Biyani's Think Tank

Concept based notes

Molecular Biophysics

[B.Sc. Biotechnology Part-I]

Pragya Dhakar

Lecturer

Deptt. of Science

Biyani Girls College, Jaipur

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Biyani Group of Colleges

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Ph : 0141-2338371, 2338591-95 • Fax : 0141-2338007

E-mail : acad@biyanicolleges.org

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

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Smita Singh

Syllabus

Section A

Introduction: Levels of molecular organization. Amino Acids, Electrolytes, Composition of primary structures of proteins, Nucleic acids.

Structure of Carbohydrates, Lipids, cofactors, vitamins and hormones.

Section B

Ramachandran or steric contour diagram and potential energy calculation of proteins, hydrogen bonding, hydrophobic interactions. Ionic interactions, disulphide bonds and their role in protein structure.

Secondary structural elements and organization of tertiary structure of proteins, helix-coil transition and zipper model, method for structural elucidation,X-ray crystallography.

General features and thermodynamic aspects of protein folding.

Section-C

General characteristics of nucleic acid structure, backbone rotation angles and steric hindrances, conformational properties of bases, stabilizing order forms, stacking interactions, A,B, and Z type double helices.

rRNA structure, tertiary structure higher organization of DNA, protein nucleic acid interactions membrane potential micelle and bilayer formation, studies of bilayer structure and function, order disorder transitions.

Section D

Interforces transport across membranes (the Nerst Planks approach end rate theory of transport)

Photochemical and photobiological phenomena, mechanism of photosynthesis, vision, absorption and fluorescence.

Section –A

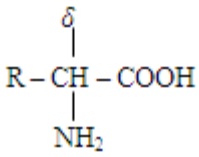
Levels of Organization

Q.1 Write an account on the different types of Amino acids?

Ans. Amino Acids:-

These are nitrogenous, compound with an acidic (carboxyl - COOH) group and a basic (amino – NH_2) group. The number of these groups varies defining the type of Amino Acids, this variation occurs at R group.

General structure of amino acids: -



In this structure R stands for the side chains that vary depending upon the type of amino acids. The α carbon is the carbon adjacent to the functional group.

As Amino acids show sterol chemical Properties this central α –Carbon is asymmetric is all amino acids except classified depending on different types of factors.

There are 3 main systems that classify amino acids.

A. On the basis of the composition of the side chain or R Group.

B. On the basis of the number of amino acid and carboxylic groups.

C. On the basis of polarity of the side chain or R-group

Classification of Amino Acids

(A) On the basis of R-Group (8Types)

1. Simple Amino Acids

(Functional group in-pt in R group)

e.g. – Glycine, alanine

2. Hydroxy Amino Acids

(OH gp. present in side chain

e.g. serine threonine

3. Sulfur – Containing Amino Acids

(Satomin inside chain

e.g. cyteine and threonine)

4. Acidic Amino Acid

(B) On the basis of no. of amino acids (3 Types)

1. Mono amino- Mono-carboxylic Amino Acids (7types)

2. Monoamino – *di* –carboxylic, amino acid

e.g. aspartic Acid, & Glutamic Acid

3. Diamino – Mono-carboxylic Amino Acids

e.g. lysine, Arginine

(C) Based on polarity of side chain (4 Types)

1. Amino Acids with non-polar R Groups e.g. Alanine, Valine, Leucine

2. Amino acids with polar but uncharged R Groups

e.g. Glycine

3. Amino Acids with negative charge e.g. (Aspartic Acid and Glutamic Acid)

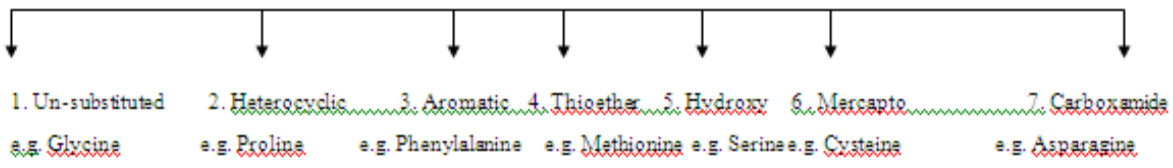
4. With positive the charge
e.g. Lysine & Arginine

e.g. cytaine and threonine)

4. With positive the charge
e.g. Lysine & Arginine

4. Acidic Amino Acid
   (with COOH gp. e.g. aspartic
   Acid glutamic acid)
5. Amino Acid Amides
   Derivatives of acidic amino acids
   Acids in which one- COOH
   has been transformed
   To (-Co NH2)
   e.g. Asparagine and Glutamine
6. Basic Amino Acids
   (amino gp. present)
   In the side chain)
   e.g. Lysine and Arginine
7. Heterocyclic Amino Acids
   (Containing ring with one 1 atom
   other than carbon)
   e.g. Tryptophan, Histidine, etc.
8. Aromatic Amino Acids
   (Benzene ring present in the side
   Chain e.g. phenylalanine and tyrosine)

Chain e.g. phenylalanine and tyrosine)



Q. 2 Determine the Composition of Primary structure of proteins?

Ans. **Proteins:**

Proteins are the polymers of amino acids joined mainly by peptide bonds. They have been stabilized by different types of bonding like covalent, hydrogen, disulfide upon the type of conformation that a protein attains which may be:

A. 1^0 or Primary structure

B. 2^0 or Secondary structure

C. 3^0 or tertiary

D. 4^0 Quaternary

Primary Structure :

It is the Amino acid sequence of its polypeptide chain and it is stabilized by disulfide linkage and covalent bonding.

