plants.

These bacteria fix free atmospheric nitrogen into nitrogenous compounds which are utilized by the plants. In return, the plant provides nutrients and protection to the bacteria. In the stomach of the cows and goat's bacteria digest cellulose enabling these animals to feed on grass. Our own intestine contains a number of harmless bacteria, for example *Escherichia coli*.

4.2.1 Acetogen

An acetogen is a microorganism that generates acetate (CH_3COO) as an end product of anaerobic respiration or fermentation. However, this term is usually employed in a more narrow sense only to those bacteria and archaea that perform anaerobic respiration and carbon fixation simultaneously through the reductive acetyl coenzyme A (Acetyl-CoA) pathway (also known as the Wood-Ljungdahl pathway). These genuine acetogens are also known as 'homoacetogens' and they can produce acetyl-CoA (and from that, in most cases, acetate as the end product) from two molecules of carbon dioxide (CO_2) and four molecules of molecular hydrogen (H_2). This process is known as acetogenesis, and is different from acetate fermentation, although both occur in the absence of molecular oxygen (O_2) and produce acetate. Although previously thought that only bacteria are acetogens, some archaea can be considered to be acetogens.

Acetogens are found in a variety of habitats, generally those that are anaerobic (lack oxygen). Acetogens can use a variety of compounds as sources of energy and carbon; the best studied form of acetogenic metabolism involves the use of carbon dioxide as a carbon source and hydrogen as an energy source. Carbon dioxide reduction is carried out by the key enzyme Acetyl-CoA synthase. Together with methane-forming archaea, acetogens constitute the last limbs in the anaerobic

food web that leads to the production of methane from polymers in the absence of oxygen. Acetogens may represent ancestors of the first bioenergetically active cells in evolution.

NOTES

Metabolic Roles

Acetogens have diverse metabolic roles, which help them thrive in different environments. One of their metabolic products is acetate which is an important nutrient for the host and its inhabiting microbial community, most seen in termite's guts. Acetogens also serve as 'Hydrogen sinks' in termite's GI tract. Hydrogen gas inhibits biodegradation and acetogens use up these hydrogen gases in the anaerobic environment to favor the biodegradative capacity of the host by reacting hydrogen gas and carbon dioxide to make acetate. Acetogens have the ability to use variety of substrates in an event where another competitor such as a methanogen makes Hydrogen gas a limiting substrate. Acetogens can use and convert alcohols, lactates and fatty acids, which are usually restricted to syntrophs, instead of just carbon dioxide and hydrogen. This enables them to take on the roles of important contributors of food chain such as primary fermenters. Acetogens can work together with methanogens, as exemplified by the conversion of carbohydrates by a *Methanosarcina barkeri* and coculture of *A. woodii*. The methanogen takes up acetate to favor the acetogen. Sometimes the interspecies transfer of Hydrogen gas between *A. woodii* and an H_2-consuming methanogen results in hydrogen gas being released from the acetogen instead of going toward acetogenesis by WLP. Acetogens are also one of the contributors to corrosion of steel. Acetobacterium woodii utilize hydrogen gas and CO_2 to make the acetate that is used as carbon source for many of the Sulfate-reducing bacteria growing with hydrogen gas and sulfate.

4.2.2 Methanogenesis

Methanogenesis is the biological production of methane mediated by anaerobic microorganisms from the Archaea domain commonly called methanogens. The production of methane is the energy yielding metabolism of methanogens and is unique to these organisms.

Methane is produced by three major pathways

1. Reduction of Carbon-dioxide
2. Fermentation of Acetate
3. Dismutation of Methanol or Methylamines

All three pathways have in common the demethylation of methyl–coenzyme M to methane and the reduction of the heterodisulfide of coenzyme M and coenzyme B catalysed by methyl–coenzyme M and heterodisulfide reductases. Investigations of the biochemistry of the pathways have revealed novel enzymes with metal and cofactor requirements that have introduced new principles of biochemistry.

NOTES

Methane producing prokaryotes are a group of archaea which are capable of reducing carbon dioxide or low molecular weight fatty acids to produce methane. The methanogens are strictly anaerobic active at redox potential between 350 – 450 mv. They can use CO_2 as an electron acceptor. They reduce CO_2 using H_2 produced in the fermentation process. They use carbon dioxide as the only carbon source and are considered as chemolithotrophic.

If carbon-dioxide is considered available in carbonate form the reaction comes to be:

$HCO_3^- + H^+ + 4H_2 \rightarrow CH_4 + 3H_2O$ ($\Delta G'O = -32.4$ kcal/mol = – 135.6 kJ/mol)

In the above equation numbered (i) ΔG'O (Called Gibbs free energy) gives the indication of energy yield of the reaction is kilocalories per mole. ÄG'O in exothermic (energy yielding) reactions is negative. The carbon dioxide is converted to methane via a pathway involving unusual coenzymes.

To begin with initially CO_2 is bound to methanofuran at the formyl reduction level and it is followed by reduction to methenyl, methylene, methyl and finally methane level, involving a number of enzymes. Transformation of CO_2 to cell material is not according to ribulose diphosphate pathway commonly found in other chemolithotrophs rather by acetyl- CoA synthase pathway.

It is significant to note that methyl group of acetate is formed by step-wise reduction of CO_2 to methyl via tetrahydro methanopterin pathway which otherwise leads to methane evolution. The methyl group gets joined to carbonyl group of acetate which is derived by the reduction of another CO_2 to CO by carbon monoxide dehydrogenase, causing catalysis of synthesis of methyl group, CO and HS – CoA to acetyl CoA that is why it has been named as acetyl CoA synthase.

The acetate in turn is processed through a reverse tricarboxylic acid cycle to give pyruvate and triose. There are the methanogens like Methanosarcina barken that can metabolize methanol, acetate and methylamines to methane and carbon-dioxide. The syntrophism also known as cross feeding between methanogens and other anaerobes like Syntrophobacter associated with methanogens broaden the range of substrates suitable for methanogens.

It is a matter of deep concern that part of methane escapes to the atmosphere and takes part in photochemical processes. Currently methane concentration of the atmosphere is estimated to be 1.7 ppm and it is increasing every year by one percent. To conclude different methanogens have evolved several metabolic reactions for generating cellular energy. All are based on anaerobic respiration and all produce methane. The metabolic pathways of methanogens produce methane.

The metabolic pathway of methanogens can be divided into three categories:

1. CO_2 Reducing Pathway
2. Methyl trophic Pathway
3. Aceticlastic Pathways

All the three pathways are represented below in Figure 4.2

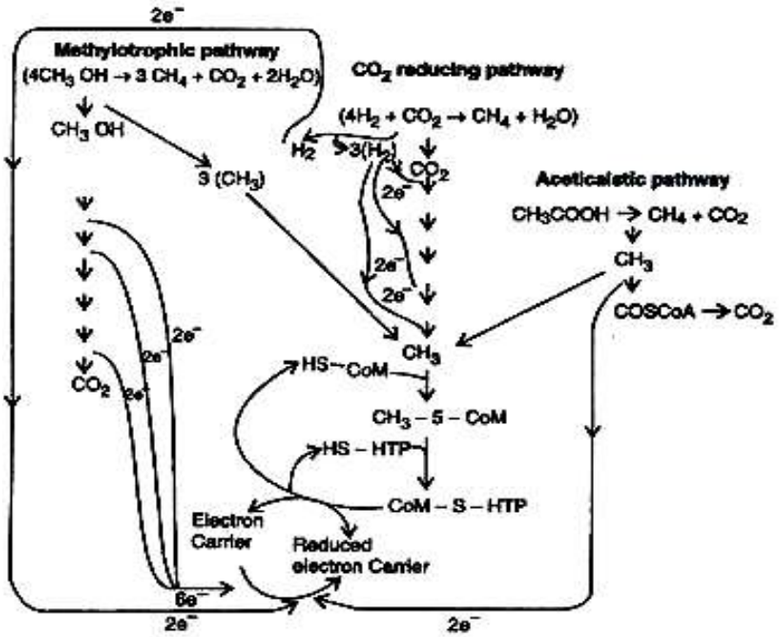


Fig. 4.2 *Representation of Methanogenesis in Archaea*

Representation of methanogenesls in archaea. Out of the three probable strategies most methanogens adopt the CO_2 reducing pathway beginning with CO_2 or formate. Some use a methylotrophic pathway starting with methonal or methylamines. Very few methanogens adopl accticlastic pathway beginning with acetate.

A limited number of methanogens are capable to oxidize secondary alcohols for CO_2 reduction to methane. The methanogens which carry out CO_2 reducing methanogenesis use a specialized anaerobic respiration pathway (Refer Figure 4.3). The reduction of carbon dioxide to methane (CH_4) takes place through a series of reductive steps which generate a methyl group. This pathway employs several reducing enzymes and coenzymes F_{420} and nickel containing coenzyme F_{430}, methanofuran, methanol protein and coenzyme M.

NOTES

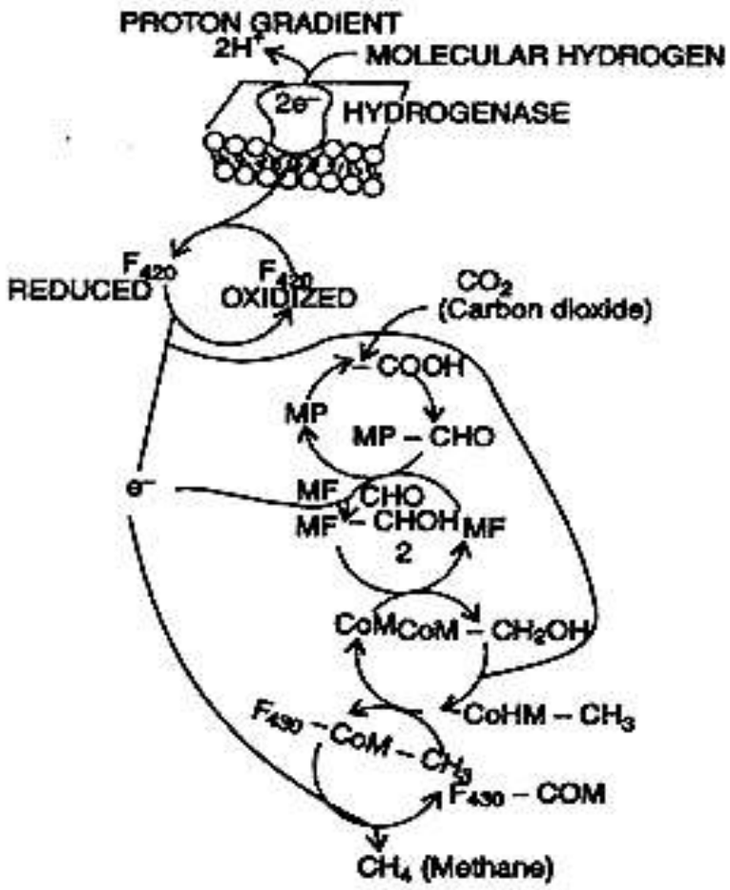


Fig. 4.3 *Conversion of Carbon-Dioxide into Methane*

Pathway showing conversion of carbon dioxide (CO_2) to methane (CH_4) by methanogen (methanogenic archaea). Factor$_{420}$ (F_{420}). Factor$_{430}$ (F_{430}) coenzyme M (CoM), methanoprotein (MP) and methanofuran (MF) are involved as electron carriers.

Check Your Progress

1. Give one difference between chemoheterotrophs and chemoautotrophs.
2. What are the favorable conditions for the survival of chemoheterotrophs?
3. Which organisms fall under chemoheterotrophs?
4. How do parasitic bacteria obtain their food ?

4.3 PHYIOLOGY AND ECONIMIC IMPORTANCE OF METHYLOTROPHS

NOTES

Methylotrophs are a diverse group of microorganisms that can use reduced one-carbon compounds, such as methanol or methane, as the carbon source for their growth; and multi-carbon compounds that contain no carbon-carbon bonds, such as dimethyl ether and dimethylamine. This group of microorganisms also includes those capable of assimilating reduced one-carbon compounds by way of carbon dioxide using the ribulose bisphosphate pathway. These organisms should not be confused with methanogens which on the contrary produce methane as a by-product from various one-carbon compounds such as carbon dioxide. Some methylotrophs can degrade the greenhouse gas methane, and in this case they are called methanotrophs. The methanotroph methylococcus capsulatus is used to degrade methane and other substrates. The abundance, purity, and low price of methanol compared to commonly used sugars make methylotrophs competent organisms for production of amino acids, vitamins, recombinant proteins, single-cell proteins, co-enzymes and cytochromes.

Metabolism

The key intermediate in methylotrophic metabolism is formaldehyde, which can be diverted to either assimilatory or dissimilatory pathways. Methylotrophs produce formaldehyde through oxidation of methanol and/or methane. Methane oxidation requires the enzyme methane monooxygenase (MMO). Methylotrophs with this enzyme are given the name methanotrophs. The oxidation of methane (or methanol) can be assimilatory or dissimilatory in nature. If dissimilatory, the formaldehyde intermediate is oxidized completely into CO_2 to produce reductant and energy. If assimilatory, the formaldehyde intermediate is used to synthesize a 3-Carbon compound for the production of biomass. Many methylotrophs use multi-carbon compounds for anabolism, thus limiting their use of formaldehyde to dissimilatory processes, however methanotrophs are generally limited to only metabolism.

Catabolism

Methylotrophs use the electron transport chain to conserve energy produced from the oxidation of compounds. An additional activation step is required in methanotrophic metabolism to allow degradation of chemically-stable methane. This oxidation to methanol is catalyzed by MMO, which incorporates one oxygen atom from O_2 into methane and reduces the other oxygen atom to water, requiring two equivalents of reducing power. Methanol is then oxidized to formaldehyde through the action of methanol dehydrogenase (MDH) in bacteria, or a non-specific alcohol oxidase in yeast. Electrons from methanol oxidation are passed to a membrane-associated quinone of the electron transport chain to produce ATP.

NOTES

In dissimilatory processes, formaldehyde is completely oxidized to CO_2 and excreted. Formaldehyde is oxidized to formate via the action of Formaldehyde dehydrogenase (FALDH), which provides electrons directly to a membrane associated quinone of the electron transport chain, usually cytochrome b or c. In the case of NAD+ associated dehydrogenases, NADH is produced.

Finally, formate is oxidized to CO_2 by cytoplasmic or membrane-bound Formate dehydrogenase (FDH), producing and CO_2 .

Anabolism

The main metabolic challenge for methylotrophs is the assimilation of single carbon units into biomass. Through de novo synthesis, Methylotrophs must form carbon-carbon bonds between 1-Carbon molecules. This is an energy intensive process, which facultative methylotrophs avoid by using a range of larger organic compounds. However, obligate methylotrophs must assimilate molecules. There are four distinct assimilation pathways with the common theme of generating one molecule. Bacteria use three of these pathways while Fungi use one. All four pathways incorporate 3 molecules into multi-carbon intermediates, then cleave one intermediate into a new molecule. The remaining intermediates are rearranged to regenerate the original multi-carbon intermediates.

Bacteria

Each species of methylotrophic bacteria has a single dominant assimilation pathway. The three characterized pathways for carbon assimilation are the ribulose monophosphate (RuMP) and serine pathways of formaldehyde assimilation as well as the ribulose bisphosphate (RuBP) pathway of CO_2 assimilation.

Ribulose Bisphosphate (RuBP) Cycle

Unlike the other assimilatory pathways, bacteria using the RuBP pathway derive all of their organic carbon from CO_2 assimilation. This pathway was first elucidated in photosynthetic autotrophs and is better known as the Calvin Cycle. Shortly thereafter, methylotrophic bacteria who could grow on reduced compounds were found using this pathway.

First, 3 molecules of ribulose 5-phosphate are phosphorylated to ribulose 1,5-bisphosphate (RuBP). The enzyme ribulose bisphosphate carboxylase (RuBisCO) carboxylates these RuBP molecules which produces 6 molecules of 3-phosphoglycerate (PGA). The enzyme phosphoglycerate kinase phosphorylates PGA into 1,3-diphosphoglycerate (DPGA). Reduction of 6 DPGA by the enzyme glyceraldehyde phosphate dehydrogenase generates 6 molecules of the compound glyceraldehyde-3-phosphate (GAP). One GAP molecule is diverted towards biomass while the other 5 molecules regenerate the 3 molecules of ribulose 5-phosphate.

NOTES

Ribulose Monophosphate (RuMP) Cycle

A new pathway was suspected when RuBisCO was not found in the methanotroph Methylomonas methanica. Through radio-labelling experiments, it was shown that M. methanica used the Ribulose monophate (RuMP) pathway. This has led researchers to propose that the RuMP cycle may have preceded the RuBP cycle.

Like the RuBP cycle, this cycle begins with 3 molecules of ribulose-5-phosphate. However, instead of phosphorylate , ribulose-5-phosphate, 3 molecules of formaldehyde form a C-C bond through an aldol condensation, producing 3 molecules of 3-hexulose 6-phosphate (hexulose phosphate). One of these molecules of hexulose phosphate is converted into GAP and either pyruvate or dihydroxyacetone phosphate (DHAP). The pyruvate or DHAP is used towards biomass while the other 2 hexulose phosphate molecules and the molecule of GAP are used to regenerate the 3 molecules of ribulose-5-phosphate.

Serine Cycle

Unlike the other assimilatory pathways, the serine cycle uses carboxylic acids and amino acids as intermediates instead of carbohydrates. First, 2 molecules of formaldehyde are added to 2 molecules of the amino acid glycine. This produces two molecules of the amino acid serine, the key intermediate of this pathway. These serine molecules eventually produce 2 molecules of 2-phosphoglycerate, with one molecule going towards biomass and the other being used to regenerate glycine. Notably, the regeneration of glycine requires a molecule of CO_2 as well, therefore the Serine pathway also differs from the other 3 pathways by its requirement of both formaldehyde and CO_2 .

Yeasts

Methylotrophic yeast metabolism differs from bacteria primarily on the basis of the enzymes used and the carbon assimilation pathway. Unlike bacteria which use bacterial MDH, methylotrophic yeasts oxidize methanol in their peroxisomes with a non-specific alcohol oxidase. This produces formaldehyde as well as hydrogen peroxide. Compartmentalization of this reaction in peroxisomes likely sequesters the hydrogen peroxide produced. Catalase is produced in the peroxisomes to deal with this harmful by-product.

Dihydroxyacetone (DHA) Cycle

The dihydroxyacetone (DHA) pathway, also known as the xylulose monophosphate (XuMP) pathway, is found exclusively in yeast. This pathway assimilates three molecules of formaldehyde into 1 molecule of DHAP using 3 molecules of xylulose 5-phosphate as the key intermediate.

DHA synthase acts as a transferase (transketolase) to transfer part of xylulose 5-phosphate to DHA. Then these 3 molecules of DHA are phosphorylated to DHAP by triokinase. Like the other cycles, 3 molecules are produced with 1

molecule being directed for use as cell material. The other 2 molecules are used to regenerate xylulose 5-phosphate.

Environmental Implications

NOTES

As key players in the carbon cycle, methylotrophs work to reduce global warming primarily through the uptake of methane and other greenhouse gases. In aqueous environments, methanogenic bacteria produce 40-50% of the world's methane. Symbiosis between methanogens and methanotrophic bacteria greatly decreases the amount of methane released into the atmosphere.

The use of methylotrophs in the agricultural sector is another way in which they can potentially impact the environment. Traditional chemical fertilizers supply nutrients not readily available from soil but have effects such as decreased crop yield, poor soil quality and plant diseases. They are also environmentally costly to produce. Methylotrophs have high potential as alternative biofertilizers and bioinoculants due to their ability form mutualistic relationships with several plant species. Methylotrophs provide plants with nutrients such as soluble phosphorus and fixed nitrogen and also play a role in the uptake of said nutrients. Additionally, they can help plants respond to environmental stressors through the production of phytohormones. Methylotrophic growth also inhibits the growth of harmful plant pathogens and induces systemic resistance. Methylotrophic biofertilizers used either alone or together with chemical fertilizers have been shown to increase both crop yield and quality without loss of nutrients.

Check Your Progress

5. What are methylotrophs?
6. What does methylotrophs produce?
7. Which enzyme is required by methane oxidation requires?
8. What is the main metabolic challenge for methylotrophs?

4.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Chemoheterotrophs, unlike chemoautotrophs, are unable to synthesize their own organic molecules. Instead, these organisms must ingest preformed carbon molecules, such as carbohydrates and lipids, synthesized by other organisms.
2. Chemoheterotrophs are only able to thrive in environments that are capable of sustaining other forms of life due to their dependence on these organisms for carbon sources.

3. Chemoheterotrophs are the most abundant type of chemotrophic organisms and include most bacteria, fungi and protozoa.
4. Parasitic bacteria obtain their food from living hosts on which these grow. Parasites which cause diseases are known as pathogens e.g., Clostridium, Mycobacterium, etc.
5. Methylotrophs are a diverse group of microorganisms that can use reduced one-carbon compounds, such as methanol or methane, as the carbon source for their growth; and multi-carbon compounds that contain no carbon-carbon bonds, such as dimethyl ether and dimethylamine.
6. Methylotrophs produce formaldehyde through oxidation of methanol and/or methane.
7. Methane oxidation requires the enzyme methane monooxygenase (MMO).
8. The main metabolic challenge for methylotrophs is the assimilation of single carbon units into biomass.

4.5 SUMMARY

- Chemoheterotrophs, unlike chemoautotrophs, are unable to synthesize their own organic molecules.
- Instead, these organisms must ingest preformed carbon molecules, such as carbohydrates and lipids, synthesized by other organisms.
- They do, however, still obtain energy from the oxidation of inorganic molecules like the chemoautotrophs.
- Chemoheterotrophs are only able to thrive in environments that are capable of sustaining other forms of life due to their dependence on these organisms for carbon sources.
- Chemoheterotrophs are the most abundant type of chemotrophic organisms and include most bacteria, fungi and protozoa.
- These bacteria obtain both carbon and energy from organic compounds such as carbohydrates, lipids and proteins.
- The carbon source as well as the source of energy are mostly the same for these bacteria. Most of the bacteria are chemo heterotrophs.
- An acetogen is a microorganism that generates acetate (CH_3COO) as an end product of anaerobic respiration or fermentation.
- However, acetogen is usually employed in a more narrow sense only to those bacteria and archaea that perform anaerobic respiration and carbon fixation simultaneously through the reductive acetyl coenzyme A (Acetyl-CoA) pathway (also known as the Wood-Ljungdahl pathway).

NOTES

- These genuine acetogens are also known as 'homoacetogens' and they can produce acetyl-CoA (and from that, in most cases, acetate as the end product) from two molecules of carbon dioxide (CO_2) and four molecules of molecular hydrogen (H_2). This process is known as acetogenesis, and is different from acetate fermentation, although both occur in the absence of molecular oxygen (O_2) and produce acetate.
- Although previously thought that only bacteria are acetogens, some archaea can be considered to be acetogens.
- Acetogens are found in a variety of habitats, generally those that are anaerobic (lack oxygen).
- Acetogens can use a variety of compounds as sources of energy and carbon; the best studied form of acetogenic metabolism involves the use of carbon dioxide as a carbon source and hydrogen as an energy source.
- Carbon dioxide reduction is carried out by the key enzyme Acetyl-CoA synthase.
- Together with methane-forming archaea, acetogens constitute the last limbs in the anaerobic food web that leads to the production of methane from polymers in the absence of oxygen.
- Acetogens may represent ancestors of the first bioenergetically active cells in evolution.
- Acetogens can use and convert alcohols, lactates and fatty acids, which are usually restricted to syntrophs, instead of just carbon dioxide and hydrogen. This enables them to take on the roles of important contributors of food chain such as primary fermenters.
- Acetogens can work together with methanogens, as exemplified by the conversion of carbohydrates by a Methanosarcina barkeri and coculture of A. woodii.
- The methanogen takes up acetate to favor the acetogen. Sometimes the interspecies transfer of Hydrogen gas between A. woodii and an H2-consuming methanogen results in hydrogen gas being released from the acetogen instead of going toward acetogenesis by WLP.
- Acetogens are also one of the contributors to corrosion of steel.
- Acetobacterium woodii utilize hydrogen gas and CO_2 to make the acetate that is used as carbon source for many of the Sulfate-reducing bacteria growing with hydrogen gas and sulfate.
- Methanogenesis is the biological production of methane mediated by anaerobic microorganisms from the Archaea domain commonly called methanogens.

- A limited number of methanogens are capable to oxidize secondary alcohols for CO_2 reduction to methane. The methanogens which carry out CO_2 reducing methanogenesis use a specialized anaerobic respiration pathway.

4.6 KEY WORDS

- **Parasitic:** These bacteria obtain their food from living hosts on which these grow. Parasites which cause diseases are known as pathogens, for example, Clostridium, Mycobacterium.
- **Saprophytic:** These bacteria obtain their food from dead and organic remains like fruits, vegetables, leaves, meat, faeces, corpses and other non-living products.
- **Putrefaction:** The anaerobic breakdown of carbohydrates is fermentation while that of proteins.
- **Symbiotic:** These bacteria live in close association with organs of other organisms (higher plants and animals) in such a way that both the concerned organism receives mutual benefit from this association.
- **Methanogenesis:** It is the biological production of methane mediated by anaerobic microorganisms from the Archaea domain commonly called methanogens.

4.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Write a short note on chemoheterotrophism.
2. What are the categorizes in which chemoheterotrophs may belong?
3. Write a short note on acetogens.
4. Write about physiology and economic importance of methylotrophs.
5. Expalin ribulose monophosphate (RuMP) cycle in brief.

Long-Answer Questions

1. Explain in detail about chemoheterotrophism.
2. Explain methanogenesis in detail.
3. What are the physiology and economic importance of methylotrophs?
4. Elaborate about metabolism, catabolism and anabolism.
5. Distinguish between RuBP and RuMP cycle.

NOTES

4.8 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

NOTES

BLOCK - II
BACTERIAL PHOTOSYNTHESIS, NITROGEN METABOLISM AND STRESS RESPONSE

UNIT 5 BACTERIAL PHOTOSYNTHESIS

Structure

5.0 Introduction
5.1 Objectives
5.2 Photosynthesis: General Introduction
5.3 Types of Bacterial Photosynthesis
5.4 Structure of Photosynthetic Pigments
5.5 Answers to Check Your Progress Questions
5.6 Summary
5.7 Key Words
5.8 Self Assessment Questions and Exercises
5.9 Further Readings

5.0 INTRODUCTION

Photosynthetic bacteria have been around for longer than the Earth's atmosphere could sustain human life. It was only recently though that scientists began to unravel the mystery of how these micro-organisms execute the mechanisms of photosynthesis. While scientists still have not been able to put all the pieces of the photosynthetic bacteria puzzle in the right places, they are actively studying them and are gaining valuable knowledge about the way they photosynthesize and how they have evolved. In fact, they believe that these micro-organisms may have had a huge impact on why the world evolved the way it did, and may show potential for life in places deemed uninhabitable, including extreme climates like Antarctica and even other planets.

Much like the name suggests, these micro-organisms are special types of bacteria that contain light absorbing pigments and reaction centers which make them capable of converting light energy into chemical energy. Cyanobacteria contain chlorophyll while other forms of bacteria contain bacteriochlorophyll. Although bacteriochlorophyll resembles chlorophyll, it absorbs light of a longer wavelength than chlorophyll. Bacteriochlorophyll a is the most common form of bacteriochlorophyll but other forms include b, c, d, e, f and g. Bacteria that contain bacteriochlorophyll do not use water as an electron donor and therefore do not produce oxygen. This is known as anoxygenic photosynthesis. Cyanobacteria

NOTES

perform photosynthesis using water as an electron donor in a similar manner to plants. This results in the production of oxygen and is known as oxygenic photosynthesis.

Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the 'halobacteria'. The cyanobacteria conduct plant photosynthesis, called oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; the extreme halophilic archaea use a type of non-photosynthetic photophosphorylation mediated by a pigment, bacteriorhodopsin, to transform light energy into ATP.

In this unit, you will study about bacterial photosynthesis, general types of microbial photosynthesis, oxygenic and anoxygenic, structure of photosynthetic pigments, chlorophylls, bacteriochlorophyll, carotenoids and phycobilins.

5.1 OBJECTIVES

After going through this unit, you will be able to:

- Discuss about bacterial photosynthesis
- Understand about general types of microbial photosynthesis, oxygenic and anoxygenic
- Explain the structure of photosynthetic pigments
- Learn about chlorophylls, bacteriochlorophyll, carotenoids and phycobilins

5.2 PHOTOSYNTHESIS: GENERAL INTRODUCTION

Although the process of photosynthesis is most commonly associated with plants and algae, much of our understanding of the molecular basis for light energy capture and photochemical energy transduction has come from studies of photosynthetic bacteria. Photosynthesis is the use of light as a source of energy for growth, more specifically the conversion of light energy into chemical energy in the form of ATP. Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the 'halobacteria' (actually archaea). The cyanobacteria conduct plant photosynthesis, called oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; the extreme halophilic archaea use a type of non-photosynthetic photophosphorylation mediated by a pigment, bacteriorhodopsin, to transform light energy into ATP.

Net equation

$6CO_2 + 12H_2O + \text{Light Energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$

Photosynthetic reactions divided into two stages

- **Light Reaction -** Light energy absorbed & converted to chemical energy (ATP, NADPH)
- **Dark Reaction -** Carbohydrates made from CO_2 using energy stored in ATP & NADPH

NOTES

5.3 TYPES OF BACTERIAL PHOTOSYNTHESIS

Five photosynthetic groups within domain Bacteria (based on 16S rRNA)

1. Oxygenic Photosynthesis

- Occurs in cyanobacteria and prochlorophytes
- Synthesis of carbohydrates results in release of molecular O_2 and removal of CO_2 from atmosphere.
- Occurs in lamellae which house thylakoids containing chlorophyll a/b and phycobilisomes pigments which gather light energy
- Uses two photosystems (PS)

  - PS II- generates a proton-motive force for making ATP.

  - PS I- generates low potential electrons for reducing power.

  Figure 5.1 shows electron flow in oxygenic photosynthesis.

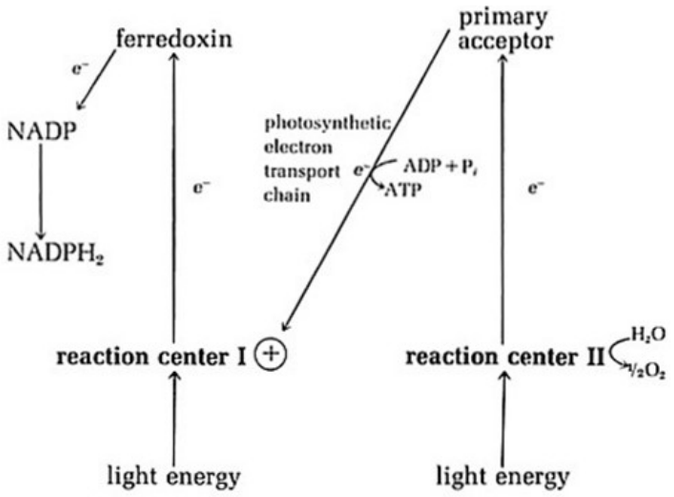


Fig. 5.1 *Electron Flow in Oxygenic Photosynthesis.*

NOTES

2. Anoxygenic Photosynthesis

- Uses light energy to create organic compounds, and sulfur or fumarate compounds instead of O_2 .
- Occurs in purple bacteria, green sulfur bacteria, green gliding bacteria and heliobacteria.
- Uses bacteriochlorophyll pigments instead of chlorophyll.
- Uses one photosystem (PS I) to generate ATP in 'cyclic' manner.

Figure 5.2 shows the cyclic flow of electrons during anoxygenic photosynthesis.

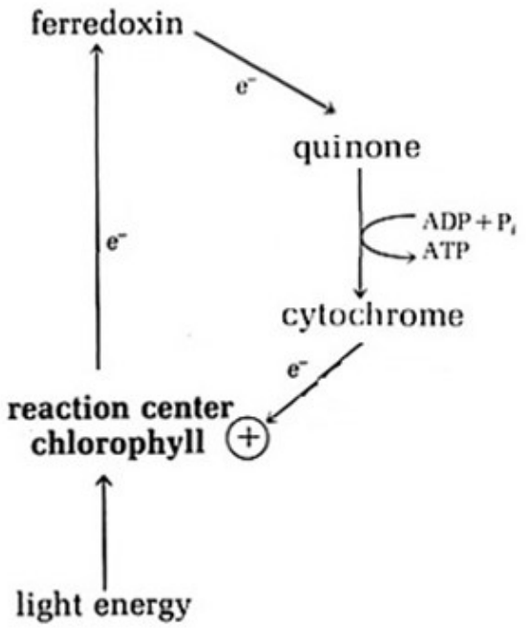


Fig. 5.2 *The Cyclical Flow of Electrons during Anoxygenic Photosynthesis.*

You can also find the comparison of electron transport pathways in oxygenic and anoxygenic photosynthesis later in Figure 5.5.

Light Reaction

The Light Reactions depend upon the presence of chlorophyll, the primary light-harvesting pigment in the membrane of photosynthetic organisms. The functional components of the photochemical system are light harvesting pigments, a membrane electron transport system, and an ATPase enzyme. The photosynthetic electron transport system of is fundamentally similar to a respiratory ETS, except that there is a low redox electron acceptor (for example ferredoxin) at the top (low redox end) of the electron transport chain, that is first reduced by the electron displaced from chlorophyll.

Check Your Progress

1. What is photosynthesis?
2. What type of prokaryotes convert light energy into chemical energy?
3. Which bacteria conduct oxygenic photosynthesis?
4. In how many stages photosynthetic reactions divided?
5. What does PS I and PS II do?

NOTES

5.4 STRUCTURE OF PHOTOSYNTHETIC PIGMENTS

There are several types of pigments distributed among various phototrophic organisms.

Chlorophyll

Chlorophyll is the primary light-harvesting pigment in all photosynthetic organisms. Chlorophyll is a tetrapyrrole which contains magnesium at the center of the porphyrin ring. It contains a long hydrophobic side chain that associates with the photosynthetic membrane. Cyanobacteria have chlorophyll a, the same as plants and algae. The chlorophylls of the purple and green bacteria, called bacteriochlorophylls are chemically different than chlorophyll in their substituent side chains. This is reflected in their light absorption spectra. Chlorophyll absorbs light in two regions of the spectrum, one around 450nm and the other between 650-750nm; bacterial chlorophylls absorb from 800-1000nm in the far red region of the spectrum.

Bacteriochlorophyll

The main light harvesting pigment in purple photosynthetic bacteria is not chlorophyll but bacteriochlorophyll (BChl), a closely related magnesium porphyrin that has a more saturated tetrapyrrole ring (Refer Figure 5.3A). This causes BChl to absorb at significantly longer wavelengths than chlorophyll in the near infrared, the absorbance spectrum being dictated by the details of the conjugated electron system of the macrocycle (Refer Figure 5.3A). Light harvesting is also carried out by a variety of carotenoids that provide the main pigmentation in the visible region of the spectrum, and so make purple bacteria purple (or a variety of other colours). Figure 5.3B shows the absorbance spectrum of photosynthetic membranes prepared from Rhodobacter (Rba.) sphaeroides; the strong absorbance in the near infrared is attributable to BChl (Qy bands) as is the band around 600 nm (Qx bands), whilst absorbance between 450-550 nm arises mainly from carotenoid. The so-called Soret bands of BChl are located in the near-UV between 300-400 nm (not shown).

NOTES

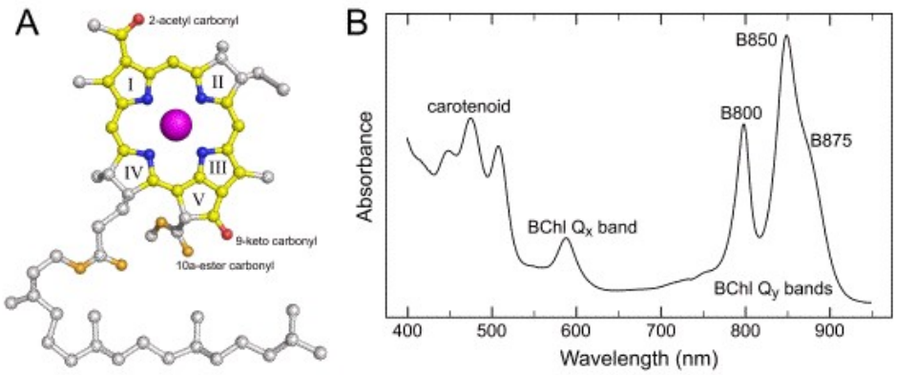


Fig. 5.3 *Pigments and Absorption.*

In the above Figure A shows the structure of bacteriochlorophyll a. Carbon and oxygen atoms that form part of the electron system are shown in yellow and red, respectively, with other carbon and oxygen atoms in white and orange. The central Mg (magenta sphere) is coordinated in-plane by the four pyrrole nitrogens (blue), plus a fifth out-of-plane ligand donated by the protein (not shown). The five rings are numbered as shown, and the labelled carbonyl groups provide additional potential points of attachment to the protein, and B shows the visible/ near-infrared absorbance spectrum of photosynthetic membranes from Rba. sphaeroides.

Carotenoids

Carotenoids are always associated with the photosynthetic apparatus. They function as secondary light-harvesting pigments, absorbing light in the blue-green spectral region between 400-550 nm. Carotenoids transfer energy to chlorophyll, at near 100 percent efficiency, from wave lengths of light that are missed by chlorophyll. In addition, carotenoids have an indispensable function to protect the photosynthetic apparatus from photo oxidative damage. Carotenoids have long hydrocarbon side chains in a conjugated double bond system. Carotenoids 'quench' the powerful oxygen radical, singlet oxygen, which is invariably produced in reactions between chlorophyll and O_2 (molecular oxygen). Some non-photosynthetic bacterial pathogens,. i.e., *Staphylococcus aureus*, produce carotenoids that protect the cells from lethal oxidations by singlet oxygen in phagocytes.

Phycobilins

Phycobiliproteins are the major light harvesting pigments of the cyanobacteria. They also occur in some groups of algae. They may be red or blue, absorbing light in the middle of the spectrum between 550 and 650nm. Phycobiliproteins consist of proteins that contain covalently-bound linear tetrapyrrole (phycobilins). They are contained in granules called phycobilisomes that are closely associated with the photosynthetic apparatus. Being closely linked to chlorophyll they can efficiently transfer light energy to chlorophyll at the reaction center.

NOTES

All phototrophic bacteria are capable of performing cyclic photophosphorylation. This universal mechanism of cyclic photophosphorylation is referred to as Photosystem I. Bacterial photosynthesis uses only Photosystem I (PSI), but the more evolved cyanobacteria, as well as algae and plants, have an additional light-harvesting system called Photosystem II (PSII). Photosystem II is used to reduce Photosystem I when electrons are withdrawn from PSI for CO_2 fixation. PSII transfers electrons from H_2O and produces O_2.

Dark Reaction

The use of RUBP carboxylase and the Calvin cycle is the most common mechanism for CO_2 fixation among autotrophs. Indeed, RUBP carboxylase is said to be the most abundant enzyme on the planet (nitrogenase, which fixes N_2 is second most abundant). This is the only mechanism of autotrophic CO_2 fixation among eukaryotes, and it is used, as well, by all cyanobacteria and purple bacteria. Lithoautotrophic bacteria also use this pathway. But the green bacteria and the methanogens, as well as a few isolated groups of prokaryotes, have alternative mechanisms of autotrophic CO_2 fixation and do not possess RUBP carboxylase.

RUBP carboxylase (ribulose bisphosphate carboxylase) uses ribulose bisphosphate (RUBP) and CO_2 as co-substrates. In a complicated reaction the CO_2 is 'fixed' by addition to the RUBP, which is immediately cleaved into two molecules of 3-phosphoglyceric acid (PGA). The fixed CO_2 winds up in the -COO group of one of the PGA molecules. Actually, this is the reaction which initiates the Calvin cycle.

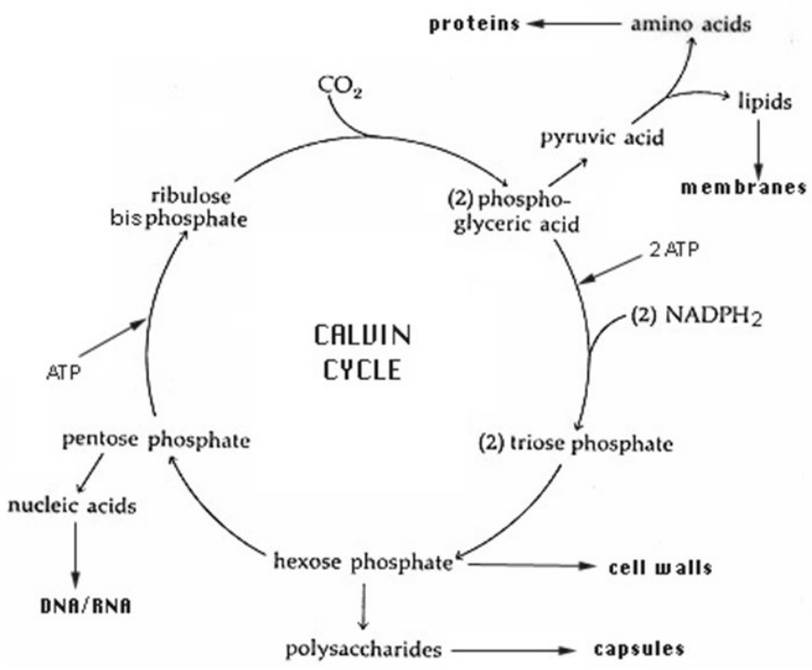


Fig. 5.4 *The Calvin Cycle*

NOTES

The Calvin cycle (Refer Figure 5.4) is concerned with the conversion of PGA to intermediates in glycolysis that can be used for biosynthesis, and with the regeneration of RUBP, the substrate that drives the cycle. After the initial fixation of CO_2 , 2 PGA are reduced and combined to form hexose-phosphate by reactions which are essentially the reverse of the oxidative Embden-Meyerhof pathway. The hexose phosphate is converted to pentose-phosphate, which is phosphorylated to regenerate RUBP. An important function of the Calvin cycle is to provide the organic precursors for the biosynthesis of cell material. Intermediates must be constantly withdrawn from the Calvin cycle in order to make cell material. In this regard, the Calvin cycle is an anabolic pathway. The fixation of CO_2 to the level of glucose (C6H12O6) requires 18 ATP and 12 NADPH2.

Most of the phototrophic prokaryotes are autotrophs, which mean that they are able to fix CO_2 as a sole source of carbon for growth. Just as the oxidation of organic material yields energy, electrons and CO_2 , in order to build up CO_2 to the level of cell material (CH_2O), energy (ATP) and electrons (reducing power) are required. The overall reaction for the fixation of CO_2 in the Calvin cycle is CO_2 + 3ATP + 2NADPH2 $\rightarrow$ CH_2O + 2ADP + 2Pi + 2NADP. The light reactions operate to produce ATP to provide energy for the dark reactions of CO_2 fixation. The dark reactions also need reductant (electrons). Usually the provision of electrons is in some way connected to the light reactions.

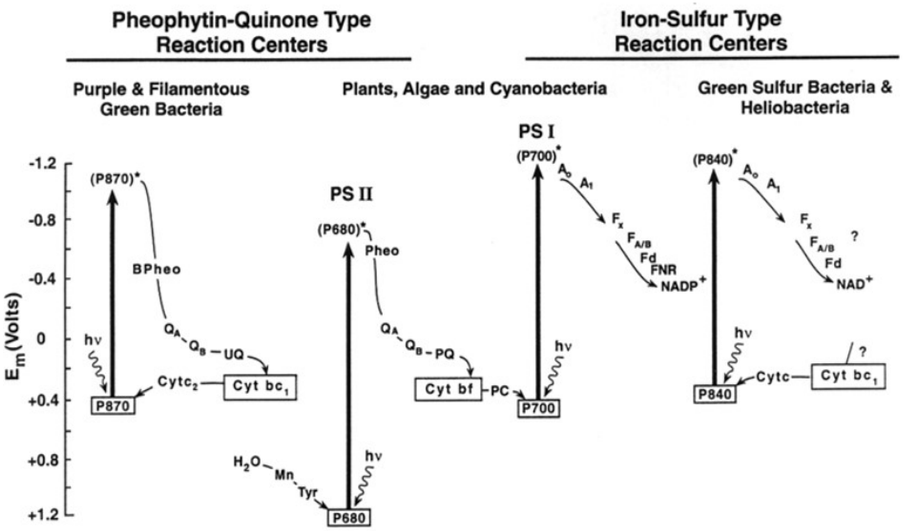


Fig. 5.5 *Comparison of Electron Transport Pathways in Oxygenic and Anoxygenic Photosynthesis*

The differences between plant and bacterial photosynthesis are summarized in Table 5.1 given below. Bacterial photosynthesis is an anoxygenic process. The external electron donor for bacterial photosynthesis is never H_2O, and therefore, purple and green bacteria never produce O_2 during photosynthesis. Furthermore, bacterial photosynthesis is usually inhibited by O_2 and takes place in microaerophilic

and anaerobic environments. Bacterial chlorophylls use light at longer wave lengths not utilized in plant photosynthesis, and therefore they do not have to compete with oxygenic phototrophs for light. Bacteria use only cyclic photophosphorylation (Photosystem I) for ATP synthesis and lack a second photosystem.

Table 5.1 *Differences between Plant and Bacterial Photosynthesis*

	Plant photosynthesis	**Bacterial photosynthesis**
Organisms	**Plants, algae, cyanobacteria**	**Purple and green bacteria**
Type of chlorophyll	Chlorophyll-a and absorbs 650-750nm	bacteriochlorophyll and absorbs 800-1000nm
Photosystem I (cyclic photophosphorylation)	present	present
Photosystem I (noncyclic photophosphorylation)	present	absent
Produces O_2	yes	no
Photosynthetic electron donor	H_2O	H_2S, other sulfur compounds or certain organic compounds

Energy Transduction In The (Anoxygenic) Photosynthetic Bacteria

The bacteria that use chlorophyll-type molecules to exploit sunlight as an energy source comprise the purple phototrophic bacteria, green sulphur bacteria, green filamentous bacteria, the heliobacteria and the cyanobacteria. All but the last of these transduce light energy into a biologically-useful form without the generation of oxygen from the oxidation of water, and are classed as anoxygenic photosynthetic bacteria. The most heavily-studied group are the purple phototrophic bacteria, for a variety of reasons including metabolic flexibility, genetic accessibility, and the relatively simple and modular nature of their photosynthetic apparatus.

In general terms, the strategy for solar energy utilization in all organisms that contain chlorophyll (Chl) or bacteriochlorophyll (BChl) is the same.

1. Light energy is captured by pigment molecules in the light harvesting or 'antenna' region of the photosystem, and is stored temporarily as an excited electronic state of the pigment.
2. Excited state energy is channeled to the reaction center region of the photosystem, a pigment-protein complex embedded in a charge-impermeable lipid bi-layer membrane.
3. Arrival of the excited state energy at a particular bacteriochlorophyll (BChl), or pair of BChls in the reaction center triggers a photochemical reaction that separates a positive and negative charge across the width of the membrane.

NOTES

4. Charge separation initiates a series of electron transfer reactions that are coupled to the translocation of protons across the membrane, generating an electrochemical proton gradient [proton motive force (pmf)] that can be used to power reactions such as the synthesis of ATP.

The minimal photosynthetic unit in purple phototrophic bacteria comprises a reaction centre (RC) surrounded by a light harvesting complex called LH1. Together these form the so-called RC-LH1 complex, and this is capable of converting light energy into a pmf in partnership with a second membrane-embedded electron transfer protein, the cytochrome (cyt) bc1 complex (termed bc1 below). In some species, light harvesting capacity is augmented by one or more types of peripheral antenna complex, termed LH2, LH3, and so on.

Peripheral Antenna - X-ray crystallography and other structural techniques have revealed that the light harvesting pigment-proteins of purple bacteria have a cylindrical architecture. In *Rhodopseudomonas (Rps.) acidophila* the peripheral LH2 complex is formed from nine copies of each of two short polypeptides, termed and ß, that each have a single membrane-spanning -helix (Refer Figure 5.6). These and ß polypeptides form two concentric protein cylinders in the membrane, between which are sandwiched the light harvesting BChls and carotenoids.

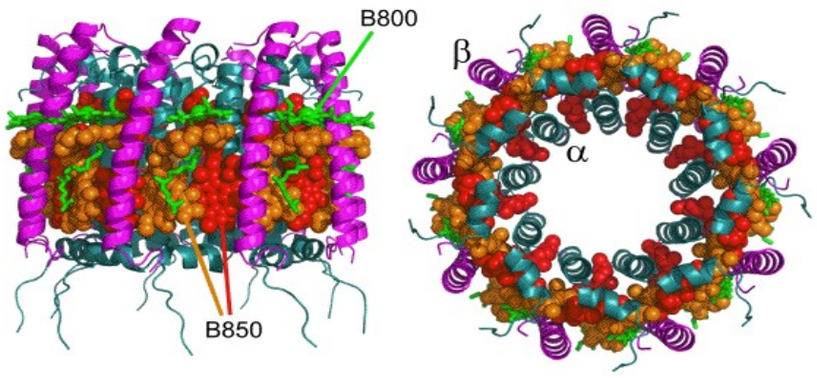


Fig. 5.6 *The LH2 Pigment-Protein from* Rhodopseudomonas Acidophila.

