

UG e-LECTURE NOTES
COURSE No. BCM-101(1+1)
COURSE TITLE: PLANT BIOCHEMISTRY

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CONTENTS

LECTURE OUTLINES	PAGE NO.
INTRODUCTION	5
CHAPTERS	
1. The Cell Structure and Function	8
2. Carbohydrates	14
3. Lipids	33
4. Amino acids and proteins	47
5. Nucleic acids	65
6. Enzymes	75
7. Biological Oxidation, Electron Transport Chain and Oxidative Phosphorylation	89
8. Metabolism	94
9. Secondary metabolites	109
REFERENCE	115
ACKNOWLEDGEMENT	116

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BCM.101(1+1)Crs-Plant Biochemistry

Lecture Outline:(Theory)

(Lecture hrs)

Introduction to biochemistry: The plant cell and its subcellular organelles-nucleus, endoplasmic reticulum, Golgi apparatus, vacuoles, mitochondria, chloroplasts, microbodies, oleosomes, cytoskeleton, cell wall and their functions.

(1)

2. Biomolecular components of plant cells:

a. Carbohydrates-Definition and classification.

Monosaccharides-stereochemistry, nomenclature, cyclic forms. Mutarotation, reactions of monosaccharides. Di-and oligo-saccharides. Plant polysaccharides, structure and role.

(3)

b. Amino acids and proteins: Importance of proteins in living systems. Diverse roles of proteins. Amino acids, the subunits of proteins. Classification, structures and chemical reactions. Protein amino acids. The peptide bond. Biological role of peptides. Protein classification. Structure of proteins-primary, secondary, tertiary and quaternary structures. Properties of proteins. Denaturation of proteins.

(3)

c. Lipids: Classification. Fatty acids-their classification and structures. Chemical properties, Triglycerols, waxes, phospholipids, sphingolipids, terpenes and sterols. Their structures and functions.

(2)

d. Enzymes: Classification and nomenclature. Factors that influence enzyme activity. Immobilization and industrial applications.

(2)

e. Nucleic acids: RNA and DNA. The constituents of nucleic acids, structures of purines and pyrimidines. Nucleosides, nucleotides. DNA-its structure. Types of RNA, their characteristics and role. Cloverleaf structure of tRNA.

(2)

3. Metabolism of biomolecules:

An overview of metabolism, catabolism and anabolism-their general characteristics.

(1)

A. Carbohydrate metabolism:

(3)

- a. Glycolysis-Pathway of glucose in the cytosolic glycolytic sequence. Energetics of glycolysis.
- b. TCA cycle-a mitochondrial reaction cycle. Oxidation of Pyruvate via TCA cycle. Energetics of TCA cycle.
- c. Electron transport and oxidative phosphorylation-the components of the electron transport chain. ATP synthesis coupled to electron transport.
- d. The pentose phosphate pathway-reactions. Role of pentose phosphate pathway.

B. Lipid metabolism: β -Oxidation of fatty acids and its energetics.

C. Amino acid metabolism: Removal of amino group of amino acids by deamination and transamination. Decarboxylation of amino acids.

4. Secondary metabolites:

(2)

A. Phenolics: Different classes of phenolics and their functions.

Polyphenols:-the tannins-classification of tannins. Phenolics and tannins as antinutritional factors. Roles of phenolics and tannins in disease/pest resistance of plants.

B. Alkaloids: Definition and occurrence of some common alkaloids of plants. Role of alkaloids as defense compounds.

C. Terpenoids: nomenclature, classification and occurrence. Functions of terpenoids. Carotenoids-their distribution and functions in higher plants.

INTRODUCTION

The term biochemistry derived from the Greek word 'Bios' meaning 'life' may be defined as the science that deals with the chemical basis of life. Thus, biochemistry is the study of chemistry of living organisms or simply it is referred to as 'biological chemistry'.

Chemistry is the fundamental science that deals with the properties and behavior of molecules. The term 'life' is used to characterize living organisms from the smallest viruses and other microorganisms to the most complex and highly evolved plants and animals including human beings. Some of the important identifying characteristics of living organisms that distinguish from nonliving things on earth are summarized below:

1. The cells of living organisms are composed of many kinds of complex lifeless molecules.
2. Each component part of a living organism appears to have a specific purpose or function.
3. Capacity to extract and transform energy from the environment.
4. Metabolism of nutrients.
5. Ability to grow in size and self-replicate precisely and
6. Response to external stimuli.

The chemical composition of living organisms is qualitatively quite different from that of the earth's crust where they live. The four most abundant elements in the earth's crust are oxygen, silicon, aluminum and iron. In contrast, the four most abundant elements in living organisms are hydrogen, oxygen, carbon and nitrogen which make up about 99 per cent of the mass of most cells. Most of the chemical components of living organisms are organic compounds of high molecular weight called the biomacromolecules. These organic compounds include carbohydrates, lipids, proteins, enzymes, nucleic acids and related compounds in varying amounts each having a specific function in the living organisms.

Biochemistry probes into the chemical changes involved right from birth, followed by the changes during growth and development and until death and dissolution of living organisms.

Biochemistry encompasses a study of chemical nature of living organisms the relationship of the living to their environment the processes by which an exchange of chemical substances takes place between the living organisms and the environment the processes by which the absorbed materials are utilized for synthetic reactions leading to growth and replenishment of tissues and multiplication of the cell and the species the metabolic breakdown of the materials to supply energy for all the above and the mechanisms which regulate with precision all these processes. All these studies come

under the purview of biochemistry. Correlation of biological functions and molecular structures is the central theme of biochemistry.

Biochemistry includes various aspects of organic chemistry in organic chemistry, physical chemistry, physics, biology and other basic disciplines. It is also interrelated with physiology, microbiology, medicine and agriculture.

Scope of biochemistry

During the early part of the twentieth century, the central theme of biochemistry was the development of the field of intermediary metabolism that is the elucidation of the pathways for the synthesis and degradation of the constituents of living organisms. Although studies concerned with intermediary metabolism continue to be important, at the present, biochemical research may be classified into the following major areas.

1. Composition and characteristics of chemical compounds of living organisms.
2. Cell ultrastructure.
3. Cellular control mechanisms.
4. Physical chemistry of biomacromolecules.
5. Structure-function, kinetics, regulation and mode of action of enzymes.
6. Intermediary metabolism.
7. Bioenergetics particularly the mechanisms of formation of adenosine triphosphate (ATP) in the process of oxidative phosphorylation.
8. The molecular basis for genetic and developmental phenomena.
9. The molecular basis for physiological phenomena including nerve conduction, muscle contraction, vision and transport across membrane
10. Role, transformation and requirement of nutrients in plants, animals and other organisms and
11. Chemistry of inheritance: structure-function and regulation of gene expression.

3. Impact on biotechnology

Recent research in biochemistry, microbiology, cell biology, molecular genetics and recombinant DNA has led to the development of a technology what is known as 'biotechnology'.

Biotechnology encompasses many facets of the management and manipulation of the biological system. In 1981, the European Federation of Biochemistry defined this branch of science as "the integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application of the capacities of microbes and culture cells". A recent offshoot of biotechnology research is genetic engineering, which involves gene splicing and recombinant DNA cloning.

There has been considerable public and political interest in this field of technology

because of reported breakthrough possibly of great significance to life on earth. Some of the applications of genetic engineering and biotechnology in various fields are listed below:

1. Agriculture	Improvement of crop plants for higher photosynthetic efficiency, nutrient and water uptake, biological nitrogen fixation, nutritional quality of cereals, resistance to pests and diseases, plant cell and tissue culture and improvement of animal stock for desirable characteristics
2. Chemical industry	Transformation of substances by biocatalysts to products such as biopolymers, antibiotics, alternative structural materials to plastics
3. Energy industry	Production of new fuel sources and improved efficiency of energy recovery from existing ones.
4. Food industry	Production of colourants, sweeteners, preservatives, etc
5. Fermentation industry	Production of beer, wine, alcohols, amino acids, vitamins, etc.,
6. Public health	Production of vaccines, drugs, growth hormones, interferons monoclonal antibodies, etc., use of bacteria in waste water treatment and recycling and pollution control

In spite of rapid advances in our knowledge of life processes in the past few decades, however, the science of biochemistry is yet to determine how the collections of lifeless molecules found in living organisms interact with each other to constitute, maintain and perpetuate the living state. Indeed, biochemistry has an important role to play in all the life processes and technological developments sue as food, energy, public health and pollution control in order to keep pace with the expanding world population.

CHAPTER 1

THE CELL STRUCTURE AND FUNCTION

1.1 The Cell

The cell is the basic structural and functional unit of life. All living-organisms are composed of cells. Bacteria and blue-green algae, the smallest free-living organisms consist of single, independent cells. Larger organisms such as higher animals and plants contain billions of cells organized into tissues with specialized functions.

In the seventeenth century when Robert Hooke was examining a thin slice of cork under the microscope, he saw that the plant tissue was divided into small compartments separated by walls. He called the compartments 'cells' meaning little rooms.

Over 150 years later, M. Schleiden proposed that the structure of all plant tissues is based upon an organization of cells. Shortly thereafter, T. Schwann proposed that all animal tissues are also organizations of cells and that the fundamental unit of life is the cell

1.2 Modern cell theory concept

Modern cell theory concept can be reduced to four fundamental statements:

1. Cells make up all living matter
2. All cells arise from other cells
3. The genetic information required during the maintenance of existing cells and the production of new cells passes from one generation to the next and
4. The chemical reactions of an organism, that is its metabolism, takes place in cells

1.3 Properties of living cells

Cells show characteristics of life that distinguish from the inanimate material found on the earth:

1. All cells are capable of metabolism: Cells can take up chemical compounds from their environment, modify them and combine them into larger, more complex molecules. The newly synthesized compounds are then used to build the complicated structures necessary for cellular integrity and function.

2. All cells are capable of energy transformation: During photosynthesis, green plant cells convert solar energy into the energy of chemical bonds of sugar molecules. All cells degrade these sugars and store the released energy in the chemical bonds of 'high energy' compounds. Cells use their stored energy to perform cellular work such as biosynthesis, division, contraction, locomotion and the transmission of electrical stimuli.

3. All cells are capable of self-directed growth and replication: By metabolic processes, cells increase their mass and at a critical time when all cellular constituents are present in the proper concentrations, cells divide, giving rise to two identical daughter cells. Each cell contains all the information necessary for perpetuating itself, and this information remains constant generation after generation.

1.4 The cell types

Cells exist in a remarkable number of sizes and shapes. Despite this immense diversity, cells can be divided by their internal organization into two basic types: prokaryotic and eukaryotic cells

1.4.1 Prokaryotic cells (Greek, pro-before, karyon-kernel)

These cells have neither membrane surrounded nuclei nor any other internal compartments enclosed by a membrane. They are the putative ancestors of all life on earth. They are relatively smaller than eukaryotic cells and usually contain only one DNA molecule. e.g. Bacteria, blue-green algae and rickettsiae.

1.4.2 Eukaryotic cells (Greek, eu-true)

In contrast to prokaryotic cells, eukaryotic cells have a welldefined membrane surrounding a central nucleus and a variety of intracellular structures and organelles. The intracellular membrane systems establish a number of distinct subcellular compartments permitting a unique degree of subcellular specialization. These cells have their DNA divided among several chromosomes. e.g. Protists, fungi, higher plants and animals. Some of the important differences between the prokaryotic and eukaryotic cells are summarized in Tables 1.1 and 1.2. The structure of an animal cell, a plant cell and a bacterium is shown in Fig. 1.1

1.5 The cell structure and function

Biochemists have long been interested in the study of the structure, chemical composition and functions of biological cells which have been greatly facilitated by two technical advances, the development of the electron microscope and of the ultracentrifuge. The structural organization of eukaryotic cells is summarized in Table 1.3.

The intracellular location of major enzymes and metabolic pathways are summarized in Table 1.4.

Table 1.1 Comparison of prokaryotic and eukaryotic cells

	Prokaryotic cells	Eukaryotic cells
Organisms	Bacteria and cyanobacteria	Protists, fungi, plants and animals
Cell size	Generally 1-10 μm	Generally 10-100 μm in linear dimension
Metabolism	Anaerobic or aerobic	Aerobic

Organelles	Few or none	Nucleus, mitochondria, chloroplasts, endoplasmic reticulum, etc., present
DNA	Circular DNA in cytoplasm	Very long linear DNA molecules containing many noncoding regions; bounded by nuclear envelope
RNA and protein	Synthesized in the same compartment	RNA-synthesized and processed in nucleus; proteins-synthesized in cytoplasm
Cytoplasm	No cytoskeleton, cytoplasmic streaming, endocytosis and exocytosis absent	Cytoskeleton composed of protein filaments; cytoplasmic streaming; endocytosis and exocytosis present
Cell division	Chromosome pulled apart by attachments to plasma membrane	Chromosome pulled apart by cytoskeletal spindle apparatus
Cellular organization	Mainly unicellular	Mainly multicellular with differentiation of many cell types

Table 1.2 Some major differences between eukaryotic and prokaryotic cells with respect to genetic organization and cytoplasmic structures

	Eukaryotes	Prokaryotes
Genetic organization		
Nucleoplasm bounded by a membrane	+	-
Number of chromosomes	>1	1 ^s
Histones in chromosomes	+	-
Nucleolus	+	-
Nuclear division by mitosis	+	-
DNA in other organelles	+	-
Fusion of gametes	+	-
Formation of partial diploids by unidirectional transfer of DNA	-	+
Cytoplasmic structures		
Endoplasmic reticulum	+	-
Golgi apparatus	+	-
Lysosomes	+	-
Mitochondria	+	-
Chloroplasts	+/-	-
Ribosomes	80 S (cytoplasmic) 70 S (organellar)	70 S
Microtubular systems	+	-
Organelles bounded by nonunit membrane	-	+/-

Presence of cell wall containing peptidoglycan	-	+/-
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^SSome genetic information may be present in separate genetic elements (plasmids) '+' or '-' indicates presence or absence of structure

Table 1.3. The structural organization of eukaryotic cells.

Cell structure	Molecular composition	Properties and functions
Cellwall (absent in animal cells)	About 20nm thick, made up of cellulose fibrils encased in a cement of polysaccharides and proteins	Protects the cell; maintains rigid cell surface
Cell membrane	About 9nm thick made up of about equal amounts of lipids and proteins, lipids are arranged in a bilayer	Transport of ions and molecules, recognition; receptors for small and large molecules; cell morphology and movement
Nucleus	About 4-6 μ m in diameter surrounded by a perinuclear envelope; contains DNA associated with histones and organized into chromosomes	DNA synthesis and repair RNA synthesis
Nucleolus	Rich in RNA	RNA processing; ribosome synthesis
Mitochondria	About 1 μ m diameter; outer and inner membranes differ in lipid composition and in enzymatic activity, enzymes of TCA cycle are largely in matrix while that of electron transport and energy conversion are in inner membrane; contains a specific type of DNA	Energy conservation cellular respiration; oxidation of carbohydrates and lipids; urea and heme synthesis; control of cytosolic [Ca ²⁺]
Chloroplast (algae and green plants only)	Membrane surrounded organelle possessess a distinctive DNA; contains green pigment called chlorophyll	Site of photosynthesis
Golgi complex	Made up of flattened, single membrane vesicles which are stacked often, some become vacuoles in which secretory products are concentrated	Export of proteins, modification and sorting of proteins for incorporation into organelles
Endoplasmic reticulum	Consists of flattened single membrane vesicles whose inner compartment, the cisternae interconnect to form channels through the cytoplasm, a number of enzymes are associated for the synthesis of sterols, triglycerols, phospholipids, etc.	Membranes synthesis; synthesis of proteins and lipids for cell organells and for export; lipid synthesis; detoxidation reactions
Microbody (Peroxisome)	Single membrane vesicles of about 0.5 μ m in diameter, contain catalase, D-amino acid	Oxidative reactions involving oxygen, utilization of

	oxidase, urate oxidase and other oxidative enzymes	hydrogen peroxide
Lysosome (animal cells and protozoans)	Single membrane vesicles 0.25 – 0.5 μm in diameter, rich in hydrolytic enzymes such as ribonuclease and phosphatase	Cellular digestion; hydrolysis of proteins, carbohydrates, lipids and nucleic acids
Ribosome	80S type, contains 50 per cent of each protein and RNA	Protein synthesis
Vacuole	Single membrane vesicles; contain dissolved sugars, salts of organic acids, proteins, mineral salts, pigments, O_2 and CO_2	Segregation of cellular waste products
Cytosol	Soluble portion of the cytoplasm; highly viscous; rich in glycolytic enzymes, metabolic intermediates and inorganic salts	Metabolism of carbohydrates, lipids, amino acids, nucleic acids, protein biosynthesis
Microtubules and micro filaments	The microtubules (25 nm diameter) and microfilaments (7 nm diameter) made up of globular subunits, tubulin and actin, respectively	Cell cytoskeleton; cell motility, cell morphology; intracellular movements

Table 1.4 Intracellular location of major enzymes and metabolic pathways

Cell structure	Major enzymes and metabolic pathways
Cytoplasm	Glycolysis; hexose monophosphate pathway; Glycogenesis and glycogenolysis; fatty acid synthesis; purine and pyrimidine catabolism; peptidases; aminotransferases, aminoacyl synthetases
Mitochondria	TCA cycle; fatty acid oxidation; amino acid oxidation; fatty acid elongation; urea synthesis; electron transport and coupled oxidative phosphorylation
Lysosomes	Lysozyme; acid phosphatases; hydrolases including proteases, nucleases, glycosidases, arylsulfurases, lipases, phospholipases and phosphatases
Endoplasmic reticulum (microsomes)	NADH- and NADH-cytochrome c reductases; cytochrome b_5 and cytochrome P450 related mixed function oxidases; glucose-6-phosphatase; esterase; β -glucuronidase; glucuronyl-transferase; protein synthetic pathways; phosphoglyceride and triacylglycerol synthesis; steroid synthesis and reduction
Golgi bodies	Galactosyl- and glucosyltransferase; chondroitin sulfotransferase; 5'nucleotidase; NADHcytochrome c reductase; glucose-6-phosphatase
Peroxisomes	Urate oxidase; D-amino acid oxidase; α -hydroxy acid oxidase; catalase; long-chain fatty acid oxidation

Nucleus	DNA and RNA biosynthetic pathways
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1.6.2. Chemical Composition

All living organisms show a remarkable similarity in the nature of the chemical substances present in them. Water is the principal constituent of living organisms. they contain about 70 per cent water by weight. The dry weight of cells is composed primarily of four classes of larger organic compounds or biomacromolecules – carbohydrates, lipids, proteins and nucleic acids, in addition, there are some small organic molecules and inorganic minerals. Though there is considerable variation in the chemical composition of different cells, an average composition may be given as in Table. 1.7

Table 1.7: Chemical composition of a cell

Components	Approx. % dry weight
Proteins	71
Lipids	12
Nucleic acids	7
Carbohydrates	5
Inorganic minerals and other materials	5

CHAPTER 2 CARBOHYDRATES

Based on chemical constitution, the carbohydrates or saccharides are most simply defined as polyhydroxy aldehydes or ketones and their derivatives. Many have the empirical formula $(\text{CH}_2\text{O})_n$ which originally suggested that they were 'hydrates' of carbon. Chemically, carbohydrates are composed of carbon, hydrogen and oxygen and most of them have hydrogen and oxygen in the ratio of 2:1 for each carbon. More complex carbohydrates may contain nitrogen, phosphorus or sulphur in addition to carbon, hydrogen and oxygen.

2.1 Occurrence

Carbohydrates are the most abundant of all biochemical compounds and constitute more than 50 percent of the total biochemical matter. They are widely distributed in plants, animals and microbes. They are synthesized in green plants and algae from water and CO_2 using solar energy in a process called photosynthesis. Included in the category of carbohydrates are the sugars, the glycogens, the starches and the celluloses. In addition, there are complex carbohydrates such as glycoproteins, glycolipids, lipopolysaccharides, etc. The major sources of some of the important carbohydrates are listed in Tables 2.1, 2.2 and 2.3.

2.2 Physiological role and biological importance

The carbohydrates serve many functions in the living organisms.

Some of their vital functions are:

1. Chief source of energy (4 kcal/g).
2. Reserve or storage forms of energy in plants (starch, inulin) and animals (glycogen).
3. Structural elements in plant cell wall (cellulose), exoskeleton of some insects and crustacea (chitin), cell walls of certain microorganisms (peptidoglycans) and skin and connective tissues of animals (mucopolysaccharides).
4. Important components of nucleic acids, co-enzymes and flavoproteins (for example, ribose) and
5. They are involved in cell recognition, contact inhibition and also have antigenic properties of blood group substances.

2.3 Classification

The carbohydrates can be classified into three main groups as: a) monosaccharides, b) oligosaccharides and c) polysaccharides, based on number of monomeric sugar units present.

Monosaccharides are the simplest sugars consisting of single polyhydroxy aldehyde or ketone group that cannot be hydrolyzed into smaller units under reasonable

mild conditions. They serve as the building-blocks for the more complex sugars.

Oligosaccharides (Greek Oligo 'few') contain from two to ten monosaccharide units joined through glycosidic linkage or bond. They are hydrolyzable into constituent monosaccharide units.

Polysaccharides are polymers of monosaccharide units joined in long linear or branched chains through glycosidic bonds. Hydrolysis of polysaccharides yields many units of constituent monosaccharides. Polysaccharides have two major biological functions: a) as a storage form of fuels and b) as structural elements in living organisms.

2.4 Monosaccharides (simple sugars)

Monosaccharides, also called as simple sugars have the empirical formula $(\text{CH}_2\text{O})_n$, where $n=3$ or larger number. They contain a short chain of carbon atoms with one carbonyl group, each of the remaining carbon atoms bearing a hydroxyl group. If the carbonyl group is an aldehyde (-CHO) the sugar is called as an aldose (name ends in 'ose') and if a ketone (C=O) it is a ketose (usually ends in 'ulose').

The simplest monosaccharides are the 3-carbon trioses glyceraldehyde and dihydroxyacetone (Fig. 2.1)

Glyceraldehyde is an aldotriose; dihydroxyacetone is a ketotriose. Other monosaccharides are tetroses (four carbons), pentoses (five carbons), hexoses (six carbons), heptoses (seven carbons) and octoses (eight carbons). Each exists in two series, ie., aldotetroses and ketotetroses; aldopentoses and ketopentoses; aldohexoses and ketohexoses, etc. The configurational relationships and the structures (open chain form) of the D-aldoses and D-ketoses are shown in Figs. 2.2 and 2.3, respectively.

Of these monosaccharides, hexoses (both aldoses and ketoses) are the most abundant. Glucose (aldohexose) is the most abundant monosaccharide; serves as the major fuel for most organisms and the basic building-block of the many oligo- and polysaccharides. However, aldopentoses are important components of nucleic acids (for example, ribose) and various polysaccharides (for example, xylose and arabinose). Trioses, tetroses and heptoses are important intermediates in carbohydrate metabolism. The major sources and functions of some important monosaccharides are given in Table 2.1.

Table 2.1 Some important monosaccharides.

Name	Major source	Function	Type	No.of carbons
Xylose (wood sugar)	Hydrolysis of wood, straw, seed hulls	Constituent of wood, straw, seed hulls	Aldopentose	5

Arabinose	Hydrolysis of gum arabic, cherry tree gum, pectin	Constituent of gum arabic	Aldopentose	5
Ribose	Hydrolysis of nucleic acids	Constituent of nucleic acids	Aldopentose	5
Glucose (dextrose)	Ripe fruits, sweet corn, honey, blood, egg yolk	Energy source	Aldohexose	6
Mannose	Hydrolysis of mannans	Constituent of mannans	Aldohexose	6
Galactose	Hydrolysis of lactose	Constituent of milk sugar	Aldohexose	6
Fructose (levulose)	Honey, sweet fruits	Energy source	Ketohexose	6

2.4.1 General properties

Some of the general properties of monosaccharides are summarized below:

1. Monosaccharides are polyhydroxy aldehydes or ketones and their derivatives having either a potentially free aldehyde or a ketone group.
2. Simplest form of carbohydrates which cannot be hydrolyzed to other sugar units under reasonably mild chemical conditions.
3. Generally monosaccharides are white crystalline solids, insoluble in ether, sparingly soluble in alcohol but readily soluble in water.
4. Most of them have a sweet taste and char when heated.
5. Those with potentially a free aldehydic or a ketonic group are able to reduce metal ions under alkaline conditions. Hence, they are excellent reducing agents.
6. Amphoteric nature i.e., they are capable of reacting as weak acids or weak bases with strong acids or alkalis to form salts.

Stereochemistry

All the monosaccharides except dihydroxyacetone contain one or more asymmetric carbon atom(s) i.e., a single carbon atom having four different substituents and thus are chiral molecules. Subcompounds are capable of existing in two or more isomeric forms that are non-superimposable mirror images of each other. Such compounds exist in right-handed forms and are called chiral (hand) compounds. This phenomenon is called chirality (handedness).

Glyceraldehyde contains only one asymmetric carbon atom (carbon atom, 2) and therefore can exist as two different stereoisomers, i.e., as D- and L- glyceraldehyde. The symbols D- and L- designate the absolute configuration of an isomer and not the sign of rotation of plane-polarized light. The structure with -OH group on the right and -CHO

group on the top of the asymmetric carbon atom is designated as D-glyceraldehyde. The structure in which the -OH group is to the left and -CHO group on the top is designated as L-glyceraldehyde (Fig. 2.4).

The D- and L-glyceraldehydes are used as reference or parent compounds for designating the absolute configuration of all stereoisomeric compounds. The term configuration refers to the special arrangement of the atoms in a molecule resulting from the double bonds and/or chiral centers. Configurational isomers cannot be interconverted without breaking one or more covalent bonds.

If a molecule has more than one asymmetric carbon atom, it can exist in 2^n stereoisomeric forms, where 'n' represents the number of asymmetric carbon atoms present. Thus, aldotrioses, aldotetroses, aldopentoses and aldohexoses have 1, 2, 3 and 4 asymmetric (chiral) carbon atoms and exist in 2, 4, 8 and 16 stereoisomeric forms respectively. For sugars having two or more asymmetric carbon atoms, the convention has been adopted that the prefixes D- and L- refer to the asymmetric carbon atom farthest removed from the carbonyl carbon atom (or penultimate carbon atom or the last but one carbon atom).

Figs. 2.2 and 2.3 show the Fischer projectional formulae of D-aldoses and D-ketoses, respectively. All have the same configuration at the asymmetric carbon atom farthest from the carbonyl carbon atom. But, the number of stereoisomeric forms depends on the number of asymmetric carbon atoms.

Enantiomers

Aldoses and ketoses of the L-series are mirror-images of their D-counterparts as shown in Fig. 2.5. These two D- and L- forms of a sugar are known as enantiomers. L-sugars are found in nature, but they are not so abundant as D-sugars.

Diastereoisomers

Two sugars having the same molecular formulae but not the mirror images of each other are known as diastereoisomers. e.g. D-glucose and D-mannose. All these sugars are not mirror images of each other (Fig. 2.2).

Epimers

Two sugars differing only in the configuration around one specific carbon atom are called epimers of each other. Thus, D-glucose and D-mannose are epimers with respect to carbon atom 2, and D-glucose and D-galactose are epimers with respect to carbon atom 4 (Fig. 2.2).

8. Optical activity

All the monosaccharides except dihydroxyacetone contain one or more asymmetric carbon atom(s) and thus are optically active. Optical activity refers to the

ability of a compound in solution to rotate the plane of polarization of plane-polarized light when observed in a polarimeter. Optical activity is shown by all compounds capable of existing in two forms that are non-superimposable mirror images of each other.

The optical activity is expressed quantitatively as the specific rotation:

$$[\alpha]_D^{25} = \frac{\text{Observed rotation (in degrees)} \times 100}{\text{Optical path length (dm)} \times \text{concentration (g/100ml)}}$$

Where, α = specific rotation in degrees at temperature usually 25°C and the wavelength of the light employed (usually the D line of sodium) is 589.3nm.