-Lys-Ala-His-Gly-Lys-Val-Lev-Gly-Ala-

Primary structure

(Amino acid sequence is a Polypeptide chain)

e.g. of Proteins sequenced –(a) insulin by Frederick Sanger – 1953

(b) β- Galactosidase in 1978

Procedure for determining Primary structure of Proteins:-

A. Prepare the protein for sequencing

B. Sequence the polypeptide chain

C. Organize the completed structure

(A) Prepare the Protein for sequencing : 4 Sub steps

(a) Determination of type of subunits → (i) C-terminal (End Group Analysis)

(b) → (ii) N- Terminal

(i) C – terminal Identification s → Chemical (Hydrozinolysis)

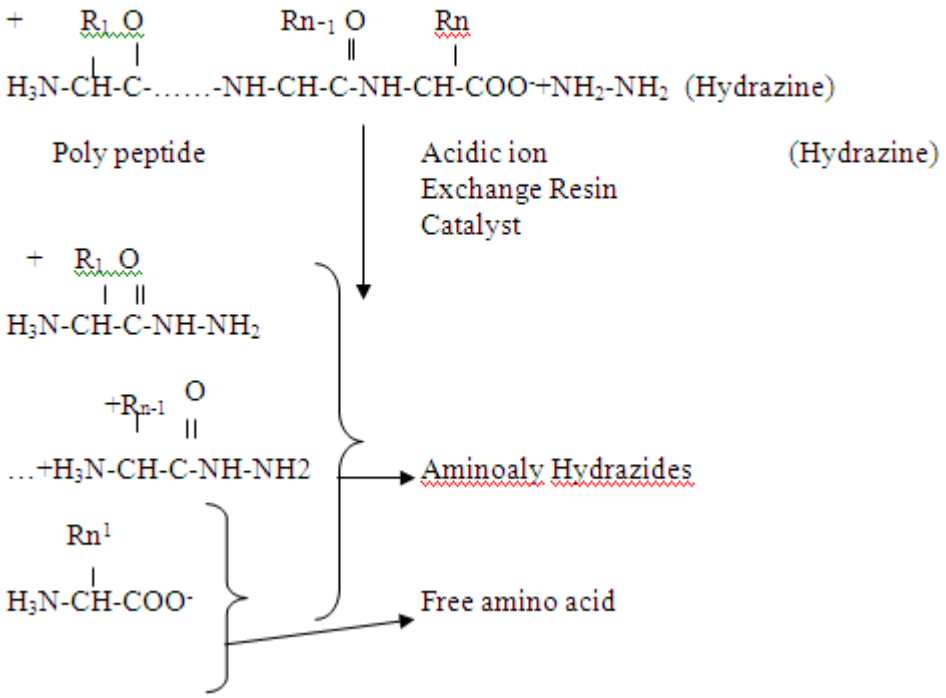
→ Enzymatic (Exopeptides)

(e.g. carboxy Peptidases)

(ii) N – terminal Identification → Chemical (Edman Degradation)

→ Enzymatic (Amino Peptidases)

(i) C-terminal identification by hydrazinolysis



II. **Enzymatic Method**

With the help of enzymes like exopeptidases like carboxy-peptidases that cleaves on external sites only.

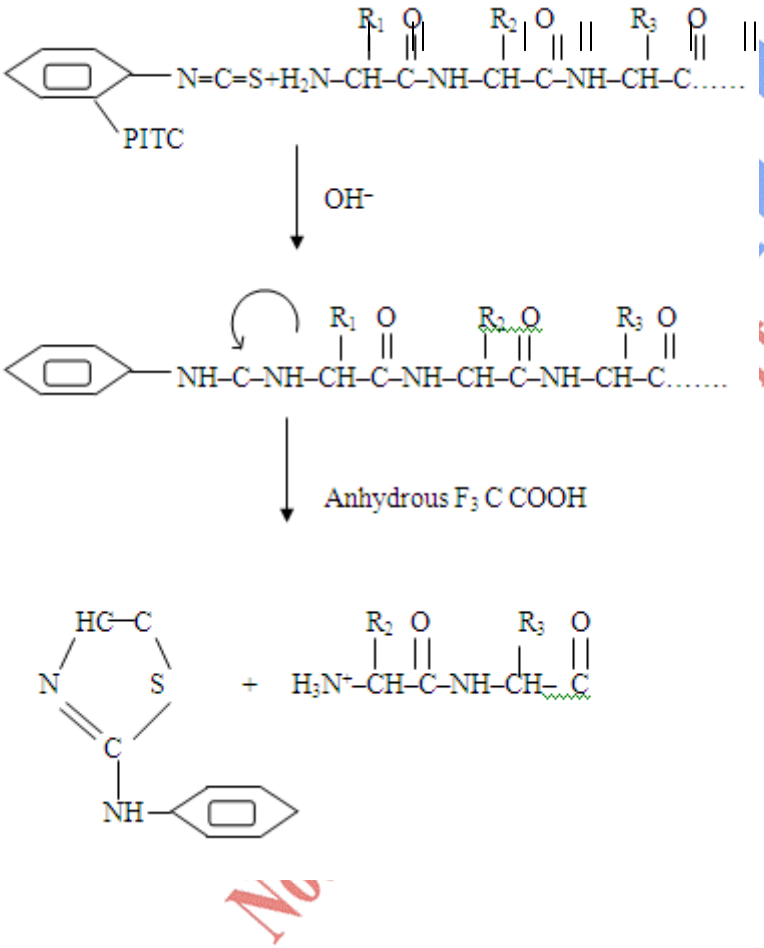
(i) N-terminal Identification

(I) Edman Degradation

Invented by Pehr Edman.

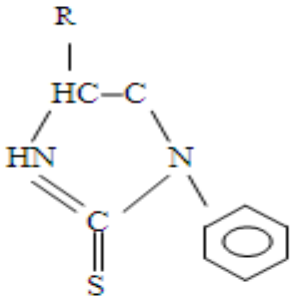
Reagent – PITC/Edman's Reagent/ Phenyl Isothiocyanate

Reaction :



Thiazolinone derivative

H^+



PTH amino acid

(phenyl thio hydantion)

(II) Enzymatic

Exo-peptidases like amino peptidases are used for N-terminal identification.