Views are parallel (left) and perpendicular (right) to the plane of the membrane. The protein scaffold comprises concentric cylinders of nine (inner), and nine ß (outer) polypeptides (teal and purple ribbons, respectively). The 18 B850 BChls (which absorb strongly at 850 nm) are shown as spheres, in alternating red and orange, and the nine B800 BChls (which absorb strongly at 800 nm) are shown as green sticks (with the central Mg shown as a sphere). The macrocycles of the B850 and B800 BChls are arranged perpendicular and parallel, respectively, to the plane of the membrane.

The BChls of LH2 form two rings that are arranged approximately in the plane of the membrane. The first of these comprises 18 BChls, one for each and ß polypeptide, oriented with the macrocycle of each BChl approximately

NOTES

perpendicular to the plane of the membrane. These 'B850' BChls have a prominent absorbance band at 850 nm. The second ring comprises nine 'B800' BChls, which are arranged with the macrocycle of each BChl parallel to the plane of the membrane, and gives rise to a prominent absorbance band at 800 nm. The LH2 complex also contains light harvesting carotenoid. Peripheral LH complexes from other species show variations on this theme, for example the LH2 from *Rhodospirillum molischianum* has eight pairs of and ß polypeptides. The LH2 from Rba. sphaeroides is very similar to that from *Rps. acidophila*, and its 850 and 800 nm absorbance bands are apparent in the spectrum.

Core Antenna - To date a high resolution X-ray crystal structure for a RC-LH1 complex has not been reported, and the most detailed information available is from a 4.8 Å structure for the RC-LH1 complex from *Rps. Palustris* (Refer Figure 5.7). This shows a central RC surrounded by a cylindrical LH1 with a roughly oval cross section in the membrane plane. The LH1 pigment-protein has a similar overall architecture to LH2, being formed from concentric cylinders of two polypeptides also called and ß. Each polypeptide has a single membrane-spanning -helix, with a ring of BChls (one per or ß polypeptide) sandwiched between the protein cylinders. LH1 is larger than LH2 to accommodate the RC in the central vestibule, with 15 pairs of and ß polypeptides in *Rps. Palustris*. LH1 contains carotenoids and single type of 'B875' BChl, arranged in a ring with each BChl macrocycle perpendicular to the membrane. The 875 nm absorbance band of the *Rba. sphaeroides* LH1 complex can be seen as a shoulder in the spectrum.

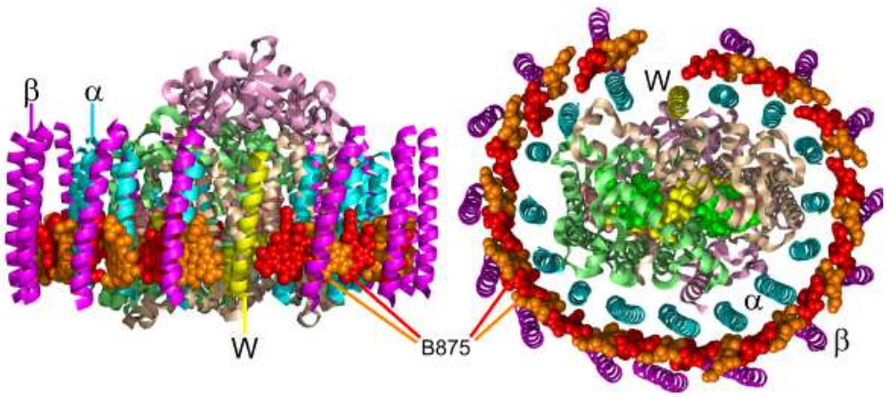


Fig. 5.7 *The RC-LH1 Pigment-Protein from Rps. Palustris.*

Views are parallel (left) and perpendicular (right) to the plane of the membrane. The protein scaffold comprises concentric cylinders of 15 (inner) and 15 ß (outer) polypeptides (teal and purple ribbons, respectively), plus a single W polypeptide (yellow ribbon). The 30 B875 BChls are shown as spheres, in alternating red and orange, their macrocycles being arranged perpendicular to the plane of the membrane. The central RC is shown as ribbons and spheres for polypeptides and cofactors, respectively (Refer Figure 5.8). The figure was constructed using PDB file 1PYH.

NOTES

In Rps. Palustris, the ring of LH1 pigment-protein does not completely surround the central RC, and an additional membrane-spanning polypeptide is present in the gap in the LH1 ring. The function of this component is unclear, but it has been speculated that it is related to the PufX polypeptide, which is a minor component of the RC-LH1 complex in Rhodobacter (Rba.) sphaeroides, and is important for efficient quinone exchange between the RC and the bc1.

Reaction Centre - The function of the carotenoids and BChls of the antenna is to feed the RC with excited state energy. The related PSO module by Yocum on RCs gives a detailed account of the structure, and mechanism of the purple bacterial RC. The following is a brief summary of the heavily-studied Rba. sphaeroides complex, included here for completeness. The module by Yocum describes the closely-related RC from *Blastochloris (Blc.)* viridis.

The Rba. *sphaeroides* RC comprises three polypeptides and ten cofactors, four BChls, two bacteriopheophytins (BPhe), which is BChl lacking the central Mg, two ubiquinones, a carotenoid and a non-heme iron (Refer Figure 5.8A). The BChl, BPhe and quinone cofactors are arranged within a protein scaffold formed by the L- and M-polypeptides in two approximately symmetrical membrane-spanning branches (Refer Figure 5.8B). The arrival of excitation energy at a 'special pair' of 870 nm-absorbing BChls (denoted P870) at the periplasmic end of the RC triggers a membrane-spanning four-step electron transfer along the A-branch of cofactors, which results in the reduction of a quinone at the so-called QB site near the cytoplasmic side of the membrane.

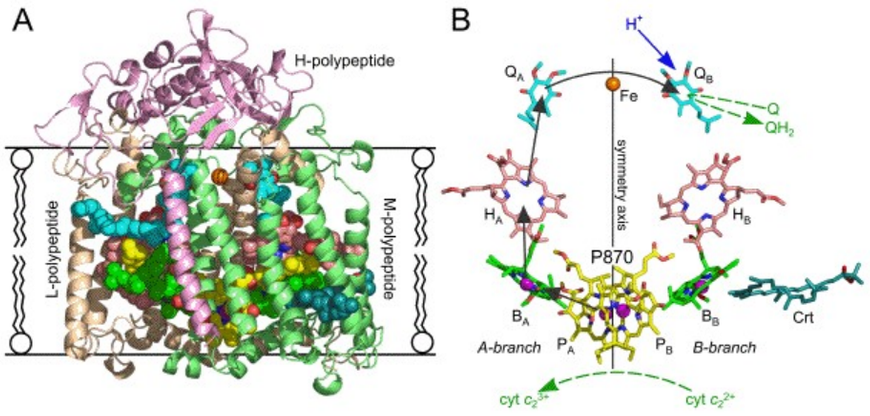


Fig. 5.8 *Overall Structure and Cofactors of the RC from Rba. Sphaeroides.*

(A) The cofactors (spheres) are encased by the largely intra-membrane L and M polypeptides (beige and light-green ribbons, respectively). The H polypeptide has an extra-membrane domain, and a single anchoring -helix (salmon ribbons).

(B) The cofactors are shown as sticks, with Mg and Fe atoms shown as purple or brown spheres, respectively. Hydrocarbon side chains of the BChl, BPhe and quinone cofactors are truncated for clarity. Carbon atoms are coloured thus: P870 BChl dimer (PA and PB) are yellow, monomeric BChls (BA and BB) are green, BPhes (HA and HB) are pink, quinones (QA and QB) are cyan, and

NOTES

carotenoid (Crt) is teal. Oxygen and nitrogens are shown in red and blue, respectively. The BChl, BPhe, and quinone cofactors are arranged in two membrane-spanning branches around an axis of two-fold pseudo-symmetry that connects P870 with the Fe atom. Black arrows show the route of electron transfer, blue arrow shows the site of H+ uptake, and dashed green arrows show Q/QH2 association/disassociation and delivery of electrons to P870+ by cyt c22+. The figure was constructed using PDB file 2BOZ.

As outlined below, the RC operates as a light-powered cytochrome c2: ubiquinone oxidoreductase, and participates in a cyclic proton motive electron transfer system with a partner ubiquinol: cytochrome c2 oxidoreductase, the bc1 (Refer Figure 5.9). This protein, which has similarities to the bc1 of mitochondria and the b6f complex of oxygenic phototrophs, catalyses a bifurcated electron transfer in which one electron removed from ubiquinol is used to reduce cyt c23+, and the second one is used to reduce quinone on the opposite side of the membrane. The X-ray crystal structure of the Rba. sphaeroides bc1 has been determined and, in common with other bc1 complexes, this protein is dimeric (Refer Figure 5.9A). At the core of the bc1 is a cyt b, comprising a membrane-spanning helix bundle encasing two hemes termed bL and bH. These form a membrane-spanning electron transfer chain that links a ubiquinol oxidase site near the periplasmic side of the membrane (Qo) with a quinone reductase site near the periplasmic side (Qi). The bc1 also contains a Rieske iron sulphur (Fe-S) protein containing a 2Fe-2S centre and a cyt c1, both of which have domains on the periplasmic side of the membrane, and a single membrane-spanning -helix. Each Rieske protein connects across the dimer, the membrane-spanning -helix associating with one monomer, and the extra-membrane domain associating with the second monomer.

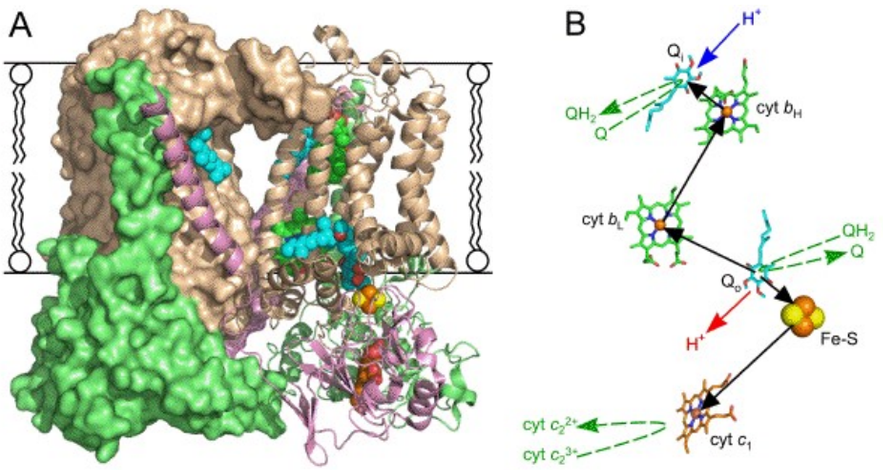


Fig. 5.9 *Overall structure and Cofactors of the Bc1 Dimer from Rba. Sphaeroides.*

(A) In the view presented, parallel to the plane of the membrane, one half is shown as a solid object, and the second half as ribbons and spheres for the protein and cofactors, respectively. Each monomer comprises a cyt b (beige), cyt c1 (green) and Rieske Fe-S protein (pink).

NOTES

(B) View in the plane of the membrane of a stick model of the cofactors of one half of the bc1 dimer. Heme Fe atoms are shown as small spheres, and the FeS centre as large spheres. Carbon atoms are coloured thus: b hemes are green, c heme is orange, and quinones are cyan. Oxygens, nitrogens, irons and sulphurs are shown in red, blue, brown and yellow, respectively. Black arrows show the routes of electron transfer, blue/red arrows show sites of proton uptake/release and dashed green arrows show association and disassociation of Q/QH2, and the reduction of cyt c23+ by cyt c1. The Figure was constructed using PDB file 2QJY, which has the inhibitor stigmatellin at the Qo site; this has been digitally replaced by quinone to construct the Figure.

4. Photosystem Mechanism

The purple bacterial photosystem is capable of harvesting light energy over a range of wavelengths. Photon absorption produces a singlet excited electronic state of the absorbing pigment, and energy harvested by a carotenoid pigment of an antenna complex is transferred to an adjacent BChl. As outlined above, the BChls have three principal absorbance bands, the Soret band in the blue/near-UV region, the Qx band in the red region, and the Qy band in the near-infrared. Energy absorbed in the Soret or Qx regions is converted to the lowest-energy Qy excited state through internal conversion.

The Qy excited state energies of the LH2 and LH1 BChls are arranged to ensure the funneling of energy into the RC. Within an individual LH2 protein, energy absorbed by the B800 BChls is passed to the ring of lower energy B850 BChls. This energy is then passed either to a neighbouring LH2 or into the ring of lower-energy LH1 B875 BChls surrounding the RC (Sundström et al., 1999) (Refer Figure 5.10). The final step involves the transfer of excited state energy to the P870 dimer of BChls in the RC, triggering photochemical charge separation. Funneling of excited state energy into the RC is therefore achieved by having red-shifted (low-energy) BChls closest to the RC.

B800 BChls of LH1 are shown in green, B850 BChls of LH2 and B875 BChls of LH1 are shown in alternating red and orange, and P870 BChls of the RC are shown in yellow. Lifetimes in picoseconds (ps) are given for the energy transfer events indicated by the arrows. Energy absorbed by antenna carotenoids is passed to neighbouring BChls. Double-headed arrows indicate femtosecond (fs) energy migration within the B850 or B875 pigment rings.

As the rate of energy transfer decreases with the sixth power of the distance between donor and acceptor, light harvesting complexes need to be tightly-packed within the membrane to ensure that excited state energy is passed efficiently to the RC with minimal losses from emission. The excited state lifetime of BChl is of the order of a few nanoseconds, which means that energy absorbed by antenna pigments has to be funneled to the RC on a time scale of a few tens to hundreds of ps. Energy transfer between weakly coupled BChls in adjacent LH complexes takes place on time scales of a few ps and, along with

NOTES

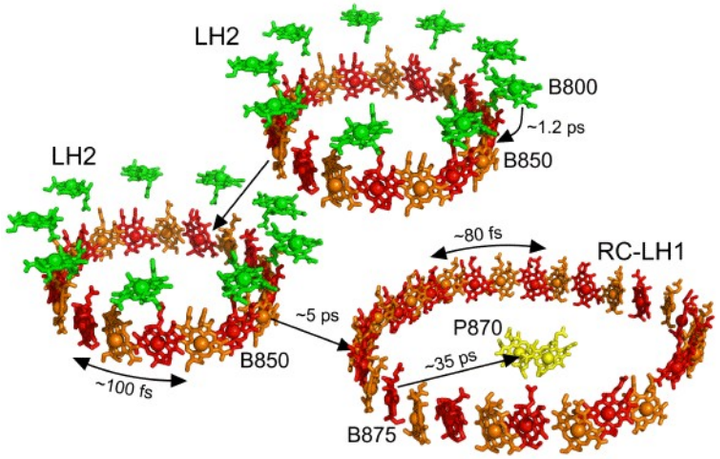


Fig. 5.10 *Energy Transfer in the Purple Bacterial Photosystem.*

B800B850 energy transfer, can be described by the Förster mechanism (non-radiative resonance energy transfer through coupling between two transition dipoles). Within the B850 and B875 rings, where the BChls are strongly coupled, the excitation is delocalized over the whole ring with extremely fast fs 'hopping times' between adjacent BChls, and this process is better described using an exciton approach involving delocalized electronic transitions.

The slowest step is the transfer of the excited state from the B875 BChls to the P870 BChl dimer (referred to as 'trapping'), which, due to the relatively large distance, takes place in around 30-50 ps. This arrangement, where the charge-separating Chls or BChls of the RC are separated from the light-harvesting Chls or BChls of the antenna by an exclusion zone formed by the protein scaffold, is also a feature of the structure of both PS1 and PS2 of oxygenic photosynthesis. This architecture appears to have two principal functions, to ensure that unproductive back transfer of excitation energy (detrapping) is slow compared to productive charge-separation (which takes place in a few ps), and to ensure that the efficiency of membrane-spanning charge separation is not interfered with by unwanted electron transfer reactions between RC and antenna BChls.

The process of photochemical charge separation is described in detail in the related module by Yocum on RCs. A summary of the reactions catalysed by the Rba. sphaeroides RC. Acquisition of excited state energy converts P870 into a sufficiently powerful reductant to be able to donate an electron to the adjacent BA BChl. This electron is subsequently passed in three steps to the quinone at the QB site near the cytoplasmic side of the membrane, the photooxidised P870 (P870+) located near the periplasmic face of the membrane being re-reduced (in Rba. sphaeroides) by a water-soluble c-type cytochrome (cyt c2).

A second light-induced charge separation results in double reduction of the QB quinone, delivery of the second electron being accompanied by the uptake of two protons from the cytoplasm to form ubiquinol [dihydroquinone (QH2)]. The

NOTES

immediate products of light absorption, energy funnelling and charge separation are therefore one QH2, which dissociates into the intra-membrane phase, and two oxidised cyt c2 (cyt c23+) which can diffuse in the periplasmic space. These mobile products are then used as substrates by the bc1 (Refer Figure 5.9).

Oxidation of QH2 takes place at a site (Qo) near the periplasmic side of the membrane and is a bifurcated reaction, one electron being used to reduce cyt c2 by passage through the so-called high-potential chain formed by the Rieske Fe-S protein and cyt c1, accompanied by the release of a proton. A notable feature of this reaction is a large scale change in the conformation of the Rieske protein, that moves the Fe-S centre away from the Qo site and towards the cyt c1 heme. This movement prevents the second electron from being passed to the Rieske Fe-S centre. Instead, accompanied by release of the second proton, the second electron is passed back across the membrane via two b-type hemes to a second quinone reductase site (Qi). Oxidation of a second QH2 at the Qo site results in the reduction of a second cyt c2 via the high potential chain, and double reduction and protonation of the quinone at the Qi site. As the sites for reduction/ protonation of quinone in both the RC and bc1 are located on the cytoplasmic side of the membrane, and the site for quinol oxidation in the latter is located on the periplasmic side, light-powered cyclic electron flow in this system is coupled to the translocation of protons from the cytoplasmic compartment into the periplasm. Figure 5.11 summarises the movement of quinone and cyt c2 between binding sites on the RC and bc1, and the proton translocation that is powered by sunlight.

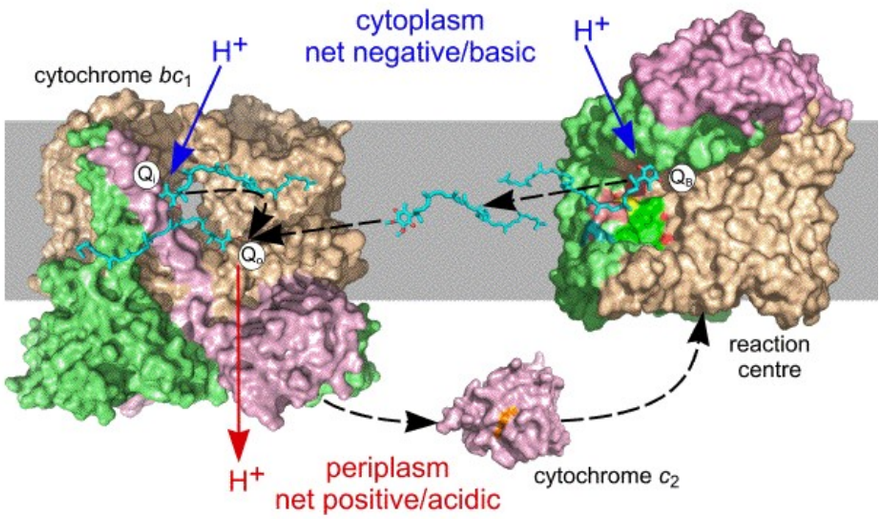


Fig. 5.11 *Light Driven Cyclic Electron Transfer and Proton Pumping.*

The RC acts in partnership with the bc1 to translocate protons across the photosynthetic membrane, transducing sunlight into the energy of the proton motive force. Dashed black arrows show the movement of reducing equivalents between the RC and bc1, or between the cytoplasmic and periplasmic side sides of the bc1, by the mobile carriers ubiquinol and cyt c2. Blue arrows show sites for the

uptake of protons from the cytoplasm, and red arrows the site of proton release into the periplasm. The migration of electrons internal to the RC and bc1 is not shown. The view of the bc1 dimer shows the Qi site of one monomer, and the Qo site of the second monomer.

NOTES

Summaries of this process from the points of view of redox potential and free energy are. Transformation of the redox potential of P870 through light absorption (Refer Figure 5.12A) triggers a cascade of redox reactions, as electrons flow via the QB site into the intra-membrane

Q-pool, through the high potential chain of the bc1, and through the cyt c2 pool to re-reduce P870+. The additional energy of the initially-formed P870* excited state (~1.45 eV for an 870 nm photon) is progressively lost as charge is separated through the protein. However, part of this energy is preserved in the form of the energy of the pmf formed through the proton translocation that is coupled to electron flow. This pmf is then used to power a variety of energy-requiring reactions, including ATP synthesis, active transport, motion of the bacterial flagellum, and so on

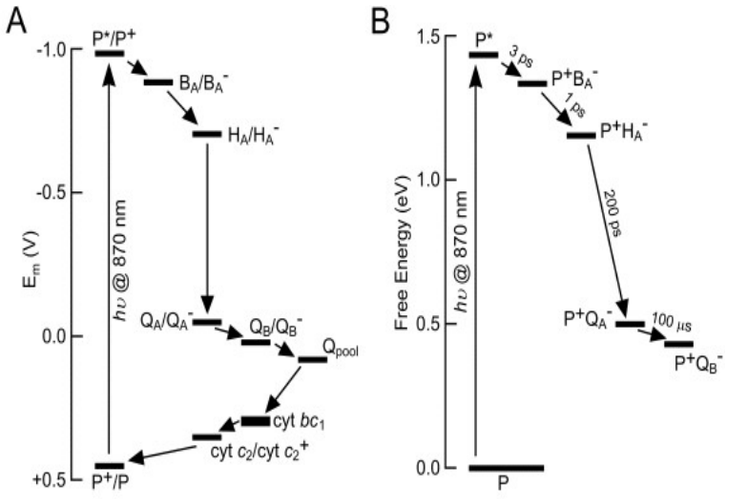


Fig. 5.12 *Redox Potential and Energetics of Electron Transfer in the Rba. Sphaeroides Photosystem.*

In the above figure A shows the formation of the excited state through light absorption transforms the redox potential of P870 (abbreviated to P in this diagram), triggering reduction of BA and subsequent electron transfer. 'cyt bc1' refers to the high-potential chain in the bc1 complex. Redox couples are written with the notation reactant/product, B shows free energy of charge separation in the RC.

Photosystem Diversity

The outline description given in the last section is based on the photosystem from Rba. sphaeroides, which has been the main workhorse for structural and functional studies. The photosystems from other species of purple bacteria operate on the

NOTES

same principles, but the details vary from organism to organism. So, for example, the first RC to yield a high resolution X-ray crystal structure, that from Blc. viridis, has an additional sub-unit comprising an extra-membrane tetra-heme cytochrome that is attached to the periplasmic side of the complex (Deisenhofer et al., 1995). One of the hemes of this cytochrome sub-unit acts as the initial reductant of the photo-oxidised primary electron donor in this RC, termed P960+, the water-soluble c-type cytochrome delivering electrons from the bc1 to this tetra-heme cytochrome sub-unit, and so only indirectly reducing P960+. This RC contains BChl b rather than BChl a, and has menaquinone in the QA quinone site.

Perhaps the greatest variety comes in the type and organization of light-harvesting complexes present in purple bacteria. It has been known for many years that many species contain one or more types of peripheral light harvesting complex, the levels of which are affected by environmental factors such as light intensity or oxygen levels. In other species, including Blc. viridis and Rsp. rubrum, this peripheral antenna is absent, the light harvesting function being the sole responsibility of the LH1 antenna that surrounds each RC.

A full understanding of the process of photosynthetic energy transduction in purple bacteria not only requires detailed information on the structure and mechanism of individual components, but also an appreciation of how these are put together to form a fully functioning photosynthetic membrane. In recent years the technique of atomic force microscopy (AFM) has provided detailed information on this aspect of the photosystem, the distinctive ring-like topography of light harvesting complexes proving particularly amenable to imaging and interpretation. In Blc. viridis, which lacks peripheral antenna complexes, the RC-LH1 complexes form very regular arrays, each unit in the array comprising a central RC surrounded by a complete ring of LH1 pigment-protein (comprised of 16 pairs of and ß polypeptides, see above).

In Rsp. photometricum, the RC-LH1 complex also has a closed ring of 16 /ß pairs, but the presence of LH2 means that the membrane is less ordered, with individual RC-LH1 complexes separated by variable amounts of smaller rings attributable to the peripheral LH2 complex. In Rps. Palustris, the photosynthetic membrane is also disordered in this way, and in agreement with the low resolution X-ray data described above, the ring of LH1 surrounding each Rps. Palustris RC is not complete, comprising only 15 /ß pairs. In Rba. blasticus, two forms of the RC-LH1 complex are apparent, a minor monomeric form comprising an RC surrounded by an incomplete ring of LH1 pigment-protein, and a major dimeric form in which two RCs are surrounded by a S-shaped antenna, both with 13 LH1 /ß pairs per RC. This dimeric arrangement was first reported for the Rba. sphaeroides RC-LH1 complex, and in this species RC-LH1 dimers are arranged in linear arrays several complexes in length, and surrounded by LH2 complexes. Although AFM has been successfully used to characterize the organization of RCs and LH complexes in photosynthetic membranes from a variety of purple bacteria, the location of the bc1 and ATP synthase in these membranes has not

been established. However, the information from AFM and kinetic spectroscopy has been used to build spectacular models of the photosynthetic membrane, which include speculations on the location of bc1 and ATP synthase.

Metabolic Diversity

The mechanistic or physiological significance of observed variations in the organization of the photosynthetic membrane is not understood, but it may be linked, at least in part, to the metabolic flexibility displayed by this group of bacteria. The species displaying the greatest flexibility, including Rba. sphaeroides and the closely related Rba. capsulatus, are capable of both photoautotrophy (carry out photosynthesis to acquire energy and fix carbon dioxide), and photoheterotrophy (photosynthesis requiring a source of carbon other than carbon dioxide), and can also grow in the dark through aerobic respiration, or anaerobic respiration with a variety of terminal electron acceptors. Fermentative growth is also well documented, as is chemolithotrophy (deriving energy from the oxidation of inorganic compounds) with hydrogen or thiosulphate as electron donors. Most purple photosynthetic bacteria can fix N_2 to form NH_3 and H_2, and some can carry out denitrification of nitrate to N_2. Some species are obligate anaerobes, but others can tolerate high levels of oxygen, although in species such as Rba. sphaeroides expression of the photosynthetic apparatus is down-regulated by oxygen. In another group, termed aerobic anoxygenic phototrophs, expression of the photosynthetic apparatus takes place only in the presence of oxygen. These bacteria are widespread with a number of intriguing characteristics, including low levels of RCs and LH complexes, repression of BChl synthesis by light, and high levels of carotenoid.

A major factor in the prominent use of Rba. sphaeroides as a model organism for studying purple bacterial photosynthesis is its metabolic flexibility. In addition to growing in the light, this species will grow in the dark in the presence of a suitable source of carbon and electrons, such as succinate or malate for example, and a suitable electron acceptor, such as oxygen. Although the expression of the photosynthetic apparatus is repressed at very high levels of oxygen, a fully-functional photosystem is assembled at moderate-to-low oxygen tensions, although it is not required for growth. As a result, it is possible to introduce potentially lethal mutations into the RC and LH complexes of the photosystem, and study the structural and functional consequences of these in proteins or membranes prepared from cells grown in the absence of light. Such experiments are not possible in obligate phototrophs, where impairment of the photosynthetic apparatus can lead to cell death, and incubation under illuminated conditions can give rise to reversion or suppression mutations.

Photosynthesis In Other Anoxygenic Photosynthetic Bacteria

Although by far the most heavily studied, the purple phototrophs are only one of several groups of bacteria that use BChl proteins to exploit sunlight as an energy source. The green non-sulphur bacteria (also called green filamentous bacteria), such as Chloroflexus (Cf.) aurantiacus, have a photosystem similar to that of purple

NOTES

photosynthetic bacteria. The RC of Cf. aurantiacus is similar to that of Rba. sphaeroides, with an intramembrane heterodimer of L and M polypeptides that scaffold the electron transfer cofactors. However, this RC lacks the H-polypeptide, both quinones are menaquinone, and the bacteriochlorin cofactors comprise three BChls and three BPhes. The RC is associated with a B808-866 antenna complex that has spectroscopic similarities to the purple bacterial LH2 antenna, but shows sequence similarities to the polypeptides of the purple bacterial LH1. In addition to possessing an intra-membrane light harvesting system, green non-sulphur bacteria possess an extensive light harvesting system called a chlorosome, which is attached to the cytoplasmic face of the photosynthetic membrane. This comprises an aggregate of up to 10,000 molecules of BChl c, with smaller amounts of protein, lipid and carotenoids, and the chlorosome transfers energy to the intra-membrane antenna via a set of baseplate proteins, and thence to the RC.

The RCs from purple bacteria and green filamentous bacteria are both classed as quinone-pheophytin type, or type-II RCs, along with the PS2 RC from oxygenic photosynthesis. The remaining anoxygenic photosynthetic bacteria, the green sulphur bacteria and heliobacteria, contain an Fe-S type or Type-I RC similar to the PS1 RC from oxygenic photosynthesis. Again the reader is referred to the module by Yocum on RCs for a description of the PS1 RC; the key differences with the type-II RCs discussed thus far, is that the membrane-spanning electron transfer chain terminates in Fe-S centers rather than dissociable quinones, and within the RC pigment-protein the central electron transfer domain is flanked by two additional symmetrical domains that house antenna BChls or Chls and carotenoids. In green sulphur bacteria, such as Chlorobium limicola, the light harvesting function carried out by the intra-membrane antenna regions of the RC is again augmented by an extra-membrane chlorosome antenna comprising largely of BChls c, d, and e. This is also connected to the intramembrane antenna via baseplate proteins, but the green sulphur bacteria also contain the Fenna-Matthews-Olson (FMO) protein that participates in energy flow from the chlorosome to the intra-membrane components. The FMO protein is water-soluble, and was the first chlorophyll protein to be structurally characterized to a high resolution by X-ray crystallography.

The final group of anoxygenic photosynthetic bacteria are the heliobacteria. These also have a Type-I RC, with a central electron transfer domain and two antenna domains, and unlike other photosynthetic bacteria this RC contains BChl g as a minor component. Heliobacteria do not have additional intra- or extra-membrane antenna complexes. A feature of the *heliobacterium* RC, shared with the Type-I RC from green sulphur bacteria, is that it is formed from a homodimer of a single polypeptide, rather than the L/M heterodimer seen in purple bacteria (or the D1/D2 and PsaA/PsaB heterodimers seen in PS1 and PS2). Another feature established in recent is years is that, in common with PS1, both branches of membrane-spanning cofactors are used to transfer electrons across the membrane during photochemical charge separation. In the type-II RCs described above, the two quinones have discrete functional roles, the QA quinone acting as a one electron relay but the QB quinone being specialized for accumulation of two electrons and undergoing double

protonation in order to generate a quinol. However, in the Type-I RCs the quinone cofactors are both non-dissociable, and on reduction pass the electron on to an Fe-S centre located on the symmetry axis. As a result, in principle, either branch of cofactors could be used to catalyse membrane-spanning electron transfer, and findings in recent years have shown that this is indeed the case.

NOTES

Check Your Progress

6. How is chlorophyll important?
7. What are bacteriochlorophylls?
8. What is the main light harvesting pigment in purple photosynthetic bacteria?
9. What is the role of carotenoids?
10. What does RUBP carboxylase use?
11. What does light energy do?

5.5 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Photosynthesis is the use of light as a source of energy for growth, more specifically the conversion of light energy into chemical energy in the form of ATP.
2. Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the 'halobacteria'.
3. The cyanobacteria conduct plant photosynthesis, called oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; the extreme halophilic archaea use a type of non-photosynthetic photophosphorylation mediated by a pigment, bacteriorhodopsin, to transform light energy into ATP.
4. Photosynthetic reactions divided into two stages:
   - Light Reaction - Light energy absorbed & converted to chemical energy (ATP, NADPH)
   - Dark Reaction - Carbohydrates made from CO_2 using energy stored in ATP & NADPH
5. Uses of two photosystems (PS)
   - PS II- Generates a proton-motive force for making ATP.
   - PS I- Generates low potential electrons for reducing power.
6. Chlorophyll is the primary light-harvesting pigment in all photosynthetic organisms.

NOTES

7. The chlorophylls of the purple and green bacteria, called bacteriochlorophylls are chemically different than chlorophyll in their substituent side chains.
8. The main light harvesting pigment in purple photosynthetic bacteria is not chlorophyll but bacteriochlorophyll (BChl), a closely related magnesium porphyrin that has a more saturated tetrapyrrole ring.
9. Carotenoids transfer energy to chlorophyll, at near 100 percent efficiency, from wave lengths of light that are missed by chlorophyll.
10. RUBP carboxylase (ribulose bisphosphate carboxylase) uses ribulose bisphosphate (RUBP) and CO_2 as co-substrates.
11. Light energy is captured by pigment molecules in the light harvesting or 'antenna' region of the photosystem, and is stored temporarily as an excited electronic state of the pigment.

5.6 SUMMARY

- The process of photosynthesis is most commonly associated with plants and algae, much of our understanding of the molecular basis for light energy capture and photochemical energy transduction has come from studies of photosynthetic bacteria.
- Photosynthesis is the use of light as a source of energy for growth, more specifically the conversion of light energy into chemical energy in the form of ATP.
- Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the 'halobacteria'.
- The cyanobacteria conduct plant photosynthesis, called oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; the extreme halophilic archaea use a type of non-photosynthetic photophosphorylation mediated by a pigment, bacteriorhodopsin, to transform light energy into ATP.
- The Light Reactions depend upon the presence of chlorophyll, the primary light-harvesting pigment in the membrane of photosynthetic organisms.
- Chlorophyll is the primary light-harvesting pigment in all photosynthetic organisms.
- Chlorophyll is a tetrapyrrole which contains magnesium at the center of the porphyrin ring. It contains a long hydrophobic side chain that associates with the photosynthetic membrane.
- Cyanobacteria have chlorophyll a, the same as plants and algae. The chlorophylls of the purple and green bacteria, called bacteriochlorophylls are chemically different than chlorophyll in their substituent side chains. This is reflected in their light absorption spectra.

NOTES

- Chlorophyll absorbs light in two regions of the spectrum, one around 450nm and the other between 650-750nm; bacterial chlorophylls absorb from 800-1000nm in the far red region of the spectrum.
- The main light harvesting pigment in purple photosynthetic bacteria is not chlorophyll but bacteriochlorophyll (BChl), a closely related magnesium porphyrin that has a more saturated tetrapyrrole ring.
- Light harvesting is also carried out by a variety of carotenoids that provide the main pigmentation in the visible region of the spectrum, and so make purple bacteria purple.
- Carotenoids are always associated with the photosynthetic apparatus. They function as secondary light-harvesting pigments, absorbing light in the blue-green spectral region between 400-550 nm.
- Carotenoids transfer energy to chlorophyll, at near 100 percent efficiency, from wave lengths of light that are missed by chlorophyll.
- Phycobiliproteins are the major light harvesting pigments of the cyanobacteria. They also occur in some groups of algae. They may be red or blue, absorbing light in the middle of the spectrum between 550 and 650nm.
- Phycobiliproteins consist of proteins that contain covalently-bound linear tetrapyrrole (phycobilins). They are contained in granules called phycobilisomes that are closely associated with the photosynthetic apparatus.
- The use of RUBP carboxylase and the Calvin cycle is the most common mechanism for CO_2 fixation among autotrophs.
- Lithoautotrophic bacteria also use this pathway. But the green bacteria and the methanogens, as well as a few isolated groups of prokaryotes, have alternative mechanisms of autotrophic CO_2 fixation and do not possess RUBP carboxylase.
- The Calvin cycle is concerned with the conversion of PGA to intermediates in glycolysis that can be used for biosynthesis, and with the regeneration of RUBP, the substrate that drives the cycle.

5.7 KEY WORDS

- **Chlorophyll:** It is the primary light-harvesting pigment in all photosynthetic organisms.
- **Photosynthesis:** It is the use of light as a source of energy for growth, more specifically the conversion of light energy into chemical energy in the form of ATP.
- **Bacteriochlorophylls:** The chlorophylls of the purple and green bacteria, called bacteriochlorophylls are chemically different than chlorophyll in their substituent side chains.

NOTES

5.8 SELF ASSESSMENT QUESTION AND EXERCISES

Short-Answer Questions

1. Write a brief note on photosynthesis.
2. In how many stages photosynthetic reactions divided?
3. Differentiate between oxygenic and anoxygenic photosynthesis.
4. Write a short note on chlorophyll.
5. Brief about carotenoids.

Long-Answer Questions

1. Write about the structure of photosynthetic pigments.
2. Explain light and dark reaction.
3. Describe about energy transduction in the (Anoxygenic) photosynthetic bacteria.
4. Write about photosystem mechanism.
5. Explain about photosystem diversity.
6. Explain about anoxygenic photosynthetic bacteria.

5.9 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 6 BACTERIAL PHOTOSYNTHESIS: MECHANISM

Structure

6.0 Introduction
6.1 Objectives
6.2 Photosynthetic Bacteria- Green Sulphur and Purple
  6.2.1 Green S Bacteria
  6.2.2 Purple S Bacteria
6.3 Photophosphorylation
6.4 Mechanism of Photosymthesis of Non-Cyclic and Cyclic Electron Transport
  6.4.1 Non-cyclic Electron Transport in Photosynthesis
  6.4.2 Cyclic Electron Transport in Photosynthesis
6.5 Answers to Check Your Progress Questions
6.6 Summary
6.7 Key Words
6.8 Self Assessment Questions and Exercises
6.9 Further Readings

6.0 INTRODUCTION

Photosynthetic bacteria have been around for longer than the Earth's atmosphere could sustain human life. It was only recently though that scientists began to unravel the mystery of how these micro-organisms execute the mechanisms of photosynthesis.

Oxygenic photosynthetic bacteria perform photosynthesis in a similar manner to plants. They contain light-harvesting pigments, absorb carbon dioxide, and release oxygen. Cyanobacteria or Cyanophyta are the only form of oxygenic photosynthetic bacteria known to date. There are, however, several species of Cyanobacteria. They are often blue-green in color and are thought to have contributed to the biodiversity on Earth by helping to convert the Earth's early oxygen-deficient atmosphere to an oxygen-rich environment. This transformation meant that most anaerobic organisms that thrived in the absence of oxygen eventually became extinct and new organisms that were dependent on oxygen began to emerge. Photosynthetic bacteria are currently being used in various applications which include water purification, bio-fertilizers, animal feed and bioremediation of chemicals among many others. They are used in the treatment of polluted water since they can grow and utilize toxic substances such as H2S or H2S203.

Sulfur bacteria are Gram-negative, anaerobic, filamentous photosynthetic bacteria. During photosynthesis, carbon dioxide reduction to carbohydrates doesn't

NOTES

utilize water as electron donor but hydrogen sulfide (H2S); hence they are called as sulfur (S) bacteria.

In this unit, you will study about photosynthetic bacteria - green and purple sulfur, photo phosphorylation, mechanism of photosynthesis non-cyclic and cyclic electron transport

6.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand about photosynthetic bacteria - green and purple sulfur
- Learn about photo phosphorylation
- Discuss about the mechanism of photosynthesis
- Discuss about non-cyclic and cyclic electron transport

6.2 PHOTOSYNTHETIC BACTERIA- GREEN SULPHUR AND PURPLE

Sulfur bacteria are Gram negative, anaerobic, filamentous photosynthetic bacteria. During photosynthesis, carbon dioxide reduction to carbohydrates doesn't utilize water as electron donor but hydrogen sulfide (H_2S); hence they are called as sulfur (S) bacteria. Depending upon their carotenoid content, S bacteria are identified as green and purple bacteria. Purple bacteria appear purple or reddish brown, green bacteria appear as yellow green, green orange or brown. The color of major photosynthetic pigment, bacteriochlorophyll is not taken into account as human eye cannot detect its absorption spectrum in infrared region. Both purple and green bacteria are important biological constituents of atmospheric sulfur cycle. It is mandatory to know reactions of sulfur cycle, to understand the role of these bacteria. Sulfur is present in the nature in amino acids cysteine and methionine, free and combined, organic and inorganic states; its transformations between oxidized and reduced states are accomplished by S bacteria. They oxidize H_2S to sulfate which is the most suitable form of essential sulfur utilized by plants. Anaerobic oxidation of sulfur with nitrate reduction is also found in some S bacteria. Sulfate is reduced and assimilated by plants and microbes in the soil. Assimilated sulfate is incorporated into proteins under aerobic conditions. Dissimilatory sulfate reduction occurs with the production of H_2S. Hydrogen is used in the reduction of sulfate to H_2S via sulphite; that means sulfate is utilized as terminal electron acceptor. Purple and green S bacteria coexist in illuminated sulfide rich anaerobic aquatic environments

6.2.1 Green S Bacteria

Green bacteria are not cyanobacteria or blue-green algae as one may assume; they different group of phototrophic bacteria from family Chlorobiaceae.

Photosynthetic pigments are bacteriochlorophylls c, d or e; *carotenoids chlorobactene, hydrochlorobactene, isorenieratene* and *â-isorenieratene.* They use H_2S, other reduced inorganic sulfur compounds and H_2 as electron donors. One interesting thing is that they cannot use sulfate as sulfur source, they require sulfide to meet biosynthetic needs when growing with H_2 as electron donor and also vitamin B12. Elemental sulfur produced from H_2S oxidation is deposited extracellularly as sulfur globules prior to its oxidation to sulfate. They cannot grow photoheterotrophically using organic compounds as their principle or sole carbon source in absence of inorganic reductant. They can photoassimilate organic compounds like acetate or pyruvate but only if H_2S and CO_2 are present at a time. They are unique and only bacteria (except some Archaebacteria) that use reductive tricarboxylic acid cycle (TCA) for CO_2 fixation; other bacteria use Calvin-Benson cycle. Important genera of green S bacteria are: *Chlorobium, Chloropseudomonas (Prosthecochloris), Pelodictyon, Clathrochloris (Acaryochloris)* and *Chlorobacterium (Chloroherpeton).* Green but non-sulfur (non-S) bacteria of family Chloroflexaceae are also present in anaerobic habitats. They are different from green S bacteria in structure, nutrition, metabolism and ecology but like green S bacteria; contain bacteriochlorophyll c or d and as major and minor pigments respectively. They are photoheterotrophs, facultative photoautotrophs or chemoheterotrophs; usually inhabit hot springs containing low organic matter. They derive organic nutrients from cyanobacteria and found always in association with cyanobacteria. Principle genera include *Chloroflexus, Oscillochloris, Chloronema and Heliothrix*. *Heliothrix* is found only in hot springs of Yellowstone National Park and Western Oregon.

6.2.2 Purple S Bacteria

Purple S bacteria are from family Chromatiaceae, strictly anaerobic, obligate photoautotrophic inhabit sulfide rich waters. Sulfide is generated by sulfate reducing bacteria such as *Desulfovibrio, Desulfococcus, Desulfomonas, Desulfosarcina, Desulfolobus, Desulfobacter* and *Desulfococcus spp*. Photosynthetic pigments are bacteriochlorophyll a or b, carotenoids of group 1, 3 and 4. H_2S, sulfur, thiosulfate, molecular hydrogen and organic compounds acts like electron donors. All purple S bacteria fix nitrogen photosynthetically. They can grow on pyruvate heterotrophically in dark. H_2S is oxidized via elemental sulfur to sulfate by Calvin-Benson cycle. It always leads to transient accumulation of elemental sulfur as sulfur globules, gas vacuoles inside the cytoplasm. They also synthesize poly-â-hydroxybutyrate (PHB) granules. These bacteria also have special cell membrane intrusions or infolding. This results in enlarged membrane area to accommodate more centers of respiratory and photosynthetic activity. Principle genera are: T*hiospirillum, Chromatium, Ectothiorhodospira, Thiocystis, Thiocapsa, Lamprocystis, Thiodictyon, Thiopedia* and *Amoebobacter spp*. Purple nonS bacteria are also present and they sensitive to H_2S. They represent principle genera *Rhodospirillum, Rhodopseudomonas, Rhodomicrobium, Rhodopila,*

NOTES

Rhodocyclus and Rhodobacter from family *Rhodospirillaceae*. They oxidize sulfide anaerobically at very low concentration in presence of light. They occur typically in freshwater habitats where sulfide is absent or present in extremely low concentration and organic matter is abundant. They can photoassimilate wide range of organic compounds like fatty acids, primary or secondary alcohols, organic acids, carbohydrates, proteins and aromatic compounds. Some species also carry out simultaneous processes of denitrification and nitrogen fixation to produce reduced and combined form of nitrogen to support cell growth.

Figure 6.1 shows the electron transport chain for purple photosynthetic and green sulfur bacteria

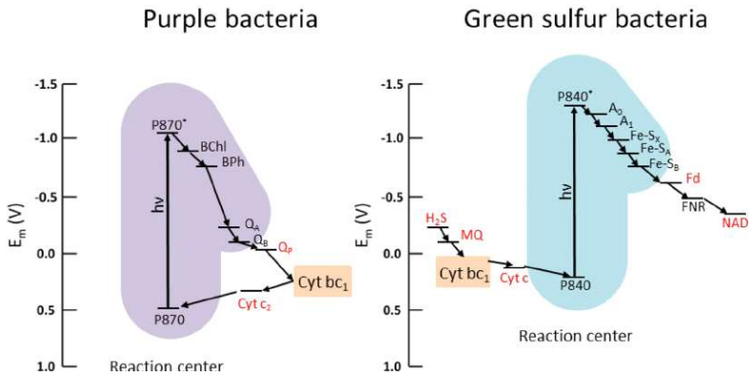


Fig. 6.1 *Electron Transport Chain Diagrams for Purple Photosynthetic and Green Sulfur Bacteria*

Ecological Importance Of Sulfur Oxidation And Reduction

Sulfur is abundant in nature but its oxidation products like sulfate is the utilizable form for plants. Sulfate anions solubilize inorganic salts containing nutrients such as phosphorous for plant use. Sulfate also prevents excessive alkalinity due to ammonia formation by microorganisms. Anaerobic oxidation of sulfur along with nitrate reduction is sometimes detrimental process which results in loss of soil fertility. H_2S produced by sulfur reduction is highly toxic to aquatic flora and fauna, often associated with fish mortality. In waterlogged anaerobic soils such as paddy fields, H_2S may damage vegetation. H_2S produced by reduction is important source of reducing power to support the growth of H_2S aerobic chemoautotrophs or anaerobic photo auto and photoheterotrophs. Sulfur reducing bacteria and phototrophic bacteria are predominant in Sulfureta habitats such as sulfate rich stagnant lakes. These bacteria promote formation of elemental sulfur from sulfates; geological sulfur deposits are formed from activities of these bacteria. S bacteria also contribute to corrosion of metal pipes.

In the above figure the molecules in red are mobile electron shuttles. Protein complexes containing multiple electron transfer steps (reaction centers and cytochromes) are highlighted.

Check Your Progress

1. What are green sulphur bacteria?
2. What are purple sulphur bacteria?
3. Why are sulphur bacteria named so?
4. Which colour does purple and green bacteria napper?
5. How is hydrogen used in photosynthesis?

6.3 PHOTOPHOSPHORYLATION

In the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called photophosphorylation. Only two sources of energy are available to living organisms: sunlight and reduction-oxidation (redox) reactions. All organisms produce ATP, which is the universal energy currency of life. Commonly in photosynthesis this involves photolysis, or photodissociation, of water and a continuous unidirectional flow of electrons from water to PS.

In photophosphorylation, light energy is used to create a high-energy electron donor and a lower-energy electron acceptor. Electrons then move spontaneously from donor to acceptor through an electron transport chain.

6.4 MECHANISM OF PHOTOSYMTHESIS OF NON-CYCLIC AND CYCLIC ELECTRON TRANSPORT

6.4.1 Non-cyclic Electron Transport in Photosynthesis

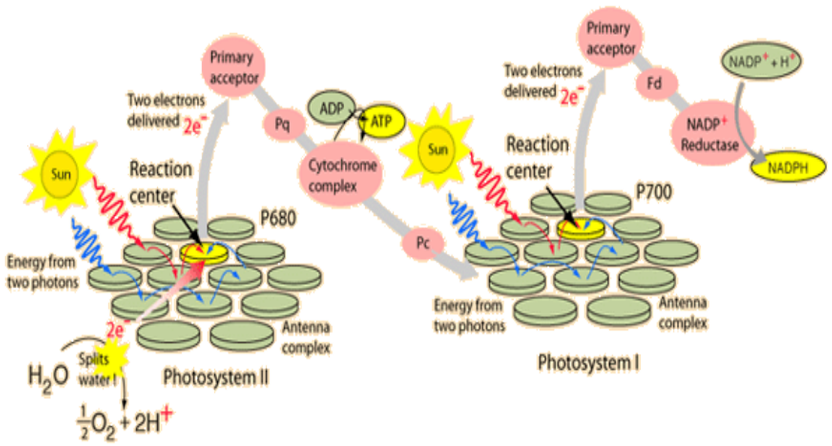


Fig. 6.2 *Non-Cyclic Electron Transport Process*

NOTES

Photophosphorylation refers to the use of light energy to ultimately provide the energy to convert ADP to ATP, thus replenishing the universal energy currency in living things. In the simplest systems in prokaryotes, photosynthesis is used just for the production of energy, and not for the building of any biological molecules. But in plants the photosynthesis process also provides energy in the form of reduced coenzymes which drive the process of biochemical synthesis culminating in the production of glucose and other sugars. This process uses both Photosystem II and Photosystem I acting in series. The above illustration is patterned after Moore and shows some of the steps of the non-cyclic electron transport process(Refer Figure 6.2)

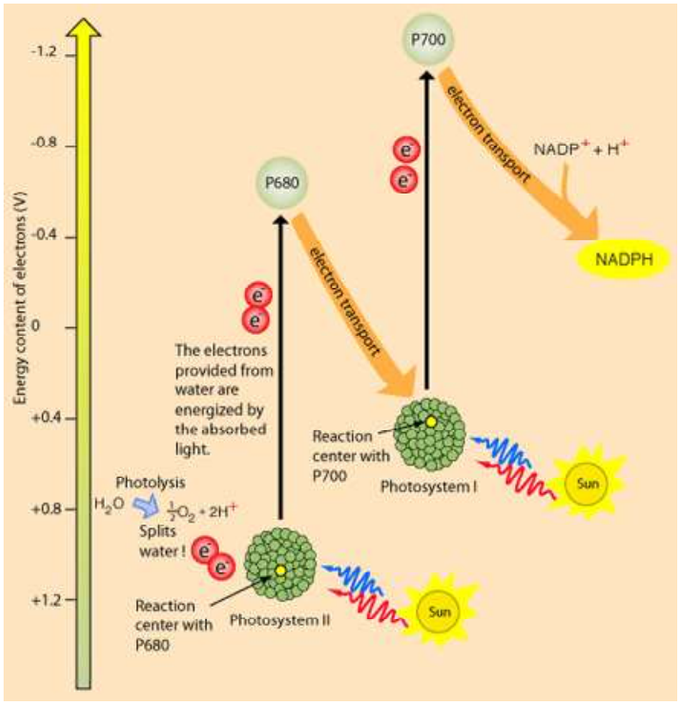


Fig. 6.3 *Energy Cycle in Living Beings*

Another way to look at this two-stage process for providing the energy for building molecules is in terms of the electron energies measured in units of the redox potential in volts. This illustration, patterned after Karp, outlines the steps in the process with the position on the vertical axis indicating the level of energy attained.