If the rotation of the beam of plane-polarized light is clockwise (to the right or rectus as the observer looks towards the light source), the enantiomer is designated as dextrarotatory (dextro, 'd' or '+' symbols) and if it is anticlockwise (to the left or sinister), the enantiomer is designated as levorotatory (levo, 'l' or '-' symbols). For example, the specific rotation of α -D-glucose is + 112.2° (dextrarotatory) and that of D-fructose is -93° (levorotatory). Thus, the symbol '+' and '-' refer to the direction of rotation of the beam of plane-polarized light but not the absolute configuration.

The D-and L-stereoisomers of any given compound have identical physical properties and identical chemical reactivities, with two exceptions: (a) they rotate the plane of plane-polarized light equally but in opposite directions and (b) they react at different rates with reagents that are themselves asymmetric. The equimolar mixture of the D- and L-stereoisomers, known as racemic mixture or racemate (designated as D L-) is optically inactive as the asymmetric carbon atom passes through a symmetrical intermediate during chemical reaction.

2.4.2 Structural aspects

1. Ring structure and mutarotation

In aqueous solution, many monosaccharides act as if they have one more asymmetric center than is given by the open chain structural formulae as in Figs. 2.2 and 2.3. D-glucose may exist in two different isomeric forms differing in specific rotation, α -D-glucose, for which $[\alpha]_D^{20} = + 112.2^\circ$, and β -D-glucose, for which $[\alpha]_D^{20} = +18.7^\circ$. These two sugars do not differ in elementary composition but differ in physical and chemical properties. When the α - and β -isomers of D-glucose are dissolved in water, the optical rotation of each gradually changes with time and approaches a final equilibrium value of $[\alpha]_D^{20} = +52.7^\circ$ (Fig. 2.6). This change, called mutarotation is due to the formation of an equilibrium mixture consisting of about one-third α -D-glucose and two thirds β -D-glucose at 20°C.

Although carbohydrates are formally aldehydes or ketones, a sugar like glucose

does not readily answer the normal reactions of aldehydes as would be expected; this is because of their existence as cyclic hemiacetals or hemiketals. Aldehydes can react with an alcohol to form a hemiacetal; similarly ketones can react with an alcohol to form a hemiketal (Fig. 2.7).

Anomers

From various chemical considerations it has been deduced that the α - and β -isomers of D-glucose are not open-chain structures in aqueous solution but six-membered ring structures formed by the reaction of the alcoholic hydroxyl group at carbon atom 5 with the aldehydic carbon atom 1 to form a hemiacetal which renders an other chiral center at carbon atom 1, also known as carbonyl carbon atom or anomeric carbon atom. Isomeric forms of monosaccharides that differ from each other only in configuration about the carbonyl carbon atom are known as anomers. Thus, D-glucose will have two anomers designated as α -D-glucose and β -D-glucose.

As hemiacetal or hemiketal formation is reversible, if one of the anomers is dissolved in water, an equilibrium mixture of the two anomers results. This interconversion between the two anomers is due to mutarotation. The cyclic hemiacetal formation in the case of glucose by the reaction of the alcoholic hydroxyl group at carbon 5 with the aldehydic carbon atom 1 results in formation of six-membered ring. The six-membered ring forms of sugars are called pyranoses because they are derivatives of the heterocyclic compound pyran (Fig. 2.8). Thus, the systematic name for the ring form of α -D-glucose is α -D-glucopyranose.

In the case of fructose, the hemiketal is formed by reaction of the hydroxyl group on carbon atom 5 with the carbonyl group at carbon atom 2 to yield a five-membered ring. The five-membered ring forms of sugars are called furanoses as they are derivatives of heterocyclic compound, furan (Fig. 2.8) as suggested by Haworth. The systematic name for the ring form of α -D-fructose is α -D-fructofuranose.

Accordingly, the Haworth perspective formulae of some monosaccharides can be written as in Fig. 2.9. Haworth formulae may be used to indicate the ring forms of monosaccharides.

The substituents on the carbon atoms are represented as extending above or below the plane of the ring. The lower half of the ring is thickened to indicate that it is the portion of the ring that is directed out of the plane of the paper towards the reader.

A few simple rules to transform Fischer's projection formula to correct Haworth perspective formula are the following:

1. If the ring closes on a -OH which is on the right of the Fischer's projection, the

hydroxymethyl group (-CH₂OH 'tail') points up; if it closes on a -OH group on the left, the tail points down

2. The ring -OHs points down if they are on the right in the Fisher's projection, and up if they are on the left
3. The -OH group on the anomeric carbon atom in the D-series will be down if it is α - and up if β -

Sugar conformation

Conformation denotes the arrangement in space of atoms in a molecule which can be achieved by rotation about single bonds. Although, the Haworth formulae give a better indication of the true structures of sugars than do the straight chain forms, they do not represent the actual conformations. Six-membered rings can exist in a number of conformations of which the chair and boat forms are comparatively stable.

In the chair form, half the bonds of the ring are oriented 'up and down, while the other half are oriented essentially in the plane of the ring. Substituents linked by bonds oriented up and down are called 'axial' while those linked by bonds in the plane of the ring are called 'equatorial' (Fig. 2.10).

2.4.3 Reactions of sugars

The carbohydrates, because of the various reactive groups present in the molecule, undergo a large number of chemical reactions.

2.4.3.1 Reactions of the carbonyl group

1. With dilute alkali

Dilute aqueous bases at room temperature cause rearrangements about the anomeric carbon atom and its adjacent carbon atom without affecting substituents at other carbon atoms. Treatment of D-glucose with dilute alkali yields an equilibrium mixture of D-glucose, D-fructose and D-mannose. This reaction involves intermediate enol forms, called enediols of the hydroxy aldehyde and hydroxy ketone structures of carbon atoms 1 and 2.

2.Reducing property of sugars

The enediols formed above are reactive species. They are good reducing sugars. When glucose is heated with an alkaline solution of Cu²⁺ ions, the Cu²⁺ is reduced to Cu⁺ which is precipitated as Cu₂O. This is the basis for the estimation of reducing sugars.

3. Reduction to alcohols

The carboxyl group of monosaccharides can be reduced by hydrogen gas in the presence of metal catalysts or by sodium amalgam in water to form the corresponding sugar alcohols (Fig. 2.11). For example, D-glucose on reduction yields D-glucitol (also

called as L-sorbitol) while D-mannose yields D-mannitol.

Sugar alcohols occur in nature, particularly in plants. One such alcohol, glycerol is an essential component of lipids. Myo-inositol, a stereoisomer of inositol is found as a component of phosphoglycerides and also in phytic acid, the hexaphosphoric ester of inositol.

4. Reactions with carbonyl reagents

The carbonyl group of monosaccharide reacts with carbonyl reagents like hydrazine, phenyl hydrazine, hydroxylamine or semicarbazide to yield crystalline hydrazone, phenylhydrazone, oxime or semicarbazide respectively. Further, reaction of hydrazone or phenylhydrazone with excess of the reagent gives rise to osazones whose characteristic crystalline structures are used to identify the sugars. The structure of the phenylosazone of D-glucose is given in Fig. 2.12.

5. Reactions with concentrated acids

Monosaccharides are generally stable to hot dilute mineral acids even on heating. When aldohexoses are heated with strong mineral acids, however, they are dehydrated, and 5-hydroxymethyl furfural is formed.

This dehydration reaction is the basis of certain qualitative tests for sugars, since the furfurals can be reacted with α -naphthol and other aromatic compounds to form characteristic coloured products.

6. Glycoside formation

Sugar hemiacetals and hemiketals can react with alcohol in the presence of a mineral acid to form anomeric α - and β -glycosides. Glycosides are asymmetric mixed acetals formed by the reaction of the anomeric carbon atom of the intramolecular hemiacetal or pyranose form of the aldohexose with a hydroxyl group of an alcohol. This is called a glycosidic bond. The anomeric carbon in such glycosides is asymmetric.

The glycoside linkage is also formed by the reaction of the anomeric carbon of a monosaccharide with a hydroxyl group of another monosaccharide to yield a disaccharide. Oligo- and polysaccharides are chains of monosaccharides joined by glycosidic linkages.

D-glucose with methanol in the presence of HCl yields methyl α -D-glucopyranoside or methyl- β -D-glucopyranoside (Fig. 2.13).

2.4.3.2 Reactions of the alcoholic group

1. Acylation

The free hydroxyl groups of monosaccharides and polysaccharides can be acylated

by reaction with an acylchloride or acetic anhydride to yield O-acyl derivatives which are useful in determining the structure. Treatment of α -D-glucose with excess of acetic anhydride yields penta-O-acetyl α -D-glucose (Fig. 2.14). The resulting esters can be hydrolyzed by alkali.

2.Methylation

The methylation of the hydroxyl group on the anomeric carbon atom occurs readily with methanol in the presence of acid to yield methyl glycosides, which are acetals. The remaining hydroxyl groups of monosaccharides require much more drastic conditions for methylation, e.g. treatment with dimethyl sulfate or methyl iodide and silver oxide which yield methyl ethers not methyl acetals. Methylation of all the free hydroxyl groups of a carbohydrate is called exhaustive methylation. These ether derivatives are resistant to hydrolysis unlike the glycosides. Methylation studies are important in structural analysis of carbohydrates.

Methylation of methyl α -D-glucopyranoside for example, yields methyl 1,2, 3, 4, 6-tetra-O-methyl-D- glucopyranoside.

3.Periodate oxidation

Periodic acid (HIO_4) will oxidize and cleave the C-C bonds which contain adjacent free hydroxyl groups or a hydroxyl group and a carbonyl grouping. For example, in the case of glycerol, C-2 is oxidized two times so that it is converted into formic acid while C-1 and C-3 are converted only to formaldehyde. In case of free sugars, like glucose, all the bonds are broken by periodate oxidation.

Periodate oxidation studies like the exhaustive methylation technique have been useful in the elucidation of the structure of carbohydrates.

2.4.4 Sugar derivatives

2.4.4.1 Sugar acids

There are three important types of sugar acids: aldonic, aldaric and uronic acids (Fig. 2.15).

1. Aldonic acids: These are obtained by the mild oxidation of aldoses when only the aldehyde group is oxidized. Halogens at pH 5.0 oxidize aldoses to the corresponding lactones which then hydrolyze slowly to give monocarboxylic acids. For example, D-glucose yields D-gluconic, acid which in phosphorylated form is an important intermediate in carbohydrate metabolism.

2. Aldaric acids: These are dicarboxylic acids derived from aldoses by oxidation. of both the aldehydic carbon atom and the carbon atom bearing the primary hydroxyl group using

a strong oxidizing agent like nitric acid. D-Glucose gives D-glucaric acid while D-galactose gives mucic acid on oxidation. Aldaric acids are sometimes useful in the identification of sugars.

3. Alduronic acids: These are sugar acids in which the terminal primary alcoholic group of sugar is oxidized to a carboxyl group; the reducing aldehydic group at the other end remains unaltered. These acids are biologically very important. The uronic acids are components of many polysaccharides.

An important sugar acid is L-ascorbic acid or vitamin C which is the γ -lactone of hexanoic acid having an enediol structure between C-2 and C-3. Ascorbic acid is unstable and readily undergoes oxidation to dehydroascorbic acid. Ascorbic acid is present in large amounts in citrus fruits and tomatoes.

2.4.4.2 Sugar phosphates

Phosphate derivatives of monosaccharides are found in all living cells, in which they serve as important intermediates in carbohydrate metabolism. Representative sugar phosphates are shown in Fig. 2.16.

2.4.4.3 Deoxy sugars

If one or more hydroxyl groups of sugars are replaced by hydrogen atoms, the resulting compounds are known as deoxy sugars. The important deoxy sugars are 2-deoxy-D-ribose, a constituent of DNA, 6-deoxy-L-mannose (L-rhamnose) which is present in many plant polysaccharides and 6-deoxy-L-galactose (L-fucose) present in many glycoproteins like the blood group substances (Fig. 2.17).

2.4.4.4 Amino sugars

These are sugars in which a hydroxyl group is replaced by an amino group. The most common sugars are D-glucosamine (2-amino-1-deoxy-D-glucose) and D-galactosamine (2-amino-2-deoxy-galactose) in which the hydroxyl group at carbon atom 2 is replaced by an amino group (Fig. 2.18). D-Glucosamine occurs in many polysaccharides of vertebrate tissues and is also a major component of chitin. D-Galactosamine is a component of glycolipids and of the major polysaccharide of cartilage, chondroitin sulfate.

Two other important derivatives of amino sugars are muramic acid and neuraminic acid (Fig. 2.19). These can be considered as six-carbon amino sugars linked to a three carbon acid. Muramic acid is D-glucosamine to which a lactic acid moiety is attached at C-3. Neuraminic acid is derived from D-mannosamine and pyruvic acid by an aldol condensation. The two compounds are usually acetylated. N-acetylmuramic acid is a component of bacterial cell wall material. The acetylated derivative of neuraminic acid (NANA), known as sialic acid, is a component of glycoproteins present in animal cell

membrane.

2.5 Oligosaccharides

These sugars consist of a short chain of 2 to 8 or 10 monosaccharide units linked by the glycosidic bond (s) with the elimination of water molecule (s). The glycosidic bond is formed most frequently between the anomeric carbon of one sugar residue and a hydroxyl group of the other sugar residue. Depending on the number of monosaccharide units that are linked, the oligosaccharides are further classified as disaccharides (two sugar units), trisaccharides (three sugar units), tetrasaccharides (four sugar units), etc. Amongst these, disaccharides are the most important class because of their biological role and relative abundance in natural products.

2:5.1 Disaccharides ($C_{12}H_{22}O_{11}$) .

These are a group of compound sugars composed of two monosaccharides linked by the glycosidic bond with the elimination of one molecule of water.

2.5.1.1 General properties

1. Those with potentially a free aldehyde or a ketone group can reduce Fehling's solution, hence are called reducing disaccharides.
2. The reducing disaccharides have most of the properties of monosaccharides i.e., they can form osazones and show mutarotation, etc.
3. Disaccharides can be hydrolyzed into their constituent monosaccharide units unlike monosaccharides.
4. Some disaccharides may exist in white crystalline solids and are soluble in water and sweet in taste.
5. Disaccharides are not fermented by yeast directly but they are first hydrolyzed to constituent monosaccharides which in turn are fermented.

The most abundant disaccharides in nature are maltose, sucrose and lactose. The major sources, structural and functional aspects of some of the disaccharides are summarized in Table 2.2.

2.5.1.2 Maltase (4-O- α -D-glucopyranosyl-D-glucopyranose)

It is a disaccharide formed by linking two units of α -D-glucose through α -1,4 glycosidic bond with the elimination of one molecule of water (Fig. 2.20). It is a reducing sugar since the -OH group bound to carbon 1 of the glucose residue is free and can exist in the aldehyde form. It exhibits mutarotation since it exists in both α - and β -forms.

It does not occur in nature but is only formed when starch is hydrolyzed by the enzyme diastase. Sprouting cereal grains have a high content of amylases which split the

starch present to dextrans and maltose. Malt prepared from sprouting barely, is an excellent, source of maltose. Starches are also split to maltose by the amylases present in human saliva and in the pancreatic secretion of man and all animals. Maltose is hydrolyzed to two units of glucose by the enzyme maltase of intestinal juice.

2.5.1.3 Sucrose (α -D-glucopyranosyl- β -D-fructofuranoside)

Sucrose or cane sugar or beet sugar or saccharose or invert sugar is a disaccharide made up of one molecule each of α -D-glucose and β -D-fructose, the linkage involving the potential aldehyde group of carbon atom 1 of glucose and the ketonic group of the carbon atom 2 of fructose (β ,2- \rightarrow 1) linkage, (Fig.2.20). It is a non-reducing sugar because of the absence of a potentially free aldehyde or ketonic group and forms no osazone. As it does not exist in α - and β -forms, it fails to exhibit mutarotation.

It is hydrolyzed by acid or enzyme sucrase (invertase) into glucose and fructose. The specific rotation of sucrose is $+66.5^\circ$ and after hydrolysis, the specific rotation of the mixture is -19.84° . Such a change in specific rotation from dextro- to levorotatory nature is called 'inversion' and hence the name 'invert sugar'. The reason for the inversion is that fructose is more strongly levorotatory (-93°) than glucose which is dextrarotatory ($+52.5^\circ$).

It is the most abundant oligosaccharide and is ubiquitous in plants. It is generally manufactured from sugarcane and sugar beet.

2.5.1.4. Lactose (4-O- β -D-galactopyranosyl-D-glucopyranose)

Lactose or milk sugar is made up of β -D-galactose and α -(in α -form) or β -(in β -form) D-glucose through β -1, 4 glycosidic bond (Fig. 2.20). It is a reducing sugar, exhibits mutarotation and forms osazone. It reduces Fehling's solution but not Barfoed's reagent and thus can be distinguished from other reducing disaccharides. It is hydrolyzed by the enzyme lactase into its constituent hexoses.

It does not ferment as easily as glucose and hence makes an ideal constituent of milk of mammals (about 5 g/100 ml milk). It is not produced in plants.

2.5.1.5 Isomaltose (4-O- α -D-glycopyranosyl-D-glucopyranose)

It is a product of partial hydrolysis of amylopectin of starch and glycogen, made up of two glucose units joined through α -1,6 linkage (Fig.2.20).

2.5.1.6 Cellobiose (4-O- β -D-glucopyranosyl-D-glucopyranose)

It is a partial hydrolytic product of cellulose, made up of two glucose units joined through β -1, 4 linkage (Fig. 2.20). It is a reducing sugar. It is probably present in only trace amounts in nature and formed during the digestion of cellulose by the cellulases of microorganisms.

2.5.1.7 Trehalose (1-O- α -D-glucopyranosyl-1- α -D-glucopyranoside)

It is made up of two glucose units linked through two anomeric carbon atoms (Fig. 2.20). It is a non-reducing sugar. It is the major carbohydrate present in insects and fungi where it serves as a storage carbohydrate from which glucose may be obtained as required.

2.5.2 Trisaccharides (C₁₈H₃₄O₁₇)

A naturally occurring trisaccharide is raffinose [α -D-galactopyranosyl-O-(1,6- α -D-glucopyranosyl-O-(1,2)- β -D-fructofuranoside] found in sugar beet, coffee and other plant materials. It is a non-reducing sugar.

Melezitose[O- α -D-glucopyranosyl(1->3)-O- β -D-fructofuranosyl(2,1)- α -D-glucopyranoside] is found in the sap of some coniferous trees.

2.5.3 Tetrasaccharides

The important one among tetrasaccharides is the stachyose derived from raffinose. Stachyose consists of galactose-galactose-glucose-fructose monosaccharide sugars linked through α -1, 6, α -1, 6 and α -1, 2 glycosidic bonds, respectively. It occurs during germination of seeds.

2.6. Polysaccharides or glycans (C₅H₁₀O₅)_n

These are complex carbohydrates which are polymerized anhydrides of a large but undetermined number of the simple sugars which are joined by glycosidic bonds. Those found in nature contain either five or six carbon monosaccharide units. The bulk of carbon found in nature exists in the form of polysaccharides. These are involved in the majority of biological processes although free monosaccharides and disaccharides occur in many biological fluids and plants.

2.6.1 General properties

Some of the important properties of polysaccharides are as follows:

1. Complex sugars of high molecular weight; polymers of several units of monosaccharides or either derivatives with linear or branched chains.
2. Upon hydrolysis by acids or enzymes, they are broken down into various intermediate products and finally into their constituent monosaccharides or their derivatives.
3. They are tasteless, apparently amorphous, some are crystalline
4. Mostly insoluble in cold water but form a sticky or gelatinous solutions
5. They differ in the nature of their recurring monosaccharides units, in the length of their chains and in the degree of branching

2.6.2 Biological role

Polysaccharides serve two main functions in the living organisms as:

1. Storage form of cellular fuel and
2. Structural elements in animal, plant and microbial systems

The major sources, structural and functional aspects of some of the polysaccharides are summarized in Table 2.3

2.6.3. Classification

Polysaccharides can be classified in many ways

A. Based on function

1. Structural polysaccharides: These polysaccharides serve as structural components of living organisms. e.g. cellulose (plant cell wall), chitin (exoskeleton of some insects), etc.

2. Storage/ reserve / nutrient polysaccharides: These polysaccharides function as reserve or storage form of fuel in living organisms e.g. starch (plants), glycogen (animal cells) etc.

Table 2.3. Structure and functions of some polysaccharides

Name	Source	Function	Monosaccharide units	Linkage	Branching
Starch (α -amylose)	Plant cell walls, rice, wheat, barley, potato	Energy storage	D-Glucose	$\alpha(1\rightarrow4)$	None
Starch (amylopectin)	Plant cell walls, rice, wheat, barley, potato	Energy storage	D-Glucose	$\alpha(1\rightarrow4)$ & $\alpha(1\rightarrow6)$	Branched ($\alpha(1\rightarrow6)$)
Glycogen	Animal cells	Energy storage	D-Glucose	$\alpha(1\rightarrow4)$ & $\alpha(1\rightarrow6)$	Highly branched ($\alpha(1\rightarrow6)$)
Cellulose	Plant cell walls, jute, cotton, straw	Structural component	D-Glucose	$\beta(1\rightarrow4)$	None
Chitin	Fungal cell walls & exoskeleton of insects & crustacea	Structural component	N-Acetyl D-Glucose amine	$\beta(1\rightarrow4)$	None
Peptidoglycan	Bacterial cell wall	Structural component	N-Acetyl D-Glucoseamine & N-acetyl muramic acid	$\beta(1\rightarrow4)$	Cross linked through peptide bonds
Dextran	Bacteria and Yeasts	Extra cellular product	D-Glucose	$\alpha-1, 6;$ $\alpha-1, 2;$ $\alpha-1, 3;$	Some branching ($\alpha-1, 6$)

Pectin	Plants	Structural component	D-Galacturonic acid & its methyl esters	α -1, 4	None
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B. Based on composition:

1. Homopolysaccharides: These are made up of single kind of monosaccharide residues or their derivatives. e.g. Starch, glycogen, cellulose, chitin, inulin, etc.
2. Heteropolysaccharides: These are made up of two or more different kinds of monosaccharide units or their derivatives. e.g. Hyaluronic acid, heparin, pectins, gums, mucilages, chondroitins, etc.

Polysaccharides are often called as glycans. Those containing glucose are called as glycans (starch and glycogen); those containing mannose are called mannans and those containing galactose units are called galactans.

2.6.4 Structural polysaccharides

2.6.4.1 Cellulose

It is the most abundant organic compound of our planet accounting for about 50 per cent of all carbon. It is the principal constituent of cell walls in higher plants forming the main structural element. It is a linear homopolymer of glucose units linked by β -1,4 glycosidic bonds (Fig. 2.21). It is insoluble in water and all organic solvents. It dissolves in conc. H_2SO_4 , on diluting the solution and boiling, glucose is formed as final product. Partial hydrolysis of cellulose yields cellobiose, a disaccharide. Cellulase, a β -glucosidase produced by many bacteria and fungi, hydrolyzes cellulose. The large amount of glucose present in cellulose is not available as a source of energy for humans due to the lack of enzymes capable of cleaving the β -1,4 bonds. However, ruminants can effectively use cellulose as they contain a large bacterial population in their rumen capable of hydrolyzing it.

In plant cell walls, cellulose microfibrils are cemented together by other substances important among them being pectin and hemicellulose. Pectins contain arabinose, galactose and galacturonic acid while hemicelluloses are homopolymers of D-xylose linked by β -1,4 bonds. The important sources of cellulose are cotton fibers (98%), jute (50-70%), wood (40-50%), algae and bacteria. Cellulose and its derivatives are widely used in textiles, films and plastics.

2.6.4.2 Chitin

It is a structural homopolysaccharide made up of N-acetyl glucosamine residues in β -1,4 linkage (Fig. 2.21). It is the principal structural polysaccharide present in the exoskeleton of crustaceous insects, earthworms and mollusks. It is the second most abundant organic substance on earth.

2.6.4.3 Peptidoglycan (murein)

It is a structural heteropolysaccharide present in bacterial cell walls. The repeating unit of peptidoglycan is the muropeptide which is a disaccharide composed of N-acetyl-D-glucosamine (NAG) and N-acetyl muramic acid (NAMA) joined by a, β -1,4 glycosidic bond. NAMA consists of a NAG unit which has its C-3 hydroxyl group joined to the hydroxyl group of lactic acid by an ether linkage.. In the peptidoglycan the carboxyl group of each lactic acid moiety is in turn linked to a tetrapeptide consisting of L-alanine, D-isoglutamine, L-lysine and D-alanine (Fig. 2.21). The terminal D-alanine residue of the side chain of one polysaccharide chain is joined covalently with the peptide side chain of an adjacent polysaccharide chain, either directly as in *E.coli* or through a short connecting peptide, e.g. The pentaglycine in *Staphylococcus aureus* (Fig. 2.21)

The peptidoglycan structure of the bacterial cell wall is resistant to the action of peptide-hydrolyzing enzymes, which do not attack peptides containing D-amino acids. However, the enzyme lysozyme, found in tears and in egg white, hydrolyzes the $\beta(1\rightarrow4)$ glycosidic bonds of the polysaccharide backbone of the peptidoglycan structure.

2.6.5 Reserve or storage polysaccharides

2.6.5.1 Starch

It is a principal storage homopolysaccharide of the plant kingdom, made up of D-glucose as repeating units. It is a mixture of two components amylose (about 20%) and amylopectin (about 80%) Amylose consists of long unbranched chains of D-glucose units which are linked by α -1, 4 glycosidic bonds (Fig 2.22). Its molecular weight ranges from a few thousands of about 500,000. It gives blue colour with iodine due to the iodine-amylose complex in which iodine molecule is occupying a position in the interior of the helical coil. Amylopectin also has a backbone of α -1, 4 linked glucose units but in addition, branched through α -1, 6 linkages (Fig. 2.22). The average length of branching is from 24 to 30 glucose residues. It gives a purple colour with iodine. Its molecular weight may range from 50,000 to 1,000,000.

Both amylose and amylopectin can be hydrolyzed by the enzymes α - and β -amylases. α -amylases cleave α -1,4 linkages at random to give the mixture of maltose and glucose units while the β -amylases, present in plants remove maltose units successively from the non reducing end. The intermediate product left after the cleavage of starch by α and β amylases is called limit dextrins. Neither of these enzymes can hydrolyze α -1,6 linkages. Microbial glucomylase can act on both α -1, 4 and α -1, 6 linkages of starch to yield glucose.

Starch forms the major source of carbohydrates in the human diet and is of great economic importance. The important source of starch are seeds, fruits, tubers, bulbs and cereal grains varying from a few per cent of over 75 per cent. It is also found in some protozoa, bacteria and algae.

2.6.5.2 Glycogen

It is the storage homopolysaccharide in animals and is often called ‘animal starch’. It is present mainly in liver, skeletal muscle and in smaller amounts in all other tissues. It is stored in liver and muscles of animals and split to glucose in the liver to maintain proper concentration of glucose in the blood to furnish energy. The amount of glycogen present in the animal varies widely among the different tissues with diet and physiological state of the body. It is also abundant in the mollusks while glycogens like polysaccharides are found in some bacteria.

Glycogen is a branched chain of D-glucose units resembling amylopectin of starch. However, the branching through α -1, 6 linkages is more extensive than amylopectin, with 8-10 glucose units between branching points. A glycogen molecule may contain as many as 30,000 glucose units. It is readily dispersed in water to form an opalescent solution which gives a reddish brown colour with iodine. It does not reduce Fehling’s solution.

2.6.5.3. Insulin

It is a storage polysaccharide in the Compositae family (artichokes, dahlias, dandelions, etc). It is a homopolymer made of D-Fructose units linked by β (2→1) bonds.

2.6.5.4 Dextrans

These are storage polysaccharides of some yeasts and bacteria. They consist of D-glucose units joined by α -1, 6 glycosidic bonds primarily with cross linkages through α -1,2 and α -1, 3 linkages.

2.6.6. Acid mucopolysaccharides

These are a group of related heteropolysaccharides usually containing two types of alternating monosaccharide units of which atleast one has an acidic group either a carboxyl or sulfuric group. When they occur as complexes with specific protein they are called mucins or mucoproteins. Mucoproteins are jelly like, sticky or slippery substances; some provide lubrication and some function as a flexible intercellular cement.