(b) **Cleavage of disulfide bonds :**

This can be done by two methods → I. Oxidative per formic acid

II. Reductive by Mercaptans

(i) Oxidative cleavage :

Performic acid converts all Cystine residues into cysteic acid residues and methionine to methionine sulfone.

i. Reductive cleavage :

Reductive cleavage of disulfide bonds by mercaptan : Reductive cleavage is most often achieved by treatment with I-mercaptoethanol or by either of the diastereiso mers dithiothreitol or dithioerythritol (Cleland's Reagent).

In order to expose all disulfide group to the reducing agent the reaction is usually carried out under conditions that denature the protein. The resulting free sulfhydryl groups are alkylated, usually by treatment with iodoacetic acid, to prevent the reformation of :

$$Cys\text{-}CH_2\text{-}SH + ICH_2COO^- \longrightarrow Cys\text{-}CH_2\text{-}S\text{-}CH_2COO^- + H\,I$$

δ-Carboxymethylcysteine

Disulfide bonds through oxidation by O_2 S- alkyl derivative are stable in air and under the condition used for the subsequent cleavage of peptide bonds.

(a) Separation, Purification and characterization of the chains – For determining amino acid sequence, acidic, basic low salt concentrations, elevated temperatures, or denaturing agents (e.g. area, minimum analog guanidinium used)

For Separation Ion exchange and gel filtration chromatography are used molecular mass of polypeptide is measured by gel filtration chromatography are SDS PAGE mass spectrometry.

(b) Amino acid composition determination :-

This done by complete hydrolysis of the polypepticle chain followed by the quantitative analysis of the liberated amino acids.

Two types of hydrolysis are done :-

(I) Chemicals (a) Acidic Hydrolysis (b) basic hydrolysis

(II) Enzymatic

(I) Chemical Hydrolysis :-

(a) Acidic Hydrolysis :

For acid catalyzed hydrolysis, the polypeptide is dissolved is GN-HCL realed in an evacuated tube to prevent the air oxidation of the sulfur- containing amino acids and heated at $100\text{-}120^0$ for 10 to 100 h.

Disadvantage :

- These harsh conditions degrade some of the amino acids like Ser, Thr and Tyr which are partially degraded.
- It also destroys tryptophan residues.
- Gln and Asn are converted to Glutamate and aspartate plus NH_4 + so that only the amount of Asx (= Asp+Asn) Glx = Glu+Gin and NH_4 + (Asn + Gln) canbe independently measured after acid hydrolysis.

(b) Base catalyzed hydrolysis

Base catalyzed hydrolysis of polypeptides in carred out in 2 to 4N Naoh at 100^0 for 4-8

Disadvantage –

Causes degradation of Cys, Ser Thr and Arg and Partially deaminates and racemizes the other amino acids.

Hence, alkaline hydrolysis is principally used to measure Tryptophan content.

II. Enzymatic Method :

The complete enzymatic digestion of a poly peptide requires mixtures of peptidases because individual peptidases do not cleave all peptide bonds. Exopeptidases and endopeptidases commonly used for this purpose. Pronase, a mixture of relatively nonopecific proteases from *Streptomyces griseus*, is also often used to effect complete proteolysis. This is used for amino acids Trp, Aln, Gln.

(a) Sequence the polypeptide Chain :-

(b) Specific peptide cleavage reaction ; Polypeptides that are longer than 40-80 residues can not be directly sequenced. Therefore, these are cleaved, either enzymatically or chemically to small fragments.

(i) Enzymatic Method : Enzyme used is trypsin a type of endopeptidase is used for the purpose having greatest specificity and is therefore the most valuable member of the arsenal of peptidases used to fragment polypeptides.

- It cleaves at C-side of the positively charged residue Arg & Lys if next residue is not pro.

- As it cleaves on positively charged amino acids, these positive sites can be added or deleted by chemical treatment. e.g pootive charge on lys is climinated by treatment with dicarboxylic anhydride such as citraconic anhydride.

(ii) Chemical method : Chemical reagents promote peptide bond cleavage at specific residues. The most useful of these, cyanogens bromide (NCBR) causes specific and quantitative cleavage on the C-side of met residues to form a peptidyl homooerine actone. The reation is performed in an acidic solvent CO.IN HCL or 70% formic acid) that denatures moot proteins so that cleavage normally occurs at all met residues.

Further separation and fortification of the peptide fragments is done which can be achieved by HPHC. Then sequence of the fragment is determined by repeated cycles of Edman degradation. For this adman and Geoffrey developed an automated device called sequenator.

Following these steps at last the peptide fragments are ordered via comparing amino acid sequences of one set of peptide fragments with those of a second set whose specific cleavage sites overlap those of the first set.

Q. 3 Write short notes on :

(a) Vitamins

(b) Hormones

Ans: **(a) Vitamins**

The concept of vitamin as introduced by **Hopkins** in collaboration with **Cashier Funk** of Poland and suggested vitamin theory according to which specific diseases Beriberi, Scurvy and Rickets are each caused by the absence from the diet of a particular nutritional factor.

Funk first time isolated the dietary factor from rice polishings which acted as antiberiberi substance. As this factor was an amine and necessary to life be introduced the term vitamin (vita = Life) on the suggestion of Dr. Max Nierenotein.

Definition: Vitamins are the substances distinct from major components of food, required in minute quantities (i.e. oligodynamic in nature) and whose absence causes specific deficiency disease.

Source _ Plants and bacteria

General Features of Vitamins :

1. Vitamins are the organic substances and functions at a very low concentration.

2. They can be fat soluble (A, D, E, K.) or water soluble (B & C) depending upon their structural components.

3.Synthesis – They are synthesized naturally by natural plants, animals and even humans, where plant synthesized all the vitamins but animals and humans can synthesize only few like vit A and vit D some microbes are also known to synthesize vitamins like vit B.