The thylakoid membranes are the location for the electron transport interactions that make available the light energy for chemical processes.

6.4.2 Cyclic Electron Transport in Photosynthesis

Photophosphorylation refers to the use of light energy to ultimately provide the energy to convert ADP to ATP, thus replenishing the universal energy currency in living things. In the simplest systems in prokaryotes, photosynthesis is used just for

the production of energy, and not for the building of any biological molecules. In these systems there is a process called cyclic photophosphorylation, which just accomplishes the ADP to ATP process for immediate energy for these cells. This process uses only Photosystem I and the chlorophyll P700.

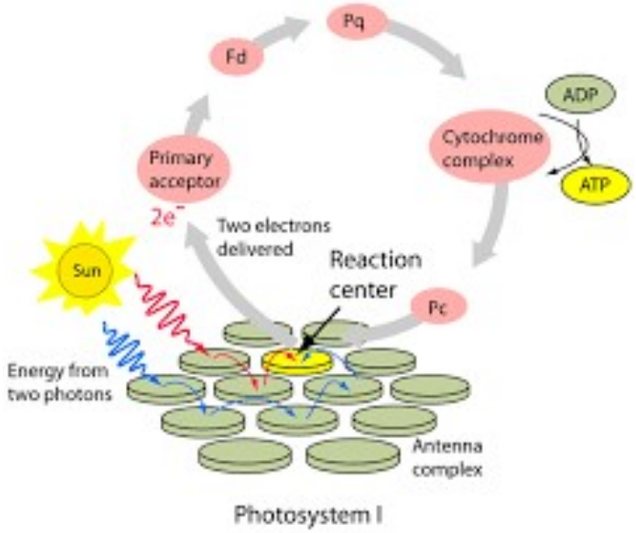


Fig. 6.4 *The Cyclic Process - Patterned after a Visualization in Moore, et al.*

The above sketch (Refer Figure 6.4) of the cyclic process is patterned after a visualization in Moore, et al. Two photons from either the red or blue end of the spectrum fit the sensitive response of the pigments. They are captured by the antenna complex and transferred to the Photosystem I reaction center, which contributes two high energy electrons to the primary electron receptor. They are passed to ferredoxin (Fd), an iron containing protein which acts as an electron carrier. A second electron carrier plastoquinone (Pq) carries the electrons to a complex of two cytochromes. In the process, energy is provided to produce a proton gradient across the membrane which can be used for the ADP to ATP conversion. The electrons are returned by plastocyanin (Pc) to the P700 pigment in the reaction center to complete the cycle.

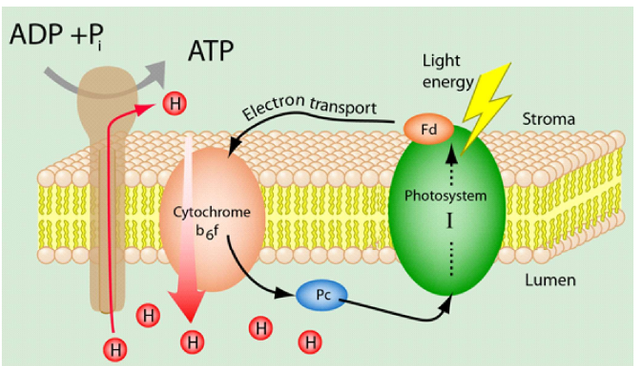


Fig. 6.5 *Lead of Karp to place the Events Relative to the Membrane*

NOTES

The above sketch (Refer Figure 6.5) follows the lead of Karp to place the events relative to the membrane. This makes it clearer that the process of producing the ATP is driven by the proton gradient. Karp points out that this cyclic photophosphorylation also takes place in isolated chloroplasts and can provide additional ATP to aid the carbohydrate synthesis taking place as result of the non-cyclic electron transport.

Check Your Progress

6. What is photophosphorylation?
7. In photophosphorylation how is light energy used?
8. What happens in cyclic process?

6.5 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Green sulfur bacteria are from Chlorobiaceae family of obligately anaerobic photoautotrophic bacteria. Together with the non-photosynthetic Ignavibacteriaceae, they form the phylum Chlorobi.
2. Purple sulfur bacteria(PSB) are the part of a group of Proteobacteria capable of photosynthesis, collectively referred to as purple bacteria.
3. During photosynthesis, carbon dioxide reduction to carbohydrates doesn't utilize water as electron donor but hydrogen sulfide (H_2S); hence they are called as sulfur (S) bacteria.
4. Purple bacteria appear purple or reddish brown, green bacteria appear as yellow green, green orange or brown.
5. Hydrogen is used in the reduction of sulfate to H_2S via sulphite; that means sulfate is utilized as terminal electron acceptor.
6. Photophosphorylation is the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called photophosphorylation. Only two sources of energy are available to living organisms: sunlight and reduction-oxidation (redox) reactions.
7. In photophosphorylation, light energy is used to create a high-energy electron donor and a lower-energy electron acceptor. Electrons then move spontaneously from donor to acceptor through an electron transport chain.
8. In cyclic process, energy is provided to produce a proton gradient across the membrane which can be used for the ADP to ATP conversion. The electrons are returned by plastocyanin (Pc) to the P700 pigment in the reaction center to complete the cycle.

6.6 SUMMARY

NOTES

- Sulfur bacteria are Gram negative, anaerobic, filamentous photosynthetic bacteria.
- During photosynthesis, carbon dioxide reduction to carbohydrates doesn't utilize water as electron donor but hydrogen sulfide (H_2S); hence they are called as sulfur (S) bacteria.
- Depending upon their carotenoid content, S bacteria are identified as green and purple bacteria.
- Purple bacteria appear purple or reddish brown, green bacteria appear as yellow green, green orange or brown.
- The color of major photosynthetic pigment, bacteriochlorophyll is not taken into account as human eye cannot detect its absorption spectrum in infrared region.
- Both purple and green bacteria are important biological constituents of atmospheric sulfur cycle.
- Sulfur is present in the nature in amino acids cysteine and methionine, free and combined, organic and inorganic states; its transformations between oxidized and reduced states are accomplished by S bacteria.
- Anaerobic oxidation of sulfur with nitrate reduction is also found in some S bacteria. Sulfate is reduced and assimilated by plants and microbes in the soil.
- Hydrogen is used in the reduction of sulfate to H_2S via sulphite; that means sulfate is utilized as terminal electron acceptor. Purple and green S bacteria coexist in illuminated sulfide rich anaerobic aquatic environments
- Green bacteria are not cyanobacteria or blue-green algae as one may assume; they different group of phototrophic bacteria from family Chlorobiaceae.
- Photosynthetic pigments are bacteriochlorophylls c, d or e; carotenoids chlorobactene, hydrochlorobactene, isorenieratene and â-isorenieratene.
- Elemental sulfur produced from H_2S oxidation is deposited extracellularly as sulfur globules prior to its oxidation to sulfate.
- Important genera of green S bacteria are: *Chlorobium, Chloropseudomonas (Prosthecochloris), Pelodictyon, Clathrochloris (Acaryochloris)* and *Chlorobacterium (Chloroherpeton)*. Green but non-sulfur (nonS) bacteria of family Chloroflexaceae are also present in anaerobic habitats.
- Principle genera include Chloroflexus, Oscillochloris, Chloronema and Heliothrix. Heliothrix is found only in hot springs of Yellowstone National Park and Western Oregon.

NOTES

- Purple S bacteria are from family Chromatiaceae, strictly anaerobic, obligate photoautotrophic inhabit sulfide rich waters. Sulfide is generated by sulfate reducing bacteria such as *Desulfovibrio, Desulfococcous, Desulfomonas, Desulfosarcina, Desulfolobus, Desulfobacter* and *Desulfococcus spp.*
- Sulfur is abundant in nature but its oxidation products like sulfate is the utilizable form for plants.
- Sulfate anions solubilize inorganic salts containing nutrients such as phosphorous for plant use.
- Sulfate also prevents excessive alkalinity due to ammonia formation by microorganisms.
- Anaerobic oxidation of sulfur along with nitrate reduction is sometimes detrimental process which results in loss of soil fertility.

6.7 KEY WORDS

- **Photosynthesis:** It is the process by which plants, some bacteria, and some protistans use the energy from sunlight to produce sugar, which cellular respiration converts into ATP, the 'fuel' used by all living things
- **Phosphorylation:** It is an important mechanism by which the activity of proteins can be altered after they are formed. A phosphate group (PO3–4) is added to a protein by specific enzymes called kinases.
- **Green sulfur bacteria:** They are from Chlorobiaceae family of obligately anaerobic photoautotrophic bacteria. Together with the non-photosynthetic Ignavibacteriaceae, they form the phylum Chlorobi.
- **Purple sulfur bacteria(PSB):** They are the part of a group of Proteobacteria capable of photosynthesis, collectively referred to as purple bacteria.

Photophosphorylation: In the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called photophosphorylation. Only two sources of energy are available to living organisms: sunlight and reduction-oxidation (redox) reactions

6.8 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Brief about photosynthesis process in bacteria?
2. Give a key difference between green sulphur bacteria and purple sulphur bacteria.

3. Define phosphorylation?
4. What is photophosphorylation?
5. Write in brief about cyclic electron transport process.

Long-Answer Questions

1. Elaborate about bacterial photosynthesis mechanism.
2. Distinguish between green sulphur bacteria and purple sulphur bacteria.
3. Write about ecological importance of sulfur oxidation and reduction.
4. Explain photophosphorylation.
5. Give a detailed overview of cyclic electron transport process.
6. Explain about non-cyclic electron transport process.

NOTES

6.9 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 7 MICROBIAL STRESS RESPONSES

NOTES

Structure

7.0 Introduction
7.1 Objectives
7.2 Microbial Stress Response
  7.2.1 Osmotic Stress
  7.2.2 Osmoregulation
7.3 Aerobic to Anerobic Transitions
7.4 Oxidative Stress
  7.4.1 Free Radicals
  7.4.2 Antioxidants
  7.4.3 Damaged Caused by Oxidative Stress
7.5 pH Stress and Acid Tolerance
7.6 Thermal Stress and Heat Shock Response
7.7 Nutrient Stress and Starvation Stress
7.8 Answers to Check Your Progress Questions
7.9 Summary
7.10 Key Words
7.11 Self Assessment Questions and Exercises
7.12 Further Readings

7.0 INTRODUCTION

To survive adverse and fluctuating conditions, microorganisms possess mechanisms to recognise diverse environmental changes and mount an appropriate response. Microorganisms frequently react simultaneously to a wide variety of stresses and the various stress response systems interact with each other by a complex of global regulatory networks. Stress response systems can play an important role in the virulence of pathogenic organisms.

When supplied with sufficient nutrients and optimal growth temperature, pH, oxygen levels, and solute concentrations, microbes will grow at a maximum growth rate characteristic for the organism. Variations in any of these parameters can affect the maximum growth rate and, thus, can represent an environmental stress for the microbe. The ability of microbes to sense and respond to impromptu alterations in the environment is crucial to their survival. In reality, conditions that allow for maximal growth rates outside the laboratory are few and far between. As a result, most bacteria live in a constant state of stress.

In this unit, you will study about microbial stress responses, osmotic stress and osmoregulation, aerobic to anaerobic transitions, oxidative stress, pH stress and acid tolerance, thermal stress and heat shock response, nutrient stress and

starvation stress, thermal stress and heat shock response, nutrient stress and starvation stress, etc.

7.1 OBJECTIVES

NOTES

After going through this unit, you will be able to:

- Understand the microbial stress responses
- Explain about osmotic stress and osmoregulation
- Discuss about aerobic to anaerobic transitions
- Understand what oxidative stress is
- Analyse the pH stress and acid tolerance
- Explain thermal stress and heat shock response
- Discuss about nutrient stress and starvation stress

7.2 MICROBIAL STRESS RESPONSE

The bacterial stress response enables bacteria to survive adverse and fluctuating conditions in their immediate surroundings. Various bacterial mechanisms recognize different environmental changes and mount an appropriate response. A bacterial cell can react simultaneously to a wide variety of stresses and the various stress response systems interact with each other by a complex of global regulatory networks.

Bacteria can survive under diverse environmental conditions and in order to overcome these adverse and changing conditions, bacteria must sense the changes and mount appropriate responses in gene expression and protein activity. The stress response in bacteria involves a complex network of elements that acts against the external stimulus. Bacteria can react simultaneously to a variety of stresses and the various stress response systems interact (cross-talk) with each other. A complex network of global regulatory systems leads to a coordinated and effective response. These regulatory systems govern the expression of more effectors that maintain stability of the cellular equilibrium under the various conditions.

Stress response systems can play an important role in the virulence of pathogenic organisms.

In bacteria some of the most important stress response systems are

- Heat shock response, controlled by the sigma factor sigma 32
- Envelope stress response, controlled mainly by the sigma factor sigma E and the Cpx two-component system
- Cold shock response, which governs expression of RNA chaperones and ribosomal factors

- General stress response, which depends on the sigma factor sigma S
- (p)ppGpp- dependent stringent response, which reduces the cellular protein synthesis capacity and controls further global responses upon nutritional downshift.

NOTES

7.2.1 Osmotic Stress

Osmotic shock or osmotic stress is a sudden change in the solute concentration around a cell, causing a rapid change in the movement of water across its cell membrane. Under conditions of high concentrations of either salts, substrates or any solute in the supernatant water is drawn out of the cells through osmosis. This also inhibits the transport of substrates and cofactors into the cell thus shocking the cell. Alternatively, at low concentrations of solutes, water enters the cell in large amounts, causing it to swell and either burst or undergo apoptosis. The rupture of bacterial or other cells in a solution following a sudden reduction in osmotic pressure. Osmotic shock is sometimes induced to release cellular components for biochemical analysis.

7.2.2 Osmoregulation

Osmoregulation is the process of maintaining salt and water balance (osmotic balance) across membranes within the body. The fluids inside and surrounding cells are composed of water, electrolytes, and nonelectrolytes. An electrolyte is a compound that dissociates into ions when dissolved in water. A nonelectrolyte, in contrast, does not dissociate into ions in water. The body's fluids include blood plasma, fluid that exists within cells, and the interstitial fluid that exists in the spaces between cells and tissues of the body. The membranes of the body (both the membranes around cells and the 'membranes' made of cells lining body cavities) are semipermeable membranes. Semipermeable membranes are permeable to certain types of solutes and to water, but typically cell membranes are impermeable to solutes.

The body does not exist in isolation. There is a constant input of water and electrolytes into the system. Excess water, electrolytes, and wastes are transported to the kidneys and excreted, helping to maintain osmotic balance. Insufficient fluid intake results in fluid conservation by the kidneys. Biological systems constantly interact and exchange water and nutrients with the environment by way of consumption of food and water and through excretion in the form of sweat, urine, and feces. Without a mechanism to regulate osmotic pressure, or when a disease damages this mechanism, there is a tendency to accumulate toxic waste and water, which can have dire consequences.

Mammalian systems have evolved to regulate not only the overall osmotic pressure across membranes, but also specific concentrations of important electrolytes in the three major fluid compartments: blood plasma, interstitial fluid, and intracellular fluid. Since osmotic pressure is regulated by the movement of

water across membranes, the volume of the fluid compartments can also change temporarily. Since blood plasma is one of the fluid components, osmotic pressures have a direct bearing on blood pressure. (Refer Figure 7.1)

NOTES

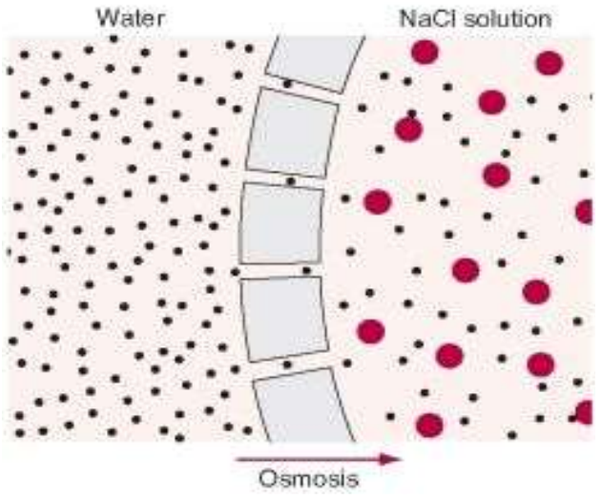


Fig. 7.1 *Osmoregulation*

Check Your Progress

1. How does bacterial cell reacts to the stress?
2. What are the most important stress response system?
3. Define osmotic stress.
4. What is osmoregulation?
5. What is electrolyte?
6. What imapct does extra and insufficient water and fluid have on kidney?

7.3 AEROBIC TO ANEROBIC TRANSITIONS

Adjustment of physiology in response to changes in oxygen availability is critical for the survival of all organisms. However, the chronology of events and the regulatory processes that determine how and when changes in environmental oxygen tension result in an appropriate cellular response is not well understood at a systems level. Therefore, transcriptome, proteome, ATP, and growth changes were analyzed in a halophilic archaeon to generate a temporal model that describes the cellular events that drive the transition between the organism's two opposing cell states of anoxic quiescence and aerobic growth. According to this model, upon oxygen influx, an initial burst of protein synthesis precedes ATP and transcription induction, rapidly driving the cell out of anoxic quiescence, culminating in the resumption of

NOTES

growth. This model also suggests that quiescent cells appear to remain actively poised for energy production from a variety of different sources. Dynamic temporal analysis of relationships between transcription and translation of key genes suggests several important mechanisms for cellular sustenance under anoxia as well as specific instances of post-transcriptional regulation.

Adaptation to varying levels of oxygen is critical for the survival of all organisms since this element is required for energy production in aerobic organisms, but is a dangerous poison for obligate anaerobes. Thus, diverse strategies have evolved for optimizing fitness under conditions of fluctuating oxygen availability. For example, anaerobic microbes have evolved specialized anoxic physiologies, including mechanisms to exclude and scavenge traces of oxygen. In contrast, facultative anaerobes such as *Escherichia coli* (*E. Coli*) flexibly transition between oxidative metabolism and anaerobic growth, using alternate respiratory enzymes when oxygen becomes limiting. Anoxia-tolerant eukaryotes, such as *Caenorhabditis elegans* enter a state of suspended animation in which energy supply and demand are drastically reduced in a regulated manner during oxygen starvation.

Understanding cellular responses to oxygen at the molecular systems level requires comprehensive and quantitative measurements of changes in parameters, such as transcription, translation, and metabolism. Transcriptome measurements are quite comprehensive, whereas current technology limits the detection of the complete microbial proteome and metabolome; for example, the highest reported coverage for microbial shotgun proteomics is 60%. Furthermore, in addition to this disparity in technical tractability, the dynamic nature of information processing at all of these levels further complicates the collective comparative analysis of global changes in transcriptome, proteome, and metabolome.

Facultative microorganisms, such as *E. coli* and *S. enterica* are capable of modifying their metabolism to accommodate growth under either aerobic or anaerobic conditions. The transition between aerobic and anaerobic metabolism is accompanied by alterations in the rate, route, and efficiency of pathways of electron flow. Figure 7.2 illustrates the basic pathways utilized by *E. coli* for aerobic versus anaerobic electron flow. Under anaerobic conditions without alternate electron acceptors, pyruvate is converted to formate, acetate, or ethanol, CO_2 and H_2 gas (mixed acid fermentation). However, the choices and energy yield become more plentiful when alternate electron acceptors are available. *E. coli*, even under aerobic conditions, synthesizes two distinct cytochrome oxidases — cytochrome *o* (*cyo* operon) and cytochrome *d* (*cyd* operon)— produced under high O_2 and low O_2 conditions, respectively. Under anaerobic conditions, at least five more terminal oxidoreductases can be produced as shown in Table 7.1.

NOTES

Table 7.1 *Characteristics of Electron Transport Systems in* E. Coli

Electron Acceptor	δG^{Oa} (kJ/mol)	Terminal Respiratory Enzyme	Operon	Chromosomal Location (min)
O_2	−233	Cytochrome *o* oxidase	*cyoABCDE*	10
O_2	−233	Cytochrome *d* oxidase	*cydAB*	17
NO_3^-	−144	Nitrate reductase	*narGHJI*	27
NO_3^-	−144	Nitrate reductase	*narZYWV*	33
DMSO	−92	DMSO/TMAO reductase	*dmsABC*	20
TMAO	−87	TMAO reductase	*torA*	28
Fumarate	−67	Fumarate reductase	*frdABCD*	94

Figure 7.2 illustrates the general metabolic pathway in the production of D-lactate by *E. coli* strains from glycerol. Relevant enzymes and genes are shown. Broken lines represent multiple steps.

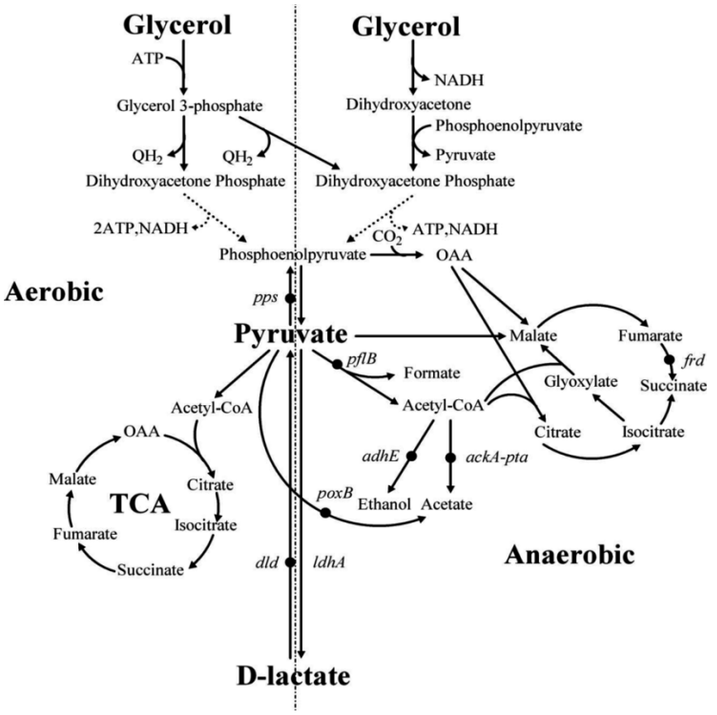


Fig. 7.2 *General Metabolic Pathway in the Production of D-lactate by E. Coli Strains from Glycerol*

NOTES

7.4 OXIDATIVE STRESS

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, is discussed in relation to its possible role in the production of tissue damage in diabetes mellitus. Important free radicals are described and biological sources of origin discussed, together with the major antioxidant defense mechanisms. Examples of the possible consequences of free radical damage are provided with special emphasis on lipid peroxidation. Finally, the question of whether oxidative stress is increased in diabetes mellitus is discussed.

7.4.1 Free Radicals

A free radical is an oxygen containing molecule that has one or more unpaired electrons, making it highly reactive with other molecules.

Oxygen by-products are relatively unreactive but some of these can undergo metabolism within the biological system to give rise to these highly reactive oxidants. Not all reactive oxygen species are harmful to the body. Some of them are useful in killing invading pathogens or microbes.

However, free radicals can chemically interact with cell components such as DNA, protein or lipid and steal their electrons in order to become stabilized. This, in turn, destabilizes the cell component molecules which then seek and steal an electron from another molecule, therefore triggering a large chain of free radical reactions.

7.4.2 Antioxidants

Every cell that utilizes enzymes and oxygen to perform functions is exposed to oxygen free radical reactions that have the potential to cause serious damage to the cell. Antioxidants are molecules present in cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves. An imbalance between oxidants and antioxidants is the underlying basis of oxidative stress.

7.4.3 Damaged Caused by Oxidative Stress

Oxidative stress leads to many pathophysiological conditions in the body. Some of these include neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, gene mutations and cancers, chronic fatigue syndrome, fragile X syndrome, heart and blood vessel disorders, atherosclerosis, heart failure, heart attack and inflammatory diseases. (Refer Table 7.2)

Table 7.2 *Oxidative Stress and its Description*

Oxidants	Description
O2-, superoxide anion	One-electron reduction state of O2, formed in many autoxidation reactions and by the electron transport chain. Rather unreactive but can release Fe2+ from iron-sulfur proteins and ferritin. Undergoes dismutation to form H2O2 spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed •OH formation.
H2O2, hydrogen peroxide	Two-electron reduction state, formed by dismutation of •O2- or by direct reduction of O2. Lipid soluble and thus able to diffuse across membranes.
OH, hydroxyl radical	Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components
ROOH, organic hydroperoxide	Formed by radical reactions with cellular components such as lipids and nucleobases.
RO, alkoxy and ROO, peroxy radicals	Oxygen centred organic radicals. Lipid forms participate in lipid peroxidation reactions. Produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.
HOCl, hypochlorous acid	Formed from H2O2 by myeloperoxidase. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine.
ONOO-, peroxynitrite	Formed in a rapid reaction between •O2- and NO•. Lipid soluble and similar in reactivity to hypochlorous acid. Protonation forms peroxynitrous acid, which can undergo homolytic cleavage to form hydroxyl radical and nitrogen dioxide.

NOTES

Check Your Progress

7. What is oxidative stress?
8. What is free radical?
9. Are oxygen by-products reactive?
10. What happens to the cell that uses enzymes?
11. What are antioxidants?
12. What is the underlying basis of oxidative stress?
13. What does oxidative stress leads to?

7.5 pH STRESS AND ACID TOLERANCE

Before defining the responses to acid stress, acid stress itself must be defined. Acid stress can be described as the combined biological effect of low pH and weak (organic) acids present in the environment. Weak acids include volatile fatty

NOTES

acids (VFAs) like butyrate, propionate and acetate produced as a result of fermentation. Weak acids in their uncharged, protonated forms can diffuse across the cell membrane and dissociate inside the cell, lowering internal pH (pHi) in the process. The lower the external pH (pHo), the more undissociated weak acid will be available (based upon pKa values) to cross the membrane and affect pHi. This means that it takes less organic acid to kill a cell at pHo 3.5 than is needed at pHo 4.4. Intracellular accumulation of weak acids is also thought to have harmful effects on the cell beyond that of acidifying pHi. It is important to remain cognizant of the relationship between pH and weak acid concentration when considering cellular acid survival strategies.

Acid Tolerance Responses of Salmonella Typhimurium

S. typhimurium possesses different low pH inducible acid survival systems depending upon whether the cells are in exponential or stationary phase. Most of our knowledge comes from studies with exponential phase cells grown in minimal glucose media. Cells actively growing at pHo 7.7 rapidly die when shifted to conditions below pHo 4. However, adapting these organisms to a mildly acidic pH (pHo 5.8) for one generation increases their tolerance to more extreme acid conditions (pHo 3). This inducible acid tolerance response (ATR) is a two-stage process involving overlapping acid protection systems triggered at different levels of acidity. Encounters with pHo 6 invoke the first stage (pre-acid shock) involving the synthesis of emergency pH homeostasis systems that alkalinize the cytoplasm during periods of extreme acid stress (pHo 3). The second stage (post-acid shock) is engaged once pHo falls below 4.5. Approximately 50 acid shock proteins (ASP) that are believed to prevent or repair macromolecular damage are induced during this stage.

Cross-protection

The ability of one stress condition to provide protection against other stresses is referred to as cross-protection. Several studies have shown that acid adaptations confer resistance to a wide range of stress conditions including heat, salt, H_2O_2, crystal violet and polymyxin B. However, adaptation to other stresses does not typically induce significant acid tolerance. This implies that exposure to acid may be perceived by bacteria as a more general stress indicator whereas heat, salt and H_2O_2 may be more specific stress signals. Furthermore, the demonstration that acid shock induces cross-resistance to a variety of stresses suggests that cells undergoing acid shock in the stomach will be well prepared to endure the environmental stresses subsequently confronted in the intestine.

Low pH and Pathogenicity

Studies by several groups have underscored the importance of low pH in the pathogenesis of enteric bacteria. For example, the infectious dose for Salmonella species is significantly decreased if stomach acidity is buffered, suggesting that the

better prepared the organism is to tolerate stomach acid, the more likely it will survive to cause disease. In addition, systems associated with Salmonella pathogenesis are induced by the low pH present in phagocytic compartments following the invasion of epithelial cells and macrophages. Various mutations that confer acid sensitivity also attenuate S. typhimurium. In *E. coli*, Poynter et al. have shown that surface attachment of the bacteria enhances acid resistance. In fact, the apparent low infectious dose of enterohemorrhagic *E. coli* disease has been correlated to its acid survival properties. It is clearly evident that a correlation exists between the response of enterobacteria to acid stress and the pathogenic process.

Check Your Progress

14. Define acid stress.
15. What composes weak acids?
16. What happens when external pH is low?
17. What is cross-protection?
18. What is heat shock in prokaryotic cell?
19. With what the structure of RNA polymerase in B resembels to?

7.6 THERMAL STRESS AND HEAT SHOCK RESPONSE

A large amount of studies has been carried out in which stress to cells was provided in the form of heat shock, i.e., exposing the cells to elevated temperatures (40-42°C, in some cases up to 60°C as well).

It is a known fact that prokaryotes possess only a single copy of a heat shock gene.

This fact, coupled with the essential function of most heat shock genes, necessitates the constitutive expression under all conditions of most prokaryotes regulatory stress genes. However, in general, eukaryotes possess at least two copies of heat shock genes. Of the two, one is under heat shock regulation and the other is under constitutive control.

Stress Gene Inductive Regulation and Control Mechanism

In prokaryotic cells the heat shock regulon is defined by the rpoH gene. Its product is a 32 KD sigma factor (σ). For instance, it has been reported that the major heat shock genes of *E. coli* are under the control of the rpoH (htpR) gene which codes for σ heat shock factor. The σ factor directs core RNA polymerase enzyme (Eσ) to recognize the promoters for heat shock genes.

NOTES

The studies on how the cell uses σ to regulate the heat shock response have provided a great deal of information about the molecular mechanisms involved in mounting a transient, global response to environmental stimuli. The evidence that in *E. coli* RNA polymerase containing σ (Eσ) is responsible for nearly all the transcription of the heat shock genes has been shown. It indicates that HSPs are required for cell growth at most temperatures.

So, the HSPs are involved in thermotolerence and in turn regulation of the heat shock response for the transcription and transition of rpoH mRNA. It also integrates the stability of σ and is regulated to provide the amount of σ required for heat shock gene expression in stress protein synthesis. The HSPs poses diverse functions at the molecular level ranging from the major *E. coli* σ factor σ (the rpoD gene product) to a cryptic form of lysyl tRNA synthetize (the lys U gene product).

It has been reported that abnormal protein synthesis under adverse conditions, like in sporulation in prokaryotic cells, is accompanied by the appearance of new RNA polymerase specificity factors that read the gene necessary for stress protein formation. It is also stated that the overall structure of RNA polymerase in B. subtitles is essentially identical to that in *E. coli* (σ_2 β σ).

However, the precise molecular weights of the various sub units differ between gram positive and gram negative bacteria. Vegetative growing Bacillus subtitles has been stated to contain multiple factors, namely σ^{55}, Qs^{37}, σ^{32} and σ^{28} with molecular weights 55, 37, 32, and 28 KD, respectively.

Each of them can bind interchangeably to the core RNA polymerase (σ_2 β β^1). This can confer upon it the ability to recognize a different set of promoter sequences. Factors σ and σ seem to be involved in switching on genes that are expressed at the very onset of sporulating protein synthesis under stressed environmental condition(s).

In exhaustion of nutritional components like carbon, nitrogen, or phosphorous, at least two new sporulation protein specific factors have been stated to be synthesized. One being σ and the other is probably σ that dissociates σ^{55} factor from the core RNA polymerase.

In this way σ replaces σ on core polymerase and permits the transcription of the sporulation protein specific promoter to start functioning. Interestingly, the 'a' factors determining the expression of specialized sets of genes are appearing as general features of many prokaryotes as diverse as *E. coli*, *Bacillus* and *Streptomyces* species.

For example, the expression of heat shock stress genes and nitrogen-regulated genes in *E. coli* is known to be dictated by specific a factors encoding by genes htpR and ntrA. Also, multiple a factors are a feature of most complex bacterial viruses such as T4, λ, T7, μ, and B. subtilis phage SPOl.

Thus, it is important to know how and which proteins play a role in stress alterations like mutations allowing cell growth at all temperatures to take place. It relates to an organism response at the cellular and molecular level when confronted with sudden changes in environment and molecular adaptation in the ability of the cells to acclimate themselves to the new environment.

Adaptive Expression

Although much information has not been accumulated on this, some light has been thrown on the E. coli cell system. In this organism three interesting twists in rpoH gene expression have been reported. In the first twist, rpoH is expressed from at least four different promoters.

In the second twist, two of the promoters are negatively regulated by the DnaA protein (required for inhibition of E. coli DNA replication as well as global regulation). The third promoter is under CAP-CAMP catabolite control, and the fourth is under the control of a more recently known σ^{24} (σ^{E}) factor. The gene coding for σ has not been identified as yet, it is, however, clears that σ^{24}-promoted transcription is accelerated at high temperature of 50°C range. It indicated that there are at least two heat shock systems in E. coli.

The conventional one is regulated by σ^{32}, and more currently known under σ^{24} regulation. Heat shock genes at high temperatures like 50°C are transcribed exclusively by either $E\sigma^{32}$ or $E\sigma^{24}$, and $E\sigma^{24}$levels, are continuously replenished by the σ^{24} -promoted transcription of the rpoH gene.

So, it is stated that heat shock gene HtrA (degP) is under the exclusive transcriptional control of $E\sigma^{24}$. Its product is essential for viability of E. coli at higher temperatures and remains under heat shock adaptive regulation. It tells that these two heat shock regulons are interconnected. The third twist promises rpoHmRNA translation under negative repression control as well. Signals affecting repressor element activity alters intracellular σ^{32} levels.

It appears that adaptive expression of stress has been known for a long time; however, its actual application in biological and physiological systems has been recognized only recently. Adaptive expressions in (a) ischemic stress associated with lethal ischemic injury, (b) pathogenesis in stress-related diseases. (c) modifying micro environment of the lipid bilayer and modulating involved enzyme activities. etc., have recently also been known.

However, report is also available regarding short-term adaptive expression in prokaryotic cells to stress conditions. It appears; therefore, that adaptive expression depends largely on the source cell as well as the stressor type. For example, the results of a recent study on vascular endothelial cells (VEC) indicated that VEC promptly responded to the stressor if it is in the form of oxidative stress.

7.7 NUTRIENT STRESS AND STARVATION STRESS

NOTES

Nutrient starvation and other environmental stresses are routine occurrences for most bacteria. Situations of true "feast" or non stress conditions are few and far between for microbes outside the laboratory. Thus, microbes are most frequently found in a state of nutrient starvation or stress-induced slow growth or no growth. Fortunately, this is the case, because given bacterial growth rates achieved under no limiting conditions in the laboratory, if microbial growth were no limiting in natural or animal host environments, the consequences would be deadly.

Starvation-Stress Response

When E. coli, Salmonella, and many other non-differentiating microbes are starved for an essential nutrient such as a carbon-energy (C) source, they respond by inducing the expression of up to 50 or so new proteins or preexisting proteins. The genetic and physiologic reprogramming that occurs is the starvation-stress response (SSR). The function of the SSR is to allow for the long-term starvation survival of the bacteria and to provide a general cross-resistance to a variety of other environmental stresses including extremes in temperature, pH, and osmolarity as well as exposure to reactive oxygen and nitrogen species and antimicrobial peptides/proteins. It should be noted that a distinction must be made between starved cells and so called stationary-phase cells. Typically, stationary-phase cells populate cultures that have stopped growing following exponential growth in rich or non-limiting media, in contrast to starved cells, which populate cultures that have ceased growing in response to exhaustion of one or more defined nutrients. For stationary-phase cells, the condition that limits growth is not necessarily defined nor is it typically limited to a single stress; for starved cells, the limitation/stress that restricts growth is defined. Another key difference is that stationary-phase cultures normally achieve a much higher cell density compared with starved cultures, which can have a significant effect on overall cellular responses and long-term survival. Furthermore, the genes/proteins expressed in stationary-phase cells may or may not overlap with those expressed in starved cells. The SSR refers specifically to the response of starved cells.

In general, for Gram-negative bacteria, starved cells are morphologically and physiologically very different from log-phase cells. The initial response to carbon-energy source limitation is to try and avoid the stress by increasing expression or expressing new uptake or scavenging systems to be able to utilize any nutrients that may become available. Persistence of the starvation-stress eventually results in a cell that is smaller, much more hardy, and metabolically efficient. This is mediated by the accumulation of at least two cellular nucleotides: cyclic 3, 5 adenosine monophosphate and guanosine 3, 5 -bis(diphosphate). In addition, at least two alternative σ factors, σ S and σ E, encoded by the rpoS and

rpoE genes, respectively, are key SSR regulators. These regulators are general factors controlling responses to starvation stress in a more global manner. However, this is not sufficient for the regulation of some stress-responsive genes. Thus, additional regulators may be involved in the regulation of specific genes, indicating a discriminating complexity for the SSR and other stress responses. Examples of some regulatory proteins include Fis, FadR (regulator of fatty acid metabolism, which binds to medium/longchain fatty acyl-CoA molecules and represses fatty acid biosynthetic genes and activates fatty acid degradation genes), Lrp (leucine-responsive protein, which controls certain aspects of amino acid metabolism), OxyR , SpvA/SpvR (regulators of the Salmonella plasmid virulence or spv genes), PhoP (response regulator that controls virulence factors),

7.8 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. A bacterial cell can react simultaneously to a wide variety of stresses and the various stress response systems interact with each other by a complex of global regulatory
2. In bacteria some of the most important stress response systems are:
   - Heat shock response, controlled by the sigma factor sigma 32
   - Envelope stress response, controlled mainly by the sigma factor sigma E and the Cpx two-component system
   - Cold shock response, which governs expression of RNA chaperones and ribosomal factors
   - General stress response, which depends on the sigma factor sigma S
   - (p)ppGpp-dependent stringent response, which reduces the cellular protein synthesis capacity and controls further global responses upon nutritional downshift.
3. Osmotic shock or osmotic stress is a sudden change in the solute concentration around a cell, causing a rapid change in the movement of water across its cell membrane.
4. Osmoregulation is the process of maintaining salt and water balance (osmotic balance) across membranes within the body.
5. An electrolyte is a compound that dissociates into ions when dissolved in water.
6. Excess water, electrolytes, and wastes are transported to the kidneys and excreted, helping to maintain osmotic balance. Insufficient fluid intake results in fluid conservation by the kidneys.
7. Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant

NOTES

defenses, is discussed in relation to its possible role in the production of tissue damage in diabetes mellitus.

8. A free radical is an oxygen containing molecule that has one or more unpaired electrons, making it highly reactive with other molecules.
9. Oxygen by-products are relatively unreactive but some of these can undergo metabolism within the biological system to give rise to these highly reactive oxidants.
10. Every cell that utilizes enzymes and oxygen to perform functions is exposed to oxygen free radical reactions that have the potential to cause serious damage to the cell.
11. Antioxidants are molecules present in cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves.
12. An imbalance between oxidants and antioxidants is the underlying basis of oxidative stress.
13. Oxidative stress leads to many pathophysiological conditions in the body. Some of these include neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, gene mutations and cancers, chronic fatigue syndrome, fragile X syndrome, heart and blood vessel disorders, atherosclerosis, heart failure, heart attack and inflammatory diseases.
14. acid stress can be described as the combined biological effect of low pH and weak (organic) acids present in the environment.
15. Weak acids include volatile fatty acids (VFAs) like butyrate, propionate and acetate produced as a result of fermentation.
16. The lower the external pH (pHo), the more undissociated weak acid will be available (based upon pKa values) to cross the membrane and affect pHi.
17. The ability of one stress condition to provide protection against other stresses is referred to as cross-protection.
18. In prokaryotic cells the heat shock regulon is defined by the rpoH gene.
19. Structure of RNA polymerase in B. subtitles is essentially identical to that in E. coli (σ_2 β σ).

7.9 SUMMARY

- The bacterial stress response enables bacteria to survive adverse and fluctuating conditions in their immediate surroundings.
- Various bacterial mechanisms recognize different environmental changes and mount an appropriate response.

NOTES

- A bacterial cell can react simultaneously to a wide variety of stresses and the various stress response systems interact with each other by a complex of global regulatory networks.
- Bacteria can survive under diverse environmental conditions and in order to overcome these adverse and changing conditions, bacteria must sense the changes and mount appropriate responses in gene expression and protein activity.
- The stress response in bacteria involves a complex network of elements that acts against the external stimulus.
- Bacteria can react simultaneously to a variety of stresses and the various stress response systems interact (cross-talk) with each other. A complex network of global regulatory systems leads to a coordinated and effective response.
- These regulatory systems govern the expression of more effectors that maintain stability of the cellular equilibrium under the various conditions.
- Osmotic shock or osmotic stress is a sudden change in the solute concentration around a cell, causing a rapid change in the movement of water across its cell membrane.
- Under conditions of high concentrations of either salts, substrates or any solute in the supernatant water is drawn out of the cells through osmosis. This also inhibits the transport of substrates and cofactors into the cell thus shocking the cell.
- Alternatively, at low concentrations of solutes, water enters the cell in large amounts, causing it to swell and either burst or undergo apoptosis.
- The rupture of bacterial or other cells in a solution following a sudden reduction in osmotic pressure. Osmotic shock is sometimes induced to release cellular components for biochemical analysis.
- Osmoregulation is the process of maintaining salt and water balance (osmotic balance) across membranes within the body.
- The fluids inside and surrounding cells are composed of water, electrolytes, and nonelectrolytes.
- An electrolyte is a compound that dissociates into ions when dissolved in water.
- A nonelectrolyte, in contrast, does not dissociate into ions in water. The body's fluids include blood plasma, fluid that exists within cells, and the interstitial fluid that exists in the spaces between cells and tissues of the body.
- The membranes of the body (both the membranes around cells and the 'membranes' made of cells lining body cavities) are semipermeable

NOTES

membranes. Semipermeable membranes are permeable to certain types of solutes and to water, but typically cell membranes are impermeable to solutes.

- Excess water, electrolytes, and wastes are transported to the kidneys and excreted, helping to maintain osmotic balance. Insufficient fluid intake results in fluid conservation by the kidneys.
- Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, is discussed in relation to its possible role in the production of tissue damage in diabetes mellitus.
- A free radical is an oxygen containing molecule that has one or more unpaired electrons, making it highly reactive with other molecules.
- Oxygen by-products are relatively unreactive but some of these can undergo metabolism within the biological system to give rise to these highly reactive oxidants.
- Every cell that utilizes enzymes and oxygen to perform functions is exposed to oxygen free radical reactions that have the potential to cause serious damage to the cell.
- Antioxidants are molecules present in cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves.
- An imbalance between oxidants and antioxidants is the underlying basis of oxidative stress.
- Oxidative stress leads to many pathophysiological conditions in the body. Some of these include neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, gene mutations and cancers, chronic fatigue syndrome, fragile X syndrome, heart and blood vessel disorders, atherosclerosis, heart failure, heart attack and inflammatory diseases.
- Weak acids include volatile fatty acids (VFAs) like butyrate, propionate and acetate produced as a result of fermentation.
- The ability of one stress condition to provide protection against other stresses is referred to as cross-protection.

7.10 KEY WORDS

- **Osmotic shock:** Osmotic shock or osmotic stress is a sudden change in the solute concentration around a cell, causing a rapid change in the movement of water across its cell membrane.
- **Osmoregulation:** Osmoregulation is the process of maintaining salt and water balance (osmotic balance) across membranes within the body.

- **Electrolyte:** An electrolyte is a compound that dissociates into ions when dissolved in water.
- **Oxidative stress:** Oxidative stress is the disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses.
- **Free radical:** A free radical is an oxygen containing molecule that has one or more unpaired electrons, making it highly reactive with other molecules.
- **Antioxidants:** Antioxidants are molecules present in cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves.
- **Acid stress:** Acid stress can be described as the combined biological effect of low pH and weak (organic) acids present in the environment.
- **Cross-protection:** The ability of one stress condition to provide protection against other stresses is referred to as cross-protection.

7.11 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Write a short note on microbial stress response.
2. Brief about osmotic stress.
3. What are free radicals?
4. What are the damages caused by oxidative stress?
5. What is pH stress?
6. What is starvation stress response?

Long-Answer Questions

1. Discuss about the microbial stress response and its types.
2. Explain about aerobic to anaerobic transitions.
3. Discuss about oxidative stress in detail.
4. Write about pH stress and acid tolerance.
5. Explain in detail about thermal stress and heat shock response.
6. Give a detailed overview of nutrient stress and starvation.

7.12 FURTHER READINGS

NOTES

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

NOTES

BLOCK - III
MICROBIAL METABOLISM

UNIT 8 NITROGEN METABOLISM

Structure

8.0 Introduction
8.1 Objectives
8.2 Process of Nitrogen Metabolism
8.3 Nitrogen Cycle
  8.3.1 Ammonification and Nitrification
8.4 Nitrogenase Enzyme
8.5 Physiology of Nitrogen Fixation in Symbiotic and Free Living Bacteria
8.6 Answers to Check Your Progress Questions
8.7 Summary
8.8 Key Words
8.9 Self Assessment Questions and Exercises
8.10 Further Readings

8.0 INTRODUCTION

Nitrogen metabolism is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure. Operation and mechanism of metabolic pathways is provided by proteins. Genetic information is stored in nucleic acid polymers. Each of the monomer of these macromolecules has an individual metabolic pathway. In addition, the monomeric nucleotides are essential for energy turnover as key intermediates in all metabolic pathways and also as second messenger molecules, often in form of cyclic nucleotides.

All nitrogen metabolism is based on a recycling of ammonia NH_3 in its neutral or charged form NH_4^+(ammonium ion). Ammonia, however, is not a major form of nitrogen on earth, instead it has to be replenished to support a growing need of life forms. As simple as it may sound, a growing bacterial culture needs raw material in form of small organic and inorganic molecules. NH_3 ultimately is derived from atmospheric N_2. In a process called nitrogen fixation, some bacterial species, the symbiotic eubacteria *Rhizobium* (in plant root nodules) and the archaea *cyanobacteria* (formerly blue-green algae) contain an enzyme complex for the reduction of molecular nitrogen to ammonia. This is the *nitrogenase complex* and contains Fe-S and Mo-Fe cofactors for the transfer of electrons from ferredoxin to N_2.

In this unit, you will study about nitrogen metabolism, nitrogen cycle – ammonification, nitrification, denitrification, nitrogen fixation, nitrogenase enzyme, physiology of nitrogen fixation in symbiotic and free living bacteria, etc.

NOTES

8.1 OBJECTIVES

After going through this unit you will be able to:

- Explain what nitrogen metabolism is
- Discuss about nitrogen cycle
- Analyse what ammonification, nitrification, denitrification and nitrogen fixation is and their significance
- Understand about nitrogenase enzyme
- Explain the physiology of nitrogen fixation in symbiotic and free living bacteria.

8.2 PROCESS OF NITROGEN METABOLISM

Like carbon, hydrogen and oxygen, nitrogen is also one of the most prevalent essential macro–elements which regulates plant growth, especially in agricultural systems. The main source of nitrogen for the construction of nitrogenous organic compounds is the atmosphere.

It occurs in such essential biomolecules as nucleic acids, proteins, some of the phytohormones and in many of the vitamins. So, nitrogen as component of these biomolecules and many other compounds is involved in most of the biochemical reactions contributing to life activities.

Plants require higher amounts of nitrogen as it is important in their structure and metabolism. Nearly, 80 per cent of the earth's atmosphere is composed of nitrogen, bathing the entire plant world, but unfortunately most plants cannot utilize it in its elementary form. For its supply they have to depend on the soil and from there they acquire nitrogen in inorganic form either as ammonium compounds or as nitrate.

Only certain prokaryotic systems can use the atmospheric nitrogen directly.

