BIO CHEMISTRY

UNIT 1 : AMINO ACIDS AND PROTEINS

Amino acids - Classification and structures; essential and non-essenntial amino acids.

Physical properties, zwitterions, isoelectric point. Methods of synthesis of amino acids (Gabriel, Strecker, malonic acid methodologies: brief outline only). Reactions of amino acids-reaction due to amino groups, reaction due to carboxylic acid group, due to presence of both amino and carboxylic acid groups, non-hydro reaction.

Proteins - Introduction and classification. Protein structure-primary, secondary, tertiary and quaternary structure, Denaturation and rematuration of proteins. Separation and purification of proteins-dialysis-gel filtration - electrophoresis.

UNIT 2 : ENZYMES

Nomenclature, classification and properties-specificity, factors influencing enzyme action. Mechanism of enzyme action - Lock and Key model.

Coenzymes - cofactors - prosthetic groups of enzymes (TPP, NAD, NADP, FAD, ATP). Their importance in enzyme action. Immobilization of enzymes. Enzyme specificity.

UNIT 3 : LIPIDS

Classification - neutral lipids, phospho lipids (lecithines, cephalins, plasmalogens) and glycolipids - importance and synthesis. Fatty acids - satured, unsaturated fatty acids. Properties - Hydrolysis-acid number, saponification number. Auto-oxidation (Rancidity), addition reactions-Iodine value, Polenske number, Reichert-Meissl number, acetyl number. Cholesterol - biosynthesis. Bile salts derived from cholesterol.

UNIT 4 : CARBOHYDRATES

Classification - reducing and non-reducing sugars. Glucose: structure-conformation - Stability. Carbohydrates of the cell membrane - starch, cellulose and glycogen. (Structure and utility) Metabolism: Glycolysis and its reversal; TCA cycle. Relation between glycolysis and respiration. Principles of bioenergetics, electron transport chain and oxidative phosphorylation. Gluconeogenesis, pentose phophate pathway.

UNIT 5 : NUCLEIC ACIDS

Nucleosides and nucleotides - purine and pyrimidine bases. Nucleic acids Difference between DNA and RNA. Classification of RNA. Biosynthesis of DNA: Replication. Biosynthesis of mRNA: Transcription. Genetic code - mutations and mutants. DNA repair. Biosynthesis of proteins. DNA sequencing and PCR, recombinant DNA technology, DNA polymorphism.

Reference

[1] Lehninger, Principles of Biochemistry, Fourth Edition by David L. Nelson and Michael M.Cox, Worth Publishers, New York, 2005.

[2] L. Veerakumari, Biochemistry, MJP publishers, Chennai, 2004.

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CHAPTER I
AMINO ACIDS AND PROTEINS

Contents

1.0 Aims and objectives

- Explains the classification and structure of amino acids
- Gives the method of synthesis of amino acids
- Explains the reactions of amino acids
- Describes the structure of protein
- Explains separation and purification of proteins.

1.1 Introduction

Amino acids, as the name implies, are organic compounds containing an amino ($-NH_2$) group and carboxyl (-COOH) group. Amino acids, in which the amino group is attached to the α - carbon atom, are called α-amino acids. They have the general formula.

1.2 Structure of amino acids

Amino acids are obtained by the hydrolysis of proteins by acids, alkalies or enzymes. About 20 amino acids are found in proteins, all of which except proline and hydroxyproline are α-amino acids. Amino acids may be designated with three or one letter abbreviated names.

Amino acid	Designation		Structure
	3 - letter	1 - letter	
Glycine	Gly	G	
Alanine	Ala	A	

1.3 Classification

1. Classification based on their incorporation in proteins

There are about 200 amino acids which are known to occur in plants and animals. However, only 20 amino acids are found in proteins. They are called proteogenic amino acids. The others which never occur in proteins are known as non- proteogenic amino acids.

Proteogenic amino acids	Non-proteogenic amino acids
Glycine	Cystine
Alanine	Histamine
Phenylalanine	Ornithine
Cysteine	β - alanine

2. Classification based on the position of amino group

Amino acids may be classified as α, β or γ - amino acids according to the position of the amino group in the carbon chain e.g.

α-Amino acids

Alanine

Phyenylalanine

β-Amino acids

β-Alanine

γ-Amino acids

Creatine

3. Classification based on their acid-base properties

On the basis of the acid-base properties in solution, amino acids are classified into three types :

i) Neutral amino acids : Most α-amino acids contain an amino group at one end and a carboxyl group at the other end. They do not contain any amino group or carboxyl group in the side chain (R). These are called neutral amino acids.

Examples

Glycine

Alanine

ii) Acidic amino acids : The amino acids containing an extra carboxyl group in the side chain are referred to as acidic acids.

Examples

Aspartic acid

Glutamic acid

iii) Basic amino acids : The amino acids containing an extra amino group in the side chain are termed basic amino acids.

Examples

Ornithine

Lysine

4. Classification based on their biological importance

Amino acids are of great biological importance. Based on their in the diet, amino acids are classified into two groups:

i) Essential amino acids : Amino acids are essential for the growth of body and its protection from diseases. Certain amino acids which are essential for the growth of body, cannot be synthesised by the body. Therefore, they must be included in the human diet. Such amino acids are referred to as essential amino acids. They are also called indispensable amino acids.

The deficiency of essential amino acids may cause various diseases like nervous breakdown, inhibition of full mental growth and their continuous deficiency may even cause death in young animals.

There are eight essential amino acids which owe special importance to human beings. They are phenylalanine, valine, leucine, isoleucine, lysine, threonine, methioine and tryptophan. These are rich in cereals, soyabeans, dhal, agathi, gingili seeds, egg, milk, cheese, meat, carrot leaves and tender coconut.

ii) Non-essential amino acids: Out of 20 amino acids which form the building blocks of proteins, 10 amino acids like glycine, alanine, cystine, tyrosine etc are synthesised within the body. Hence, they need not be included in the diet. These amino acids are termed non-essential amino acids.

5. Classification based on their structure

In this scheme, amino acids are classified on the basis of the structure of the side chains.

i) Aliphatic amino acids : They include amino acids with aliphatic side chains.

Alanine Valine

Leucine Isoleucine

ii) Aromatic amino acids : These are amino acids containing aromatic rings in the side chain.

Phenylalanine Tyrosine

Proline Tryptophan

iii) Hydroxy amino acids : These amino acids contain hydroxyl (-OH) groups in the side chain

Serine Threonine

iv) Sulphur containing amino acids : These amino acids contain sulphur atom in the side chain.

Cysteine Methionine

v) Dicarboxylic amino acids (Acidic amino acids): These are amino acids containing acidic (carboxylic) groups in the side chain

Aspartic acid Glutamic acid

vi) Diamino acids (Basic amino acids) : These amino acids contain two amino groups, one in the main an the other in the side chain.

Ornithine Lysine

1.4 Physical properties

Optical activity

All the naturally occuring α-amino acids except glycine possess atleast one asymmetric carbon atom (chiral centre) and hence optically active.

Glycine α-Amino acid

1.4.1 Zwitter ions : Amino acids are colourless crystalline solids. They readily dissolve in water and their aqueous solutions reacts both with mineral acids and strong bases. This amphoteric character of amino acids is due to their dipolar or zwitter ions formed by the transfer of a proton from the acidic - COOH group to basic - NH_2 group

(Amino acid) (Zwitter ion)

1.4.2 Isoelectric point : Amino acids are hydrophilic colloids. Depending upon the pH of the medium, they move either towards the anode or towards the cathode when placed in an electric field.

In acid solution (low pH), the zwitter ion takes up a proton from the acid and exists as cation. So, it migrates towards the cathode.

(Zwitter ion) (cation)

In alkaline solution (high pH) the zwitter ion donates a proton to the alkali and exists as anion. So, it migrates towards the anode.

(zwitter ion) (anion)

1.5 Methods of synthesis of α - amino acids

1.5.1 Gabriel's phtalimide synthesis.

Bear yields are obtained when α-halogen substituted acids are treated with potassium phthalimide and the product hydrolysed.

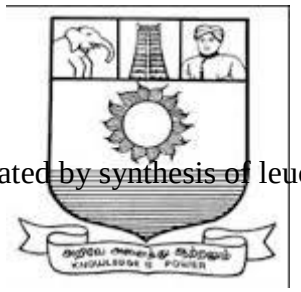
The phthalic acid obtained may again be converted to potassium phthalimide.

1.5.2 Strecker's synthesis:

The method consists in treating a cyanohydrin with concentrated ammonia and hydrolysing the amino nitrile.

In paractice the amino nitrile (aminocyanide) is prepared in one step by treating the oxo compound with a mixture of amonium chloride and potassium cyanide.

1.5.3 Malonic acid methodologies



The method has been illustrated by synthesis of leucine.

α-Bromo-isocaproic acid

1.6 Reactions of amino acids

1.6.1 (i) They form salt with strong inorganic acids.

Hydrochloride of α-amino acid

(ii) They can be acetylated with acetyl chloride or acetic anhydride

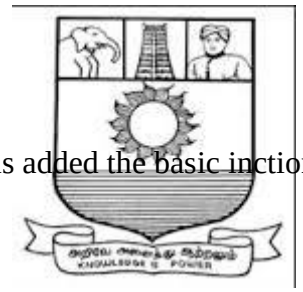
Similarly, benzoylation can also be done using benzoyl chloride

(iii) On treatment with nitrous acid, they form α-hydroxy acids. As one mole of nitrogen is eliminated for each free amino group, this forms the basis of Van Slyke method for determination of free - NH_2 groups in proteins.

α-Hydroxy acid

(iv) When treated with chloroform and alcoholic caustic potash they give carbylamine reaction

(v) When excess of formaldehyde is added the basic inction of amino group is blocked



This reaction forms the basis of formal titration method due to sorenson. The product obtained has a free carboxyl group which can be titrated using standard alkali and phenolphathalein indicator.

1.6.2 Reaction due to Carboxyl group

(i) With alkalies salts are formed

(ii) Suspension of the amino acid in acetyl chloride, when treated with PCl_5, yield the hydrochloride of acid chloride.

(iii) Amino acids when heated with alcohol in presence of dry hydrogen chloride form ester hydrochlorides.

The free acid is obtained when ester hydrochloride is hydrolysed by sodium carbonate solution.

(iv) When dry distilled or better boiled with barium hydroxide, the amino acid is decarboxylated

(v) They are reduced to amino alcohol with lithium aluminium hydride

(vi) Amino acids form chelate compounds with heavy metal salts. Thus when copper oxide is heated with water solution of glycine, a chelate complex in form of deep blue needles is obtained.

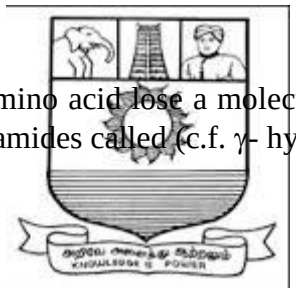
This reaction can be used for isolation purification and calorimetric estimation of α-amino acids.

1.6.3 Reactions due to both the -NH_2 and COOH groups

(i) When α-amino acids are heated two molecules of water are lost between two molecules of acid and cyclic diamides, called diketopiperazines, are obtained (c.f.α - hydroxy acids).

(ii) When heated, β-amino acid lose a molecule of ammonia to give α, β - unsaturated acids (c.f. β - hydroxy acids).

(iii) On being heated γ - and δ- amino acid lose a molecules of water in between -NH_2 and COOH groups to give cyclic amides called (c.f. γ- hydroxy acids).



1.6.4 Nin-hydrin reaction:

Nin-hydrin (2,2 -dihydroxyindane -1,3-dione) is a chemical used to detect α-aminoacid and also free amino and carboxylic acid groups on proteins and peptides. When reacting with these free amines, a deep blue or purple colour is produced. Nin-hydrin is most commonly used to detect finger prints, as it react with free alpha amino group (NH_2-CH_2-COOH). This group is present in all amino acids, proteins or peptides.

1.7 Introduction of proteins

Proteins are complex organic nitrogenous substances found in animal and plant tissues. They contain carbon, hydrogen, oxygen and nitrogen. Some proteins also contain sulphur, phosphorus and iron. The presence of nitrogen is significant because it distinguishes proteins from carbohydrates and fats. The nitrogen content of most of the proteins is about 16 per cent. Proteins are so essential to life that an animal or plant can survive without fat or carbohydrate but not without protein. Hence the name protein, which is derived from the Greek word proteios meaning first rank or to be first.

Proteins are built from 20 simpler compounds called amino acids. The amino acids contain a basic amino (NH_2) group at one end and an acid (COOH) group at the other end.

The amino acids in the protein molecule are linked together through peptide (-CO NH-) bonds.

1.7.1 Classification of proteins

Proteins are classified in two ways :

(A) Classification based on solubility and shape : On the basis of their solubility and shape, proteins are classified into two groups-fibrous proteins and globular proteins.

1. Fibrous proteins : These are linear polymers which exist in the form of fibers. They are insoluble in common solvents like water but soluble in concentrated acids and alkalies. Examples : Collagen, fibroin, keratin.

2. Globular proteins : These are water soluble proteins. These are cross - liked polymers wherein the peptide chains are folded tightly to give spherical or globular shape to the molecules. Examples : Enzymes, hormones, haemoglobin, myoglobin.

B) Classification based on chemical structure : On the basis of their chemical structure, proteins are divided into three groups-simple proteins, conjugated proteins and derivated proteins.

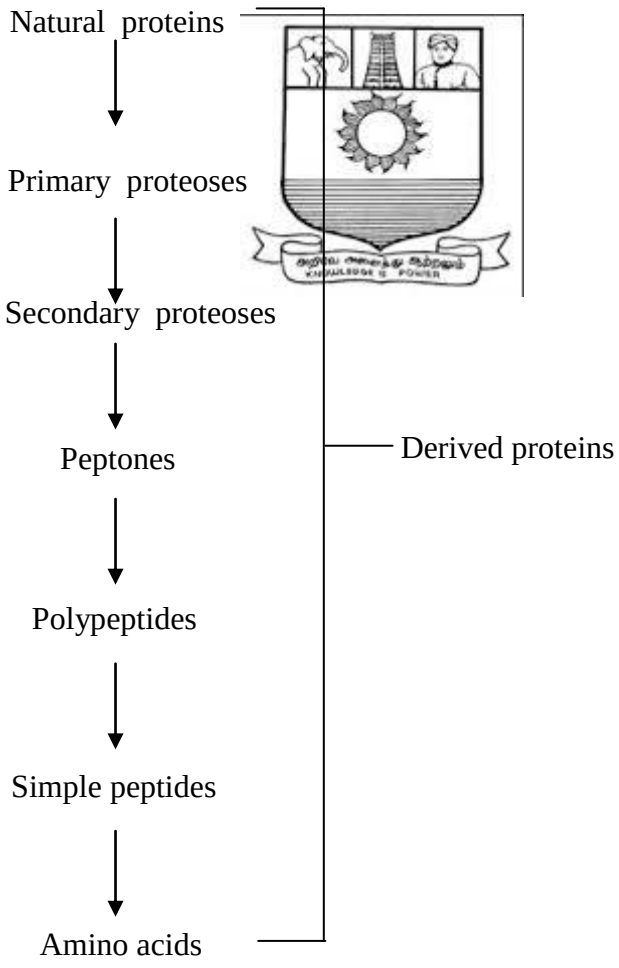
1. Simple proteins : The proteins which give only amino acids or their derivatives on hydrolysis are called simple proteins. Based on their solubility, simple proteins are further classified into 7 groups.

	Protein	**Examples**
i)	Albumins	Serum albumin, egg albumin
ii)	Globulins	Serum globulin, tissue globulin
iii)	Glutelins	Glutenin, oryzenin
iv)	Prolamins	Zein, hordein
v)	Albuminoids	Keratin, collagen, fibroin
vi)	Histones	Nucleic acid
vii)	Protamins	Salmine, sturine

2. Conjugated proteins : These are proteins which contain a non-protein part. The non-protein part of the conjugated proteins is known as the prosthetic group. So, the conjugated proteins on hydrolysis yield non-protein substances in addition to amino acids. Depending upon the nature of prosthetic group, conjugated proteins are further divided into 5 groups.

	Protein	Prosthetic group	Examples
i)	Glycoproteins	Carbohydrate	Mucin, serum albumin
ii)	Phosphoproteins	Phosphoric acid	Caesin, vitellin
iii)	Lipoproteins	Lipid	Lipoproteins of blood serum
iv)	Nucleoproteins	Nucleic acid	Nuclein
v)	Chromoproteins	Carotenoid pigment	Rhodopsin
vi)	Metalloproteins	Metal	Chlorophyll, haemoglobin

3. Derived proteins : These are the intermediate products obtained by the stepwise hydrolysis of natural proteins by acids, alkalies or enzymes.



1.8 Protein structure

Linderstom-Lang proposed four levels of structural organisation for proteins based on the degree of complexity of their molecules. They are :

i) Primary structure
ii) Secondary structure
iii) Teritiary structure
iv) Quaternary structure

Fibrous proteins are relatively simple molecules with primary and secondary levels of structure. On the other hand, globular proteins have high complexity due to all the four levels of structural organisation.

1.8.1 Primary structure

Amino acids are the building blocks of proteins. These amino acids are arranged in a regular manner through peptide (-CO-NH-) bonds in the form of a linear chain called polypeptide chain.

The order or sequence of aminoacids in the polypeptide chain is referred to as the primary structure. For example, oxytocin contains nine amino acid residues - cysteine (2 molecules), glycine, leucine, isoleucine, proline, asparagine, glutamine and tyrosine. The sequence of amino acid is found to be as shown below :

Proteins are degraded into smaller fractions by partial hydrolysis with enzymes or chemicals like cyanogen bromide and the N-terminal and C-terminal amino acids in the smaller fragments are determined. This is called End-group analysis.

End-group analysis

i) Determination of N-terminal amino acid
(Sanger's method)

2,4-dinitrofluorobenzene (DNFB) is called Sanger's reagent. It reacts with N-terminal amino acid to form dinitrophenyl amino acid (DNP - amino acid) and other free amino acids. The DNP-amino acid is coloured and can be easily identified by chromatography.

ii) Determination of C-terminal amino acid
(Hydrazinolysis method)

When a peptide is heated with anhydrous hydrazine, all amino acid residues except the C-terminal one are converted into the corresponding hydrazides. The free C-terminal amino acid is eluted and identified by cation- exchange chromatography.