4. They have also been synthesized artificially

5. They are non antigenic in nature.

6. Are effective when taken orally.

Classification :

Vitamins have been classified into two large groups :

1. Fat soluble vitamins :- A,D,E and K
2. Water soluble vitamins :- B and C

(i) Fat soluble vitamins

These are oily substances, not readily soluble in H_2O and their biochemical functions are not well known. These include Vit A, D, E and K. They play more specialized rules in certain groups of animals and in particular type of activities.

e.g. They function in the formation of a visual pigment. (Vit. A) in the absorption of calcium and phosphorus from the vertebrate intestine (Vit D) protecting mitochondrial system from inactivation (vit E) or in the formation of a blood clotting factor in vertebrates (Vit K)

Chemical Structure: The 4 fat soluble vitamin can be regarded as lipids vitamin A, E and K are terpenoids, and vitamin D is a steroid. All four are Isoprenoid compounds, since they are synthesized biologically from units of isoprene, a building block of many naturally – occurring oily, greasy or rubbery substance of plant origin.

(ii) Water soluble vitamins – They are also kla universal vitamins as they perform same function independent of their occurrence they are catalytic factors and as such form of all links in biochemical reaction. Characteristic of all living objects.

e.g. Thiamine is required whenever sugars are oxidized aerobically to release energy. The biochemical or coenzyme functions of nearly all of these are known. They mainly include vitamins of B complex s/a B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11 & B12 and Vit C.Choline, inositol, P-amino-benzoic acid, bioflavonoid and L-lipoid acid are frequently included in this category The B series of vitamins being water soluble and extractable are required daily in meager amounts (in mg) for normal growth and good health of humans many other organism.

(b) Hormones

Definition :

Are substances which, produced in any part of an organism, is transferred to another part and there influences a specific physiological process. The term hormone, was first used by William M. Bayliss and Ernest H starling.

Features:

-The tissues and organs where hormones are produced are C/a effectors and where they exert their influence as targets.

-They have low molecular weight and diffuse readily. Based on their site of action they can be local or general.

-Non specificity and cross effects Hormones are not specific for the organism in which they are produced and may influence bodily procedures in other individuals also.

e.g. Adrenalin also influence protozoan and crustacean besides man and other vertebrates. Their cross effects have also been found b/w plants and animals.

General functions:

Hormones carry out several functions ranging from growth, vegetative and sexual development , cellular oxidation to thermal production.

The general functions of protein can be discussed under following heads:

(1) Regulatory or homeostatic function:

The hormones have regulatory effects on the competition of the body fluids, the rate of guesses exchanges and the activity of the vascular system and the central nervous system (CNS). The body Fluids are maintained at a constancy in the composition in a normal individual for the conduction various activity such environment within the cell bas been termed internal milieu.

Homeostasis can be defined as the tendency the maintain uniformity or stability in the internal environment of the organism or stability in the internal environment of the organism and to maintain the normal composition of the body fluids. Hormones play important and decisive role fluids. Hormones play an important and decisive role in homeostatic regulation of internal milieu.

3. **Permissive function:**

Endocrine glands affect a number of processes, but these glands also affect the functioning of one another thus certain hormones require the presence of another hormone for the expression of their activity. This help in maintaining a perfect hormonal balance.

3. **Integrative functions :**

The Integrative functions of the hormones in case of endocrine system are slow and steady whereas those of the nervous system are rapid. This results into the emergence of a new discipline of science neuroendocrinology.

4. **Morphogenetic functions:**

Hormones, govern the ontogenetic develop of an individual from the embryonic to the adult stage.

Classification:

Type of Hormones: Depending upon their components they can be classified into three main types :

(a) Steroid Hormones :

These include sex hormone, and hormones from adrenal cortex. These are synthesized by mammals by the ovary (or testis) adrenal cortex corpus luteum and the placenta

e.g. Ovarian hormones: Estrone, β estradiol

Testicular Hormones – Testosterone, androsterone

(b) Peptide Hormones :

They are protein in nature and include hormones like insulin and glucagon secreted by pancreas. Other hormones include hormones of hypophysis e.g. Thyotropin, Gonedotropin etc.

(c) Amino acid derivative :

It includes hormones like thyroidal or adrenal medullary– Trioidodhydronine, from thyroidal, hormone while adrenalin from – adrenal medullarly.

Q. 4 What are carbohydrates? Describe the structures of different types of carbohydrates?

Ans. **Definition**

Carbohydrates can be defined as Polyhydroxy aldehydes or ketones and their derivatives.

(a) Monosaccharide's

(b) Oligosaccharides

(c) Poly saccharides

1. Homopoly /saccharides) 2. Heteropoly (saccharides)

(a) Monosaccharide's

These are compounds which poses a free aldehyde (-cho) or ketone (> O) group and 2 or more hydroxy (-OH) groups

They can be subdivided into :-

Trioses (3 carbon compound)

Tetroses (4 carbon compound)

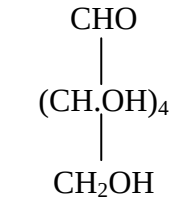
Pentoses (5 carbon compound)

Hexoses (6 carbon compound)

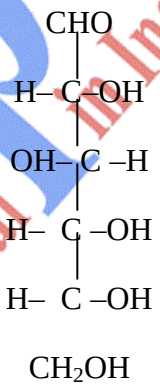
Heptoses (7 carbon compound)

Structural forms :

- Structural elucidations of monosaccharide show different forms describing not only the formula but also the other aspects and features like stability of these carbohydrates.
- These structural conformation can be understood by taking examples of various types of monosaccharides .
- Glucose: Linear form given by fitting and Baeyer, it tells us the presence of aldehyde and 5 –OH groups.
- Fisher – projection formula



Fitting - Baeyer Formula



Fisher projection formula

Fisher projection formula gives much more details compared to fitting and Baeyer like the presence of 4 asymmetric carbon atom in the molecular. It is also useful in indicating configurational differences among sugars.

Ring form :

Another way of presenting structural formula of carbohydrates is ring form .These rings of glucose molecule is formed as a results reaction of aldehyde and ketone with group of alcohols forming hemiacetals and hemiketals respectively. For large molecules where number of carbon atom are higher molecule where number of carbon atom are higher than 4 the reaction takes place within the molecule to form 5 or 6 membered ring.

It glucose molecule angles of tetrahedral carbon atom tend to bend it forming a ring.