The most abundant acid mucopolysaccharide is hyaluronic acid present in cell coats and in connective tissues of vertebrates. The repeating unit of hyaluronic acid is a disaccharide made up of D-glucotronic acid and N-acetyl –D-glucosamine in β (1->3) linkage (Fig. 2.23). Each disaccharide unit is attached to the next by β (1->4) linkages, hence hyaluronic acid contains alternating β (1->3) and β (1->4) linkages

Table 2.4. Structure and function of acid mucopolysaccharides

Name	Source	Function	Monosaccharide units	Linkage
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Hyaluronic acid	Higher animals	Component of connective tissues & viscous fluids of the body	D-Glucuronic acid & N-acetyl-D-glucosamine	$\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$
Chondroitin sulfate	Higher animals	Component of cartilage, tendons & skin	D-glucuronic acid, N-acetyl-D-galactosamine with sulfate esters at 4 th or 6 th carbon atom of galactosamine	$\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$
Heparin	Mast cells, lungs	Anticoagulant properties	L-Iduronic acid, D-glucuronic acid & N-sulfo-D-glucosamine with several O-sulfate groups	$\alpha(1\rightarrow4)$

Another acid mucopolysaccharide is chondroitin, which is nearly identical in structure to hyaluronic acid the only difference is that it contains N-acetyl-D-galactosamine instead of N-acetyl-D-glucosamine residues. The sulfuric acid derivatives, chondroitin-4-sulfate (chondroitin A) and chondroitin-6-sulfate (chondroitin C) are major structural components of cell coats, cartilage, bone, cornea and other connective tissues in vertebrates.

2.7 Glycoproteins

Glycoproteins are molecules composed of covalently joined protein and carbohydrates. The carbohydrate is attached to the polypeptide chains of the protein in a series of reactions that are enzymatically catalyzed after the protein component is synthesized.

Glycoproteins in cell membranes apparently have an important role in the group behavior of cells and other biological functions of the membrane. They form a major part of the mucus that is secreted by epithelial cells, where they perform an important role in lubrication and in the protection of tissues lining the body's ducts. Many other proteins secreted from cells into extracellular fluids are glycoproteins. These proteins include hormone proteins found in blood, such as follicle stimulating hormone (FSH), lutenizing hormone (LH), chorionic gonadotropin; and plasma proteins such as immunoglobulins etc. Glycoproteins are also one of the major components of the cell coats of higher organisms.

The carbohydrate percent within glycoproteins is highly variable. Some glycoproteins such as IgG contain low amounts of carbohydrate (4 %). Human ovarian cyst glycoprotein is composed of 70 per cent carbohydrate and human gastric

glycoprotein is 82 per cent carbohydrate. Glycoproteins having a very high content of carbohydrate are called proteoglycans.

CHAPTER 3

LIPIDS

The term 'lipid' (Greek, 'lipos'-fat) was proposed by the biochemist Bloor to describe a naturally occurring heterogeneous group of organic compounds such as fatty acids, acylglycerols, phosphoglycerides, steroids, terpenes and prostaglandins which are soluble in nonpolar organic solvents such as chloroform, ether, benzene and hexane but insoluble or only sparingly soluble in water. They are the components of living systems consisting of basically carbon, hydrogen and oxygen ; in addition some have nitrogen and phosphorus.

Occurrence

Fats and oils are widely distributed in nature in both plant and animal tissues. They occur in relatively high concentration in seeds of certain plants (oilseeds) where they function to supply food for use of the growing seedlings. Animals store deposits of fats in their adipose tissues; these stored fats constitute a reserve which can be used as the source of energy.

3.2 Physiological role and biological importance

The general and important functions of some classes of lipids in biological systems are as follows:

1. Major sources of metabolic energy in animals, insects, birds and high lipid containing seeds
2. Basic structural components of cell membranes
3. As a protective water proof coating on the surface of cuticle of leaves or fruits of plants, feathers of birds and as insect secretions
4. As cell surface components concerned in cell recognition, species specificity and tissue immunity
5. Intense biological activity – some have profound biological activity; they include some of the vitamins and hormones
6. Fats stored subcutaneously in warm blooded animals serve as insulation against an unfavorable environment and also fatty tissues around vital organs give protection against mechanical injuries and
7. As activators of enzymes – for example, phosphatidylcholine micelles for activation of microsomal enzymes

3.3 General properties

Although the properties vary from one class to other, some of the general properties of lipids are:

1. Soluble in nonpolar solvents but only sparingly soluble in water
2. Greasy or fat-like in nature and show translucent properties
3. Polar lipids are amphipathic (Greek, amphi, double) i.e., one end of a lipid molecule, the head, is polar or ionic and therefore, hydrophilic the other end, the tail (hydrocarbon) is nonpolar and therefore hydrophobic.
4. Most lipids contain fatty acids. The glyceride esters of saturated fatty acids are usually liquids at room temperature and
5. Fats and oils containing unsaturated fatty acids slowly become rancid when exposed to light, heat, moisture and air.

3.4 Classification

Although there are different ways of classification, a useful classification of lipids by Bloor is as follows:

1. **Simple lipids:** These are esters of fatty acids with alcohols; saponifiable; include the most abundant of all lipids such as fats and oils or triglycerides and the less abundant waxes.
2. **Compound or complex lipids:** These are esters of fatty acids containing other groups in addition to alcohol and fatty acids; saponifiable; include phosphoglycerides and sphingolipids.
3. **Derived lipids:** These are derived from the hydrolysis of above two classes of lipids; nonsaponifiable (except fatty acids); include fatty acids, sterols, terpenes and fat-soluble vitamins.

3.5 Fatty acids

Most lipids contain fatty acids which may be obtained from complete hydrolysis of simple and compound lipids. They may be defined as the aliphatic monocarboxylic acids consisting of an alkyl hydrophobic tail (hydrocarbon) and a terminal hydrophilic (polar head i.e., carboxyl group) group with a basic formula.



A large number of fatty acids have been identified of which few are common and biologically important. Fatty acids occur as saturated, unsaturated, branched and cyclic acids. Most naturally occurring fatty acids, whether saturated or unsaturated are straight chain compounds with an even number of carbon atoms. Although the range of chain length is great (2 to >8- carbons), the most common chain lengths are C16, C18 and C20. The lower members (C4-C10) occur mainly in milkfat, those of intermediate length (C10-C14) and higher members (C16-C20) are found in most animal and vegetable fats. Fatty acids with an odd number of carbon atoms occur only in trace amounts in terrestrial

animals and in marine organisms. The structure and important sources of some of fatty acids are represented in Table 3.1.

Nomenclature

The most abundant fatty acids have common (trivial) name and systemic name that have been accepted for use in the official nomenclature. In IUPAC (International Union of Pure and Applied Chemistry) nomenclature, the carboxyl carbon is C-1 and other carbons are numbered sequentially. The carbon adjacent to the carboxyl carbon is designated as α and the other carbons are designated as β , γ , δ etc. The carbon farthest from the carboxyl carbon is ω carbon. For example the nomenclature of lauric acid (a 12 carbon fatty acid) is shown in Fig 3.1.

Table 3.1 Structure and source of some fatty acids of biological importance

Common (trivial name)	Systematic name	Structure	No. of carbons	Good source
Saturated fatty acids				
Butyric	Tetranoic acid	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$	4	Butter, milk fat
Caproic	Hexanoic acid	$\text{CH}_3(\text{CH}_2)_4\text{COOH}$	6	Butter, coconut oil, palmoil
Caprylic	Otanoic acid	$\text{CH}_3(\text{CH}_2)_6\text{COOH}$	8	Coconut oil, palm oil
Capric	Decanoic acid	$\text{CH}_3(\text{CH}_2)_8\text{COOH}$	10	Coconut oil, palm oil
Lauric	Dodecanoic acid	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	12	Laurel, coconut oil, palm oil
Myristic	Tetradecanoic acid	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	14	Butter, woolfat, nutmeg
Palmitic	Hexadecanoic	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	16	Animal plant and bacterial fats
Stearic	Octadecanoic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	18	Animal, plant and bacterial fats
Arachidic	Eicosanoic acid	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	20	Peanut oil,

				groundnut oil
Unsaturated fatty acids				
Palmitoleic	9-Hexa-decenoic acid	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	16	Sardine Oil
Oleic	9-Octa-decenoic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	18	Olive oil
Linoleic	9,12-Octa-decadienoic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	18	Cottonseedoil, soybeanoil, linseed oil
Linolenic	9,12,15-Octadecatrienoic acid	$\text{CH}_3(\text{CH}_2\text{CH}=\text{CH}_3(\text{CH}_2)_7\text{COOH}$	18	Linseed oil
Arachidonic	Eicosatetraenoic acid	$\text{CH}_3(\text{CH}_2)_3(\text{CH}_2\text{CH}=\text{CH})_4(\text{CH}_2)_3\text{COOH}$	20	Animal fats, adrenal phosphatides

Saturated fatty acids do not contain double bonds in the carbon chain. Monounsaturated fatty acids usually contain a cis-olefinic bond in a limited number of preferred positions in the chain. Polyunsaturated fatty acids have two to six cis double bonds. Most of these double bonds have a cis-configuration. The positions of the double bonds of unsaturated fatty acids are indicated by the symbol Δ^n where the subscript indicates the position of the first carbon in the double bond, numbering from the carboxyl carbon as C-1. Thus the C18 fatty acid has one cis double bond between carbons 9 and 10 is called cis Δ^9 octadecenoic acid.

3.5.2 General properties

1. Conformation: Saturated and unsaturated fatty acids have quite different conformations. In saturated fatty acids, the hydroxyl carbon tails are flexible and can exist in a very large number of conformations because each single bond in the backbone has complete freedom of rotation. The fully extended form shown in Fig 3.2 the minimum energy form, is the most probable conformation of saturated fatty acids. Unsaturated fatty acids on the other hand show one or more rigid kinks contributed by the nonrotating double bond(s). The cis-configuration of the double bonds produces a bend of about 30° in the aliphatic chain, whereas the trans-configuration more nearly resembles the extended form of saturated chains (Fig. 3.2).

The cis-forms of unsaturated fatty acids can be converted into trans forms by heating with certain catalysts. In this way oleic acid can be readily converted to its trans-isomer elaidic acid, which has a much higher melting point.

2. Solubility: Because of the essentially hydrocarbon nature of the common fatty acids, they exhibit very low solubility in water. The low molecular weight fatty acids (acetic acid and butyric acid) are miscible with water, whereas fatty acids with more than six carbons (caproic acid) are essentially insoluble in water, but are soluble in many nonpolar solvents.

3. Melting point: The saturated fatty acids in the series up to 10-carbon member (capric acid) are liquids at room temperature and volatile with steam (called as volatile fatty acids) and the members having more than 10-carbons are solids under similar condition and nonvolatile with steam (called as non-volatile fatty acids). A decrease in chain length and an increase in degree of unsaturation of the chain will lower the melting point of a fatty acid.

4. Micelles : Since a fatty acid consists of a hydrophilic (polar carboxyl group) head group and a hydrophobic (hydrocarbon) tail, when placed in an aqueous solution they become arranged spontaneously such that their hydrocarbon tails avoid the water and their polar head groups are in contact with it to form a micelle.

5. Reactions: The carboxyl group of the fatty acids will undergo most of the chemical reactions common to other carboxylic acids and can be esterified and reduced. The unsaturated fatty acids undergo addition reactions at their double bonds which are susceptible to chemical oxidation.

3.5.3 Essential fatty acids

Some of the unsaturated fatty acids like linoleic and linolenic acids are required in the diet of mammals as they cannot be synthesized by them. Such fatty acids are called as essential fatty acids.

3.6 Glycerol

Glycerol is a trihydric alcohol and is a constituent of all fats and oils. It has the ability to react with three molecules of fatty acids to form a triple ester called a triglyceride. Glycerol and fatty acids are the backbone structures of all fats and oils.

3.7 Triglycerides or acylglycerols

These are the most abundant group of naturally occurring lipids. Chemically they are the esters of fatty acids with the trihydric alcohol, glycerol. One, two or all the three of the hydroxyl groups of glycerol can be esterified to give rise to mono- di- and triglycerides, respectively. While the mono- and diglycerides are important intermediates in the metabolism of lipids, most of the natural lipids are triglycerides. In the glycerides, fatty acids are linked to glycerol by an ester bond between the hydroxyl groups of glycerol and the carboxyl groups of the fatty acids.

According to the identity and position of three fatty acids esterified to glycerol, triglycerides can be classified as below:

1. **Simple triglycerides:** These contain single kind of fatty acids in all the positions eg. Tristearin, tripalmitin and triolein.
2. **Mixed triglycerides:** These contain two or more kinds of fatty acids e.g. β -oleo- α,α' stearopalmitin or 1-stearo-2-oleo-3-palmitin.

Most of the triglycerides which occur in nature are mixed triglycerides. All fats and oils from both animal and plant origin are triglycerides. Those obtained from animal fats contain a higher percentage of saturated fatty acids while those of plant origin are rich in unsaturated fatty acids.

3.7.1 Biological importance

Triglycerides are the main storage form of energy in higher animals, migratory birds and oilseeds. They have the highest calorific value (about 9.0 kcal/g) in contrast to carbohydrates and proteins (about 4.0 kcal/g each). This is because they are more highly reduced than the latter two. Fats also serve as thermal insulators showing the loss of heat through the skin and regulate body temperatures and afford protection to vital organs.

3.7.2. General properties

Some of the general properties of triglycerides can be summarized as below :

1. **Specific gravity :** It is lower than that of water
2. Greasy or fat like and show translucent properties
3. **Solubility:** All triglycerides are insoluble in water and do not tend by themselves to form highly dispersed micelles. They are soluble in nonpolar solvents.
4. **Melting point:** It is determined by their fatty acid composition. In general, the melting point increases with the increasing carbon chain length of the saturated fatty acid components whereas it decreases with increase in degree of unsaturation.
5. They exist in the solid or liquid form, depending on the nature of the constituent fatty acids. Most plant triglycerides have low melting points and are liquids at room temperature since they contain a large proportion of unsaturated fatty acids (oils). In contrast, animal triglycerides contain a higher proportion of saturated fatty acids resulting in higher melting points and thus at room temperature they are solids or semi-solids (fats).
6. **Optical activity:** Although glycerol itself is optically inactive, carbon atom 2 becomes asymmetric whenever the fatty acid substituents on carbon atoms 1 and 3 are different.

7. **Autooxidation:** Fats and oils rich in unsaturated fatty acids slowly oxidize when exposed to atmospheric oxygen, light, heat and moisture and develop off-flavor and off-odour.

3.7.3 Chemical reactions

1. **Hydrolysis:** All triglycerides on hydrolysis yield three molecules of fatty acids and one molecule of glycerol when boiled with acids or bases or by the action of enzymes called lipases.

2. **Saponification:** Hydrolysis of triglycerides or fats and oils by alkali is called saponification. The free fatty acids formed by the hydrolysis of triglycerides react with excess of alkali to form metallic salts called soaps. Sodium and potassium salts of fatty acids are soluble in water (soft soaps) but other metal salts such as calcium, magnesium and barium are water insoluble (hard soaps).

3. **Oxidation:** Many fats and oils when stored for long time they often become rancid-develop off-flavor and off-odour. One type of rancidity, called hydrolytic rancidity is caused by the growth of microorganisms which secrete lipases and split triglycerides into mono- and diglycerides, glycerol and fatty acids. If fatty acids of low molecular weight are released, they impart unpleasant taste and odour. This kind of rancidity which commonly occurs in butter can be reduced by refrigeration (butter is stored at low temperature), by exclusion of water or destroying the microorganisms.

The other type of rancidity called the oxidative rancidity occurs due to the autooxidation of the unsaturated fatty acids at their double bonds yielding short chain acids and aldehydes having rancid taste and odours. This can be prevented by the addition of compounds like vitamin E (antioxidant).

In case of oxidative rancidity, oxygen adds to the olefinic bonds of unsaturated fatty acids to produce either cleavage or polymerization. The slow oxidation of unsaturated fatty acids in edible fats is associated with a cleavage type of reaction.

4. **Hydrogenation:** Hydrogen can be made to combine with unsaturated fats and oils to produce hydrogenated shortening. This reaction is used to enhance the keeping quality of vegetable oils used for food, e.g., vanaspati. The reaction of hydrogen with fats and oils is used commercially to produce hydrogenated shortening and oleomargarine.

5. **Halogenation:** Unsaturated fatty acids, either free or combined in lipids react with halogens like bromine and iodine which get decolorized. These halogens add at the carbon double bonds.

3.7.4 Analytical constants of fats and oils

Fats and oils can be characterized to know about the nature of the fatty acids, carbon chain length and degree of unsaturation. The following quantitative analytical constants are used.

- 1. Saponification number or value:** It is the number of milligrams of KOH required to completely saponify one gram of fat or oil. The higher the saponification number, the shorter the average carbon chain length of the fatty acids in a fat or an oil.
- 2. Iodine number or value:** It is the number of grams of iodine absorbed by 100 grams of a fat or oil. It is a measure of degree of unsaturation of the fatty acids in a fat or an oil.
- 3. Acid value:** It is the number of milligrams of KOH required to neutralize the free fatty acids in one gram of a fat or oil. It is a measure of free fatty acid content in a fat or oil.

Table 3.2 shows the analytical constants of some oils and fats from different sources.

3.8 Waxes

These are water-insoluble, solid esters of higher fatty acids with long chain monohydroxylic fatty alcohols or sterols. They differ from fats and oils in that glycerol is replaced by high molecular weight alcohols or sterols. They are the less abundant class of lipids; saponifiable in nature and very resistant to atmospheric oxidation. Because of these properties, they are used in furniture and automobile polishes.

Examples of true waxes are beeswax, carnauba wax (from the carnauba plant) and spermaceti (sperm whale wax). Beeswax and spermaceti are composed mainly of palmitic acid esterified with either hexacosanol ($C_{26}H_{53}OH$) or triacontanol ($C_{30}H_{61}OH$). Carnauba wax, the hardest known wax consists of fatty acids esterified with tetracosanol ($C_{24}H_{49}OH$) and tetratriacontanol ($C_{34}H_{69}OH$).

Waxes are found as protective coatings on skin, fur and feathers of animals and birds and on leaves and fruits of higher plants and on exoskeleton of many insects.

3.9 Phosphoglycerides (glycerol phosphatides)

Phosphoglycerides also called glycerol phosphatides or loosely referred to as phospholipids or phosphatides are the second large class of complex lipids occurring both in plant and animal tissues especially in the most active tissues of animal body such as brain and liver, very small amounts elsewhere in cells. They consist of phosphorus and nitrogen in addition to glycerol, fatty acids and a polar head group.

3.9.1 Structure

In phosphoglycerides one of the primary hydroxyl groups of glycerol is esterified to phosphoric acid, the other hydroxyl groups are esterified to fatty acids. The parent compound of the series is thus the phosphoric ester of glycerol. This compound has an

asymmetric carbon atom and can be designated as either D-glycerol-1-phosphate or L-glycerol-3-phosphate. Because of this ambiguity, the stereochemistry of glycerol derivatives is based on the stereospecific numbering (S_n) of the carbon atoms, (Fig 3.5). The isomer of glycerol phosphate found in natural phosphoglycerides is called S_n -glycerol-3-phosphate; it belongs to the L-stereochemical series.

The parent compound of the phosphoglycerides is phosphatidic acid (Fig 3.5) formed by esterification of the two fatty acid residues at carbon atoms 1 and 2 of S_n -glycerol-3-phosphate with no polar alcohol head group. It occurs in only very small amounts in cells, but it is an important intermediate in the biosynthesis of phosphoglycerides.

In addition to phosphatidic acid, phosphoglycerides contain polar head group, namely an alcohol designated X-OH, whose hydroxyl group is esterified to the phosphoric acid (Fig 3.5).

3.9.2 Important types

The most abundant phosphoglycerides in higher plants and animals are phosphatidylethanolamine and phosphatidylcholine (Table 3.3), which contain as polar head groups the amino alcohols, ethanolamine and choline, respectively (the old trivial names are cephalin and lecithin, respectively). These two phosphoglycerides are major components of most animal cell membranes.

In phosphatidylserine, the hydroxyl group of the amino acid L-serine is esterified to the phosphoric acid. In phosphatidylinositol, the head group is the six carbon cyclic sugar alcohol myo-inositol. In phosphatidylglycerol, the head group is a molecule of glycerol (Table 3.3). Phosphatidylglycerol is often found in bacterial membranes as an amino acid derivative, particularly of L-lysine, which is esterified at the 3' position of the glycerol head group.

Closely related to phosphatidylglycerol is the more complex lipid cardiolipin (diphosphatidylglycerol) which consists of a molecule of phosphatidylglycerol in which the 3'-hydroxyl group of the second glycerol moiety is esterified to the phosphate group of a molecule of phosphatidic acid (Table 3.3). Cardiolipin is abundantly present in the cell membranes of bacteria and in the inner membrane of mitochondria.

Plasmalogens differ from all the other phosphoglycerides described above. One of the two hydrocarbon tails is contributed by a long chain fatty acid esterified to the 2 position of glycerol, but the other is a long aliphatic chain in *cis* α , β -unsaturated ether linkage at the 1 position (Fig 3.6). Ethanolamine is the most common polar head group. They are abundant in the membrane of muscle and nerve cells.

3.9.3 General properties

Some of the important properties of phosphoglycerides are summarized below:

1. Pure phosphoglycerides are white waxy solids. On exposure to air they darken and undergo chemical changes because of the tendency of their polyunsaturated fatty acids are preoxidized by atmospheric oxygen which result in polymerization.
2. **Solubility:** soluble in most non-polar solvents containing some water and are best extracted from cells and tissues with chloroform-methanol mixtures.
3. **Micelles:** When placed in water, they appear to dissolve, but only very minute amounts go into true solution, most of the 'dissolved' lipid is in the form of micelles.
4. **Polarity:** Because of the presence of polar head group, they are polar lipids.
5. **Saponification:** Mild alkaline hydrolysis of phosphoglycerides yields the fatty acids as soaps.
6. **Hydrolysis:** Phosphoglycerides can be hydrolyzed by specific enzymes called phospholipases. Phospholipase A₁ specifically removes the fatty acid from the 1 position and phospholipase A₂ from the 2 position. Phospholipase B (a mixture of phospholipase A₁ and A₂) catalyzes successive removal of the two fatty acids of phosphoglycerides. Phospholipase C hydrolyzes the bond between phosphoric acid and glycerol, while phospholipase D removes the polar head group to leave a phosphatidic acid.

3.9.4 Biological importance

Phosphoglycerides serve many biological functions some of which are as follows :

1. Major components of biological membranes and subcellular organelles
2. Regulation of the permeability of cell membrane
3. Maintenance of protoplasmic structure in view of their ability to form emulsions
4. Transport of other lipids in the blood stream and regulation of fat metabolism by activating certain enzymes and
5. As donors of arachidonic acid for the synthesis of prostaglandins and thromboxanes

3.10 Sphingolipids

Sphingolipids are also a group of complex lipids found abundantly in brain and nervous tissues and are apparently lacking in plants and microorganisms. They also occur in blood and nearly all of the tissues of human beings. Various sphingolipids are components of the plasma membrane of practically all cells.

All sphingolipids contain three characteristic building-block components ; one molecule of a fatty acid, one molecule of sphingosine or its derivative and a polar head group.

Sphingosine (4-sphingenine) is a C18 amino alcohol containing a long unsaturated hydrocarbon tail. In mammals sphingosine and dihydrosphingosine (sphinganine) (Fig 3.8) are the major bases of sphingolipids, in higher plants and yeasts phytosphingosine (4-hydroxysphinganine) is the major base and in marine invertebrates doubly unsaturated bases such as 4,8-sphingadiene are common.

The sphingosine base is connected at its amino group by an amide linkage to a long saturated or monounsaturated fatty acid. The resulting compound, which has two non-polar tails and is called a ceramide (Fig 3.8) is the characteristic parent compound of all sphingolipids. Different polar head groups are attached to the hydroxyl group at the 1 position of the sphingosine base.

3.10.1 Common types

The different classes of sphingolipids are discussed below:

1. Sphingomyelins: These are principal structural lipids of the membranes of nerve tissue. They are the only class of sphingolipids that are consisting of phosphorylcholine or phosphorylethanolamine as polar head groups, hence are called phospholipids. They are formed by the esterification of the primary alcoholic group of ceramide with phosphorylcholine or ethanolamine. They have physical properties very similar to those of phosphatidylethanolamine and phosphatidylcholine. The most common fatty acids in sphingomyelin are palmitic, stearic, lignoceric and nervonic acid.

2. Neutral glycosphingolipids: These are abundant in nerve tissues, especially brain. In these lipids, the primary alcoholic group of the ceramide is linked to a hexose, frequently D-galactose (hence called as galactolipids or galactocerebroside. (Fig 3.10), less commonly D-glucose and lactose. They are important cell-surface components in animal tissues and also concerned with blood-group specificity.

3. Acidic glycosphingolipids (gangliosides): These are present in high concentration in the ganglia of the central nervous system particularly in the nerve endings. They are glycosylceramides (containing short oligosaccharide chains) containing one or more sialic acid molecules (N-acetylneuraminic acid or NANA) attached to galactose units (Fig. 3.11).

They function in the transmission of nerve impulses across synapses and are also believed to be present at receptor sites for acetylcholine and other neurotransmitter substances. Some of them are concerned in blood-group specificity.

3.11 Terpenes

Terpenes are derived class of lipids which are non-saponifiable in nature. They are constructed of multiples of the five-carbon hydrocarbon, isoprene (2-methyl-1, 3-

butadiene) (Fig 3.12). Terpenes containing two, three, four, six and eight isoprene units are called mono-, sesqui-, di-, tri- and tetraterpenes, respectively. They may be either linear or cyclic.

The successive isoprene units of terpenes are usually linked molecules, in a head-to-tail arrangement particularly in the linear segments, but sometime in tail-to-tail arrangement (Fig.3.12).

Biological importance

A larger number of terpenes have been identified with important biological functions some of which are listed below:

1. The mono-terpenes geraniol, limonene and menthol are major components of oil of geranium, lemon oil and mint oil, respectively.
2. The diterpene phytol, a linear terpenoid alcohol is a component of the photosynthetic pigment chlorophyll.
3. The triterpene squalene is an important precursor in the biosynthesis of cholesterol.
4. The β -carotene, an important carotenoid (tetraterpene) is the precursor of vitamin A.
5. Natural rubber and gutta-percha are polyterpenes.
6. The most important class of terpenes are the members of the group of fat-soluble vitamins, namely vitamins A, E and K and
7. Another class of terpenoid compounds functioning as coenzymes is the ubiquinone or coenzyme Q family of compounds, which function as hydrogen carriers for biological oxidations in the mitochondria. Analogous compounds, called plastoquinones are found in the chloroplasts, where they function in photosynthesis.

3.12 Sterols (steroids)

These are the important non-saponifiable and most abundant groups of derived lipids. They are wax-like solid alcohols which occur free and as fatty acid ester in animals, plants and microorganisms. In contrast to most lipids, they contain a ring structure, the cyclopentanoperhydrophenanthrene nucleus (Fig 3.13).

In the structure shown above, IV is a cyclopentane ring, and rings I, II and III fused together, constitute a perhydrophenanthrene ring. Different steroids vary in respect of the side chain attached on carbon 17. All steroids originate from the linear triterpene squalene. Steroids occur in plants, animals, fungi and yeasts but are absent in bacteria.

3.12.1 Common sterols

1. **Cholesterol (C₂₇H₄₅OH):** It is a principal animal sterol (Fig. 3.14) present in relatively high concentration in nerve tissues and in bile. It occurs only rarely in higher plants.
2. **Lanosterol:** It is the first important steroid product of cyclization of squalene, which in animal tissues is the precursor of cholesterol. It is found in the waxy coating of wool in esterified form (Fig 3.15).
3. **Ergosterol (C₂₈H₄₃OH):** This is one of the most important phytosterols occurring in plants (Fig 3.16). When exposed to ultraviolet light it yields vitamin D₂ (calciferol).

Other plant sterols include stigmasterol, β -sitosterol and spinasterol which have an extra methyl or ethyl group and may have an additional point of unsaturation in the hydrocarbon chain at the 17 position.