By determining both N- and C-terminal groups, it is possible to determine the amino acid sequence. Consider a tetrapeptide, consisting of amino acids A, B, C and D. The N-terminal method is applied to find the N-terminal amino acid (say, A). The process is repeated with the tripeptide left behind and if B and C are N-terminal acids, then D is the C-terminal amino acid. Now, the amino acid sequence of the tetrapeptide is

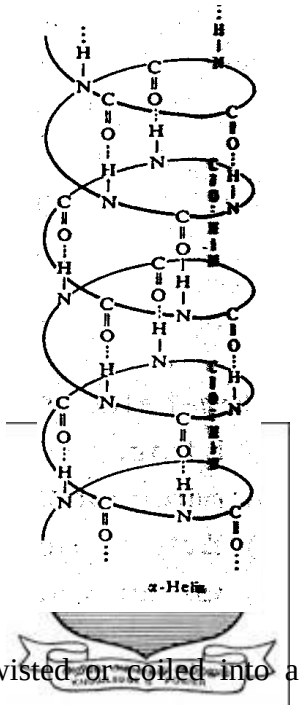
Primary structure of Haemoglobin : Haemoglobin is made up of four polypeptide chains- two α-chains and two β chains. Each α chain contains 141 amino acid residues. Each β chain 146 amino acids. Thus, haenoglobin has a total of 574 amino acid residues.

α chain -Leu - Ser - Ala - Leu - Ser - Asp - Leu - His - Ala -

β Chain -Phe - Ala - Thr - Leu - Ser - Glu - Leu - His - Cys -

1.8.2 Secondary structure

The arrangement of the polypeptide chain in space (conformation) is called the secondary structure of proteins. In order to explain the stability of the polypeptide chain against the steric strain imposed by the bulky groups (R) when arranged in a linear fashion, Linus Pauling proposed a longitudinally coiled conformation called α-helix. The salient features of the model are :



i). The polypetide chain is twisted or coiled into a α-helix (spiral). It may be right handed or left handed.

ii). The right handed α-helix is the conformation of all naturally occuring proteins. The α-helix is so named because of the mobility of α-carbon atoms.

iii). The helical arrangement brings the N-H group of one amino acid into close proximity of the C = O group of the fourth amino acid in the chain.

iv). Hydrogen bonds are formed between the N-H and C = O groups.

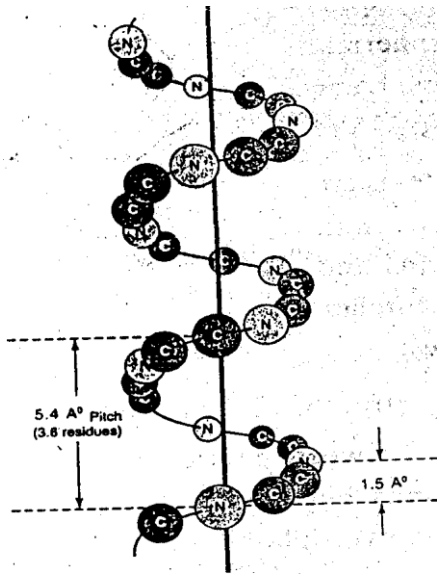
v). The bulky groups (side chains) are projected away from the helix so that the helix is rigid and free from steric strain.

vi). The α-helix is stabilised by

- Hydrogen bonds
- Hydrophobic interactions.
- Elecrtostatic interactions and
- Van der waal's forces

vii). Each turn of the helix contains 3.6 amino acid residues and is 5.4 A° long

viii). The distance between two successive amino acid resiudes is 1.5 A°.

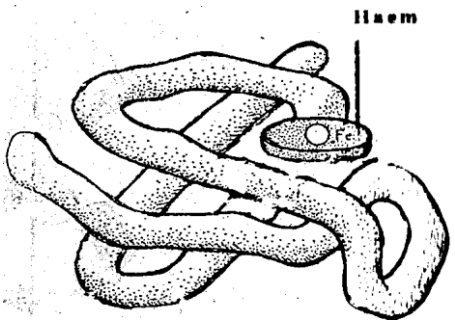


1.8.3 Tertiary structure

Each secondary structure (α-helix) is a compact unit called domain. The folding of domains in large proteins to form a compact structure of definite shape is termed tertiary structure. The folding brings together active amino acids, which are otherwise scattered along the chain and may form cavity. The folding occurs in such a way as to expose polar hydrophilic groups to the surface and non-polar hydrophobic groups to the interior. In proteins consisting of a single polypeptide chain (e.g myoglobin), the tertiary structure determines the overall shape of the molecule.

Myoglobin is extremely compact with a very little empty space inside. It has spherical shape to which a haem group is bound.

Fibrous proteins are less compact than globular proteins and form long thin threads (rod -like shape).



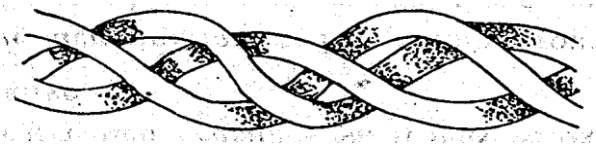
The tertiary structure is stabilised by

i) H-bonds between peptide groups
ii) Ionic bonds between COO^- and $^+NH_3$ groups
iii) Non-ionic hydrophobic bonds between non-polar R groups
iv) Disulphide bonds between sulphur containing anion acid residues.

1.8.4 Quaternary structure

Proteins such as haemoglobin are oligomeric (multi-chain) in nature. Each chain in the molecule has its own characteristic tertiary structure and is called a subunit. These subunits or polypeptide chains are held together by cross linked groups or aggregated through non-covalent forces such as hydrogen bonds, ionic bonds and hydrophobic interactions of form quaternary structure. For example, haemoglobin contains four polypeptide chains held together by non-covalent bonds. In haemoglobin each subunit contains a heme group. It has a globular shape.

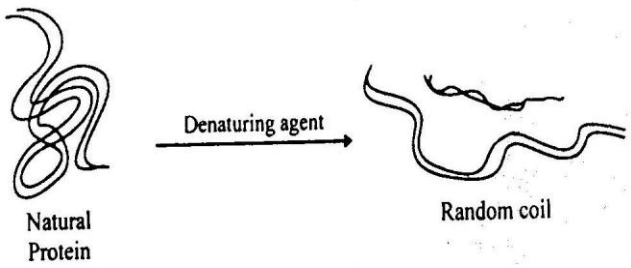
Collagen is a fibrous protein. It consists of three polypcptide chains which are coilcd as shown below:



1.9 Denaturation and Renaturation:

It is found that protein lose their physiological activity and other properties when heated or treated with acids. This causes deformation of protein structure is known as denaturation. Deformation may also be caused by some bacteria and on shaking with alcohol. Coagulation of egg white by the action of heat and coagulation of milk in presence of an acid (lemon juice) to form cheese are some examples of denaturation of protein.

The denaturation does not distrupt primary structure of protein but it disrupt the tertiary structure. Peptide chains are mainly linked by hydrogen bonds and salt bridges have weak associations and easily distrupted on heating. Similarly, changes in pH have greatest distruptive effect on hydrogen bonding as well as salt bridges in proteins. Amino acid chains on terminal ends get positively charged on protonation by acid. Due to the presence of similar charges on such two peptide chains repel each other which causes uncoil of the protein molecule and hence denaturation. This process does not change primary structure but there is alternation in secondary and tertiary structure. The protein molecule uncoil from a specific conformation into a random conformation that results to the coagulation of protein in a solution.



2. Renaturation. The reverse of denaturation is known as renaturation. However, denaturation of protein may not be always reversible. For example, globular protein of egg white on boiling coagulates into a rubber like insoluble mass which cannot turn back to original protein conformation. This is called irreversible denaturation. In case of reversible denaturation the protein can be brought back to its original shape by saturating with soluble

salts like. Magnesium sulphate, ammonium sulphate, etc. This is a reversible process and original form of protein can be regained again.

The process of renaturation is generally very slow and sometimes may not occut at all. For example, when denaturation is effected by heat, it does not renature on quick cooling but on slow cooling renaturation often occurs. Such renaturation is called annealling.

1.10 Separation and purification of porteins

1.10.1 Dialysis

Proteins can be separated from small molecules by dialysis through a **semi-permeable membrane** such as cellophane (cellulose acetate). **Pores** in the membrane allow molecules up to approximately 10 kDa to pass through, whereas larger molecules are retained inside the dialysis bag. As most proteins have molecular masses greater than 10kDa, this technique is not suitable for fractionating proteins, but is often used to remove small molecules such as salts and ammonium sulfate from a protein solution. It should be noted that at equilibrium, the concentration of small molecules inside a dialysis bag will be equal to that outside and so several changes of the surrounding solution are often required to lower the concentration of the small molecule in the protein solution sufficiently.

1.10.2 Gel filtration chromatography

In gel filtration chromatography (**size exclusion chromatography** or **molecular sieve chromatography**), molecules are separated on the basis of their **size and shape.** The protein sample in a small volume is applied to the top of a column of **porous beads** (diameter 0.1 mm) that are made of an insoluble but highly hydrated polymer such as polyacrylamide (Bio-Gel). Small molecules can enter the **pores** in the beads whereas larger or more elongated molecules cannot. The smaller molecules therefore have a larger volume of liquid accessible to them; both the liquid surrounding the porous beads and that inside the beads. In contrast, the larger molecules have only the liquid surrounding the beads accessible to them, and thus move through the column faster, emerging out of the bottom (**eluting**). The smaller molecules move more slowly through the column and elute later. Beads of differing pore sizes are available, allowing proteins of different sizes to be effectively separated. Gel filtration chromatography is often used to de-salt a protein sample.

1.10.3 Electrophoresis

When placed in an **electric field,** molecules with a net charge, such as proteins, will move towards one electrode or the other, a phenomenon known as **electrophoresis.** The greater the net charge the faster the molecule will move. In **polyacrylamide gel electrophoresis (PAGE)** the electrophoretic separation is carried out in a gel which serves as a molecular sieve. Small molecules move readily through the pores in the gel, whereas larger molecules are retarded. The gels are commonly made of **polyacrylamide** which is chemically inert and which is readily formed by the polymerization of acrylamide. The pore sizes in the gel can be controlled by choosing appropriate concentrations of acrylamide and the cross-linking reagent, methylene bisacrylamide. The higher the concentration of acrylamide used, the smaller the pore size in the final gel. The gel is usually cast between two glass plates separated by a distance of 0.5-1.0 mm. The protein sample is added to wells in the top of the gel, which are formed by placing a plastic comb in the gel solution before it sets. A blue dye

(bromophenol blue) is mixed with the protein sample to aid its loading on to the gel. Because bromophenol blue is a small molecule, it also migrates quickly through the gel during electrophoresis and so indicates the progress of electrophoresis.

Check Your Progress

1. Protein is polymer of .....
2. Protein give purple colour when treated with .....
3. The main structural feature of proteins is ..... linkage
4. Hydrolysis of proteins in the presence of enzymes produces .....

1.11 Sum up

Amino acids are biologically important organic compounds containing amine ($-NH_2$) and carboxylic acid (-COOH) functional groups, usually along with a side chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen oxygen and nitrogen though other elements are found in the side-chains of certain amino acids. Proteins are the buliding blocks of life. Every cell in the human body contains protein. The basic structure of protein is a chain of amino acids.

1.12 Key words

Amino acids - Amino acids are organic compounds containing an amino ($-NH_2$ group) and a carboxyl (-COOH) group.

Zwitter ions - A dipolar ion, with spatially separated positive and negative charges

Denaturation - Partial or complete unfolding of the specific nature conformation of a polypeptide protein or nucleic acid.

Dialysis - Proteins can be separated from small molecules by dialysis through a semi - permeable membrane which has poles that allow small molecules to pass through but not proteins

Electrophoresis- When placed in an electric field, molecules with a net charge such as proteins, will move towards one electrode or the other. This phenomenon is known as electrophoresis.

1.13 Question for Discussion

1. What are zwitter ions?
2. What is meant of denaturation of proteins
3. Which note on dialysis.
4. Give the different classification of proteins
5. Explain the primary and secondary structure of protein.
6. Explain Electrophoriesis.
7. Wirte note on Gel filtration method for the separation of proteins.

Check Your Progress : Model Answers

1. Amino acid
2. Ninhydrin solution
3. Peptide
4. Amino acid

1.14 Suggested readings

[1] Lehninger, Principles of Biochemistry, Fourth Edition by David L. Nelson and Michael M.Cox, Worth Publishers, New York, 2005.

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CHAPTER II

ENZYMES

CONTENTS

2.0 Aims and objectives

- Gives an idea about the nomenclature of enzymes
- Explans the factors influencing enzyme action
- Describes the mechanism of enzyme action
- Explains the immobilisation of enzymes
- Explains the various coenzymes.

2.1 Introduction

Enzymes are orgainc catalysts produced by living cells. They accelerate a wide variety of chemical reactions which occur in biological systems. Thus, enzymes are biocatalysts.

2.2 Nomenclature

Enzymes are named by adding the suffix-ase to the name of substrate on which it acts as catalyst.

Name of Substrate	Name of Enzyme
Lipid	Lipase
Amylum	Amylase

Some enzymes were given trivial names e.g pepsin, trypsin etc.

2.3 Properties of enzymes

- ❖ Most of the enzymes are colourless solids, but some are yellow, bule, green or greenish brown.
- ❖ They are mostly soluble in water or dilute salt solutions, but they can be precipitated out of their aqueous solutions by protein precipitating agents.
- ❖ They are colloidal in nature and do not pass through dialysing membranes, although the prosthetic groups of enzymes (or coenzymes) can be easily separated by dialysis from the proteinoid part (or apoenzyme).
- ❖ They usually contain C, H, N and S, although phosphorus and metallic ions are also present occasionally.
- ❖ They have high molecular weights. Pepsin has, for example, a molecular weight of 39,200.
- ❖ As catalysts, they are effective in very small amounts. The number of moles of substrate converted by one mole of an enzyme per minute is termed the turnover number of the enzyme and the turnover number of enzymes range from 100 to 3,000,000.
- ❖ Most of the enzymes get inactivated, presumably through denaturation, when heated above 80°C. The optimum temperature for enzyme action is about 20 to 40°C.
- ❖ They shown an extraordinary specificity of action. They may be specific for particular substrate or a particular type of reaction. (e.g., urease hydrolyses urea only;

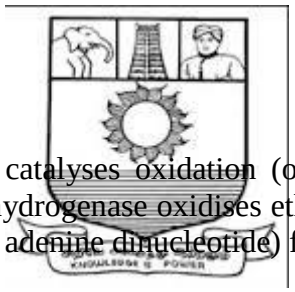
phosphatases hydrolyse the esters of phosphoric acid only; etc.), they may show a relative specificity (e.g., Pepsin is most active for those peptide links in which the amino group belongs to an aromatic amino acid and the carboxyl group is derived from a dicarboxylic amino acid); or they may exhibit stereospecificity (e.g., maltase hydrolyses α-glycosides only whereas emulsin hydrolyses β-glycosides only), lactic acid dehydrogenase catalyzes oxidation of L-lactic acid only).

- Enzyme actions are greatly influenced by pH variations. The optimum pH for most of the enzyme actions is about 7.
- Enzyme actions are usually carried out in dilute solutions, as high concentration of the solution of the substrate renders the enzymes inactive.

2.4 Classification

The International Union of Biochemistry (IUB) classified enzymes into 6 groups :

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases (Synthetases)



1. Oxidoreductases

These are enzymes which catalyses oxidation (oxidases) or reduction (reductases) reaction. For example, Alcohol dehydrogenase oxidises ethanol into acetaldehyde. It requires the coenzyme NAD^+ (nicotinamide adenine dinucleotide) for its activity.

2. Transferases

These enzymes are concerned with the transfer of a group of atoms from one compound to another. Example: Glutamate oxaloacetate transminase catalyses the transfer of an amino group from glutamic acid to oxaloacetic acid. It requires pyridoxal phosphate (PLP) as coenzyme for its activity.

3. Hydrolases

These are enzymes which catalyse hydrolysis. Lipase, for example, hydrolyses glycerides (esters) into glycerol and fatty acids

4. Lyases

These include enzymes that catalyse removal of groups like H_2O, CO_2, NH_3 etc from substrates

5. Isomerases

These enzymes catalyse the interconversion of isomers.

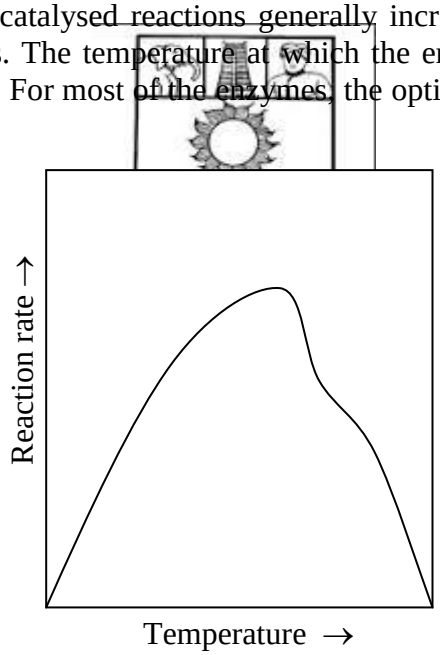
6. Ligases

These enzymes catalyse synthesis of compounds. They link two substrates using ATP or GTP. For example, glutamine synthetase is a ligase (or synthetase) which catalyses the synthesis of glutamine from glutamate and NH_3

2.5 Factors affecting Enzyme action

1. Temperature

The rate of enzyme catalysed reactions generally increases with temperature upto a maximum and then declines. The temperature at which the enzyme activity is maximum is called optimum temperature. For most of the enzymes, the optimum temperature lies between 20°C and 40°C.



Enzyme	Optimum temperature
Plant urease	60°C
Digestive enzymes	40°C
Human enzymes	37°C

At higher temperatures, the enzymes are denatured and their catalytic activity is lost. The effect of temperature on the rate of enzymatic reactions is measured in terms of

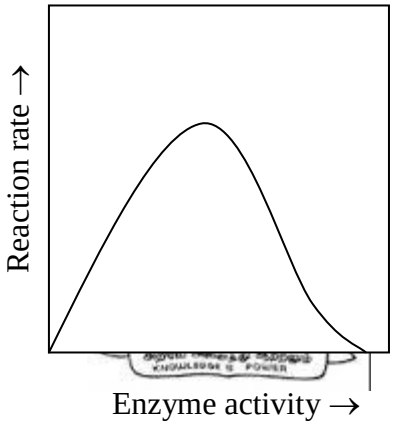
temperature coefficient, Q_{10}. It is defined as the ratio of the reaction rate at temperature t + 10°C to that at t°C.

$$Q_{10} = \frac{K_{t+10}}{K_t}$$

2. pH of the medium

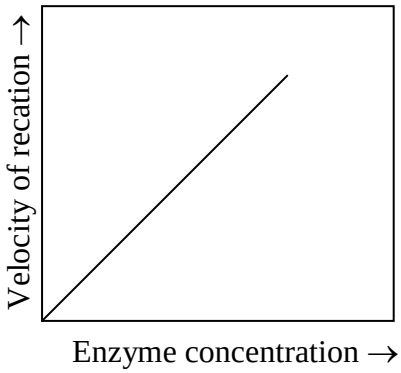
Most enzymes have a characteristic pH at which their activity is maximum. This is termed optimum pH. Above and below this pH, the enzyme activity declines. Most of the enzymes act effectively in the pH range of 5 to 9.