In a stable ecological system, the atmospheric nitrogen is converted into a metabolically useful form by a few prokaryotic life styles for supply to higher plants and animals. Ammonia, the most useful form, is initially converted to glutamate and glutamine and then to other nitrogen-containing compounds required for the growth and maintenance of the life of plants.

Check Your Progress

1. What is nitrogen metabolism?
2. Define nitrogen fixation?
3. What is the main source of nitrogen in the environment?
4. What is the total composition of nitrogen in the environment?

NOTES

8.3 NITROGEN CYCLE

The other sources of nitrogen in the soil are nitrate (NO_3), ammonium ions (NH_4+) or organic nitrogen, which are obtained from the nitrogen cycle and farm manure. Nitrate is converted to ammonia prior to metabolism. Ammonium ions and organic nitrogen in the form of amino acids mobilized from the proteins or partially destroyed proteins, can be absorbed directly by the roots of the plants.

With death and decay of organic systems the amino acids, purines and pyrimidine's are cycled back to ammonia, some of which is lost from the nitrogen cycle by the processes which convert it to NO_2 – and then to nitrogen gas or to NO_3 –. So, nitrogen metabolism includes both anabolic and catabolic processes.

The anabolic processes are

- Nitrogen fixation
- Amino acid synthesis
- Protein synthesis

The catabolic processes are

- Proteolysis and amino acid destruction
- De-nitrification
- Nitrification

Therefore, it is evident that nitrogen is available in several forms for the plant system, like nitrate, ammonia, organic nitrogen and molecular nitrogen in the environment. The continuous inter-conversion of these forms to maintain the constancy of the amount of nitrogen in atmosphere, by physical and biological processes constitute the nitrogen cycle (Refer Figure 8.1).

NOTES

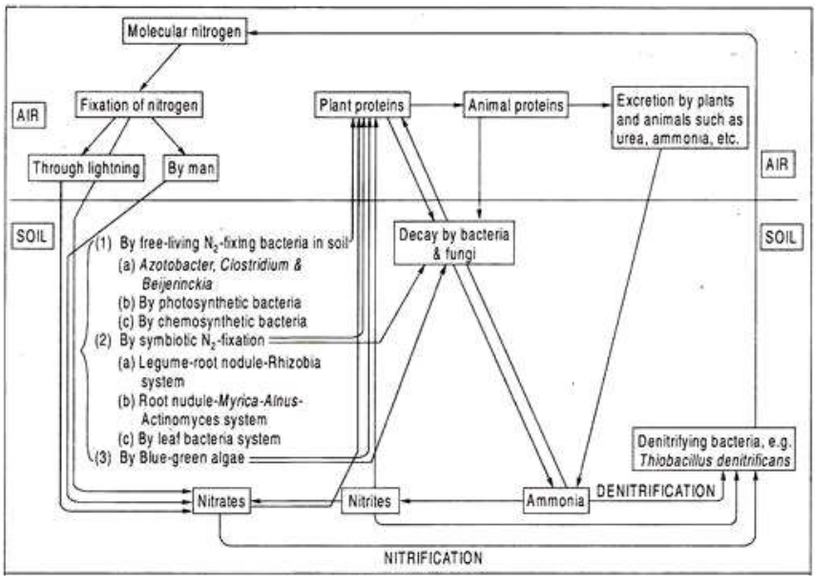


Fig. 8.1 *Nitrogen Cycle*

It essentially involves

- Ammonification and nitrification
- De-nitrification
- Nitrogen fixation

8.3.1 Ammonification and Nitrification

Conversion of organic nitrogen to NH^+ by soil microbes is called ammonification. The organic nitrogen in the soil comes from the animal excreta as well as from the decaying plant and animal remains.

In warm (30°-35°C) and moist soils at about neutral pH, ammonia is further oxidized first to produce nitrite and then to nitrate within a few days of its formation. This oxidation is known as nitrification. Certain microorganisms are responsible for carrying out both these processes.

The ammonifying saprophytic bacteria are *Bacillus mycoides*, *B. vulgaris* and *B. ramosus*. Certain fungi and actinomycetes can also release ammonia from the natural organic compounds. Two genera of chemoautotrophic bacteria, Nitrosomonas and Nitrobacter, are responsible for nitrification.

Nitrosomonas converts ammonia only into nitrite which is then further converted to nitrate by Nitrobacter as follows

$$NH_3 + \tfrac{3}{2}O_2 \longrightarrow NO_2^- + H_2O + H^+,\ \Delta F' = -66{,}500 \text{ cal}$$

$$NO_2^- + \tfrac{1}{2}O_2 \longrightarrow NO_3^-,\ \Delta F' = -17{,}500 \text{ cal}$$

Both the reactions are exergonic; the energy released through the oxidation of ammonia or of nitrite is utilized by the bacteria in their chemosynthesis.

NOTES

Nitrate Assimilation

Nitrate which is the most abundant form of nitrogen in the soil, and the root systems of higher plants absorb it in the form of NO_3^-. Since it cannot be directly used by the plants, it must first be reduced to the NH_3 level before incorporation into the nitrogenous compounds.

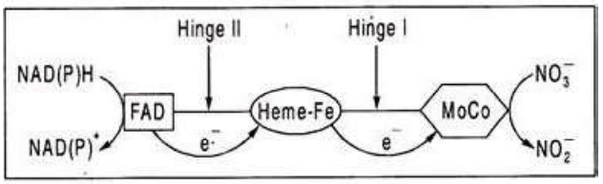
For the first time, nitrate is reduced to nitrite by the enzyme nitrate reductase, requiring respiratory energy according to the following reaction:

$$\underset{+5}{NO_3^-} + NADH\ (NADPH) + H^+ \longrightarrow \underset{+3}{NO_2^-} + NAD^+\ (NADP^+) + H_2O$$

(+ 5 and + 3 are oxidation numbers of nitrogen)

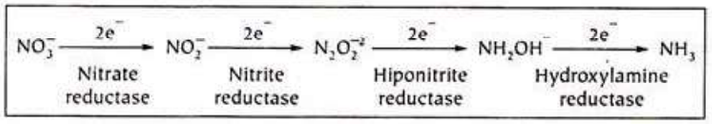
The nitrate reductases have been purified from soybean leaves, Neurospora and bacteria like E. coli, Pseudomonas aeruginosa, etc. This enzyme is a complex metalloenzyme that form homodimers. It has binding sites for NAD (P) H and for nitrate.

Three cofactors —FAD, heme-Fe and molybdenum cofactor (MoCo) — form the redox centers that facilitate the chain of electron transfer reactions as follows:



MoCo is a molybdenum ion complexed with an organic molecule pterin, which acts as a metal chelator. Each nitrogen reductase (NR) sub unit is 1000 amino acid long and contains all three cofactors.

Most plant NR use NADH, but some use either NADH or NADPH. The genes for nitrate reductase from several higher plants have been cloned. The process is repeated in the further reduction of nitrite through the intermediates, hyponitrite and hydroxylamine, to form NH_3.



Each step involves the addition of two electrons by reduced NAD^+ ($NADP^+$). This reduction process of NO_3^- to NH_3 and its incorporation into the

NOTES

cellular proteins by aerobic microorganism and higher plants, is referred to as nitrate assimilation.

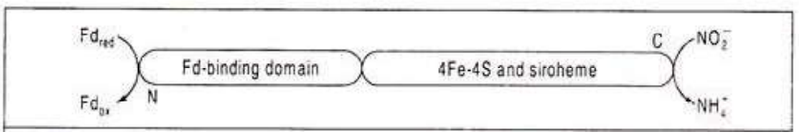
Nitrite reductase (NiR) transfers electrons from ferredoxin to nitrite as follows:

$$NO_2 + 6Fd_{red} + 8H^+ + 6e \rightarrow NH_4 + 6Fd_{ox} + 2H_2O$$

The source of electrons is reduced ferredoxin (Fd_{red}), produced in chloroplasts by photosynthetic non cyclic electron transfer. In non-photosynthetic tissues nitrite reduction also utilizes Fd_{red} in plastids.

Where NADPH produced from oxidative pentose phosphate pathway reduces ferredoxin by an enzyme Fd-$NADP^+$ reductase. The enzyme NiR consists of a single polypeptide containing two prosthetic groups: an iron sulfur center (Fe_4S_4) and a specialized heme (siroheme).

The enzyme is a nuclear-encoded protein with an N-terminal transit peptide that is cleaved from the mature enzyme. The precursor peptide is targeted to the plastids by the transit peptide. NiR is a monomer of 60 to 70 kDa molecular mass having functional domains and cofactors that shuttle electrons from Fd_{red} to nitrite.



The two functional domains are bridged by a sulfur ligand. NiR is regulated transcriptionally in coordination with NR. As nitrite is toxic, cells must contain enough NiR to reduce all the nitrite produced by NR.

De-Nitrification

The conversion of nitrate and nitrite into ammonia, nitrogen gas and nitrous oxide (N_2O) is called de-nitrification. The process ending in the release of gaseous nitrogen into the atmosphere, completes the nitrogen cycle.

In this process through a series of reactions nitrates are reduced to ammonia and free nitrogen by the anaerobic bacteria like Pseudomonas denitrificans, Bacillus subtilis. Thiobacillus denitrificans. Micrococcus, Azotobacter, Clostridium, etc. These bacteria use NO_3^- as an electron acceptor during respiration instead of O_2, thus obtaining energy for survival.

Nitrogen Fixation

Nitrogen fixation is defined as the conversion of elementary di-nitrogen (N_2O) into organic form to make it available for plants. Rotation of leguminous and non-leguminous field crops in the ancient agricultural practice was based on the observation that this process gave a better yield if the non- leguminous crops grew following the leguminous crops.

NOTES

Nobody, however, realized that the benefit was related to fixation of air nitrogen until Baussingault in 1838 established that the soil fertility increased due to some bacteria found in root nodules of leguminous crops as well as in soil and that these bacteria were capable of fixing atmospheric nitrogen.

Nitrogen fixation is of two types:

Non-biological nitrogen fixation and biological nitrogen fixation.

Non-Biological or Physical Nitrogen Fixation

Nitrogen is an extremely stable molecule. It exists in di-nitrogen form (N_2) having a triple bond (N = N) between two nitrogen atoms. The nitrogen bond has the shortest length of 1.095 Å, the highest ionization potential (15.58 eV) and the highest stretching frequency. For this reason, it is highly resistant to chemical attack.

To break this triple bond about 225 kcal of energy is required but it is very difficult to achieve. In the fertilizer industry nitrogen is reduced to ammonia at very high temperature and pressure over an iron catalyst.

$$N_2 + 3H_2 \underset{300\ atm}{\overset{500°C}{\rightleftharpoons}} 2NH_3$$

Nitrogen may also be fixed through the electrical discharges that occur during lightning. In this process, the atmospheric nitrogen combines with oxygen to produce oxides of nitrogen which are subsequently hydrated by water vapor and carried to earth as nitrites and nitrates by rain.

$$N_2 + O_2 \longrightarrow 2NO \xrightarrow{O_2} 2NO_2$$

Biological Nitrogen Fixation

During the process of evolution some bacterial species have acquired the capacity to reduce nitrogen to ammonia, a process governed by a set of genes called the nitrogen fixation (nlf) genes These species are termed 'nitrogen-fixing' organisms. Such organisms include either non-symbiotic microorganisms that can live independently or certain bacteria living in symbiosis with higher plants.

The former group encompasses certain species of heterotrophic bacteria, both aerobic (Azotobacter sp.) and anaerobic (Clostridium sp.); photosynthetic bacteria (Rhodospirillum sp.) and several blue-green algae (Cyanophyta).

The symbiotic system consists of bacteria of the genus Rhizobium together with many members of the family Leguminosae, such as peas, beans, clovers, soybean, etc., to form an important nitrogen-fixing cooperative. An essential feature of the symbiotic fixation is the development of nodules on the roots of the plants.

NOTES

Check Your Progress

5. What are the two processes that occur in nitrogen metabolism?
6. What are catabolic processes?
7. Name anabolic processes.
8. What is ammonification?
9. What is the source of organic nitrogen?
10. What is de-nitrification?
11. Define nitrogen fixation.

8.4 NITROGENASE ENZYME

There are numerous types of enzymes, complexes, and other material that operate in living organisms and can be important to their survival.

Nitrogenase, is one enzyme that is produced by certain types of bacteria and is vital to their existence and growth. Nitrogenase is a unique enzyme with a crucial function that is distinct to bacteria that utilize it, has unique structure and symmetry, and is sensitive to other compounds that inhibits its functioning. It is 'an enzymatic complex which enables fixation of atmospheric nitrogen'. The unique structure of nitrogenase is almost completely known because of the extensive research that has been done on this enzyme. Nitrogenase can also bind to compounds other than nitrogen gas, which can inhibit and decrease its production of ammonia to the rest of the organism's body. Without proper functioning, the bacteria that utilize nitrogenase would not be able to survive, and other organisms that depend on these bacteria would also die.

Nitrogenase is unique in its ability to fix nitrogen, so that it is more reactive and able to be applied in other reactions that help organisms grow and thrive. However, nitrogen gas, N_2, is an inert gas that is stabilized by its triple bond , and is difficult for living organisms to use as a source of nitrogen because the molecule's stability. Nitrogenase is used to separate nitrogen gas, N_2, and transforms it into ammonia, NH_3 in the reaction.

In the form of ammonia organisms have a useable source of nitrogen that is more reactive and can be used to create proteins and nucleic acids that are also necessary for the organism. According to the Peters and Szilagyi, 'Three types of nitrogenase are known, called molybdenum (Mo) nitrogenase, vanadium (V) nitrogenase and iron-only (Fe) nitrogenase' and the molybdenum nitrogenase,crystal

NOTES

structure, is the one that has been studied the most of the three. The nitrogenase enzyme breaks up a diatomic nitrogen gas molecule using a large number of ATP and 8 electrons to create two ammonia molecules and hydrogen gas for each molecule of nitrogen gas . As a result, the bacteria that utilize this enzyme must expend much of their energy, in the form of ATP, so that they will constantly obtain a steady source of nitrogen. Without nitrogenase's function of fixing nitrogen gas into ammonia, then organisms would not be able to thrive since they would not receive a source of nitrogen for other important reactions.

Many details have been discovered over time about the functioning of nitrogenase, but there has yet to a complete agreement on the structure of nitrogenase. The iron-molybdenum cofactor center of the nitrogenase enzyme consists of iron, sulfur, molybdenum, a homocitrate molecule, a histadine amino acid and a cysteine amino acid. Nitrogenase contains various molecules that have different conformations throughout the enzyme giving nitrogenase a unique symmetry.

Nitrogenase is not easily used because of its sensitivity to other compounds, which prevents it from being easily used by other organisms. Nitrogenase contains several iron atoms that could become disrupted if it were to become oxidized by any oxygen gas. In addition, 'nitrogenase has the ability to bond acetylene and carbon monoxide' making the three molecules (acetylene, carbon monoxide, and nitrogen gas) competitive substrates that bind to nitrogenase. The restrictive functionality of nitrogenase makes it only possible for anaerobic organisms to utilize nitrogenase. Rhizobium, normally found in plant roots, is an example of an anaerobic family of bacteria that utilizes nitrogenase. They exist in a symbiotic relationship with leguminous plants to produce nitrogen in exchange for other nutrients and protection from oxygen and other compounds that could inhibit nitrogenase's operation. These limitations of nitrogenase prevent it from being commonly used among organisms, but it is an important element to the bacteria that make use of this enzyme.

Nitrogenase is a unique enzyme that is critical to the bacteria that utilizes this enzyme. Its function is necessary for anaerobic bacteria since it is their main source of nitrogen. However, the high cost of ATP and electrons is another restrictive reason why more organisms do not produce their own source of nitrogen. The specific connections within nitrogenase cause it to lack any symmetric elements in its structure. More research must be done before the complete understanding of nitrogenase and its structure, such as with the possibility and identity to a central atom within nitrogenase. Nitrogenase is a sensitive enzyme that can easily be inhibited by oxygen and other compounds and affects its ability to fix nitrogen. It is the major reason why nitrogenase is strictly found in anaerobic bacteria that is able to utilize nitrogenase for its source of nitrogen. Nitrogenase is simply one enzyme among the multitudes of other enzymes and complexes that is vital strictly to the organisms that utilize it.

NOTES

Check Your Progress

12. What is nitrogenase enzyme?
13. Give a key function of nitrogenase enzyme.
14. How is nitrogenase enzyme used?
15. What does iron-molybdenum cofactor center of the nitrogenase enzyme consists of?
16. What does nitrogenase enzyme do after forming bond with acetylene and carbon monoxide?

8.5 PHYSIOLOGY OF NITROGEN FIXATION IN SYMBIOTIC AND FREE LIVING BACTERIA

Symbiotic N_2-Fixing Bacteria

Symbiotic bacteria is the one which forms association with either plant , animal or fungus in which bacteria as well as the host, both are benefited, example - Rhizobium bacteria forms association with the root nodules of higher plants , plant provide shelter to the bacteria and bacteria helps in nitrogen fixation.

The heterotrophic bacteria that fix di-nitrogen gas (N_2) from the atmosphere in plant root nodules (symbiotic bacteria) have a mutually beneficial relationship with their host plants. Legumes (pod-bearing plants, such as peas, beans, alfalfa and clovers, etc.) had a beneficial effect upon both companion and whatever crop was planted next in the same soil. It is evident that the fixation of atmospheric nitrogen in the legume is due to the formation of root nodules.

Symbiotic bacteria initially start by infecting root hairs, causing an invagination (enclosing-like sheaths) inward through several cells. Surrounding plant cells proliferate quickly, perhaps because of auxin, a phytohormone produced by the infecting bacteria.

As the bacteria enter the nodule cells, they form enclosing membranes and produce meta-hemoglobin, an oxygen-carrying pigment (the nodule may be pink in cross-section). The hemoglobin like material may be an oxygen sink or trap to keep the bacteria in an anaerobic environment, which is necessary for N_2 fixation.

The di-nitrogen (N_2) fixation is performed by the enzymes nitrogenase. This enzyme lowers the activation energy (the energy requires to perform the reaction). The fixation proceeds in reduction stages from di-nitrogen (N = N) through uncertain intermediates HN=NH and H_2N-NH_2 to produce 2 NH_3.

Finally, the ammonium is transformed into some organic compounds such as amino acids. All of this will take place when the nitrogen is bonded to the enzyme(s).

NOTES

The lifetime of a bacterium may be only a few hours and the bodies of a portion of the bacterial population are continuously dying, decomposing, and releasing NH_4^+ and NO_3^- ions for the utilization by the host plant. Most of the nitrogen fixed is excreted by the bacteria and made available to the host plant and to the other plants growing nearby. The well-known symbiotic bacteria belong to the genus Rhizobium.

Symbiotic heterotrophic bacteria specific to the crop to be grown are frequently applied or inoculated, in a dried powdered from to the crop seed to ensure that nitrogen fixing organisms are present. The same bacterial species will not inoculate all legumes. Sesbania rostrata (dhaincha) was found to form nodules both in roots and stems and it is most important host plant for the symbiotic N_2-fixation.

Recently some plants have been found to have symbiotic relationship with different N_2-fixing bacteria, including blue green bacteria (cyanobacteria), are Digitaria (grass species), water fern for example, azolla (with blue green bacteria), Gunnera macrophylla (with blue green bacteria).

It has been also reported that bacteria of the genus Klebsiella have been found to be associated in N_2-fixation with various grasses (non-legumes) but none has yet proven to be symbiotic. In addition, may other non-leguminous plants have symbiotic N_2-fixing nodulation (for example, Alnus species, Casuaraina equisetifolia, etc.)

Since the number of host plants is limited, cross inoculation groups have been established. A cross-inoculation group refers to a collection of leguminous species that are capable of developing nodules when exposed to bacteria obtained from the nodules of any member of that particular plant group.

Non-Symbiotic N_2-Fixing Bacteria:

The non-symbiotic nitrogen fixing bacteria do not require a host plant. In 1891, Winogradsky observed that when soil was exposed to the atmosphere, the nitrogen content of the soil was recorded to be increased.

The anaerobic bacterium Clostridium pasteurianum was found responsible for such an increase of the nitrogen content in soil. In 1901, Beijerinck proved that there were also free-living aerobic bacteria, Azotobacter chroococcum that could fix atmospheric nitrogen.

Another bacterial group, Granulobacter (purple colour) obtains nitrogen directly from the atmosphere. The amounts of atmospheric nitrogen fixed by these bacteria are largely variable because of divergent nature of soils.

In aerobic soils of tropical climatic regions, the acid tolerant N_2-fixer Azotobacter beijerinckia is most abundant Azospirillum spp. also fix N_2-non-symbiotically and help to many crops for their growth and yield.

8.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

NOTES

1. Nitrogen metabolism is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure.
2. In a process called nitrogen fixation, some bacterial species, the symbiotic eubacteria *Rhizobium* (in plant root nodules) and the archaea *cyanobacteria* (formerly blue-green algae) contain an enzyme complex for the reduction of molecular nitrogen to ammonia.
3. The main source of nitrogen for the construction of nitrogenous organic compounds is the atmosphere.
4. Nearly, 80 per cent of the earth's atmosphere is composed of nitrogen, bathing the entire plant world, but unfortunately most plants cannot utilize it in its elementary form.
5. Nitrogen metabolism includes both anabolic and catabolic processes.
6. The catabolic processes are
   - Proteolysis and amino acid destruction
   - De-nitrification
   - Nitrification
7. The anabolic processes are
   - Nitrogen fixation
   - Amino acid synthesis
   - Protein synthesis
8. Conversion of organic nitrogen to NH^+ by soil microbes is called ammonification.
9. The organic nitrogen in the soil comes from the animal excreta as well as from the decaying plant and animal remains.
10. The conversion of nitrate and nitrite into ammonia, nitrogen gas and nitrous oxide (N_2O) is called de-nitrification.
11. Nitrogen fixation is defined as the conversion of elementary di-nitrogen (N_2O) into organic form to make it available for plants.
12. Nitrogenase, is one enzyme that is produced by certain types of bacteria and is vital to their existence and growth
13. Nitrogenase is a unique enzyme with a crucial function that is distinct to bacteria that utilize it, has unique structure and symmetry, and is sensitive to other compounds that inhibits its functioning.

14. Nitrogenase is used to separate nitrogen gas, N_2, and transforms it into ammonia, NH_3 in the reaction.
15. The iron-molybdenum cofactor center of the nitrogenase enzyme consists of iron, sulfur, molybdenum, a homocitrate molecule, a histadine amino acid and a cysteine amino acid
16. Nitrogenase forms bond with acetylene and carbon monoxide and make the three molecules (acetylene, carbon monoxide, and nitrogen gas) competitive substrates that bind to nitrogenase.

8.7 SUMMARY

- Nitrogen metabolism is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure.
- Operation and mechanism of metabolic pathways is provided by proteins.
- The monomeric nucleotides are essential for energy turnover as key intermediates in all metabolic pathways and also as second messenger molecules, often in form of cyclic nucleotides.
- All nitrogen metabolism is based on a recycling of ammonia NH_3 in its neutral or charged form NH_4^+(ammonium ion).
- Ammonia, is not a major form of nitrogen on earth, instead it has to be replenished to support a growing need of life forms.
- NH_3 ultimately is derived from atmospheric N_2.
- In a process called nitrogen fixation, some bacterial species, the symbiotic eubacteria *Rhizobium* (in plant root nodules) and the archaea *cyanobacteria* (formerly blue-green algae) contain an enzyme complex for the reduction of molecular nitrogen to ammonia.
- Like carbon, hydrogen and oxygen, nitrogen is also one of the most prevalent essential macro-elements which regulates plant growth, especially in agricultural systems.
- Plants require higher amounts of nitrogen as it is important in their structure and metabolism. Nearly, 80 per cent of the earth's atmosphere is composed of nitrogen, bathing the entire plant world, but unfortunately most plants cannot utilize it in its elementary form.
- Only certain prokaryotic systems can use the atmospheric nitrogen directly.
- Ammonia, the most useful form, is initially converted to glutamate and glutamine and then to other nitrogen-containing compounds required for the growth and maintenance of the life of plants.

NOTES

- The other sources of nitrogen in the soil are nitrate (NO_3), ammonium ions (NH_4^+) or organic nitrogen, which are obtained from the nitrogen cycle and farm manure.
- Nitrate is converted to ammonia prior to metabolism. Ammonium ions and organic nitrogen in the form of amino acids mobilized from the proteins or partially destroyed proteins, can be absorbed directly by the roots of the plants.
- Conversion of organic nitrogen to NH^+ by soil microbes is called ammonification. The organic nitrogen in the soil comes from the animal excreta as well as from the decaying plant and animal remains.
- Nitrate which is the most abundant form of nitrogen in the soil, and the root systems of higher plants absorb it in the form of NO_3^-. Since it cannot be directly used by the plants, it must first be reduced to the NH_3 level before incorporation into the nitrogenous compounds.
- The conversion of nitrate and nitrite into ammonia, nitrogen gas and nitrous oxide (N_2O) is called de-nitrification. The process ending in the release of gaseous nitrogen into the atmosphere, completes the nitrogen cycle.
- Nitrogen fixation is defined as the conversion of elementary di-nitrogen (N_2O) into organic form to make it available for plants.
- Rotation of leguminous and non-leguminous field crops in the ancient agricultural practice was based on the observation that this process gave a better yield if the non- leguminous crops grew following the leguminous crops.
- Nitrogen is an extremely stable molecule. It exists in di-nitrogen form (N_2) having a triple bond (N = N) between two nitrogen atoms. The nitrogen bond has the shortest length of 1.095 Å, the highest ionization potential (15.58 eV) and the highest stretching frequency. For this reason, it is highly resistant to chemical attack.
- The symbiotic system consists of bacteria of the genus Rhizobium together with many members of the family Leguminosae, such as peas, beans, clovers, soybean, etc. to form an important nitrogen-fixing cooperative.
- An essential feature of the symbiotic fixation is the development of nodules on the roots of the plants.

8.8 KEY WORDS

- **Nitrogen metabolism:** Nitrogen metabolism is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure.

- **Ammonification:** Conversion of organic nitrogen to NH^+ by soil microbes is called ammonification.
- **De-nitrification:** The conversion of nitrate and nitrite into ammonia, nitrogen gas and nitrous oxide (N_2O) is called de-nitrification.
- **Nitrogen fixation:** Nitrogen fixation is defined as the conversion of elementary di-nitrogen (N_2O) into organic form to make it available for plants.
- **Nitrification:** Nitrification is the biological oxidation of ammonia or ammonium to nitrite followed by the oxidation of the nitrite to nitrate. The transformation of ammonia to nitrite is usually the rate limiting step of nitrification.

8.9 SELF ASSESSMENT QUESTION AND EXERCISES

Short-Answer Questions

1. Write a short note on nitrogen metabolism.
2. What is nitrogen cycle? Name the processes that occur in nitrogen cycles.
3. Differentiate between catabolic and anabolic process.
4. Write a short note on nitrogen fixation.
5. What is nitrate assimilation?

Long-Answer Questions

1. Discuss in detail the mechanism of nitrogen metabolism.
2. Explain nitrogen cycle in detail.
3. Explain the anabolic process along with all the phases of it.
4. Write in detail about nitrogenase enzyme.
5. Distinguish between symbiotic and asymbiotic bacteria.
6. Write about the physiology of nitrogen fixation in symbiotic and asymbiotic bacteria.

8.10 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

NOTES

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 9 AEROBIC RESPIRATION

NOTES

Structure

9.0 Introduction
9.1 Objectives
9.2 Aerobic Respiration
- 9.2.1 Stage I of Respiration: Glycolysis (EMP-Pathway of Glycolytic Breakdown)
- 9.2.2 Stage II of Respiration: Pyruvate Oxidation or Conversion of Pyruvic Acid to Acetyle Coenzyme A
- 9.3.3 Stage III of Respiration: TCA Cycle or Krebs Cycle

9.3 Glyoxylate Cycle
- 9.3.1 Overview of the Glyoxylate Cycle
- 9.3.2 Steps Involved in Glyoxylate Cycle
- 9.3.3 Significance of Glyoxylate Cycle
- 9.2.4 Stage IV of Respiration: Terminal Oxidation

9.4 Answers to Check Your Progress Questions
9.5 Summary
9.6 Key Words
9.7 Self Assessment Questions and Answers
9.9 Further Readings

9.0 INTRODUCTION

Living things need energy for everything they do, including growing and reproducing. This energy comes from glucose. All animals and humans get glucose through eating it, but plants need to make their own glucose during photosynthesis.

Plants and animals transport glucose and oxygen to tiny structures in their cells, called mitochondria. Here, the glucose and oxygen take part in a chemical reaction. The reaction is called aerobic respiration, and it produces energy which transfers to the cells.

Aerobic respiration makes two waste products: carbon-dioxide and water. Animals remove carbon dioxide from their bodies when they breathe out. In daytime, plants use some of this carbon dioxide for photosynthesis. At night, they release the carbon dioxide to their surroundings.

Aerobic respiration uses oxygen to break down glucose, amino acids and fatty acids and is the main way the body generates adenosine triphosphate (ATP), which supplies energy to the muscles. After glycolysis (the anaerobic breakdown of glucose into pyruvate, pyruvate is converted to acetyl CoA in the matrix of the energy-transferring mitochondria, via the link reaction. Next is the Krebs cycle, which occurs twice per glucose molecule, producing – among other chemicals that feed into the aerobic part of the process – more ATP.

NOTES

In this unit, you will study about aerobic respiration, TCA cycle, intracellular location and reactions, amphibolic reactions and glyoxylate cycle.

9.1 OBJECTIVES

After going through this unit you will be able to:

- Understand about aerobic respiration and its mechanism
- Learn about TCA cycle
- Know about intracellular location and reactions
- Explain amphibolic reactions
- Discuss about glyoxylate cycle

9.2 AEROBIC RESPIRATION

Aerobic Respiration is the process by which the energy from glucose is released in the presence of oxygen. It takes place only if oxygen is available.

For instance, if glucose were oxidized, the result would be energy, carbon dioxide and water. Take a look at the chemical formula given below.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \text{Energy (ATP)}$$

In simple words, Glucose + Oxygen = Carbon-dioxide + Water + Energy (ATP)

In brief, aerobic respiration helps in release of maximum energy and also gets rid of carbon-dioxide and excess water.

The four stages involved in the aerobic respiration process are

1. Glycolysis (or EMP-Pathway of Glycolytic Breakdown)
2. Pyruvate Oxidation or Conversion of Pyruvic Acid to Acetyl Coenzyme A
3. TCA Cycle or Krebs Cycle
4. Terminal Oxidation

9.2.1 Stage I Of Respiration: Glycolysis (EMP-Pathway Of Glycolytic Breakdown)

Although glycolysis occurs in all organisms except primitive bacteria, it has been studied in greatest detail in yeast and animal muscle cells. The same sequence of reactions is found in both types of cells. The chemical reactions of glycolysis were discovered mainly by the efforts of G.G. Embden (1874-1933), Otto Meyerhof (1884 -1951) and J.K. Parnas (1884 -1949), and hence this pathway is also generally referred to as EMP-pathway. Within a period of about 10 years following 1914 the essential features of the EMP pathway were established. It is common to the majority of living organisms, both plants and animals.

NOTES

It occurs chiefly in the soluble part of the cytoplasm ,i.e., cytosol, rather than being associated with mitochondria or other organelles. This is first stage of respiration, which includes a sequence of chemical reactions through which glucose is incompletely oxidized to yield the 3-carbon compound pyruvic acid (Glycolysis = lysis or splitting of glucose).

The essential feature of glycolysis is the breakdown of 6-carbon sugar glucose into two 3-carbon fragments (Pyruvic acid molecules). Glycolysis takes place in ten consecutive steps, each catalyzed by a different enzyme complex (Refer Figure 9.1). Biochemical steps 1, 3 & 10 are irreversible in glycolysis.

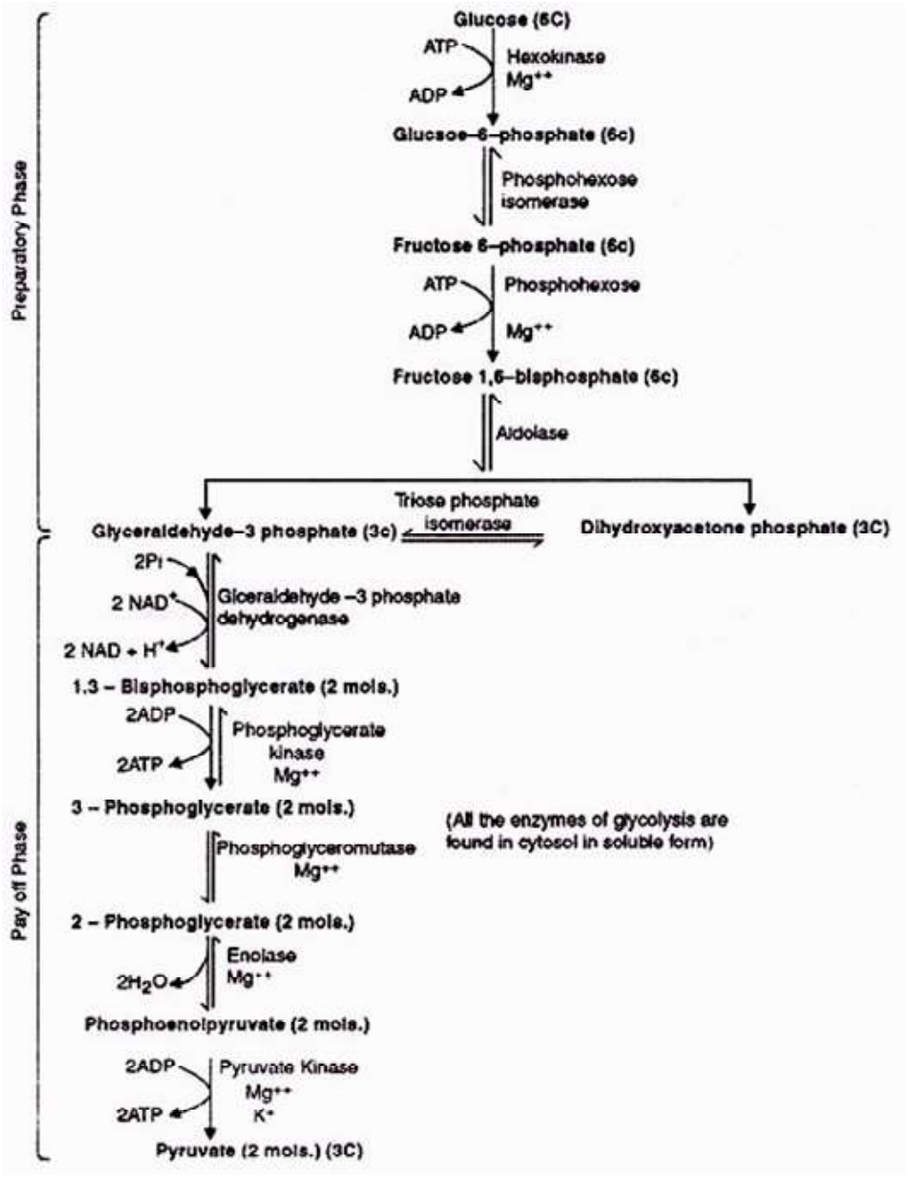


Fig. 9.1 *Glycolysis/EMP Pathway*

NOTES

First Half of Glycolysis (Preparatory Phase)

Step 1 (1st Phosphorylation)

In order to obtain energy in the form of ATP (Adenosine triphosphate) from the breakdown of glucose, it is first necessary to 'spark off' the reaction sequence by putting ATP into it. During step 1 the primary alcohol group (- CH_2OH) at position C-6 of glucose reacts with the terminal phosphate group of ATP, forming glucose-6-phosphate and ADP (Adenosine diphosphate). The reaction is catalysed by enzyme hexokinase and activator Mg^{++} ions.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

Step 2 (Isomerization)

During second step of reactions the glucose-6-phosphate is rearranged (isomerized) to form fructose-6-phosphate. In this reaction, the aldehyde group (- CHO) at C-1 is reduced to a primary alcohol group (- CH_2OH) as a result of simultaneous oxidation of the secondary alcohol group (> CHOH) at C-2 to a keto group (> CO). This isomeric change is catalysed by the enzyme phosphohexoisomerase.

$$\text{Glucose 6-phosphate} \underset{\text{Isomerase, } Mg^{2+}}{\overset{\text{Phospholexore}}{\rightleftharpoons}} \text{Frucotse-6-phosphate}$$

Fructose 6-phosphate can also be produced directly by phosphorylation of fructose with the help of enzyme fructokinase.

Step 3 (2nd Phosphorylation)

The formation of a primary alcohol group at C-1 makes possible step 3, which is a repetition of the phosphorylation effected in step 1. Fructose -6-phosphate is again phosphorylated in the presence of one ATP molecule. The reaction is catalysed by an enzyme kinase and Mg^{++} cofactor, forming fructose-1-6-diphosphate (phosphate attached at C-1 position) and ADP. This reaction is also reversible.

$$\text{Fructose 6-phosphate} + \text{ATP} \underset{\text{kinasw} + Mg^{2+}}{\overset{\text{Phosphofructo}}{\rightleftharpoons}} \text{Frucotse 1-6-diphosphate} + \text{ADP}$$

Step 4 (Splitting of Hexose Molecule)

We have seen that the first three steps of glycolysis have thus converted, at the expense of two molecules of ATP, a molecule of glucose into one of fructose- 1-6-diphosphate, with a phosphate group at each end. Fructose-1-6-diphosphate is now split into two smaller fragments.

In this step the 6-carbon sugar, fructose-1-6-diphosphate, is split between C-3 and C-4 to form two 3-carbon fragments, one an aldehyde (glyceraldelyde-3-phosphate) and the other a ketone (dihydroxyacetone-phosphate). This reaction is carried out under the influence of an enzyme aldolase.

NOTES

The EMP pathway is common to a great many microorganisms as well as higher forms. The enzyme fructose diphosphate aldolase (also called as fructose biphosphate aldolase) is one of the most critical steps in the pathway. In the absence of this enzyme, glucose or other hexose sugars must be metabolized via one of several alternative pathways. (Not discussed here).

$$\text{Fructose 1 : 6-phosphate} \xrightleftharpoons{\text{Aldolase}} \text{Glyceraldehyde 3-phosphate (GAP)}$$

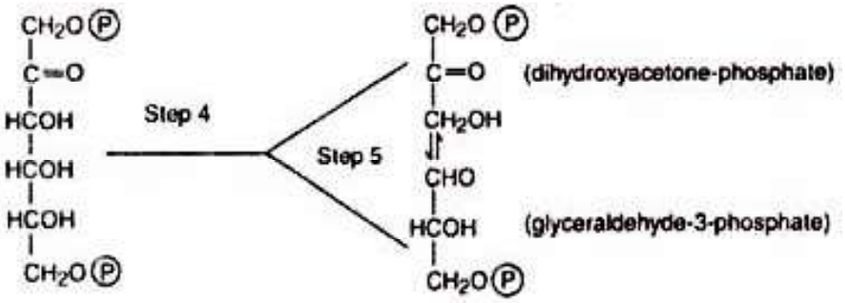
or

Phosphoglyceraldehyde (= PGAL) + Dihydroxyacetone-3-phosphate

$$\text{Dihydroxy acetone - 3 - phosphate} \xrightleftharpoons[\text{isomerase}]{\text{Iriose phosphate}} \text{Glyceraldehyde 3-phosphate}$$

Step 5: Isomerization

These two fragments, which may collectively be called triose phosphates, are readily inter-convertible by an isomerization under the effect of an enzyme complex Phospho-triose- isomerase. Mostly dihydroxyacetone- phosphate is converted to glyceraldehyde-3-phosphate.



Second Half of Glycolysis (Pay off Phase)

In the second half of glycolysis, comprising steps 6 to 10, there is a net gain of ATP, achieved by the oxidation of glyceraldehyde-3-phosphate, one of the triose phosphates formed in step 4. Because the two triose phosphates are inter-convertible, both follow the same pathway and steps 6 to 10 are therefore repeated twice to complete the breakdown of one molecule of glucose.

Step 6 (Dehydrogenation and Phosphorylation)

From the point of view of energy production step 6 is very important one. During this step glyceraldehyde-3-phosphate is simultaneously oxidized and phosphorylated, and the energy liberated by the oxidation of the aldehyde group is conserved to form the high-energy compound 1, 3-diphosphoglycerate, i.e., it has a high free energy of hydrolysis.

NOTES

The electrons removed from the aldehyde group during its oxidation are accepted by the coenzyme NAD^+ (Nicotinamide adenine dinucleotide), which is thereby reduced to give $NADH_2$ ($NADH + H^+$). The NAD^+ is bound to the enzyme glyceraldehyde-3-phosphate dehydrogenase which catalyzes the overall reaction.

In this reaction one inorganic H_3PO_4 group combines at C-1 of 3-phosphoglyceraldehyde (GAP) by non-enzymatic condensation to from 1-3-di-phosphoglyceraldehyde, and then two hydrogen atoms are removed by NAD^+ coenzyme 1 under the effect of enzyme glyceraldehyde-3-phosphate dehydrogenase to yield NADH and 1, 3-diphosphoglyceric acid (1, 3-PGA). In this dehydrogenation energy is carried along with H atoms into $NADH_2$.

$$\text{Glyceraldehyde 3-phosphate} + \text{H3PO4} + \text{NAD} \underset{\text{Dchydtogenase}}{\overset{\text{Glyceraldehyde Phosphate}}{\rightleftharpoons}} \text{1 : 3 diphosphoglycerate}$$

Step 7 (Formation of ATP): In this step the high-energy phosphate on C-1 of 1, 3-diphosphoglyceric acid is transferred to ADP to form ATP and 3-phosphoglyceric acid (3-PGA) under the effect of phosphotransferase enzyme. The overall result of steps 6 and 7, therefore, is that the energy liberated during the oxidation of an aldehyde group (– CHO in glyceraldehyde-3-phosphate) to a carboxylic acid group (– COOH in 3-phosphoglyceric acid) is conserved as the phosphate bond energy of an ATP molecule.

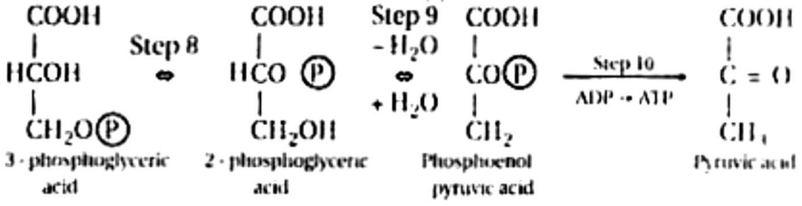
The direct synthesis of ATP from metabolites is called substrate level phosphorylation.

$$\text{1 : 3-diphospholgycerate} + \text{ADP} \underset{\text{kinase + Mg}}{\overset{\textit{Phospheglycerate}}{\rightleftharpoons}} \text{3-phophoglycerate} + \text{ADP}$$

Step 8 (Isomerization): During this step, 3-phosphoglyceric acid from step 7 undergoes an intramolecular rearrangement (isomerization) with the help of an enzyme phospho-glycero-mutase by transfer of phosphate group C-3 to C-2 position thus forming 2-phosphoglyceric acid (2-PGA).

$$\text{3-phosphoglycerate} \underset{}{\overset{\text{Phosphoglycefomutase}}{\rightleftharpoons}} \text{2-phosphoglycerate}$$

Step 9 (Dehydration): Now the 2-PGA loses one molecule of water under effect of an enzyme enolase to from another high-energy phosphate compound, phosphoenol pyruvic acid (PEP), which acts as a second source of ATP in glycolysis. Mg^{2+} is required for this process.



NOTES

Net Products of Glycolysis

In glycolysis two molecules of ATP are consumed during double phosphorylation of glucose to form fructose 1: 6 diphosphates. In return four molecules of ATP are produced by substrate level phosphorylation (conversion of 1: 3 diphosphoglycerate to 3-phosphoglycerate and phosphoenol pyruvate to pyruvate). Two molecules of NADH2 are formed at the time of oxidation of glyceraldehyde 3-phosphate to 1: 3 diphosphoglycerate.

The net reaction is as follows:

$$\text{Glucose} + 2NAD^+ + 2\,ADP + 2H_3PO4 \rightarrow 2\,\text{Pyruvate} + 2\,NADH + 2H^+ + 2\,ATP$$

Each NADH is equivalent to 3ATP, so that the net gain in glycolysis is 8ATP.

9.2.2 Stage II of Respiration: Pyruvate Oxidation or Conversion of Pyruvic Acid to Acetyle Coenzyme A

Although not itself part of the TCA cycle, the oxidative decarboxylation of pyruvic acid to acetyl CoA is the essential step by which the partially oxidized glucose molecule enters the cycle. The reaction is very complicated and involves the participation of several enzymes and coenzymes, collectively called as pyruvate dehydrogenase complex.

The most important coenzyme in this complex is coenzyme A. From the point of view of its involvement in the oxidative decarboxylation of pyruvate, the most significant feature of the coenzyme A molecule is that it is terminated by a – SH group because it is here that an acetyl group becomes attached. The molecule can thus conveniently be written in equations as CoA-SH. The oxidation which accompanies the removal of CO_2 from pyruvic acid requires the presence of NAD as the oxidizing agent.

The overall reaction may be written as follow:

$$\underset{\text{Pyruvic acid}}{CH_3COCOOH} + \underset{\text{Cocenzyme A}}{CoA - SH} + NAD^+ \rightarrow \underset{\text{Accetyl Conenzyme A}}{CH_3CO - 8 - CoA} + CO_2 + NADH + H^+$$

Acetyl CoA, which is the end product of this reaction, is another high energy compound. The energy locked in the bond between the acetyl group and the coenzyme A is ultimately responsible for driving the TCA cycle. Acetyl CoA being high energy compound acts as a link between EMP Pathway and TCA Cycle.

9.3.3 Stage III of Respiration: TCA Cycle or Krebs Cycle

In most eukaryotes, including all the higher plants and higher animals, respiratory breakdown of pyruvate (i.e., pyruvic acid) occurs chiefly within the mitochondria by a cyclic pathway, variously known as the citric acid cycle, the tri-carboxylic acid cycle, the TCA cycle, or the Krebs cycle, after Sir Hans Adolf Krebs, a German (later British) biochemist who proposed its basic features in 1937.

NOTES

Krebs was awarded Nobel Prize in 1953 for this work. The reaction sequence is called a 'cyclic' because, unlike the reactions of the glycolytic pathway, the end product (here, the 4-carbon oxaloacetic acid) combines with more of the starting material (here acetyl CoA) and reenters the reaction sequence as soon as it is formed.

The sequence is called the TCA cycle because the first intermediate in the cycle is a tri-carboxylic acid. Many prokaryotes (bacteria and blue-green algae) also have the TCA cycle, but others do not have all the necessary enzymes.

The TCA cycle, in combination with the closely linked cytochrome system, causes the complete oxidation of pyruvate to form water and carbon-dioxide. Free oxygen is required, and energy is liberated. Much of the energy is transferred into ATR but some of it is lost as heat 15 molecules of ATP can be produced from ADP and phosphate ion through the respiration of one molecule of pyruvic acid (i.e., 30 molecules of ATP from 1 mole of glucose) by the TCA cycle and the associated cytochrome system.

The successive reactions of the TCA cycle is outlined as follows

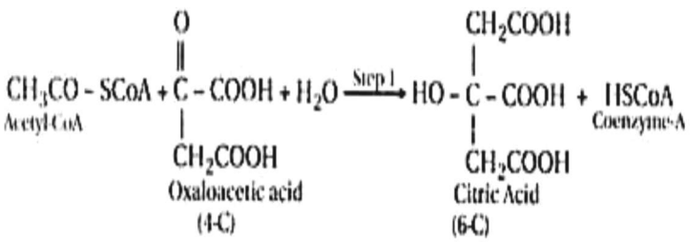
The TCA cycle can be divided into nine main steps, although some of them involve more than one chemical reaction (Refer Figure 9.2)

Step 1 (Condensation)

In the initial step, Acetyl-CoA reacts with oxaloacetic acid (a 4-carbon compound) in the presence of a condensing enzyme to produce citric acid (a 6-carbon compound) and to regenerate coenzyme A, which is reused to form acetyl-CoA.

$$\text{Oxaloaceticacid} + \text{Acetyl-CoA} \xrightarrow{H_2O} \text{Citric acid} + \text{CoA}$$

One ATP supplies the necessary energy, being converted into ADP and phosphate ion in the process. The requisite enzyme is called citrate synthase.



Step 2 (Dehydration and Hydration): During next step citric acid undergoes isomerization. In the presence of enzyme aconitase citric acid is first dehydrated to form cis-aconitic acid, and then the latter is again hydrated to form iso-citric acid. This is an isomeric Change

$$\underset{(6-C)}{\text{Citric acid}} \xrightarrow{-H_2O} \underset{(6-C)}{\text{Cis-aconitic acid}} \xrightarrow{+H_2O} \underset{(6-C)}{\text{Isocitric acid}}$$

Step 3 (Dehydrogenation): Isocitric acid is oxidized to oxalosuccinic acid, another 6-carbon compound, by the conezyme NAD. Here another pair of reactive, energy

bearing atoms are transferred to NAD, forming NAD.2H. The requisite enzyme is called isocitrate dehydrogenase.