3.12.2 Biological importance

Steroids include bile acids (cholic and dexoycholic acid); androgen or male sex hormone (testosterone); the estrogen or female sex hormone (estrone and β -estradiol); the progestational hormone (progesterone); the adrenocortical hormones (corticosterone and aldosterone); and an insect molting hormone (ecdysone). Among the most important steroids are a group of compounds having vitamin D activity.

3.13 Prostaglandins

They are found in most animal tissues and are extremely potent. They are C₂₀ unsaturated hydrocarboxylic acids with a cyclopentane ring in the molecule. The parent compound is prostanic acid. The best known prostaglandins are E₁, E_{1 α} , and F_{1 α} and F_{2 α} abbreviated as PGE₁, PGF_{1 α} and PGF_{2 α} , respectively.

Prostaglandins have wide range of physiological activities. Among them are their effects on blood pressure, blood clotting, gastric secretion, contraction of smooth muscle and reproduction. They also have hormonal or regulatory activities. They are synthesized from C₂₀ polyunsaturated fatty acids, like arachidonic acid. The structure of prostanic acid and a representative prostaglandin – PGE₁, are shown in Fig 3.17 .

3.14 Lipoproteins

Certain lipids associate with specific proteins to form lipoprotein system. There are two major types, transport lipoproteins and membrane system. In these systems the lipids and proteins are non-covalently joined but are held together largely by hydrophobic interactions between the non-polar portions of the lipid and the protein components.

Lipids are transported in the blood by the plasma lipoproteins of which there are four different classes differing in density.

3.14.1 Biological membranes

Membranes are a conspicuous feature of cell structure; in some eukaryotic cells the different membrane systems may make up as much as 80 per cent of the total dry cell mass. Membranes serve not only as barriers separating aqueous compartments with different solute composition but also as the structural base to which certain enzymes and transport systems are firmly bound. They are very thin (about 8 nm) and flexible.

CHAPTER 4 AMINO ACIDS AND PROTEINS

4.1 Amino acids

Amino acids may be defined as low molecular weight organic compounds which contain an acidic carboxyl group (-COOH), basic amino group (-NH₂) and a side chain group (usually denoted by R). Since invariably all the amino acids possess both a carboxyl group and an amino group, the nature of the R group is the criterion for differentiating different types of amino acids. All proteins in all species ranging from bacteria to man are made up the same set of 20 α -amino acids as building-blocks through peptide bond formation.

Fig 4.1 shows the general structural formula of the 20 α -amino acids commonly found in proteins.

The carbon immediately adjacent to the carboxyl group is called the α -carbon atom. All the 20 amino acids except proline and hydroxyproline possess common α -carboxyl and α -amino groups. All the amino acids consist of carbon, hydrogen, oxygen and nitrogen.

4.1.1 Standard or protein amino acids

The 20 α -amino acids found in proteins are referred to as the standard amino acids or protein amino acids, which are listed in Table 4.1 along with their structural formulae. The amino acids are ordinarily designed by three-letter symbols, but a set of one-letter symbols has also been adopted to facilitate comparative display of amino acid sequence of homologous proteins.

4.1.2 Essential or indispensable amino acids

Those amino acids which cannot be synthesized by mammals but required to be supplemented through diet are called as essential amino acids. Vertebrates are not able to synthesize all the common amino acids; for example man and the albino rat can make only 10 of the 20 amino acids required as building-blocks of proteins. The remainder, the essential or nutritionally indispensable amino acids must be obtained from plants or other sources.

The 10 amino acids listed in Table 4.2 are known to be essential for rats and children but for adult human beings arginine and histidine are not essential.

Table 4.2 Amino acids indispensable for man and growing albino rat

Lysine	Methionine
Arginine	Threonine
Histidine	Leucine
Tryptophan	Isoleucine

Phenylalanine	Valine
---------------	--------

4.1.3 The rare amino acids of proteins

In addition to the 20 standard amino acids, several others of relatively rare occurrence have been isolated from hydrolyzates of some specialized types of proteins. All are derivatives of some standard amino acids. Among them is 4-hydroxyproline, derivative of proline found in abundance in the fibrous protein, collagen and in some plant proteins. Hydroxylysine, the 5-hydroxy derivative of lysine is present in collagen. The unusual amino acids ϵ -N-methyllysine, ϵ -N-trimethyllysine and methylhistidine, the methyl derivatives of standard amino acids have been found in certain proteins.

4.1.4 Non-protein amino acids

In addition to the 20 common and several rare amino acids of proteins, over 150 other amino acids are known to occur biologically in free or combined form but never in proteins. Most derivatives of the L- α -amino acids found in proteins, but β -, γ - and δ amino acids are also known (Fig. 4.3).

Some nonprotein amino acids are important precursors or intermediates in metabolism. Thus β -alanine is a building-block of the vitamin pantothenic acid; homocysteine and homoserine are intermediates in amino acid metabolism; citrulline and ornithine are intermediates in the synthesis of arginine, γ -aminobutyric acid functions as a chemical agent in transmission of nerve impulses. Some non protein amino acids have the D-configuration, for example D-glutamic acid found in the cell walls of many bacteria, D-alanine found in the larvae or pupae of some insects and D-serine, found in the earthworm.

4.1.5 The stereochemistry

All standard amino acids except glycine have at least one asymmetric (chiral) α -carbon atom attached to four different groups of atoms and consequently exist as stereoisomers. A solution of one member of a stereoisomer pair rotates the plane of polarized light either to the right ('+' or dextrorotatory) or to the left ('-' or levorotatory). A solution of the other member of the pair rotates the plane of polarized light by the same amount, but in opposite direction.

The amino acid stereoisomers can be classified as D- or L- by comparing the orientation of the four substituents around the asymmetric carbon (absolute configuration) with the reference compounds D- and L-glyceraldehyde (Fig 4.4).

The amino acids found in proteins are usually of L-configuration. Racemic mixtures contain equal amounts of the L- and D-isomers. Some amino acids of the D-configuration do occur in living matter (e.g. in bacteria cell wall and some antibiotics) but not in proteins.

4.1.6 The acid-base properties

All the crystalline standard amino acids have relatively high melting or decomposition points usually above 200°C. They are much more soluble in water than in non-polar solvents. The R groups on the standard amino acid confer specific properties on each. The properties may depend on the solution pH.

Amino acids in neutral aqueous solution or in the crystalline phase exist as dipolar (zwitterions) molecules (presence of both positive and negative charges on the same molecule) which are electrically neutral in nature (Fig.4.5).

The charge properties of amino acids are determined by the number of ionizable groups they contain. Free carboxyl and free amino groups are capable of losing and gaining hydrogen ions (H^+). Thus altering the charge on the molecule, this depends on hydrogen ion concentration of the solution. In a neutral solution (the physiological pH of 7.0), the amino groups pick up an H^+ and become positively charged while carboxyl groups lose an H^+ and are therefore negatively charged. With the exception of proline and hydroxy proline, all amino acids contain a minimum of one free amino group and one free carboxyl group and have at least one positive and one negative charge at pH 7.0.

The net charge on any amino acid at a given pH is the total of the positive and negative charges on the molecule (Table 4.3). In summary, at low pH's, amino acids accept the maximum number of hydrogen ions and behave as positively charged (cations) as possible and at high pHs, they accept the minimum number of hydrogen ions and behave as negatively charged (anions) as possible. The charge properties of lysine and aspartic acid in solution at different pH's are shown in Fig.4.7.

4.1.7 Classification

Amino acids can be classified in two ways-based on structure and polarity.

A. Based on structure

Based on structure, amino acids are grouped into three classes as:

I. Aliphatic amino acids: These are straight or open- chain amino acids which are further subdivided into four groups as:

1. Monoamino monocarboxylic (neutral) amino acids: These consist of one amino and one carboxyl groups and hence are neutral to litmus, e.g. glycine, alanine, valine, leucine, isoleucine, serine and threonine.

2. Monoaminodicarboxylic (acidic) amino acids: These consist of one amino and two carboxyl groups and hence are acidic to litmus, e.g. aspartic acid and glutamic acid.

3. Monocarboxylicdiamino (basic) amino acids: These consist of one carboxyl and two amino groups and hence are basic to litmus e.g. lysine, arginine and histidine.

4. Sulphur-containing amino acids: These consist of one or more sulphur atoms, e.g. cysteine, cystine and methionine.

II. Aromatic amino acids: These contain an aromatic ring in the molecule, e.g. phenylalanine and tyrosine.

III. Heterocyclic amino acids: These contain an heterocyclic nucleus in the molecule e.g., histidine, tryptophan, proline and hydroxyproline.

B. Based on polarity

1. Nonpolar or hydrophobic R groups: These amino acids are relatively less soluble in water than those with polar R groups. e.g., alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan and proline. The least hydrophobic member of this class is alanine which is near the border line between the non-polar amino acids and those with uncharged polar R groups.

2. Uncharged polar R groups: These are relatively more soluble in water than the first group because of the presence of polar R group (indicated within brackets) e.g., glycine (H^+), serine (OH^-), threonine (OH), tyrosine (phenolic hydroxyl) and cysteine ($-\text{SH}$ or thiol group). Glycine is the border line member of this class and is sometimes even grouped in the first category. The remaining amino acids are weakly hydrophilic as their R groups do not ionize at physiological pH values and hence remain uncharged. Cysteine and tyrosine have the most polar substituents of this class of amino acids. Cysteine often occurs in protein in its oxidized form, cystine.

3. Positively charged R group: Amino acids such as lysine, histidine and arginine (basic amino acids) having positively charged R group at physiological pH belong to this category and are strongly hydrophilic. The positive charge arises because these amino acids possess nitrogen-containing R groups ($\text{C}-\text{NH}_2$ group in lysine, guanidino group in arginine and imidazole group in histidine) that accept protons in aqueous solution at neutral or acidic pH.

4. Negatively charged R groups: Amino acids namely, aspartic acid and glutamic acid (acidic amino acids) each with a second carboxyl group in addition to α -carboxyl group

give up a proton in aqueous solution at neutral or basic pH and hence possess an extra negative charge at physiological pH. Because of this, they are strongly electrophilic.

4.1.8 Reactions

The characteristic organic reactions of amino acids are those of the α -carboxyl group, the α -amino group and the functional groups present in different side chains (R). Some of their reactions are given below.

4.1.8.1 Reactions of the carboxyl group

1. Esterification: The carboxyl group of an amino acid may be esterified with alcohols. The reaction is often used as a means of protecting the carboxyl group of amino acids in the chemical synthesis of peptides.

2. Reduction: The carboxyl group of an amino acid can be reduced with the potent reducing agent lithium borohydride to yield the corresponding primary alcohol. This reaction is useful in the study of amino acid sequence.

3. Sorenson's formal titration: The carboxyl group of an amino acid cannot be directly titrated against alkali because of the formation of dipolar ion. In the presence of excess of neutralized formaldehyde, the amino acid solution can be directly titrated against alkali as the amino group which interferes with carboxyl group is converted into a mono- and subsequently into a dimethylol derivative of amino acid.

4.1.8.2 Reactions of amino group

1. Reaction with nitrous acid (Van Slyke reaction): The amino group of an amino acid will react with the strong oxidizing agent nitrous acid (HNO_2) to liberate N_2 . This reaction which is stoichiometric is important in the estimation of α -amino groups in amino acids. The amino acids proline and hydroxyproline do not react. The products are the corresponding α -hydroxy acid and N_2 gas, which can be measured manometrically.

2. Reaction with ninhydrin: The amino group of amino acids on heating with ninhydrin (oxidizing agent) undergoes oxidation to form ammonia, CO_2 and the aldehyde:

A second equivalent of ninhydrin (oxidized) then reacts with the reduced ninhydrin and NH_3 formed in above equation to produce a highly colored product (Ruheman's purple). This reaction is very widely used to estimate amino acids quantitatively in very small amounts. A purple color is given in the ninhydrin reaction by all the amino acids and peptides having a free α -amino group, whereas, proline and hydroxyproline in which the

α -amino group is substituted (imino acids) yield derivative with a characteristic yellow color.

3. **Reaction with 1-fluoro-2,4-dinitrobenzene (FDNB):** The compound FDNB (Sanger's reagent) reacts with the free amino group on the NH_2 -terminal end of polypeptide as well as amino groups of free acids and produces the bright yellow colored dinitrophenyl (DNP)-amino acid derivative. This reaction can be used to identify the N-terminal amino acid of a peptide or protein.

4. **Reaction with dansyl chloride (5-dimethylamino-naphthalene-1-sulfonyl chloride) :** The amino group of both free amino acids and peptide chains will react with dansyl chloride to produce a dansyl amino acid derivative which fluoresces under UV light. This reaction may be also to find out N-terminal amino acid of a peptide or protein.

5. **Reaction with phenylisothiocyanate (Edman's reaction):** Phenylisothiocyanate reacts with α -amino group of an amino acid (or peptide) to form the corresponding phenylthiocarbamyl amino acid. In anhydrous acid this compound cyclises to form a phenylthiohydantoin which is stable in acid. This reaction is used both to degrade a polypeptide chain and to identify the N-terminal amino acid in the peptide.

4.1.8.3 Reactions of the R group

Amino acids also show qualitative color reactions typical of certain functions present in their R group e.g. the thiol group of cysteine, the phenolic hydroxyl group of tyrosine and the guanidinium group of arginine.

1. **Oxidation of cysteine to cystine:** The thiol group of cysteine is highly susceptible to atmospheric oxygen in the presence of iron salts or by other mild oxidizing agents. The oxidation product is cystine in which the disulfide bond constitutes a covalent bridge between two residues of cystine. Cystine plays an important role in protein structure since its disulfide group serves as a covalent cross link between two polypeptide chains or between two points as a single chain.

2. **Reaction of cysteine with Ag^+ :** The thiol group of cysteine reacts with heavy metal ions such as Hg^{2+} and Ag^+ to form mercaptides.

4.2 Peptides

Amino acids react with one another through the α -carboxyl group of one molecule and the α -amino group of a second to form a peptide. The amide linkage between the two amino acids is called a peptide bond ($-\text{CONH}$) which is the backbone of the polypeptide chain. The R groups of the amino acids are never involved in the formation of peptide bonds; they remain free, extending out from the long, linear backbone of the chain. Peptide bonds are strong, covalent linkages which are not easily broken. They can

be cleaved in enzymatic reactions or chemically by heating the protein with strong acid or alkali.

The product formed when two amino acids are joined is called a dipeptide (Fig.4.9). Similarly, higher peptides are formed by linking three (tripeptide), four (tetrapeptide), etc. amino acids.

If the peptide contains 2-10 amino acid residues, it is called as an oligopeptide (Fig.4.10). Ordinarily, a peptide with more than ten residues is termed a polypeptide. All the proteins are polypeptides. The α - carboxyl group of one amino acid is linked to the α -amino group of the next successively through peptide bonds in all the proteins.

Most peptides consist of a linear chain-like assembly of amino acids with two terminal residues, one terminal residue possessing a free amino group (N-terminal) and the other a free carboxyl group (C-terminal).

4.2.1 Nomenclature

The amino acids which are linked from left to right are named with 'yl' endings until last amino acid which retains its usual ending. For example, the full and abbreviated name for a tripeptide is given below:

Full name	Abbreviated name
Valyl-aspartyl-lysine	Val-asp-lys

4.2.2 Properties

Peptides undergo the same kinds of chemical reactions as those given by the free α -amino acids. One widely used color reaction of peptides and proteins that is not given by free amino acids is the biuret reaction (Fig.4.11). Treatment of a peptide or a protein with Cu^{2+} in alkaline solution yields a purple Cu^{2+} -peptide complex. A peptide with three or more amino acids can participate in this reaction. It is used in the quantitative estimation of peptides and protein. In solution (at physiological pH near 7.0), the terminal groups of a peptide will be in the ionized state.

4.2.3 Important peptides

Table 4.4 shows some of the biologically important peptides with functions.

In addition to the large number of different short peptides identified as partial hydrolysis products of proteins, many peptides not derived from proteins have been found in living organisms. Such non-protein peptides usually differ structurally from those derived from proteins. For example, the tripeptide glutathione, found in all cells of higher animals, contains a glutamic acid residue joined in an unusual peptide linkage involving its γ -carboxyl rather than the α -carboxyl group. The muscle dipeptide carnosine contains a β -amino acid. Oxytocin and vasopressin are two peptide hormones secreted by the

posterior lobe of the pituitary gland. Many antibiotics are peptides or derivatives of peptides including gramicidin. The structures of these peptides are shown in Fig. 4.12.

Table 4.4 : Some important naturally occurring peptides

Peptide	No. of amino acid residues	Biological importance
Glutathione (γ -glutamyl - cysteinyl-glycine)	3	Biological reducing agent
Vasopressin	9	Water balance hormone
Oxytocin	9	Stimulates uterine contraction
Gramicidin A	10	Antibiotic
Glucagon	29	Enhances blood sugar
Adrenocorticotropic hormone (ACTH)	39	Stimulates cortex of adrenal glands
Insulin	51	Regulates blood sugar level

4.3 Proteins

The term 'protein' (Greek, proteios – primary or first) was suggested by Berzelius in a letter to Mulder in 1838 to those organic nitrogenous compounds found in the cells of all living organisms.

Proteins are intimately associated with all physical and chemical activity of the cell. It is indeed unlikely that any chemical reaction is carried out in living tissues without the participation of proteins. One of the principles of the molecular logic of the living state is that all the living organisms contain organic macromolecules built according to a common plan.

Proteins represent the most abundant as well as the most varied class of macromolecules consisting nearly 50 per cent or more of the dry weight of the cell. They consist of about 50 per cent carbon, 7 per cent hydrogen, 20-23 per cent oxygen, 16 per cent nitrogen (average) and 0-3 per cent sulphur. In addition, some proteins contain phosphorus, iron, zinc, copper, magnesium, etc. For example, a single cell of the bacterium *Escherichia coli* contains about 3000 different proteins and there are as many as 50,000 different proteins in an organism as complex as a human being. In view of the biological importance, it is necessary to examine the various aspects of proteins in greater detail.

4.3.1 Physiological role and biological importance

Proteins play vital and diversified roles, some of which are listed in Table 4.6.

4.3.2 Physico-chemical properties

Some of the physico-chemical properties and reactions of proteins are summarized below:

1. All proteins are of high molecular weight, complex in structure composed of several units of 20 amino acids as building-blocks which are linked by peptide bonds. Hence, proteins are polypeptides with one or more chains.

2. Excepting the chromo-proteins, they do not have any characteristic color, odour, or taste. They occur as families.
3. **Solubility behaviour:** A protein in solution shows changes in solubility as a function of pH, ionic strength, temperature and the dielectric properties of the solvent. These properties are widely exploited in purification of proteins and enzymes.
4. **Denaturation:** It is unfolding of the characteristic native folded structure of the polypeptide chain of globular protein molecules into randomly coiled structure (Fig.4.13). In general a denatured protein is less soluble than the native protein. But, the most significant consequence of denaturation is that the protein usually loses its three-dimensional structure and biological activity and in case of enzymes loss of enzymatic activity has been recorded.

Many cases have now been observed in which an unfolded protein molecule spontaneously returns to its native biologically active form with the removal of denaturants or denaturing agents (agents which cause denaturation of proteins) in the test tube, a process called renaturation (Fig.4.13). This has been demonstrated in case of enzyme pancreatic ribonuclease by Anfinsen and his colleagues. If the denatured protein is an enzyme, its catalytic activity returns on renaturation, without change in the specificity of the reaction catalyzed. However, renaturation of a denatured protein cannot evoke any biological activity that was not present in the original protein. These facts indicate that the sequence of amino acids in the polypeptide chain contain the information required to specify its native folded conformation.

The rigid structure of a globular protein is held in shape by three main types of cross linkages: S-S (disulfide) linkages, salt bridges (between aspartic, glutamic acid and lysine, arginine) and hydrogen bonds (between peptide linkages: $-C=O \dots H-N-$, and similar polar groups). When a protein is denatured, some or all of these cross linkages are split and the specific internal structure of the native protein is lost. Under mild denaturing conditions (extreme temperature and pH) no covalent bonds in the backbone of the polypeptide chain are broken, the primary structure remains intact. Most globular proteins undergo denaturation when heated above 60 to 70°C.

Table- 4.5 shows some of the denaturing agents (physical and chemical) and conditions which cause protein denaturation.

Table 4.5: Protein denaturants

Agent	Probable cause of denaturation
Physical agents	
pH	Change in ionization of R groups of amino acids of proteins
Heat	Thermal splitting of salt bridges, 'melting' ice crystal bound water
Shaking or stirring	Unfolding of peptide chains (protein monolayer)

	forms at surface and in foam)
Ultrasonic waves	Mechanical agitation, thermal effects, release of (O) from water
UV light and X-rays	Absorption of energy, splitting of bonds
Chemical agents	
Mineral acids and alkalis	Cleavage of salt bridges
Urea, acetone and alcohols	Cleavage of hydrogen bonds
Guanidine HCl	Liberation of hydrogen bonds
Tungstic, picric, trichloroacetic acids	Cleavage of salt bridge

5. **Precipitation :** Proteins are precipitated from solution by heavy metal ions (AgNO_3 , CuSO_4 , lead acetate, mercuric chloride), alkaloidal reagents (trichloroacetic acid, picric acid, metaphosphoric acid), and concentrated salt solution [$(\text{NH}_4)_2\text{SO}_4$, Na_2SO_4 , NaCl]. The precipitation is the result of the destabilization of protein-solvent interaction.

6. **Absorbancy:** Most proteins have distinct light absorption maxima at 280 nm due primarily to the presence of tyrosine, tryptophan and phenylalanine. This property can be used to measure the amount of protein in a given sample (at levels of 0.1 to 0.5 mg/ml).

7. **Hydrolysis:** Proteins can be hydrolyzed completely to yield a mixture of constituent amino acids as end-products by an acid (6M HCl for 12-36 hours at 100-110°C), a base (2M NaOH) or by enzymes (proteases). Hydrolysis of proteins is brought about by breaking of peptide bonds by the addition of water molecules. Hydrolysis with HCl completely destroys tryptophan if it is present in protein. Base hydrolysis is more destructive of other amino acids. A nondestructive method involves the use of enzymes that catalyze the hydrolysis of peptide bonds in a stepwise reaction.

8. **Acid-base properties:** Acid-base properties of proteins largely depend upon the types of ionizable R groups in their polypeptide chains. Since proteins differ in amino acid composition and sequence, each protein has distinctive acid-base properties. Proteins similar to amino acids can also be titrated with an acid or base. The shape of the titration curve reflects the number and types of ionizing groups. The titration curve indicates that pK' values for some types of R groups differ slightly from pK' s of those groups in free amino acids, which is due to the electrostatic interaction of the neighbouring charges. The titration curve also shows the isoelectric pH at which the molecule carries no net charge and fails to migrate in an electric field. The isoelectric pH will be relatively high, above pH 7.0, if the protein has a relatively high content of basic amino acids (lysine and arginine) while it will be relatively low if the protein has maximum number of acidic amino acid residues (aspartic acid and glutamic acid). Most globular proteins have isoelectric pH's between 4.5 and 6.5.

The titration curve of a protein also indicates the sign and magnitude of its net electric charge at any given pH. At any pH above its isoelectric pH, it has a net negative charge and will move towards the anode. Similarly, at any pH below its isoelectric pH, the protein has a net positive charge and will move towards the cathode. These acid-base properties are directly exploited for separating and analyzing protein mixture using electrophoresis and ion-exchange chromatography.

Proteins because of their weak acidic and basic groups, act as buffers. For example, proteins in blood assist the bicarbonate and phosphate buffer systems in maintaining the pH of 7.4.

9. **Reactions:** Functional groups such as α -carboxyl group, α -amino group and R groups present in proteins undergo reactions similar to those of free amino acids. Thus, proteins can be acylated, arylated, alkylated, their carboxyl groups can be reduced and their sulphhydryl groups can be oxidized. Unlike free amino acid, proteins answer the two most important color reactions, namely biuret reaction (Fig.4.11) and Folin-Ciocalteu Reaction (FCR) which are the bases for quantitative determination of protein and are widely used methods.

In FCR method (Lowry's method) proteins react with the Folin- Ciocalteu reagent to give a blue colored complex. The color is formed due to reaction of copper in alkaline medium with protein (as in the biuret test) and the reduction of phosphomolybdate (which is present in FCR) by tyrosine and tryptophan present in the protein. This method is a more sensitive method and now extensively used in protein estimation.

4.3.3 Classification

Proteins can be classified in various ways based on function, composition and structure. Many of the proteins, but not all are distinguished on the basis of their function and this is given in Table 4.6.

Based on composition, proteins can be categorized into general classes:

1. **Simple proteins:** These proteins yield only α -amino acids on complete hydrolysis.
2. **Complex or conjugated proteins:** These proteins give in addition to amino acids an organic or inorganic non-protein moiety called prosthetic group on hydrolysis.

The simple proteins are further sub-classified on the basis of solubility in various solvents (Table 4.7).

Table 4.6: Representative proteins in the functional classes

Functional class	Example	Occurrence or biochemical function
Catalytic	Enzymes	Hydrolysis, oxidation, reduction synthesis and

		degradation of macromolecules etc.
Structural	Collagen	Framework of bone, tendons and connective tissue
	Glycoproteins	Cell coats and wall
	α -Keratin	Component of hair, wool, skin. Nails, feathers and hoofs
	Sclerotin	Exoskeletons of insects
	Fibroin	Component of silk of cocoons, spider webs
	Viral-coat	Sheath around nucleic acid protein
Contractile	Actin and myosin	Contraction of muscle fibres
	Dynein	Cilia and flagella
Protective	Antibodies	Natural defense in vertebrates
	Fibrinogen	Precursor of fibrin in blood clotting
Transport	Hemoglobin	Transport of O ₂ in vertebrate blood
	Serum albumin	Transport of fatty acids in blood
Respiratory	Cytochrome	Mitochondrial electron transport chain
Hormonal	Insulin	Regulates blood glucose level
	Growth hormone	Stimulates growth of bones
Storage	Gliadin	Wheat seed protein
	Zein	Corn seed protein
	Casein	A milk protein
	Ferritin	Iron storage in spleen
	Ovalbumin	Egg-white protein
Toxins	Ricin	Toxic protein in castor bean
	Diphtheria toxin	Bacterial toxin
	Gossypin	Toxic protein in cotton seed
Vision	Rhodopsin	Visual cycle in eye
Membrane	Na ⁺ -K ⁺ ATPase	Active transport

Table 4.7: Sub-classification of simple proteins

Name	Example and source	Soluble in	Other special features
Albumins	Ovalbumin (egg white) Serum albumin (blood)	Water and salt solution	Coagulated by heat
Globulins	Serum globulin (blood) Edestin (hemp seed)	Dilute salt solution	Coagulated by heat
Glutelins	Glutenin (wheat) Oryzenin (rice) Glutelin (corn)	Dilute acids and alkalies	Coagulated by heat
Prolamines	Gliadin (wheat) Zein (corn)	60-80% alcohol	Not coagulated by heat; rich in proline and glutamic acid
Protamines	Sturin and salmin (sperm)	Water, dilute	Not coagulated by heat;

	of fish)	acids and ammonia	strongly basic in reaction due to rich in diamino acids
Histones	Histone (thymus)	Water and dilute acids	Not coagulated by heat; basic in nature due to rich in diamino acids
Scleroprotein (albuminoids)	Elastin- (tendons) Keratin (hair)	Insoluble in common solvents	Hydrolyzed by long boiling with strong acid

The conjugated proteins are also sub-classified based on the chemical nature of the prosthetic group (Table 4.8).