Enzyme	Optimum pH
Pepsin	1.2
Trypsin	8 - 9
Salivary amylase	7



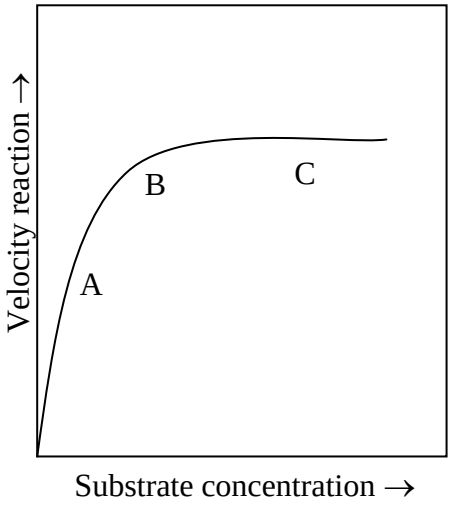
3. Enzyme concentration

Increase in enzyme concentration has been found to increase proportionately the velocity of the reaction. This is because of the provision of additional catalytic sites to which the substrate may bind with concomitant rate enhancement. This property is utilized in determining the level of serum enzymes for the diagnosis of diseases.



4. Substrate concentration

An increase in the substrate concentration increases the enzyme activity and velocity of the enzymatic reaction till a maximum is reached. A rectangular hyperbola is obtained when the velocity of reaction is plotted against the substrate concentration. The graph shows three distinct phases:



i). At low substrate concentration, the rate of the reaction is directly proportional to the substrate concentration (A). i.e the reaction is first order with respect to the substrate.

ii). At extremely high substrate concentration, the reaction rate approaches a maximum (V_{max}). This is the phase C at which the reaction rate is independent of the substrate concentration. i.e the reaction is zero order with respect to the substrate. This is due to the fact that the available active sites of all the enzyme molecules are occupied by the substrate.

iii). Between the extremeties (phase B) the reaction follows both first and zero order kinetics. At this phase, the substrate concentration is not directly proportional to the enzyme activity.

5. Enzyme activators

There are some inorganic ions which accelerate enzyme activity. These are called enzyme activators. For example, Cu^{2+} ions activate phenol oxidase while amylase requires Cl^- ions for its optimum activity.

The metal ions may be bound with the enzymes either loosely or tightly. When the metal ions are held loosely, the enzymes are called metal activated enzymes (e.g. Enolase, Mg^{2+}). The enzymes in which the metal ions are tightly bound are called metalloenzymes.

Examples

Alcohol dehydrogenease	Zn^{2+}
Phenol oxidase	Cu^{2+}

6. Enzyme inhibitors

Certain substances bind with the enzyme and bring about a decrease in catalytic activity of that enzyme. Such substances are called enzyme inhibitors. For example, the activity of acetylcholine esterase is decreased by succinylcholine.

2.6 Mechanism of Enzyme action

An enzyme catalysed reaction proceeds through the formation of an enzyme-substrate complex

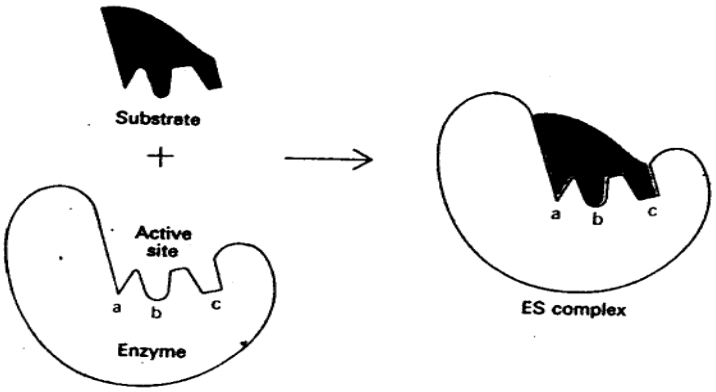
$$E + S \rightarrow ES$$

The complex then breaks down to give the products of reaction. The enzyme is released and can be used over and over again.

$$ES \rightarrow E + \text{Products}$$

2.6.1 Lock and key model

The Lock and Key model proposed by Emil Fischer explains the action of many enzymes. This model assumes that the enzyme is a rigid three-dimensional body with specific regions on the surface. These are called active centres or active sites which bind the substrate. Only the substrate having a matching shape will fit into the site just as a proper key can fit into a lock.



Active sites : The active site of an enzyme is the region that binds the substrate to form an enzyme-substrate complex. The salient features of the active sites are :

1. The active site is very small compared of the total volume of an enzyme
2. The active site is a rigid three-dimensional body with cavity or cleft into which the substrate is bound.
3. The specificity of binding depends on the precisely defined arrangement of atoms in an active site.
4. Substrates are bound to enzymes by relatively weak forces.

2.7 Coenzymes

Definition

Coenzymes may be defined as non-protein, heat stable, low molecular weight organic substances necessary for the activity of enzymes.

The protein part of enzyme is known as apoenzyme. The entire enzyme system consisting of the apoenzyme and coenzyme is called holoenzyme

Apoenzyme + Coenzyme = Holonezyme.

The coenzymes are linked to their apoenzymes through non-covalent forces. For example, the coenzyme ATP is attached to its apoenzyme hexokinase through noncovalent forces. When the coenzymes are tightly attached to their apoenzymes through covalent bonds, the coenzymes are termed prosthetic groups. For example, iron protoporphyrin (haem) is the prosthetic group in haemoglobin (apoenzyme).

Example

Many coenzymes are derivatives of water soluble B- complex vitamins.

Vitamin	Coenzyme
Thiamin (B_1)	Thiamin pyrophosphate (TPP)
Riboflavin (B_2)	Flavin mononucleotide (FMN) (or) Flavin adenine dinucleotide (FAD)
Riboflavin (B_2)	Niacinamide adenine dinucleotide (NAD) (or) Diphospopyridine nucleotide (DPN)
Niacin (B_5)	Niacinamide adenine dinucleotide phosphate (NADP)
Pyridoxine (B_6)	Pyridoxal phosphate (PLP)
Pantothenic acid (B_3)	Acetyl coenzyme A

2.8 Prosthetic groups and their importance in enzyme action

2.8.1 Thiamin pyrophosphate (TPP)

Structure

Role

TTP is involved in the transfer of acyl and carboxyl groups. For example, it serves as a coenzyme for the enzyme pyruvate dehydrogenase which brings out the oxidative decarboxylation of pyruvate to give acetaldehyde.

Mechanism of action

The proton at C-2 in the thiazole ring is ionised to yield a carbanion. The carbanion of TPP thus formed reacts with the carboxyl carbon of the pyruvate to give CO_2 and hydroxyethyl TPP. The hydroxy ethyl group then undergoes hydrolysis to yield acetadehyde.

R

2.8.2 Nicotinamide Adenine Dinucleotide (NAD^+) or Coenzyme I

Structure

Role

NAD^+ is mainly involved in hydrogen transferring reactions. That is, it serves as the coenzyme for many oxidoreductases. For example, malate dehydrogenase which is an important enzyme in TCA cycle requires NAD^+ for its activity.

Mechanism of action

H is transferred from the substrate (malate) to the pyridine nucleus of NAD^+ is reduced to NADH.

2.8.3 NADP

Nicoinamide adenine dinucleotide (NAD^+) and **nicotinamide adenine dinucleotide phosphate ($NADP^+$)** coenzymes are based on a common structure consisting of the base adenine, two ribose sugars linked by phosphate groups and a nicotinamide ring. $NADP^-$ differs from NAD^- in having an additional phosphate group attached to one of the ribose sugars. These two coenzymes share a common function as they both act as carriers of

electrons and are involved in oxidation-reduction reactions. NAD^- is more commonly used in **catabolic** (breakdown) reactions, whilst $NADP^-$ is used in **anabolic** (biosynthetic) reactions. The reactive part of both molecules is the **nicotinamide ring** which exists in either a reduced or an oxidized form, and so acts to accept or donate electrons in an enzymatic reaction. The reaction also involves the transfer of protons, according to the equation:

$$NAD^+ + H^+ + 2e^- \equiv NADH$$

2.8.4 FAD

Flavin adenine dinucleotide (FDA) and **flavin mononucleotide (FMN)** are also carriers of electrons and have related chemical strutures. Both of these coenzymes consists of a **flavine mononucleotide unit** which contains the reactive site. FAD has an additional sugar group and an adenine base which complete its structure. FAD and FMN react with two protons, as well as two electrons, in alternating between the reduced and oxidized state:

$$FAD + 2H^- + 2e^- \equiv FADH_2$$

2.8.5 ATP

Adenosine triphosphate (ATP) is a nucleoside triphosphate used in cells as a coenzyme often called the 'molecular unit of currency' of intracellular energy transfer. ATP transports chemical energy within cells for metabolism. It is one of the end products of photophosphorylation, cellular respiration and fermentation and used by enzymes and structural proteins

One molecule of ATP contains three phophate groups and it is produceed by a wide variety of enzymes including ATP synthese from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and various phosphate group donors. Substrate -level phosphorylation, oxidative phosphorylation in cellular respiration and photophosphorylation in photosynthesis are three major mechanisms of ATP biosynthesis.

2.9 Immobilization of Enzymes

Enzymes can be preserved for long periods without loss of activity by grafting them on a solid matrix. Now, the enzymes are said to be immobilished. Beaded gels and cyanogen bromide -activated sepharose are widely used for the immobilization of enzymes.

Glucose oxidase (GOD) and peroxidase (POD) are coated on a strip of paper and the immobilished enzymes are used in clinical laboratory for the detection of glucose on urine.

The intensity of the blue colour depends upon the concentration of glucose. Hence, glucose in urine may be detected and estimated by this method.

Immobilized enzymes have a variety of advantages over the free enzymes :

1. They can be stored for long periods without loss of activity.
2. Stability is generally increased to thermal denaturation and, in the case of proteases, to autodigestion
3. They can be recovered from the reaction mixtures in pure state and reused many times.
4. Immobilized enzymes like GOD and POD are used for the detection and estimation of glucose in urine.

Industrial applications of Enzymes

Enzymes are widely used in food, pharmaceutical and chemical industries.

Commodity produced	Enzyme used
Food Industry	
1. Curd from milk	Lactobacillus acidophilus
2. Chees from milk	Streptococcus themorphilus
3. Fermenting rice for delicious idlies	Leucanostoc mesenteroides

2.10 Enzyme specificity

Enzymes are highly specific in their action. Each enzyme is capable of bringing out only one or a small group of reactions. Thus, specificity is an important criterion of enzyme action. The specificity may be of various types :

1. Reaction specificity
2. Substrate specificity
   i) Absolute specificity
   ii) Relative specificity
   iii) Group specificity
   iv) Stereospecificity

1. Reaction specificity

Different enzymes bring out different reactions on the same substrate e.g

2. Substrate specificity

Substrate specificity varies from enzyme to enzyme. This is of several types :

i) Absolute specificity : Certain enzymes act on only one substrate. For example, urease acts only on urea to give ammonia and carbon dioxide

ii) Relative specificity : some enzymes act on structurally related substrates with different speeds. For example, D-aminoacid oxidases act on all D-amino acids, but they can bring about oxidative deamination of D-tyrosine very rapidly and less so the other D-amino acids

iii) Group specificity : Some enzymes act on specific bonds on different compounds. For example, pepsin hydrolyses peptide bonds preferentially involving tryptophan, tryrosine, phenylalanine and leucine while Trypsin acts best on peptide bonds involving - COOH group of arginine and lysine.

iv) Stereosepecificity : Specific isomers act on only one stereoisomer an exhibit stereosepecificity. For example, L-amino acid oxidase acts on L-amino acids whereas D-amino acid oxidase acts on D-amino acids. Similarly, fumarase catalyses the interconversion of fumeric acid and L-malic acid. It has no effect on the interconversion of maleic acid and D-malic acid.

Check your progress

1. Enzymes are .....
2. Coenzyme derived from pantothenic acid is .....
3. Enzyme reactions are highly stereo .....
4. ..... transports chemical energy within cells for metabolism.

2.11 Sum up

Enzymes are biocatalysts. They promote chemical reactions. Enzymes retain their identity at the end of the reactions as in the begining. All enzymes are proteins and exhibit all

the properties of proteins. Many enzymes require the presence of certain non-protein compounds which help in accelerating the enzyme action. They are called as coenzymes or prosthetic groups.

2.12 Key words:

Enzyme : A protein that catalyses a specific chemical reaction. It does not affect the equilibrium of the catalysed reaction, it enhance the rate of a reaction by providing a reaction path with lower activation energy

Coenzyme : An organic cofactor required for the action of certain enzymes; often contains a vitamin as a component.

NAD, NADP : Nicotinamide containing coenzymes functioning as carriers of hydrogen atoms and electrons in some oxidation-reduction reactions.

Prosthetic group : A metal ion or an organic compound (other than an amino acid) that is convalently bound to a protein and is essential for its activity.

2.13 Question for Discussion

1. What are coenzymes
2. Give the different classification of enzymes
3. Explain Lock and key model of enzymes action
4. Write note an NAD and NADP
5. Explain the different types of enzyme specificity.
6. Write note on immobilization of enzymes.

Check Your Progress : Model answer

1. Protein
2. Acetyl coenzyme A
3. Specific
4. ATP

2.14 Suggested Readings:

[1] J. L. Jain, Biochemistry, Sultan Chand and Co.1999.

[2] David Hames and Nigel Hooper, Biochemistry

[3] C.B. Power and G.R. Chatwal, Biochemistry.

CHAPTER III

LIPIDS

CONTENTS

3.0 Aims and objecitives

- Explains the classification of lipids
- Gives idea about the types of fatty acids
- Describes the method of analysis of fatty acids
- Explains the biosynthesis of cholesterol
- Explains the bile salts derived from cholesterol.

3.1 Introduction

Lipids are a heterogeneous group of compounds which are relatively insoluble in water but soluble in non-polar organic solvents such as ether, chloroform and benzene. The term lipid means non-polar organic solvent. A widely used universal lipid solvent is chloroform + methanol (2:1). On hydrolysis, lipids yield fatty acids which are utilised by the living organisms. Thus, they are the esters of fatty acids. Oils, fats, steroids, waxes and related compounds are example of lipids.

Lipids are widely distributed throughout the plant and animal kingdom. In plants, they occur in the seeds, nuts and fruits. In animals, they are stored in adipose tissues, bone marrows and nervous tissues.

3.2 Classification

Lipids are broadly classified into three groups :

1. Simple lipids
2. Complex lipids
3. Derived lipids

1. Simple lipids

Simple lipids are the esters of fatty acids with alcohols. They are further classified as follows:

a) Oils and Fats :

These are the esters of fatty acids with glycerol. Hence, they are also called triglycerides. There is no difference between an oil and a fat as far as the chemical properties are concerned. They are the triglycerides of fatty acids. The difference between an oil and a fat lies only in their melting points. Those melting above 20°C are called fats while those melting below 20°C are oils.

b) Waxes : These are the esters of fatty acids with high molecular weight monohydric alcohols. Some of the important alcohols and acids found in waxes are tabulated below:

Alcohols	Acids
Lauryl $C_{12}H_{25}OH$	Myristic $C_{13}H_{27}COOH$

Cetyl $C_{16}H_{33}OH$	Palmitic $C_{15}H_{31}COOH$
Octodecyl $C_{18}H_{37}OH$	Cerotic $C_{25}H_{51}COOH$
Caranaubyl $C_{24}H_{49}OH$	
Ceryl $C_{26}H_{53}OH$	Melissic $C_{29}H_{59}COOH$
Myricyl $C_{30}H_{61}OH$	

Bee wax, spermaceti, sperm oil and wool wax are some naturally occurring waxes. Cholesteryl palmitate is the wax found in blood plasma.

2. Complex lipids

The lipids having some additional groups besides fatty acids and alcohols are called complex or compound lipids. The additional group may be a phosphate, nitrogenous base, carbohydrate or protein. They are further classified as follows:

a) Phospholipids : Lipids containing a phosphoric acid residue in addition to fatty acids and alcohol are known as phospholipids. There are two types of phospholipids.

i) Glycerophospholipids : These are phospholipids in which the alcohol is glycerol. Example : Lecithin, Cephalin.
ii) Spingophospholipids : These are phospholipids containing sphingosine as the alcohol. Example : sphingomyelin.

b) Glycolipids : Lipids containing a glucose or galactose unit in addition to fatty acids and spingosine alcohol are called glycolipids. Examples : cerebroside, gangliosides etc.

c) Lipoproteins : These are complexes of lipids with proteins which occur in milk, blood serum and egg yolk.

3. Derived lipids

These are the hydrolysis products of simple and complex lipids. They include fatty acids, glycerol, steroids (e.g. cholesterol), fat soluble vitamins, hormones, carotenoids, hydrocarbons (e.g. pentacosane in bee wax), fatty aldehydes and ketones.

3.2.2 Phospholipids

Phospholipids are complex lipids containing phosphoric acid in addition to fatty acid, nitrogenous base and alcohol. Based on the nature of alcohol moiety, phopholipids are classified into three groups:

1. Glycerophosphatides
2. Phosphospingosides
3. Phosphoinositides

1. Glycerophophatides

There are phospholipids in which the alcohol is glycerol. This includes lecithins, cephalins, phosphatidyl cerine and plasmalogens.

a) Lecithins (or Phosphatidyl cholines)

Occurrence : Lecithins occur in all cell membranes, particularly rich in liver.

Composition : Lecithins contain glycerol, fatty acid, phosphoric acid and a quaternary base, choline. The structural moiety without choline is called phosphatidic acid. Hence, lecithins are also known as phosphatidyl cholines. A large number of lecithins exist differing in their fatty acid moiety. The fatty acids commonly occurring in lecithins are palmitic, stearic, oleic, linolenic and arachidonic acids. The lecithins in human RBC membranes are reported to exist in more than 20 forms.

Structure : The structures of α and β forms of lecithin are shown below:

Functions :

1) Lecithins are important constituents of lipoproteins, particularly chylomicrons.
2) Lecithins play an important role in fat metabolism in the liver. It helps emulsification of lipid-water mixtures, a prerequisite in the digestion as well as absorption of lipids from the gastrointestinal tract.
3) In the plasma, they serve the very useful function of keeping cholesterol and its ester in the dissolved state.
4) The toxicity of the venoms of bee and cobra is partly due to the presence of lecithinase, an enzyme which hydrolyses lecithins to lysolecithin which brings about rapid haemolysis.

b) Cephalins (or Phosphatidyl ethanolamines)

Occurrence : Cephalins occur together with lecithins in animal tissues and are particularly concentrated in the brain. They are also present in the erythrocyte and in soyabean oil.

Composition : Cephalins which are also known as phosphatidyl ethanolamines contain glycerol, fatty acid, phosphoric acid and a base ethanolamine (in the place of choline of lecithins)

Structure : Cephalins are structurally similar to lecithins.

Functions : Similar to lecithins

c) Phosphatidyl cerine

Occurrence : Phosphatidyl cerine occurs in lesser amount in all animal tissues.