The aldehyde group and the alcohol group of carbon 5 or carbon 4 readily approach each other forming intramolecular hemiacetals. Similarly the keto group of a ketose can also

approach the alcohol

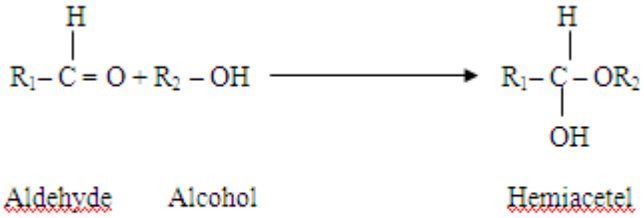


Fig: Formation of a hemiacetal

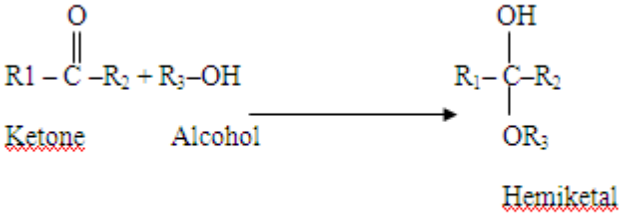
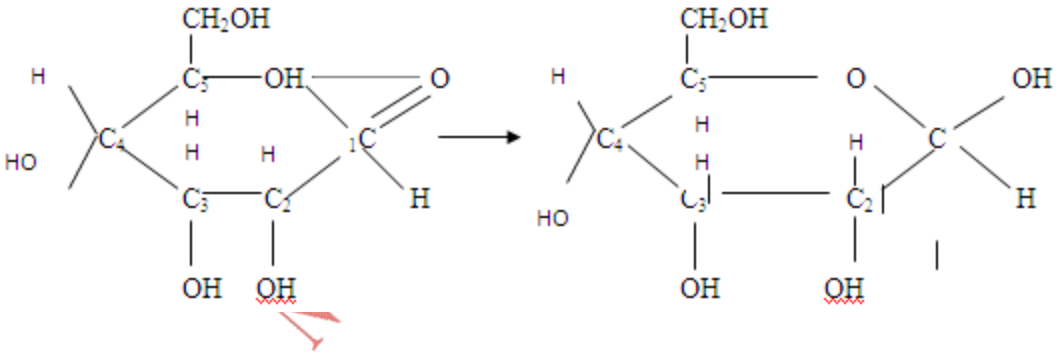
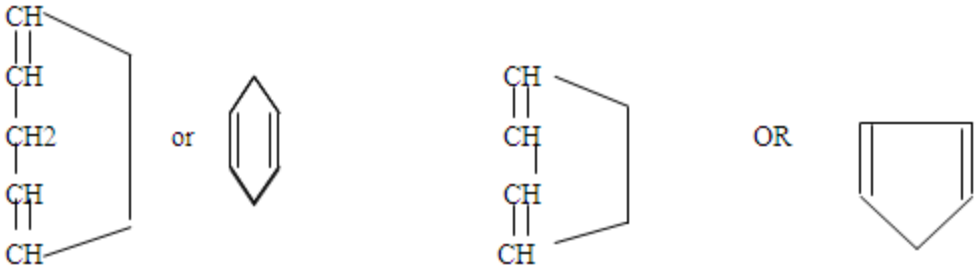


Fig: Formation of a hemiketal

The group of either carbon 5 or carbon 4 form intramolecular hemiketals. This results in the formation of either a 6 – membered ring (pyranose form), or a S-membered ring (furanose form). A 7- membered ring becomes too strained to allow participation of the –oH group of carbon 6 of aldohexoses with the –CHO group in ring formation.



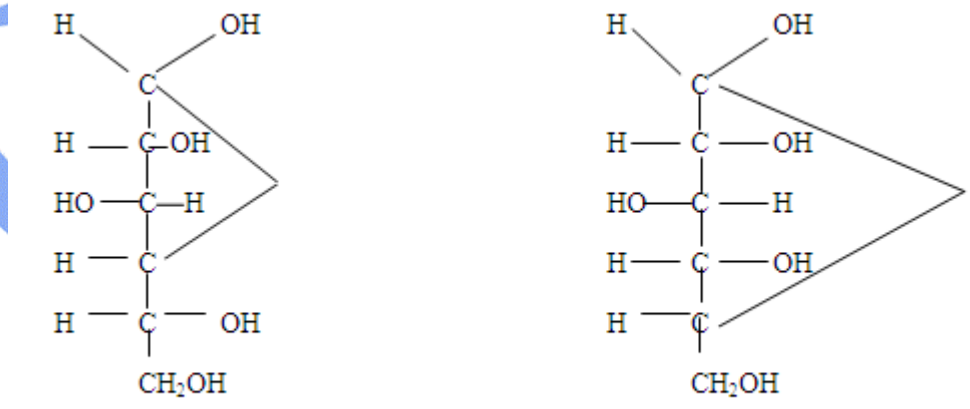
These sugar structure are named as pyran furan. The pyranose form of the sugars are more stable than furanose form in solution



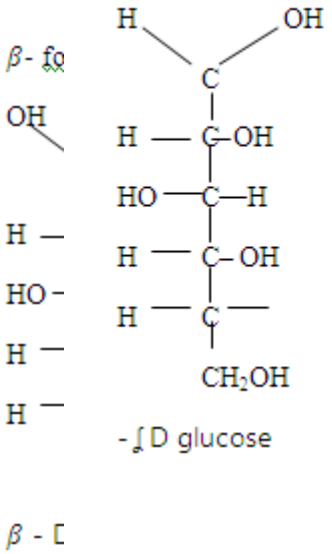
Pyran Furan

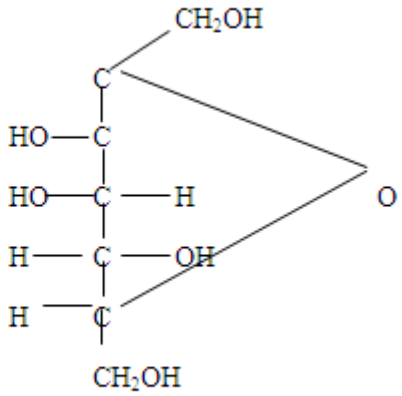
Formation of ring structure in glucose results in a new centre of symmetry i.e. the carbon 2 in ketose. This shows that the ring form of glucose can exist as either of the 2 isomeric form termed and theβ isomers. These two isomers are diastereoisomers rather than enantiomers because the β form of D-glucose is not a mirror image of the α form. They are also k/a anomers as they differ in configuration only around the hemicecetal or anomeric carbon atom.