NOTES

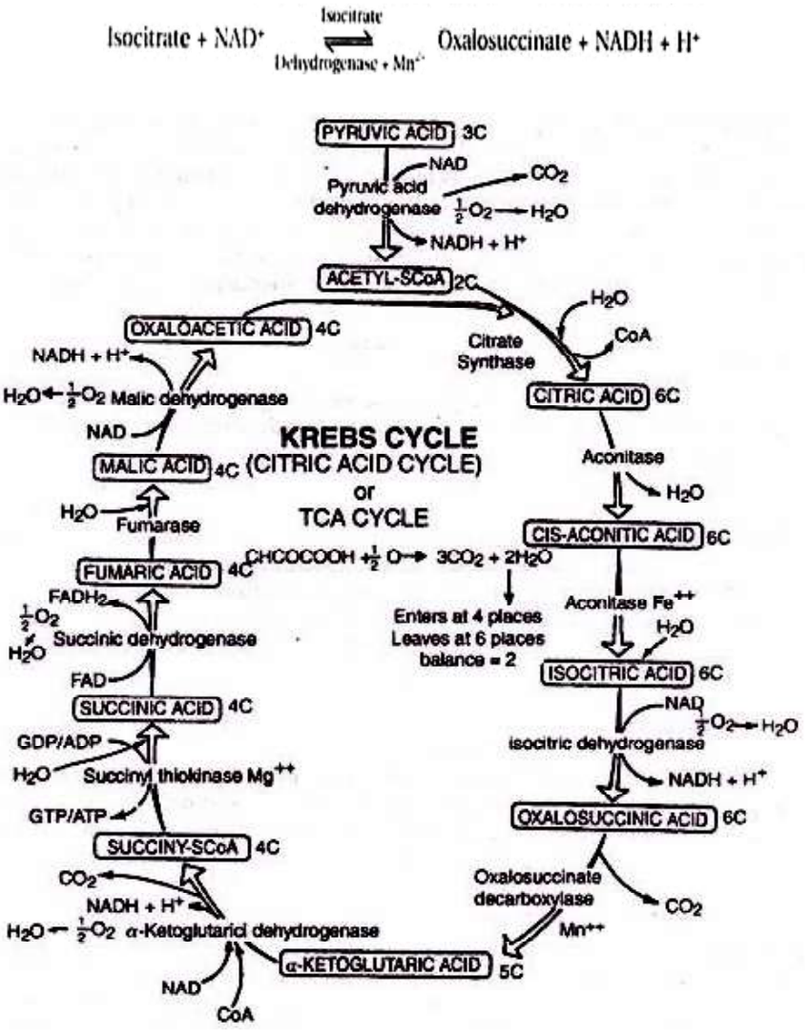


Fig. 9.2 *Diagrammatic Representation of Oxidative Decarboxylation of Pyruvic Acid and Different Chemical Reactions in Krebs Cycle from Acetyl CoA*

Step 4 (Decarboxylation): Being highly unstable, the oxalosuccinic acid is converted to á- ketoglutaric acid, a 5-carbon compound, and a molecule of CO_2 is released in the process. The responsible enzyme is oxalosuccinate decarboxylase, acting in the presence of manganese ions.

$$\text{Oxalosuccinate} \xrightarrow{\text{Decarboxylase}} \alpha\text{-Ketoglutarate} + CO_2 \uparrow$$

Step 5 (Decarboxylation and Dehydrogenation): á-ketoglutaric acid, which resembles pyruvic acid in having a keto group (> CO) next to its acidic group (-COOH), undergoes an oxidative decarboxylation reaction with CoA, similar to that undergone by pyruvic acid, the product being a 4-carbon succinyl ~S-CoA.

NOTES

This is a high energy compound analogous to acetyl ~S-CoA formed from pyruvic acid. A molecule of CO_2 is produced in the formation of succinyl ~S-CoA, a molecule of water is taken on, and another pair of reactive hydrogen atoms are transferred to NAD, forming NAD.2H.

$$\alpha\text{-Ketoglutarate} + CoA + NAD^+ \xrightarrow[\text{dehydrogenase}]{\alpha\text{-ketoglutarate}} \text{Succinyl CoA} + NADH + H^+ + CO_2 \uparrow$$

Step 6 (Formation of ATP/GTP): Succinyl-CoA releases succinate (succinic acid) another 4- carbon compound, and CoA. The CoA is immediately available again for either of its two reactions in the TCA cycle or for other uses. A molecule of ATP is formed from ADP and phosphate ion in this step, through the intervention of another phosphate carrier called guanosine di-phosphate (GDP).

The breakdown of succinyl-CoA to succinate and CoA releases energy which is used to attach a phosphate ion to GDP forming GTP The phosphate group is then transferred from GTP to ADR forming ATP The enzyme governing this step is called succinyl-CoA synthetase (or Succmyl Thiokinase).

$$\text{Succinyl CoA} + GDP/ADP + H_3PO_4 \xrightarrow[\text{Thiokinase}]{\text{Succinyl}} \text{Succinate} + CoA + GTP/ATP$$

The remaining three steps of the TCA cycle serve to regenerate oxaloacetic acid, the initial 4-carbon acceptor of Acetyl-CoA, from succinate. This involves the oxidation of the hydrocarbon group (CH_2) in the α position (i.e., next to the –COOH group) of succinate to a carbonyl group (> CO).

Step 7 (Dehydrogenation): Succinate forms fumarate (fumaric acid) another 4-carbon compound, and releases another pair of reactive hydrogen atoms, which are fed directly into the cytochrome system (discussed in subsequent paragraphs) instead of being transferred to NAD.

The requisite enzyme is called succinate dehydrogenase. The two reactive hydrogen atoms are accepted by a coenzyme called FAD (The only functional difference between NAD and FAD in the present context is that the subsequent oxidation of each molecule of reduced FAD is coupled to the formation of two molecules of ATP, whereas the oxidation of reduced NAD results in the formation of three molecules of ATP).

$$\text{Succinate} + FAD \underset{\text{Dehydrogenase}}{\overset{\text{Succinic}}{\rightleftharpoons}} \text{Fumarate} + FADH_2$$

Step 8 (Hydration): Fumarate (fumaric acid) forms malate (malic acid), another 4- carbon compound, taking on a molecule of water in the process. The governing enzyme is called fumarase. No significant energy change occurs in this step. This reaction is just molecular rearrangement by the addition of the elements of water across the double bond to form malate.

$$\text{Fumarate} + H_2O \overset{\text{Fumarase}}{\rightleftharpoons} \text{Malate}$$

NOTES

Step 9 (Dehydrogenation): In the final step malic acid gives rise to oxaloacetate (oxaloacetic acid) and releases two more reactive hydrogen atoms, which are accepted by NAD^+ to form $NADH_2$+ The oxaloacetic acid, in turn, is ready to react with another molecule of Acetyl-CoA (fed in from the breakdown of pyruvic acid) completing the TCA cycle. The requisite enzyme is called malate dehydrogenase.

$$\text{Malate} + \text{NAD(P)}^+ \underset{\text{Dehydrogenase}}{\overset{\text{Malic}}{\rightleftharpoons}} \text{Oxaloacetate} + \text{NAD(P)H} + \text{H}^+$$

Oxaloacetic acid picks up another molecule of activated acetate to repeat the cycle.

A molecule of glucose yields two molecules of $NADH_2$,2ATP and two pyruvates while undergoing glycolysis. The two molecules of pyruvate are completely degraded in Krebs cycle to form two molecules of ATP, $8NADH_2$ and 2 $FADH_2$

$$\text{Glucose} + 4\ \text{ADP} + 4\ \text{H}_3\text{PO}_4 + 10\ \text{NAD} + 2\text{FAD} \longrightarrow 6\text{CO}_2 + 4\ \text{ATP} + 10\ \text{NADH} + 10\ \text{H}^+ + 2\ \text{FADH}_2$$

In the above description TCA cycle has been described with reference to glucose, but it must be remembered that the significance of this cycle is not confined to the respiration of glucose only. All food materials, after they have been broken down into their constituent units (for example, storage polysaccharides into monosaccharides; proteins into amino acids, etc.) are incompletely oxidized to form, apart from CO_2 and H_2O, one of only three possible substances, all of which are intermediates in the TCA cycle. The entire Krebs cycle may be represented by the following equation —

$$2\ \text{Acetyl coenzyme A} + 8\ \text{H}_2\text{O} + 6\text{NAD}^+ + 2\text{FAD}^+ + 2\text{GDP} + 2\text{P}i \longrightarrow 4\text{CO}_2 + 2\text{H}_2\text{O} + 6\text{NADH} + 2\text{FADH}_2 + 2\text{GTP} + 6\text{H}^+$$

Like glycolysis. Krebs cycle also produces only two high-energy phosphate molecules. It, however, forms 6 NADH and 2 $FADH_2$, as compared to only 2, NADH formed in glycolysis. The 6 NADH and 2 $FADH_2$ provide energy (22 ATP) to the cell in electron transmitter chain.

9.2.4 Stage IV of Respiration: Terminal Oxidation

It is the name of oxidation found in aerobic respiration that occurs towards the end of catabolic process and involves the passage of both electrons and protons of reduced coenzymes to oxygen.

$$\text{FADH}_2 \rightleftharpoons \text{FAD} + 2\text{H}^+ + 2\text{e}^-; \qquad \frac{1}{2}\ \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \longrightarrow \text{H}_2\text{O}$$

Terminal oxidation consists of two processes:

(i) Electron transport and

(ii) Oxidative phosphorylation.

NOTES

(i) Electron Transport Chain (ETC) or Electron Transport System (ETS)

You have seen that the glucose molecule is completely oxidized by the end of the citric acid cycle. But the energy is not released unless NADH and $FADH_2$ are oxidized through the electron transport system (ETS) or Electron Transport Chain (ETC) or Mitochondrial Respiratory Chain.

Here, oxidation of a compound means removal of electrons from it. This is usually accompanied by removal of hydrogen. Reduction means addition of electrons to a compound, usually accompanied by addition of hydrogen.

The metabolic pathway through which the electron passes from one carrier to another is called electron transport system (ETS) or Electron transport chain (ETC) (Refer Figure 9.3), and it is operative in the inner mitochondrial membrane. Four enzyme complexes (Complex I, II, III & IV) (Refer Figure 9.4) are involved in electron transport.

Electrons from NADH produced in the mitochondrial matrix during citric acid cycle are oxidized by an NADH dehydrogenase (Complex I), and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via $FADH_2$ that is produced during oxidation of succinate (i.e., succinic acid) through the activity of the enzyme, succinate dehydrogenase (Complex II), in the citric acid cycle. The reduced ubiquinone (ubiquinol) is then oxidized with the transfer of electrons to cytochrome-C via cytochrome bc_1 complex (Complex III).

Cytochrome-C is a small protein attached to outer surface of the inner membrane and acts as a mobile carrier for transfer of electrons between complex III and IV Complex IV refers to cytochrome c oxidase complex containing cytochromes a and two copper (cu) centres.

When the electrons pass from one carrier to another via complex I to IV in the electron transport chain, they are coupled to ATP synthase (Complex V) for the production of ATP from ADP and inorganic phosphate (pi).

The number of ATP molecules synthesized depends on nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$ produces 2 molecules of ATR During electron transfer, the hydrogen atoms split into protons and electrons.

The electrons are carried by the cytochromes. They recombine with their protons before the final stage, when hydrogen atom is accepted by oxygen to form metabolic water. ($2H + O \rightarrow 2H_2O$). Although the aerobic process of respiration takes place only in the presence of oxygen, the role of oxygen is limited to the terminal stage of the process.

Yet, the presence of oxygen is essential, since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor.

The whole process by which oxygen effectively allows the production of ATP by phosphorylation of ADP, is called oxidative phosphorylation.

Details of oxidative phosphorylation follow

NOTES

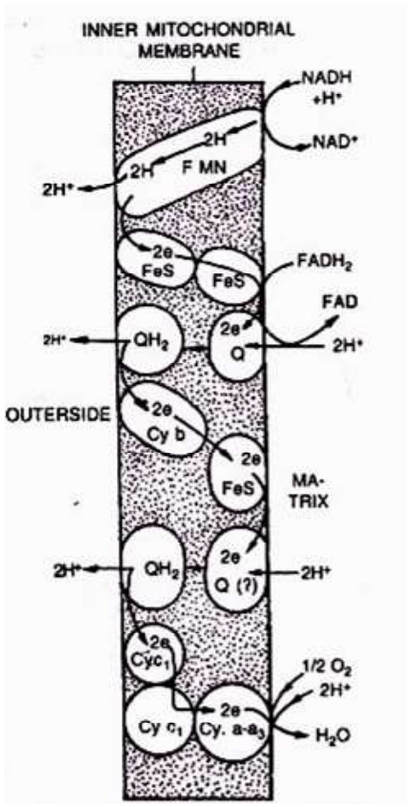


Fig 9.3 *Diagram to show Mitochondrial Electron Transport Chain (ETC)*

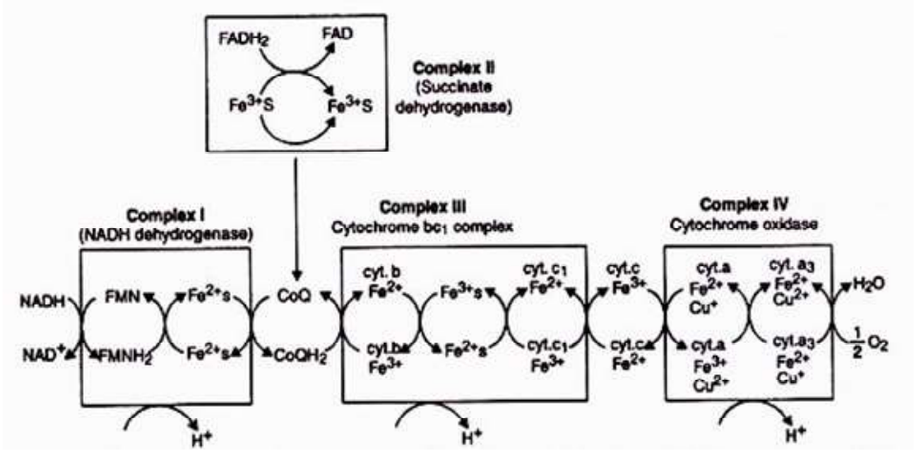


Fig 9.4 *Diagram Representing Four Enzyme Complexes Successively Associated with Electron Transport*

NOTES

(ii) Oxidative Phosphorylation or Chemiosmotic Synthesis of ATP: Mitchell's Hypothesis

Oxidative phosphorylation is the synthesis of energy rich ATP' molecules with the help of energy liberated during oxidation of reduced co-enzymes ($NADH_2$, $FADH_2$) produced in respiration. The enzyme required for the synthesis is called ATP synthetize. (Refer Figure 9.5)

It is considered to be the fifth complex (complex V) of electron transport chain. Synthesis of ATP molecules takes place through the process of chemiosmosis. Peter Mitchell in 1961 put forward chemiosmotic hypothesis in mitochondrial, etc. He got the Nobel Prize in 1978 for this hypothesis.

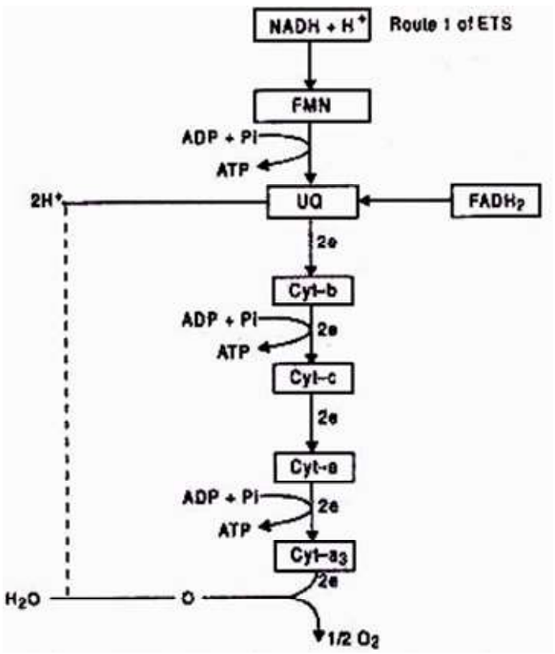


Fig 9.5 *Diagram to Explain Terminal Oxidation and Oxidative Phosphorylation*

According to this hypothesis, ATP synthesis involves two steps

(i) Creation of H^+ Gradient

(ii) Proton Flow

(i) Creation of H^+ Gradient

The energy released during electron transfer passes H^+ ions or protons from the matrix through the inner mitochondrial membrane into the inter-membrane space between the outer and inner membranes. This raises the concentration of protons outside the inner membrane above that in the matrix.

Higher concentration of positively charged protons in the inter-membrane space creates an electric potential across the inner mitochondrial membrane as the matrix now becomes negative with respect to the inter-membrane space. The

proton concentration gradient and the membrane electric potential are together referred to as the proton motive force.

(ii) Proton Flow

Proton motive force causes the flow of protons from the inter-membrane space across the inner mitochondrial membrane down their electrochemical gradient into the matrix. Protons pass through the channel in F_0 - F_1 ATPase particle, where F_1 catalyzes the synthesis of ATP from ADP and Pi with the electrical kinetic energy released by the downhill flow of protons.

The transfer of energy from electron flow to electrochemical proton gradient and then to the phosphate bonds of ATP is called oxidative phosphorylation. The ETS is, therefore, also known as oxidative phosphorylation pathway. The method of forming ATP by using chemical (H^+) flow through a membrane is termed chemiosmotic ATP synthesis (Refer Figure 9.6).

Route 1 of ETS

The electrochemical gradient created as an electron pair passes from $NADH_2$ to oxygen is sufficient to drive the synthesis of 3 ATP molecules.

Route 2 of ETS

The electron pair from $FADH_2$ passes over only a part of the electron transport chain. Therefore, fewer H^+ ions are added to the gradient. The smaller gradient generates only 2 ATP molecules for each pair of electrons.

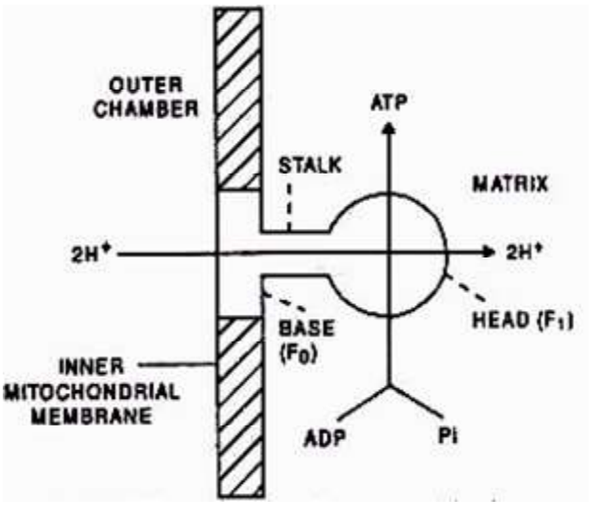


Fig. 9.6 *ATP Synthesis by F0-F1 in an Oxysome Present in the Inner Membrane of Mitochondrion*

Shuttle System

The $NADH_2$ formed during glycolysis is in the cytoplasm. It must reach the inner membrane of mitochondria where ETS is located so that ATP may be produced. The inner mitochondrial membrane is impermeable to $NADH_2$. A special electron

NOTES

carrier system, called the shuttle system, located in the mitochondrial membrane, picks up the electrons from the hydrogen's of NADH present in the cytoplasm, transfers them across the mitochondrial membranes, and delivers them to the electron carriers inside the mitochondrion.

There are two distinct shuttle systems: the less efficient glycerol-phosphate shuttle present in the skeletal muscle, brain cells and most of the eukaryotic cells, and the more efficient malate-aspartate shuttle present in the heart, liver and kidney cells (Refer Figure 9.7). The less efficient shuttle transfers electrons from NADH to FAD, reducing the latter to $FADH_2$. The $FADH_2$ produces only 2 ATP molecules as stated above.

The more efficient shuttle transfers electrons from NADH of cytoplasm to NAD of mitochondrion, reducing it to NADH. The latter introduces its electron pairs into route 1 of ETS. In this route, 3 ATP molecules are formed per pair of electrons. The shuttle continuously transfers electrons from cytoplasm to the mitochondria and does not allow the accumulation of hydrogen molecules in the cytoplasm.

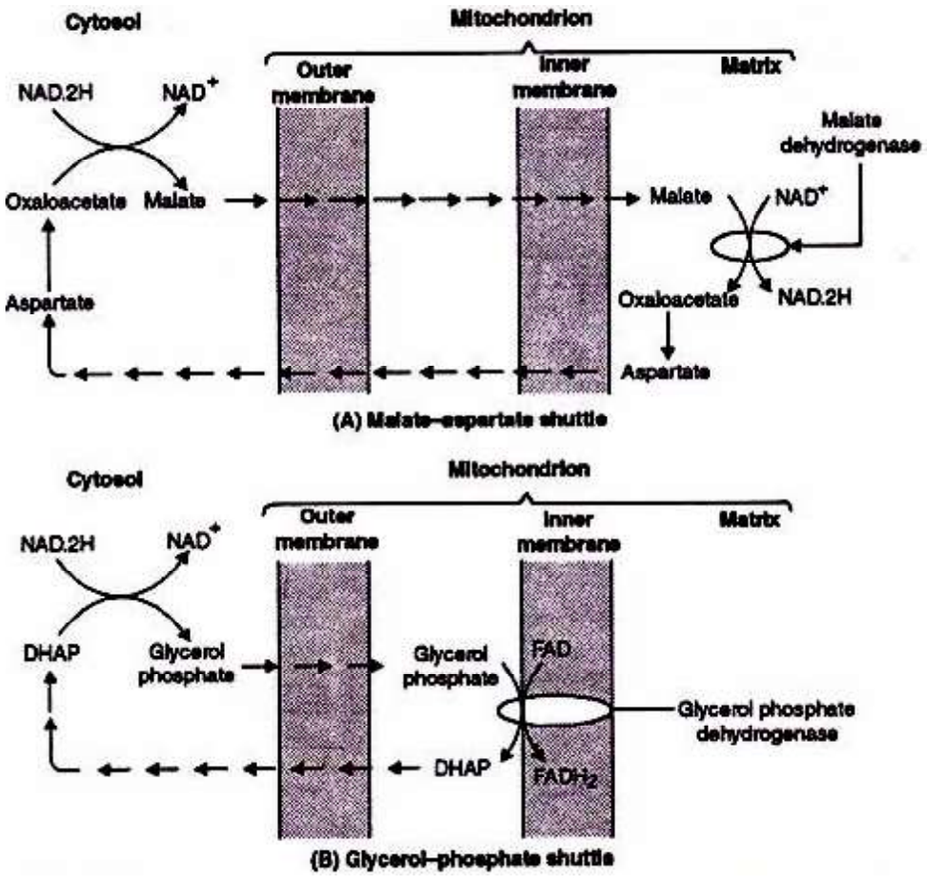


Fig 9.7 Shuttle System

Check Your Progress

1. What is aerobic respiration?
2. Write the reaction for aerobic respiration.
3. How is aerobic respiration useful?
4. What are the four stages that are involved in aerobic respiration process?

NOTES

9.3 GLYOXYLATE CYCLE

The glyoxylate cycle (Refer Figure 9.8) , a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi. The glyoxylate cycle centers on the conversion of Acetyl-CoA to succinate for the synthesis of carbohydrates. In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available. The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis. In recent years, however, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals and suggests that animals encode alternative enzymes of the cycle that differ in function from known MS and ICL in non-metazoan species

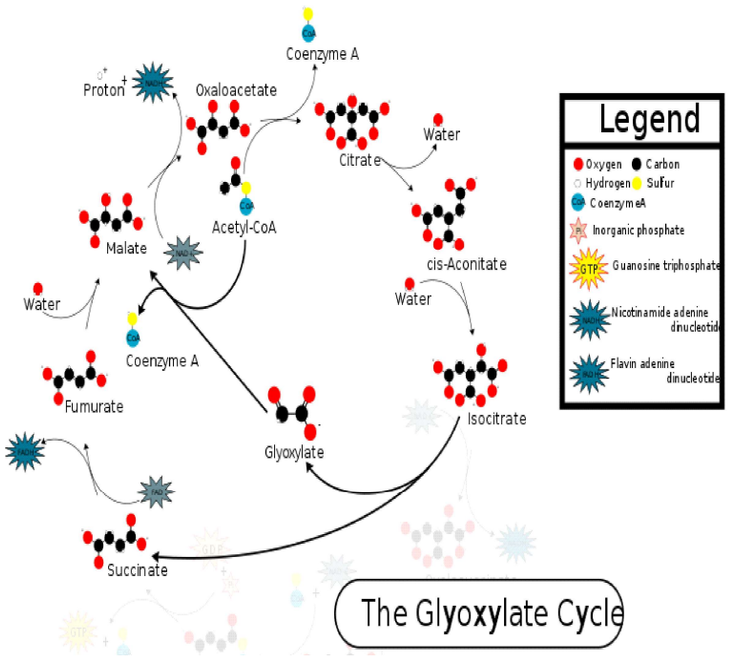


Fig. 9.8 *The Glyoxylate Cycle*

NOTES

9.3.1 Overview of the Glyoxylate Cycle

It had been observed by many plant physiologists that during the germination of fatty seeds, the fat content decreased with a simultaneous increase in sucrose (i.e., carbohydrates). This apparent conversion of fats into sucrose remained a mystery till 1957 when Kornberg and Krebs discovered that a strain of bacterium Pseudomonas could readily convert ^{14}C-labelled acetic acid into labelled malic acid and citric acid (these are intermediates of Krebs Cycle) which involved the following reactions:

(1) Acetyl-CoA combined with Oxaloacetic acid to form Citric Acid.

Acetyl-CoA + Oxaloacetic acid $\rightarrow$ Citric Acid + CoA

(Acetic acid first reacted with Coenzyme-A to form Acetyl CoA).

(2) Acetyl CoA reacted with glyoxylic acid in the presence of the enzyme malate synthetase to produce Malic acid.

Acetyl CoA + Glyoxylic Acid $\rightarrow$ Malic Acid + CoA

The glyoxylic acid was obtained through the breakdown of Isocitric Acid (an intermediate of Krebs' Cycle) by the enzyme Isocitratase

Isocitric acid $\rightarrow$ glyoxylic acid + succinic acid

On the bases of the above reactions Kornberg and Krebs (1957) framed a cycle which is called as Glyoxylic acid cycle or Glyoxylate Cycle through which the fats could be converted into sucrose (i.e., carbohydrate) during the germination of fatty seeds in plants.

The glyoxylate cycle (which is intimately associated with Krebs Cycle) is now known to occur in many other bacteria, yeasts, molds, and higher plants and is completed in glyoxysomes, mitochondria and cytosol.

9.3.2 Steps Involved in Glyoxylate Cycle

Various steps of this cycle (Refer Figure 9.9) occurring in higher plants especially during the germination of fatty seeds are as follows:

(a) Reactions in Glyoxysome

(i) Acetyl-CoA produced after the α-oxidation of fatty acids (in glyoxysomes) condenses with oxaloacetic acid to form Citric Acid.

NOTES

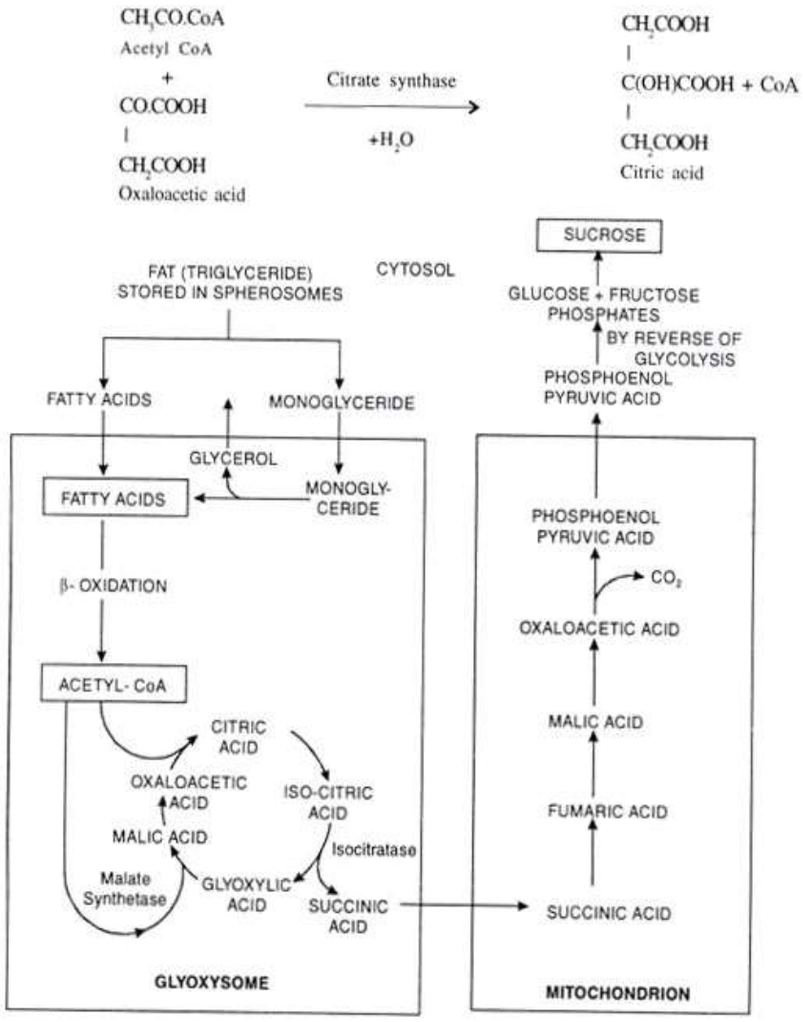
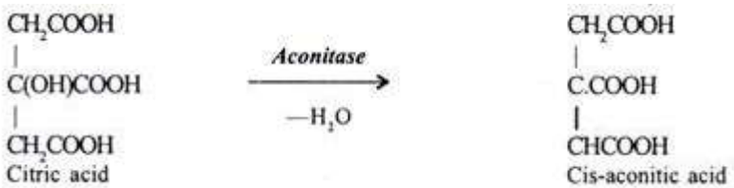


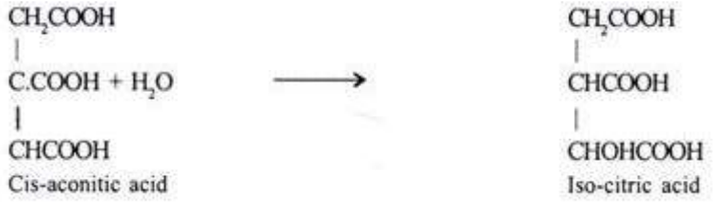
Fig 9.9 *Glyoxylate Cycle*

(ii) Citric acid is dehydrated to produce cis-Aconitic Acid in the presence of Aconitase.

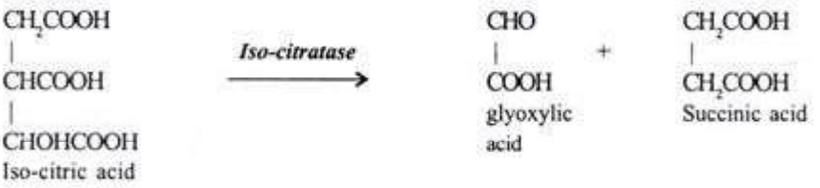


NOTES

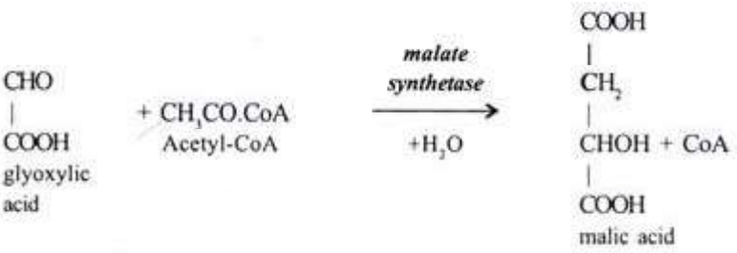
(iii) cis-Aconitic acid reacts with one molecule of H_2O to form Iso-citric acid.



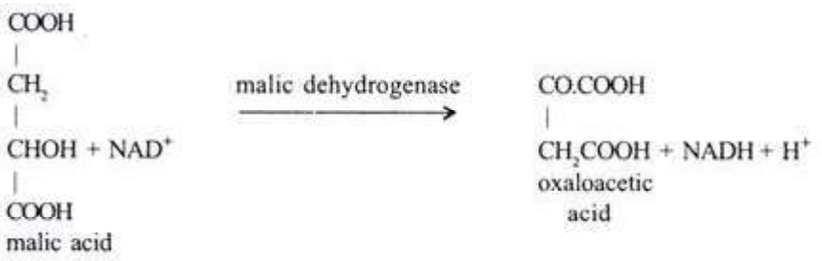
(iv) Iso-citric acid is broken down into glyoxylic acid and succinic acid by the enzyme Isocitratase.



(v) Glyoxylic acid combines with acetyl CoA (produced after the â-oxidation of fatty acids) in the presence of Malate synthetase to produce Malic acid.



(vi) Malic acid is oxidised into oxaloacetic acid in the presence of Malic dehydrogenase and the coenzyme NAD.

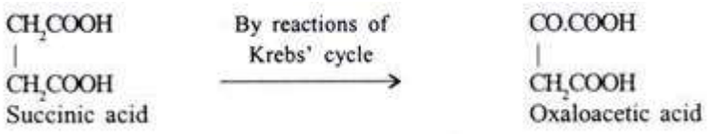


Oxaloacetic acid thus produced combines with Acetyl-CoA to regenerate citric acid.

NOTES

(b) Reactions in Mitochondrion

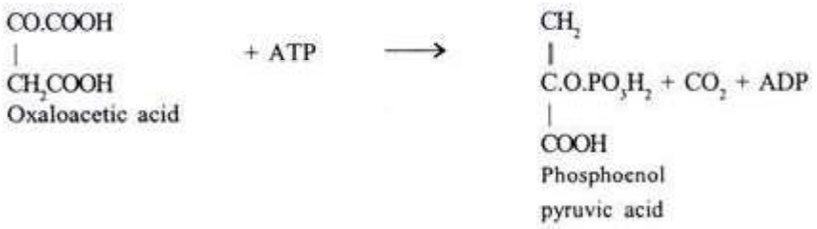
(vii) Succinic acid (produced in reaction iv) Moves into mitochondrion and is converted into oxaloacetic acid as in the Krebs cycle.



The conversion of succinic acid into oxaloacetic acid does not take place in glyoxysomes because of the absence of appropriate enzymes (i.e., succinic dehydrogenase and fumarase) in the latter. It takes place in mitochondria.

(vii) Oxaloacetic acid produced in the above step

(viii) is decarboxylated in the presence of ATP to form phosphoenol pyruvic acid. This reaction also takes place in mitochondria)



(c) Reactions in Cytosol

(ix) Phosphoenol pyruvic acid moves into cytosol and by the reverse reactions of glycolysis and with slight modification is converted into the glucose and fructose phosphates.

(x) Finally, glucose and fructose phosphates are converted into sucrose

After the conversion of fats into carbohydrates is completed, the glyoxysomes disappear from the cells.

9.3.3 Significance of Glyoxylate Cycle

(1) During the germination of fatty seeds, the fats which are insoluble are hydrolysed into fatty acids and glycerol. Fatty acids after P-oxidation produce Acetyl-CoA units which synthesize sucrose (which is soluble) through glyoxylate cycle. Soluble sucrose is then supplied to different growing regions of the young germinating seedling till it develops its own photosynthetic system.

(2) Those micro-organisms which can grow on ethyl alcohol or acetate as a sole source of energy and carbon make use of this cycle in synthesizing longer carbon chains.

(3) The glyoxylate cycle is an example of gluconeogenesis.

NOTES

Check Your Progress

5. What is glyoxylate cycle?
6. Is glyoxylate cycle present or absent in animals?
7. What happens to Acetyl-CoA produced after the â-oxidation?
8. What happens when Malic acid is oxidised into oxaloacetic acid?
9. Why does conversion of succinic acid into oxaloacetic acid does not take place in glyoxysomes?

9.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Aerobic Respiration is the process by which the energy from glucose is released in the presence of oxygen. It takes place only if oxygen is available.
2. The reaction for aerobic respiration i s-

$$C_6H_{12}O_6 + 6O2 = 6CO_2 + 6H_2O + \text{Energy (ATP)}$$

3. Aerobic respiration helps in release of maximum energy and also gets rid of carbon-dioxide and excess water.
4. The four stages involved in the aerobic respiration process are
   - Glycolysis (or EMP-Pathway of Glycolytic Breakdown)
   - Pyruvate Oxidation or Conversion of Pyruvic Acid to Acetyl Coenzyme A
   - TCA Cycle or Krebs' Cycle
   - Terminal Oxidation.
5. The glyoxylate cycle , a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi.
6. The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis.
7. Acetyl-CoA produced after the α-oxidation of fatty acids (in glyoxysomes) condenses with oxaloacetic acid to form Citric Acid.
8. Malic acid is oxidised into oxaloacetic acid in the presence of Malic dehydrogenase and the coenzyme NAD.
9. The conversion of succinic acid into oxaloacetic acid does not take place in glyoxysomes because of the absence of appropriate enzymes (i.e., succinic dehydrogenase and fumarase) in the latter. It takes place in mitochondria.

9.5 SUMMARY

NOTES

- Living things need energy for everything they do, including growing and reproducing. This energy comes from glucose. All animals and humans get glucose through eating it, but plants need to make their own glucose during photosynthesis.
- Plants and animals transport glucose and oxygen to tiny structures in their cells, called mitochondria. Here, the glucose and oxygen take part in a chemical reaction. The reaction is called aerobic respiration, and it produces energy which transfers to the cells.
- Aerobic respiration makes two waste products: carbon-dioxide and water.
- Animals remove carbon dioxide from their bodies when they breathe out. In daytime, plants use some of this carbon dioxide for photosynthesis. At night, they release the carbon dioxide to their surroundings.
- Aerobic respiration uses oxygen to break down glucose, amino acids and fatty acids and is the main way the body generates adenosine triphosphate (ATP), which supplies energy to the muscles.
- After glycolysis (the anaerobic breakdown of glucose into pyruvate, pyruvate is converted to acetyl CoA in the matrix of the energy-transferring mitochondria, via the link reaction.
- Next is the Krebs cycle, which occurs twice per glucose molecule, producing – among other chemicals that feed into the aerobic part of the process – more ATP.
- Aerobic Respiration is the process by which the energy from glucose is released in the presence of oxygen. It takes place only if oxygen is available.
- Although glycolysis occurs in all organisms except primitive bacteria, it has been studied in greatest detail in yeast and animal muscle cells.
- It occurs chiefly in the soluble part of the cytoplasm ,i.e., cytosol, rather than being associated with mitochondria or other organelles. This is first stage of respiration, which includes a sequence of chemical reactions through which glucose is incompletely oxidized to yield the 3-carbon compound pyruvic acid (Glycolysis = lysis or splitting of glucose).
- In order to obtain energy in the form of ATP (Adenosine triphosphate) from the breakdown of glucose, it is first necessary to 'spark off ' the reaction sequence by putting ATP into it.
- During second step of reactions the glucose-6-phosphate is rearranged (isomerized) to form fructose-6-phosphate.

NOTES

- The electrons removed from the aldehyde group during its oxidation are accepted by the coenzyme NAD^+ (Nicotinamide adenine dinucleotide), which is thereby reduced to give $NADH_2$ ($NADH + H^+$). The NAD^+ is bound to the enzyme glyceraldehyde-3-phosphate dehydrogenase which catalyzes the overall reaction.
- Krebs was awarded Nobel Prize in 1953 for this work. The reaction sequence is called a 'cyclic' because, unlike the reactions of the glycolytic pathway, the end product (here, the 4-carbon oxaloacetic acid) combines with more of the starting material (here acetyl CoA) and reenters the reaction sequence as soon as it is formed.
- Oxidative phosphorylation is the synthesis of energy rich ATP' molecules with the help of energy liberated during oxidation of reduced co-enzymes ($NADH_2$, $FADH_2$) produced in respiration. The enzyme required for the synthesis is called ATP synthetize.
- The energy released during electron transfer passes H^+ ions or protons from the matrix through the inner mitochondrial membrane into the inter-membrane space between the outer and inner membranes. This raises the concentration of protons outside the inner membrane above that in the matrix.
- Proton motive force causes the flow of protons from the inter-membrane space across the inner mitochondrial membrane down their electrochemical gradient into the matrix.
- The glyoxylate cycle, a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi.
- The glyoxylate cycle centers on the conversion of Acetyl-CoA to succinate for the synthesis of carbohydrates.
- In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available.

9.6 KEY WORDS

- **Aerobic Respiration:** Aerobic Respiration is the process by which the energy from glucose is released in the presence of oxygen.
- **Isomerization:** Isomerization is the process by which one molecule is transformed into another molecule which has exactly the same atoms, but the atoms have a different arrangement.
- **Decarboxylation:** Decarboxylation is a chemical reaction that removes a carboxyl group and releases carbon dioxide (CO_2).

NOTES

- **Hydration: Hydration** is the process of replacing water in the body. This can be done through drinking water, eating ice chips, eating foods that have high water content, drinking other fluids or an intravenous or IV line. Being dehydrated is when your body is lacking the water levels necessary to function optimally.
- **Dehydrogenation:** Dehydrogenation is a chemical reaction that involves the removal of hydrogen from an organic molecule. It is the reverse of hydrogenation.
- **Phosphorylation:** Phosphorylation is an important mechanism by which the activity of proteins can be altered after they are formed.
- **Mitochondria:** Mitochondria are rod-shaped organelles that can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP).

9.7 SELF ASSESSMENT QUESTIONS AND ANSWERS

Short-Answer Questions

1. Write in brief about glycolysis or EMP-Pathway of glycolytic breakdown.
2. Write a short note 1st and 2nd phosphorylation.
3. Explain splitting of hexose molecule.
4. What are the net products of glycolysis?
5. Brief about TCA Cycle or Krebs Cycle.
6. Explain in short about the formation of ATP/GTP.

Long-Answer Questions

1. Explain in detail about the four stages involved in the aerobic respiration.
2. What are the two stages of glycolysis? Explain in detail.
3. Write a note on pyruvate oxidation or conversion of pyruvic acid to acetyl coenzyme A.
4. Discuss in detail about TCA cycle and its stages in detail.
5. Describe in detail about Electron Transport System (ETS).
6. Write about Mitchell's Hypothesis in detail.
7. Give a detailed overview of the glyoxylate cycle.

9.9 FURTHER READINGS

NOTES

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 10 MECHANISM OF SUBSTRATE

NOTES

Structure

10.0 Introduction
10.1 Objectives
10.2 Mechanisms of Substrate-Level Phosphorylation
10.3 Respiratory Electrons Transport in Mitochondria and Bacteria
10.4 Mechanism of Oxidative Phosphorylation
10.5 Answers to Check Your Progress Questions
10.6 Summary
10.7 Key Words
10.8 Self Assessment Questions and Exercises
10.9 Further Readings

10.0 INTRODUCTION

Substrate level phosphorylation is when ADP is converted to ATP by the direct transfer of a phosphate group. The phosphate group is donated or transferred from a phosphorylated intermediate. This is in contrast to oxidative phosphorylation, where a chemiosmotic gradient is used to power the phosphorylation process.A simple way to think of the difference is that in substrate level, the phosphorylation is a direct process, with no middle man. The phosphate comes off of one molecule and is directly transferred to another. The energy for the phosphorylation of ADP is provided directly from breaking the high energy phosphate bond on the intermediate molecule. This is referred to as reaction coupling.

Oxidative phosphorylation has middle men in the form of NADH and the electron transport enzymes. The phosphate comes from a pool of inorganic phosphates instead of directly from another molecule, and the energy to phosphorylate the ADP comes from the proton gradient, not from coupled reactions.The most famous examples of substrate level phosphorylation are found in the glycolysis pathway. The ATP are produced in the pay off phase of glycolysis by the action of the enzymes phosphoglycerate kinase and pyruvate kinase.

In this unit, you will study about the mechanisms of substrate, level phosphorylation, respiratory electron transport in mitochondria and bacteria , mechanism of oxidative phosphorylation, etc.

NOTES

10.1 OBJECTIVES

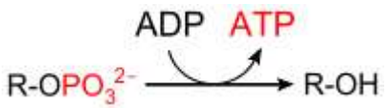
After going through this unit you will be able to:

- Understand the mechanisms of substrate
- Know about level phosphorylation
- Explain respiratory electron transport in mitochondria and bacteria
- Discuss the mechanism of oxidative phosphorylation

10.2 MECHANISMS OF SUBSTRATE-LEVEL PHOSPHORYLATION

Substrate-level phosphorylation is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl (PO_3) group to ADP or GDP from another phosphorylated compound.

Unlike oxidative phosphorylation, oxidation and phosphorylation are not coupled in the process of substrate-level phosphorylation, and reactive intermediates are most often gained in the course of oxidation processes in catabolism. Most ATP is generated by oxidative phosphorylation in aerobic or anaerobic respiration while substrate-level phosphorylation provides a quicker, less efficient source of ATP, independent of external electron acceptors. This is the case in human erythrocytes, which have no mitochondria, and in oxygen-depleted muscle.



Substrate-level phosphorylation occurs in the cytoplasm of cells during glycolysis and in mitochondria either during the Krebs cycle or by MTHFD1L, an enzyme interconverting ADP + phosphate + 10-formyltetrahydrofolate to ATP + formate + tetrahydrofolate (reversibly), under both aerobic and anaerobic conditions. In the pay-off phase of glycolysis, a net of 2 ATP is produced by substrate-level phosphorylation.

Glycolysis

The first substrate-level phosphorylation occurs after the conversion of 3-phosphoglyceraldehyde and Pi and NAD+ to 1,3-bisphosphoglycerate via glyceraldehyde 3-phosphate dehydrogenase. 1,3-bisphosphoglycerate is then dephosphorylated via phosphoglycerate kinase, producing 3-phosphoglycerate and ATP through a substrate-level phosphorylation.

The second substrate-level phosphorylation occurs by dephosphorylating phosphoenolpyruvate, catalyzed by pyruvate kinase, producing pyruvate and ATP.

During the preparatory phase, each 6-carbon glucose molecule is broken into two 3-carbon molecules. Thus, in glycolysis dephosphorylation results in the production of 4 ATP. However, the prior preparatory phase consumes 2 ATP, so the net yield in glycolysis is 2 ATP. 2 molecules of NADH are also produced and can be used in oxidative phosphorylation to generate more ATP.

Mitochondria

ATP can be generated by substrate-level phosphorylation in mitochondria in a pathway that is independent from the proton motive force. In the matrix there are three reactions capable of substrate-level phosphorylation, utilizing either phosphoenolpyruvate carboxykinase or succinate-CoA ligase, or monofunctional C1-tetrahydrofolate synthase.

Phosphoenolpyruvate Carboxykinase

Mitochondrial phosphoenolpyruvate carboxykinase is thought to participate in the transfer of the phosphorylation potential from the matrix to the cytosol and vice versa. However, it is strongly favored towards GTP hydrolysis, thus it is not really considered as an important source of intra-mitochondrial substrate-level phosphorylation.

Succinate-CoA Ligase

Succinate-CoA ligase is a heterodimer composed of an invariant á-sub-unit and a substrate-specific ß-sub-unit, encoded by either SUCLA2 or SUCLG2. This combination results in either an ADP-forming Succinate-CoA ligase (A-SUCL, or a GDP-forming succinate-CoA ligase (G-SUCL). The ADP-forming succinate-CoA ligase is potentially the only matrix enzyme generating ATP in the absence of a proton motive force, capable of maintaining matrix ATP levels under energy-limited conditions, such as transient hypoxia.

Monofunctional C1-Tetrahydrofolate Synthase

This enzyme is encoded by MTHFD1L and reversibly interconverts ADP + phosphate + 10-formyltetrahydrofolate to ATP + formate + tetrahydrofolate.

Other Mechanisms

In working skeletal muscles and the brain, Phosphocreatine is stored as a readily available high-energy phosphate supply, and the enzyme creatine phosphokinase transfers a phosphate from phosphocreatine to ADP to produce ATP. Then the ATP releases giving chemical energy. This is sometimes erroneously considered to be substrate-level phosphorylation, although it is a transphosphorylation.

NOTES

Importance of substrate-level phosphorylation in anoxia.

During anoxia, provision of ATP by substrate-level phosphorylation in the matrix is important not only as a mere means of energy, but also to prevent mitochondria from straining glycolytic ATP reserves by maintaining the adenine nucleotide translocator in 'forward mode' carrying ATP towards the cytosol.

Check Your Progress

1. What is substrate-level phosphorylation?
2. Where does substrate-level phosphorylation occur?
3. When does first substrate-level phosphorylation occur?
4. What happens in second substrate-level phosphorylation?
5. How is ATP generated?
6. what does mitochondrial phosphoenolpyruvate carboxykinase do?

10.3 RESPIRATORY ELECTRONS TRANSPORT IN MITOCHONDRIA AND BACTERIA

The electron transport chains of bacteria (prokaryotes) operate in plasma membrane (mitochondria are absent in prokaryotes). Some bacterial electron transport chains resemble the mitochondrial electron transport chain. Paracoccus denitrificans is a gram-negative, facultative anaerobic soil bacterium.

It is a model prokaryote for studies of respiration. When this bacterium grows aerobically, its electron transport chain possesses four complexes that correspond to the mitochondrial chain.

But, when this bacterium grows anaerobically with nitrate as its electron acceptor, the chain is structured quite differently. Since most bacteria grow anaerobically using different variety of electron acceptor substances, the bacterial electron transport chains are frequently very different.

Bacterial electron transport chains vary in their electron carriers (for example, in their cytochromes) and are usually extensively branched. Electrons often enter at several points and leave through several terminal oxidases. Bacterial electron transport chains are usually shorter and possess lower phosphorus to oxygen (P/O) ratios than mitochondrial transport chain.