Composition : It has the same composition as lecithins except that the base is serine, an amino acid. Thus, it is an aid phospholipid.

Structure

d) Plasmalogens

Occurrence : Plasmalogens occur abundantly in brain and muscles. They also occur in the seeds of higher plants.

Composition : Plasmalogens contain glycerol, fatty acid, phosphoric acid and a base choline or ethanolamine. Thus, they resemble lecithins and cephalins. But, one of the fatty acids is replaced by a long chain aliphatic aldehyde. The aldehyde is in the enolic form (RCH = CHOH) and combines with the α-carbon of glycerol by an ether linkage to form CH_2OH=CHR

Structure

2. Phosphospingosides

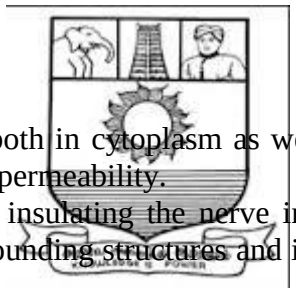
These are phospholipids in which the alcohol is sphingosine or sphingol, a complex amino alcohol. Fatty acid, choline and phosphoric acid are the other constitutents. These are also known as sphingophospholipids or simply sphingolipids.

Example : Sphingomyelin

Sphingomyelin is an important constituent of myelin and present in large amounts in brain and nervous tissues. They apparently lack in plants and microorganisms. The disease called Niemann-Pick's disease is due to the accumulation of large amounts of sphingomyelin in the spleen, liver and brain as a consequence of metabolic defect.

3. Phosphoinositides

These are phospholipids in which the base is replaced by inositol, a cyclic hexahydric alcohol. These are also known as phosphatidyl inositols, Monophosphotidyl inositols are widely distributed in animals and plants but diphosphatidyl inositols have been reported to be present in brain tissues only.



Functions of phospholipids

1) Phospholipids are present both in cytoplasm as well as cell membranes and regulate cell activity and membrane permeability.
2) They are of importance in insulating the nerve impluse (like the rubber around an electric wire) from the surrounding structures and in channelling out the enzymes into distinct groups.
3) They participate in cellular respiration.
4) They are essential for the synthesis of lipoproteins which take part in the transport of lipids from and to the liver.
5) Phospholipids participate in the absorption of fat from the intestine.
6) Phospholipids prevent accumulation of fat in the liver
7) They help in removing cholestrol from the body
8) Dipalmitoyl phosphatidyl chlorine a phospholipid, acts as a lung surfactant to lower surface tension.
9) Cephalins participate in blood clotting.
10) Phosphatidyl inositols are involved in signal transmission across cell membrances.

3.2.3 Glycolipids

Lipids containing spingosine, a carbohydrate (glucose or galactose) and fatty acid are called glycolipids (or) glycosphingolipids. They are widely distributed in the white matter of the brain and in the myelin sheaths of nerves. Gaucher's disease is due to the accumulation of a large amount of glycolipids in the liver and spleen.

Examples : Cerebrosides, Gangliosides.

1. Cerebrosides : These are glycosphingolipids containing ceramide (sphingosine attached to fatty acid) and one or more sugar residues. In glucocerebroside, a glucose unit forms a β-glycosidic bond with the ceramide portion of the molecule. Similarly, galactose forms galactocerbroside.

The cerebrosides occur primarily in the brain and at nerve synapses

2. Gangliosides :

These are complex glycosphingolipids derived from glucocerebroside. They contain one or more residues of sialic acid in the carbohydrate moiety. In human brain, the gangliosides contain N-acetylneuraminic acid as the sialic acid component. Gangliosides are present in high concentration in nervous tissues such as brain. They appear to have receptor and other functions. For example, a ganglioside named G_{M1} is the receptor in human intestine for cholera toxin. The structure of G_{M1} ganglioside is shown below:

Cer	=	Ceramide (Acyl sphingosine)
Glc	=	Glucose
Gal	=	Galactose
GalNAc	=	N-Acetylgalactosamine
NeuAc	=	N-Acetylneuraminic acid

Gangliosides accumulate in brain in Tay – Sach's disease due to genetic lack of the enzyme required for their degradation.

Functions of Glycolipids

1. Glycolipids are the constituents of cell membranes
2. They are responsible for the blood group specificity and tissue and organ specificity
3. They are also responsible for tissue immunity and for cell-cell recognition sites.
4. They participate in transmission of nerve impulses across synapses.
5. They act as receptors for acetylcholine and other neuro transmitter substances.

3.3 Fatty acids

The naturally occurring oils and fats are hydrolysed to yield long chain monocarboxylic acids called fatty acids. Fatty acids occurring in higher animals tend to have chain lengths of 16, 18 or 20 carbon atoms except in milk fat, where the saturated fatty acids contain 14-10 carbon atoms. Oleic acid is the most abundant fatty acid in humans. Butyric acid with four carbon atoms is normally the shortest fatty acid found in human fat.

Classification

Fatty acids are classified on the basis of chemical composition and physical properties into four types:

1. Saturated fatty acids
2. Unsaturated fatty acids
3. Unusual fatty acids
4. Essential fatty acids

3.3.1 Saturated fatty acids

These are the fatty acids which have only single bonds in the hydrocarbon chain. Their general formula is $CH_3(CH_2)_n COOH$. They contain an even number of carbon atoms, because they are synthesised from two-carbon units. The major saturated fatty acids found in plants and animals are listed below:

Name	Formula	Source
Butyric acid	$CH_3 (CH_2)_2COOH$	Butter
Caproic acid	$CH_3(CH_2)_4COOH$	Butter
Caprylic acid	$CH_3(CH_2)_6COOH$	Butter, Coconut oil, Palm oil
Capric acid	$CH_3(CH_2)_8COOH$	Coconut oil, Palm oil

3.3.2 Unsaturated fatty acids

These are the fatty acids which contain one or more double bonds in their hydrocarbon chains. The general formula of these acids is $R\text{-}CH{=}CH(CH_2)_n$ COOH. The unsaturated fatty acids have been found to be more common in living organisms than the saturated acids. They may have an odd or an even number of carbon atoms. Odd- numbered fatty acids are more common in plants, but most of the fatty acids in animals are even-numbered. The unsaturated fatty acids are further divided into two types:

a) Monounsaturated fatty acids which contain only one double bond.

Examples

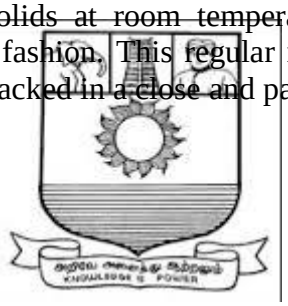
Acid	Source
Palmitoleic acid	Milk fat,
$CH_3(CH_2)_5CH = CH(CH_2)_7COOH$	Sardine oil

b) Polyunsaturated fatty acids which contain more than one double bonds. The double bonds may be conjugated or non-conjugated.

Acid	Source
Linoleic acid	Linseed oil,
$CH_3(CH_2)_4(CH = CH(CH_2)_2\,(CH_2)_6COOH$	Soyabean oil

3.4 Properties

1) Saturated fatty acids are solids at room temperature with the hydrocarbon chain arranged in regular zig-zag fashion. This regular nature of their hydrocarbon chains allows the molecules to be packed in a close and parallel alignment



2) Unsaturated fatty acids exhibit geometric isomerism. Most unsaturated acids occur in the relatively less stable cis isomeric form rather than the stable trans form. The cis double bond interrupts the regular packing of the chains. Thus, unsaturated fatty acids are all liquids at room temperature.

3) Fatty acids are the building blocks of phospholipids and glycolipids.

3.4.1 Hydrolysis

The esters of glycerol and fatty acids are called triacyl glycerols or triglycerides or neutral fats

Triacyl glycerols (oils or fats) are hydrolysed by acid or enzyme lipase of pancreatic juice to fatty acids and glycerol.

3.4.2 Acid number

Definition :

The acid number or acid value is defined as the number of milligrams of potassium hydroxide required to neutralise the free fatty acids in 1 gram of oil or fat.

Significance :

Acid number is used to determine rancidity due to free acid. A high acid value indicated a stale oil or fat stored under improper conditions.

3.4.3 Saponification number

Definition :

The saponification number is defined as the number of milligrams of potassium hydroxide required for the complete hydrolysis (saponifiation) of 1 gram of oil or fat.

Determination :

A known weight of the oil or fat is refluxed with a known excess of alcoholic KOH solution in a boiling water bath. The reaction takes about an hour for completion. The solution is then cooled and the unused KOH is titrated with standard HCl using phnolphthalein indicator. A blank titration is conducted simultaneously with the same amount of alcoholic KOH solution without the oil or fat. The difference in titre values gives the amount KOH used.

Saponification value = $\frac{(V_2 - V_1) \times N_{HCl} \times 56}{W}$

Where, $V_2 - V_1$ = difference in titre values in ml
W = weight of oil taken

Significance :

1) The saponification value is a measure of the nature of fatty acid present in an oil or fat. The oil with short chain fatty acids (e.g. butyric acid) has high saponification value. Butter, for example, contains butyric value is, therefore, useful in checking the purity of butter.
2) Saponification value is used to find out the suitability of an oil for soap making.

3.4.4 Rancidity:

On prolonged exposure to mosit air, naturally occurring edible oils and fats, especially those from animal sources, loose the taste and develop an unpleasant odour. This process is called rancidity. This is due to the partial hydrolysis of the oil or fat and some degree of oxidation of the unsaturated fatty acids at the double bond. The rancid oil or fat is unfit to eat.

Rancidity is of two types:

i) Hydrolytic rancidity :

This is due to the partial hydrolysis of oils or fats at the site of the ester linkages to yield short chain fatty acids which have bad odours. This is brought about by certain enzymes like lipase. Hence, hydrolytic rancidity is also known as lipolysis.

ii) Oxidative rancidity :

Oxidation of the unsaturated fatty acids present in oils or fats by atmospheric air at the C=C positions yield peroxides which decompose into aldehydes and ketones of bad tastes and odours.

Prevention of rancidity :

Vegetable oils and fats contain certain substances like vitamin E, vitamins C, phenols, hydroquinone, tannins etc which are anti-oxidants and therefore prevent development of rancidity. Hence, vegetable oils and fats can be preserved for a longer period than animal fats.

Rancidity of oils and fats, particularly animal fats, may be prevented by the following methods:

i. Proper storage in refrigerators or air-tight containers
ii. Addition of antioxidants like phenols, propyl gallate, butylated hydroxy anisole (BHA), butylated hydroxy anisole (BHA), butylated hydroxytouene (BHT), pyrogallol, hydroquinone etc.

3.5. Analysis of oil or fat

3.5.1 Addition reaction

Fats containing unsaturated fatty acids, readily add on elements such as halogens and hydrogen at their double bonds.

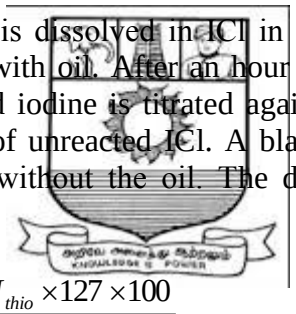
3.5.2 Iodine number

Definition:

The iodine number is defined as the number of grams of iodine or ICl absorbed by 100 grams of oil.

Determination :

A weighed samples of oil is dissolved in ICl in acetic acid (Wij's solution). Wij's solution reacts faster than free I_2 with oil. After an hour when the reaction is complete, KI solution is added and the liberated iodine is titrated against standard thiosulphate solution. The titre value gives the amount of unreacted ICl. A blank titration is carried out with the same volume of Wij's solution without the oil. The difference in titre values gives the amount of ICl consumed by the oil.



$$\text{Iodine value} = \frac{(V_2 - V_1) \times N_{thio} \times 127 \times 100}{W \times 1000}$$

Where, $V_2 - V_1$ = difference in titre values in ml

W = weight of oil taken

Significance :

The iodine value is a measure of the extent of unsaturation of the fatty acids and hence the drying power of an oil. It is used to find out suitability of an oil for paint making. Linseed oil is used in paints because of its high drying power. This oil has a high iodine value.
It is also valuable in checking adulteration of oils.

3.5.3 Polenske number

It is the number of millilitres of 0.1 N KOH required to neutralise the insoluble fatty acids (i.e. those which are not volatile with steam distillation) obtained from 5 gm of fat.

3.5.4 Reichert-Meissl (R.M) number

Definition:

The R.M value or number is defined as the number of millitres of 0.1 N KOH solution required to neutralise the steam volatile water soluble fatty acids obtained by the hydrolysis of 5 grams of the oil or fat.

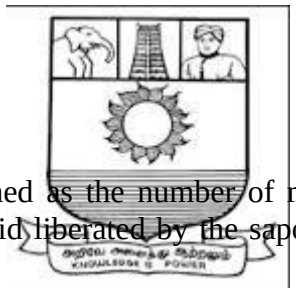
Determination :

A 5 gram sample of the oil or fat is introduced into a flask and treated with NaOH in glycerol. Saponification occurs rapidly forming glycerol and sodium soaps. The soaps are neutralised with sulphuric acid and the mixture steam distilled. On condensation of the distillate, the insoluble fatty acids precipitate. The ppt is filtered off and the filtrate is titrated against standard N/10 KOH solution.

Significance :

The R.M. value is useful for ascertaining the purity of butter and ghee.
It is also valuable in checking adulteration of butter with coconut oil and ghee with vanaspathi.

3.5.5 Acetyl number



Definition :

The acetyl number is defined as the number of milligrams of potassium hydroxide required to neutralise the acetic acid liberated by the saponification of 1 gram of acetylated oil or fat.

Singificance :

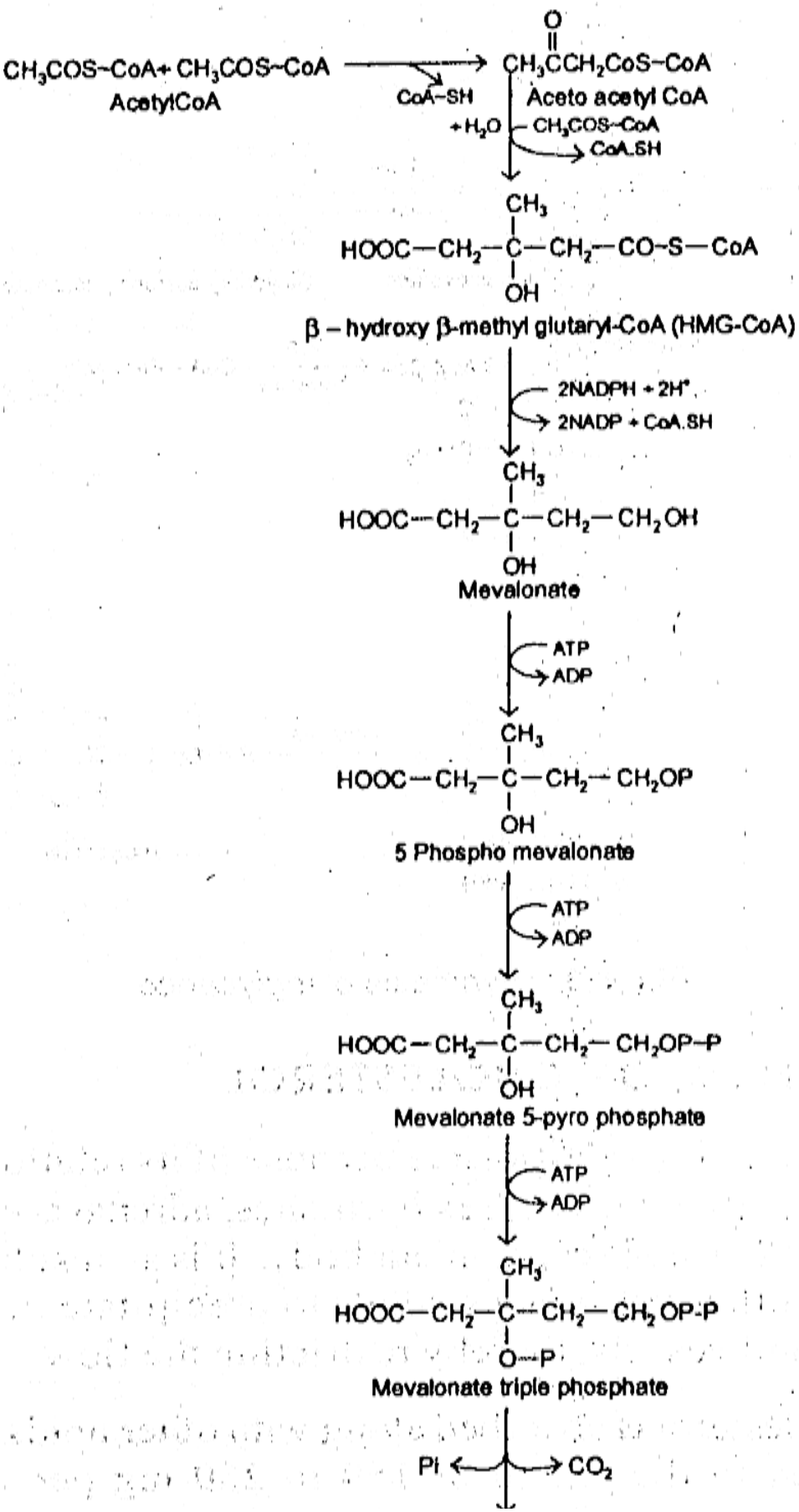
Acetyl number indicates the amount of hydroxylated acid present in a given oil or fat.

3.6 Cholesterol

Cholesterol is of major significance because of its relationship to many physiologically active steroids, sex hormones, adrenal cortex hormones, bile salts, etc. which are present in our body. It is and insoluble substance and along with other substances, tends to precipitate in and along the lining of the blood vessels, there by restricting the flow.