Table 4.8 Sub-classification of conjugated proteins

Conjugated protein	Prosthetic group	Example
Lipoproteins	Lipid	Plasma β_1 -lipoproteins
Glycoproteins	Carbohydrate	Immunoglobulins
Nucleoproteins	Nucleic acid	Ribosomes
Flavoproteins	FAD	Succinate dehydrogenase
Phosphoproteins	Phosphoric acid	Casein (milk)
Chromoproteins	Colored substance	Hemoglobin (contains heme)
Metalloproteins	Metal ion	Ferritin (contains ferric hydroxide)

A third and useful way of classification of protein is based on conformation (overall three dimensional structures) as shown below:

1. **Fibrous proteins:** These proteins are composed of polypeptide chains arranged in parallel along a single axis to yield long fibres or sheets. They are insoluble in water and physically tough. e.g. collagen, the main protein constituent of connective tissue and bone, elastin, the main protein constituent of elastin tissue, the keratins of hair, skin, feather, horn and nails and fibroin, the major constituents of silk.
2. **Globular proteins:** The polypeptide chains of these proteins are tightly folded into compact spherical or globular shapes. They are soluble in water and usually show mobile or dynamic functions in the cell e.g. most of the enzymes, hormones and antibodies.

4.3.4 Structure of proteins

As mentioned earlier, proteins are linear chains of amino acids linked by peptide bonds between the carboxyl (-COOH) group of one amino acid and the amino (-NH₂)

group of the next. The free amino and carboxyl groups at opposite ends of the polypeptide chain are termed the N-terminus (amino terminus) and C-terminus (carboxyl terminus), respectively. A simple general structural formula for proteins would be as shown in Fig 4.14. Normally, the structure of a protein is discussed under four levels of organization:

a) the primary structure refers to the unique sequence of amino acid residues in the polypeptide chain which are covalently linked by peptide bonds and to the position of the disulfide bonds which form cross-links within or between peptide chains; (b) the secondary structure denotes certain repeating hydrogen bonded conformation patterns most common are of which are α -helix and β -pleated sheet; (c) the tertiary structure refers to the complete three dimensional architecture of the protein, including the orientation of prosthetic group, if present; and (d) the quaternary structure represents the non-covalent association of folded polypeptide subunits into a multi-sub-unit protein. These structures are discussed in greater detail.

4.3.4.1 Primary structure

It is the simplest level of structural organization and in some respects is the most important, since the conformation and function of a protein are determined by its primary structure. Insulin, a peptidal hormone involved in the regulation of glucose metabolism was the first polypeptide to be sequenced in the early 1950s at Cambridge University in England by Frederick Sanger. The two chains of insulin, A and B consist of 21 and 30 amino acid residues, respectively (Fig.4.15). Today, the amino acid sequences of many proteins are known.

The amino acid sequence can have a profound influence on the three-dimensional structure and biological activity. Even a change in one amino acid residue may adversely affect the biological activity of a protein. This is well illustrated in hemoglobin, the oxygen carrying protein, within red blood cells. The normal adult hemoglobin molecule (HbA) contains four polypeptide chains, two identical α -chains and two identical β -chains. Sickle cell anemia, an inherited disease of humans is characterized by low oxygen-binding capacity. This malfunction is due to the presence of an abnormal hemoglobin molecule (HbS) in the sickle cells as a particular glutamic acid residue (the sixth amino acid from N-terminal end) is replaced by a valine residue in both the β -chains of HbS. Despite this small difference, the HbS molecule does not function properly. It is apparent, however, that certain amino acid residues in a particular protein are more important than others in determining the structure and hence the function.

4.3.4.2 Secondary structure

As mentioned earlier, the secondary structure of protein refers to the conformational patterns of the polypeptide chain. The structural characteristics and arrangement of the peptide bond which repeats itself along the chain were obtained from

the X-ray diffraction studies of simple crystalline peptides by L.Pauling and R.B.Corey. According to these studies, two major types of conformations that are known to occur in naturally occurring polypeptide chains are the α -helix and the β -pleated sheet structure.

1. **The α -helix:** In polypeptide chains composed of L-amino acids, the most common helical orientation is called the right-handed α -helix (Fig.4.16) where the oxygen of each α -carboxyl group is hydrogen bonded to the amide nitrogen of the third amino acid from it along the polypeptide chain. Each peptide bond remains planar and the side chains of the amino acids project outward from the axis of the helix. The group on the α -carbon of the amino acid are perpendicular to the main axis of the helix and there are 3.6 amino acid residues per turn of the helix. The pitch of the helix (one complete turn) is 5.4\AA and the distance between each amino acid residue along the axis is 1.5\AA .

The percentage of α -helix content in globular proteins varies from 0-80 per cent while it would be 100 per cent in some fibrous proteins. The best examples of an α -helical structure are the keratins, myosin and fibrinogen. Charged residues, bulky residues and glycine tend to destabilize an α -helix and most neutral, less bulky residues favor a helical structure.

2. **Pleated sheet structure:** The second readily identified, hydrogen-bond stabilized structure found in proteins is the β -pleated sheet. A polypeptide having the appropriate sequence forms an extended conformation known as the β -conformation. A series of such polypeptide chains can associate via inter-chain hydrogen bonds to form a stable structure known as a β -pleated sheet. This type of secondary structure differs from the α -helix in that the hydrogen bonds are not parallel to the long axis of the polypeptide chain, but are perpendicular to it.

Two types of β -pleated sheets are possible. In one, the β -parallel pleated sheet, the hydrogen bonded polypeptide chains run in the same direction relative to their N-terminal and C-terminal ends (Fig.4.17). In the second, the antiparallel β -pleated sheet, the two hydrogen bonded chains run in opposite directions (Fig.4.17). Although both types occur, the latter is more stable because the C=O and -NH are oriented for maximum interaction. The best example of β -structure is silk fibroin. In such β -keratins, there are no cystine cross linkages between the side-to-side chains and the polypeptide chains are usually oriented in opposite (antiparallel) directions.

3. **Triple helix:** Collagen found in skin, tendons and cartilage is one of the best studied fibrous proteins. The recurring structural unit in collagen is the tropocollagen molecule. In tropocollagen, three left-handed helices (these are not α -helices as observed in α -keratins) are coiled around each other with a right-handed twist to form a triple helix (Fig.4.18) which is held together by interchain hydrogen bonds involving hydroxyproline and covalent links via modified lysine residues. Other distinguishing features in tropocollagen from α -helical conformation are the absence of intrachain

hydrogen bonds, a different number of residues per turn (pitch) and the presence of large number of proline and hydroxyproline residues.

4.3.4.3 Tertiary structure

The tertiary structure refers to the specific three-dimensional structure of the polypeptide chains of the protein. Detailed knowledge of the three-dimensional structure of proteins has been made available by the application of X-ray diffraction methods.

The tertiary structure includes the conformational relationship in space of the side chain groups to the peptide chain and the geometric relationship of the distant regions of the polypeptide chain to each other. For example, the α -helix does not completely describe the structure of fibrous proteins stabilized by numerous disulfide bonds between the individual chains constituting the tertiary structure. However, most soluble proteins (globular proteins) do not form long extended filaments like the fibrous proteins but are tightly folded into a compact structure, usually of a spherical shape. The globular protein has a distinctive three-dimensional structure that often includes localized region of α -helical conformation and β -structure. The biological properties of the proteins are a direct consequence of the three-dimensional structure.

The tertiary structure of globular proteins is determined by the amino and sequence (i.e. the primary structure) which is supported by numerous experimental observations: a) the denaturation of some globular proteins is reversible as shown by Anfinsen's experiments (b) X-ray analysis reveals that the R groups of globular proteins interact to maintain the three-dimensional structure, and (c) homologous proteins show similar tertiary structures.

The tertiary structure of globular proteins is maintained by four types of interactions between amino acid residues (Fig.4.19): covalent bonds (cystine cross-links), intra- and inter-chain hydrogen bonds, ionic bonds (electrostatic interactions) and association of hydrophobic R groups.

Using X-ray diffraction techniques, three-dimensional structure of some of the proteins like myoglobin and hemoglobin were established by John Kendrew and Max Perutz, respectively.

4.3.4.4 Quaternary structure

Many proteins are composed of more than one polypeptide chains, such multimeric proteins are called oligomeric proteins. The chains may be identical or different. Each chain is called a subunit or a protomer of the protein. Subunits generally

occur in even numbers. The spacial arrangement of subunits in a protein represents the fourth level of structure, known as quaternary structure. The polypeptide chains are not covalently bound together but are stabilized by the same weak forces which produce tertiary structures.

Hemoglobin, an oligomeric protein was the first protein for which a complete quaternary structure was determined. This protein has a total of four subunits, tow identical α - and two identical β -chains, each possessing a characteristic tertiary structure. These four chains are oriented in a specific arrangement relative to each other to form the quaternary structure of the hemoglobin molecule (Fig 4.20). The identical subunits of the protein may or may not be active when dissociated from the large unit.

CHAPTER 5 NUCLEIC ACIDS

Nucleic acids were first discovered in 1868 by Friedrich Meischer, a Swiss medical chemist during his studies on the chemistry of living cells. Using pus from discarded hospital bandages as the material for investigation, he managed to isolate a compound from the nuclei of pus cells and designated it as nuclein. On analysis of this material it was found to contain carbon, hydrogen, oxygen, nitrogen and phosphorous. Later studies by Altmann revealed that nuclein has acidic properties and therefore suggested that the compound be named as nucleic acid. Though it was later found that they occur both in the nucleus and cytosol of cells, the name has been retained.

Nucleic acids are high molecular weight biopolymers of nucleotides as repeating units which are linked by characteristic 3'-5' phosphodiester bonds.

5.1 Occurrence and biological role

Nucleic acids are one of the major components of all cells, making up from 5 to 15 per cent of their dry weight. They are also present in viruses. They are responsible for the storage and transmission of genetic information and translation of this information for a precise synthesis of proteins characteristics of the individual cell. The other biological role of different types of nucleic acids is discussed in subsequent pages.

5.2 Types

Based on the nature of sugar moiety and the nitrogenous bases present, two types of nucleic acids are recognized: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

5.3 Components

Complete hydrolysis of nucleic acids by acids, bases or specific enzymes yields three characteristics components: (a) heterocyclic basis (b) sugar and (c) phosphoric acid. The difference in hydrolysis products of RNA and DNA is given in Table 5.1. **Hydrolytic products of RNA and DNA**

Components	RNA	DNA
Acid	Phosphoric acid	Phosphoric acid
Sugar	D-Ribose	2'-Deoxy-D-ribose
Bases		
Purines	Adenine	Adenine
	Guanine	Guanine
Pyrimidines	Cytosine	Cytosine

	Uracil	Thymine
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5.3.1 Purine and pyrimidine bases

Two classes of heterocyclic nitrogenous bases are found in nucleic acids – those related to purine and pyrimidine. The principal purine bases found in both DNA and RNA are adenine (A) and guanine (G).

While cytosine is present both in DNA and RNA, uracil is present only in RNA and thymine in DNA. A few other pyrimidine bases occur in some nucleic acids eg. 5-methylcytosine in wheat germ and 5-hydroxymethylcytosine in some viruses.

Both the purine and pyrimidine bases exhibit lactam-lactim tautomerism as illustrated for uracil. At neutral and acid pH values, the lactam form predominates.

5.3.2 Sugars

The sugar present in nucleic acid is a pentose. In RNA the sugar is D-ribose and in DNA, as its name implies, the sugar is 2'-deoxy-D-ribose and both are present in the β -furanose form.

5.4 Nucleosides

The nucleosides are compounds formed by linking purine and pyrimidine bases to either D-ribose or 2'-deoxy-D-ribose in a N- β -glycosidic bound. The point of attachment of the bases to the sugar is N-9 of the purines or N-1 of the pyrimidines to C-1 of D-ribose or 2'-deoxy-D-ribose. The carbon atoms of the sugar are designated by prime numbers (i.e., C-1', C-5'), while the atoms in the bases lack the prime sign. Table 5.2 lists the trivial names of the purine and pyrimidine nucleosides which are related to the bases that occur in RNA and DNA.

Table 5.2 : Names of nucleosides

Components	RNA	DNA
Adenine	Adenosine	2'-Deoxyadenosine
Guanine	Guanosine	2'-Deoxyguanosine
Uracil	Uridine	2'-Deoxyuridine
Cytosine	Cytidine	2'-Deoxycytidine
Thymine	Thymine ribionucleoside	2'-Deoxythymidine

5.5 Nucleotides

These are phosphoric acid esters of nucleosides in which phosphoric acid is esterified with one of the hydroxyl groups of D-ribose (2',3' and 5' hydroxyl group) or 2'-deoxy-D-ribose (3' and 5' hydroxyl group). Therefore, 2', 3' or 5' ribonucleoside monophosphate and 3' or 5' deoxyribonucleoside monophosphates can be formed. However, the 5' position is most commonly phosphorylated.

Nucleoside monophosphates can be linked to a phosphate or pyrophosphate group through anhydride bonds to give nucleoside di- and triphosphate, respectively. The compounds are called acids or mono-, di- or triphosphate accordingly. The structure of the mono-, di-, and triphosphates of adenosine are shown in Fig. 5.7 as examples.

In the absence of oxygen atom at C-2' of the sugar ring, the structures are correspondingly known as deoxyadenosine-5'-monophosphate (dAMP), deoxyadenosine-5'-diphosphate (dADP) and deoxyadenosine-5'-triphosphate (dATP), respectively. A list of biologically important nucleotides is presented in Table 5.3

Table 5.3 : Biologically important nucleotides and their nomenclature.

Ribonucleotides	Deoxyribonucleotides
Adenosine-5'-monophosphate (adenylic acid; AMP)	Deoxyadenosine-5'-monophosphate (deoxyadenylic acid; dAMP)
Guanosine-5'-monophosphate (guanylic acid; GMP)	Deoxyguanosine-5'-monophosphate (deoxyguanylic acid; dGMP)
Cytidine-5'-monophosphate (Cytidylic acid; CMP)	Deoxycytidine-5'-monophosphate (deoxycytidylic acid ; dCMP)
Uridine-5'-monophosphate (Uridylic acid; UMP)	Deoxythymidine-5'-monophosphate (deoxythymidylic acid ; dTMP)

Each of the 5'-monophosphates exists as the 5'-diphosphate and 5'-triphosphate. Thus as an example, there occurs GMP, GDP, GTP, dGMP, dGDP and dGTP.

An important discovery has been the identification of cyclic nucleotides. An important cyclic nucleotide is 3',5'-cyclic adenosine monophosphate (3',5-cyclic AMP or cAMP) which is called a second messenger plays a key role in the biochemical action of a number of hormones.

5.5.1 Metabolic functions

All types of cells contain a wide variety of nucleotides and their derivatives. Nucleotides serve many functions some of the which are as follows:

1. Role in energy metabolism: ATP is the main form of chemical energy available to the cells. It is generated in cells by oxidative phosphorylation and substrate level phosphorylation. ATP is utilised to drive metabolic reactions, as a phosphorylating agent, and is involved in such processes as muscle contraction, active transport and maintenance of cell membrane integrity. As a phosphorylating agent ATP serves as the phosphate donor for the generation of the other nucleoside-5'-triphosphates (e.g. GTP, UTP, CTP).

2. Monomeric units of nucleic acids: The nucleic acids, DNA and RNA are composed of monomeric units of the nucleotides as building-blocks.

3. Physiological mediators: cAMP plays an important role as a 'second messenger' in epinephrine-and glucagon-mediated control of glycogenolysis and glycogenesis. cGMP acts as a mediator of cellular events. ADP is important for normal platelet aggregation and hence blood coagulation.

4. Components of coenzymes: Coenzymes such as NAD^+ , FAD and coenzyme A are important metabolic constituents of cells and are involved in many metabolic pathways.

5. Activated intermediates: The nucleotides also serve as carriers of activated intermediates required for a variety of reactions. UDP-glucose is a key intermediate in the synthesis of glycogen and glycoproteins. CTP is utilized to generate CDP-choline, CDP-ethanolamine which are involved in phospholipid metabolism.

6. Allosteric effectors: Many of the regulated steps of the metabolic pathways are controlled by the intracellular concentrations of nucleotides.

5.6 Deoxyribonucleic acid (DNA)

In prokaryotic cells, which contain only a single chromosome essentially all the DNA is present as a single double-helical, i.e., two-stranded macromolecule exceeding 2×10^9 in molecular weight. In eukaryotic cells, which contain several chromosomes, there are several DNA molecules. In diploid eukaryotic cells nearly all the DNA molecules are present in the cell nucleus, where they are combined with basic proteins called histones. In addition to the nuclear DNA, diploid eukaryotic cells also contain very small amounts of DNA in the mitochondria and chloroplasts which differ in base composition and molecular weight and free of protein complexes.

In bacteria, the DNA molecule which makes up about 1 per cent of the cell weight is found in the nuclear zone; it is usually attached at a single point, to an infolding of the cell membrane called a mesosome. In bacteria no protein is associated with the DNA. Sometimes small circular, double stranded extrachromosomal DNA molecules are found in the cytoplasm of bacteria, called plasmids. The DNAs isolated from different organisms and viruses normally have two strands in complementary double-helical arrangement.

5.6.1 Biological role

The functions of DNA may be summarized as below:

1. It contains the genetic information that is transmitted from generation to generation, which is achieved by self-replication of DNA during cell growth and division so that two daughter double helical molecules of DNA are obtained, each identical to parent DNA.

2. It expresses its encoded genetic information for the synthesis of RNA and proteins for metabolic function and control of all cellular activities. The genetic information is carried in the form of genes and expressed at appropriate times.

A gene is defined as a sequence of bases in DNA which specifies the complete amino acid sequence of polypeptide chain or the base sequence of an RNA molecule (rRNA, tRNA). The sequence that specifies a polypeptide chain is commonly called a structural gene.

Most genes of eukaryotic organisms do not consist of a single continuous sequence which is transcribed into mRNA. Rather, they are interrupted by regions called introns which do not specify the protein product. The regions which are transcribed and specify the final protein product are called as exons.

5.6.2 Characteristics and properties

1. **Genetic material:** DNA is the molecule of heredity and is responsible for the progeny to have the same characteristics as their parents.

2. **DNA content:** The DNA content of a cell is remarkably constant for each species (except in germ cells and when chromosomal variation occurs) and cannot be altered by environmental circumstances, with change in age or nutritional status.

3. **Base composition:** DNA isolated from different tissues of the same organism has the same base composition. The base composition of DNA varies from one species to the other; while the DNA from closely related species has more or less similar base composition.

In nearly all DNAs, the number of adenine residues is equal to the number of thymine residues i.e., $A=T$, and the number of guanine residues is equal to the number of cytosine residues, i.e., $G=C$ or $A+G = T+C$ or

$$\frac{A+G}{T+C} = 1$$

4. **Effect of pH:** DNA is a polybasic acid due to the presence of phosphate groups which are fully ionized at physiological pH. Because of negative charges present DNA binds strongly to histones and cations like Na^+ and Mg^{2+} . pH also affects the stability of the double helical structure of DNA. The hydrogen bonded base pairs are stable between pH 4.0 and pH 10.0. Outside these limits, their hydrogen bonds break and the complementary strands separate from each other, a process known as denaturation.

5. **Effect of temperature :** when highly polymerized double stranded DNA is slowly heated, the double helix 'melts', as a result the double-stranded structure is converted to a random coil over a range of a few degrees of temperature. This transition from a helix to

a coil results in increase in absorbance. The midpoint temperature (T_m) is the melting temperature of the helix of specific DNA polymer. The T_m 's of different DNA's increase linearly as a function of the percentage of G-C base pairs.

6. **Absorbance:** The purine and pyrimidine bases found in the DNA and also RNA, strongly absorb ultraviolet radiation of wave-length at 260 nm. This property is used to identify and estimate nucleic acids. The high molecular weight DNA typically has an optical density at 260 nm which is about 35-40 per cent less than the optical density expected from adding up the individual absorbances of bases in the DNA. The phenomenon is called the hypochromic effect which is explained by the fact that in a helical structure, the bases are stacked one above the other. Interaction of π electrons between the bases then results in a decrease in absorbancy.

7. **Hydrolysis:** Gentle acid hydrolysis of DNA at pH 3.0 causes selective hydrolytic removal of all its purine bases without affecting the pyrimidine-deoxyribose bonds or the phosphodiester bonds of the backbone. The resulting DNA derivative devoid of the purine bases is called an apurinic acid. Selective removal of the pyrimidine bases by hydrazine produces apyrimidic acid. DNA is not hydrolyzed by dilute alkali unlike RNA because of no 2'-hydroxyl groups.

Enzymes that hydrolyze the phosphodiester bonds of nucleic acid are collectively known as nucleases. Nucleases can be classified by their point of attack upon the polynucleotide chain. Those that attack the polymer at either its 3' or 5' terminus and sequentially remove nucleotide residues one at a time or as small oligonucleotides are known as exonucleases; those that attack within the chain are called endonucleases. The nucleases listed in Table 5.4 illustrate some of the diverse properties of these enzymes.

5.6.3 Structure

5.6.3.1 Covalent backbone structure

The monomeric units of DNA are called deoxyribonucleotides which are linked through a 3'-5'-phosphodiester bond with the phosphate group serving as a bridge between C-3' of one nucleotide and C-5' of the adjacent nucleotide. The alternating sugar-phosphate groups perform a structural role and serve as the backbone of the molecule. Different bases (A, G, C and T) are attached to the sugar residues and it is the precise sequence of bases along the polynucleotide chain that constitutes the primary structure of DNA. The bases of the nucleotide units are not present in the backbone structure but constitute distinctive side chains, just as the R groups of amino acid residues are the distinctive side chains of polypeptides.

The covalent backbone structure of a DNA chain and the segment of a DNA molecule are shown in Fig. 5.10

5.6.3.2 Shorthand representation

The covalent structure and base sequence of polynucleotide chains is often schematized as shown in Fig. 5.11. These diagrams are also useful in indicating the specific bonds cleaved during chemical or enzymatic hydrolysis of nucleic acids. The nucleosides of RNA are symbolized by A, U, G and C those of DNA by dA, dT, dG and dC. The letter p designates a terminal phosphate group, a hyphen as internal phosphate group. When p appears to the left of a nucleoside symbol, the phosphate is esterified to the 5' position when it appears at the right of the nucleotide symbol, the phosphate is esterified to the 3' position. Thus pA is adenosine 5'phosphate and Ap is adenosine – 3' phosphate. Oligonucleotides are conveniently symbolized as shown in the examples in Fig.5.11 To symbolize a DNA sequence the base symbols are prefixed by d, as in dA-T-G-Cp. The 3' terminus of an oligonucleotide is that end at which the terminal nucleoside is attached by its 5' carbon to the phosphoric group of the preceding nucleotide in the main chain.

5.6.3.3. Double helical structure (the Watson-Crick model)

In 1953, J. D Watson and F.H.C. Crick proposed double helical structure of DNA molecule (Fig.5.12) based on a) X-Ray diffraction analysis data on DNA fibers by R. Franklin and M.H.F. Wilkins b) The data of E.Chargaff and others on base pairing and equivalence and c) titration data which suggested that the long polynucleotide chains were held together through hydrogen bonding between base pair residues.

In summary, the salient features of the Watson - Crick Model of double helical structure of DNA are as follows.

1. DNA is a double helix made up of two polynucleotide chains which are wound into a right handed double helix about a central axis.
2. The two polynucleotide chains are antiparallel i.e. the phosphodiester bonds in one chain run in 5'→3' direction and in other chain run in 3'→5' direction.
3. Each polynucleotide chain consists of an alternate sugar-phosphate backbone lying on the outer side of the helix.
4. The purine and pyrimidine bases are stacked one above the other in pairs in the interior of the double helix, nearly perpendicular to the long axis of the helix. The distance between successive base pairs is 3.4\AA and a complete turn of about 10 base pairs. The diameter of the helix is 20\AA . One of the major forces holding the two chains together is the hydrogen bonds between base pairs (Fig.5.13). Thymine is always base paired with adenine by two hydrogen bonds (A=T) and cytosine is always base paired with guanine (G=C) by three hydrogen bonds. Thus, the two chains in a DNA molecule are not identical but complementary with respect to base pairing arrangement. In addition to the hydrogen bonding, there is also a vertical interaction between the stacked bases through hydrophobic forces which contribute significantly to the stability of the helical structure.

5.7 Ribonucleic acid (RNA)

The second nucleic acid component, RNA occurs in all prokaryotic and eukaryotic cells and in some viruses. RNA is present mainly in the cytosol. Unlike DNA, RNA is essentially a single stranded molecule. However, evidence indicates that there may be regions in the molecule where it folds back on itself to form helical segments held together through base pairing. The Watson- Crick base pairing can occur between adenine and uracil, and between guanine and cytosine. Some of these secondary structures are hairpin stems and loops that play a part in protein -RNA recognition.

5.7.1 Covalent backbone structure

The covalent backbone structure of RNA consists of a linear polymer or ribonucleotide units linked by 3'→5' phosphodiester bonds (Fig.5.14). The structure of RNA is very similar to that of single strand of DNA. Thus, like DNA, RNA is made up of a sugar, phosphate backbone with phosphodiester bonds linking the 3' hydroxyl group of one ribonucleotide to the 5'-hydroxyl group of the adjacent ribonucleotide.

Structurally, RNA differs from DNA in three important ways:

1. The sugar group of RNA is ribose, not 2' deoxyribose.
2. Thymine is replaced by uracil (U) as one of the four common bases. The common bases in RNA are adenine, uracil, guanine and cytosine. However, in some species of RNA, unusual bases are also present.
3. RNA molecules are usually single-stranded while DNA molecules are usually double-stranded.

5.7.2 Types

Although DNA is the primary genetic material within the cells, many RNA molecules participate in the processes by which this genetic information is expressed. Within a given cell, RNA molecules are found in multiple copies and in multiple forms.

Based on cellular location and function, three major types of RNA have been identified in all cells: (a) messenger RNA (mRNA), (b) ribosomal RNA (rRNA) and (c) transfer RNA (tRNA). The different types of RNAs are discussed below.

5.7.2.1 mRNA

This is also known as a template RNA. It is synthesized in the nucleus during the process of transcription. It has a primary structure complementary to a portion of one of the strands of DNA; as for example a segment of DNA-like A-T-C-A it is transcribed into U-A-G-C in mRNA structure. In bacterial cells, mRNA is highly unstable i.e., it is constantly being degraded and re-synthesized. In eukaryotic cells, the turnover rate of mRNA is much lower because of the presence of a long, sequence of about 200 successive adenylate residues at the 3' end which plays a role in the processing or transport of mRNA from the nucleus to the ribosomes.

5.7.2.2 rRNA

This is also known as a high- molecular RNA and constitutes upto 65 per cent of the mass of ribosomes. The major portion of the total RNA of a cell is ribosomal RNA. There are three types of rRNA, viz., 5S, 15S and 23S in prokaryotic cells and four types – 5S, 5.8S, 18S and 28S in eukaryotic cells. A few of the bases in rRNA are methylated. The rRNA is presumed to play a role in maintenance of structure of ribosomes.

5.7.2.3 tRNA

This is also known as a soluble and acceptor RNA because of its high solubility and acceptor and transfer of amino acid carrying function in the synthesis of protein, respectively. This RNA consists of some of the unusual nucleotide residues like – ribothymidylic acid (T), pseudouridylic acid (), dihydouracil (D) and methylguanine (mG). Additional evidence suggests that 60-70 per cent of the tRNA exists as a helical ‘cloverleaf’ structure with the anticodon (i.e., the nucleotide triplet necessary for the positioning of the specific RNA in the mRNA template during protein synthesis) located in the central petal of cloverleaf. The secondary (cloverleaf structure) and tertiary structure of tRNA are shown in Fig .

The physico-chemical properties of different RNAs are present in Table

Table: Physico-chemical properties of different RNAs

Type of RNA	Cellular content (%)	Size and sedimentation coefficient	Site of synthesis	Helix strands	Conformation	Functions
mRNA (cytoplasmic)	5-10	Depends on protein size, 1000 to 10,000 nucleotides	Nucleoplasm	Single	Extended	Transfer of genetic information from nucleus to cytoplasm of from gene to ribosome
rRNA (cytoplasmic)	75-80	28S, 5400 nucleotides; 18S, 2100 nucleotides; 5.8S, 158 nucleotides; 5S, 120 nucleotides	Nucleous Nucleous Nucleous Nucleoplasm	Single – double	Extended compact	Structural framework for ribosomes

tRNA (cyto- plasmic)	10-15	65 – 110 nucleotides 4S	Nucleo- plasm	Single – double	Clover- leaf	Transfer of amino acids to mRNA ribosome complex and correct sequence insertion
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5.7.2.4 Other types

In addition to the above three types of RNA molecules, eukaryotic cells contain a population of large, nuclear RNA molecules having widely varying molecular weights. These heterogenous nuclear RNA (hnRNA) molecules are the precursors of mature mRNA. Another group of small nuclear RNA (snRNA) molecules are bound to proteins in complexes known as small nuclear ribonucleoprotein particles (snRNPs or Snurps) which play an important part in the synthesis of mRNA.