Thus bacterial (prokaryotic) and mitochondrial (eukaryotic) electron transport chains differ in details of construction although they operate employing the same fundamental principles. For convenience, a simplified view of the electron transport chain of *Escherichia coli* is being given here as to show these differences (Refer Figure 10.1).

NOTES

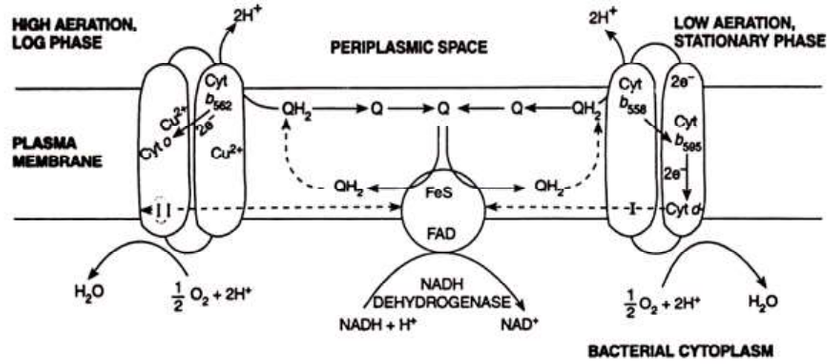


Fig. 10.1 *Electron Transport Chain of Escherichia Coli*

Electron transport chain of *E. Coil* that operates in aerobic conditions. NADH is the electron donor. Ubiquinone (Q) is the connecting link between NADH dehydrogenase with two terminal oxidase systems of the two branches, cytochrome *d* branch (shown as I) and cytochrome *o* branch (shown as II).

Although the electron transport chain of *E. coli* transports electrons from NADH (NADH is the electron donor) to acceptors and moves protons (H^+) across the plasma membrane similar to mitochondrial electron transport chain, it is quite different from the latter in its construction. *E. coli* transport chain is short, consists of two branches (cytochrome d branch and cytochrome o branch), and a quite different array of cytochromes (for example, Cyt b_{558}, Cytb_{562}, Cyt d, Cyt o).

Coenzyme Q (ubiquinone) carries electrons and donates them to both branches, but the branches operate under different growth conditions. The cytochrome d branch shows very high affinity for oxygen and operates at low oxygen levels (low aeration) usually when the bacterium is in stationary phase of growth.

This branch is not as efficient as the cytochrome o branch because it does not actively pump protons to periplasmic space.

The cytochrome o branch shows moderately high efficiency for oxygen and operates at high oxygen concentrations (high aeration). This branch operates normally when the bacterium is in log phase of its growth ,i.e., growing rapidly, and actively pumps protons (H^+) in the periplasmic space.

Check Your Progress

7. Where does electron transport chains of bacteria occur?
8. Where does bacterial electron transport chains vary?
9. Is bacterial (prokaryotic) and mitochondrial (eukaryotic) electron transport chains similar?
10. What does Coenzyme Q carry?

10.4 MECHANISM OF OXIDATIVE PHOSPHORYLATION

NOTES

Most of the usable energy obtained from the breakdown of carbohydrates or fats is derived by oxidative phosphorylation, which takes place within mitochondria. For example, the breakdown of glucose by glycolysis and the citric acid cycle yields a total of four molecules of ATP, ten molecules of NADH, and two molecules of $FADH_2$. Electrons from NADH and $FADH_2$ are then transferred to molecular oxygen, coupled to the formation of an additional 32 to 34 ATP molecules by oxidative phosphorylation. Electron transport and oxidative phosphorylation are critical activities of protein complexes in the inner mitochondrial membrane, which ultimately serve as the major source of cellular energy (Refer Figure 10.2).

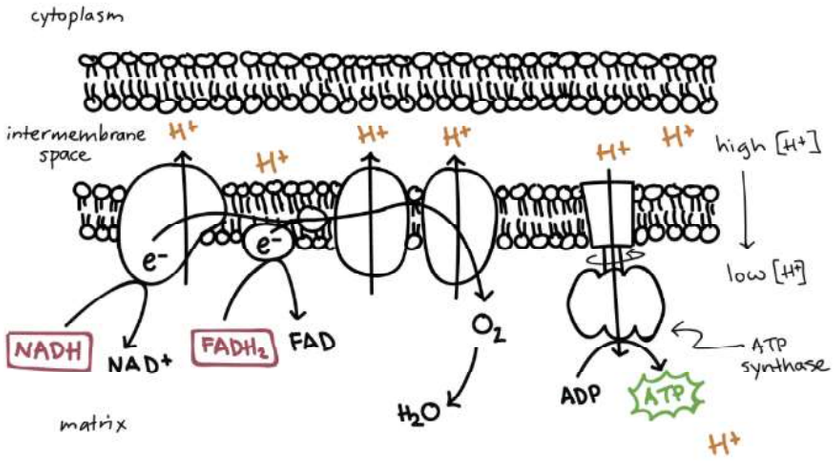


Fig. 10.2 *Oxidative Phosphorylation*

The Electron Transport Chain is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called chemiosmosis. Together, the electron transport chain and chemiosmosis make up oxidative phosphorylation. The key steps of this process, shown in simplified form in the diagram above, include:

Delivery of Electrons by NADH and $FADH_2$

Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD^{+}++ and FAD, which can be reused in other steps of cellular respiration.

NOTES

Electron Transfer and Proton Pumping

As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump $H^{-}++$ ions, moving them out of the matrix and into the intermembrane space. This pumping establishes an electrochemical gradient.

Splitting of Oxygen to form Water

At the end of the electron transport chain, electrons are transferred to molecular oxygen, which splits in half and takes up $H^{-}++$ to form water.

Gradient-driven Synthesis of ATP

As $H^{-}++$ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthase, which harnesses the flow of protons to synthesize ATP.

The Electron Transport Chain

The electron transport chain is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I to IV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane (Refer Figure 10.4).

As the electrons travel through the chain, they go from a higher to a lower energy level, moving from less electron-hungry to more electron-hungry molecules. Energy is released in these 'downhill' electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the intermembrane space, forming a proton gradient. (Refer Figure 10.3).

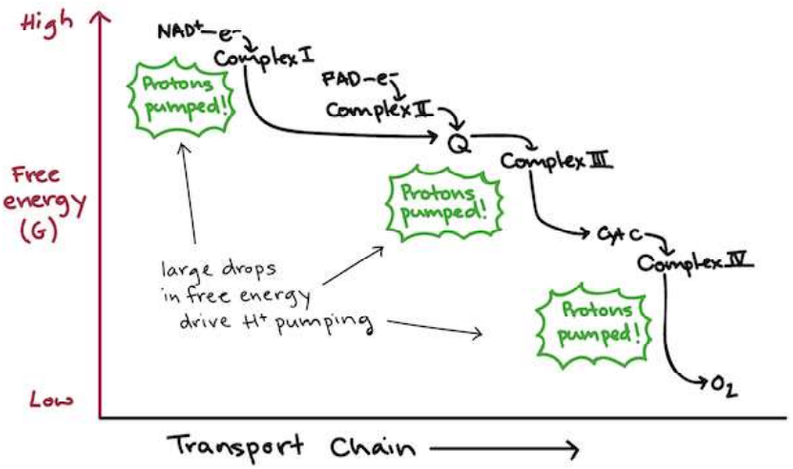


Fig. 10.3 *Release of Energy to form Protein Gradient*

NOTES

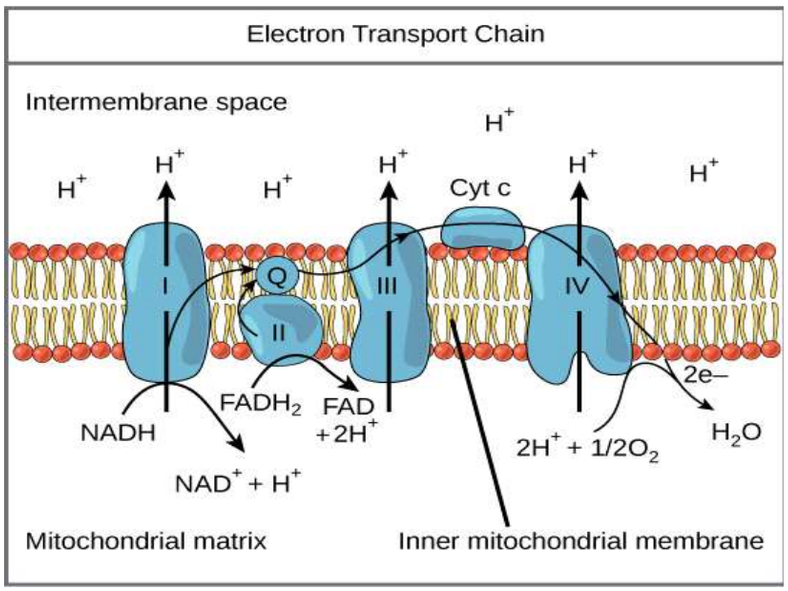


Fig. 10.4 *Electron Transport Chain*

Image of the electron transport chain. All the components of the chain are embedded in or attached to the inner mitochondrial membrane. In the matrix, NADH deposits electrons at Complex I, turning into NAD+ and releasing a proton into the matrix. $FADH_2$ in the matrix deposits electrons at Complex II, turning into FAD and releasing 2 H+. The electrons from Complexes I and II are passed to the small mobile carrier Q. Q transports the electrons to Complex III, which then passes them to Cytochrome C. Cytochrome C passes the electrons to Complex IV, which then passes them to oxygen in the matrix, forming water. It takes two electrons, 1/2 O2, and 2 H+ to form one water molecule. Complexes I, III, and IV use energy released as electrons move from a higher to a lower energy level to pump protons out of the matrix and into the intermembrane space, generating a proton gradient.

All of the electrons that enter the transport chain come from NADH and $FADH_2$ molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.

NADH is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD–++. As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix into the intermembrane space.

$FADH_2$ is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane.

Because of this 'bypass', each $FADH_2$ molecule causes fewer protons to be pumped (and contributes less to the proton gradient) than an NADH.

Beyond the first two complexes, electrons from NADH and $FADH_2$ travel exactly the same route. Both complex I and complex II pass their electrons to a small, mobile electron carrier called ubiquinone (Q), which is reduced to form QH_2 and travels through the membrane, delivering the electrons to complex III. As electrons move through complex III, more H^{-}++ ions are pumped across the membrane, and the electrons are ultimately delivered to another mobile carrier called **cytochrome C** (**cyt C**). Cyt C carries the electrons to complex IV, where a final batch of H^{-}++ ions is pumped across the membrane. Complex IV passes the electrons to O_2, which splits into two oxygen atoms and accepts protons from the matrix to form water. Four electrons are required to reduce each molecule of O_2, and two water molecules are formed in the process.

The electron transport chain has two important functions:

- **Regenerates Electron Carriers**

NADH and $FADH_2$ pass their electrons to the electron transport chain, turning back into NAD^{-}++ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.

- **Makes a Proton Gradient**

The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H^{-}++ in the intermembrane space and a lower concentration in the matrix. This gradient represents a stored form of energy, and, as we'll see, it can be used to make ATP.

Chemiosmosis

Complexes I, III, and IV of the electron transport chain are proton pumps. As electrons move energetically downhill, the complexes capture the released energy and use it to pump H^{-}++ ions from the matrix to the intermembrane space. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the proton-motive force (Refer Figure 10.5).

Like many other ions, protons can't pass directly through the phospholipid bilayer of the membrane because its core is too hydrophobic. Instead, H^{-}++ ions can move down their concentration gradient only with the help of channel proteins that form hydrophilic tunnels across the membrane.

In the inner mitochondrial membrane, H^{-}++ ions have just one channel available: a membrane-spanning protein known as **ATP synthase**. Conceptually, ATP synthase is a lot like a turbine in a hydroelectric power plant. Instead of being turned by water, it's turned by the flow of H^{-}++ ions moving down their

NOTES

electrochemical gradient. As ATP synthase turns, it catalyzes the addition of a phosphate to ADP, capturing energy from the proton gradient as ATP.

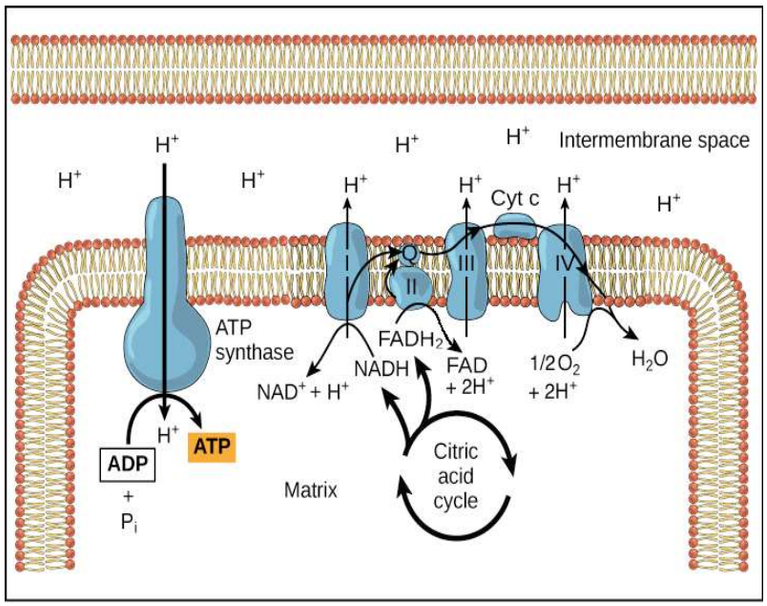


Fig. 10.5 *Chemiosmosis Process*

The electron transport chain and ATP synthase are embedded in the inner mitochondrial membrane. NADH and $FADH_2$ made in the citric acid cycle (in the mitochondrial matrix) deposit their electrons into the electron transport chain at complexes I and II, respectively. This step regenerates NAD+ and FAD (the oxidized carriers) for use in the citric acid cycle. The electrons flow through the electron transport chain, causing protons to be pumped from the matrix to the intermembrane space. Eventually, the electrons are passed to oxygen, which combines with protons to form water. The proton gradient generated by proton pumping during the electron transport chain is a stored form of energy. When protons flow back down their concentration gradient (from the intermembrane space to the matrix), their only route is through ATP synthase, an enzyme embedded in the inner mitochondrial membrane. When protons flow through ATP synthase, they cause it to turn (much as water turns a water wheel), and its motion catalyzes the conversion of ADP and Pi to ATP.

This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis**. More broadly, chemiosmosis can refer to any process in which energy stored in a proton gradient is used to do work. Although chemiosmosis accounts for over 80% of ATP made during glucose breakdown in cellular respiration, it's not unique to cellular respiration. For instance, chemiosmosis is also involved in the light reactions of photosynthesis.

What would happen to the energy stored in the proton gradient if it weren't used to synthesize ATP or do other cellular work? It would be released as heat,

and interestingly enough, some types of cells deliberately use the proton gradient for heat generation rather than ATP synthesis. This might seem wasteful, but it's an important strategy for animals that need to keep warm. For instance, hibernating mammals (such as bears) have specialized cells known as brown fat cells. In the brown fat cells, uncoupling proteins are produced and inserted into the inner mitochondrial membrane. These proteins are simply channels that allow protons to pass from the intermembrane space to the matrix without traveling through ATP synthase. By providing an alternate route for protons to flow back into the matrix, the uncoupling proteins allow the energy of the gradient to be dissipated as heat.

Check Your Progress

11. What is electron transport chain?
12. What does reduced electron carry?
13. What does electron transport chain compriese of?
14. What is NADH?
15. Why $FADH_2$ cannot transfer electrons?

10.5 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Substrate-level phosphorylation is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl (PO_3) group to ADP or GDP from another phosphorylated compound.
2. Substrate-level phosphorylation occurs in the cytoplasm of cells during glycolysis and in mitochondria either during the Krebs cycle.
3. The first substrate-level phosphorylation occurs after the conversion of 3-phosphoglyceraldehyde and Pi and NAD+ .
4. The second substrate-level phosphorylation occurs by dephosphorylating phosphoenolpyruvate, catalyzed by pyruvate kinase, producing pyruvate and ATP.
5. ATP can be generated by substrate-level phosphorylation in mitochondria in a pathway that is independent from the proton motive force.
6. Mitochondrial phosphoenolpyruvate carboxykinase is thought to participate in the transfer of the phosphorylation potential from the matrix to the cytosol and vice versa.
7. The electron transport chains of bacteria (prokaryotes) operate in plasma membrane (mitochondria are absent in prokaryotes). Some bacterial electron transport chains resemble the mitochondrial electron transport chain.

NOTES

8. Bacterial electron transport chains vary in their electron carriers and are usually extensively branched. Electrons often enter at several points and leave through several terminal oxidases. Bacterial electron transport chains are usually shorter and possess lower phosphorus to oxygen (P/O) ratios than mitochondrial transport chain.
9. No, Bacterial (prokaryotic) and mitochondrial (eukaryotic) electron transport chains differ in details of construction although they operate employing the same fundamental principles.
10. Coenzyme Q (ubiquinone) carries electrons and donates them to both branches, but the branches operate under different growth conditions.
11. The electron Transport Chain is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions.
12. Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD^{-}++ and FAD, which can be reused in other steps of cellular respiration.
13. The electron transport chain is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I to IV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.
14. NADH is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD^{-}++.
15. $FADH_2$ is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane.

10.6 SUMMARY

- Substrate level phosphorylation is when ADP is converted to ATP by the direct transfer of a phosphate group.
- The phosphate group is donated or transferred from a phosphorylated intermediate. This is in contrast to oxidative phosphorylation, where a chemiosmotic gradient is used to power the phosphorylation process.
- The phosphate comes off of one molecule (the intermediate) and is directly transferred to another (the ADP).

- The energy for the phosphorylation of ADP is provided directly from breaking the high energy phosphate bond on the intermediate molecule. This is referred to as reaction coupling.
- Oxidative phosphorylation has middle men in the form of NADH and the electron transport enzymes.
- The phosphate comes from a pool of inorganic phosphates instead of directly from another molecule, and the energy to phosphorylate the ADP comes from the proton gradient, not from coupled reactions.
- The most famous examples of substrate level phosphorylation are found in the glycolysis pathway.
- The ATP are produced in the pay off phase of glycolysis by the action of the enzymes phosphoglycerate kinase and pyruvate kinase.
- Substrate-level phosphorylation is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl (PO_3) group to ADP or GDP from another phosphorylated compound.
- Unlike oxidative phosphorylation, oxidation and phosphorylation are not coupled in the process of substrate-level phosphorylation, and reactive intermediates are most often gained in the course of oxidation processes in catabolism.
- Most ATP is generated by oxidative phosphorylation in aerobic or anaerobic respiration while substrate-level phosphorylation provides a quicker, less efficient source of ATP, independent of external electron acceptors.
- The first substrate-level phosphorylation occurs after the conversion of 3-phosphoglyceraldehyde and Pi and NAD+ to 1,3-bisphosphoglycerate via glyceraldehyde 3-phosphate dehydrogenase. 1,3-bisphosphoglycerate is then dephosphorylated via phosphoglycerate kinase, producing 3-phosphoglycerate and ATP through a substrate-level phosphorylation.
- During the preparatory phase, each 6-carbon glucose molecule is broken into two 3-carbon molecules.
- ATP can be generated by substrate-level phosphorylation in mitochondria in a pathway that is independent from the proton motive force.
- Mitochondrial phosphoenolpyruvate carboxykinase is thought to participate in the transfer of the phosphorylation potential from the matrix to the cytosol and vice versa.
- The electron transport chains of bacteria (prokaryotes) operate in plasma membrane (mitochondria are absent in prokaryotes). Some bacterial electron transport chains resemble the mitochondrial electron transport chain.
- Paracoccus denitrificans is a Gram-negative, facultative anaerobic soil bacterium.

NOTES

- Bacterial (prokaryotic) and mitochondrial (eukaryotic) electron transport chains differ in details of construction although they operate employing the same fundamental principles.
- Coenzyme Q (ubiquinone) carries electrons and donates them to both branches, but the branches operate under different growth conditions.
- Most of the usable energy obtained from the breakdown of carbohydrates or fats is derived by oxidative phosphorylation, which takes place within mitochondria.

10.7 KEY WORDS

- **Glycolysis:** Glycolysis is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate.
- **Phosphorylation:** Phosphorylation is an important mechanism by which the activity of proteins can be altered after they are formed.
- **Mitochondria:** Mitochondria are rod-shaped organelles that can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP).
- **Electron transport chain:** The electron transport chain is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I to IV.
- **Chemiosmosis:** Chemiosmosis is the process in which energy from a proton gradient is used to make ATP.

10.8 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Write a short note on glycolysis.
2. What is phosphoenolpyruvate carboxykinase?
3. Brief about succinate-CoA ligase.
4. Write a short note on oxidative phosphorylation.
5. What is electron transport chain?

Long-Answer Questions

1. Give a detailed overview in the mechanism of substrate-level phosphorylation.
2. Elaborate about respiratory electron transport in mitochondria and bacteria.

3. Discuss about oxidative phosphorylation.
4. Explain about electron transport chain.
5. Write a note on chemiosmosis.

NOTES

10.9 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 11 ANAEROBIC RESPIRATION

NOTES

Structure

11.0 Introduction
11.1 Objectives
11.2 Anaerobic Respiration
11.3 Common Types of Anaerobic Respiration
11.4 Nitrate Reduction and Denitrification
11.5 Sulflate and Sulphur Reduction
11.6 Carbonate (CO_2) Respiration
  11.6.1 Acetogenesis
  11.6.2 Methanogenesis
11.7 Answers to Check Your Progress Questions
11.8 Summary
11.9 Key Words
11.10 Self Assessment Questions and Exercises
11.11 Further Readings

11.0 INTRODUCTION

Respiration is the process through which the energy stored in fuel is converted into a form that a cell can use. Typically, energy stored in the molecular bonds of a sugar or fat molecule is used to make ATP, by taking electrons from the fuel molecule and using them to power an electron transport chain. Respiration is crucial to a cell's survival because if it cannot liberate energy from fuel to drive its life functions, the cell will die.

Anaerobic respiration is a form of respiration that is contrasted with aerobic respiration in that it does not use oxygen. It is an alternative method of respiration that is used when oxygen is not present and derives energy from other chemicals nearby or within the body. Aerobic respiration produces more energy and is 15 times more efficient than anaerobic respiration. This is why most large creatures breathe to take in oxygen. Instead of oxygen, anaerobic cells use substances such as sulfate, nitrate, sulfur, and fumarate to drive their cellular respiration.

Molecular oxygen is the most efficient electron acceptor for respiration, due to its nucleus' high affinity for electrons. However, some organisms have evolved to use other oxidizers, and as such, these perform respiration without oxygen. These organisms also use an electron transport chain to generate as much ATP as possible from their fuel, but their electron transport chains extract less energy than those of aerobic respiration because their electron acceptors are weaker. Many bacteria and archaea can only perform anaerobic respiration. Many other organisms can perform either aerobic or anaerobic respiration, depending on whether oxygen is present.

In this unit, you will study about anaerobic respiration, sulphate, nitrate, carbonate respirations, ecological significance of sulphate, nitrate, carbonate respirations, etc.

NOTES

11.1 OBJECTIVES

After going through this unit, you will be able to:

- Explain anaerobic respiration
- Learn about sulphate, nitrate, carbonate respirations
- Analyse the ecological significance of sulphate, nitrate, carbonate respirations

11.2 ANAEROBIC RESPIRATION

Anaerobic respiration is an alternate mode of energy generation in which an exogenous electron acceptor other than O_2 is used in electron transport chain leading to a proton motive force.

In contrast to aerobic respiration where O_2 is used as electron acceptor, the electron acceptors used in anaerobic respiration include nitrate, sulphate, carbonate, ferric ion and even certain organic compounds (for example, fumarate, chlorate, trimethylamine oxide, etc.)

The use of these alternate electron acceptors allows microorganisms to respire in environments where oxygen is absent. Because the solubility of oxygen in water is rather low, and because oxygen is in such high demand as an electron acceptor by aerobic organisms, anaerobic respirations are thus ecologically extremely important for anaerobic organisms, the prokaryotes. Anaerobic respiration, therefore, is a hallmark of prokaryotes and is rare in eukaryotes (Refer Figure 11.1).

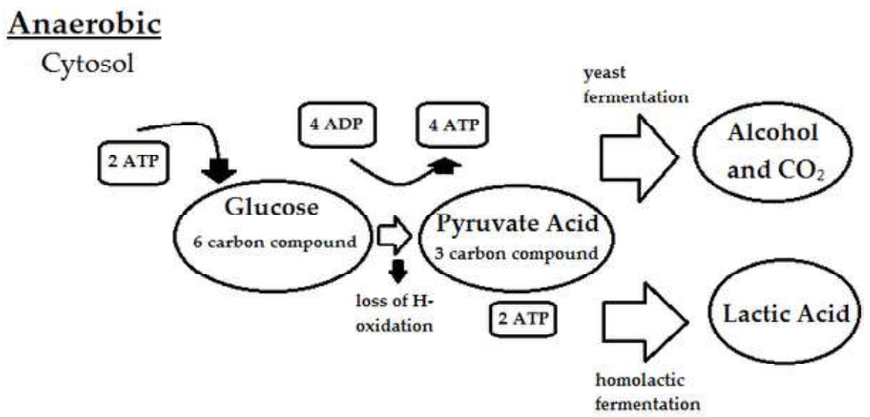


Fig. 11.1 *Anaerobic Respiration: A Molecule other than Oxygen is used as the Terminal Electron Acceptor in Anaerobic Respiration*

NOTES

Obligate Anaerobes and Facultative Aerobes

Most of the microorganisms are obligate anaerobes because they carry out only anaerobic respiration and are unable to use O_2. They are so sensitive to oxygen that they are inhibited or even killed by oxygen. So far as is known, obligate anaerobes belong to three groups of microorganisms: a wide variety of prokaryotes, a few fungi, and a few protozoa.

One of the best-known group of obligately anaerobic bacteria belongs to the genus Clostridium, which are widespread in soil, lake sediments, and intestinal tracts and are often responsible for spoilage of canned foods.

Other obligately anaerobic bacteria occur among the methanogens (for example, Methanobacterium formicicum) and many other species of archaebacteria, sulphate reducing and homoacetogenic bacteria, and many of the bacteria present in the animal gut.

Among obligate anaerobes, there are some bacteria that have ability to tolerate oxygen and grow in its presence even though they cannot use it. Such obligate anaerobes are called aerotolerant anaerobes (for example, Streptococcus pyogenes).

Facultative aerobes are those bacteria that carry out aerobic respiration if oxygen is present in the environment, but switch over to an alternate electron acceptor to carry out anaerobic respiration when oxygen is depleted from the environment. An example of facultative aerobe is *Escherichia coli.*

Assimilative and Dissimilative Metabolism

All microorganisms need sources of N, S, and C for growth. When an inorganic compound such as nitrate (NO^-_3), sulphate (SO^{2-}_4) or CO_2 is reduced for use as a nutrient source, it is said to be 'assimilated' and the reduction process is called assimilative metabolism.

But when such inorganic compounds are used as electron acceptors for energy metabolism in anaerobic respiration, they are said to be 'dissimilated', and the reduction process is called dissimilative metabolism.

Assimilative and dissimilative metabolisms differ markedly from one another. In assimilative metabolism, only comparatively lesser amount of the compounds (NO^-_3 SO^{2-}_4or CO_2) is reduced to fulfill the requirements for cell growth and reduced atoms are eventually converted to macromolecules that constitute the cell material.

In dissimilative metabolism, a comparatively large amount of electron acceptors (NO^-_3 SO^{2-}_4 or CO^{2-}_3, etc.) are reduced, and the reduced product is excreted by the organism into the environment as waste product.

Assimilative metabolism is carried out by many organisms (many bacteria, archaebacteria, fungi, algae, and higher plants), whereas only a restricted variety

of organisms, primarily the prokaryotic microorganisms, carry out dissimilative metabolism.

Check Your Progress

1. What is anaerobic respiration?
2. Which are the electrons acceptors used in anaerobic respiration?
3. Name the best-known group of obligately anaerobic bacteria. Where are they found?
4. Define assimilative metabolism.
5. What happens in dissimilative metabolism?
6. What are the organisms that carry assimilative metabolism

NOTES

11.3 COMMON TYPES OF ANAEROBIC RESPIRATION

Since a variety of compounds are used as exogenous electron acceptors by anaerobic microorganisms during dissimilative metabolism for energy conservation because of their varying ecological behaviors (obligate and facultative), they have adopted varying routes for anaerobic respiration. In the light of this, the anaerobic respiration can be categorized into different types. A summary of the most common types of anaerobic respiration is given in Table below 11.1.

Table 11.1 *Most Common Types of Anaerobic Respiration*

	Type	Overall process	Microorganisms involved
1.	Nitrate respiration (denitrification)	$NO_3^- \longrightarrow NO_2^-, N_2O, N_2$	Facultative aerobes (e.g., *Escherichia coli, Par denitrificans, Pseudomonas stutzeri*)
2.	Sulphate respiration (Sulphate reduction)	$SO_4^{-2} \longrightarrow H_2S$	Obligate anaerobes (e.g., *Desulfovibrio desulfi Desulfobacter*)
3.	Carbonate respiration	$CO_2 \longrightarrow CH_3COOH$ (Acetic acid)	Obligate anaerobes (homoacetogenic bacteria *Acetobacterium woodii* and *Clostridium aceticum*
		$CO_2 \longrightarrow CH_4$(Methane)	Obligate anaerobes (methanogenic archae bacteria *Methanobacterium, Methanobrevibacter, Methan* etc.)
4.	Sulphur respiration	$S^0 \longrightarrow H_2S$	Obligate anaerobes (e.g., *Desulfurococcus, Pyro* and facultative aerobes.
5.	Iron respiration	$Fe^{3+} \longrightarrow Fe^{2+}$ (Ferric) (Ferrous)	Obligate anaerobes and facultative aerobes (*She Geobacter, Geospirillum*, and *Geovibrio*)
6.	Fumarate respiration	Fumarate ⟶ Succinate	Facultative aerobes (*Wolinella succin Desulfovibrio gigas*, some clostridia, and *Esc coli*)

NOTES

Anaerobic respiration is the formation of ATP without oxygen. This method still incorporates the respiratory electron transport chain, but without using oxygen as the terminal electron acceptor. Instead, molecules such as sulfate (SO_4^{2-}), nitrate (NO_3^-), or sulfur (S) are used as electron acceptors. These molecules have a lower reduction potential than oxygen; thus, less energy is formed per molecule of glucose in anaerobic versus aerobic conditions.

Many different types of electron acceptors may be used for anaerobic respiration. Denitrification is the utilization of nitrate (NO_3^-) as the terminal electron acceptor. Nitrate, like oxygen, has a high reduction potential. This process is widespread, and used by many members of Proteobacteria. Many denitrifying bacteria can also use ferric iron (Fe^{3+}) and different organic electron acceptors.

Sulfate reduction uses sulfate (SO_2^{-4}) as the electron acceptor, producing hydrogen sulfide (H_2S) as a metabolic end product. Sulfate reduction is a relatively energetically poor process, and is used by many Gram-negative bacteria found within the δ-Proteobacteria. It is also used in Gram-positive organisms related to Desulfotomaculum or the archaeon Archaeoglobus.

Sulfate reduction requires the use of electron donors, such as the carbon compounds lactate and pyruvate (organotrophic reducers), or hydrogen gas (lithotrophic reducers). Some unusual autotrophic sulfate-reducing bacteria, such as *Desulfotignum phosphitoxidans,* can use phosphite (HPO_3^-) as an electron donor. Others, such as certain *Desulfovibrio* species, are capable of sulfur disproportionation (splitting one compound into an electron donor and an electron acceptor) using elemental sulfur (S0), sulfite (SO_3^{-2}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulfate (SO^{2-}).

Acetogenesis is a type of microbial metabolism that uses hydrogen (H_2) as an electron donor and carbon dioxide (CO_2) as an electron acceptor to produce acetate, the same electron donors and acceptors used in methanogenesis.

Ferric iron (Fe^{3+}) is a widespread anaerobic terminal electron acceptor used by both autotrophic and heterotrophic organisms. Electron flow in these organisms is similar to those in electron transport, ending in oxygen or nitrate, except that in ferric iron-reducing organisms the final enzyme in this system is a ferric iron reductase. Since some ferric iron-reducing bacteria (for example, *G. metallireducens*) can use toxic hydrocarbons (for example, toluene) as a carbon source, there is significant interest in using these organisms as bioremediation agents in ferric iron contaminated aquifers.

Other inorganic electron acceptors include the reduction of Manganic ion (Mn^{4+}) to manganous (Mn^{2+}), Selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) to selenium (Se), Arsenate (AsO_4^{3-}) to arsenite (AsO_3^{3-}), and Uranyl (UO_2^{2+}) to uranium dioxide (UO_2)

Organic compounds may also be used as electron acceptors in anaerobic respiration. These include the reduction of fumarate to succinate, Trimethylamine N-oxide (TMAO) to trimethylamine (TMA), and Dimethyl sulfoxide (DMSO) to Dimethyl sulfide (DMS).

NOTES

11.4 NITRATE REDUCTION AND DENITRIFICATION

Denitrification is a type of anaerobic respiration that uses nitrate as an electron acceptor. In anaerobic respiration, denitrification utilizes nitrate (NO_3^-) as a terminal electron acceptor in the respiratory electron transport chain. Denitrification is a widely used process; many facultative anaerobes use denitrification because nitrate, like oxygen, has a high reduction potential

Denitrification is a microbially facilitated process involving the step wise reduction of nitrate to nitrite (NO_2^-) nitric oxide (NO), nitrous oxide (N_2O), and, eventually, to dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. The complete denitrification process can be expressed as a redox reaction: $2\ NO^{3-} + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$.

Protons are transported across the membrane by the initial NADH reductase, quinones and nitrous oxide reductase to produce the electrochemical gradient critical for respiration. Some organisms (for example*, E. coli*) only produce nitrate reductase and therefore can accomplish only the first reduction leading to the accumulation of nitrite. Others (for example*, Paracoccus denitrificans* or *Pseudomonas stutzeri*) reduce nitrate completely. Complete denitrification is an environmentally significant process because some intermediates of denitrification (nitric oxide and nitrous oxide) are significant greenhouse gases that react with sunlight and ozone to produce nitric acid, a component of acid rain. Denitrification is also important in biological waste water treatment, where it can be used to reduce the amount of nitrogen released into the environment, thereby reducing eutrophication.

Denitrification takes place under special conditions in both terrestrial and marine ecosystems. In general, it occurs where oxygen is depleted and bacteria respire nitrate as a substitute terminal electron acceptor. Due to the high concentration of oxygen in our atmosphere, denitrification only takes place in anaerobic environments where oxygen consumption exceeds the oxygen supply and where sufficient quantities of nitrate are present. These environments may include certain soils and groundwater, wetlands, oil reservoirs, poorly ventilated corners of the ocean, and in sea floor sediments.

NOTES

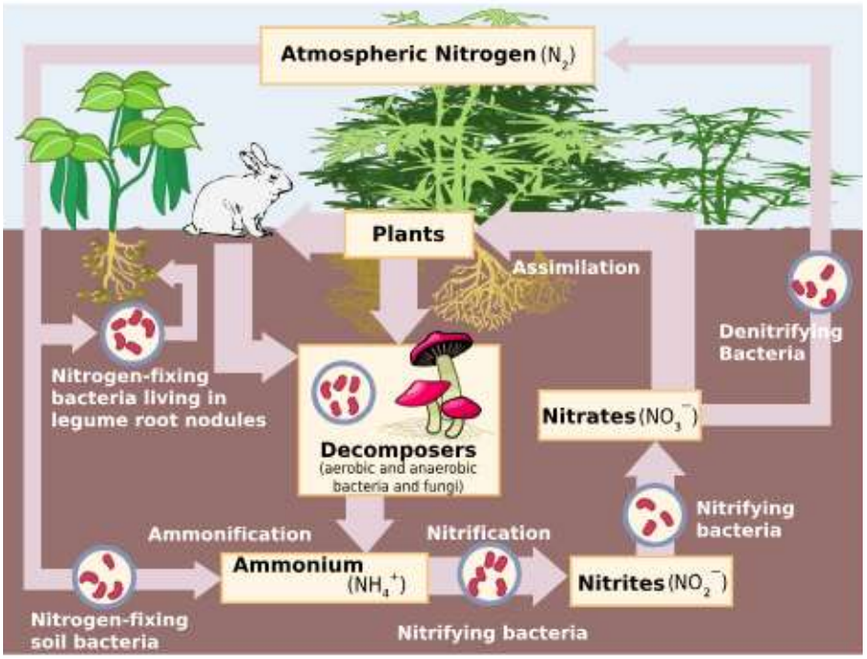


Fig. 11.2 *The Role of Soil Bacteria in Nitrogen Cycle*

The role of soil bacteria in the Nitrogen cycle: Denitrification is an important process in maintaining ecosystems. Generally, denitrification takes place in environments depleted of oxygen. (Refer Figure 11.2).

Denitrification is performed primarily by heterotrophic bacteria (for example, *Paracoccus denitrificans*), although autotrophic denitrifiers have also been identified (for example, *Thiobacillus denitrificans*). Generally, several species of bacteria are involved in the complete reduction of nitrate to molecular nitrogen, and more than one enzymatic pathway have been identified in the reduction process.

Rhizobia are soil bacteria with the unique ability to establish a N_2-fixing symbiosis on legume roots. When faced with a shortage of oxygen, some *Rhizobia* species are able to switch from O_2-respiration to using nitrates to support respiration.

The direct reduction of nitrate to ammonium (dissimilatory nitrate reduction) can be performed by organisms with the nrf- gene. This is a less common method of nitrate reduction than denitrification in most ecosystems. Other genes involved in denitrification include nir (nitrite reductase) and nos (nitrous oxide reductase), which are possessed by such organisms as *Alcaligenes faecalis*, *Alcaligenes xylosoxidans*, *Pseudomonas spp*, *Bradyrhizobium japonicum*, and *Blastobacter denitrificans*.

Check Your Progress

7. Define denitrification.
8. How is nitrate used as in denitrification anaerobic respiration?
9. What is redox reaction and how does it occur in anaerobic respiration?
10. How are protons transported across the membrane?
11. Which organisms perform denitrification?
12. What are Rhizobia ?

NOTES

11.5 SULFLATE AND SULPHUR REDUCTION

Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain. Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain. Compared to aerobic respiration, sulfate reduction is a relatively energetically poor process, though it is a vital mechanism for bacteria and archaea living in oxygen-depleted, sulfate-rich environments.

Many sulfate reducers are organotrophic, using carbon compounds, such as lactate and pyruvate (among many others) as electron donors, while others are lithotrophic, and use hydrogen gas (H_2) as an electron donor. Some unusual autotrophic sulfate-reducing bacteria (for example, *Desulfotignum phosphitoxidans*) can use phosphite (HPO^{3-}) as an electron donor, whereas others (for example, *Desulfovibrio sulfodismutans, Desulfocapsa thiozymogenes,* and *Desulfocapsa sulfoexigens*) are capable of sulfur disproportionation (splitting one compound into two different compounds, in this case an electron donor and an electron acceptor) using elemental sulfur (S0), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulfate (SO_4^{2-}).

Before sulfate can be used as an electron acceptor, it must be activated. This is done by the enzyme ATP-sulfurylase, which uses ATP and sulfate to create adenosine 52 -phosphosulfate (APS). APS is subsequently reduced to sulfite and AMP. Sulfite is then further reduced to sulfide, while AMP is turned into ADP using another molecule of ATP. The overall process, thus, involves an investment of two molecules of the energy carrier ATP, which must to be regained from the reduction.

All sulfate-reducing organisms are strict anaerobes. Because sulfate is energetically stable, it must be activated by adenylation to form APS (adenosine 52-phosphosulfate) to form APS before it can be metabolized, thereby consuming

NOTES

ATP. The APS is then reduced by the enzyme APS reductase to form sulfite (SO_3^{2-}) and AMP. In organisms that use carbon compounds as electron donors, the ATP consumed is accounted for by fermentation of the carbon substrate. The hydrogen produced during fermentation is actually what drives respiration during sulfate reduction.

Sulfate-reducing bacteria can be traced back to 3.5 billion years ago and are considered to be among the oldest forms of microorganisms, having contributed to the sulfur cycle soon after life emerged on Earth. Sulfate-reducing bacteria are common in anaerobic environments (such as seawater, sediment, and water rich in decaying organic material) where they aid in the degradation of organic materials. In these anaerobic environments, fermenting bacteria extract energy from large organic molecules; the resulting smaller compounds (such as organic acids and alcohols) are further oxidized by acetogens, methanogens, and the competing sulfate-reducing bacteria.

Many bacteria reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction. By contrast, sulfate-reducing bacteria reduce sulfate in large amounts to obtain energy and expel the resulting sulfide as waste; this is known as 'dissimilatory sulfate reduction'. Most sulfate-reducing bacteria can also reduce other oxidized inorganic sulfur compounds, such as sulfite, thiosulfate, or elemental sulfur (which is reduced to sulfide as hydrogen sulfide).

Toxic hydrogen sulfide is one waste product of sulfate-reducing bacteria; its rotten egg odor is often a marker for the presence of sulfate-reducing bacteria in nature. Sulfate-reducing bacteria are responsible for the sulfurous odors of salt marshes and mud flats. Much of the hydrogen sulfide will react with metal ions in the water to produce metal sulfides. These metal sulfides, such as ferrous sulfide (FeS), are insoluble and often black or brown, leading to the dark color of sludge. Thus, the black color of sludge on a pond is due to metal sulfides that result from the action of sulfate-reducing bacteria.

Black sludge: The black color of this pond is due to metal sulfides that result from the action of sulfate-reducing bacteria. (Refer Figure 11.3).

Some sulfate-reducing bacteria play a role in the anaerobic oxidation of methane ($CH_4 + SO^{2-}_4 \rightarrow HCO_3- + HS- + H_2O$). An important fraction of the methane formed by methanogens below the seabed is oxidized by sulfate-reducing bacteria in the transition zone separating the methanogenesis from the sulfate reduction activity in the sediments. This process is also considered a major sink for sulfate in marine sediments. In hydrofracturing fluids used to frack shale formations to recover methane (shale gas), biocide compounds are often added to water to inhibit the microbial activity of sulfate-reducing bacteria in order to avoid anaerobic methane oxidation and to minimize potential production loss.



Fig. 11.3 *Black Sludge in a Pond*

Sulfate-reducing bacteria often create problems when metal structures are exposed to sulfate-containing water. The interaction of water and metal creates a layer of molecular hydrogen on the metal surface. Sulfate-reducing bacteria oxidize this hydrogen, creating hydrogen sulfide, which contributes to corrosion. Hydrogen sulfide from sulfate-reducing bacteria also plays a role in the biogenic sulfide corrosion of concrete, and sours crude oil.

Sulfate-reducing bacteria may be utilized for cleaning up contaminated soils; some species are able to reduce hydrocarbons, such as benzene, toluene, ethylbenzene, and xylene. Sulfate-reducing bacteria may also be a way to deal with acid mine waters.

Check Your Progress

13. How is sulphate used as in sulphate reduction?
14. Define assimilatory sulfate reduction.
15. Which bacteria causes the odors of salt marshes and mud flats?
16. What causes black sludge on ponds?
17. How are sulfate-reducing bacteria used?

11.6 CARBONATE (CO_2) RESPIRATION

Two major groups of obligate anaerobic prokaryotes (homoacetogens and methanogens) use CO_2 as an electron acceptor and hydrogen (H_2) as a major

NOTES

electron donor for energy conservation during anaerobic respiration. Homoacetogens and methanogens carry out the processes of acetogenesis and methanogenesis, respectively, and an overview of these processes is shown in Figure 11.4.

Acetogenesis generates ion gradients of H^+ (proton motive force) or Na^+ (sodium motive force) while methanogenesis generates ion gradients of H^+ (proton motive force) across the plasma membrane during electron transport. These forces drive ATP synthesis via ATPase. Acetogenesis also involves ATP synthesis via substrate-level phosphorylation.

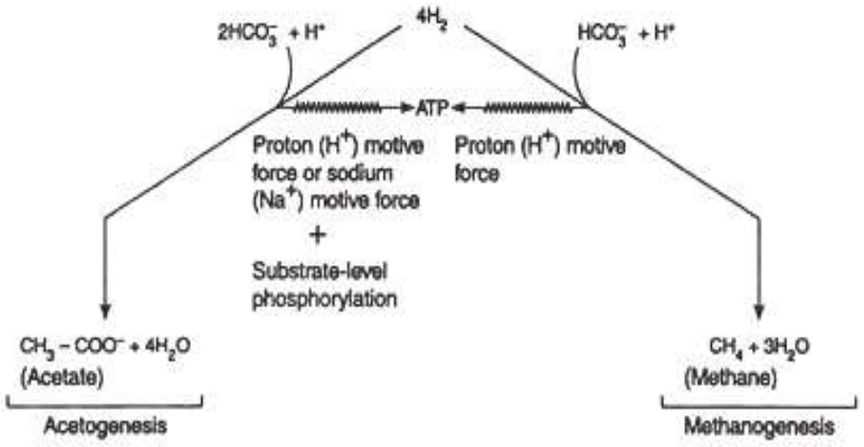


Fig 11.4 *Overview of Acetogenesis and Methanogenesis*

11.6.1 Acetogenesis

Acetogenesis is carried out by homoacetogens (Acetoanaerobium noterae, Acetobactericum woodii, Clostridium aceticum, Clostridium thermoaceticum, Clostridium formicoacetium, and many others).

These bacteria reduce CO_2 to acetate by Acetyl-CoA pathway involving two linear pathways—one molecule of CO_2 is reduced to methyl group of acetate, and the other molecule of CO_2 is reduced to the carbonyl group of acetate—followed by their assembly at the end to form Acetyl-CoA, which then is converted to acetate (Refer Figure 11.5).

In Acetyl-CoA pathway of CO_2 reduction to acetate, the ATP is synthesized when a sodium (Na^+) gradient is established generating sodium motive force across the plasma membrane. The energized state of plasma membrane allows for ATP synthesis via the action of a Na^+-powered ATPase enzyme. Additional ATP is also synthesized via substrate-level phosphorylation during the conversion of Acetyl-CoA to acetate via acetyl-phosphate.

NOTES

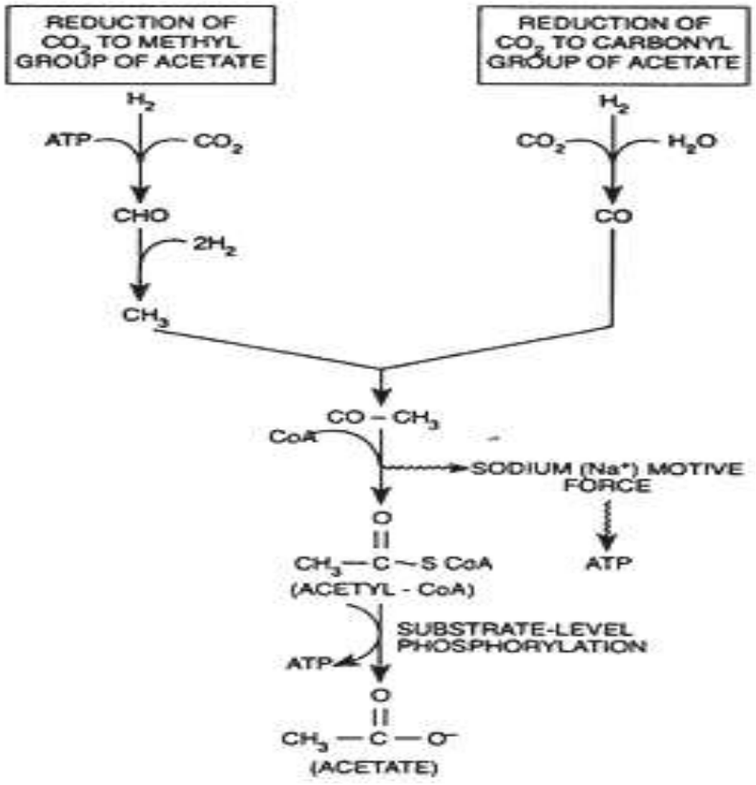


Fig 11.5 *Production of CO_2 to Acetate via Acetyl CoA Pathway*

11.6.2 Methanogenesis

Methanogenesis, or biomethanation, is a form of anaerobic respiration that uses carbon as the terminal electron acceptor, resulting in the production of methane. The carbon is sourced from a small number of low molecular weight organic compounds, such as carbon dioxide, acetic acid, formic acid (formate), methanol, methylamines, dimethyl sulfide, and methanethiol. The two best described pathways of methanogenesis use carbon dioxide or acetic acid as the terminal electron acceptor:

Methanogenesis of Acetate: Acetate is broken down to methane by methanogenesis, a type of anaerobic respiration. (Refer Figure 11.6).

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

$CH_3COOH \rightarrow CH_4 + CO_2$

The biochemistry of methanogenesis is relatively complex. It involves the coenzymes and cofactors F420, coenzyme B, coenzyme M, methanofuran, and methanopterin.