3.6.1 Biosynthesis of cholesterol

Cholesterol is synthesised in the body from two-carbon units in the form of acetyl CoA formed either from fatty acid or from the metabolism of carbohydrate through pyruvate. The reaction describes as follow as :


CH3COS~CoA + CH3COS~CoA
AcetylCoA
CoA-SH
CH3CCH2CoS~CoA
Aceto acetyl CoA
+H2O
CH3COS~CoA
CoA.SH
HOOC—CH2—C(CH3)(OH)—CH2—CO~S—CoA
β – hydroxy β-methyl glutaryl-CoA (HMG-CoA)
2NADPH + 2H+
2NADP + CoA.SH
HOOC—CH2—C(CH3)(OH)—CH2—CH2OH
Mevalonate
ATP
ADP
HOOC—CH2—C(CH3)(OH)—CH2—CH2OP
5 Phospho mevalonate
ATP
ADP
HOOC—CH2—C(CH3)(OH)—CH2—CH2OP~P
Mevalonate 5-pyro phosphate
ATP
ADP
HOOC—CH2—C(CH3)(O~P)—CH2—CH2OP~P
Mevalonate triple phosphate
PI
CO2

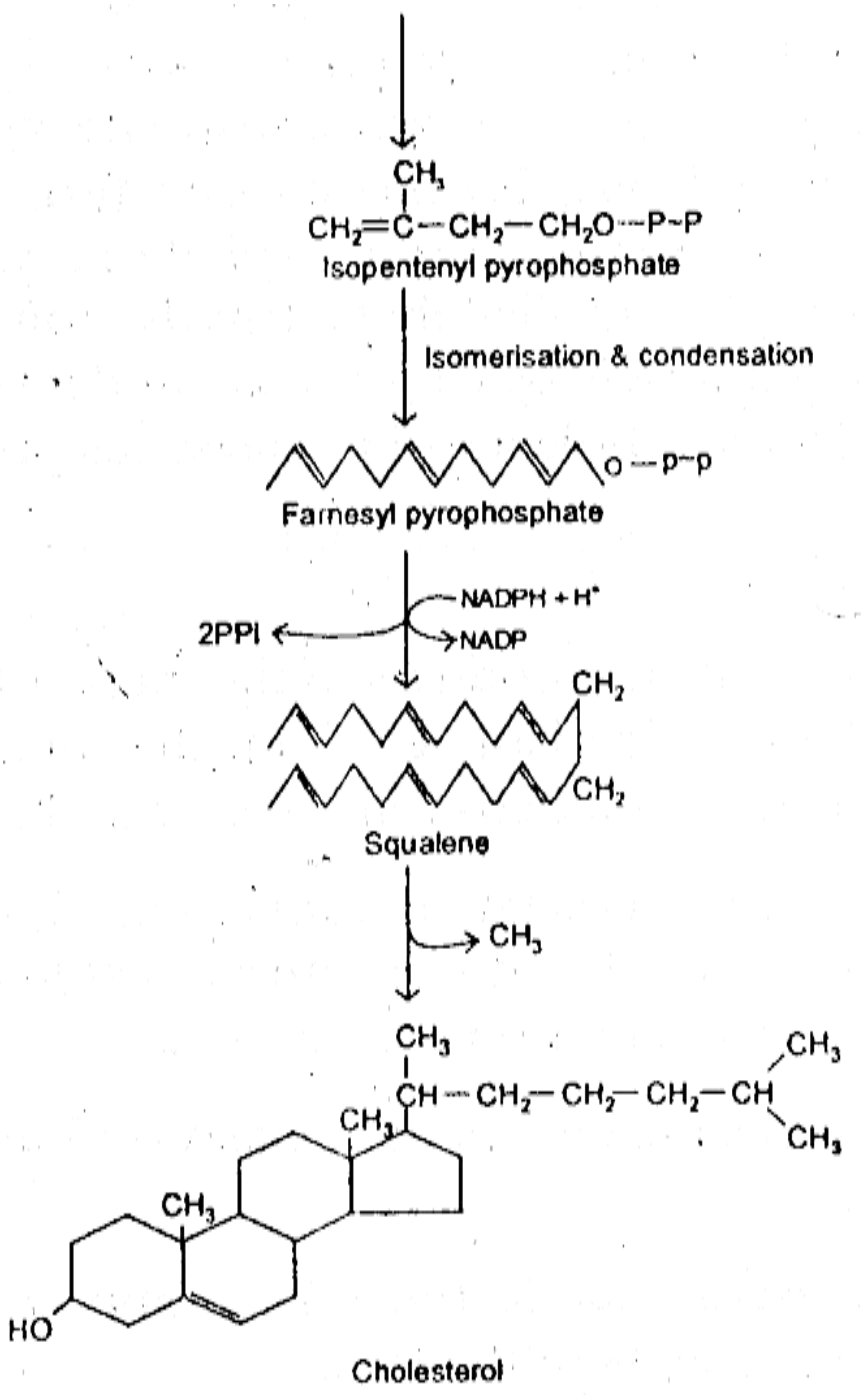


Figure Biosynthesis of cholesterol

Two molecules of acetyl CoA condense to form β-hydroxy β-methyl glutaryl CoA which in turn, gives rise to the intermediate compound called mevalonic aicd. Mevalonic acid is phosphorylated three times in succession by ATP, forming first a monophosphomevalonic acid, next a diphosphomevalonic acid and finally triple phosphorylated mevalonic acid, a transient intermediate which simultaneously loses a molecule of phosphate and CO_2 to form isopentenyl pyrophosphate which can also exist in an isomeric form 3,3-dimethylallyl pyrophosphate. These compounds are said to be the forerunners of many important biological compounds including carotenoid pigments and cholesterol. One molecule of 3,3methylallyl pyrophosphate now reacts with one molecule of isopentenyl pyrophosphate to yield geranyl

pyrophosphate which with another molecule of isopentenyl pyrophosphate forms farnesyl prophosphate with the removal of inorganic pyrophosphate at each stage. The two molecules of farnesyl pyrophosphate finally condense to form the hydrocarbon squalene which, by ring closure and loss of methyl groups, is readily converted into cholesterol by enzymes present in the liver.

3.6.2 Bile Acids or Bile Salts

Bile acids are imporatant end products of the metabolism of cholesterol in liver. The four bile acids isolated from the human bile are cholic acid, deoxycholic acid, chenodeoxycholic acid and lithocholic acid.

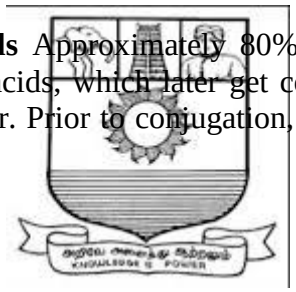
Cholic acid is present in largest amounts in the bile itself, forming a maximum of 60% of the total. It is a saturated sterol having three OH groups on the nucleus in positions 3, 7 and 12.

Deoxycholic acid lacks the OH group in position 7 and constitutes 25% of the total.

Chenodeoxycholic acid lacks the OH group in position 12 and constitutes about 15 to 20% of the total.

Lithocholic acid has only one OH group in position 3.

Route of Formation of Bile acids Approximately 80% of the cholesterol metabolised is converted by liver tissues to bile acids, which later get conjugated in the liver with glycine and taurine by enzymes of the liver. Prior to conjugation, the bile acids are activated to acyl CoA.



Intestinal bacteria act upon the unabsorbed bile acids and the products are excreted in the faeces. Thus, it may be seen that bile represents the main route for the excretion of cholesterol, which is held in solution by the emulsifying action of bile salts. Under abnormal conditions, this can lead to the formation of cholesterol stones (calculi) in the biliary passages like gall bladded and common bile duct and cause obstruction to the circulation of bile pigments resulting in obstructive jaundice.

Check Your Progress

1. Food is stored in the living organisms as .....
2. The degree of unsaturation of an oil is measured in terms of .....
3. Cholesterol is a ..... lipid
4. Glycerophophatides are phospho lipids in which the alcohol is .....
5. ..... is a essential lipid synthesized in liver

3.7 Sum up

Lipids are a heterogeneous group of compounds which are relatively insoluble in water but soluble in non polar organic solvents such as ether, benzene etc. on hydrolysis

lipids yield fatty acids which are utilised by the living organisms. Thus they are the esters of fatty acids. Oils, fats, steroids, waxes and related compounds are examples of lipids. Fats are used to synthesize phospholipids. Phospholipids are powerful emulsifying agents and are essential for the digestion and absorption of fats. Cholesterol is an essential lipid synthesized in the liver.

3.8 Key words:

Fatty acid : A long chain aliphatic carboxylic acid found in natural fats and oils. Also a component of membrane phospholipids and glycolipids.

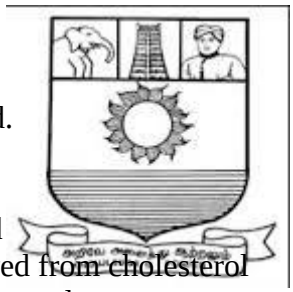
Lipid : A small water insoluble biomolecule generaly containing fatty acid and glycerol.

Bile salts : Amphipathic steriod derivatives with detergent properties, participating in digestion and absorption of lipids.

Glycolipid : A lipid containing a carbohydrate group.

Phospholipid : A lipid containing one or more phosphate group.

3.9 Questions for Discussion



1. What is glycolipid?
2. Write note on saturated fatty acid.
3. Define (i) iodine number
   (ii) saponification of lipid
4. Explain the classification of lipid
5. Give the different bile salts derived from cholesterol
6. Explain the biosynthesis of cholesterol.

Check Your Progress : Model answer

1. Fat
2. Iodine number
3. Neutral
4. Glycerol
5. Cholesterol

Suggestion Readings

[1] L. Veerakumari, Biochemistry, MJP publishers, Chennai, 2004.
[2] Robert L. Caret, Katherine J. Denniston, Joseph J. Topping, Principles and Applications of organic and biological chemistry, WBB publishers, USA, 1993.
[3] J. L. Jain, Biochemistry, Sultan Chand and Co.1999.

CHAPTER IV
CARBOHYDRATES

CONTENTS

4.0 Aims and objectives

- Explains classifications of carbohydrates
- Explains glycolysis and its reversal
- Describes TCA cycle
- Gives the idea of prinicples of bioenergetics
- Explains oxidative phosphorylation.

4.1 Introduction

Carbohydrates are compounds normally characterised by having carbon, hydrogen and oxygen. The basic units of the carbohydrates are monosaccharides, and glucose is the most important monosaccharide. Carbohydrates are chemically described as polyhydric alcohols having potentially active aldehyde and ketone groups.

Biological significance

i) Carbohydrates supply the major portion of energy required by living cells.
ii) Certain products of carbohydrate metabolism act as catalysts to promote oxidation of foodstuffs.
iii) Certain carbohydrates can be used as the starting material for the biosynthesis of compounds such as fatty acids and amino acids.

Chemical characteristics of carbohydrates

Chemically, carbohydrates contain carbon, hydrogen and oxygen. All simple sugars contain a potential aldehyde or ketone group. All compound sugars, which are made up of simple sugar molecules, also contain the potential aldehyde group either in the free form or in the combined form. The importance of potential aldehyde or ketone group is that they are associated with reducing properties.

4.2 Classification of carbohydrates

Carbohydrates are classified into four major groups:

1. Monosaccharides

Monosaccharides contain only one molecule of sugar and they cannot be broken into simpler substances by hydrolysis. E.g. glucose, fructose, etc.

2. Oligosaccharides

Oligosaccharides yield 2 to 10 monosaccharides on hydrolysis. E.g. raffinose, stachylose and verbacose.

3. Disaccharides

Disaccharides ($C_{12}H_{22}O_{11}$) yield two molecules of monosaccharides on hydrolysis. E.g. sucrose, lactose and maltose.

4. Polysaccharides

Polysaccharides $(C_6H_{10}O_5)_n$ yield more than 10 molecules of monosaccharides on hydrolysis. E.g. starch, glycogen, cellulose, etc.

Monosaccharides

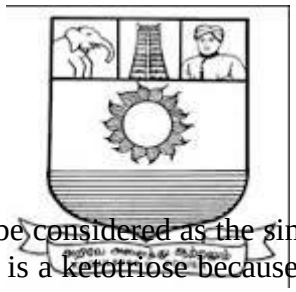
Monasaccharides are further classified according to the number of carbon atoms.

1. Diose

It contains two carbon atoms. E.g. glycolaldehyde ($CH_2OH.CHO$). It has an aldehyde group and therefore it is referred to as aldose.

2. Triose

Trioses contain 3 carbon atoms ($C_3H_6O_3$). They are considered as true carbohydrates because they contain polyhydroxylic groups.



Glyceraldehyde which can be considered as the simplest monosaccharide, contains an aldehyde group. Dihydroxyacetone is a ketotriose because it is a triose and contains a ketone group.

3. Tetroses

They contain four carbon atoms ($C_4H_8O_4$). E.g. erythrose, threose and erythrulose.

4. Pentoses

They contain five carbon atoms ($C_5H_{10}O_5$). E.g. ribose, deoxyribose, xylulose and arabinose.

5. Hexoses

They are physiologically important compounds. They contain six carbon atoms ($C_6H_{12}O_6$).e.g. glucose, fructose, galactose, mannose, etc.

Glucose, galactose and mannose are aldohexoses each having an aldehyde group. Fructose is a ketohexose having a ketone group. Glucose and fructose occur freely in plants and fruits. Galactose is a component of lactose, which is a disaccharide present in millk. Polysaccharides containing mannose are found in ivory nuts, orchid tubers and yeast.

Monosaccharides are, thus, more active reducing agents than the disaccharides.

Carbohydrates with free carbonyl groups or in hemiacetal form give positive tests to these reagents without having been hydrolyzed first and are referred to as '**reducing**' sugars; others (i.e., the acetal types) are then '**nonreducing**' sugars (Table 1)

Differences between reducing and nonreducing sugars

Sl.No	Reducing sugar	Nonreducing sugar
1	Carbohydrates with a free aldehyde (at C-1) or a free ketone (at C-2) group.	Aldehyde or ketone group is not free but instead utilized in bond formation
2	They are in hemiacetal or hemiketal form.	They are in acetal or ketal form.
3	Do exhibit mutarotation.	Do not exhibit mutarotation.
4	Do form osazones with phenyl hydrazine.	Do not form osazones.
5	Do form oximes with hydroxylamine. Examples - Glucose, Fructose, Lactose, Maltose, Cellobiose	Do not form oximers. Examples- Sucrose, Glycogen, Inulin

4.3 Glucose

4.3.1 Structure

The straight chain configuration of glucose is

In the above configuration, the -OH group attached to the asymmetric carbon (C-5) farthest from the carbonyl group (C-1) is in the right. This is called D-configuration. Further, the natural glucose is dextro-rotatory (+). For these reasons, natural glucose of dextrose is designated as D(+) glucose.

Cyclic structure

The open chain structure of glucose fails to explain the following observations:

1. Glucose contains a-CHO group but it does not form addition compounds with ammonia and sodium bisulphite and gives no violet colour with Schiff's reagent.
2. When treated with methyl alcohol saturated with HCl gas, glucose forms two isomeric methyl glucosides (α and β).
3. Glucose displays muta rotation, i.e. when D-glucose is dissolved in water, the specific rotation decreases from +110° to +52.5°.

The above observations clearly indicate that glucose does not have free -CHO group. According to Tollen's the -CHO and -OH group on C-5 interact to give six membered hemiacetal ring. When this happens, the carbonyl carbon (C-1) becomes asymmetric and two isomeric forms are possible for glucose. These are called α and β glucose.

[D] Pyranose structure

In 1926, Haworth proved that α - and β-glucose exist as a six-membered heterocyclic pyran ring called pyranose structure or Haworth's projection formula.

The configurations are still better represented by the conformational structures as shown below:

4.4 Carbohydrates of the cell membrane

Carbohydrates which contain more than 10 monosaccharide units are known as polysaccharides.

Example : Starch, cellulose, glycogen, inulin etc.

4.4.1 Starch :

a) Source :

Plant materials such as roots, tubers, stem, vegetables, fruits and cereals are the main sources of starch.

b) Structure :

Starch is the nutritional reservoir in plant. Starch is a homopolysaccharide consists of only α-D-glucose. Two chief constituents of starch are (i) Amylose (15-20%) and (ii)Amylopectin (80 -85%)

Amylose forms the inner portion of the starch grain and is soluble in water. It is a linear, non branched polymer of glucose. The glucose residues are united by $\alpha(1 \rightarrow 4)$ linkage. The molecular weight of amylose is 60,000.

Amylose forms the outer covering of the starch grain and is insoluble in water. It is a highly branched polymer of glucose. The glucose residues are united by $\alpha(1 \rightarrow 4)$ linkages in the chains and by $\alpha(1 \rightarrow 6)$ at the branch points. Its molecular weight is ≈2, 00, 000. It is like glycogen except its lower degree of branching.

Uses :

Starch is used

i) as food material
ii) for the manufacture of glucose and alcohol
iii) in paper industry
iv) in textile industry
v) in printing
vi) to prepare starch acetate, nitrostarch etc.
vii) for making adhesives
viii) as an indicator.

4.4.2 Cellulose

Cellulose is a fibrous, tough, water-insoluble substance, found in the protective cell walls of plants. It is a linear, unbranched homopolysaccharide of 10, 000 or more D-glucose units connected by β-1-4 glycosidic bonds. Due to β linkages, D-glucose chains in cellulose assume an extended conformation and undergo side-by-side aggregation by cross-links of hydrogen bonds into insoluble fibrils.

Uses

Cellulose is used (i) as a food for herbivores. (ii) in the manufacture of rayon and explosives (iii) in making photographic film. (iv) in the manufacture of toothbrush and comb (v) in the manufacture of cellophane etc.

4.4.3 Glycogen

It is the main carbohydrate storage substance of animals and fungi. It is made up of molecules similar to amylopectin but with more numerous side chains.

Uses :

(i) Excess carbohydrate in the body are deposited as glycogen. (ii) Animal glycogen is used as food.

4.5 Glycolysis

The glucose that diffuses into the cells finds its way into the mitochondria and undergoes anaerobic reactions. The anaerobic reactions occur in the absence of oxygen and these changes constitute glycolysis. During glycolysis, glucose is spilt into two molecules of pyruvic acid. In muscles, glycolysis results in lactic acid. In yeast and anaerobic bacteria, glycolysis results in the formation of ethyl alcohol and carbon dioxide.

4.5.1 Glycolysis and its reversal

Glycolysis is defined as the sequence of reactions converting glucose into pyruvate or lactate with the production of ATP. It is also known as Embden - Meyerhof pathway.

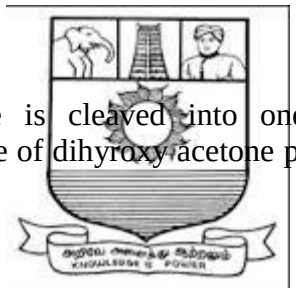
Reactions of glycolysis :

1. Glucose is converted into glucose-6-phosphate by enzyme hexokinase or glucokinase in the presence of ATP and Mg^{2+} ions. It is an irreversible step.

2. Glucose-6-phosphate undergoes isomerization to give fructose-6-phosphate in the presence of enzyme phosphohexose isomerase and Mg^{2+}. It is a reversible step.

3. Fructose-6-phophate is phosphorylated to fructose 1,6-biphosphate by phosphofructkinase (PFK). This is the key enzyme in the control of glycolysis. This is an irreversible step. This enzyme is inhibited by citrate.

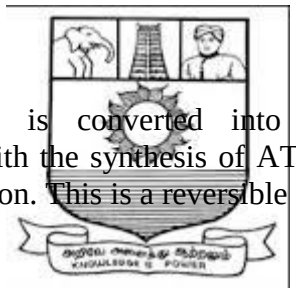
4. Fructose 1, 6-biphosphate is cleaved into one molecule of glyceraldehyde-3-phosphate and one molecule of dihyroxy acetone phosphate by enzyme aldolase. This is a reversible step.



5. Dihydroxy acetone phosphate is isomerized into glyceraldehyde-3-phosphate by enzyme triose phosphate isomerase. This is a reversible reaction. Thus from one molecule of glucose, 2 molecules of glyceraldehyde-3-phosphate are obtained. This enzyme is inhibited by bromohydroxy acetone phosphate.