5.8 Nucleoproteins

Certain nucleic acids occur in cells in non-covalent association with specific proteins as supramolecular complexes, of these complexes, the ribosome and the viruses are the best understood. Ribosomes are ribonucleoproteins particles found in all types of cells, they are essential in the biosynthesis of proteins.

Virus particles, or virions are composed primarily of nucleic acid and a number of specific protein subunits. Viruses infect animal plant and bacterial cells. The bacterial viruses are also called bacteriophages. Virus particles are capable of attaching and delivering their nucleic acid, the infectious portion of the virion, into the cytoplasm. The viral nucleic acid then monopolizes the biosynthetic machinery of the host cell, forcing it to synthesize the molecular components of virus molecules rather than the normal host cells nucleic acids and proteins.

All plant viruses contain RNA and are either rod-like helices, as in tobacco mosaic virus (TMV) or icosahedral as in tomato bushy stunt virus. Animal viruses contain either DNA or RNA. The bacterial viruses widely studied are those of *E. coli* cells such as the DNA bacteriophages T2, T4, T6, ϕ X174 and λ , and the RNA bacteriophages Q β , MS2 and R17

CHAPTER 6 ENZYMES

It is well known that highly complex synthetic and breakdown reactions take place much more rapidly and easily by the living organism. In the absence of the cell these chemical reactions would proceed too slowly. The principal agents which participate in the precise and orderly transformations and regulation of the chemical reactions in the cell belong to a group of proteins named enzymes.

An enzyme is a protein that is synthesized in a living cell and catalyzes or speeds up a thermodynamically possible chemical reaction. The enzymes in one way modify the equilibrium constant (K_{eq}) or the free energy change (ΔG) of a reaction.

6.4 Terminology

Some of the terms used in enzymology are defined below:

Substrate: The substrate acted upon by the enzyme.

Product: The substrate formed as a result of the enzymatic action

Active site or catalytic site: the site on the enzyme wherein the substrate is bound and is converted into products

Regulatory site: The site other than the active site on the enzyme wherein the effector or modulator is bound and controls the rate of enzyme catalyzed reaction.

Effector or modulator: The substance which binds at an allosteric site (the site other than the catalytic site) of the regulatory enzyme and may stimulate or inhibit the rate of enzyme catalyzed reaction

Holoenzyme: A completely catalytically active enzyme.

Holoenzyme =	Apoenzyme +	Cofactor
(active)	(inactive)	(inactive)

Cofactor: The non-protein component of the enzyme molecule required for complete activity. Cofactors can be classified into three groups; coenzymes, prosthetic groups and metal ions.

Apoenzyme: The protein component of the enzyme.

Coenzyme: The non-protein organic molecule that is not covalently bound and can be readily dissociated from the protein component of the enzyme by dialysis.

Prosthetic group: The non-protein component that is covalently bound and not readily dissociated from the protein component of the enzyme by dialysis.

Zymogen or proenzyme : The precursor form of the enzyme in an inactive form.

Activator: Any substance that increases the rate of an enzyme-catalyzed reaction.

Inhibitor: Any substance that reduces or inhibits the rate of an enzyme-catalyzed reaction.

Turnover number (molar activity): The number of molecules transformed into product (s) per minute by one molecule of the enzyme.

Specific activity: The amount of enzyme units present per milligram of protein.

Enzyme unit: The amount of enzyme which transforms one micromole of substrate per minute under defined assay conditions.

Michaelis-Menten constants (K_m): The substrate concentration at half the maximal velocity of the enzyme-catalyzed reaction.

6.5 General characteristics

The general characteristics of enzymes are as follows:

6.5.1 Specialized proteins

All the enzymes as far as known are specialized proteins that catalyze biochemical reactions. Enzymes show all the properties of protein, i.e., like proteins, enzymes are chemically made up of amino acids as building-blocks linked by peptide bonds; can be hydrolyzed to yield a mixture of constituent amino acids; lose catalytic activity if subjected to extreme pH, temperature, strong acids or bases, organic solvents or other conditions which denature protein and give typical color tests like biuret and FCR (Folin-Ciocalteu reagent) reactions.

6.5.2 Biological organic catalysis

Enzymes are also referred to as biological organic catalysis as:

1. They enhance the rate of specific chemical reactions.
2. They do not shift the equilibria of the reactions they catalyze.
3. They are regenerated during the course of the reaction and
4. They are effective in concentrations that are minute as compared with the concentrations of substrates undergoing reaction.

How an enzyme enhances the rate of chemical reactions?

For a chemical reaction $A + B \rightarrow C + D$ to occur, three criteria must be met:

1. The reactants, called substrates (A, B) must collide
2. The molecular collision must occur with the correct orientation, and
3. The reactants must have sufficient energy. This energy is called the activation energy which is the amount of energy required to bring all the molecules at a given temperature to the transition state. More specifically, it is the difference in energy between the ground state of reactants and the transition state.

Enzymes enhance the rate of chemical reaction by decreasing the activation energy of the reaction (Fig .6.1) and with a high probability of correct orientation of reactants. The transition state refers to the state at which all molecules at any given instant possess

enough energy to attain an activated condition before the reactants can be converted to products (C, D). This state is at the top of the energy barrier separating the reactants and products.

In the normal course, not all molecules acquire sufficient energy to attain the transition state by collision. There are two ways of increasing the reaction rate by:

- a) Increasing the temperature which increases thermal motion and energy of molecules capable of entering the transition state, and
- b) Lowering the energy of the transition state by the addition of catalysts.

In the enzyme-catalyzed reaction, the lowering of activation energy is achieved by the formation of activated ES (enzyme-substrate) complex by the combination of the enzyme with the substrate. This is clearly represented in Fig(6.1) where, (1) is the energy of activation, the difference in the energy level of reactants, A and B and the transition state for uncatalyzed reaction, (2) it is energy of activation for the formation of ES complex and is much less than (1) and (3), is the difference in energy levels between the reactant and product, i.e., overall free energy change of the reaction which remains the same in both catalyzed and uncatalyzed reactions.

6.5.3 Specificity

Unlike inorganic catalysts enzymes, the biological organic catalysts are more specific toward their substrates and for the type of reactions that catalyze. The term specificity refers to the affinity of the enzyme towards its substrate.

6.5.3.1 Types of specificity

Enzymes exhibit different types of specificity

1. **Absolute specificity:** Some enzymes act on only one substrate. Such enzymes are said to exhibit absolute specificity. For example, succinic dehydrogenase, a key enzyme of TCA cycle catalyzes only the oxidation of succinate to fumarate.
2. **Absolute group specificity:** Some other enzymes act on a very small group of substrates having the same functional group but at different rates. Such enzymes are said to exhibit absolute group specificity. For example, alcohol dehydrogenase oxidizes both ethanol and methanol which have common hydroxyl group. Similarly, hexokinase not only phosphorylates glucose but also fructose and mannose.
3. **Relative group specificity:** Some other enzymes exhibit relative group specificity. A given enzyme can act upon more than one group of substrates. For example, trypsin catalyzes the hydrolysis of both ester and amide bonds.
4. **Stereospecificity:** Many other enzymes show stereospecificity i.e., a given enzyme can act upon only particular stereoisomer. For example, L-amino acid oxidase acts only on L-amino acid but not on its D-form of amino acid. D-amino oxidase acts only on D-amino acid but not on its L-form.

The enzymes are so specific since the active site of each enzyme has the proper shape, size and charge to bind certain substructure only and to catalyze the conversion of these substrates to specific products.

6.6 Active site

Enzyme –catalyzed reactions occur at an asymmetric pocket of the enzyme called the active site. The conformation and chemical composition of the active site determines the specificity of enzymatic catalysis. Theoretically, the active site can be subdivided

a) A binding site, which includes the amino acid residues come into contact with the substrate, and (b) a catalytic site, which includes residues directly responsible for catalysis. However, the binding and bond-breaking processes are important in catalysis by enzymes.

In all cases where conformations of enzymes have been determined by X-ray crystallography, the active site has been found to a relatively small area of the enzyme surface. Furthermore, the active site is a specific three – dimensional region having a unique arrangement of amino acid side chains, which are often contributed by amino acids situated quite far apart on the linear sequence of polypeptide chain. For example, the groups on the active site of enzyme lysozyme are contributed by the side chains of glutamate 35, aspartate – 52, tryptophan – 62 and 63 and aspartate – 101 (number after each amino acid refers to its location along the polypeptide chain). The rest of the polypeptide chain may be extremely important in maintaining the correct three – dimensional conformation of the active site.

6.7 Theories for enzyme – substrate binding

Two theories have been proposed to explain interaction of substrate and enzyme.

6.7.1 Lock and key model

According to the lock and key model proposed by Emil Fisher in 1894, the substrate and enzyme have structural complementarity and fit together like lock and key i.e., the active site of the enzyme has a complementary shape of the substrate to form enzyme- substrate complex (Fig.6.2). This model has proved to be essentially correct in the case of enzymes known to exhibit absolute specificity.

6.7.2 Induced-fit theory

This theory proposed by D.E.Koshland in 1968 suggests that the substrate binds at the active site of the enzyme and then modifies the shape of the active site so that it becomes complementary for the substrate binding (Fig.6.3). For example, binding of substrate to lysozyme takes place in this way.

6.8 Nomenclature

6.8.1 Naming of enzymes

In the past enzymes were named in a haphazard manner as an when they were discovered. The ways in which enzymes are or have been named are listed below:

1. The first enzymes studied have been named for their colour their localization within the body or after the person who discovered them. However, this nomenclature had not been agreeable to many.

2. Later, many enzymes have been named by adding the suffix “ase” to the name of the substrate, for example, urease catalyzes the hydrolysis of arginine to ornithine and urea and so on. However, this nomenclature has not been always practicable.

3. Further, a systematic classification of enzymes has been adopted on the recommendation of an International Enzyme Commission as listed in the 1973 edition of Enzyme Nomenclature with a few exceptions. According to this, enzymes have been classified into six major classes and sets of classes based on the nature and type of reactions catalyzed. According to this, each enzyme is assigned:

A recommended name: It is usually short and appropriate everyday use.

A systematic name: It identifies the reaction the enzymes catalyzes and

A classification number: It is used where accurate and unambiguous identification of an enzyme is required, as in research journals, abstracts and indexes.

An example is given by the enzyme catalyzing the reaction:



The recommended name: creatine kinase

The systematic name: ATP: creatine phosphotransferase

Classification number : EC 2.7.3.2, where EC stands for Enzyme Commission, the first digit (2) for the major class name (transferases), the second digit (7) for the subclass (phosphotransferases), the third digit (3) for the sub-subclass (phosphotransferases with a nitrogenous group as acceptor) and the fourth digit (2) designates creatine kinase.

6.8.2 Enzyme classification

Enzymes can be classified into six major classes based on the nature and type of reactions catalyzed as given below:

1. **Oxidoreductases:** These enzymes catalyze oxidation or reduction reactions by transfer of hydrogen or electrons, e.g., succinic dehydrogenase.

2. **Transferases:** These enzymes are involved in transferring functional groups between donors and acceptors. The amino, acyl, phosphate, one-carbon and glycosyl groups are the major moieties that are transferred e.g., glutamic pyruvic transaminase.

3. **Hydrolases:** This group of enzymes can be considered as special class of transferases in which the donor group is transferred to water. The generalized reaction

involves the hydrolytic cleavage of C-O, C-N, O-P and C-S bonds. The cleavage of the peptide bond by peptidases is good example of this reaction. The proteolytic enzymes are a special class of hydrolases called peptidases.

4. **Layses:** These enzymes remove the groups of water, ammonia or CO₂ from the substrate to cleave double bond or conversely, add these groups to double bonds e.g., fumarase.

5. **Isomerases:** These are a very heterogeneous group of enzymes that catalyze isomerizations (i.e., structural rearrangements within a molecule) of several types. These include cis-trans, keto-enol, and adose-ketose interconversion. Isomerases that catalyze inversion at asymmetric carbons are either epimerases or racemases. Mutases involve the intramolecular transfer of a group such as phosphoryl group.

6. **Liagases (synthetases):** These enzymes are involved in synthetic reactions where two molecules are joined together at the expense of breakdown of nucleoside-triphosphates. The formation of aminoacyl tRNAs, acetyl coenzyme A, glutamine, and the addition of CO₂ to pyruvate are reactions catalyzed by ligases, e.g. pyruvic carboxylase.

The six major classes of enzymes and the type of reactions catalyzed are summarized in Table .6.1

Table 6.1 : Classification of enzymes and types of reactions catalyzed

S.N.	Enzyme class	Reactions catalyzed
1.	Oxidoreductases	Oxidation and reduction of substrates (usually involve hydrogen transfer)
	Dehydrogenases	Transfer of hydrogen atoms from substrate to NAD*
	Oxidases	Transfer of hydrogen atoms from substrate to oxygen
	Oxygenases	Partial incorporation of oxygen to substrate
	Peroxidases	Transfer of electrons from substrate to hydrogen peroxide
2.	Transferases	Transfer of a chemical group (such as a methyl group, amino group, phosphate group from one molecule to another
	Phosphorylases	Addition of orthophosphate to substrate
	Transaminases	Transfer of amino group from one substrate to another
	Kinases	Transfer of phosphate from ATP to substrate
3.	Hydrolases	Cleavage of bonds by the addition of water
	Phosphatases	Removal of phosphate from substrate
	Peptidases	Cleavage of peptide bonds

4.	Lyases	Addition of groups to double bond (-C=C-, C=O, -C=N-)
	Decarboxylases	Removal of carbon dioxide from substrate
5.	Isomerases	Rearrangement of atoms of a molecule
6.	Liagases	Formation of new bonds using energy from (simultaneous) breakdown of ATP
	Synthetases	Joining two molecules together

6.10 Factors affecting the rate of enzyme-catalyzed reactions

Several factors are known to influence the rate of enzyme-catalyzed reaction, chief of them being :

1. Enzyme concentration
2. Substrate concentration
3. Temperature
4. pH and
5. Activators and inhibitor

6.10.1 Effect of enzyme concentration

With few exceptions, the initial velocity (V_0) of enzymatic reactions bears a linear relationship with the concentration of the enzyme [E], provided other conditions do not act as limiting factors. If an excess substrate is present, doubling the enzyme concentration usually doubles the rate of formation of end products. This usually applies only at the start of the reaction, for the end-products of the reaction often have an inhibitory effect on the enzyme, and decrease its efficiency. Fig 6.5 is a graph which relates enzyme concentration to enzyme activity, all other factors being held constant. The dotted part is hypothetical, and almost impossible to attain *in vitro*; it may occur to a limited extent in the living cell.

6.10.2 Effect of substrate concentration

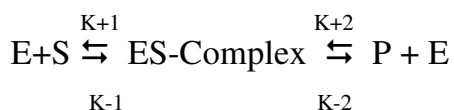
The concentration of substrate [S] affects the rate of the enzyme-catalyzed reaction. When the enzyme concentration is kept constant and the substrate concentration varied, the rate of enzyme-catalyzed reaction increases linearly upto a certain concentration of substrate after which a point is finally reached, where the enzyme is saturated with substrate. When this saturation point is reached, further increases in substrate concentration have no influence on rate of the enzyme-catalyzed reaction which is due to saturation of all the active sites of the enzyme by the substrate and partly due to more rapid accumulation of end-products causing inhibition of enzyme. The typical shape of curve obtained in this case is a nonlinear hyperbolic relationship between V_0 and substrate concentration (Fig 6.6).

Pioneering work on the kinetic studies of enzyme-catalyzed actions was made by A. Brown (1902) and V. Henri (1903). This was further developed by L. Michaelis and M.L. Menten in 1903 and by G.E. Briggs and J.B.S.Haldane in 1926.

To account for hyperbolic relationship. Brown put forward hypothesis that the enzyme (E) reversibly combines with the substrate (S) to form an enzyme-substrate (ES) complex which is decomposed to yield product (P) and the free enzyme in its original form.



Based on the work of Briggs and Henri, Michaelis and Ment derived a mathematical equation which is consistent with the empirical data represented in Fig . They assumed that a rapid equilibrium exists between E, S and ES and that ES-complex breakdown of ES to E and S. This plot is based on the following equation for the behavior of a simple enzyme-catalyzed reaction in which one substrate is converted to one product.



Where k_{+1} , k_{-1} , k_{+2} and k_{-2} are the velocity constants for the above four steps. By measuring the initial rate of reaction, the rate constant k_{-2} may be ignored because not enough products will be present to make the reverse reaction proceed at significant rate.

The equation they obtained is called Michaelis-Menten equation which is,

$$V_o = \frac{V_{\max} [S]}{K_m + [S]}$$

Where V_o = Initial velocity of the enzymatic reaction

V_{\max} = Maximal velocity at infinite substrate concentration,

[S] = Substrate concentration and

K_m = Michaelis – Menten constant

In Michaelis – Menten equation, when $[S] = K_m$, then

$$V_o = \frac{V_{\max} [S]}{[S] + [S]}$$

$$\text{Or } V_o = \frac{V_{\max}}{2}$$

Hence, K_m can be defined as the concentration of the substrate at which the reaction rate is half the maximal velocity. Usually, K_m has dimension moles per liter and its independent of enzyme concentration.

Determination of K_m and V_{max}

The hyperbolic curve of the V_o versus $[S]$ plot (Fig.6.6) does not permit the determination of exact values of K_m and V_{max} . This limitation can be overcome by plotting the same kinetic data in different ways. The most popular approach is simple by taking reciprocal of both sides of Michaelis-Menten equation to yield a straight-line rate equation, called a Lineweaver-Burk equation which is,

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

By plotting $1/V_o$ versus $1/[S]$, a straight line is obtained having a slope of K_m/V_{max} , an intercept of $1/V_{max}$ on $1/v_o$ axis and an intercept of $-1/K_m$ on $1/[S]$ axis. Such a plot is called a Lineweaver- Burk plot or double reciprocal plot (Fig. 6.7). By determining the value of $1/V_{max}$, K_m can be determined. K_m can also be directly determined by extrapolation of the line to meet the $1/(S)$ axis where the intercept is $-1/K_m$.

The double-reciprocal plot has the advantage that measurement of the velocity of the reaction can be made at a number of substrate concentrations and then extrapolated back to infinite substrate concentrations at the intercept, which gives a straight-line so that accurate values of V_{max} and K_m can be determined. This also gives variable information on enzyme inhibition.

Significance of K_m and V_{max}

The K_m value which is expressed in molar concentration is characteristic of each individual enzyme. K_m is not a fixed value may vary with structure of substrate, pH and temperature. For enzyme having more than one substrate, each substrate has a characteristic K_m value. K_m helps to evaluate the specificity of action of given enzyme towards similar substrates. The substrate with the lowest K_m value has highest apparent affinity for the enzyme. It also establishes an approximate value for the intercellular level of substrate as the enzymes are not necessarily saturated with their substrates. Knowledge of the K_m of an enzyme can be of consideration value in investigations of metabolic control.

V_{max} represents the efficiency of enzyme action and can be used to compare the catalytic efficiency of different enzymes.

6.10.3 Effect of temperature

As with most chemical reactions, the rate of an enzyme-catalyzed reaction increases with temperature, however, in the case enzymes there is a temperature at which thermal denaturation of protein causes a loss of activity and the rate begins to slow down. For each enzyme there is an optimum temperature at which it is more active and its activity slows down when the temperature is changed in either direction away from this optimum. This is because, temperature changes cause slight changes in the three-dimensional shape of protein, increases in temperature may cause the protein to loosen up and become less compact. Even small changes in the dimensions of its active site can make an enzyme a less efficient catalyst.

The velocity of a chemical reaction is related to temperature according to Vant Hoff's law which states that a rise of 10°C will double the speed of a chemical reaction.

$$\text{Temp. coeff. (Q}_{10}\text{)} = \frac{\text{Velocity at (T+10}^{\circ}\text{)}}{\text{Velocity at T}^{\circ}}$$

The temperature coefficient is usually expressed by the symbol, Q_{10} which is also applicable to enzyme-catalyzed reactions. For most enzyme-catalyzed reactions, Q_{10} is approximately 2 at lower temperatures, but gradually drops off until the rate is 1 (or lower) at higher temperatures. Fig.6.8 shows the general effect of temperatures (bell shaped curve) on most enzyme-catalyzed reactions. Most enzymes show a temperature optimum between 25 and 37°C but are inactivated at temperatures above 55°C although enzymes in thermophilic bacteria are active even at temperature exceeding 55°C .

6.10.4 Effect of pH

The activity of the enzyme is markedly influenced by the pH of the medium in which the reaction occurs. It is usually found that activity is shown over a limited range of pH and within this range, a bell-shaped activity curve (Fig) is often observed when enzyme activity is plotted against pH. Every enzyme has a characteristic pH optimum wherein the activity is at maximum and the activity decreases on either side of this value. Most enzymes have pH optimum in the region 6-8, which, however, varies with the source of the enzyme, the kind of substrate, the kind of buffer and the temperature. For example, pepsin has a pH optimum around 2, for enzymes of plants and fungi it is 4.0-6.5 and for most enzymes of higher animals it is 6.5-8.0.

At extreme pH values protein denaturation will occur with concomitant loss of enzyme activity. Slight changes in pH which do not denature the protein, may alter the state or degree of ionization on the R groups within the active site, thereby reducing the enzymes ability to bind the substrate and catalyze the reaction. Changes in pH can also alter the charges of the substrate molecules, thus changing the character of the enzyme-substrate complex.

6.10.5 Effect of activators and inhibitors

Activators are specific chemical compounds which accelerate the rate of enzyme-catalyzed reactions. Activators include metal ions, coenzymes and prosthetic groups. Many enzymes are activated by specific metals which are readily removed from the protein. These latter proteins are called metalloenzymes. The ions most commonly found in enzymes and an example of each are : Mg^{2+} in most phosphotransferases; Zn^{2+} in alcohol dehydrogenase; Mn^{2+} in arginase; Fe^{2+} in ferredoxin; and Cu^{2+} in cytochrome C oxidase.

Substances that reduce the activity of an enzyme are known as inhibitors. The different classes and mode of action inhibitors are discussed below.

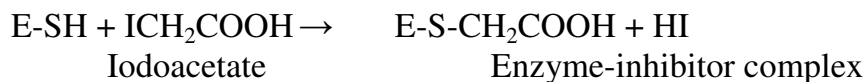
6.11 Enzyme inhibition

Enzyme inhibition refers to the phenomenon of decrease in the rate of enzyme-catalyzed reactions by specific substances known as inhibitors which include drugs, antibiotics, poisons, antimetabolites as well as products of enzymatic reactions. Inhibition studies are useful in understanding the specificity and the nature of functional groups at the active site of the enzyme and also the enzyme regulation.

Two general classes of inhibition are recognized namely, irreversible and reversible inhibitions.

6.11.1 Irreversible inhibition

This type of inhibition is brought about by an irreversible inhibitor which forms a covalent bond with a specific function, usually an amino acid residue, which may, in some manner, be associated with the catalytic activity of the enzyme. For example, iodoacetate reacts irreversibly with the essential sulfhydryl group of triosephosphate dehydrogenase.



The other example is irreversible inhibition of serine protease such as chymotrypsin by diisopropylphosphofluoridate (DIPF) which phosphorylates specific serine residue to yield inactive enzyme.

6.11.2 Reversible inhibition

This kind of inhibition is reversible in nature as the inhibitor which binds does not form a covalent bond with the enzyme molecule. Three distinct types of reversible inhibition are known and they are discussed below:

6.11.2.1 Competitive inhibition

As the name indicates, the competitive inhibitor (I) which has a structure similar to that of natural substrate (S) of the enzyme (E) reversibly binds at the active site of the enzyme to form an enzyme inhibitor (EI) complex analogous to enzyme-substrate (ES) complex. Thus, the inhibitor competes with the natural substrate for binding at the active site.

Competitive inhibition can be overcome by increasing the substrate concentration which causes the reaction sequence to swing to the right in the above equation.

A classical example of competitive inhibition is that of succinic dehydrogenase (SDH) which readily oxidizes succinic acid to fumaric acid, by malonic acid.

Another example of competitive inhibition is provided by sulpha drugs used to combat microbial infection in humans. These drug are structurally related to p-aminobenzoic acid (PABA), a vital precursor in the microbial biosynthesis of folic acid and inhibit the synthesis of this vitamin.

6.11.2.2 Non-competitive inhibition

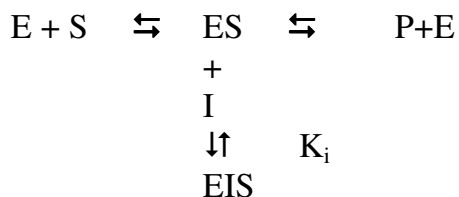
This type of inhibition is brought about by the inhibitors than bind reversibly with either the free enzyme or the enzyme substrate complex as shown below;

Non-competitive inhibition differs from competitive inhibition in that the inhibitor can combine with ES, and S can combine with EI to form both instance EIS. This type of inhibition is not completely reversed by high substrate concentration since the closed sequence of reaction will occur regardless of the substrate concentration.

The examples of non-competitive inhibitors are heavy metal ions and organic molecules which bind reversibly with sulphhydryl groups of cysteine residues in the enzymes.

6.11.2.3 Uncompetitive inhibition

Compounds that combine only with the enzyme-substrate complex but not with the enzyme are called uncompetitive inhibitors. This inhibition is not overcome by high substrate concentrations. The sequence of reaction is



An example of this type of inhibitor is α -ketoglutarate which inhibits the mouse brain γ -aminobutyrate amino transferase.

6.12 Use and application of enzymes

Enzymes are widely used in various fields due to their efficiency and specificity. Some of the commercial uses of enzymes are listed in Table 6.5

Table 6.5 Some commercial uses of enzymes

Field of application	Purpose	Enzyme used
Fermentation industry	Bread making and brewing	Amylases and proteases
	Clarification of beers and wines	Papain
Food industry	Production of sweeteners and sugar syrups	Amylase β -galactosidase, glucose isomerase
	Meat tenderizing	Papain, trypsin, chymotrypsin and other proteolytic enzymes from microbes
	Cheese making	Rennin / rennet
	Processing and clarification of fruit juices	Pectinases
Leather industry	Hydrolysis of proteins of hairs	Proteases
Paper industry	Preparation of partially hydrolyzed starch as surface coating	Amylases
Textile Industry	Removal of starch size before dyeing	Amylase
Pharmaceutical industry	As digestive capsules	Pepsin, a mixture of amylase, trypsin and lipase
Photography	Recovery of waste gelatin	Ficin
Forensic laboratory	Blood alcohol treatment	Alcohol dehydrogenase
Detergents	As biological detergents for partial degradation of polysaccharides and proteins from garments	A mixture of amylase and alkaline and neutral proteases
Medical application	Clearing of blood clots	Streptokinase
	Diagnosis of liver disease	Serum aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase
	Diagnosis of heart disease	Creatine kinase, aspartate amino-transferase, lactate

		dehydrogenase
	As therapeutic agents	Proteolytic enzymes

6.13 IMMOBILIZED ENZYMES

Immobilized enzymes are those purified enzymes which are attached to an insoluble support that will stabilize and permit the continuous use of enzymes for various commercial applications.

Immobilization of enzymes will also increase the efficiency and also reduce the cost of production of industrially important products that are beneficiary to the mankind.

6.14 METHODS OF IMMOBILIZATION

There are a number of methods of enzyme immobilization. Some of the methods are as follows:

1. Adsorption

The purified enzymes are adsorbed to insoluble support materials using alumina, bentonite, cellulose anion and cation exchangers, resins, glass, hydroxyapatite, kaolinite, etc.,

2. Covalent linkage

Insoluble support materials polyacrylamide, nylon, sephadex, silica glass beads and bifunctional reagents like 1, 5- difluoro-2,4-dinitro-benzene, dimethyladipalmidate, glutaraldehyde, etc., are employed for enzyme immobilization by this method the support materials require activation before enzyme attachment.