Those anomers having same configuration at both the anomeric heving sameconfiguratino at both the numeric penultimate carbon atom c/d as ∫ -form while in ∫ form the configuration would be different at both these carbon atom.
Configuration of atom at both these carbon canomeric and penultimate is crythro (written on the same side of the structure in) £ form and threo (written in opposite) in β-form. The £ form of D- glucose in both pyroase and furanose rings can be represented as :-
∫ - forms :-



∫ - D Glucofuranose ∫ - D Glucopyranose





β - [

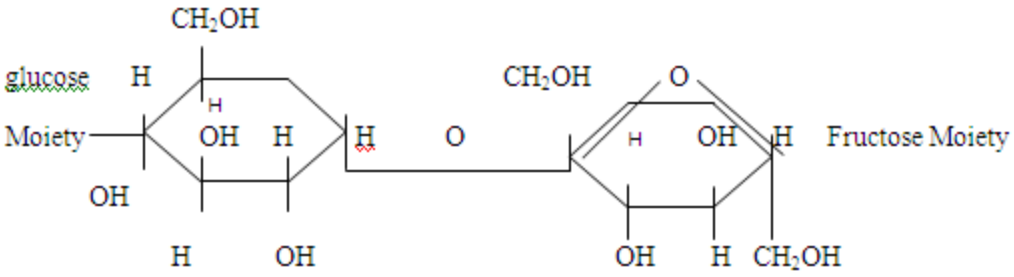
Oligosaccharides : These carbohydrates consist of more than 2 and upto 10 monosaccharide molecules. e.g. sucrose, maltose, cellobiose etc.

They may be reducing or non-reducing depending upon the type of linkage. The reducing sugars show C1-C4 and C1-C6 glycosidic linkage. While nonreducing, sugar consist of C1-C1 and C1-C2 glycosidic linkage.

Structure of oligosaccharides:

Sucrose

Fisher Formula



Hawarth Formula

Polysaccharides :

These are high molecular weight carbohydrates which on hydrolysis yield mainly monosaccharides or products related to monosaccharide.

e.g. Inulin : storage form of carbohydrates. It is stored in tubes and roots of these plants.

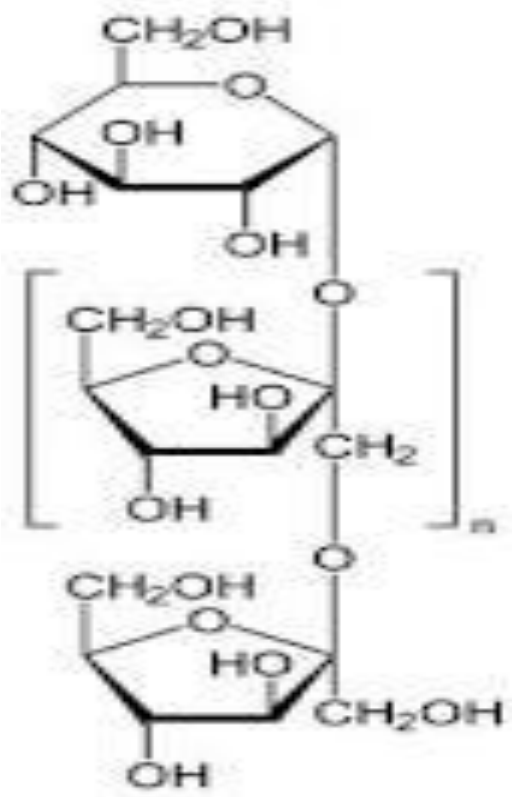


Fig.: Structure of Inulin

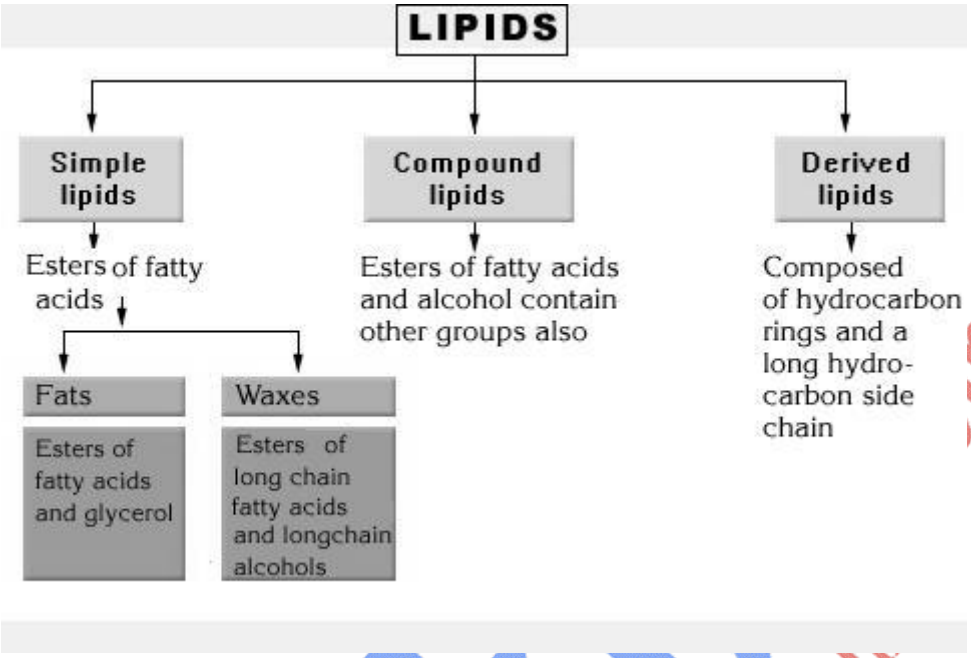
Q .5 Give a general account on lipids?