6. Glyceraldehyde-3-phosphate is converted into 1,3-biphosphoglycerate (1,3 - BPG) by enzyme glyceraldehyde-3-phosphate dehydrogenase in the presence of NAD^+. This is a reversible reaction. This reaction involves both oxidation and phosphorylation. This enzyme is inhibited by iodoacetate and arsenite.

7. 1,3- biphospho glycerate is converted into 3-phosphoglycerate by enzyme phosphoglycerate kinase with the synthesis of ATP. This reaction is an example for substrate level phophorylation. This is a reversible reaction.



8. 3-phosphoglycerate is converted into 2-phosphoglycerate by enzyme phosphoglyceromutase. It is reversible reaction.

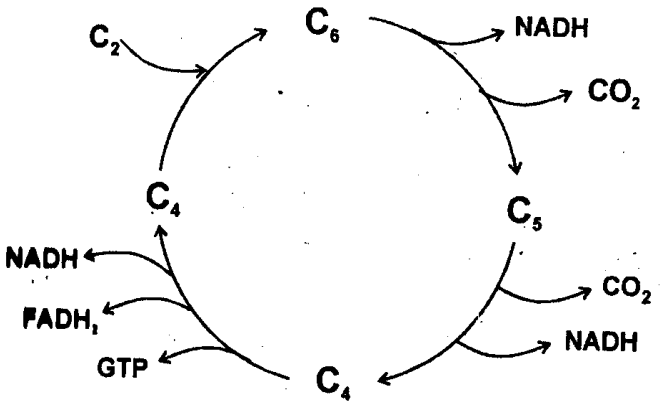
9. 2-phosphoglycerate is converted into a high energy compound phosphoenol pyruvate by enzyme enolase. This enzyme is inhibited fluoride ions.

10. Phosphoenolpyruvate is converted into pyruvate by enzyme pyruvate kinase with the synthesis of ATP. This reaction is another example for substrate level phosphorylation. This is an irreversible reaction.

4.5.1 TCA cycle

Acetyl CoA condenses with oxaloacetable to form citric acid. By a series of cyclic reaction the acetyl unit is completely oxidised to CO_2 and generates again oxaloacetate. The entire cycle is known as TCA cycle (or) citric acid cycle (or) Krebs cycle.

The overall pattern of the TCA cycle can be given as follows:



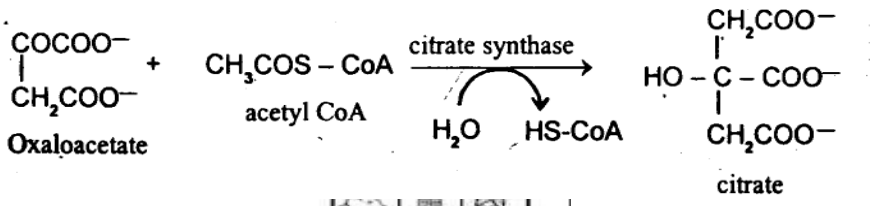
C_2 - acetyl CoA
C_6 - citric acid
C_5 - α-ketoglutarte
C_4 - oxalo acetate

Significance of TCA cycle :

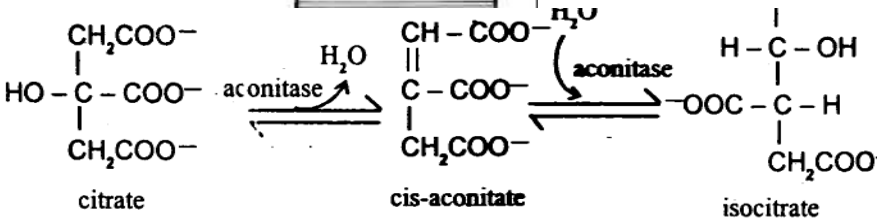
1. TCA cycle is the final common pathway for the oxidation of the fuel molecules such as amino acids, fatty acids and carbonhydrates.
2. The cycle also provides intermediates for biosynthesis of proteins and nucleic acids.
3. In eukaryotes the reaction of TCA cycle occur in mitochondria.
4. Components of TCA cycle control the key enzymes of othe pathways.
5. It is a source for reduced coenzymes.

Reaction of TCA cycle :

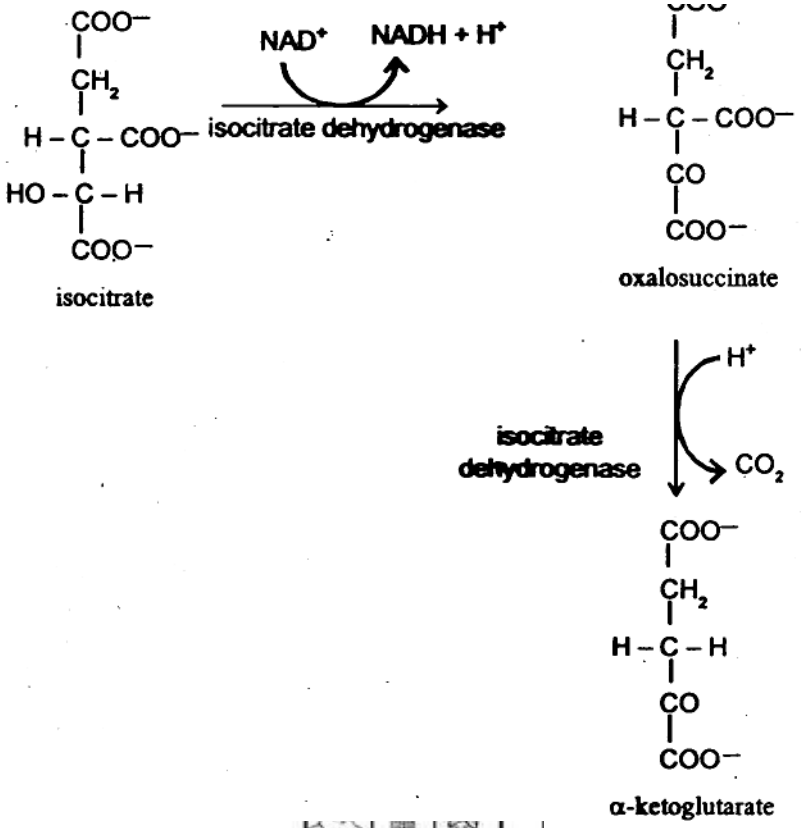
1. Oxaloacetate reacts with acetyl CoA and H_2O to yield citrate and CoA. This reaction which is an aldol condensation followed by a hydrolysis is catalysed by citrate synthase.



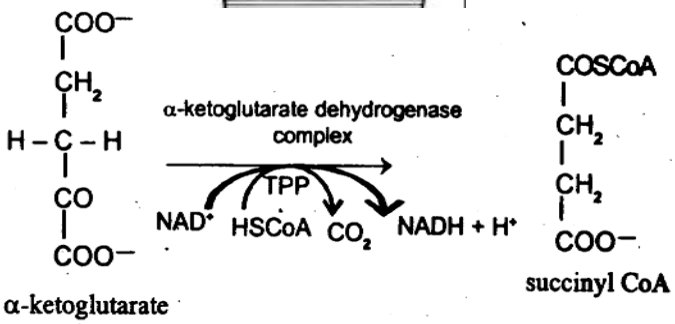
2. Citrate is isomerized into isocitrate by enzyme aconitase. The isomerization is accomplished by a dehydration followed by a hydration step.



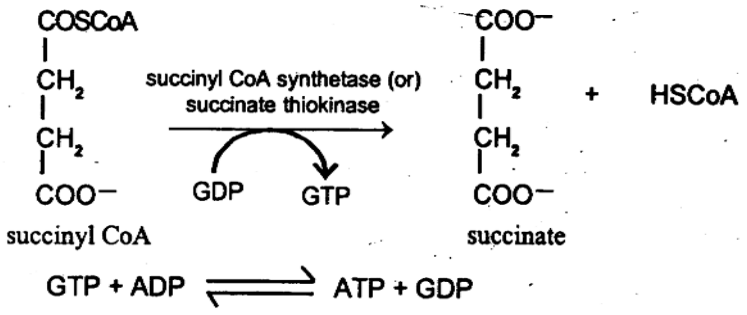
3. Isocitrate undergoes oxidative decarboxylation to give α-ketoglutarte by enzyme isocitrate dehydrogenase.



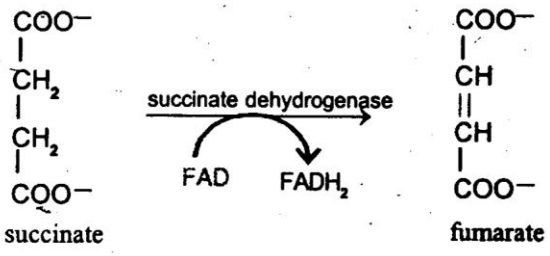
4. α-ketoglutarte undergoes oxidative decarboxylation to give succinyl CoA by enzyme α-ketoglutarte dehydeogenase complex.



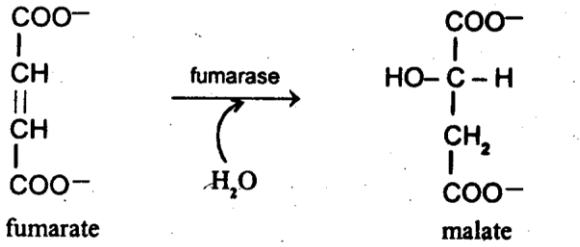
5. Succinyl CoA is converted to succinate by succinyl CoA synthetase (succinate thiokinase). This reaction is coupled with phosphorylation of GDP to GTP. This is substrate level phosphorylation.



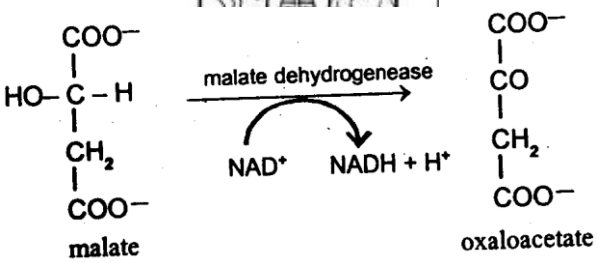
6. Succinate is oxidised to fumarate by enzyme succinate dehydrogenase.



7. Fumarate is converted to malate by enzyme fumarase.

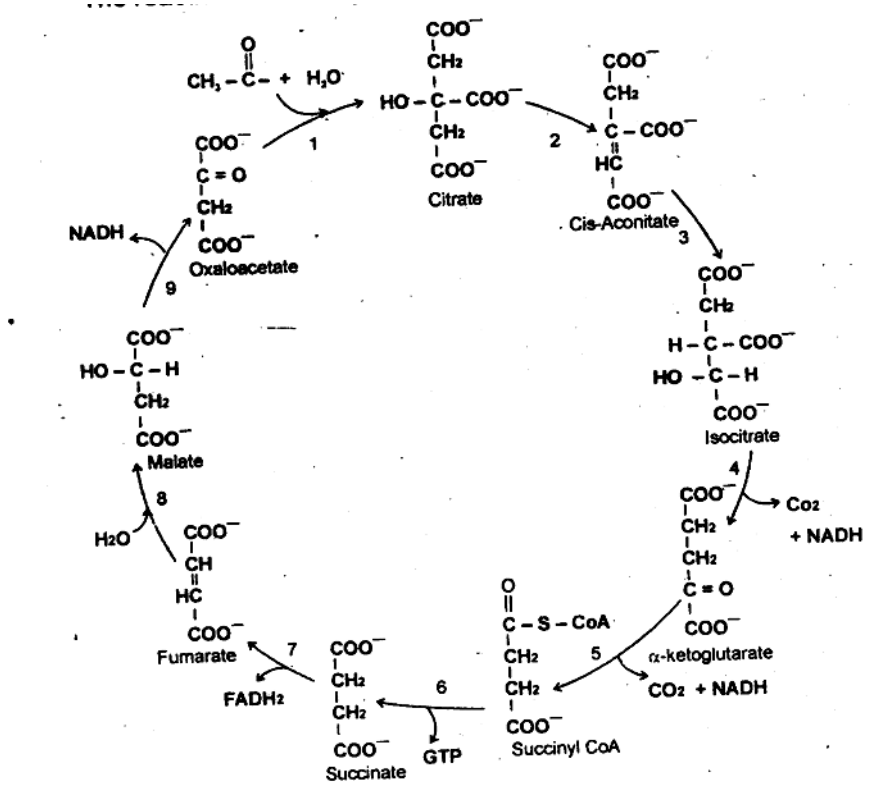


8. Malate is oxidised to oxaloacetate by enzyme malate dehydrogenease.



The net reaction of the citric acid cycle is

The reactions of TCA cycle may be summarised as follows :



(1) Citrate synthase
(2) Aconitase
(3) Aconitase
(4) Isocitrate dehydrogenase
(5) α-ketoglutarate dehydrogenase complex.
(6) Succinyl CoA synthetase
(7) Succinate dehydrogenase
(8) Fumarase
(9) Malate dehydrogenase

4.5.2 Relation between glycolysis and respiration

1. Glycolysis takes place in all cells of the body. The enzymes of this pathway are present in the cytoplasm of the cell.
2. Glycolysis can operate under aerobic or anaerobic conditions. Lactate is the end product under anaerobic condition, pyruvate is the end product under aerobic condition.
3. It is an emergency energy yielding patyway in the absence of O_2.
4. Glycolysis is the only source of energy for cells lacking mitochondria such as erythocytes.
5. This pathway is considered as the preliminary step before complete oxidation.
6. Glycolysis is very essential for brain which is dependent on glucose for energy.
7. Intermediates formed in glycolysis are useful for the synthesis of non -essential amino acids and glycerol.
8. Most of the reactions in this pathway are reversible, which are also used for gluconeogenesis.

4.6 Principle of Bioenergetics

Bioenergetics is a field in bio chemistry that concerns energy flow through living systems. This is an active area of biological research that includes the study of the transformation of energy in living organisms and the study of thousands of different cellular process such as cellular respiration and the many other metabolic process that can lead to production and utilization of energy in forms such as ATP molecules. Utilization of chemical energy from such molecular bond rearrangement powers biological process in very biological organism.

Types of reactions

1. Exergonic is a spontaneous reaction that releases energy. It is thermodynamically favoured. On the course of reaction, energy needs to be pur in this activation energy drives the reactants from a stable state to a highly energetic unstable configuration. These reactants are usually complex molecules that are broken in to simples products. The entire reaction is negative and equal to -ΔG because energy is lost from the bonds formed by the products.

2. Endergonic is an anabolic reaction that consumes energy. It has a positive ΔG because energy is required to break bonds. The free energy (ΔG) gained or lost in a reaction can be calculated ΔG = AH - TAS where G - Gibbs free energy H - enthalpy T-Temperature and S - entropy.

4.7 Electron transport chain

The oxidation of reduced substrate is done by a number of electron acceptors constituting the electron transport system. The different components of the system are nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), and the cytochromes. When a reduced substrate loses hydrogen, it breaks up into proton and electron. NAD and FAD accept both these components of hydrogen molecule, while the cytochromes allow only the electrons to pass through and the protons are liberated in the substrate. Oxygen forms the terminal constitutent of the electron transport system; it is the ultimate recipient of electrons and picks up the hydrogen proton from the substrate, and water is formed. Electron transport system plays a significant role in the energetics of cellular respiration. During this process, energy is dissipated, which when sufficient for the synthesis of ATP, brings about oxidative phosphorylation. Mitochondria contain most of the hydrogen and electron carries. The most accepted sequence of electron carriers in the mitochondria is shown in figure.

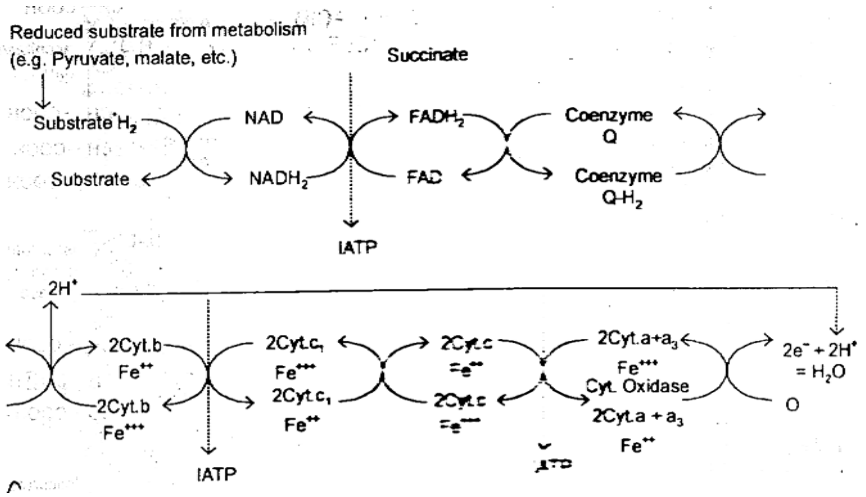


Figure : Transport of hydrogen and electron through different carriers of electron transport chain

The electron is initially accepted by NAD and gets reduced to NADH. This reduced NADH is re-oxidised by FAD, which accept the electron from NADH and gets reduced to FADH. Flavoproteins may also be directly reduced by the substrate without involvement of pyridine system. For example, succinic dehydrogenase, which is a flavoprotein bearing FAD as its prosthetic group, is reduced directly by succinic acid without involving pyridine nucleotides.

4.8 Oxidative phosphorylation

As a result of biological oxidations, the energy initially existing in the chemical bonds of various metabolites is released as free energy, a substantial portion of which is trapped under suitable conditions in the form of high-energy phosphate bond in ATP. This process is known as oxidative phosphorylation. In some of the biological oxidations some other high-energy phosphate bond compound is formed in the begining, which is later capable of transfering its bond-energy together with the phosphate group to ADP forming ATP. In some other oxidations, the energy is not trapped at all and it is lost as heat energy. A major portion of biochemically useful energy, contained in the ATP molecules, is mainly derived from electron transport chain oxidation.

In the mitochondria, the energy liberated is immediately trapped in the presence of adenosine diphosphate (ADP) and inorganic phosphate (Pi) resulting in the formation of adenosine triphosphate (ATP). Formation of ATP is thus an endergonic reaction, the energy for which is supplied by the electron transport chain oxidation. Formation of one mole of ATP from ADP requirese approximately 8 Kcals energy under physiological conditions. Obviously, ATP formation is not possible at the sites where energy release is lesser than this value. Under physiological chain and these sites are

i) between NAD and flavins,
ii) between cytochrome b and cytochrome c_1 and
iii) between cytochrome c and cytochrome a_3.

Substrates involving the entire respiratory chain for oxidation can form three moles of ATP per mole of hydrogen removed. Most of the biological oxidations involve the entire

respiratory chain. Succinate requries FAD as inital oxidising agent; hence, during oxidation of succinate by the respiratory chain, the first site of phosphorylation is bypassed, and hence, only two moles of ATP are produced per mole of succinate oxidised.