NOTES

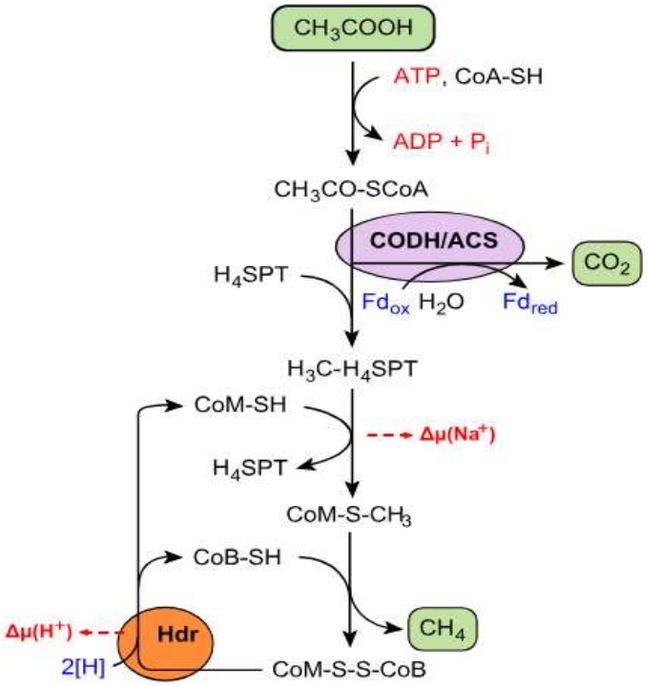


Fig. 11.6 *Methanogenesis of Acetate*

Microbes capable of producing methane are called methanogens. They have been identified only from the domain Archaea – a group that is phylogenetically distinct from eukaryotes and bacteria – though many live in close association with anaerobic bacteria. The production of methane is an important and widespread form of microbial metabolism, and in most environments, it is the final step in the decomposition of biomass.

During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, and nitrate) become depleted, while hydrogen (H_2), carbon dioxide, and light organics produced by fermentation accumulate. During advanced stages of organic decay, all electron acceptors become depleted except carbon dioxide, which is a product of most catabolic processes. It is not depleted like other potential electron acceptors.

Only methanogenesis and fermentation can occur in the absence of electron acceptors other than carbon. Fermentation only allows the breakdown of larger organic compounds, and produces small organic compounds. Methanogenesis effectively removes the semi-final products of decay: hydrogen, small organics, and carbon dioxide. Without methanogenesis, a great deal of carbon (in the form of fermentation products) would accumulate in anaerobic environments.

Methanogenesis also occurs in the guts of humans and other animals, especially ruminants. In the rumen, anaerobic organisms, including methanogens, digest cellulose into forms usable by the animal. Without these microorganisms, animals such as cattle would not be able to consume grass. The useful products of

methanogenesis are absorbed by the gut. Methane is released from the animal mainly by belching (eructation). The average cow emits around 250 liters of methane per day. Some, but not all, humans emit methane in their flatus!

Some experiments even suggest that leaf tissues of living plants emit methane, although other research indicates that the plants themselves do not actually generate methane; they are just absorbing methane from the soil and then emitting it through their leaf tissues. There may still be some unknown mechanism by which plants produce methane, but that is by no means certain.

Methane is one of the earth's most important greenhouse gases, with a global warming potential 25 times greater than carbon dioxide (averaged over 100 years). Therefore, the methane produced by methanogenesis in livestock is a considerable contributor to global warming.

Methanogenesis can also be beneficially exploited. It is the primary pathway that breaks down organic matter in landfills (which can release large volumes of methane into the atmosphere if left uncontrolled), and can be used to treat organic waste and to produce useful compounds. Biogenic methane can be collected and used as a sustainable alternative to fossil fuels.

Check Your Progress

18. What does anaerobic prokaryotes use as an electron acceptor ?
19. How is acetogenesis carried out?
20. Define methanogenesis.
21. What happens during decay process?
22. Where does methanogenesis occur?
23. Which gas is known as one of the most important greenhouse gas?

11.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Anaerobic respiration is a form of respiration that is contrasted with aerobic respiration in that it does not use oxygen.
2. In contrast to aerobic respiration where O_2 is used as electron acceptor, the electron acceptors used in anaerobic respiration include nitrate (NO^-_3)), sulphate (SO^{2-}_4), carbonate (CO_2), ferric ion (Fe^+_3), and even certain organic compounds like fumarate, chlorate, trimethylamine oxide, etc.
3. One of the best-known group of obligately anaerobic bacteria belongs to the genus Clostridium, which are widespread in soil, lake sediments, and intestinal tracts and are often responsible for spoilage of canned foods.

NOTES

4. When an inorganic compound such as nitrate (NO^{-}_{3}), sulphate (SO^{2-}_{4}) or $CO_2$is reduced for use as a nutrient source, it is said to be 'assimilated' and the reduction process is called assimilative metabolism.
5. In dissimilative metabolism, a comparatively large amount of electron acceptors (NO^{-}_{3} SO^{2-}_{4} or CO^{2-}_{3}, etc.) are reduced, and the reduced product is excreted by the organism into the environment as waste product.
6. Assimilative metabolism is carried out by many organisms (many bacteria, archaebacteria, fungi, algae, and higher plants), whereas only a restricted variety of organisms, primarily the prokaryotic microorganisms, carry out dissimilative metabolism.
7. Denitrification is a type of anaerobic respiration that uses nitrate as an electron acceptor.
8. In anaerobic respiration, denitrification utilizes nitrate (NO_3^-) as a terminal electron acceptor in the respiratory electron transport chain. Denitrification is a widely used process; many facultative anaerobes use denitrification because nitrate, like oxygen, has a high reduction potential.
9. Denitrification is a microbially facilitated process involving the step wise reduction of nitrate to nitrite (NO_2^-) nitric oxide (NO), nitrous oxide (N_2O), and, eventually, to dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. The complete denitrification process can be expressed as a redox reaction: $2\ NO^{3-} + 10\ e^{-} + 12\ H^{+} \rightarrow N_2 + 6\ H_2O$.
10. Protons are transported across the membrane by the initial NADH reductase, quinones and nitrous oxide reductase to produce the electrochemical gradient critical for respiration.
11. Denitrification is performed primarily by heterotrophic bacteria (e.g. *Paracoccus denitrificans*), although autotrophic denitrifiers have also been identified.
12. *Rhizobia* are soil bacteria with the unique ability to establish a N_2-fixing symbiosis on legume roots. When faced with a shortage of oxygen, some *Rhizobia* species are able to switch from O_2-respiration to using nitrates to support respiration.
13. Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain.
14. Many bacteria reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction.
15. Sulfate-reducing bacteria are responsible for the sulfurous odors of salt marshes and mud flats.
16. The black color of sludge on a pond is due to metal sulfides that result from the action of sulfate-reducing bacteria.

NOTES

17. Sulfate-reducing bacteria may be utilized for cleaning up contaminated soils; some species are able to reduce hydrocarbons, such as benzene, toluene, ethylbenzene, and xylene. Sulfate-reducing bacteria may also be a way to deal with acid mine waters.
18. Two major groups of obligate anaerobic prokaryotes (homoacetogens and methanogens) use CO_2 as an electron acceptor and hydrogen (H_2) as a major electron donor for energy conservation during anaerobic respiration.
19. Acetogenesis is carried out by homoacetogens like Acetoanaerobium noterae, Acetobactericum woodii, Clostridium aceticum, Clostridium thermoaceticum, Clostridium formicoacetium, and many others.
20. Methanogenesis, or biomethanation, is a form of anaerobic respiration that uses carbon as the terminal electron acceptor, resulting in the production of methane.
21. During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, and nitrate) become depleted, while hydrogen (H2), carbon dioxide, and light organics produced by fermentation accumulate.
22. Methanogenesis also occurs in the guts of humans and other animals, especially ruminants. In the rumen, anaerobic organisms, including methanogens, digest cellulose into forms usable by the animal. Without these microorganisms, animals such as cattle would not be able to consume grass.
23. Methane is one of the earth's most important greenhouse gases, with a global warming potential 25 times greater than carbon dioxide, averaged over 100 years.

11.8 SUMMARY

- Respiration is the process through which the energy stored in fuel is converted into a form that a cell can use.
- Typically, energy stored in the molecular bonds of a sugar or fat molecule is used to make ATP, by taking electrons from the fuel molecule and using them to power an electron transport chain.
- Respiration is crucial to a cell's survival because if it cannot liberate energy from fuel to drive its life functions, the cell will die.
- Anaerobic respiration is a form of respiration that is contrasted with aerobic respiration in that it does not use oxygen.
- Aerobic respiration produces more energy and is 15 times more efficient than anaerobic respiration. This is why most large creatures breathe to take in oxygen. Instead of oxygen, anaerobic cells use substances such as sulfate, nitrate, sulfur, and fumarate to drive their cellular respiration.
- Molecular oxygen is the most efficient electron acceptor for respiration, due to its nucleus' high affinity for electrons.

NOTES

- Some organisms have evolved to use other oxidizers, and as such, these perform respiration without oxygen.
- These organisms also use an electron transport chain to generate as much ATP as possible from their fuel, but their electron transport chains extract less energy than those of aerobic respiration because their electron acceptors are weaker.
- Many bacteria and archaea can only perform anaerobic respiration. Many other organisms can perform either aerobic or anaerobic respiration, depending on whether oxygen is present.
- Anaerobic respiration is an alternate mode of energy generation in which an exogenous electron acceptor other than O_2 is used in electron transport chain leading to a proton motive force.
- In contrast to aerobic respiration where O_2 is used as electron acceptor, the electron acceptors used in anaerobic respiration include nitrate (NO_3^-)), sulphate (SO^{2-}_4), carbonate (CO_2), ferric ion (Fe^+_3), and even certain organic compounds like fumarate, chlorate, trimethylamine oxide, etc.
- Most of the microorganisms are obligate anaerobes because they carry out only anaerobic respiration and are unable to use O_2. They are so sensitive to oxygen that they are inhibited or even killed by oxygen.
- One of the best-known group of obligately anaerobic bacteria belongs to the genus Clostridium, which are widespread in soil, lake sediments, and intestinal tracts and are often responsible for spoilage of canned foods.
- Other obligately anaerobic bacteria occur among the methanogens and many other species of archaebacteria, sulphate reducing and homoacetogenic bacteria, and many of the bacteria present in the animal gut.
- Among obligate anaerobes, there are some bacteria that have ability to tolerate oxygen and grow in its presence even though they cannot use it. Such obligate anaerobes are called aerotolerant anaerobes.
- Facultative aerobes are those bacteria that carry out aerobic respiration if oxygen is present in the environment, but switch over to an alternate electron acceptor to carry out anaerobic respiration when oxygen is depleted from the environment. An example of facultative aerobe is *Escherichia coli.*
- All microorganisms need sources of N, S, and C for growth. When an inorganic compound such as nitrate (NO^-_3), sulphate (SO^{2-}_4) or $CO_2$is reduced for use as a nutrient source, it is said to be 'assimilated' and the reduction process is called assimilative metabolism.
- But when such inorganic compounds are used as electron acceptors for energy metabolism in anaerobic respiration, they are said to be 'dissimilated", and the reduction process is called dissimilative metabolism.

NOTES

- Organic compounds may also be used as electron acceptors in anaerobic respiration. These include the reduction of fumarate to succinate, Trimethylamine N-oxide (TMAO) to trimethylamine (TMA), and Dimethyl sulfoxide (DMSO) to Dimethyl sulfide (DMS).
- In anaerobic respiration, denitrification utilizes nitrate (NO_3^-) as a terminal electron acceptor in the respiratory electron transport chain. Denitrification is a widely used process; many facultative anaerobes use denitrification because nitrate, like oxygen, has a high reduction potential
- Denitrification is a microbially facilitated process involving the step wise reduction of nitrate to nitrite (NO_2^-) nitric oxide (NO), nitrous oxide (N_2O), and, eventually, to dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. The complete denitrification process can be expressed as a redox reaction: $2\ NO^{3-} + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$.
- Denitrification takes place under special conditions in both terrestrial and marine ecosystems.
- *Rhizobia* are soil bacteria with the unique ability to establish a N_2-fixing symbiosis on legume roots. When faced with a shortage of oxygen, some *Rhizobia* species are able to switch from O_2-respiration to using nitrates to support respiration.
- Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain.
- Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain.
- Compared to aerobic respiration, sulfate reduction is a relatively energetically poor process, though it is a vital mechanism for bacteria and archaea living in oxygen-depleted, sulfate-rich environments.
- Many bacteria reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction.

11.9 KEY WORDS

- **Anaerobic respiration:** Anaerobic respiration is a form of respiration that is contrasted with aerobic respiration in that it does not use oxygen.
- **Denitrification:** Denitrification is a type of anaerobic respiration that uses nitrate as an electron acceptor.
- **Sulfate reduction:** Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain.
- **Methanogenesis :** Methanogenesis is a form of anaerobic respiration that uses carbon as an electron acceptor and results in the production of methane.

- **Methanogens:** Microbes capable of producing methane are called methanogens.

NOTES

11.10 SELF ASSESSMENT QUESTION AND ANSWERS

Short-Answer Questions

1. Write a short note on anaerobic respiration.
2. Brief about assimilative and dissimilative metabolism.
3. Name few common types of anaerobic respiration.
4. What is sulphate reduction?
5. Write short note on nitrate reduction and denitrification.
6. How does soil bacteria help in the nitrogen cycle?
7. What is black sludge?

Long-Answer Questions

1. Elaborate about anaerobic respiration.
2. Discuss about sulfate and sulfur reduction.
3. Explain about carbonate (CO_2) respiration.
4. Elaborate about sulphate reduction.
5. Write about methanogenesis.

11.11 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

BLOCK - IV
BACTERIAL TRANSPORT AND QUORUM SENSING

NOTES

UNIT 12 BIOENERGITICS

Structure

12.0 Introduction
12.1 Objectives
12.2 Bioenergitics
12.3 Entropy
12.4 Enthalpy
12.5 Electron Carriers
12.6 Artificial Electron Donors
12.7 Inhibitors
12.7.1 Uses of Inhibitors
12.8 Uncouplers
12.9 Phosphorylation
12.9.1 Control of Phosphorylation
12.10 Bond Energy (E)
12.11 Answers to Check Your Progress Questions
12.12 Summary
12.13 Key Words
12.14 Self Assessment Questions and Exercises
12.15 Further Readings

12.0 INTRODUCTION

Bioenergetics is the branch of biochemistry that focuses on how cells transform energy, often by producing, storing or consuming adenosine triphosphate (ATP). Bioenergetic processes, such as cellular respiration or photosynthesis, are essential to most aspects of cellular metabolism, therefore to life itself. This energy is used to drive chemical reactions and help store and process information, which is essential in propagating life. Energy may be obtained from sunlight, in which case the organisms are referred to as phototrophs, or it may be extracted from chemicals, in which case the organisms are referred to as chemotrophs. Because energy may not be available at all times to fuel these life processes, organisms have adapted mechanisms to couple chemical reactions so that exergonic reactions can provide energy for those that are endorgernic.

Environmental materials that an organism intakes are generally combined with oxygen to release energy, although some can also be oxidized anaerobically by various organisms. The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively

NOTES

weak compared with the chemical bonds holding carbon dioxide and water together. The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen. The oxidation releases energy because stronger bonds have been formed. This net energy may evolve as heat, which may be used by the organism for other purposes, such as breaking other bonds to do chemistry required for survival.

In this unit, you will study about bioenergetics, entropy, enthalpy, electron carriers, artificial electron donors, inhibitors, uncouplers, energy bond and phosphorylation, etc.

12.1 OBJECTIVES

After going through this unit you will be able to:

- Explain what bioenergetics is
- Discuss the various terms like entropy, enthalpy, electron carriers, etc.
- Understand about artificial electron donors
- Define what are inhibitors and uncouplers
- Discuss about energy bond and phosphorylation

12.2 BIOENERGITICS

Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems. This is an active area of biological research that includes the study of the transformation of energy in living organisms and the study of thousands of different cellular processes such as cellular respiration and the many other metabolic and enzymatic processes that lead to production and utilization of energy in forms such as adenosine triphosphate (ATP) molecules. That is, the goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics.

Bioenergetics is the part of biochemistry concerned with the energy involved in making and breaking of chemical bonds in the molecules found in biological organisms. It can also be defined as the study of energy relationships and energy transformations and transductions in living organisms. The ability to harness energy from a variety of metabolic pathways is a property of all living organisms. Growth, development, anabolism and catabolism are some of the central processes in the study of biological organisms, because the role of energy is fundamental to such biological processes. Life is dependent on energy transformations; living organisms survive because of exchange of energy between living tissues/ cells and the outside environment. Some organisms, such as autotrophs, can acquire energy from sunlight without needing to consume nutrients and break them down. Other organisms,

NOTES

like heterotrophs, must intake nutrients from food to be able to sustain energy by breaking down chemical bonds in nutrients during metabolic processes such as glycolysis and the citric acid cycle. Importantly, as a direct consequence of the First Law of Thermodynamics, autotrophs and heterotrophs participate in a universal metabolic network—by eating autotrophs, heterotrophs harness energy that was initially transformed by the plants during photosynthesis.

In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work (such as mechanical work) or for other processes (such as chemical synthesis and anabolic processes in growth), when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy.

Adenosine triphosphate (ATP) is the main 'energy currency' for organisms; the goal of metabolic and catabolic processes are to synthesize ATP from available starting materials , and to break- down ATP into adenosine diphosphate (ADP) and inorganic phosphate by utilizing it in biological processes. In a cell, the ratio of ATP to ADP concentrations is known as the 'energy charge' of the cell. A cell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation.

Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is hydrolyzed (broken down by water) to adenosine diphosphate and inorganic phosphate. Here it is the thermodynamically favorable free energy of hydrolysis that results in energy release; the phosphoanhydride bond between the terminal phosphate group and the rest of the ATP molecule does not itself contain this energy. An organism's stockpile of ATP is used as a battery to store energy in cells. Utilization of chemical energy from such molecular bond rearrangement powers biological processes in every biological organism.

Living organisms obtain energy from organic and inorganic materials, i.e. ATP can be synthesized from a variety of biochemical precursors. For example, lithotrophs can oxidize minerals such as nitrates or forms of sulfur, such as elemental sulfur, sulfites, and hydrogen sulfide to produce ATP. In photosynthesis, autotrophs produce ATP using light energy, whereas heterotrophs must consume organic compounds, mostly including carbohydrates, fats, and proteins. The amount of energy actually obtained by the organism is lower than the amount present in the food; there are losses in digestion, metabolism, and thermogenesis.

Environmental materials that an organism intakes are generally combined with oxygen to release energy, although some can also be oxidized anaerobically by various organisms. The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively weak compared with the chemical bonds holding carbon dioxide and water

NOTES

together. The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen (the materials are oxidized slowly enough that the organisms do not actually produce fire). The oxidation releases energy because stronger bonds (bonds within water and carbon dioxide) have been formed. This net energy may evolve as heat, which may be used by the organism for other purposes, such as breaking other bonds to do chemistry required for survival.

(i) Free energy (ΔG) is the useful energy also known as the chemical potential.

(ii) The first law of thermodynamics states that 'the total energy of a system plus its surroundings remains constant'. This is also the laws of conservation of energy. Energy may be transferred from one part to another or may be transformed into an-other form of energy.

(iii) The second law of thermodynamics states that 'the total entropy of a system must increase if a process is to occur spontaneously'.

Check Your Progress

1. What is bioenergetics?
2. Name some of the central processes in the study of biological organisms.
3. What is the main energy currency for the organisms?
4. What is the major aim of metabolic and catabolic processes?
5. How do living organisms produce ATP?
6. What is stored as an energy of an organism?
7. How do living organisms obtain energy?
8. State first and second law of thermodynamics.

12.3 ENTROPY

Entropy represents the extent of disorder of the system and becomes maximum when it approaches true equilibrium. Under constant temperature and pressure, the relationship between the free energy change (ΔG) and the change in entropy (ΔS) is given by the following equation which combines the two laws of thermodynamics.

$$\Delta G = \Delta H - T\Delta S$$

where ΔH = the change in enthalpy (heat) and T = the absolute temperature. Under biochemical reactions AH is approximately equal to AE.

12.4 ENTHALPY

NOTES

In a process carried out at constant volume (for example, in a sealed tube), the heat content of a system is equal to internal energy (E), as no PV (pressure volume) work is done. But, in a constant pressure process, the system also expends energy in doing PV work.

Therefore, the total heat content of a system at constant pressure is equivalent to the internal energy (E) plus the PV (pressure volume product) energy. This is called as enthalpy and is represented by the symbol H. Thus, enthalpy may be defined by the equation,

$H = E + PV$

In simpler words, enthalpy is the total heat content of a system. It reflects the number and kinds of chemical bonds in the reactants and products. Like internal energy, enthalpy is also a function of state and therefore, it is not possible to quantify the absolute enthalpy. However, a change in enthalpy (ΔH) accompanying a process can be measured accurately. Thus,

$\Delta H = HP – Hr$

(where p = products; r = reactants)

The unit of ΔH is joules/mole (or calories/mole)

The reactions which are accompanied by release of heat energy are called as exothermic reactions. In such cases, there is negative change in enthalpy ($-\Delta H$) from reactants to products. For example, in combustion of glucose to CO_2 + H_2O, large amount of heat is released. Therefore, this is an exothermic reaction with $-\Delta H$. melting of ice into liquid water and its subsequent vaporization into water vapours absorb considerable heat from the surroundings, therefore this is an endothermic reaction with $+ \Delta H$.

12.5 ELECTRON CARRIERS

Electron carriers are important molecules in biological systems. They accept electrons and move them as part of the electron transport chain, transferring the electron, and the energy it represents, to power the cell. Electron carriers are vital parts of cellular respiration and photosynthesis. In each reaction, when a molecule donates an electron it is said to be oxidized and the electron acceptor is said to be reduced. Oxidation-reduction reactions always happen in matched pairs; no molecule can be oxidized unless another is reduced.

Following are the types of electron carriers

- **Flavin Adenine Dinucleotide or FAD**, consists of riboflavin attached to an adenosine diphosphate molecule. It is capable of accepting and

NOTES

donating one or two electrons. It is often bound firmly to an enzyme site, so not very much free FAD exists in a cell. Some FAD is formed during the citric acid cycle of cellular respiration. One function of FAD is to donate electrons to oxidative phosphorylation, where the electrons from the reduced form of FAD are transferred to create adenosine triphosphate (ATP), and the energy currency of the cell. This process happens in the mitochondria of the cell.

- **Nicotinamide Adenine Dinucleotide**, abbreviated NAD, nicotinamide adenine dinucleotide consists of two ribose sugar rings attached to each other with phosphate groups, with an adenine unit on one side and a nicotinamide ring on the other. The nicotinamide ring is the active part of the molecule, accepting and donating two electrons. NAD acts as an electron acceptor during glycolysis and the citric acid cycle of cellular respiration and donates them to oxidative phosphorylation. The closely related nicotinamide adenine dinucleotide phosphate (NADP) is produced in the light reactions of photosynthesis and consumed in the Calvin cycle.
- **Coenzyme Q** also known as ubiquinone, has ten repeating isoprene units. This makes it soluble in fats rather than water, meaning it is valuable in moving electrons across the phospholipid mitochondrial membranes. Like FAD, coenzyme Q can accept one or two electrons. Coenzyme Q is integral to the first three steps of the electron transport chain, moving electrons across the inner mitochondrial membrane as part of oxidative phosphorylation.
- **Cytochrome C** is important at the end of the electron transport chain in the mitochondria, or in bacterial cells, which do not have mitochondria. Cytochromes are proteins forming a matrix around an iron atom, known as the heme group. The heme group accepts electrons one at a time until it has four stored in the protein matrix. Cytochrome C is a free molecule, which allows it to move electrons between the larger and less mobile electron carrier molecules. Cytochrome C transports electrons between the third and final steps of the electron transport chain.

Check Your Progress

9. What is entropy?
10. What is enthalpy?
11. What are exothermic reactions?
12. Define the term electron carriers.
13. Expand FAD and also state its function.
14. What is the importance of Cytochrome C?

12.6 ARTIFICIAL ELECTRON DONORS

NOTES

The use of artificial electron donors has been one of the biochemical approaches to the elucidation of complex electron transport chains in biological membranes. In photosynthesis it started with Hill's discovery that ferric salts could induce light-dependent oxygen evolution in cell-free leaf extracts. Since then the results of this approach has taught about the sequence, about the energy-conserving steps, and more recently about the topography of the photosynthetic electron transport chain in the chloroplast membrane. It has also made possible the design of assays for parts of the chain in a physiologically nonfunctional state, i.e. for the reaction center complexes, either during biochemical isolation or during biogenesis of the photosynthetic apparatus.

12.7 INHIBITORS

An inhibitor is any agent that interferes with the activity of an enzyme. Inhibitors may affect the binding of enzyme to substrate, or catalysis (via modification of the enzyme's active site), or both. Researchers use enzyme inhibitors to define metabolic pathways and to understand enzyme reaction mechanisms. Many drugs are designed as inhibitors of target enzymes. Inhibition is also a natural phenomenon. Cells regulate metabolic pathways by specific inhibition of key enzymes.

Enzyme inhibitors are divided into two classes, irreversible and reversible. Irreversible inhibition implies destruction or permanent modification of chemical groups in the enzyme. In contrast, reversible inhibitors form a complex with the enzyme that can dissociate and release the active enzyme. An enzyme E can bind either to substrate S, to form an ES complex (which can go on to products) or to inhibitor I, to form the complex EI.

There are two major types of reversible inhibitors, competitive and non-competitive. Competitive inhibition can be reversed by increasing substrate concentration, whereas noncompetitive inhibition cannot be reversed by adding more substrate.

The classic example of competitive inhibition is inhibition of succinate dehydrogenase, an enzyme, by the compound malonate. Hans Krebs first elucidated the details of the citric acid cycle by adding malonate to minced pigeon muscle tissue and observing which intermediates accumulated after incubation of the mixture with various substrates. The structure of malonate is very similar to that of succinate. The enzyme will bind malonate but cannot act further on it. That is, the enzyme and inhibitor form a nonproductive complex. We call this competitive inhibition, as succinate and malonate appear to compete for the same site on the enzyme. With competitive inhibition, the percent of inhibition is a function of the ratio between inhibitor and substrate, not the absolute concentration of inhibitor.

NOTES

Noncompetitive inhibition is not reversed by adding more substrate. The inhibitor binds at a site on the enzyme other than the substrate binding site. The inhibitor can bind to either free enzyme, forming EI, or to the ES complex, forming EIS. Both EI and EIS are nonproductive.

Irreversible inhibitors are those that permanently disable the enzyme. The complex EI or EIS cannot dissociate, so that these are dead-end complexes. When an irreversible inhibitor is added to the enzyme-containing solution, inhibition may not be complete immediately, but increases gradually with time, as more and more enzyme molecules are modified.

12.7.1 Uses of Inhibitors

Among the irreversible inhibitors are organophosphorus compounds, which inhibit the enzyme acetylcholinesterase and similar enzymes. Organophosphorous compounds include nerve gases (such as sarin), that work on the human nervous system, and insecticides like malathion.

Many therapeutic drugs are enzyme inhibitors. Important examples are penicillin, which inhibits an enzyme necessary for bacterial cell wall synthesis, and aspirin, an inhibitor of the synthesis of molecules that mediate pain and swelling. More recent examples are drugs used in the treatment of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) that prevent maturation of the virus by inhibiting the HIV protease, and drugs that lower cholesterol by inhibiting a key step in cholesterol biosynthesis.

Check Your Progress

15. What are inhibitors?
16. Give a major difference between irreversible and reversible inhibitor.
17. Give an example of competitive inhibitor.
18. What happens when an irreversible inhibitor is added to the enzyme containing solution?

12.8 UNCOUPLERS

Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis without affecting the respiratory chain and ATP synthase (H(+)-ATPase). Miscellaneous compounds are known to be uncouplers, but weakly acidic uncouplers are representative because they show very potent activities. The most potent uncouplers discovered so far are the hindered phenol SF 6847, and hydrophobic salicylanilide S-13, which are active in-vitro at concentrations in the 10 nm range. For induction of uncoupling, an acid dissociable group, bulky

hydrophobic moiety and strong electron-withdrawing group are required. Weakly acidic uncouplers are considered to produce uncoupling by their protonophoric action in the H (+)-impermeable mitochondrial membrane. For exerting these effects, the stability of the respective uncoupler anions in the hydrophobic membrane is very important. High stability is achieved by delocalization of the polar ionic charge through uncoupler (chemical)-specific mechanisms. Such an action of weakly acidic uncouplers is characteristic of the highly efficient membrane targeting action of a nonsite-specific type of bioactive compound.

12.9 PHOSPHORYLATION

Certain organic phosphates, formed in the tissues out of anabolic chemical reaction, play a key role in the energy transactions of living organisms. The importance of organic phosphates in metabolism was first indicated by the studies of Harden and Young, who found that fermentation of glucose by cell-free yeast juice in-creased rapidly when inorganic phosphorus is added to the medium, which was later converted to organically bound phosphate.

The work of Embden, Meyerhof, Cori and their associates show that organic phosphates concerned with the metabolism of glucose by yeast are also intermediates in the metabolism of glycogen and glucose in muscle and other tissues.

The presence of ATP, ADP, CP and other high-energy phosphate compounds in the tissue and their participation in different metabolic processes indicate the importance of organic phosphates in metabolism. The sugar phosphates, although not high-energy compounds, are obligatory intermediates. Their formation involves phosphorylation by the high-energy compound ATP in presence of suitable enzymes.

The term phosphorylation includes all chemical reactions in the body which require combination with phosphoric acid. The reverse changes are called dephosphorylation, where phosphoric acid is dissociated from the compound.

These two processes are probably the reversible reactions of the same enzyme system.

1. Physiological Importance of Phosphorylation

Phosphoric acid enters into the composition of cell protoplasm. Hence, phosphorylation is an essential chemical process for all cells. In addition to this it takes an essential part during absorption and metabolism of different foodstuffs.

2. Oxidative Phosphorylation

This reaction can take place only in presence of intact oxidation process and the energy-yielding (oxidation) mechanism is coupled with energy-harnessing (phosphorylation) mechanism. Uncoupling agents like 2, 4-dinitrophenol inhibit phosphorylation but not oxidation. They elevate the oxygen consumption rate. The second stage in phosphorylation process is transfer of phosphate group from

ATP to other intermediates. Oxidative phosphorylation takes place in the cristae of the mitochondria.

12.9.1 Control of Phosphorylation

NOTES

i. Enzymes

The enzymes that take part in this process are phosphorylase, phosphatase, etc.

ii. Hormones

- Adrenal cortex is believed to be directly responsible for phosphorylation. Glucocorticoids inhibit phosphorylation adrenalectomy accelerate it.
- Anterior pituitary, by its adrenocorticotrophic hormone, may exert a superior control on phosphorylation, through adrenal cortex. The marasmic condition that develops in diseases of adrenal cortex may be partly explained by the consequent disturbance of phosphorylation, which affects absorption, metabolism and nutrition of the body. Growth hormone affects phosphorylation in a similar way as glucocorticoids.

iii. Inorganic Salts

Sodium may have some effects on phosphorylation.

It is believed that adrenal cortex controls phosphorylation by helping the enzymes responsible for the process. But it is also known that many of the defects, seen in diseases of the adrenal cortex, are rectified by giving enough NaCl. For instance, the defective fat absorption in adrenalectomised animals improves by giving NaCl.

It is interesting to note that it is the same adrenal cortex which also controls Na metabolism. From these observations it may be suggested that adrenal cortex controls phosphorylation not by a direct action on the enzymes but by an indirect influence on Na metabolism.

It is also known that phospholipids are antagonistic to cholesterol. These two compounds are always found to remain together. It is also interesting to note that adrenal cortex which controls phosphorylation and therefore phospholipid formation controls metabolism of sterols.

12.10 BOND ENERGY (E)

Bond energy (E) is defined as the amount of energy required to break apart a mole of molecules into its component atoms. It is a measure of the strength of a chemical bond. Bond energy is also known as bond enthalpy (H) or simply as bond strength.

Bond energy is based on an average of bond dissociation values for species in the gas phase, typically at a temperature of 298 Kelvin. It may be found by measuring or calculating the enthalpy change of breaking a molecule into its component atoms and ions and dividing the value by the number of chemical bonds. For example, the enthalpy change of breaking methane (CH_4) into a carbon atom and four hydrogen ions, divided by four (the number of C-H) bonds, yields the bond energy.

Bond energy is not the same thing as bond-dissociation energy. Bond energy values are an average of the bond-dissociation energies within a molecule. Breaking subsequent bonds requires a different amount of energy.

Check Your Progress

19. What does uncouplers of oxidative phosphorylation in mitochondria?
20. What indicates the importance of organic phosphates in metabolism?
21. Define phosphorylation.
22. How many types of phosphorylation are there? Name them.
23. Define bond energy.

12.11 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems.
2. Growth, development, anabolism and catabolism are some of the central processes in the study of biological organisms, because the role of energy is fundamental to such biological processes.
3. Adenosine triphosphate (ATP) is the main "energy currency" for organisms.
4. The goal of metabolic and catabolic processes are to synthesize ATP from available starting materials (from the environment), and to break- down ATP (into adenosine diphosphate (ADP) and inorganic phosphate) by utilizing it in biological processes.
5. Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is hydrolyzed (broken down by water) to adenosine diphosphate and inorganic phosphate.
6. An organism's stockpile of ATP is used as a battery to store energy in cells. Utilization of chemical energy from such molecular bond rearrangement powers biological processes in every biological organism.

NOTES

7. Living organisms obtain energy from organic and inorganic materials; i.e. ATP can be synthesized from a variety of biochemical precursors. For example, lithotrophs can oxidize minerals such as nitrates or forms of sulfur, such as elemental sulfur, sulfites, and hydrogen sulfide to produce ATP.
8. The first law of thermodynamics states that 'the total energy of a system plus its surroundings remains constant'. This is also the laws of conservation of energy. Energy may be transferred from one part to another or may be transformed into an-other form of energy.

   The second law of thermodynamics states that "the total entropy of a system must increase if a process is to occur spontaneously".
9. Entropy represents the extent of disorder of the system and becomes maximum when it approaches true equilibrium.
10. The total heat content of a system at constant pressure is equivalent to the internal energy (E) plus the PV (pressure volume product) energy. This is called as enthalpy and is represented by the symbol H. Thus, enthalpy may be defined by the equation, $H = E + PV$.
11. The reactions which are accompanied by release of heat energy are called as exothermic reactions.
12. Electron carriers are important molecules in biological systems. They accept electrons and move them as part of the electron transport chain, transferring the electron, and the energy it represents, to power the cell.
13. Flavin Adenine Dinucleotide or FAD, consists of riboflavin attached to an adenosine diphosphate molecule. It is capable of accepting and donating one or two electrons.
14. Cytochrome C is important at the end of the electron transport chain in the mitochondria, or in bacterial cells, which do not have mitochondria.
15. Enzyme inhibitors are divided into two classes, irreversible and reversible.
16. Irreversible inhibition implies destruction or permanent modification of chemical groups in the enzyme. In contrast, reversible inhibitors form a complex with the enzyme that can dissociate and release the active enzyme.
17. The classic example of competitive inhibition is inhibition of succinate dehydrogenase, an enzyme, by the compound malonate.
18. When an irreversible inhibitor is added to the enzyme-containing solution, inhibition may not be complete immediately, but increases gradually with time, as more and more enzyme molecules are modified.
19. Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis without affecting the respiratory chain and ATP synthase (H(+)-ATPase).

20. The presence of ATP, ADP, CP and other high-energy phosphate compounds in the tissue and their participation in different metabolic processes indicate the importance of organic phosphates in metabolism
21. The term phosphorylation includes all chemical reactions in the body which require combination with phosphoric acid.
22. These two processes are probably the reversible reactions of the same enzyme system.
    - Physiological Importance of Phosphorylation:
    - Oxidative Phosphorylation
23. Bond energy (E) is defined as the amount of energy required to break apart a mole of molecules into its component atoms. It is a measure of the strength of a chemical bond. Bond energy is also known as bond enthalpy (H) or simply as bond strength.

12.12 SUMMARY

- Bioenergetics is the branch of biochemistry that focuses on how cells transform energy, often by producing, storing or consuming adenosine triphosphate (ATP).
- Bioenergetic processes, such as cellular respiration or photosynthesis, are essential to most aspects of cellular metabolism, therefore to life itself. This energy is used to drive chemical reactions and help store and process information, which is essential in propagating life.
- Energy may be obtained from sunlight, in which case the organisms are referred to as phototrophs, or it may be extracted from chemicals, in which case the organisms are referred to as chemotrophs.
- Environmental materials that an organism intakes are generally combined with oxygen to release energy, although some can also be oxidized anaerobically by various organisms.
- The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively weak compared with the chemical bonds holding carbon dioxide and water together.
- The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen.
- Growth, development, anabolism and catabolism are some of the central processes in the study of biological organisms, because the role of energy is fundamental to such biological processes.
- Life is dependent on energy transformations; living organisms survive because of exchange of energy between living tissues/ cells and the outside

NOTES

environment. Some organisms, such as autotrophs, can acquire energy from sunlight (through photosynthesis) without needing to consume nutrients and break them down.

- Other organisms, like heterotrophs, must intake nutrients from food to be able to sustain energy by breaking down chemical bonds in nutrients during metabolic processes such as glycolysis and the citric acid cycle.
- Importantly, as a direct consequence of the First Law of Thermodynamics, autotrophs and heterotrophs participate in a universal metabolic network—by eating autotrophs (plants), heterotrophs harness energy that was initially transformed by the plants during photosynthesis.
- In a cell, the ratio of ATP to ADP concentrations is known as the 'energy charge' of the cell. A cell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation.
- The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively weak compared with the chemical bonds holding carbon dioxide and water together.
- The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen (the materials are oxidized slowly enough that the organisms do not actually produce fire).
- The oxidation releases energy because stronger bonds (bonds within water and carbon dioxide) have been formed. This net energy may evolve as heat, which may be used by the organism for other purposes, such as breaking other bonds to do chemistry required for survival.
- The first law of thermodynamics states that 'the total energy of a system plus its surroundings remains constant'. This is also the laws of conservation of energy. Energy may be transferred from one part to another or may be transformed into an-other form of energy.
- Entropy represents the extent of disorder of the system and becomes maximum when it approaches true equilibrium.
- The total heat content of a system at constant pressure is equivalent to the internal energy (E) plus the PV (pressure volume product) energy. This is called as enthalpy and is represented by the symbol H. Thus, enthalpy may be defined by the equation, $H = E + PV$.
- Electron carriers are important molecules in biological systems. They accept electrons and move them as part of the electron transport chain, transferring the electron, and the energy it represents, to power the cell.
- Cytochrome C is important at the end of the electron transport chain in the mitochondria, or in bacterial cells, which do not have mitochondria.

- There are two major types of reversible inhibitors, competitive and non-competitive. Competitive inhibition can be reversed by increasing substrate concentration, whereas noncompetitive inhibition cannot be reversed by adding more substrate.
- When an irreversible inhibitor is added to the enzyme-containing solution, inhibition may not be complete immediately, but increases gradually with time, as more and more enzyme molecules are modified.
- Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis without affecting the respiratory chain and ATP synthase (H(+)-ATPase).
- The presence of ATP, ADP, CP and other high-energy phosphate compounds in the tissue and their participation in different metabolic processes indicate the importance of organic phosphates in metabolism.
- Bond energy (E) is defined as the amount of energy required to break apart a mole of molecules into its component atoms. It is a measure of the strength of a chemical bond. Bond energy is also known as bond enthalpy (H) or simply as bond strength.

12.13 KEY WORDS

- **Bioenergetics:** Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems.
- **Entropy:** Entropy represents the extent of disorder of the system and becomes maximum when it approaches true equilibrium.
- **Enthalpy**: The total heat content of a system at constant pressure is equivalent to the internal energy (E) plus the PV (pressure volume product) energy. This is called as enthalpy and is represented by the symbol H. Thus, enthalpy may be defined by the equation, $H = E + PV$.
- **Exothermic reaction:** The reactions which are accompanied by release of heat energy are called as exothermic reactions.
- **Inhibitors:** An inhibitor is any agent that interferes with the activity of an enzyme. Inhibitors may affect the binding of enzyme to substrate, or catalysis via modification of the enzyme's active site, or both.
- **Phosphorylation:** The term phosphorylation includes all chemical reactions in the body which require combination with phosphoric acid.
- **Bond energy (E):** Bond energy (E) is defined as the amount of energy required to break apart a mole of molecules into its component atoms. It is a measure of the strength of a chemical bond. Bond energy is also known as bond enthalpy (H) or simply as bond strength.

12.14 SELF ASSESSMENT QUESTION AND ANSWERS

NOTES

Short-Answer Questions

1. Write a short on bioenergetics.
2. What is entropy?
3. Define enthalpy.
4. Write in short about electron carriers.
5. What is the function of inhibitors?
6. Write a short note on bond energy.

Long-Answer Questions

1. Discuss about bioenergetics and its importance.
2. Distinguish between entropy, enthalpy and bond energy mentioning their significance.
3. Discuss in detail about electron carriers and its types.
4. Explain about inhibitors and its characteristics.
5. Describe in detail about phosphorylation.
6. Write in detail about bond energy.

12.15 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 13 TRANSPORT ACROSS MEMBRANE

NOTES

Structure

13.0 Introduction
13.1 Objectives
13.2 Transport Across Membrane
13.3 Answers to Check Your Progress Questions
13.4 Summary
13.5 Key Words
13.6 Self Assessment Questions and Exercises
13.7 Further Readings

13.0 INTRODUCTION

In cellular biology, membrane transport refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them. The regulation of passage through the membrane is due to selective membrane permeability - a characteristic of biological membranes which allows them to separate substances of distinct chemical nature. In other words, they can be permeable to certain substances but not to others.

The movements of most solutes through the membrane are mediated by membrane transport proteins which are specialized to varying degrees in the transport of specific molecules. As the diversity and physiology of the distinct cells is highly related to their capacities to attract different external elements, it is postulated that there is a group of specific transport proteins for each cell type and for every specific physiological stage. This differential expression is regulated through the differential transcription of the genes coding for these proteins and its translation, for instance, through genetic-molecular mechanisms, but also at the cell biology level: the production of these proteins can be activated by cellular signaling pathways, at the biochemical level, or even by being situated in cytoplasmic vesicles.

In this unit, you will study about how transport across membrane occurs, diffusion, osmosis, active transport and group translocation.

13.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand how transport across membranes occurs
- Define the term diffusion and osmosis

- Explain how diffusion and osmosis takes place
- Discuss what is active transport
- Explain what is group translocation

NOTES

13.2 TRANSPORT ACROSS MEMBRANE

Uptake of the required nutrients by the microbial cell is important. Since microorganisms live in nutrient poor habitats, they must be able to transport nutrients from dilute solutions into the cell against concentration gradient. Finally, they must pass through a selectively permeable plasma membrane. Microorganisms use different transport mechanisms like facilitated diffusion, active transport and group translocation. Eukaryotic microorganisms do not employ group translocation but take up nutrients by endocytosis.

Movement of materials across the plasma membrane is mostly done by two processes

Passive Process: Substances cross the area from an area of high concentration to an area of low concentration without any expenditure of energy (ATP). For example, simple diffusion, osmosis and facilitated diffusion.

Active Process: The cell must use energy (ATP) to move substances from areas of low concentration to areas of high concentration. For example, group translocation.

Passive Processes

Passive or Simple Diffusion

Often called diffusion, is the process in which molecules move from a region of higher concentration to one of lower concentration. The rate is dependent on the size of the concentration gradient between a cell's exterior and its interior. Very small molecules such as water and oxygen and carbon dioxide move across membranes by simple or passive diffusion. Larger molecules, ions, and polar substances do not cross membranes by this method.

Osmosis

*It i*s the net movement of solvent molecules across a selectively permeable membrane from an area in which the solvent molecules are highly concentrated to an area of low concentration until equilibrium is reached. In living systems, the chief solvent is water. The three types of solutions which are normally found are isotonic, hypotonic and hypertonic.

Facilitated Diffusion

The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called permeases which are embedded in the

plasma membrane. Because a carrier aids the diffusion process, it is called as facilitated diffusion. Carrier proteins also resemble enzymes in their specificity for the substances to be transported; each carrier is selective and will transport only closely related solutes. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside. Two widespread major intrinsic protein channels in bacteria are aquaporins that transport water and glycerol facilitators which aid glycerol diffusion. The carrier protein complex spans the membrane (Refer Figure 13.1). After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. The carrier would subsequently change back to its original shape and be ready to pick up another molecule. The mechanism is driven by concentration gradients and therefore is reversible.

For example, Glycerol is transported by facilitated diffusion in *E. coli, Salmonella typhimurium, Pseudomonas, Bacillus* and many other bacteria. This is prominent in eukaryotes where it is used to transport a variety of sugars and amino acids.

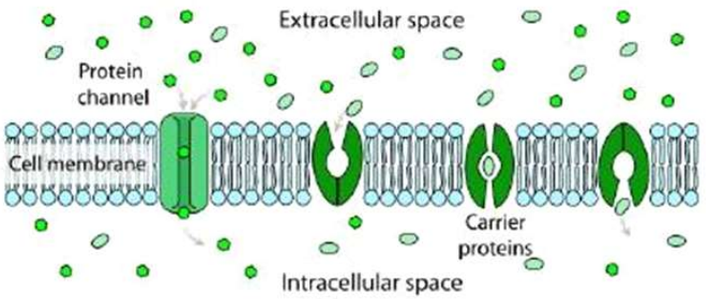


Fig. 13.1 *Facilitated Diffusion*

The carrier proteins aid in the release of solute molecules from extracellular space to the intracellular space.

Active Transport

Active transport is the transport of solute molecules to higher concentrations or against a concentration gradient, with the use of metabolic energy input. It resembles facilitated diffusion in the involvement of protein carrier activity, but differs in its use of metabolic energy and in its ability to concentrate substances. One example of active transport is binding protein transport systems or ATP-binding cassette transporters (ABC transporters) are active in bacteria, archaea and eukaryotes.

ABC Transporters

These transporters consist of two hydrophobic membrane spanning domains associated on their cytoplasmic surfaces with two nucleotide-binding domains. The membrane spanning domains from a pore in the membrane and the nucleotide binding domains bind and hydrolyze ATP to drive uptake. ABC transporters employ

NOTES

special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria or are attached to membrane lipids on the external face of the gram positive plasma membrane. These binding proteins, bind the molecule to be transported and then interact with the membrane transport proteins to move the solute molecule inside the cell (Refer Figure 13.2). *E. coli* transports a variety of sugars (arabinose, maltose, galactose, and ribose) and amino acids (glutamate, histidine, leucine) by this mechanism.

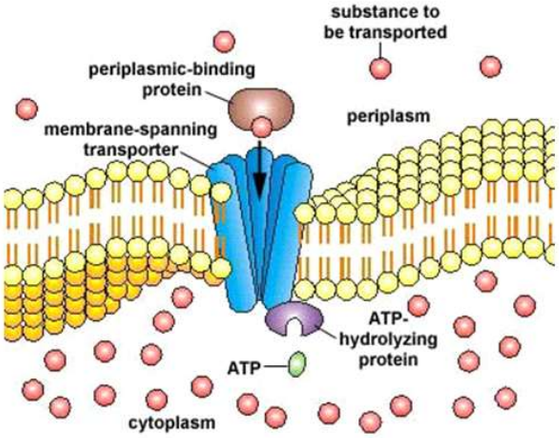


Fig. 13.2 *ABC Transporter System*

Eukaryotic ABC transporters are sometimes of great medical importance. Some tumor cells pump drugs out using these transporters. Cystic fibrosis results from a mutation that inactivates an ABC transporter that acts as a chloride ion channel in lungs.

Bacteria also use Proton gradients generated during electron transport to drive active transport. The lactose permease of *E.coli* transports a lactose molecule inward as a proton simultaneously enters the cell. Such linked transport of two substances in the same direction is called Symport (Refer Figure 13.3). *E. coli* also uses proton symport to take up amino acids and organic acids like succinate and malate.

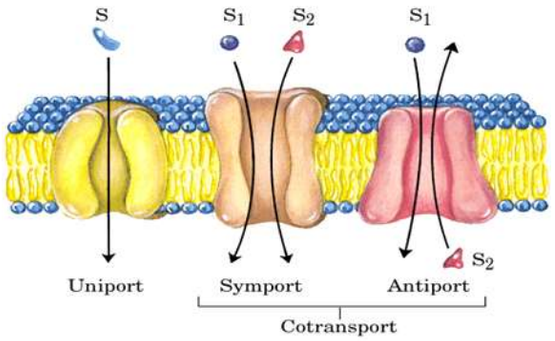


Fig. 13.3 *Comparison of Three Different Types of Transport Mechanisms*

NOTES

A proton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient. In *E. coli,* sodium transport system pumps sodium outward in response to the inward movement of protons. Such linked transport in which the transported substances move in opposite directions is called Antiport (Refer Figure 13.4). The sodium gradient generated by this proton antiport system then drives the uptake of sugars and amino acids. *E.coli* has at least transport systems for the sugar galactose.

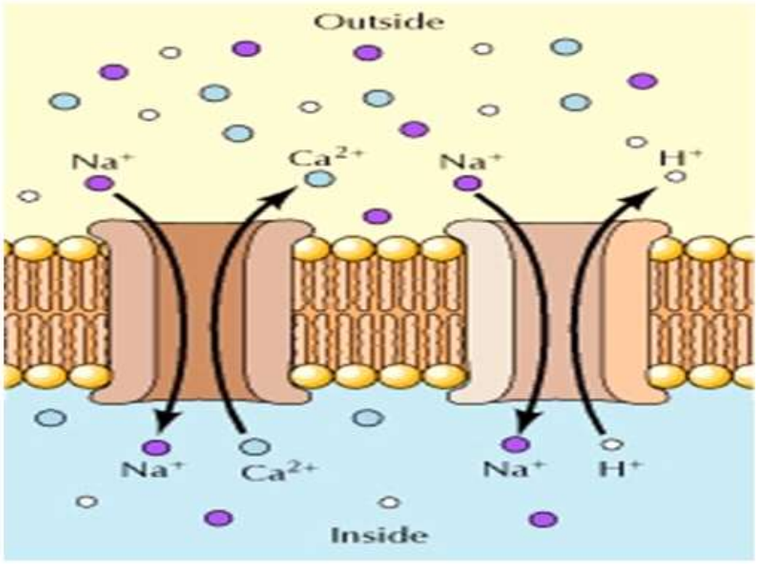


Fig. 13.4 *Antiport Mechanism*

The above figure shows the linked transport of two substances in opposite direction.