Ans : One of the biologically important molecule lipids are polymers of fatty acids.

In addition to fatty acids they also consist of alcohol as their major component which is mostly a glycerol.

The major types of lipids:

Lipids have been divided into 3types.



Biological functions of lipids.

1. **Membranes**

Eukaryotic cells are membrane-bound organelles that carry out different biological functions. The glycerophospholipids are the main structural component of biological membranes, such as the cellular plasma membrane and the intracellular membranes of organelles; in animal cells the plasma membrane physically separates the intracellular components from the extracellular environment.

2. **Energy storage**

Triglycerides, stored in adipose tissue, are a major form of energy storage both in animals and plants. The adipocyte, or fat cell, is designed for continuous synthesis and breakdown of triglycerides in animals, with breakdown controlled mainly by the activation of hormone-sensitive enzyme lipase. The complete oxidation of fatty acids provides high caloric content, about 9 kcal/g, compared with 4 kcal/g for the breakdown of carbohydrates and proteins. Migratory birds that must fly long distances without eating use stored energy of triglycerides to fuel their flights.

3. Signaling

The lipid signaling is a vital part of the cell signaling. Lipid signaling may occur via activation of G protein-coupled or nuclear receptors, and members of several different lipid categories have been identified as signaling molecules and cellular messengers. Examples : sphingosine-1-phosphate, a sphingolipid derived from ceramide that is a potent messenger molecule involved in regulating calcium mobilization, cell growth, and apoptosis; diacylglycerol (DAG) and the phosphatidylinositol phosphates (PIPs), involved in calcium-mediated activation of protein kinase C etc.

4. Other functions

The "fat-soluble" vitamins (A, D, E and K) – which are isoprene-based lipids are essential nutrients stored in the liver and fatty tissues, with a diverse range of functions. Acyl-carnitines are involved in the transport and metabolism of fatty acids in and out of mitochondria, where they undergo beta oxidation. Polyprenols and their phosphorylated derivatives also play important transport roles, in this case the transport of oligosaccharides across membranes.

5. Metabolism

The major dietary lipids for humans and other animals are animal and plant triglycerides, sterols, and membrane phospholipids. The process of lipid metabolism synthesizes and degrades the lipid stores and produces the structural and functional lipids characteristic of individual tissues.

Section- B

Structure of Proteins

Q.1 Describe the secondary and tertiary structure of proteins giving an example for each?

Ans. Based on the degree of complexity of their molecule proteins have been classified into 4 basic structural levels of organization.

These structural levels were first defined by Linderstrom lang and are often referred to as primary, secondary, tertiary and quaternary. Three of these structural levels (Primary, secondary and tertiary) can exist in molecules composed of a single polypeptide chain, whereas the fourth involves (i.e. quaternary) interactions of polypeptides within a multi chained protein molecule.

2. Secondary structure / 2^0 Structure of proteins:-

Definition:

In a polypeptide chain folding and hydrogen bonding between neighboring amino acids result in the formation of a rigid and tubular structure called a helix. This constitutes the secondary structure of proteins which refers to the steric or spatial relationship of amino acids that are near to each other in the amino acid sequence. Based on the nature of hydrogen bonding (Whether inter or inter molecular) Pauling and corey (1951) identified two regular types of secondary structure in proteins.

A. Alpha helix (α -Helix)

B. Beta Bleated Sheet (β- Bleated sheet)

A. α -helix

A helical structure was given by Pauling and very planar peptide bonds would form a right handed helical structure by simple twists about the α -carbon-to-nitrogen and the α -carbon-to-carboxy corbon bonds. They called this helical structure as α -helix. The helix is so named because of the mobility of α -carbon atoms.

Features of α -helix :

The α -helix is a rod like structure. The tightly coiled polypeptide main chain form the inner part of the rud, and the side chains extend outward in a helical array. The α -helix is stabilized by hydrogen bonds between the NH & CO groups of the main chain.

- The CO group of each amino acid is hydrogen bonded to the NH group of the amino acid that is situated four residues ahead in the linear sequence. Thus, all the main chain CO & NH group are hydrogen-bonded.

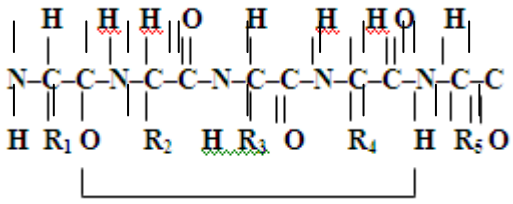
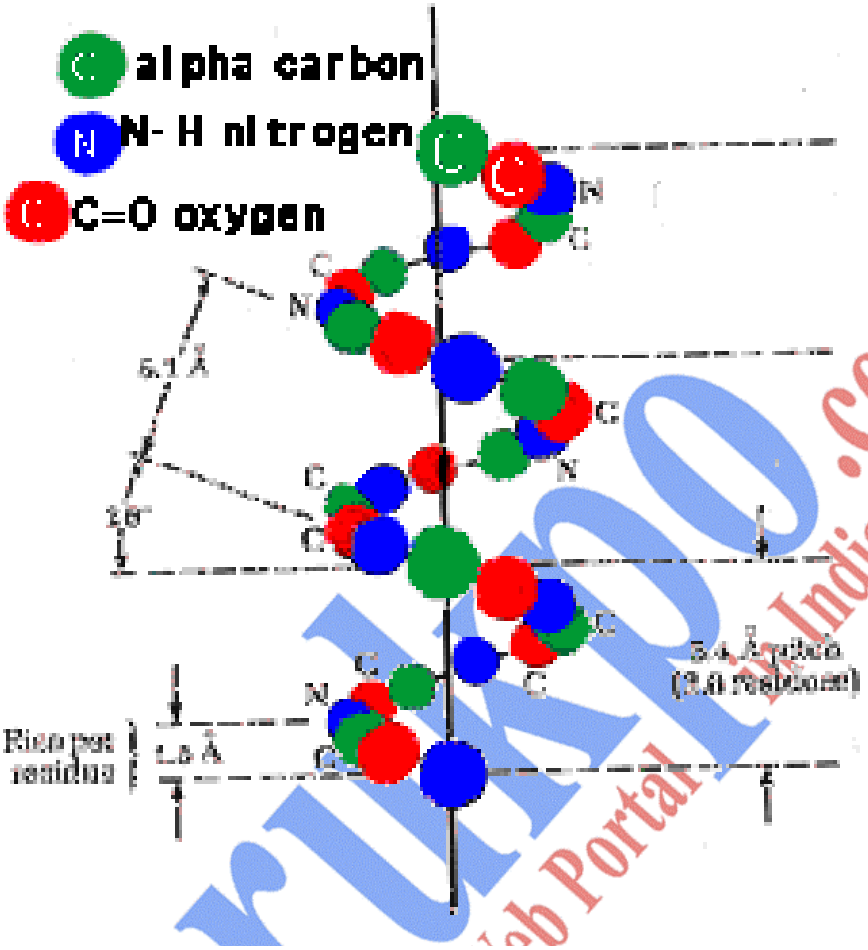