4.9 Gluconeogenesis

The process of synthesis of glucose from non-carbohydrate sources is known as **gluconeogenesis**. This process mostly occurs in the liver and kidney tissues at a basal rate but becomes very active when diet is not able to meet the carbohydrate requirement of the body at the desired rate. This process is particularly requirement of the body at the desired rate. This process is particularly required for most tissues which are exclusively dependent on glucose for their energy supply, e.g. central nervous system, RBC and adrenal medulla. The daily requirement of CNS and RBC for glucose is about 1 40g and 30g respectively.

The most common gluconeogenic substances are lactic acid and glycerol. Besides these, other substances such as propionic acid, certain amino acids such as glutamic acid, glycine, serine, aspartic acid, α-keto acids such as pyruvic acid, α-ketoglutaric acid and oxaloacetic acid are also converted into glucose. These substances at some stage of their metabolism are linked with glycolytic or citric acid cycle reactions and by reversal of these reactions, these can be ultimately converted into glucose or glycogen. In the normal course, when the demand of sugar is fulfilled, the rate of gluconeogenesis is decreased. Glucocorticoids, glucagons and catecholamines increase gluconeogenesis, whereas insulin suppresses it.

The process of gluconeogenesis plays various important roles in the body.

i. It helps in the regulation of the blood sugar level at times when dietary carbohydrates are not able to meet body carbohydrate requirement fully. By reguating blood sugar level, it also regulates the minimum required level of glycogen in the liver and muscle tissues. Also, it protects delicate organs like the brain against the harmful effects that might occur due to hypoglycemia.
ii. Gluconeogenesis brings about proper disposal of lactic acid produced by the muscles during and after exercise and glycerol produced in the adipose tissue due to turnover of the fats and prevents their wastage.

The linking site of various gluconeogenic substances in glycolysis and citric acid cycle are reversible, hence at the time of emergency, biosynthesis of fats and proteins from carbohydrates or vice versa is possible through these sites. Thus, a dynamic equilibrium is established among carbohydrates, fats and proteins.

4.10. Pentose phosphate pathway

The alternate aerobic pathway, also known as hexose monophosphate shunt, Warburg-Dickens-Lipmann pathway or pentose phosphate pathway is a cyclic mechanism analogous to tricarboxylic acid cycle in which O_2 is utilised in the early part of the reactions. Energetically also, this pathway is considered more efficient because, it yields more than 30 molecules of ATP for each molecule of glucose oxidised. It has been postulated that this could be a major pathway for utilisation of glucose in the cornea, lens of the eye, lac tating mammary gland, adipose tissue, liver and foetal heart.

The sequence of reactions begins with glucose-6-phposphate, which follows an areobic pathway, instead of the usual glycolytic pathway (Figure 4.5). The reactions of the pathway cab be divided into two broad phases. (i) Conversion of hexose to pentose (ii) Conversion of pentose to hexose.

Conversion of hexose to pentose

i. Glucose -6- phosphate is oxidised to 6-phosphogluconic acid by glucose – 6-phosphodehydrogenase and coenzyme NADP.

$$Glucose-6-p+NADP \xrightarrow[dehydrogenase]{Glucose-6-phospho} 6-Phosphogluconic\ acid+NADPH+H^{+}$$

ii. 6-Phosphogluconic acid is then oxidised by 6-phodphogluconate dehydrogenase and NADP and subsequently decarboxylated to form ribulose -5-phosphate.

$$6-Phosphogluconic\ acid+NADP \xrightarrow[dehydrogenase]{Phosphogluconate} Ribulose-5-phosphate+NADPH+H^{+}$$

Ribulose -5-Phosphate is coverted to xylulose-5-phosphate by epimerase

$$Ribulose-5-phosphate \xleftrightarrow[Isomerase]{Phosphoribo} Ribulose-5-phosphate$$

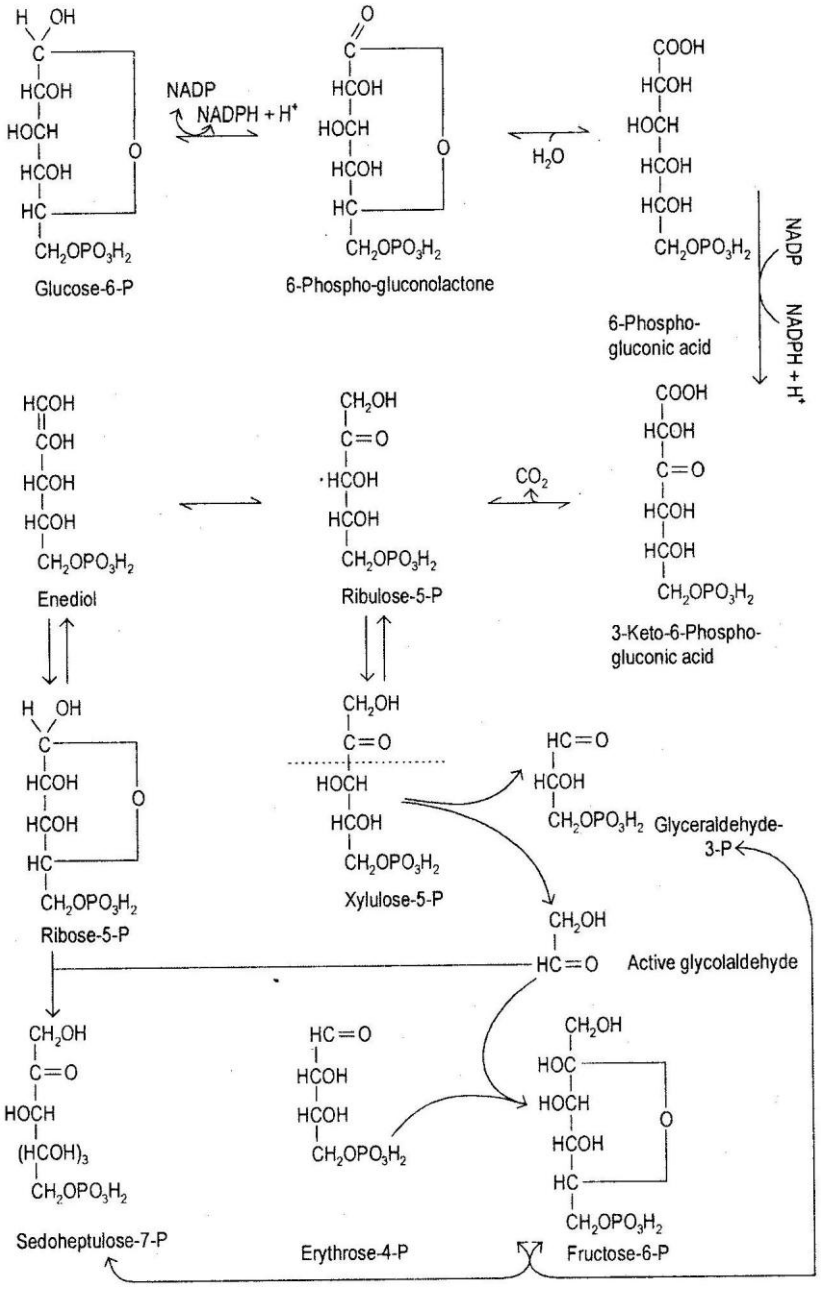
iii. Ribulose-5-phosphate is converted to xylulose -5- phosphate by epimerase.

$$Ribulose-5-phosphate \xrightarrow{Epimerase} Xylulose-5-phosphate$$

Conversion of pentose to hexose

iv. Xylulose-5-phosphate undergoes transketolation to form glyceraldehyde-3-phosphate. During this reaction a ketol group (active glycolaldehyde) is removed from xylulose-5-phosphate by enzyme transketolase. TPP and Mg^{++}ion act as confactors.

$$Xylulose-5-phosphate \xrightarrow[TPP,Mg^{++}]{Transketolase} Glyceraldehyde-3-phosphate+active\ glycolaidehyde$$



v. The ketol fragment removed from xylulose-5-phosphate condenses with ribose-5-phosphate to form a seven carbon sugar sedoheptulose, by the action of a transketolase.

$$Ketol + Ribose-5-phosphate \xrightarrow{Transketolase} Sedoheptulose$$

vi. Finally, a transaldolase transfers the dihudroxy-acetone moiety of sedoheptulose to glyceraldehyde-3-phosphate to form the hexose, fructose-6-phosphate leaving a tetrose residue, erythrose-4-phosphate. Fructose-6-phosphate can be readily converted to glucose-6-phosphate.

$$Giyceraidehyde-3-phosphate+Sedoheptulose \rightarrow Fructose-6-phosphate+ erythrose-4-phosphate$$

vii. Addition of a ketol fragment to erythrose-4-phosphate by transketolase reaction, will give fructose-6-phosphate again.

$$Ketol+Erythrose-4-phodphate \rightarrow Fructose-6-phosphate$$
$$Fructose-6-phosphate \rightarrow Glucose-6-phosphate$$

The cycle repeats starting with glucose-6-phosphate, which is regenerated at the end of each cycle.

Check Your Progress

1. An example of polyccharide is .....
2. α-D glucose and β -D glucose differ from each other due to difference in .....
3. Inulin is an example of ..... polyccharide
4. Brain gets energy from glucose through .....
5. Acetyl coenzyme is completely oxidised to carbon dioxide by a series of reaction called ..... cycle.

4.11 Sum up

Carbohydrates are widely distributed in plants and animals. Cellulose of wood and paper, starch present in cereals, cane sugar and milk sugar are all examples of carbohydrates. Carbohydrates contain carbon, hydrogen and oxygen. They usually contain an aldehyde (-CHO) or keto (-CO-) group along with a number of hydroxyl groups. Carbohydrates supply the major postion of energy required by living cells. carbohydrate metabolism act as catalysts to promote oxidation of food stuffs.

4.12 Key words:

Glycolysis : The catabolic pathway by which a molecule of glucose is broken down into two molecules of pyruvate.

Citric acid cycle : A cyclic enzymatic reaction for the oxidation of acetyl residues to carbon dioxide, in which formation of citrate is the first step also known as the krebs cycle or tricarboxylic acid cycle

Oxidative phosphorylation: The enzymatic phosphorylation of ADP to ATP coupled to electron transfer from a substrate to molecular oxygen.

Pentose phosphate pathway : A pathway that serves to interconvert hexoses and pentose and is a sources of reducing equivalents and pentose for biosynthetic process present is most organims. Also called the phorphogluconate pathway.

Electron transport : Movement of electrone from substrates to oxygen promoted by the respiratory chain

4.13 Questions for Discussion

1. Discuss the structure of Glucose
2. What is Glycolysis. Discuss the different reactions of glycolysis.
3. Discuss in detail the reaction of TCA cycle.
4. Give the principles of bioenergetics
5. What is oxidative phosphorylation.
6. Write note on glyconeogenesis
7. Explain pentose phosphate pathway.

Check Your Progress : Model answer

1. Starch
2. Configuration
3. Homo
4. Glycolysis
5. TCA

4.14 Suggested Readings

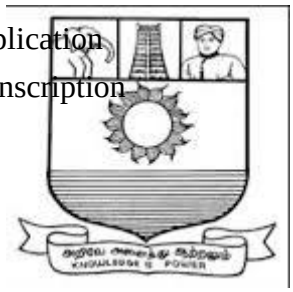
[1] L. Veerakumari, Biochemistry, MJP publishers, Chennai, 2004.

[2] David Hames and Nigel Hooper, Biochemistry

[3] C.B. Power and G.R. Chatwal, Biochemistry.

CHAPTER V
NUCLEIC ACIDS

CONTENTS



5.0 Aims and objectives

- Explains nucleosides and nucleotides
- Describes the structure of DNA and RNA
- Describes the biosynthesis of DNA and RNA
- Explains DNA sequencing and Recombinant DNA technology
- Explains the genetic code.

5.1 Introduction

Nucleic acids are the principal genetic or hereditary materials of all living organisms. They are usually present in the cell as nucleoproteins (conjugated proteins with a nucleic acid as the prosthetic group). In the eukaryotic cells of plants and animals, nucleic acids are found in the nucleus and hence the name. The prokaryotic cells (cells which do not contain a nucleus) contain them in the cytoplasm. In bacteria, they are solely present in cytoplasm. There are two types of nucleic acids :

i) Ribonucleic acid- RNA
ii) Deoxyribonucleic acid – DNA

Chemical composition

Chemically, nucleic acids are macromolecules (polymers) in which the repeating units (monomers) are nucleotides. Thus, nucleic acids are polynucleotides. The molecular weigths of nucleic acids are in the order 30, 000 to several millions.

Components of Nucleic acids

Nucleic acids are made up of three chemical units:

i) Nitrogenous base
ii) Pentose sugar
iii) Phosphoric acid

5.2 Nitrogenous bases

These are the nitrogen containing heterocyclic bases present in nucleic acids. There are two types of nitrogenous bases is nucleic acids. They are

i) Purines – e.g adenine (A) and guanine (G)
ii) Pyrimidines – e.g cytosine (C), thymine (T) and uracil (U)

All the nucleic acids contain adenine, guanine and cytosine. DNA but not RNA contains thymine also. RNAs but not DNA contain uracil also.

Structure of Nitrogenous bases

2. Pentose sugar

Nucleic acids contain a pentose sugar. DNA contains deoxyribose whereas ribose is the pentose sugar of RNA. The difference between the two sugars is that in deoxyribose there is no –OH group in carbon atom 2. The absence of oxygen as –OH group makes DNA more stable than RNA.

3. Phosphoric acid

Phosphoric acid or phosphate group is the third component of nucleic acids. It is attached to the sugar at carbon atoms 3 or 5 to form 3' – phosphoster and 5' –phosphoester respectively

5.3 Nucleosides

A nucleoside is a chemical unit formed by the combination of a pentose sugar and a nitrogenous base (purine or pyrimidine)

Pentose sugar + Nitrogenous base $\rightarrow$ Nucleoside + H_2O

Thus, a nucleoside is a N-glycoside in which the sugar component is ribose or deoxyribose and the non-sugar component (aglucone) is a purine or pyrimidine base.

There are two types of nuclesides :

i) Ribonucleosides
ii) Deoxyribonucleosides.

1. Ribonucleosides

Nucleosides containing ribose sugar are called ribonucleosides. The base and sugar are connected throug a N-glycosidic bond.

Examples

Adenosine (Adenine + Ribose)

2. Deoxyribonucleosides

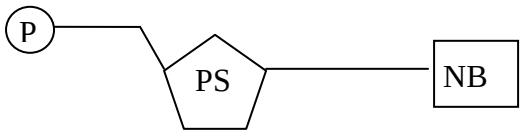
Nucleosides containing deoxyribose sugar are called deoxyribonucleosides. The base and sugar are united through a N-glycosidic bond.

Examples

Deoxyadenosine (Adenine + Deoxyribose)

5.4 Nucleotides

Nucleotides are the building blocks of nucleic acids. These are nothing but phosphoesters of nucleosides (nucleoside monophosphates). Thus, a nucleotide is composed of three units : pentose sugar, nitrogenous base and a phophate group. In each nucleotide, the pentose sugar is attached to a phosphate group at one side and a nitrogenous base at the other side.



PS = Pentose sugar
P = Phosphate group
NB = Nitrogenous base

There are two types of nucleotides,

i) Ribonucleotides
ii) Deoxyribonucleotides

1. Ribonucleotides

A ribonucleotide is formed by the reaction between the ribose sugar portion of a nucleoside and the phosphoric acid. The 3' or 5' OH group of the sugar is phosphorylated to give the corresponding nucleotide.

Examples

Adenosine monophosphate (AMP)
(Adenylic acid)

2. Deoxyribonucleotides

These are deoxyribonucleosides whose 3' or 5' –OH group of the sugar is phosphorylated.

Examples

Deoxyadenosine monophoshate (dAMP)
(Deoxyadenylic acid)

5.5 Nucleic acid
5.5.1 Structure of DNA

1. Primary structure

i) DNA is a polymer of deoxyribonucleotides. Therefore, it is otherwise known as polydeoxyribonucleotide. It contains four deoxyribonucleotides viz.

a) Deoxyadenosine monophosphate
b) Deoxyquanosine monophosphate
c) Deoxycytidine monophosphate
d) Deoxythymidine monophosphate.

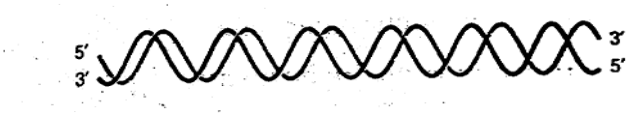
The monocleotides are held together by 3'-5' phosphodiester bonds and the base sequence constitutes the primary structure of the polynucleotide.

ii) The DNA molecule possesses polarity as indicated by the labelled 3' –and 5' – attached phosphates.

2. Secondary structure

(Watson and Crick model)

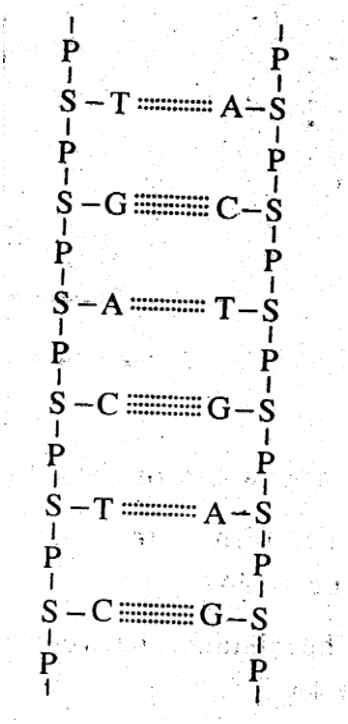
i) A DNA molecule has two long polynucleotide chains called strands.
ii) The two strands are twisted around each other in opposite direction to form a double α-helix.
iii) The two strands are antiparallel to each other i.e one runs from 5' to 3' direction and the other runs from 3' to 5' direction.
iv)



v) The two strands are held together by hydrogen bonds between the bases. A purine of one strand pairs with a pyrimidine of another. For example, adenine (A) of one strand pairs with thymine (T) of another strand.

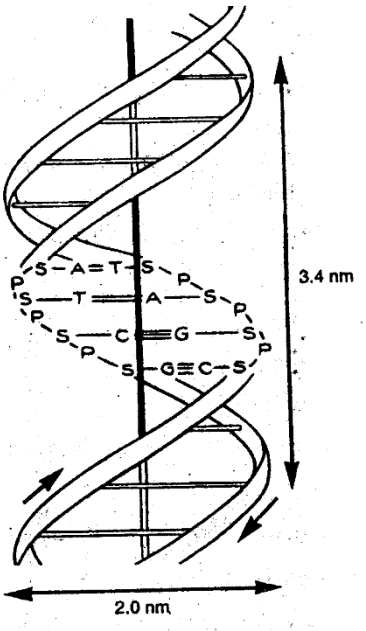
Similarly, guanine (G) pairs only with cytosine (C)

vi) The two strands are complementary to each other i.e the sequence of bases in one strand is determining the sequence of bases in the complementary pair. For example, if the sequence of bases in one strand is TGACTC, then the sequence of bases in the second strand will be ACTGAG.



vii) DNA is a right handed double helix. This means that each strand will appear to follow a clockwise path moving away from a viewer looking down the helical axis.

viii) The structure of DNA is analogous to a coiled ladder. The sugar- Phosphate backbones are at the outer edge and the base pairs in the central core of the molecule.



ix) The width of the double helix is 20A°

x) Each turn of the double helix contains 10 base pairs and is 34A° long.

xi) The distance between two successive nucleotides is 3.4A°.