Group Translocation

In active transport, solute molecules move across a membrane without modification. Many prokaryotes also take up molecules by group translocation, a process in which a molecule is transported into the cell while being chemically altered. For example, Phosphoenolpyruvate: Sugar phosphotransferease system (PTS). It transports a variety of sugars while phosphorylating them using phosphoenolpyruvate (PEP) as the phosphate donor.

PEP + Sugar (outside) $\rightarrow$ Pyruvate + Sugar-P (inside)

In *E. coli* and *Salmonella typhimurium*, it consists of two enzymes and a low molecular weight heat stable protein (HPR). HPR and enzyme I (EI) are cytoplasmic. Enzyme II (EII) is more variable in structure and often composed of three sub units or domains. EIIA is cytoplasmic and soluble. EIIB also is hydrophilic but frequently attached to EIIC, a hydrophobic protein that is embedded in the membrane. A high energy phosphate is transferred from PEP to enzyme II (EII)

NOTES

with the aid of enzyme I (EI) and HPR. Then a sugar molecule is phosphorylated as it is carried across the membrane by enzyme II (EII). Enzyme II (EII) transports only specific sugars and varies with PTS, whereas enzyme I (EI) and HPR are common to all PTS. (Refer Figure 13.5).

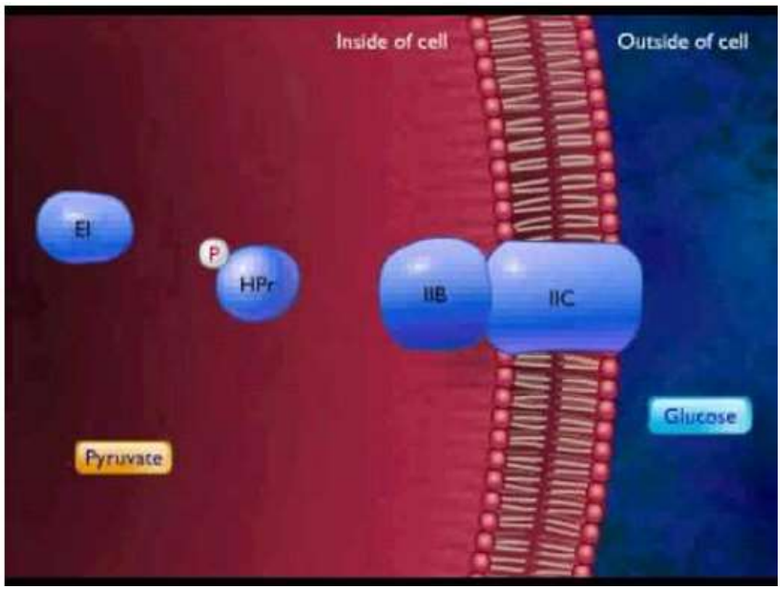


Fig. 13.5 *Group Tanslocation: Bacterial PTS Transport*

PTS's are widely distributed in prokaryotes. Aerobic bacteria lack PTS's. Genera *Escherichia, Salmonella, Staphylococcus* and other facultative anaerobic bacteria have phosphotransferase systems; some obligate anaerobic bacteria (*Clostridium*) also have PTS's. Many carbohydrates are transported by these systems. *E. coli* takes up glucose, fructose, mannitol, sucrose, N-acetylglucosamine, cellobiose and other carbohydrates by group translocation.

Iron Uptake

All microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric ion (Fe^{3+}) and its derivatives, which leave little free, iron available for transport. Many bacteria and fungi have overcome this difficulty by secreting siderophores. Siderophores – are low molecular weight molecules that are able to complex with ferric ion and supply it to the cell. These are either hydroxamates or phenolates-catecholates. Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by *E.coli*. Microorganisms secrete siderophores when little iron is available in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore receptor protein. The iron is either released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. In *E.coli*, the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter. After the iron

has entered the cell, it is reduced to the ferrous form (Fe^{2+}). Iron is so crucial to microorganisms that many use more than one route of iron uptake to ensure an adequate supply.

NOTES

Check Your Progress

1. What is membrane transport?
2. Define osmosis.
3. Define diffusion.
4. Differentiate between symport and antiport.
5. What is permeases?

13.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Membrane transport refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them.
2. Osmosis is the movement of water across a membrane from an area of low solute concentration to an area of high solute concentration.
3. Diffusion is a process of passive transport in which molecules move from an area of higher concentration to one of lower concentration.
4. The term symport is used to denote an integral membrane protein that simultaneouly transports two substances across membrane in the same direction. An antiport is an integral membrane transport protein that simutaneously transports two different molecules, in opposite directions, across the membrane.
5. The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called permeases which are embedded in the plasma membrane.

13.4 SUMMARY

- In cellular biology, membrane transport refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them.
- The regulation of passage through the membrane is due to selective membrane permeability - a characteristic of biological membranes which allows them to separate substances of distinct chemical nature.

NOTES

- The movements of most solutes through the membrane are mediated by membrane transport proteins which are specialized to varying degrees in the transport of specific molecules.
- As the diversity and physiology of the distinct cells is highly related to their capacities to attract different external elements, it is postulated that there is a group of specific transport proteins for each cell type and for every specific physiological stage.
- This differential expression is regulated through the differential transcription of the genes coding for these proteins and its translation, for instance, through genetic-molecular mechanisms, but also at the cell biology level: the production of these proteins can be activated by cellular signaling pathways, at the biochemical level, or even by being situated in cytoplasmic vesicles.
- Uptake of the required nutrients by the microbial cell is important. Since microorganisms live in nutrient poor habitats, they must be able to transport nutrients from dilute solutions into the cell against concentration gradient.
- Finally, they must pass through a selectively permeable plasma membrane. Microorganisms use different transport mechanisms like facilitated diffusion, active transport and group translocation.
- Eukaryotic microorganisms do not employ group translocation but take up nutrients by endocytosis.
- The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called permeases which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called as facilitated diffusion.
- Carrier proteins also resemble enzymes in their specificity for the substances to be transported; each carrier is selective and will transport only closely related solutes.
- As there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside. Two widespread major intrinsic protein channels in bacteria are aquaporins that transport water and glycerol facilitators which aid glycerol diffusion.
- Active transport is the transport of solute molecules to higher concentrations or against a concentration gradient, with the use of metabolic energy input.
- It resembles facilitated diffusion in the involvement of protein carrier activity, but differs in its use of metabolic energy and in its ability to concentrate substances.

NOTES

- ABC transporters employ special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria or are attached to membrane lipids on the external face of the gram positive plasma membrane.
- A proton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient.
- All microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric ion (Fe^{3+}) and its derivatives, which leave little free, iron available for transport.
- Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by *E.coli*.
- Microorganisms secrete siderophores when little iron is available in the medium.
- The iron is either released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter.
- In *E.coli*, the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter.

13.5 KEY WORDS

- **Arabinose:** Arabinose is an aldopentose – a monosaccharide containing five carbon atoms, and including an aldehyde (CHO) functional group.
- **Mannitol:** Mannitol is an osmotic diuretic used to remove excess water and toxins from the body in patients with kidney disease. It is also used in the treatment of cerebral edema.
- ***Diffusion:*** Diffusion is a process of passive transport in which molecules move from an area of higher concentration to one of lower concentration.
- ***Osmosis:*** Osmosis is the movement of water across a membrane from an area of low solute concentration to an area of high solute concentration.
- **Permeases:** The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called permeases which are embedded in the plasma membrane.
- **Symport:** The term symport is used to denote an integral membrane protein that simultaneouly transports two substances across membrane in the same direction.
- **Antiport:** An antiport is an integral membrane transport protein that simutaneously transports two different molecules, in opposite directions, across the membrane.

13.6 SELF ASSESSMENT QUESTION AND ANSWERS

NOTES

Short-Answer Questions

1. Write in brief about transport across membranes.
2. Write a short note on active transport.
3. What are ABC transpoters?
4. Write a short note on group translocation.
5. What are proton gradient?

Long-Answer Questions

1. Discuss about membrane transport and its mechanism.
2. Give a detailed overview of active transport.
3. Explain in detail about facilitated diffusion and its mechanism.
4. Explain the process of group translocation.
5. Discuss in detail about iron uptake.

13.7 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/

UNIT 14 QUORUM SENSING

NOTES

Structure

14.0 Introduction
14.1 Objectives
14.2 Quorum Sensing
14.3 Mechanism and Signalling Molecules
14.4 Answers to Check Your Progress Questions
14.5 Summary
14.6 Key Words
14.7 Self Assessment Questions and Exercises
14.8 Further Readings

14.0 INTRODUCTION

Many bacteria use a cell-cell communication system called quorum sensing to coordinate population density-dependent changes in behavior. Quorum sensing involves production of and response to diffusible or secreted signals, which can vary substantially across different types of bacteria. In many species, quorum sensing modulates virulence functions and is important for pathogenesis. Over the past half-century, there has been a significant accumulation of knowledge of the molecular mechanisms, signal structures, gene regulons, and behavioral responses associated with quorum-sensing systems in diverse bacteria. More recent studies have focused on understanding quorum sensing in the context of bacterial sociality. Studies of the role of quorum sensing in cooperative and competitive microbial interactions have revealed how quorum sensing coordinates interactions both within a species and between species. Such studies of quorum sensing as a social behavior have relied on the development of synthetic ecological models that use nonclonal bacterial populations. In this review, we discuss some of these models and recent advances in understanding how microbes might interact with one another using quorum sensing. The knowledge gained from these lines of investigation has the potential to guide studies of microbial sociality in natural settings and the design of new medicines and therapies to treat bacterial infections.

In this unit, you will study about quorum sensing, mechanism of quorum sensing, significance of signaling molecules.

14.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand what quorum sensing is
- Discuss the mechanism of quorum sensing
- Explain the significance of signaling molecules

14.2 QUORUM SENSING

NOTES

Quorum sensing (QS) is a cell-to-cell communication mechanism used by bacteria to regulate the expression of specific sets of genes in response to alterations in population density. The perception of population density is mediated by signaling molecules called autoinducers (AIs) synthesized by individual cells. Bacterial growth leads to a proportional increase in the AI extracellular concentration. Once a critical threshold is reached (namely quorum), the bacterial population detects the AI and responds to it through the coordinated expression of specific genes.

QS controls activities that are unproductive when conducted by an individual cell, but become effective when performed collectively. A wide range of biological processes are regulated by QS, including bioluminescence, gene transfer, sporulation, antibiotic production and resistance, biofilm formation, pathogen/host interaction and virulence factor secretion.

A diverse chemical vocabulary has been developed by bacteria, so that Gram-negative and Gram-positive bacteria signal QS through structurally distinct molecules. Gram-negative bacteria utilize small organic molecules as AIs, mainly N-acyl homoserine lactones (AHLs). Due to their amphiphilic nature, AHLs diffuse freely across the bacterial plasma membrane. On the contrary, Gram-positive bacteria communicate using oligopeptides named autoinducing peptides (AIPs). These are typically 5 to 17 amino acids in length which may present unusual modifications in their sequences. In contrast to Gram-negative AHLs, AIPs are actively secreted into the extracellular environment in a process facilitated by cell surface transporters.

The detection of AIs and the resulting changes in gene expression are specific to each QS system. At sufficiently high concentrations, Gram-negative AHLs bind to cytoplasmic receptors that also act as transcription factors. The receptor: AI complex then modulates transcription of QS-responsive genes. On the other hand, Gram-positive AIPs are detected by two-component signal transduction systems composed of a sensor kinase and a response regulator. When the quorum threshold is reached, AIPs bind to extracellular domains of specific membrane histidine kinase receptors. This interaction activates the kinase activity of the receptor, which phosphorylates itself and a cognate cytoplasmic response regulator allowing the expression of QS genes. Certain Gram-negative bacterial QS systems also use histidine kinase receptors that function similarly to those described for Gram-positive bacteria. Moreover, Gram-positive AIPs can also be transported back into the cell cytoplasm and interact with specific transcription factors leading to the expression of particular QS-controlled genes.

QS was first described in the marine Gram-negative Bacterium *Vibrio fischeri*, a symbiont of the Hawaiian bobtail squid, where it controls bioluminescence. This regulatory system is composed of a receptor (LuxR), a target DNA (*lux* operon) and an AI (N-(3-oxohexanoyl)-homoserine lactone).

The AI, which is synthesized by the enzyme LuxI, diffuses freely between the intra- and extracellular environments. When the bacterial population density increases, the AI binds to the cytoplasmic protein LuxR. This LuxR: AI complex then binds to the operator sequence in the *lux* operon and activates the expression of bioluminescence genes, including the luciferase enzyme.

Several clinically-relevant bacteria regulate virulence factor production by QS systems, and multidrug resistance is characteristic of these infections. Thus, the development of antimicrobials that target QS pathways represents an important therapeutic strategy, since QS disturbance attenuates the bacteria pathogenicity without being lethal. In addition, a significant advantage of this approach is the absence of selective pressure that results in drug resistance, often seen in traditional antibiotic therapies.

Check Your Progress

1. What is quorum sensing ?
2. How is the perception of population density mediated ?
3. What does QS do?
4. What does Gram-negative bacteria utilize ?
5. How do Gram-positive bacteria communicate?

14.3 MECHANISM AND SIGNALLING MOLECULES

Gram-negative LuxR Type Receptor

Gram-negative LuxR-type receptors comprise about 250 amino acid residues arranged into two functional modules. The N-terminal ligand-binding domain (LBD) and the C-terminal DNA-binding domain (DBD). In the absence of the AI, most LuxR-type proteins do not fold correctly, which leads to proteolytic degradation or accumulation in inclusion bodies, while LuxR:AI complexes are stable and can exert their cellular functions.

On the structural aspect, very little is known about the large family of LuxR-type QS receptors to date. Currently, only TraR (*Agrobacterium tumefaciens*), LasR (*Pseudomonas aeruginosa*) (Bottomley et al. 2007), QscR (*P. aeruginosa*), CviR (*Chromobacteriumviolaceum*) and SdiA (*Escherichia coli*) have had their three-dimensional structures determined (Refer Table 14.1). However, just TraR, QscR, CviR and SdiA have been crystallized as full-length proteins. For LasR, only the LBD structure has been reported. The receptors tertiary structures are quite similar but their quaternary organization is highly dynamic, making it difficult to establish a general mechanism for their function. This highlights the need for further structural studies on other AHL-binding QS systems.

NOTES

Table 14.1 *Three-dimensional Structures of Quorum Sensing Receptors*

	QS receptor	Microorganism	Oligomerization state	PDB id
Gram-negative	TraR	*Agrobacterium tumefaciens*	Dimer (holo)	1H0M
				1L3L
	LasR	*Pseudomonas aeruginosa*	Dimer (holo)	2UV0
			Dimer (holo)	3IX3
				3IX4
				3IX8
				3JPU
	CviR	*Chromobacterium violaceum*	Dimer (holo)	3QP1
				3QP2 3QP4 3QP5 3QP6
				3QP8
	QscR	*Pseudomonas aeruginosa*	Dimer (holo)	3SZT
	SdiA	*Escherichia coli*	Dimer (holo)	4LFU
				4LGW
			Monomer (holo)	2AVX
Gram-positive	PRGX	*Enterococcus faecalis*	Tetramer (apo)	2AXU
			Dimer (holo)	2AXZ
				2AW6
	P-LCR	*Bacillus thuringiensis*	Dimer (holo)	2QFC
			Dimer (apo)	4FSC
			Dimer (holo)	3U3W
	NPRR	*Bacillus cereus*	Tetramer (holo)	4GPK

TraR

The plant pathogen *Agrobacterium tumefaciens* is able to transfer the tumor-inducing plasmid responsible for plant crown gall disease by controlling the LuxI/LuxR-type QS system TraI/TraR. The TraR: N-(3-oxo-octanoyl)-L-homoserine lactone complex binds to promoter elements on the Ti plasmid called *Tra boxes*, activating conjugal genes transcription.

The structure of TraR bound to its cognate AI and its specific DNA sequence was solved by x-ray crystallography. The ternary complex structure reveals an asymmetric homodimer where one monomer is more elongated than the other. Such arrangement is possible due to differences in conformation of a linker region that leads to distinct interactions between the N- and C-terminal domains of each monomer. Surprisingly, the AI is not directly involved in receptor dimerization. Instead, the AI is completely buried in an enclosed cavity present in each N-terminal ligand-binding domain . In addition, a helix-turn-helix (HTH) motif mediates the interaction of the C-terminal domain with the DNA duplex.

NOTES

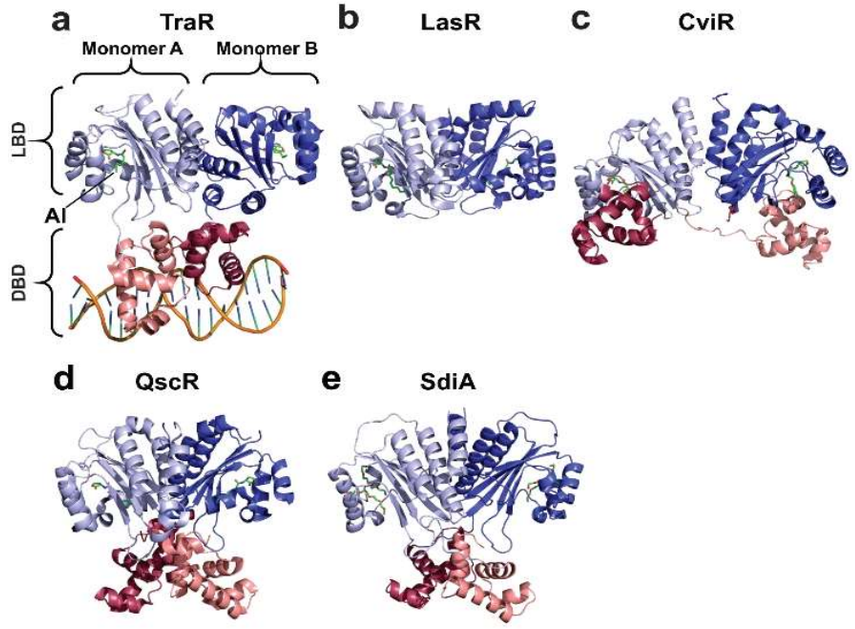


Fig. 14.1 *Three-dimensional Structures of Gram-negative LuxR-type QS Receptors.*

The above Figure is divided into 5 parts where A shows the ribbon diagram of the TraR:3-oxo-C8- HSL: DNA ternary complex structure (PDB: 1H0M); B shows the ribbon diagram of the LasR-LBD:3-oxo-C12-HSL complex structure (PDB: 2UV0); C shows the ribbon diagram of the CviR: chlorolactone complex structure (PDB: 3QP5); D shows ribbon diagram of the QscR:3- oxo-C12-HSL complex structure (PDB: 3SZT). E shows ribbon diagram of the SdiA dimer structure.

The AI-binding site is occupied by two low-molecular weight PEG molecules derived from the crystallization condition. All structures are oriented in respect to the LBD structure of TraR monomer A. N-terminal ligand-binding domains are colored in blue, while C-terminal DNA-binding domains are colored in salmon. Monomers are represented either by light or dark colors. AI structures are displayed in stick representation and colored in green.

The N-terminal ligand-binding domain (1-162) is composed of a five-stranded antiparallel β-sheet surrounded by three α-helices on each side. The AI interaction site is located between the central β-sheet and helices α3 (54-64), α4 (70-79) and α5 (97-107). A cluster of aromatic and hydrophobic amino acids forms a cavity that accommodates the AI without any solvent contact. Hydrogen bond interactions with residues Trp57 and Asp70 stabilize the AI molecule in the cavity. The homoserine lactone (HSL) portion of the AI interacts with residues Trp57, Asp70, Val72, Val73, Trp85, Phe101, Tyr102, Ala105 and Ile110, while the acyl moiety makes hydrophobic contacts with residues Tyr53, Leu40, Tyr61 and Phe62. The dimer interface is located on the opposite side of the AI interaction

NOTES

site. Hydrophobic interactions between the two α6 helices from each monomer stabilize the TraR dimer.

The C-terminal DNA-binding domain (176-243) consists of four α-helices: α7 (176-188), α8 (119-198), α9 (203-217), and α10 (223-230). The typical HTH motif is stabilized by a large hydrophobic cluster and a conserved salt bridge between residues Glu178 and Arg215. The DNA-binding domain is a dimer in the crystal and interacts with two binding sites on T*ra box*. Helices α8 and α9 directly interact with DNA, working as 'scaffold' and 'recognition' sites, respectively. Five residues from helix α9 make direct contacts with the major groove of the DNA. The specificity of this interaction is mediated by the side chains of two neighboring arginine residues, Arg206 and Arg210.

The most striking feature of the [TraR:3-oxo-C8-HSL]$_2$: *Tra box* structure is that the AI is completely embedded in a hydrophobic cavity in the N-terminal domain. This suggests a key role played by the AI in the correct folding of the receptor. The current hypothesis for TraR ligand-binding activation includes the participation of the AI in the stabilization of nascent TraR. Once properly folded, TraR becomes dimeric and protease-resistant. Receptor dimerization through the exposure of hydrophobic amino acids in helix a6 results in specific interaction of the DNA-binding domain dimers with *Tra box* sequences.

LasR

The LuxI/LuxR-homologous QS system LasI/LasR is responsible for QS-coordinated activities in *P. aeruginosa*. Thus, structure resolution of the LasR regulator was an important step toward a molecular understanding of QS signal transduction in this microorganism. The crystal structure of the LasR ligand-binding domain (1-175) in complex to its cognate AI N-(3-oxododecanoyl)-L-homoserine lactone reveals a symmetrical dimer with one AI deeply inserted in each monomer. The overall structure of the LasR monomer is very similar to that of TraR, consisting of a three α-helices/five-stranded antiparallel β-sheet/three α-helices sandwich.

As observed for other LuxR-type receptors, a cavity formed between the β-sheet and helices α3, α4 and α5 hides the AI from the solvent. The HSL moiety of the AI is stabilized by six hydrogen bond interactions with residues Tyr56, Trp60, Arg61, Asp73, Thr75 and Ser129. These residues are strictly conserved among the LuxR-type family, reflecting a common mode of interaction with the AI head group. On the other hand, the acyl chain extends into a pocket formed by a cluster of hydrophobic residues. Some of them only occur in LasR, such as Leu40, Tyr47, Cys79 and Thr80. These structural features provide the molecular basis for the high ligand specificity of LasR. Moreover, structural differences in the mode of interaction of TraR and LasR with their cognate AIs provide additional information about the specificity of QS receptors.

Despite the similarities among the monomer structures of TraR and LasR ligand-binding domains, their quaternary organization differ considerably. The most

NOTES

notable difference between the structures of TraR and LasR is the dimerization interface. In both structures, helix α9 is responsible for the majority of hydrophobic contacts and hydrogen bond interactions that contribute to dimer stabilization. However, the two α9 helices are oriented parallel to each other in the structure of TraR, while they are almost perpendicular in LasR. This causes a 90° shift in orientation of one monomer with regards to the other when comparing both structures. This difference in orientation of the N-terminal domains may lead to a difference in conformation of the two C-terminal domains and result in a distinct mode of DNA recognition by LasR. Nevertheless, the model of AI-induced activation that arises from the structure of the LasR:3-oxo-C12-HSL complex is reminiscent to that of other LuxR-type receptors and requires the binding of the AI to nascent LasR and the stabilization of its fold. Subsequently, LasR dimerization can occur, enabling specific binding to DNA promoters and transcriptional activation of QS-controlled genes.

CviR

The human pathogen *Chromobacterium violaceum* uses QS to control biofilm formation, cyanide production and purple pigment violacein synthesis. The cognate AI of the LuxR-type receptor CviR is the *N*-(hexanoyl)-L-homoserine lactone molecule. The crystal structure of full-length CviR bound to its antagonist (chlorolactone-CL) was determined. The structures of the independent domains of CviR resemble those of TraR, however, their overall organization is markedly different. In contrast to TraR, the CviR:CL complex shows a cross-sub unit architecture where the DNA-binding domain of each monomer is positioned underneath the ligand-binding domain of the opposite monomer, making extensive LBD-DBD interactions . This cross-sub unit conformation leads to a separation of the two DNA-binding helices in each C-terminal domain by about 60 Å. This distance is twice the ~30 Å separation required for efficient operator binding, which may explain, at a molecular level, the decreased DNA-binding affinity displayed by the CviR:CL complex. Because the full-length CviR structure was determined in complex with its antagonist, it is possible that the cross-sub unit structure may be a consequence of this interaction. Therefore, the accepted hypothesis for CviR inhibition by CL involves the binding of CL to the AI cavity and the induction of a closed conformation unable to bind DNA.

QscR

QscR is a LuxR homolog also found in *P. aeruginosa* that binds various AHLs including 3-oxo-C12-HSL, which is recognized by LasR. The crystal structure of full-length QscR bound to its AI showed a symmetrical dimer with a domain configuration different than those observed in the previously reported structures of LuxR-type receptors.

QscR contains general structural features that are characteristic of the LuxR family. It is formed by an N-terminal ligand-binding domain and a C-terminal

NOTES

DNA-binding domain. The LBD adopts the typical α-β-α sandwich architecture composed of a five-stranded β-sheet surrounded by five α-helices, while the DBD is formed by a canonical HTH motif. The AI binding site is located in a hydrophobic cavity formed between the β-strand and helices α3, α4 and α5. Nonetheless, QscR forms a symmetrical homodimer with a unique cross-sub unit configuration.

The HSL moiety of the AI makes a number of hydrogen bond interactions with QscR conserved residues Tyr58, Trp62, and Asp75, as well as the non conserved residues Ser38 and Ser56. In addition, numerous hydrophobic contacts between the AI acyl chain and QscR residues Arg42, Tyr52, Val78, Leu82, and Ile125 are observed. These residues are in close proximity to the cross-sub unit LBD-DBD dimerization interface and thus may be involved in QscR ligand-binding specificity. Interestingly, the conformation of the AI molecule and the shape of the binding pocket in QscR are similar to those of LasR but are distinct from TraR and CviR, suggesting that QscR and LasR recognizes their cognate AHLs in similar ways.

The cross-sub unit configuration of the QscR:3-oxo-C12-HSL dimer is a remarkable feature among the members of the LuxR-type family. In this conformation, the LBD of one monomer makes extensive interactions with the LBD and the DBD of the other monomer. Polar and hydrogen bond contacts between residues Glu84, Ser147, and Lys121 of one monomer and the same residues in the opposite monomer stabilize the LBD-LBD dimer interface. In addition, hydrogen bond interactions between residues Arg42 and Arg79 of monomer A and Asn237 of monomer B stabilize the LBD-DBD interface. The LBD-LBD dimerization interface of QscR resembles that of LasR and is distinct from those of TraR and CviR. On the other hand, the QscR DBDs form a dimer that is most similar to the one seen in TraR. This suggests that, in contrast to CviR, the cross-sub unit structure of QscR is prompt for DNA binding.

SdiA

The QS receptor SdiA is found in commensal and pathogenic bacteria of the intestine, such as *Escherichia coli*. The structure of full length SdiA was determined by x-ray crystallography and the crystal structures of SdiA have shown a similar fold to the mean NMR structure of the SdiA LBD reported previously.

Like other LuxR-type receptors, SdiA is formed by two functionally different domains: An N-terminal domain that binds the AI and a C-terminal domain that recognizes specific DNA sequences. These domains are connected by a linker and form a symmetrical dimer. The LBD exhibits an α/β/α topology composed of α-helices surrounding each side of a central antiparallel five-stranded β-sheet, which has a concave surface, where the AI-binding site is located. The DBD has four α-helices and is characterized by a typical HTH DNA-binding motif formed by helices α8 and α9. The overall folding of the SdiA LBD and DBD are well conserved when compared to other LuxR-type receptors, as opposed to the orientation of the two domains, which is significantly different. This observation is

NOTES

in agreement with the theory proposed by a previous study of an evolutionary combination of the two ancestral domains. To date, it is still unclear why each domain of LuxR-type receptors presents different relative orientations. It is assumed that each receptor defines the relative orientation of each domain since the residues located in the interdomain interface are not conserved among LuxR-type proteins. The conformation and function of the DBD remain unchanged upon AHL binding, which suggests that AHL may regulate the transcriptional activity of SdiA by increasing its stability.

The *E. coli* genome does not code for a LuxI-type QS synthetase, and it is known that SdiA is able to respond to indole as well as various AHL molecules released from other bacterial species. Therefore, SdiA may aid in space and competition with other microorganisms by being capable of recognizing a variety of AIs. This broad ligand-binding specificity of SdiA, compared to other LuxR-type receptors, may be due to a wide AI-binding site that is open to the solvent and can easily accommodate a wide range of ligands. Nevertheless, several studies have reported that *E. coli* SdiA has some selectivity to AI, especially for ligands with short chain length. This may occur because the occluded residues Phe59 and Gln72 of the AI-binding cavity limit the chain length of the acyl moiety to eight carbon atoms.

Dimeric SdiA has two Cys residues in the DBD conferring inter-cysteine distance within the range of disulphide bond formation (5.7 Å), which suggests that an inter sub unit disulphide bond may be relevant to modulation of DNA-binding activity. Furthermore, the binding affinity of SdiA for the *uvrY* promoter and the transcriptional activity of SdiA may be reduced under oxidative conditions. This cross-talk between QS and ROS signaling has also been reported in the QS arg system, which is characterized by an intramolecular disulfide redox switch.

The structural analysis of the intact *E.coli* SdiA provides an understanding not only of its broad ligand selectivity, but also for the general role of AHL-dependent QS receptors, where it is likely that regulation of SdiA may occur through multiple independent signals involving not only AIs but also the oxidative state of the cell.

Gram-positive RNPP QS Receptors

Gram-positive bacteria utilize oligopeptides as QS signaling molecules named autoinducing peptides (AIPs). These are grouped according to the subcellular localization of their cognate receptors, at the cell surface or inside the cell. The first group consists of peptides that interact with the external domain of membrane histidine kinases, resulting in further signaling to a final cytoplasmic effector, called indirect QS systems. The second group reflects a direct QS system, comprising peptides that are reimported from the vicinity and regulate the QS response by interacting directly with cytoplasmic receptors. The AIP-binding receptors of the direct QS systems have been grouped in the new RNPP protein family (Rap, NprR, PlcR and PrgX). Rap proteins are phosphatases and transcriptional anti-activators, while NprR, PlcR, and PrgX are DNA-binding transcription factors.

NOTES

The RNPP protein family is characterized by a C-terminal domain presenting several tetratricopeptide repeats (TPRs), a structural motif of 34 residues that mediates protein-protein and protein-peptide interactions. With the exception of Rap proteins, all RNPP members contain an N-terminal HTH-type DNA-binding domain and exhibit transcriptional activity. To date, the only Gram-positive AIP-binding QS receptors that were described at the molecular level are PrgX (*Enterococcus faecalis*), PlcR (*Bacillus cereus*) and NprR (*Bacillus cereus*). Although the AIP-binding mode is similar among the RNPP family members, the peptide-induced conformational changes are specific for each receptor.

PrgX

The QS receptor PrgX regulates the pCF10 plasmid conjugative transfer in *Enterococcus faecalis*. PrgX recognizes two DNA binding sites on pCF10, leading to transcriptional repression of the *prgQ* operon. AIP binding causes a decrease in the PrgX oligomerization state that alleviates transcriptional repression, resulting in expression of conjugation genes. The three-dimensional structures of full-length PrgX in its free form and bound to its cognate AIP cCF10 were determined by x-ray crystallography. Structure determination of PrgX: cCF10 complexes were essential to elucidate the mechanism by which AIP binding leads to receptor activation.

The crystal structures of apo-PrgX reveal a symmetrical tetramer composed by two homodimers that interact in a tail-to-tail manner (Refer Figure 14.2a). Each dimer cooperatively binds to one operator site on the *prgQ* operon. In the repressed state, both DNA-binding sites are occupied by the PrgX tetramer. PrgX adopts a helical structure that can be divided into three functional modules: a N-terminal DNA-binding domain, a central dimerization and AIP-binding domain, as well as a regulatory C-terminal domain (Refer Figure 14.2a).

The above Figure is divided into 3 parts: (a) Ribbon diagram of apo-PrgX (PDB: 2AXU) (left) and the PrgX: cCF10 complex (PDB: 2AXZ) (right) structures; (b) Ribbon diagram of the PlcR: PapR complex structure (PDB: 2QFC); (c) Ribbon diagram of the NprΔHTH : NprX complex structure (PDB: 2GPK). N-terminal DNA-binding domains are colored in blue, central dimerization and AIP-binding domains are colored in salmon, and regulatory C-terminal domains are colored in orange. Monomers are represented either by light or dark colors. AI structures are displayed in stick representation and colored in green.

The N-terminal domain (1-68) is composed by five α-helices, including α1 (3-15), α2 (18-24), α3 (28-37), α4 (43-55) and α5 (57-65) . DNA binding is mediated by helices α2 and α3 that form a typical HTH motif. The PrgX dimer adopts a crossed-sub unit architecture, where the N-terminal domain of one monomer simultaneously interacts with the N-terminal and central domains of the other monomer Structural comparison between PrgX and other prokaryotic transcriptional regulators enabled the construction of a model for the PrgX: DNA complex. In this model, the DNA duplex loops around the PrgX tetramer, interacting with the swapped N-terminal domains of each dimer.

NOTES

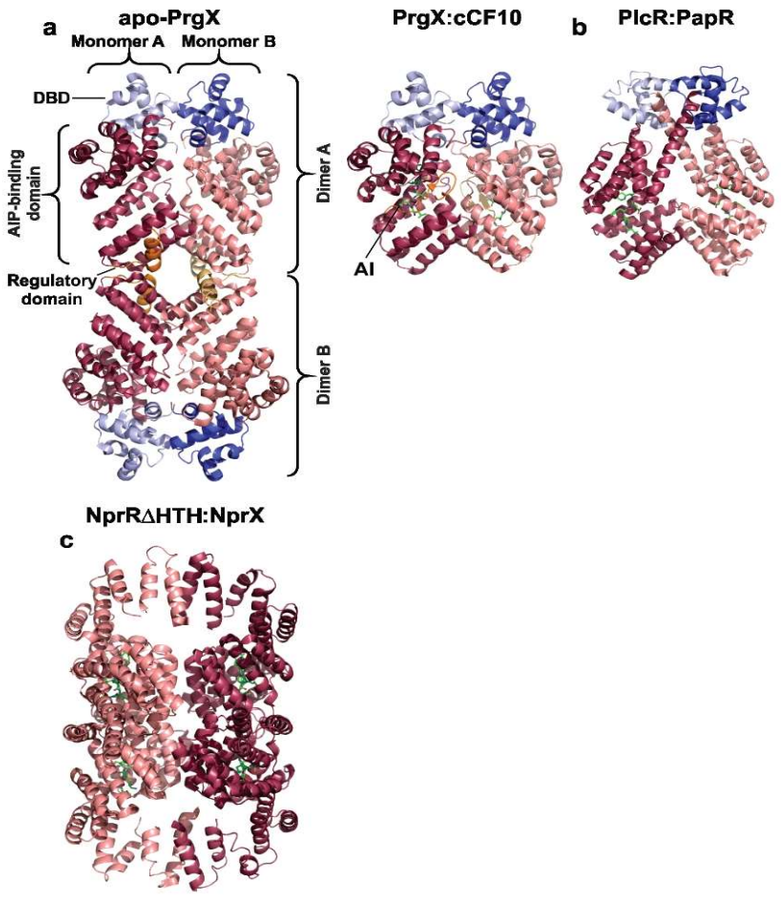


Fig. 14.2 *Three-dimensional Structures of Gram-positive RNPP QS Receptors.*

The central domain (69-283) consists of 11 antiparallel α-helices arranged in a two-layered super helix that mediates both receptor dimerization and AIP binding. The dimerization interface is composed by helices α14 and α15 from each monomer. Residues Leu234, Ile263, Ile266, and Ile267 form a hydrophobic core surrounded by hydrogen bond interactions that stabilize the PrgX dimer. In addition, the PrgX tetramer is stabilized by van der Walls and hydrogen bond interactions between the C-terminal domain loop (287-294) of one dimer and a loop comprising residues 248-255 of the opposite dimer. The cCF10 peptide binds into a pocket in PrgX composed by helices α6, α8, α10, α12, α14 and α16. Residues Ile82, Phe86, Ile200, Ile283 and Leu282, located on each side of the AIP-binding site, establish hydrophobic interactions with cCF10 side chains.

The C-terminal domain (287-305) consists of a loop region and helix α17 that regulates the prgQ operon transcription. The crystal structures of PrgX: cCF10 complexes reveal that cCF10 binding induces a conformational change in the C-terminal domain loop. In the bound conformation, this loop is rotated 120o away

NOTES

from the tetramerization interface, leading to disruption of the PrgX tetramer. cCF10-induced tetramer dissociation decreases the affinity of PrgX for the second operator site, resulting in disruption of the DNA-loop structure, RNA polymerase binding, and increased expression of prgQ-encoded genes.

PlcR

The QS receptor PlcR regulates pathogenicity in Bacillus cereus. In the presence of its cognate AIP PapR, PlcR activates the expression of virulence factors. The structure of PlcR bound to PapR was determined by x-ray crystallography. The PlcR:PapR complex structure displays a symmetrical dimer that is most similar to the dimeric unit of the PrgX:cCF10 tetramer structure. Each PlcR monomer is composed of an N-terminal DNA-binding domain and a regulatory C-terminal AIP-binding and dimerization domain.

The N-terminal domain consists of a 5-helix HTH motif that mediates DNA interaction. Helix α3 inserts itself into the DNA major groove, working as recognition helix. As in PrgX, the PlcR dimer adopts a crossed conformation formed by two monomers with their N-terminal domains swapped. Conversely, PlcR N-terminal domains are directly attached to the first C-terminal domain helix, resulting in higher mobility than in PrgX.

The C-terminal regulatory domain is strikingly similar to the central domain of PrgX. It consists of eleven α-helices forming five tetratricopeptide repeats (TPR) and a capping C-terminal helix arranged in a superhelix . The dimer interface is composed by the two TPR domains. The AIP-binding site is located in the middle of the TPR superhelix, between helices α5 and α7. Residues Asn159, Asn201 and Lys204 make hydrogen bond interactions with the backbone of PapR. Moreover, hydrophobic interactions between two PapR Phe residues and PlcR side chains from the binding pocket stabilize the AIP-bound conformation.

In contrast to PrgX, PlcR lacks the C-terminal domain loop that mediates receptor tetramerization. Consequently, apo-PlcR is dimeric in solution, with an overall dimension comparable to the crystallographic PlcR: PapR dimer, as revealed by SAXS measurements. In contrast, the PlcR: PapR complex is capable of forming higher order oligomers in solution. This leads to the current hypothesis regarding the activation mechanism of PlcR by PapR. In this hypothesis, PapR binding increases the curvature of the TPR domain of PlcR. This domain closure separates the two HTH motifs and triggers PlcR multimerization. In this PlcR: PapR multimer, all the HTH domains are mobile and thus suited for DNA binding.

NprR

Adaptive gene expression response of Bacillus cereus is controlled by the QS receptor NprR. The crystal structure of a truncated form of NprR, named NprRΔHTH (HTH motif deleted), in complex to its cognate AIP NprX has been determined.

NOTES

The structure of the NprRΔHTH: NprX complex reveals a tetramer, consisting of a dimer of dimers. Each dimer comprises two monomers arranged in a head-to-tail fashion . The NprRΔHTH:NprX monomer is composed of 18 α-helices arranged in a two-layered, right-handed superhelix formed by 9 TPR motifs. The dimer interface is mainly stabilized by van der Waals interactions between residues located in loops α8-α9 from one monomer and α10-α11 from the other monomer. On the other hand, the tetramer interface is stabilized by hydrophobic contacts between residues located on helices a9 and α18 from opposite dimers.

As in other RNPP family members, the AIP binds into a cleft located in the concave side of the TPR domain. NprX backbone atoms make van der Waals and hydrogen bond interactions with NprR amino acids from the binding pocket. In addition, a hydrogen bond between NprX Asp residue and the side chain of the conserved NprR Asn126 stabilizes the complex, playing a key role in the mechanism of NprR activation upon NprX binding.

In contrast to the NprRΔHTH: NprX tetramer structure, apo-NprR, both full-length and NprRΔHTH, is a dimer in solution, as evidenced by size-exclusion chromatography and dynamic light scattering. NprX binding shifts the dimer-tetramer equilibrium toward the higher order state. Therefore, NprX binding induces the association of two apo-NprR dimers, resulting in the formation of an active NprR: NprX tetramer. Comparison of the NprRΔHTH: NprX structure with the structures of the RNPP QS receptors PrgX and PlcR bound to their cognate AIPs enabled the building of a model for the full-length NprR: NprX complex. In this model, the two HTH-type DNA-binding domains point toward opposite sides of the tetramer, suggesting that NprR and PrgX interact with their target DNA sequences in a similar manner.

Check Your Progress

6. What does Gram-negative LuxR-type receptors comprises of ?
7. What does plant pathogen *Agrobacterium tumefaciens* transfer ?
8. What is N-terminal ligand-binding domain composed of ?
9. What does C-terminal DNA-binding domain consists of ?
10. What is the most striking feature of the $[TraR:3\text{-}oxo\text{-}C8\text{-}HSL]_2$?

14.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Quorum sensing (QS) is a cell-to-cell communication mechanism used by bacteria to regulate the expression of specific sets of genes in response to alterations in population density.

NOTES

2. The perception of population density is mediated by signaling molecules called autoinducers (AIs) synthesized by individual cells.
3. QS controls activities that are unproductive when conducted by an individual cell, but become effective when performed collectively.
4. Gram-negative bacteria utilize small organic molecules as AIs, mainly N-acyl homoserine lactones (AHLs).
5. Gram-positive bacteria communicate using oligopeptides named autoinducing peptides (AIPs).
6. Gram-negative LuxR-type receptors comprise about 250 amino acid residues arranged into two functional modules.
7. The plant pathogen *Agrobacterium tumefaciens* is able to transfer the tumor-inducing plasmid responsible for plant crown gall disease by controlling the LuxI/LuxR-type QS system TraI/TraR.
8. The N-terminal ligand-binding domain (1-162) is composed of a five-stranded antiparallel β-sheet surrounded by three α-helices on each side.
9. The C-terminal DNA-binding domain (176-243) consists of four α-helices: α7 (176-188), α8 (119-198), α9 (203-217), and α10 (223-230). The typical HTH motif is stabilized by a large hydrophobic cluster and a conserved salt bridge between residues Glu178 and Arg215.
10. The most striking feature of the [TraR:3-oxo-C8-HSL]$_2$: *Tra box* structure is that the AI is completely embedded in a hydrophobic cavity in the N-terminal domain. This suggests a key role played by the AI in the correct folding of the receptor.

14.5 SUMMARY

- Quorum sensing (QS) is a cell-to-cell communication mechanism used by bacteria to regulate the expression of specific sets of genes in response to alterations in population density.
- The perception of population density is mediated by signaling molecules called autoinducers (AIs) synthesized by individual cells.
- Bacterial growth leads to a proportional increase in the AI extracellular concentration. Once a critical threshold is reached (namely quorum), the bacterial population detects the AI and responds to it through the coordinated expression of specific genes.
- QS controls activities that are unproductive when conducted by an individual cell, but become effective when performed collectively.

NOTES

- A wide range of biological processes are regulated by QS, including bioluminescence, gene transfer, sporulation, antibiotic production and resistance, biofilm formation, pathogen/host interaction and virulence factor secretion.
- A diverse chemical vocabulary has been developed by bacteria, so that Gram-negative and Gram-positive bacteria signal QS through structurally distinct molecules.
- Gram-negative bacteria utilize small organic molecules as AIs, mainly N-acyl homoserine lactones (AHLs). Due to their amphiphilic nature, AHLs diffuse freely across the bacterial plasma membrane.
- Gram-positive bacteria communicate using oligopeptides named autoinducing peptides (AIPs).
- These are typically 5 to 17 amino acids in length which may present unusual modifications in their sequences. In contrast to Gram-negative AHLs,
- AIPs are actively secreted into the extracellular environment in a process facilitated by cell surface transporters.
- The detection of AIs and the resulting changes in gene expression are specific to each QS system. At sufficiently high concentrations, Gram-negative AHLs bind to cytoplasmic receptors that also act as transcription factors.
- The receptor: AI complex then modulates transcription of QS-responsive genes. On the other hand, Gram-positive AIPs are detected by two-component signal transduction systems composed of a sensor kinase and a response regulator.
- When the quorum threshold is reached, AIPs bind to extracellular domains of specific membrane histidine kinase receptors. This interaction activates the kinase activity of the receptor, which phosphorylates itself and a cognate cytoplasmic response regulator allowing the expression of QS genes.
- Certain Gram-negative bacterial QS systems also use histidine kinase receptors that function similarly to those described for Gram-positive bacteria.
- Gram-positive AIPs can also be transported back into the cell cytoplasm and interact with specific transcription factors leading to the expression of particular QS-controlled genes.
- QS was first described in the marine Gram-negative Bacterium *Vibrio fischeri*, a symbiont of the Hawaiian bobtail squid, where it controls bioluminescence. This regulatory system is composed of a receptor (LuxR), a target DNA (*lux* operon) and an AI (N-(3-oxohexanoyl)-homoserine lactone).

NOTES

- The AI, which is synthesized by the enzyme LuxI, diffuses freely between the intra- and extracellular environments. When the bacterial population density increases, the AI binds to the cytoplasmic protein LuxR.
- This LuxR: AI complex then binds to the operator sequence in the *lux* operon and activates the expression of bioluminescence genes, including the luciferase enzyme.
- Several clinically-relevant bacteria regulate virulence factor production by QS systems, and multidrug resistance is characteristic of these infections.
- The development of antimicrobials that target QS pathways represents an important therapeutic strategy, since QS disturbance attenuates the bacteria pathogenicity without being lethal.
- A significant advantage of this approach is the absence of selective pressure that results in drug resistance, often seen in traditional antibiotic therapies.
- Gram-negative LuxR-type receptors comprise about 250 amino acid residues arranged into two functional modules.
- The N-terminal ligand-binding domain (LBD) and the C-terminal DNA-binding domain (DBD).
- In the absence of the AI, most LuxR-type proteins do not fold correctly, which leads to proteolytic degradation or accumulation in inclusion bodies, while LuxR:AI complexes are stable and can exert their cellular functions.
- On the structural aspect, very little is known about the large family of LuxR-type QS receptors to date.
- Currently, only TraR (*Agrobacterium tumefaciens*), LasR (*Pseudomonas aeruginosa*) (Bottomley et al. 2007), QscR (*P. aeruginosa*), CviR (*Chromobacteriumviolaceum*) and SdiA (*Escherichia coli*) have had their three-dimensional structures determined.
- However, just TraR, QscR, CviR and SdiA have been crystallized as full-length proteins. For LasR, only the LBD structure has been reported.
- The receptors tertiary structures are quite similar but their quaternary organization is highly dynamic, making it difficult to establish a general mechanism for their function.

14.6 KEY WORDS

- **Quorum sensing:** Quorum sensing (QS) is a cell-to-cell communication mechanism used by bacteria to regulate the expression of specific sets of genes in response to alterations in population density.

- **Gram-positive bacteria:** Gram-positive bacteria are bacteria that give a positive result in the Gram stain test, which is traditionally used to quickly classify bacteria into two broad categories according to their cell wall.
- **Gram-negative bacteria:** Gram-negative bacteria are bacteria that do not retain the crystal violet stain used in the gram-staining method of bacterial differentiation. They are characterized by their cell envelopes, which are composed of a thin peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membraneand a bacterial outer membrane.
- **Autoinducers:** Autoinducers are signaling molecules that are produced in response to changes in cell-population density. As the density of quorum sensing bacterial cells increases so does the concentration of the autoinducer.

14.7 SELF ASSESSMENT QUESTION AND ANSWERS

Short-Answer Questions

1. Write in brief about QS.
2. What is TraR?
3. Brief about LasR.
4. Write about PrgX.
5. Give a general difference Gram-positive bacteria and Gram-negative bacteria.

Long-Answer Questions

1. Write about Quorum sensing in detail.
2. Discuss about Gram-negative LuxR Type receptor.
3. Discuss about Gram-positive RNPP QS receptors.
4. Explain the mechanism of PlcR.
5. Give a detailed overview of PrgX.
6. Write about LasR giving its detailed overview.

14.8 FURTHER READINGS

Deb, A.C. 2006. *Fundamentals of Biochemistry*. Kolkata: New Central Book Agency Pvt. Ltd.

Voet, Donald and Judith Voet. G. 2011. *Biochemistry*, 4th Edition. New York: John Wiley & Sons, Inc.

NOTES

Stryer, L. 2010. *Biochemistry*. 7th Edition. New York: W.H. Freeman and Company.

Satyanarayana, U. and U. Chakrapani. 2013. *Biochemistry*, 4th Edition. Kolkata: Book & Allied Pvt. Ltd.

Jain, J. L. 2008. *Fundamentals of Biochemistry*, 5th Edition. New Delhi: S. Chand & Company Ltd.

Moat, A.G. and W. Foster. 2002. *Microbial Physiology*. 4th Edition. New York: John Wiley & Sons, Inc.

https://nptel.ac.in/