3. Matrix entrapment

This method involves the polyacrylamide gels polymerized in a solution containing the enzyme.

4. Encapsulation

Here, the enzymes are enclosed in a capsule usually generated from cellulose nitrate or nylon which is permeable to small ions and molecules.

1.15. Use and applications of immobilized enzymes

The use of immobilized enzymes may be broadly classified into preparative and analytical.

The preparative type includes production of sugar syrups from corn starch, preparation of fructose- glucose mixture, large scale preparation of L-amino acids from racemic D,L- amino acids, antibiotics, hormones, interferons, etc.,

Analysis of blood sugar using immobilized glucose dehydrogenase is an example of analytical type of application. This enzyme oxidizes D-glucose to D-glucono- δ -lactone in the presence of NAD^+ which is reduced to NADH and H^+ . The NADH formed is measured spectrophotometrically.

CHAPTER 7

BIOLOGICAL OXIDATION ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

7.1 Biological oxidation

Oxidation may be defined as the addition of oxygen ($2 \text{Mg} + \text{O}_2 \rightarrow 2 \text{MgO}$), the loss of hydrogen ($\text{CH}_3\text{CHO} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2\text{H}^+$) or by the most general definition: the loss of electrons. Thus, in the first two examples, a magnesium atom also loses two electrons to oxygen, and a carbon atom also loses two electrons to oxygen in the formation of magnesium oxide and acetic acid, respectively.

7.2. Electron Transport chain (ETC)

In electron transport chain or respectively chain, the oxygen-dependent oxidation reduction reactions involve intermediate electron carriers intervening in the flow of electrons between reduced metabolite and the electron acceptor, the oxygen. Each intermediate electron carrier would first participate in its oxidized state as an acceptor of electrons and then be converted to its reduced state. In the reduced state the carrier, as a donor, would then transfer electrons to the next carrier, as a donor, would then transfer electrons to the next carrier in its oxidized state and in doing so, it would be reconverted back to original oxidized state. The final carrier would transfer electrons to molecular oxygen, the terminal electron acceptor in respiration which would be reduced to water.

7.2.1 Components of the electron transport chain

Oxidation-reduction enzymes generally are more complex in structure and mechanism. Most of the members of the electron-transport chain are embedded in the mitochondrial inner membrane and exceedingly difficult to extract in soluble form and purify.

There are five different kinds of electron carriers that participate in the transport of electrons from reduced substrates as they are oxidized in mitochondria. These are (a) pyridine nucleotide, NAD^+ ; (b) flavin nucleotides, FMN or FAD, (c) coenzyme Q or ubiquinone, (d) a family of cytochrome (cyt. b, c, c_1 , a, a_3) and (e) non-heme iron-sulfur proteins. These components are discussed below.

7.2.1.1 Nicotinamide adenine dinucleotide (NAD^+)

The NAD^+ is the initial electron acceptor in the oxidation of several substrates like isocitrate, malate, α -ketoglutarate, pyruvate and glutamate among others. The structure of NAD^+ is shown in Fig .

In these reactions, only the nicotinamide moiety of NAD^+ is reversibly oxidized and reduced as shown in Fig.8.1

Out of two electrons and two protons removed from substrates two electrons and one proton combine with NAD^+ and the second proton is released in the medium. The enzymatic reduction of pyridine is ring of NAD^+ or NADP^+ is accompanied by a spectral change and is stereospecific.

7.2.1.2 Flavoproteins

The prosthetic groups FMN and FAD (Fig.) are tightly bound to their dehydrogenases (flavoproteins). The active portion of FMN or FAD that participates in the oxidoreduction is the isoalloxazine ring of the riboflavin moiety, which is reversibly reduced.

These flavoproteins accept two electrons and a proton from NADH or two electrons and two protons from succinate. The transfer of electrons from NADH of flavin coenzyme is shown in Fig.8.2.

7.2.1.3 Ubiquinone (coenzyme Q)

It is a lipid-soluble electron-carrying coenzyme also participates in the transport of electrons from organic substrates to oxygen in the respiratory chain of mitochondria. This coenzyme is a reversibly reducible quinone with a long isoprenoid side chain. It occurs in animals, plants and microbes. Several ubiquinons are known differing only in the length of isoprenoid side chain which has 6 isoprene units in some microbes and 10 in the mitochondria of animal tissues. In plant tissues the closely related plastoquinones perform similar functions in photosynthetic electron transport. The oxidized and reduced forms of ubiquinone are shown in Fig.8.3

7.2.1.4 Cytochromes

The cytochromes are electron-transferring heme-proteins containing iron-porphyrin as prosthetic groups and are found only in aerobic cells. All cytochromes undergo reversible Fe (II) – Fe (III) valence changes during their catalytic cycles. Their reduced forms cannot be oxidized by molecular oxygen, with the exception of the terminal cytochrome of mitochondrial respiration, namely, cytochrome a, or cytochrome c oxidase which also contains tightly bound copper. There are at least five different types of cytochromes (b, c, c_1 , a and a_3) in the mitochondria of mammals.

The porphyrins are considered as derivatives of parent tetrapyrrole compound, porphyrin. The porphyrins are named and classified on the basis of their side-chain substituents. Of these protoporphyrin IX, generally known as heme (Fig 8.4) is the prosthetic group of cytochromes b, c and c_1 . The protoporphyrin IX forms quadridentate chelate complex with Fe (II).

Cytochromes a and a_3 , together called cytochrome c oxidase, the respiratory enzyme contain different iron-porphyrin prosthetic group called heme A (Fig 8.4). The cytochromes are distinguished from one another by their characteristics absorption spectra and variation in redox potentials.

The function of cytochromes is to participate in electron transport reactions with the iron atom of the heme group undergoing a reversible oxidation-reduction.

7.2.1.5 Non-heme iron-sulfur proteins

This type of protein was encountered as ferredoxin in plants, in nitrogen fixation and photosynthesis before it was recognized to function in mitochondrial electron transport. Most of the iron present in many mitochondria is complexed to protein other than cytochromes and these are called non-heme iron proteins. The iron-sulfur proteins contain iron and acid-labile sulfur in equimolar amount (Fig 8.5). The iron atoms, usually two or more, are arranged in an iron-sulfide bridge that in turn is bonded to cysteine residues in protein. All the Fe S-proteins are characterized by low E_o' -values indicating a role as electron carriers. In the oxidized state, both iron atoms in the model are in Fe^{3+} state. When reduced, one iron becomes Fe^{2+} .

7.2.2 The pathway of electron transport

The sequence of electron-transfer reactions in the respiratory chain from NADH to oxygen (Fig 8.6) is now fairly well established NADH is the form in which electrons are collected from any different substrates through the action of NAD^+ -linked dehydrogenases. These electrons funnel into the chain via the flavoprotein in NADH dehydrogenase. On the other hand, other respiratory substrates are dehydrogenated by flavin-linked dehydrogenases, such as succinate dehydrogenase and acyl-CoA dehydrogenase which funnel electrons into the chain via ubiquinone. NAD^+ and ubiquinone thus serve to collect reducing equivalents from respiratory substrates oxidized by pyridine-linked and flavin-linked dehydrogenases. From ubiquinone, electrons are transferred through a specific order of the cytochrome carriers b , c_1 , c and aa_3 and ultimately delivered to O_2 , the terminal electron acceptor.

The points of entry of electrons from various substrates and the sites of inhibition of electron transport by rotenone, amytal and cyanide are shown in Fig 8.6 . The symbols FP designates flavoprotein; FP_1 – NADH dehydrogenase; Fe-S – iron sulfur centers (their positions in chain are still uncertain); Q-ubiquinone (coenzyme Q).

The sequence of electron carriers shown in Fig 8.6 is supported by many types of evidences. However, only two important evidences will be mentioned. First, the sequence is consistent with the standard redox potentials of the different electron carriers, which become more positive as electrons move from substrate to O_2 . Second, the intact mitochondria could be very carefully disrupted to sub-mitochondrial fragments which are still capable of complete electron transport. Further, disruption of these fragments yields

four separate respiratory complexes (I, II, III IV), each of which is capable of catalyzing a portion of the complexes *in vitro* indicate that complexes, I, III and IV will transfer electrons from NAD^+ to O_2 while complexes II, III and IV are required for the oxidation of succinate.

7.2.3 Inhibitors of electron transport

Inhibitors that block specific carriers in the electron-transport chain have yielded valuable information on the sequence of electron carriers in the respiratory chain. Three inhibitors have been found to block electron transport – rotenone and amytal act between NADH and ubiquinone of the chain and are believed to act on NADH dehydrogenase; and antibiotic antimycin A blocks the chain between cytochromes b to c and the third inhibitor cyanide blocks the chain from cytochrome aa_3 to oxygen, inhibiting cytochrome c oxidase enzyme (Fig 8.6).

7.3 Oxidative phosphorylation

As mentioned earlier, electron transport from NADH to oxygen proceeds with a very large decrease in free energy, which is the part conserved by the coupled phosphorylation of ADP to ATP in a process known as oxidative phosphorylation. While, the oxidative phosphorylation is associated with electron transport, the substrate level phosphorylation involves synthesis of ATP from ADP and energy rich compounds such as 1,3-diphosphoglycerate and phosphoenolpyruvate in glycolysis.

Oxidative phosphorylation is fundamental to all aspects of cellular life in aerobic organisms since it is the principal route of ATP synthesis. Mitochondria, present in all aerobic eukaryotic cells are the sites of oxidative phosphorylation. The enzymes of electron transport and oxidative phosphorylation are located in inner membrane of mitochondria.

Two striking characteristics of the electron transport process relevant to the mechanism of energy conservation during electron transport are a large number of sequential electron-transferring steps resulting in stepwise release of energy and the H^+ ions are absorbed and released at some of these steps suggesting that proton exchanges are involved in energy conservation.

7.3.1 Coupling of oxidative phosphorylation to electron transport

As depicted in Fig 8.6 earlier, the ATP formation from ADP is coupled to the electron transport chain. Three molecules of ATP are synthesized at three separate points in the chain when electrons flow from NADH to oxygen in the chain. These sites have been established by measurement of p/O ratios with different substrates in the presence and absence of respiratory inhibitors. The p/O ration corresponds to the number of molecules of inorganic phosphate (p_i) used up per gram atom of oxygen consumed. The

p/O ration is 3.0 for NAD^+ linked electron transport and 2.0 for flavin-linked electron transport.

The three sites of ATP synthesis in the respiratory chain are a site I, between NAD^+ and ubiquinone, (b) site II, between cytochromes b and c, and (c) site III, between cytochrome a and oxygen.

7.3.2. Uncoupling and inhibition of oxidative phosphorylation

Oxidative phosphorylation is influenced by a number of chemical agents, which can be grouped into three major classes; (a) the uncoupling agents which allow electron transport to continue but prevent the phosphorylation of ADP to ATP, e.g. 2,4-dinitrophenol (b) agents which inhibit both electron transport and phosphorylation e.g., oligomycin, and (c) ionophores which inhibit oxidative phosphorylation by utilizing the energy of electron transport for the cation transport as they complex with K^+ , Na^+ or other cations e.g., gramicidin, valinomycin, etc. (antibiotics).

7.3.3 Mechanism of oxidative phosphorylation

Three major hypotheses have been proposed to explain the coupling of phosphorylation to electron transport : (a) the chemical-coupling hypothesis which postulates formation of a high-energy covalently bonded intermediate, (b) the conformational-coupling hypothesis which postulates an intermediate high-energy conformational state, and (c) the chemiosmotic-coupling hypothesis which is most consistent, postulates that an electrochemical gradient of H^+ ion across the inner membrane of mitochondria is the coupling vehicle.

CHAPTER 8 METABOLISM

One of the main attributes of living matter is its ability to extract, transform and use energy from the environment for maintenance, growth and development. This is achieved by a constant breakdown, turnover and resynthesis of the macromolecular components of living organisms. During digestion, the complex mixture of carbohydrates, fats and proteins are broken down into a simpler form glucose, amino acids, fatty acids and glycerol which can be absorbed by the organism to maintain its orderly structure at the expense of energy derived from these nutrients. This principle is manifested in the process of metabolism.

The sum total of all the enzymatic reactions occurring in the cell is collectively called metabolism (Greek –‘metabolos’ means changeable). The reaction sequences occurring within organisms in an orderly and regulated way are known as metabolic pathways and the compounds formed as a result of metabolism are called metabolites. Because metabolism proceeds in a stepwise manner through many intermediary metabolites, the term intermediary metabolism is used to denote the chemical pathways of metabolism.

8.1 Functions of metabolism

Cellular metabolism has four specific functions

1. To obtain chemical energy, generally as ATP through the degradation of energy-rich nutrient biomolecules from the environment or through capture of solar energy.
2. To transform nutrient biomolecules into the building-blocks or precursors needed for the synthesis of macromolecular cell components.
3. To assemble such building-blocks into proteins, nucleic acids, lipids and other cell components and
4. To form and degrade biomolecules required in specialized functions of cells.

8.2 Phases of metabolism

Reactions comprising the total metabolism of any cell can be broadly divided into two phases-catabolism and anabolism.

8.2.1 Catabolism

It refers to all the degradative reactions involving degrading or breakdown of various complex molecules to smaller and simple molecules. The chemical energy of metabolites is conserved in the form of ATP.

8.2.2 Anabolism

It refers to all the synthetic reactions involving synthesis of various molecules from smaller and simpler precursor molecules. This process requires the input of chemical energy in the form of ATP.

In living systems both catabolism and anabolism occur concurrently and simultaneously. The energy released during catabolism is required for anabolic reactions and for many other cellular activities like muscular contraction, active transport, osmotic works, catabolic reactions are oxidative whereas anabolic reactions are reductive in nature.

The pathway of reactions that is accessible to both catabolism and anabolism is called amphibolic pathway (Greek- 'amphi' means 'both') which can be used catabolically to bring about completion of the degradation of small molecules derived from catabolism or can be used anabolically to furnish small molecules as precursors in biosynthetic reactions.

8.3 Stages of metabolism

Catabolic and anabolic reactions take place in three major stages (Fig 9.1). In stage I of catabolism, large nutrient molecules are degraded to their major building-blocks. Thus, polysaccharide are degraded to yield hexoses or pentoses; lipids to yield fatty acids, glycerol and other components; and proteins to yield their component amino acids. In stage II of catabolism, the many different products of stage I are collected and converted into smaller number of still simpler intermediates. Thus, the hexoses, pentoses and glycerol are degraded via the three-carbon intermediate pyruvic acid to yield a single two-carbon species, the acetyl group of acetyl-CoA. Similarly, the various fatty acids and amino acids are broken down to form acetyl-CoA and few other products. Finally, the acetyl groups of acetyl-CoA as well as other products of stage II, are channeled into stage III, the final common catabolic pathway, in which they are ultimately oxidized into carbon dioxide and water.

During anabolism, the intermediates formed in stage III are converted into building-block molecules of stage II which are finally assembled into macromolecules of stage I.

The pathways of catabolism differ from the pathways of anabolism. Whereas, catabolic pathways are convergent with many nutritional macromolecules being converted to common end products biosynthetic pathways are divergent, with a few precursors being converted to many different products.

8.4 Metabolism of carbohydrates

Carbohydrates are the major components of diet in terms of bulk and supply of energy. In man's food the chief source of carbohydrates is starch, the polysaccharide produced by plants, especially the cereal crops, during photosynthesis. In animals, carbohydrate is stored as glycogen, primarily in the liver (2-8%) and muscle (0.5-1%). Glucose is the compound formed from both starch and glycogen on metabolism.

8.4.1 Anaerobic metabolism of carbohydrates—glycolysis and alcoholic fermentation

The term 'glycolysis' is derived from the Greek words 'glycos', sugar (sweet) and 'lysis', dissolution. The sequence of reactions by which glucose is degraded anaerobically to pyruvate with the concomitant production of ATP is called glycolysis. Glycolysis is one of the several catabolic pathways known generally as anaerobic fermentations, by which many organisms extract chemical energy from various organic fuels in the absence of molecular oxygen. It is the most ancient type of biological mechanism for obtaining energy from nutrient molecules.

Moreover, in most animals glycolysis serves as an important emergency mechanism capable of yielding energy for short periods when oxygen is not available. Most higher animals have retained the capacity for anaerobic degradation of glucose to pyruvate and then to lactate as a preparatory pathway for aerobic metabolism of glucose, which harvests most of the energy contained in glucose.

8.4.1.1 Overall view of glycolysis

The reaction sequence of glycolysis is given in Fig 9.2. Glycolysis is catalyzed by the consecutive action of a group of 11 enzymes which have been isolated from the cytosol of cells and most of them have been crystallized and studied thoroughly.

There are two major stages of anaerobic glycolysis. In the stage I, glucose is activated prior to cleavage into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. In this process two molecules of ATP are consumed. The stage I also serves as a preparatory or collection phase, in which a number of different hexoses and polysaccharides such as glycogen and starch after phosphorylation, enter the glycolytic sequence and are converted into a common product glyceraldehyde-3-phosphate. Stage II of glycolysis is the common pathway for all sugars; in this stage II, triose-phosphate is converted into lactate. During this process, there is oxidation of glyceraldehyde-3-phosphate, followed by other rearrangements resulting in the production of four molecules of ATP, thus producing a net gain of two molecules of ATP per molecule of glucose degraded to lactate.

Under anaerobic conditions, the reactions of glycolysis account for the formation of lactate from glucose (or glycogen or other hexoses or pentoses) in some cells and for the

production of alcohol from glucose in others, many microorganisms use the same pathway in the different fermentations they bring about; the reactions are identical upto the formation of pyruvate and the only difference is the way pyruvate is converted to other products.

The overall balanced equation for glycolysis can be written as:
 $\text{Glucose} + 2 \text{ P} + 2 \text{ ADP} \rightarrow 2 \text{ lactate} + 2 \text{ ATP} + 2 \text{ H}_2\text{O}$

8.4.1.2 Energetics of glycolysis

The gain or loss of ATP from the reactions of the glycolytic pathway are indicated in Table 9.1

8.4.1.3 Importance and regulation of glycolysis

Glycolysis is the central pathway of glucose metabolism in most organisms, including microorganisms, plants and humans. It is only the first stage in the overall degradation of glucose to CO_2 and H_2O . Under anaerobic conditions, one glucose molecule is degraded into two pyruvate molecules releasing two ATP molecules. Under aerobic conditions, pyruvate can be oxidized to CO_2 and H_2O with a release of large quantity of free energy that is also captured in ATP molecules. Hexoses, other than glucose, such as fructose and galactose, are enzymatically converted into intermediates of the glycolytic pathway as are certain pentoses. An ancillary function of glycolysis is the production of 2,3 – diphospho-D-glycerate, an allosteric inhibitor of hemoglobin.

Regulation of glycolysis occurs primarily by inhibition of the allosteric enzymes hexokinase, phosphofructokinase and pyruvate kinase, each of which catalyzes a thermodynamically irreversible reaction of glycolysis.

8.4.1.4 Alcoholic fermentation

In organisms like brewer's yeasts, which ferment glucose to ethanol and CO_2 rather than to lactic acid, the fermentation pathway is identical to that described for glycolysis except for the terminal step catalyzed by lactate dehydrogenase, which is replaced by two other enzymatic steps.

In the first step, pyruvate is decarboxylated to acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase which is absent in animal tissues:

In the final step of alcoholic fermentation, acetaldehyde is reduced to ethanol regenerating NAD^+ from NADH formed during anaerobic glycolysis in the presence of alcohol dehydrogenase.

The overall balanced equation for alcoholic fermentation can be written as:



The energy-conserving steps leading to ATP formation are identical in both glycolysis and alcoholic fermentation Table 9.1

Table 9.1: Energy production during glycolysis and alcoholic fermentation

Reaction step	ATP/hexose molecules
Glucose → glucose-6-phosphate	-1
Fructose -6-phosphate → fructose-1, 6-diphosphate	-1
(2) 1,3-Diphosphoglycerate → (2) 3-phosphoglycerate	+2
(2) Phosphoenolpyruvate → (2) pyruvate	+2
Net yield	+2 ATP

‘+’ : ATP produced ; ‘-’ : ATP consumed

8.4.2 Glucogenesis

In most cells, the conversion of glucose or glucose-6-phosphate to pyruvate, catalyzed by the glycolytic enzymes, is the central pathway of carbohydrate catabolism, under either aerobic or anaerobic conditions. In a comparable manner, the reverse process, the conversion of pyruvate to glucose-6-phosphate, is the central pathway in the biosynthesis of carbohydrates by many different organisms.. The biosynthesis of glucose from smaller carbon fragments such as pyruvate and lactate is termed glucogenesis. Since pyruvate can also be derived from non-carbohydrate compounds such as amino acids, glucose synthesis from pyruvate in such cases is known as gluconeogenesis (neo means ‘new’ and genesis means ‘formation’) indicating the formation of glucose from new non-carbohydrate precursors.

Glucogenesis involves reactions that bring about the condensation of two three-carbon molecules to yield one six carbon molecule. Seven reactions of glycolysis are common for both glycolysis and glucogenesis and they are reversible. Three enzymes of the glycolytic pathway, namely hexokinase, phosphofructokinase and pyruvate kinase are not utilized by the process of glucogenesis which, on the other hand, requires the involvement of four new enzymes, glucose-6-phosphatase, fructose diphosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase. The summary of glucogenesis pathway is given in Fig 9.3 .

8.4.3 TCA cycle

The TCA cycle also known as citric acid cycle is a cyclic sequence of reactions of almost universal occurrence in aerobic organisms. It is the process in which acetate (in the form of acetyl -CoA) is oxidized completely to CO₂ and H₂O. The electrons removed

from the substrates as they are oxidized are transferred through a series of electron carrying proteins to molecular oxygen via electron transport chain to form water and large quantity of chemical energy in the form of ATP is produced in the process known as oxidative phosphorylation. All these processes take place within the same organelle, the mitochondrion in eukaryotic organisms unlike the glycolytic reactions which occur in the cytosol of the cell.

H.A. Krebs, in 1937 postulated the citric acid cycle involving cycle of reactions which accounted for the oxidation of pyruvic acid to CO_2 and H_2O . Hence, the TCA cycle is also known as Krebs cycle in his honour.

8.4.3.1 Reactions of the TCA cycle

The enzymatic reactions of the TCA cycle take place within the inner mitochondrial compartment, whereas others are attached to the inner mitochondrial membrane. Some of the enzymes also occur in the cytosol of some tissues.

The oxidation of pyruvate to acetyl-CoA

Pyruvate which is formed by anaerobic breakdown of glucose via glycolytic sequence undergoes oxidative decarboxylation to yield a acetyl-CoA catalyzed by pyruvate dehydrogenase complex (PDC) which is localized in the mitochondrial membrane of higher organisms.

This reaction itself is not part of the TCA cycle but is obligatory for the entry of all carbohydrates (via pyruvate) to the TCA cycle.

The pyruvate dehydrogenase complex is a multienzyme complex composed of three enzymes. a) Pyruvate dehydrogenase b) dihydrolipoyl transacetylase and c) dihydrolipoyl dehydrogenase. In the initial reaction, pyruvic acid is decarboxylated to form CO_2 and a acetyl complex of TPP that is bound to pyruvate dehydrogenase. The two carbon acetyl group is next transferred to an oxidized lipoic acid moiety that is covalently bound to the enzyme, dihydrolipoyl transacetylase. In the third reaction, the acetyl group is transferred to coenzyme A to form acetyl -CoA, which dissociates from the enzyme in a free form. The reduced lipoic acid moiety of the dihydrolipoyl dehydrogenase is then reoxidised to the cyclic lipoyl form by the third enzyme of the complex, dihydrolipoyl dehydrogenase, a flavoprotein that contains FAD. Finally, the reduced flavin co-enzyme is reoxidized by NAD^+ and NADH is produced. The reactions involved in TCA cycle are shown in fig 9.4

8.4.3.2 Energetics of the TCA cycle

The overall reaction of the TCA cycle may be written as

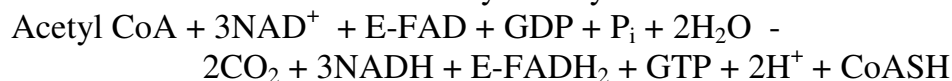


Table 9.2 summarizes the steps in glycolysis and the Krebs cycle that provide energy in the form of ATP. Under aerobic conditions, approximately 40 percent of the energy theoretically obtainable from glucose is captured in ATP. Under anaerobic conditions there is only about 1/18 of this yield.

8.4.3.3 Regulation of the TCA cycle.

The flow of pyruvate in to the TCA cycle is regulated through the activities of the enzymes as summarized below.

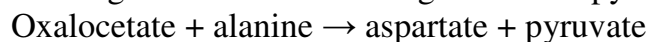
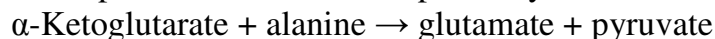
Table 9.2. Production of energy during the cellular oxidation of glucose

Reaction step	ATP used (-) or formed (+) per glucose molecule		Comments
	Anaerobic	Aerobic	
Glycolysis			
Glucose → glucose-6-phosphate	-1	-1	
Fructose-6-phosphate → fructose-1, 6-diphosphate	-1	-1	
(2) Glyceraldehyde-3-phosphate → (2)I, 3-diphosphoglycerate	0	+6	2NADH = 6ATP (Oxidative phosphorylation)
(2)I, 3-Diphosphoglycerate → (2) 3-Phosphoglycerate	+2	+2	(substrate level phosphorylation)
(2) Phosphoenolpyruvate → (2) Pyruvate	+2	+2	
Pyruvate → acetyl-CoA	0	+6	Glucose → 2 pyruvate 2NADH=6ATP
TCA cycle			
Isocitrate → α-ketoglutarate	0	+6	2NADH =6ATP
α-ketoglutarate → succinyl-CoA	0	+6	2NADH =6ATP
Succinyl-CoA → Succinate	0	+2	2GTP=2ATP
Succinate → fumarate	0	+4	2FADH ₂ = 4ATP
Malate → Oxaloacetate	0	+6	2NADH = 6ATP
Total Net →	+2	+38	(all by oxidative phosphorylation) except succinyl CoA to succinate step

1. The activity of the pyruvate dehydrogenase complex which furnished a major portion of the acetyl-CoA input into the cycle is diminished by the ATP dependent phosphorylation of the dehydrogenase component and is activated by dephosphorylation of the phosphoenzyme.
2. The concentration of oxaloacetate and its condensation with acetyl-CoA to yield citrate catalyzed by citrate synthetase which is regulated by the negative modulators succinyl-CoA, NADH and fatty acyl-CoA.
3. The activity of NAD^+ - linked isocitrate dehydrogenase is regulated by ADP, the positive modulator.
4. The activity of α -ketoglutarate dehydrogenase is regulated by succinyl-CoA (product inhibition) and
5. The activity of succinate dehydrogenase is promoted by high concentrations of succinate, phosphate and ATP and is inhibited by oxaloacetate.

The TCA cycle is also regulated by the concentrations of its various intermediates because some of the reactions also function in bio-synthesis of other compounds as summarized below:

1. α -ketoglutarate and oxaloacetate are enzymatically transaminated into glutamate and aspartate amino acids, respectively.



2. Citrate serves as a precursor of extramitochondrial acetyl-CoA for fatty acid biosynthesis through the ATP-citrate lyase reaction.



3. Succinyl-CoA can be removed from the cycle for heme biosynthesis.
4. In plants and some microorganisms, a modification of the TCA cycle (the glyoxalate cycle) allows the synthesis of citric acid cycle intermediates from acetyl-CoA.

In order to maintain the flow of acetyl-CoA into the cycle, intermediates must be replenished by special enzymatic reactions called anaplerotic ('filling-up') reactions. This occurs through two specialized enzymatic reactions catalyzed by pyruvate carboxylase and phosphoenolpyruvate carboxykinase. The former transforms pyruvate to oxaloacetate and the latter transforms phosphoenolpyruvate to oxaloacetate.