5.5.2 Functions of DNA

i) DNA is the genetic material capable of storing and transmitting genetic information (heredity character) from parents to their off springs.
ii) All cellular functions are under the control of DNA
iii) DNA acts as a template for the synthesis of mRNA by a process called transcription. The mRNA thus produced helps protein synthesis in the cytoplasm by translation in combination with tRNA

$$DNA \xrightarrow{transcription} mRNA \xrightarrow{translation} Protein$$

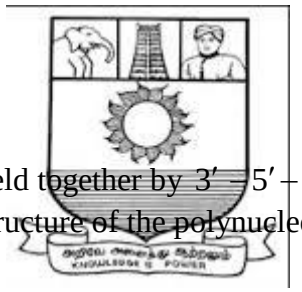
iv) DNA acts as a cofactor in the synthesis of ATP which in turn is necessary for the production of RNA and protein in the nucleus.
v) DNA produces mutations resulting in new characters.

5.5.3 Structure of RNA

1. Primary structure

RNA is a polymer of ribonucleotides. Thus, it is polyribonucleotide. It contains four types of ribonucleotides viz.

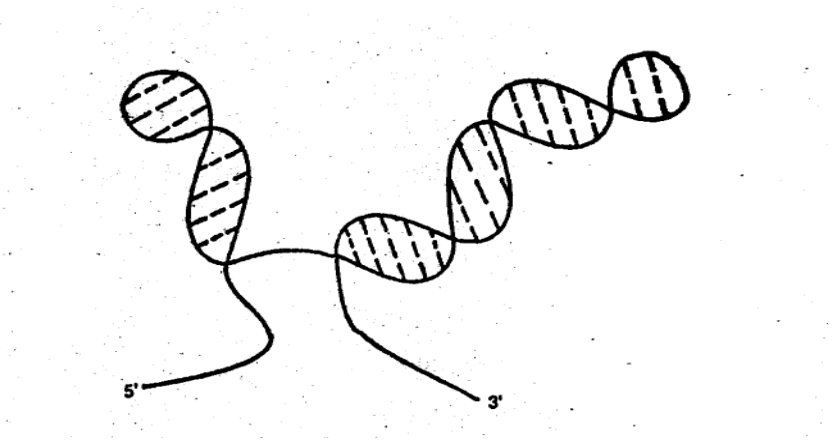
Adenosine monophosphate
Guanosine monophosphate
Uridine monophosphate
Cytidine monophosphate



The mononucleotides are held together by $3' \rightarrow 5'$ – phosphodiester bonds and the base sequence constitutes the primary structure of the polynucleotide.

2. Secondary structure

RNA occurs as a single strand molecule. There is internal hydrogen bonding within the chain to keep it in a coiled or helical form. The coiled strand folds back on itself and thus acquires double-stranded characteristics.



5.6 Difference between DNA and RNA

Sl.No	DNA	RNA
1.	General genetic material present predominantly in nucleus	Genetic material of some viruses present in cytoplasm
2.	The sugar moiety is deoxyribose	The Sugan moiety is ribose
3.	Exists as double strand helix	Exists as single strand helix
4.	Consists of adenine, guanine, cytosine and thymine	Consists of adenine guanine, cytosine and uracil
5.	Base pairing occurs throught the molecule	Base pairing occurs only in the helical region
6.	Contains a few unusual bases	Contains more unusual bases
7.	There is only one type of DNA	There are three types of RNA
8.	Denatured on heating	Stable towards heat
9.	Consists of larger number of nucleotides upto 4.3 million	Consists of a fewer number of nucleotide upto 12,000
10.	Genetic messages are encoded in DNA	RNA transmits messages encoded in DNA into proteins
11.	Does not help enzymatic reactions	Helps some enzymatic reactions

5.7 Classification of RNA

There are three primary types of RNA. The are

i) Ribosomal RNA (rRNA)
ii) Messenger RNA (mRNA)
iii) Transfer RNA (tRNA)

Ribosomal RNA (rRNA)

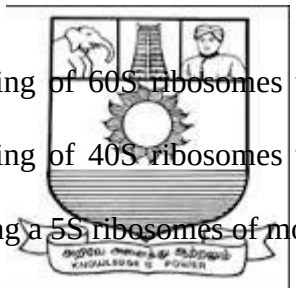
Occurrence

Ribosomal RNA constitutes about 80% of the total RNA of the cell. It occurs as nucleoprotein in the minute round particles called ribosomes located in cytoplasm.

Nature

Ribosomal RNA is a high molecular weight polynucleotide. Depending upon the size of the ribosome moiety (measured in Swedberg unit 'S') and molecular weight, there are types of rRNA in three mammalian cells.

They are



i) High MW rRNA consisting of 60S ribosomes with molecular weight less than a million.
ii) High MW rRNA consisting of 40S ribosomes with molecular weight less than a million.
iii) Low MW rRNA containing a 5S ribosomes of molecular weight 120.

Functions

i) rRNA plays a prominent role in binding m-RNA to ribosomes and its translation to tRNA.
ii) An rRNA component is found to perform the peptidyl transferase activity. Thus it acts as an enzyme called ribozyme.

Messenger RNA (mRNA)

Occurrence

This is so named because it is the type of RNA which carries message for protein synthesis from DNA (gene) to the sites of protein formation (ribosomes). Only about 5% of total cellular content of RNA is mRNA. mRNA is formed from DNA by transcription in the nucleus and then migrated into the cytoplasm and attached itself to a number of ribosomes.

Nature

The messenger RNA is very large in size containing several thousands of nucleotides. It has a molecular mass of about 5,00,000 to 4 million. In mRNA, no base pairing takes place. In fact, base pairing in mRNA destroys its biological activity. There may be 1000 to 10,000 different species of mRNA in a cell. They differ only in the sequence of their bases and in their length.

Function

mRNA serves as a messenger by carrying genetic information from DNA to the sites of protein synthesis (ribosomes).

Transfer RNA (tRNA)

Occurrence

Transfer RNA constitutes about 10-15% of total RNA of the cell

Nature

Transfer RNA is the smallest of the RNA species containing approximately 75 nucleotides with a molecular weight of 25,000. It has a cloverleaf structure with four attachement sites or arms.

Function

The rRNA molecules serve as adapters for the translation of the information in the mRNA into specific amino acids for protein synthesis.

5.8 Biosynthesis of DNA-Replication

According to Watson and Crick, the **hereditary information** is transmitted to the off spring by the phenomenon known as **DNA replication.** It involves the biosynthesis of new molecule of DNA in such a way that can preserve this sequence of base and conserves the information for distribution to off spring cells. waston and Crick speculated that during DNA **replication** that probably occurs during **interphase of mitosis**, two stands uncoil and each strand acts as a **template** for the formation of new complementary strand.

The deoxynucleoside triphosphates that presumably exist in the free state in ghe nucleus are some how attracted to the single-stranded, uncoiling, DNA. In this way, two new strands are formed that are complementary to the two parental strands. The new daughter molecules begin to be formed assume the helical configuration of the parent molecule. Each molecule consists one old parental strand and one newly sythesized strand, this mechanism is termed as **semiconservative.**

5.9 Synthesis of mRNA

Molecules of **mRNA** are synthesized by the **transcription** of **DNA**. A small part of the DNA double helix unwind, and one of the two strands serves as a template for

complementary ribonuleotide to line up. Sugar-phosphate bond fomation occurs in $5' \rightarrow 3'$ sense. This process is catalysed by enzymes called **RNA polymerases**. Therefore, complete synthesis of **mRNA** requries four major ribonucleoside **triphosphates,** as template and **RNA polymerase** (enzyme) :

The completed **mRNA** molecule does not remain in double helix with DNA but separates and migrates from the nucleus. The DNA then returns to its stable double helical structure.

5.10 Genetic code

The genetic code is the rules that specify how the nucleotide sequence of an mRNA is translated into the amino acid sequence of a polypeptide. The nucleotide sequence is read as triplets called codons. The codons UAG, UGA and UAA do not specify amino acids and are called termination codons or stop codons. AUG codes for methionine and also acts as an inititation (Start) codon.

The genetic code is a triplet code

During translation, the sequence of an mRNA molecule is read from its 5' end by ribosomes which then synthesize an appropriate polypeptide. Both in prokaryotes and in eukaryotes, the DNA sequence of a single gene is **colinear** with the amino acid sequence of the polypeptide it encodes. In other words, the nucleotide sequence of the coding DNA strand, 5' to 3', specifies in exactly the same order the amino acid sequence of the encoded polypeptide, N-terminal to C-terminal. The relationship between the nucleotide sequence of the mRNA and the amino acid sequence of the polypeptide is called **the genetic code**. The sequence of the mRNA is read in groups of three nucleotides called codons, with each codon specifying a particular amino acid. However, three codons, UAG, UGA and UAA, do not encode an amino acid. Whenever one of these codons is encountered by a ribosome, it leads to termination of protein synthesis. Therefore these three codons are called **termination codons** or **stop codons**. The codon AUG codes for methionine. Although methionine is found at internal positions in polypeptide chains, all eukaryotic polypeptides also start with methionine and all prokaryotic polypeptides start with a modified methionine (N-formyl methionine; see Topic H2). Therefore the frist AUG codon that is read by the ribosome in an mRNA is called the **initiation codon** or **start codon**.

Since RNA is composed of four types of nucleotides, there are 4^3 = 64 possible codons, that is 64 possible triplets of ucleotides with different sequences. However, only 20 amino acids are commonly found in proteins so that, in most cases, a single amino acid is coded for by several different codons. The genetic code is therefore said to be **degenerate.** In fact, only methionine and tryptophan are represented by a single codon. As a result of the

genetic code's degeneracy, a mutation that changes only a single nucleotide in DNA (point mutation), and hence changes only a single nucleotide in the corresponding mRNA, often has no effect on the amino acid sequence of the encoded polypeptide.

For many years it was thought that the genetic code is 'universal', namely that all living organisms used the same code. Now we know that the genetic code is almost the same in all organisms but there are a few differences. A few examples are given below (N denotes any of the four nucleotides A, G, C or U):

mitochondria	AUA = Met not Ile
mitochondria	UGA = Trp not **Stop**
some animal mitochondria	AGA and AGG = **Stop** not Arg
plant mitochondria	CGG = Trp not Arg
yeast mitochondria	CUN = Thr not Leu

some unicellular organisms are also now known to use a varient genetic code. For example:

some ciliated protozoa	UAA and UAG = Glu not

5.10.1 Mutation

The base sequence of m-RNA is controlled by DNA and pairing of codon and anticodon determines the production of specific protein. The change of even a single base in mRNA or t-RNA results to a permanent change of structure of protein. This phenomenon is known as mutation.

5.10.2 Mutants

In biology and especially genetics, a mutant is an organism or a new genetic character arising from an instance of mutation, which is a base pair sequence change within the DNA of a gene or chromosome of an organism. The blue lobster is an example of a mutant.

5.11 DNA repair

DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. In human cells both normal metabolic activities and environmental factors such as uv light and radiation can cause DNA damage resulting in as many as 1 million individual molecular lesions per cell per day. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell ability to transcribe the gene that the affected DNA encodes. DNA repair process is constantly active as it reponds to damage in the DNA structure.

5.12 Biosynthesis of proteins

Proteins in the body are constantly broken down and synthesised. Synthesis of plasma proteins, tissue proteins, enzymes and hormones takes place constantly. The sites of protein synthesis within the cells are ribosomes which are present in the cytoplasm.

The following steps are involved in the biosynthesis of proteins.

i. Transcription
ii. Attachment of mRNA with 30S ribosomes and formation of polyribosome
iii. Transfer of amino acids to the site of protein synthesis .
   a). Activation of amino aicds
   b). Attachment of activated amino acid to tRNA
iv. Initiation of protein synthesis
v. Elongation of polypeptide chain
vi. Termination and release of polypeptide chain
vii. Modification of released polypeptide

5.13 DNA sequencing

DNA can be sequenced by the chemical method or the chain termination procedure. the latter is now the standard method; the (single-stranded DNA to be sequenced serves as the template for the synthesis of a complementary strand when supplied with a specific primer and E. coli DNA polymerase I.

Four incubation mixtures are set up, each containing the DNA template, a specific DNA primer, E.coli DNA polymerase I and all four deoxyribonucleoside triphosphates (dNTPs). In addition, each mixture contains a different dideoxynucleoside triphosphate analog, ddATP, ddCTP, ddGTP or ddTTP. Incorporation of a dideoxy analong prevents further elongation and so produces a chain termination extension product. The products are electrophoresed on a polyacrylamide gel and the DNA sequence is read from the band pattern produced.

Automated DNA sequencing

Automated DNA sequencing uses the chain termination method but with an oligonucleotide primer labeled with a fluorescent dye. Each of the four reactions receives a primer labeled with a different dye. After incubation, the reaction mixtures are pooled and electrophoresed on one lane of a polyacrylamide gel. The order in which the different fluorescently labeled termination products elute from the gel gives the DNA sequence. More advanced systems use multiple capillary sets in which sample preparation, loading and data analysis are automated for maximum throughput.

5.13.1 PCR

The polymerase chain reaction (PCR) allows an extremely large number of copies to be synthesized of any given DNA sequence provided that two oligonucleotide primers are available that hybridize to the flanking sequences on the complementary DNA strands. The reaction requires the target DNA, the two primers, all four deoxyribonuleosides triphosphates and a thermostable DNA polymerase such as Taq DNA polymerase. A PCR cycle consists of three steps; denaturation, primer annealing and elongation. This cycle is repeated for a set number of times depending on the degree of amplification required.

Principles of PCR

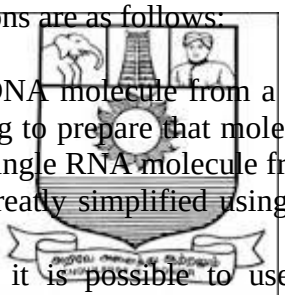
- **Denaturation.** The reaction mixture is heated to 95°C for a short time period (about 15-30 sec) to denature the target DNA into single strands that can act as templates for DNA synthesis.

- Primer annealing. The mixture is rapidly cooled to a defined temperature which allows the two primers to bind to the sequences on each of the two strands flanking the target DNA. This **annealing temperature** is calculated carefully to ensure that the primers bind only to the desired DNA sequences. One primer binds to each strand. The two parental strands do not reanneal with each other because the primers are in large excess over parental DNA.
- **Elongation.** The temperature of the mixture is raised to 72°C (usually) and kept at this temperature for a pre-set period of time to allow DNA polymerase to elongate each primer by copying the single-stranded template strands have been made partially double stranded. The new strand of each double-stranded DNA extends for a variables distance downstream.

The three steps of the PCR cycle are repeated. Thus in the second cylce, the four strands denature, bind primers and are extended. No other reactants need to be added. The three steps are repeated once more for a third cycle and so no for a set number of additional cycles. By the third cycle, some of the PCR products represent DNA sequence only between the two primer sites and the sequence does not extend beyond these sites.

Applications of PCR

PCR already has very widespread applications, and new uses are being devised on a regular basis. Some of the applications are as follows:



- PCR can amplify a single DNA molecule from a complex mixture, largely avoiding the need to use DNA cloning to prepare that molecule. Variants of the technique can similary amplify a specific single RNA molecule from a complex mixture.
- DNA sequecing has been greatly simplified using PCR, and this application is now common.
- By using suitable primers, it is possible to use PCR to create point mutations, deletions and insertions of target DNA which greatly facilitates the analysis of gene expression and function.
- PCR is exquisitely sensitive and can amplify vanishingly small amounts of DNA. Thus, using appropriate primers, very small amounts of specified bacteria and viruses can be detected in tissues, making PCR invaluable for medical diagnosis.
- PCR is now invaluable for characterzing medically important DNA samples. Because of its extreme sensitivity, PCR is now fundamentally important to forensic medicine. It is even possible to use PCR to amplify the DNA from a single human hair or a microscopic drop of blood left at the scene of a crime to allow detailed characterization.

5.13.2 Recombinant DNA technology

Recombinant DNA (r DNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources creating sequences that would not otherwise be found in the genome. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure. They differ only in the nucleotide sequence with on that identical overall structure.

5.13.3 DNA Polymorphism

DNA polymorphism is any difference in the nucleotide sequence between individuals. These differences can be single base pair changes, deletions, insertions, or even changes in the number of copies of a given DNA sequence.

Check Your Progress

1. Purines and pyrimidines are the ..... bases present in nucleic acids
2. The conjugated proteins which determine heredity is .....
3. Two strands of DNA are held together by ..... between purines and pyrimidines
4. DNA acts as a template for the synthesis of mRNA by a process called .....
5. ..... is a chemical unit formed by the combination of a pentose sugar and a nitrogenous base]

5.14 Sum up

Nucleic acids are biopolymers of high moleculars weight with mononucleotides as their repeating units. The nucleic acid contains carbon, hydrogen, oxygen, nitrogen and phophorus. There are two kinds of nucleic acids deoxysibonucleic acid (DNA) and ribonucleic acid (RNA). DNA is found mainly in the chromatin of the cell nucleus where as most of the RNA is present in the cell cytoplasm and a little in the nucleolus. Nucleic acids play a vital role in the biosynthesis of cellular proteins.

5.15 Key words

RNA **:** A polyribonucleotide of a specific sequence linked by successive 3,5. Phosphodiester bond.

DNA : A polynucleotide having a specific sequence of deoxyrionucleotide units covalently joined through 3' - 5' - phosphodiester bonds; serves as the carrier of genetic information.

Nucleoside : A compound consisting of a purine or pyrimidine base covalently linked to a pentose.

Nucleotide : A nucleoside phosphorylated at one of its pentose hydroxyl groups.

Transcription: The enzymatic process whereby the genetic information contained in one strand of DNA is used to specify a complementary sequence of bases in an mRNA chain.

5.16 Question for Discussion

1. What are nucleosides and nucleotides?
2. Explain the structure of DNA
3. Give the different functions of DNA
4. Explain the structure of RNA
5. Discuss the biosynthesis of DNA

6. Write note on Genetic code
7. Define mutation and mutants.
8. What is DNA polymorphism
9. Write note on DNA repair
10. Explain DNA sequencing.

Check Your Progress : Model answers

1. Heterocyclic
2. Nucleoproteins
3. Hydrogen bond
4. Transcription
5. Nucleoside

5.17 Suggestion Readings

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