The TAC cycle is the final common pathway for the oxidation of the major fuel molecules of the cell. The oxidation products of carbohydrates, fatty acids and amino acids converge in this pathway. Thus, it plays a central role in metabolism (Fig 9.5).

8.4.4 The pentose phosphate pathway

Although the main pathway of glucose metabolism is via glycolysis and the Krebs cycle, plants, bacteria and mammals do have another series of enzymes which permit direct oxidation without the anaerobic breakdown of glucose. This has been called the pentose phosphate pathway also known as the phospho-gluconate pathway or hexose monophosphate (HMP) shunt or direct oxidative pathway or the Warburg-Lipmann-Dickens pathway. The term shunt indicates a bypass and in some ways this pathway is a bypass to glycolysis.

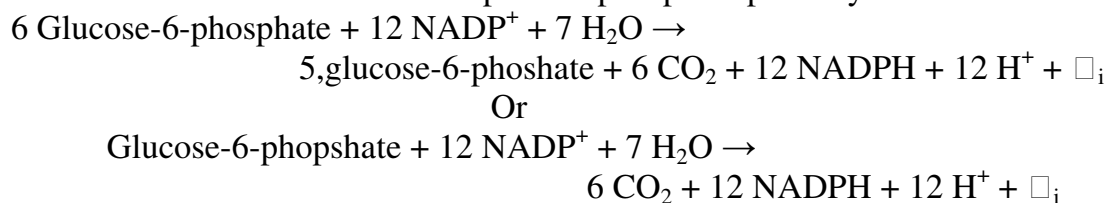
8.4.4.1 Reactions of pentose phosphate pathway

In this pathway molecular oxygen is introduced in the first step and NADP^+ acts the first electron acceptor. Electrons are then transferred in the usual fashion to molecular oxygen via electron transport chain.

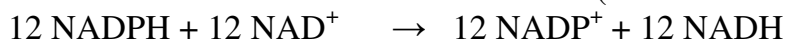
The eight reactions of the pathway can be divided into three types (a) oxidative, (b) isomerizing and (c) rearrangement. In the oxidative step, glucose-6-phosphate is converted into ribulose-5-phosphate; in the isomerizing steps, ribulose-5-phosphate is converted into ribiose-5-phosphate and xylulose-5-phosphate; and in the rearrangement steps, there is an interconversion of three-, four-, five-, six- and seven-carbon sugars in a series of non-oxidative reactions involving transaldolation and transketolation reactions. The pathway is summarized in Fig 9.6 .

8.4.4.2 Energetics of pentose phosphate pathway

The overall reaction of the pentose phosphate pathway is:



In this pathway, for six units of glucose-6-phosphate, the net chemical effect is seen to be the same as the combined operation of glycolysis and TCA cycle, namely the equivalent of one hexose unit is oxidatively degraded to 6CO_2 . The pathway is equally effective in conserving chemical energy. The 12 molecules of NADPH on reoxidation to NADP^+ by NAD^+ give rise to 12 units of NADH which through oxidative phosphorylation yields about the same number of ATP molecules ($12 \text{ NADH} = 36 \text{ ATP molecules}$).



8.4.4.3 Significance of the pentose phosphate pathway

Apart from its energetics point of view, this pathway has other significant metabolic functions.

1. The NADPH which is generated through this pathway is required in reductive biosynthesis of fatty acids and steroids.
2. It supplies ribose needed for nucleic acid biosynthesis
3. The ribose-5-phosphate which is produced as an intermediate is utilized for photosynthetic formation of glucose from CO₂ in some plants.
4. It also supplies erythrose-4-phosphate required for the biosynthesis of phenylalanine, tyrosine and tryptophan.
5. In humans, the shunt pathway is operative in liver where there is considerable biosynthetic activity and in red blood cells where NADPH is required to maintain high levels of reduced glutathione, a biological reducing agent.
6. This pathway also makes possible interconversion of various three, four, five, six, and seven carbon sugars and connects all such sugars metabolically with the glycolytic sequence.

8.5 Metabolism of lipids

Dietary lipids are a complex mixture of the neutral lipids (triacylglycerols), phospholipids, sterols and other complex lipids found in plant and animal tissues used as food. Lipids are stored mainly as triacylglycerols in plants and animals. Catabolism of these lipids provides one of the most efficient means by which living organisms derive energy for growth and maintenance. Lipids have got the highest calorific value of foods - 9k cal per gram as against 4 kcal per gram for each carbohydrates and proteins. Fatty acids provide upto 30 per cent of the total calorie requirements in humans on a normal diet. During fasting, they become virtually sole source of energy.

8.5.1. The Pathway of fatty acid oxidation – the Knoop's β -oxidation scheme

The essential features of the pathway for fatty acid oxidation were demonstrated by the German biochemist F.Knoop in 1904, who observed that when rabbits were fed on even carbon fatty acid labelled with a phenyl group at the methyl terminal (ω -carbon) atom, phenylacetic acid was excreted in the urine, regardless of the length of the carbon chain of the acid fed. On the other hand, when ω -phenyl derivatives of odd carbon fatty acids were fed, benzoic acid appear was excreted (fig. 9.9) (Phenylacetic acid and benzoic acid appear in the urine as esters of D-glucuronic acid). These results suggested that ω -phenyl fatty acids are degraded by oxidative removal of successive two-carbon fragments starting from the carboxyl end.

From these experiments, Knoop postulated that fatty acids are oxidized by β -oxidation, i.e., oxidation at the β carbon to yield a β -keto acid, which was assumed to undergo cleavage to form acetic acid and a fatty acid shorter by two carbon atoms.

8.5.2 Fatty acid oxidation cycle

The fatty acid oxidation occurs in the mitochondria and results in the complete breakdown of fatty acid to acetyl-CoA which is subsequently oxidized to CO₂ and H₂O via the TCA cycle. The β -oxidation of fatty acid is summarized in Fig .

The individual reactions of β -oxidation are briefly discussed below. Before oxidation, long-chain fatty acids from the cytosol must undergo a rather complex enzymatic activation, followed by transport across the mitochondrial membrane into the major compartment.

Activation of fatty acids

The first important step in fatty acid oxidation is the activation of the acid. It is achieved by converting the acid to the thiol ester of coenzyme A by ATP-dependent fatty acid thiokinase (acyl-CoA synthetase).

The activation of fatty acid occurs in the cytosol but the subsequent oxidation occurs in the mitochondria. The fatty acyl-CoA is not capable of crossing the inner mitochondrial membrane. This permeability barrier is overcome by the participation of carnitine, present in the inner membrane space; which serves as an acyl group carrier. The fatty acyl group from acyl-CoA is transferred to carnitine by the enzyme carnitine acyltransferase.

Acylcarnitine crosses the inner mitochondrial membrane with the help of a transport protein and in the matrix, is converted into acyl-CoA under the influence of acyl transferase. Inside the mitochondria, acyl-CoA compounds are degraded through the action of four enzymes which are described below:

Step 1: Dehydrogenation of fatty acyl-CoA

The acyl-CoA derivative formed by the thiokinase reaction is oxidized to the trans- α - β -unsaturated acyl-CoA derivative, catalyzed by acyl-CoA dehydrogenase.

The enzyme is a flavoprotein and is associated with FAD and three electron transferring flavoproteins. The dehydrogenation occurs at the α - and β -carbon atoms (carbon 2 and 3) of acyl-CoA.

Step 2: Hydration of Δ^2 -enoyl-CoA

In this step, the double bond of the Δ^2 -trans-enoyl-CoA ester is hydrated to form 3-hydroxy-acyl-CoA by the enzyme enoyl-CoA hydratase. The hydration is stereospecific.

Step 3: Oxidation of hydroxyacyl-CoA

In the next step of the fatty acid oxidation cycle, the L(+)-3-hydroxyacyl-CoA is oxidized to form β -ketoacyl-CoA derivative by 3-hydroxyacyl-CoA dehydrogenase which requires NAD⁺ and is absolutely specific for the L-stereoisomer.

Step 4: Cleavage of β -ketoacyl-CoA

In the last step of the fatty acid oxidation cycle the cleavage of the β -ketoacyl moiety in the presence of coenzyme A yields acetyl-CoA and another acyl-CoA containing two fewer carbon atoms than the original fatty acid, catalyzed by acetyl-CoA acyltransferase (thiolase).

The acetyl-CoA residues formed during fatty acid catabolism enter the TCA cycle for complete oxidation to CO_2 and H_2O while the acyl-CoA goes through β -oxidation cycle.

8.5.3 Energetics of β -oxidation of fatty acid

Each turn of this fatty acid degradation cycle yields a molecule of acetyl-CoA and produces one molecule of FADH_2 and one of $\text{NADH} + \text{H}^+$. However, one molecule of ATP is conserved in the initial activation step of fatty acid.

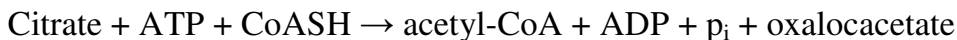
The overall reaction for the degradation of a long-chain saturated fatty acid, such as palmitic acid (C_{16}) can be written as follows:

The total energy yield is 130 molecules of ATP per molecule of palmitic acid on complete oxidation to CO_2 and H_2O .

8.5.4 Fatty acid biosynthesis

The ultimate source of all the carbon atoms of fatty acids is acetyl-CoA formed in the mitochondria by the oxidative decarboxylation of pyruvate (TCA cycle), the oxidative degradation of some of the amino acids or by the β -oxidation of long chain fatty acids.

The fatty acid biosynthesis takes place in the cytosol. Acetyl-CoA itself cannot pass out of the mitochondria into the cytosol; however, its acetyl group is transferred through the membrane in other chemical forms. As pointed out earlier, citrate formed in mitochondria from acetyl-CoA and oxaloacetate, may pass through the mitochondrial membrane to the cytoplasm via the tricarboxylate transport system. In the cytosol acetyl-CoA is regenerated from citrate by ATP-citrate lyase (citrate cleavage enzyme) which catalyzes the reaction.



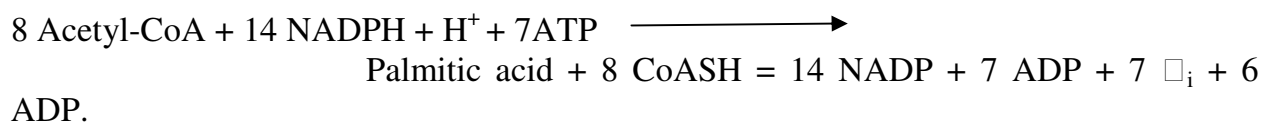
Acetyl-CoA is now ready to serve as a substrate for fatty acid synthesis. The synthesis of a long-chain saturated fatty acid, palmitic acid is given in Fig .

Overall view

The long-chain fatty acid palmitic acid is synthesized by a complex of enzymes, the fatty-acid synthase system of the cytosol, which employs a pantetheine-containing protein, acyl carrier protein (ACP), as an acyl-group carrier. Malonyl-CoA, formed from acetyl-CoA and HCO_3^- by acetyl-CoA carboxylase, is the direct precursor of seven of the eight two-carbon units of palmitic acid. Reduction of acetoacetyl-S-ACP to the β -hydroxy derivative and the dehydration of the latter to the Δ^2 -unsaturated compound is followed by reduction to butyryl-S-ACP at the expense of NADPH. The butyryl-S-acyl carrier protein (butyryl-S-ACP) is then recycled by condensation with a second unit of malonyl-S-ACP. This pattern repeats itself until palmitoyl-S-ACP is formed in seven cycles. At this point the ACP group is removed to yield free palmitic acid by the action of a thioesterase.

8.5.5. Energetics of fatty acid synthesis

The overall reaction for the synthesis of palmitic acid by this pathway may be written as:



The 14 NADPH molecules required for the reductive steps in the synthesis of palmitic acid arise largely from the NADP^+ -dependent oxidation of glucose-6-phosphate via the phosphogluconate pathway. The NADPH for fatty acid biosynthesis in plants arises through photoreduction of NADP^+ .

8.5.6. Comparative aspects of fatty acid oxidation and synthesis

Some salient differences between fatty acid oxidation and synthesis are summarized below:

1. Fatty acid synthesis occurs in the cytosol in contrast to oxidation which occurs in the mitochondrial matrix.
2. The intermediates in synthesis are covalently linked to the SH group of an acyl carrier protein whereas the intermediates of oxidation are bonded to CoASH.
3. The reductant in synthesis is NADPH while the oxidants in the oxidation are NAD^+ and FAD.
4. Many of the enzymes of synthesis in higher animals are part of a multienzyme complex while degradative enzymes do not appear to be associated.
5. During the synthesis, the β -hydroxyacyl intermediate formed is the D-isomer, while during oxidation it is the L-isomer.
6. The two carbon units used in the elongation of the growing fatty acid chain are derived from acetyl-CoA. But the actual donor is malonyl-S-ACP. The elongation

reaction is driven by the release of CO_2 . In the oxidation the product released is acetyl - CoA

8.6 Degradation of amino acids

The degradation of amino acids mainly consists of two: parts the removal of the amino group and the breakdown of the resulting carbon skeleton. The carbon skeleton of amino acids can be converted to acetyl -CoA, pyruvate and /or TCA cycle intermediates. Fig. 9.13 gives an illustration on how the carbon skeletons of amino acids enter the energy-producing pathway, the TCA cycle.

The removal of amino groups from amino acids constitutes the first stage of catabolism and is generally accomplished by the two enzymatic pathways a) transamination and b) oxidative deamination.

8.6.1. Transamination

A transamination reaction involves the transfer of an amino group from a donor amino acid to an acceptor α -keto acid to yield the α -keto acid of the donor amino acid and the amino acid of the original α -keto acid. The reaction is catalyzed by an enzyme called aminotransferase or transaminase that requires a metal ion and PLP for activity.

Most transaminases require α -ketoglutarate as the major amino group acceptor for most of the amino acid transaminations and the α -ketoglutarate is aminated to glutamic acid. In mammals, this occurs in the cytosol. The glutamate so formed then enters the mitochondrial matrix through a specific membrane transport system. In the mitochondrial matrix, glutamate is either directly deaminated or becomes the amino group donor for the resynthesis of amino acids. A prominent transaminase in animal tissues is aspartate amino transferase (aspartate transaminase) which catalyzes the reversible reaction.

8.6.2 Oxidative deamination

Glutamate formed by the action of transaminases may undergo rapid oxidative deamination catalyzed by NAD^+ or NADP^+ - linked glutamate dehydrogenase, which is present in both the cytosol and mitochondria of the liver.

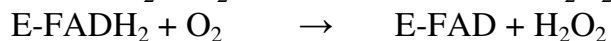
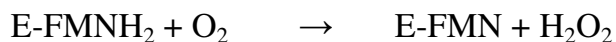
The ammonium ions liberated in the above reaction represent the α -amino groups collected from other amino acids. The enzyme can use either NAD^+ or NADP^+ as electron acceptor, but NAD^+ is preferred. The NADH formed is ultimately oxidized by the electron transport chain. Glutamate dehydrogenase plays a central role in amino acid deamination because in most organisms glutamate is the only amino acid that has such an active dehydrogenase activity.

Many organisms contain flavin-linked amino acid oxidases, which also catalyze oxidative deamination of amino acids but their role is considered as minor. One utilizes FMN as the prosthetic group, specific for L-amino acids and is called L-amino acid oxidase.

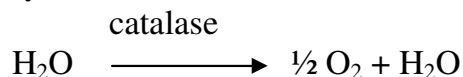
The other flavoenzyme functioning in oxidative domination is FAD-linked D-amino acid oxidase which catalyzes the oxidation of D-amino acids.

Both L- and D-amino acid oxidases are found in liver and kidneys.

The reduced forms of the L- and D-amino acid oxidases can react directly with molecular oxygen to form hydrogen peroxide and regenerate the oxidized forms of the enzymes.



The toxic hydrogen peroxide is decomposed to water and oxygen by catalase, a heme-containing enzyme.



8.6.3 Decarboxylation

The process of decarboxylation involves the α -carboxyl group of amino acids and is catalyzed by pyridoxal phosphate-dependent decarboxylases.

CHAPTER 9

SECONDARY METABOLITES IN PLANTS:

Plants produce a large and diverse group of organic compounds that appear to have direct function in growth and development. These substances are known as Secondary metabolites; secondary products or natural products. These secondary metabolites, unlike primary metabolites such as amino acids, simple carbohydrates or membrane lipids, chlorophyll, etc., have no recognized roles in processes such as photosynthesis, respiration, solute transport etc. Secondary metabolites also differ from primary metabolites in having a restricted distribution in the plant kingdom, i.e. particular secondary metabolites are often found only in one plant species or in taxonomically related group of species; while primary metabolites are found throughout the plant kingdom.

Recently; secondary metabolites have been suggested to have important ecological functions in plants. Chief among these is protection against herbivory and infection against microbial compounds.

We shall be dealing with only three groups of secondary metabolites:

9.1 Phenolics

Plants produce a large variety of secondary products that contain a phenol group - a hydroxyl functional group on an aromatic ring.

These substances are classified as phenolic compounds. Plant phenolics are a chemically heterogeneous group: some are soluble in organic solvents, some are water-soluble. Carboxylic acids and glucosides and others are large insoluble polymers. In keeping with their chemical diversity, phenolics play a variety of roles in plants. Plant phenolics are biosynthesized by different routes. However, two basic routes are involved - the shikimic acid pathway and the malonic acid pathway. The biosynthetic routes will not be considered in detail.

The shikimic acid pathway is present in plants, fungi and bacteria; but is not found in animals. The most abundant class of secondary phenolics in plants is derived from phenylalanine by the elimination of an ammonia molecule to form cinnamic acid.

Phenylalanine ammonia lyase (PAL) is probably the most studied enzyme in plant secondary metabolism. Trans-cinnamic acid and its derivatives are simple phenolic compounds called phenyl-propanoids.

Simple phenolic compounds are widespread in vascular plants. Many simple phenolics have important roles in plants as defenses against insect herbivores and fungi. Of special interest are the furanocoumarins. These compounds are nontoxic until they are activated by light. Sunlight in the UV-A region (320-400nm) activate some furanocoumarins to a high energy electronic state. These activated furanocoumarins can insert themselves (intercalate) into DNA double helix and bind to cytosine and thymine, thus blocking transcription and repair and leads to eventual cell death. Phototoxic furanocoumarins are especially abundant in the Umbelliferae family, e. g., celery and parsley.

Plants release a variety of primary and secondary metabolites into the environment from leaves, roots and decaying litter. These compounds have various effects on neighbouring plants, this phenomenon is called allelopathy. Generally speaking, "allelopathy" refers to the harmful effects of plants on their neighbours. The significance of allelopathy in plant-plant interactions is still a debated subject. In spite of lack of supporting evidence, allelopathy is currently of great interest because of its potential agricultural applications. Reduction in crop yields by weeds or old crop residues may sometimes be due to allelopathy.

9.2 Lignins

Lignin is a branched polymer of phenylpropanoid groups. Lignin has both primary and secondary roles in plants. Lignin is generally formed from three different phenyl propanoid alcohols-coniferyl, coumaryl and sinapyl alcohols. However, its precise structure is not known because it is difficult to extract lignins from plants where it is covalently bound to cellulose and other cell wall polysaccharides. Lignins form important constituents of cell walls within supporting and conducting tissues (eg., xylem cells). These tissues enable vascular plants to develop into large upright structures such as trees.

Lignin also has significant protective functions in plants. Its physical toughness deters feeding by animals, and its chemical durability makes it relatively indigestible to herbivores. The binding of lignin to cellulose and protein also decreases their digestibility. Lignification blocks the growth of pathogens and is a frequent response to infection or wounding.

9.3 Flavonoids

Flavonoids are one of the largest groups of plant phenolics flavonoids are classified into different groups including the anthocyanins, the flavones the flavonols and the isoflavones. The flavonoids contain -OH and sugar substituents in a basic skeleton of 15-C atoms.

The anthocyanins are coloured compounds and are responsible for the red, pink, purple and blue colours of plant parts. Colours in plants attract animals and insects for pollination and seed dispersal-visual and olfactory signals are provided. Anthocyanins are glycosides.

Flavones and flavonols absorb light at shorter wavelengths than anthocyanins and so are not visible to the human eye. However, insects such as bees can see farther into the UV range than humans and respond to these compounds as attractant cues. Flavones and flavonols are not restricted to flowers, they are also present in green leaves. Flavonoids and flavonols protect cells from excess UV-B radiations (280-320nm). The flavonols and flavonoids accumulate in the epidermal layers of leaves and stems and strongly absorb radiation in the UV-B region and allow the visible wavelengths to pass through uninterrupted.

Recently, it has been found that flavones and flavonoids secreted into the soil by legume roots mediate the interaction of legumes and nitrogen-fixing symbionts.

Isoflavones or isoflavonoids are mostly found in legumes and have several different biological activities. Some, such as the carotenoids, have strong insecticidal action. Others have antiestrogenic effects and so cause infertility in mammals.

Isoflavonoids are best known for their role as phytoalexins-these are antimicrobial compounds synthesized in response to bacterial or fungal infection and help to limit the spread of the invading pathogen.

9.4 Tannins:

Tannins are polymers of plant phenolics. The term, “tannin” was first used to describe compounds that could convert raw animal hides into leather the process is called tanning. Tannins bind to collagen in animal hides and increase their resistance to heat, water and microbes. Tannins are water-soluble polyphenols which cause protein precipitation from aqueous solutions.

Condensed tannins are polymers of flavonoid units. Hydrolysable tannins are heterogeneous polymers containing phenolic acids, especially gallic acid and simple sugars. The molecular weights of tannins range from 600-3000.

Tannins are feeding repellants to a great diversity of animals. Tannins bind to salivary proteins and cause a sharp, astringent taste in the mouth so animals such as cattle, deer and apes avoid plants or parts of plants which have high tannin. There are sorghum varieties high in tannin that are bird resistant, for the same reason. Plant tannins also serve as defense against microorganisms. The wood of many trees contain high amounts of tannins, this prevents fungal and bacterial decay.

Tannins are general toxins that significantly reduce the growth and survival of many herbivores when added to their diets. Since growth is reduced, they are considered as antimicrobial factors. The antinutritional effect is due to the ability of tannins to bind to proteins. In the gut of herbivorous and animals and animals tannins form complexes with proteins and decreases the digestibility of proteins.

Some unripe fruits have very high tannin levels that may be concentrated in the outer cell layers. The levels of tannins get reduced during ripening.

9.5 Terpenes:

The terpenes or terpenoids, are the largest group of secondary products. Terpenes are a diverse group of substances, generally insoluble in water.

All terpenes are derived from 5-C isoprene units as seen earlier under “Lipids”. Terpenes decompose at high temperatures to give isoprene. Terpenes are also referred to as isoprenoids.

The classification of terpenes into mono-, di-, tri--- etc, terpenes have already been dealt with under ‘lipids’ and will not be repeated here.

Terpenes are toxins and feeding deterrents to a large number of plant feeding insects and mammals, and thus, they appear to play important defensive roles in the plant kingdom

Many monoterpenes are agents of insect toxicity e. g., Pyrethroids which occur in leaves and flowers of Chrysanthemum species show striking insecticidal activity- the pyrethroids are monoterpenes. Synthetic pyrethroids are popular components of commercial insecticides of their low persistence in the environment and their negligible toxicity to mammals.

In conifers such as pine and fir monoterpenes accumulate in resin ducts and so are found in conifer resins eg. α - & β - pinene, limonene. These compounds are toxic to various insects including, which are serious pests of conifers.

Plants such as sunflower contain sesquiterpene lactones in glandular hairs. These compounds are antiherbivore agents and act as strong feeding repellents to many herbivorous insects and mammals, Gossypol of cotton is a defensive sesquiterpene derivative and is responsible for resistance of cotton to insects.

Many diterpenes have been shown to be toxins and feeding deterrents to herbivores. Phorbol and its derivatives are diterpenes- they are produced by certain plants. Phorbols are severe skin irritants and internal toxins to mammals. Phorbol type diterpenes are currently of great interest as model tumor promoters in studies of carcinogenesis in animals. The diterpene taxol from Pacific Yew, is a powerful new

anticancer drug. Triterpenes are structurally diverse and include the steroids, some steroids are defensive secondary products eg. Phytoecdysones - these are plant steroids with structures similar to insect molting hormones. Ingestion of phytoecdysones by insects disrupts molting and other developmental processes, often with lethal consequences.

Limonoids are triterpenes found in citrus fruits- the bitter substances. Azadirachtin is a complex limonoid of neem tree – it exerts a variety of toxic effects on insects and is a potential commercial insect control agent.

Cardenolides are glycosides belonging to the triterpene group. They are bitter to taste and are extremely toxic to higher animals. In humans they have dramatic effects on heart muscle. In controlled doses cardenolides extracted from species of foxglove (*Digitalis*) are used to treat heart disease because they slow and strengthen the heart beat. Saponins are also triterpenes, they have soap like properties. They are active against vertebrate herbivores.

Carotenoids are found in all photosynthesis organisms and play a role in photosynthesis. The light energy they absorb is rapidly transferred to chlorophyll. Carotenoids are accessory pigments in photosynthesis. Carotenoids also have a role in photoprotection they rapidly quench or dissipate energy stored in chlorophyll in the excited state so that it cannot react with molecular oxygen.

Chlorophylls absorb energy and if this energy cannot be stored by certain mechanisms the photosynthetic membrane can be easily damaged. The photoprotection mechanisms act as a safety valve, dissipating excess energy before it can cause damage. If the excited pigment molecules are not quenched they react with molecular oxygen, which is extremely reactive. This reacts with cellular components, especially lipids. Carotenoids act by quenching the excited state of chlorophylls.

Polyterpenes – Rubber is the best known. Rubber occurs as small particles suspended in a milky fluid called latex which is found in long vessels known as laticifers. The laticifers, in addition to rubber may also contain triterpenes, which act as defense compounds.

9.6 Alkaloids:

These are large family of N-containing secondary metabolites found in 15-20% of vascular species. More than 1200 alkaloids are known. Alkaloids include N-containing natural products which are not otherwise classified as peptides, non- protein amino acids, amines, cyanogenic glycosides, glucosinolates and fungal antibiotics are therefore included as alkaloids.

They have striking pharmacological effects on vertebrates. Alkaloids have also been detected in bacteria fungi and even in animals. The N atom of alkaloids is usually part of a heterocyclic ring.

Most alkaloids are alkaline and at cytosolic pH they are found with their N atom protonated. Hence, alkaloids are positively charged and are generally water- soluble.

Alkaloids are usually synthesized from one of a few amino acid precursors, particularly aspartic acid, lysine, tyrosine, tryptophan.

Specific alkaloid types are restricted to particular systematic units and are therefore important in taxonomy and phylogeny of plants. Most alkaloids are synthesized in the cytoplasm. The vacuole is the major compartment of alkaloid storage. Alkaloids such as morphine are found in latex of plants. Here the alkaloids are sequestered in small latex vesicles.

Alkaloids are stored predominantly in tissues which are important for survival and reproductive, which include actively growing young tissues, root, stem, bark, flowers seedlings and photosynthetically active tissues.

The main function of alkaloids is that of chemical defense against herbivores. In the leguminosae, quinolizidine alkaloids are the most important defence chemicals against insects and other herbivores. In some cases alkaloids play a role in antimicrobial defense (against bacteria, fungi and viruses) and even in the interaction of one plant and another (allelopathy -they influence the germination of other plants seeds)

Alkaloids can be regarded as multipurpose compounds which may be active in more than one environmental interaction. In addition, they act as degradable N transport and N storage compounds.

Alkaloids repel or deter the feeding of many animals. To human and other vertebrates many alkaloids have a bitter or pungent taste. If ingested they are toxic. In microorganisms and competing plants alkaloids intoxication results in growth reduction and antibiosis. Many alkaloids are used by man as medicinal compounds eg. morphine.

REFERENCE

The UG e-lecture notes of course no. BCM- 101 (1+1) titled “PLANT BIOCHEMISTRY” has been prepared following the book “Textbook of Biochemistry” authored by Dr.S.K.Thimmaiah and published by M/s HIMALAYA PUBLISHING HOUSE, MUMBAI (FIRST EDITION 1997).The students may refer this book for all the structures of biomolecules, figures,tables,etc., mentioned in the above e-lecture notes.

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