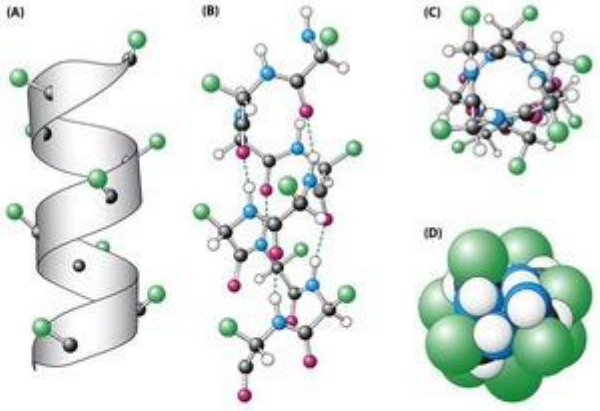
Fig :- Diagram showing that in the α _ helix, the co-group of residue n is hydrogen bonded to the NH group of residue (n+4). The hydrogen bonding occurs spontaneously and as a result a polypeptide can assume a rod-like structure with well defined dimensions.

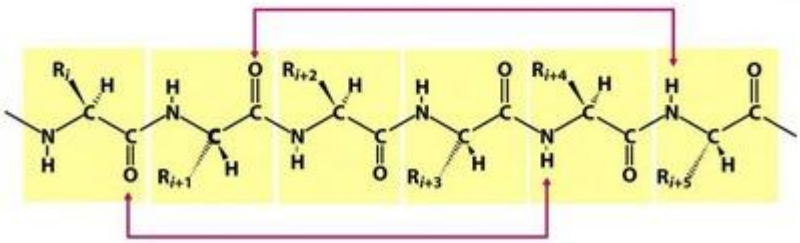


The Alpha-Helix

Features:

- NH-CO H-bonding
- rise = 1.5 Å
- rotation = 100°/a.a.
- 3.6 a.a/turn
- pitch = 5.4 Å
- H-bonding every 4 a.a.
- side chains project out from central axis





Here letters N - stands for nitrogen

R - represents α -carbon with a side chain attached.

C - Carbon

The red shaded circles represent atoms back of the plane of the paper while the white circles indicate atoms above the plane.

α -helix can be right handed or left handed.

e.g. α -Keratin

α -helix occurs in the protein α -keratin, found in skin and it's appendages S/a hair, nails and feathers & constitutes almost the entire dry weight of hair, wool, feathers, nails, claws, quills, reales, hours, hooves, tortoise shell and much of the outer layer of skin. The basic structured unit of α-keratin consist of 3 right handed helical polypeptides in a left handed coil that is stabilized by crosslinking disulfide bonds.

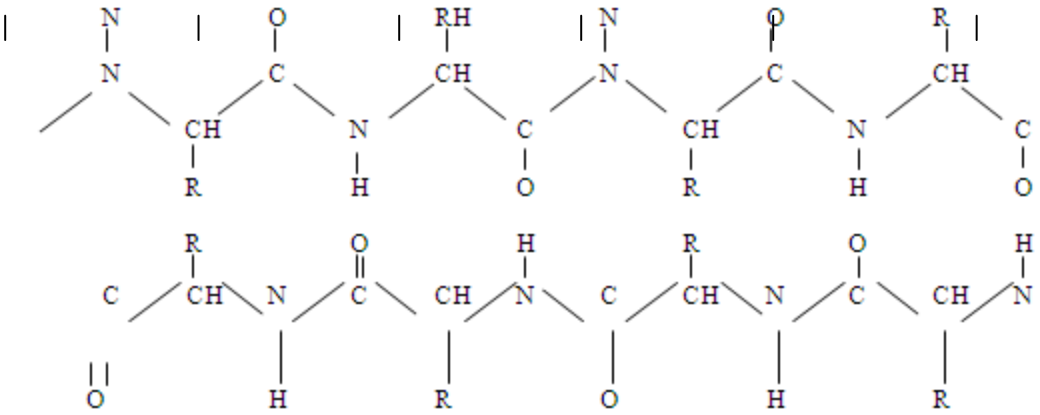
According to **Pauling** and **Corey** in α Keratin the backbone is arranged in a helical having about **3.6 amino acid** residues per turn. The R groups of amino acids extend outward from backbone of the helix. In such a structure the repeat unit, consisting of a single complete turn of the helix, extends about **0.54 nm (5.4 A)** along the long axis, corresponding closely by the major periodicity of **0.50 to 0.55 nm** deduced from X-ray pattern of natural α -keratins. The **rise per residue 10** about **0.15 cm**, corresponding to the minor periodicity of 0.15 nm also observed in the diffraction patterns. Such an α -helix permits the formation of intrer chain hydrogen bonds between successive coils of the helix, parallel to the long axis of the helix and extending between the hydrogen atom attached to the electronegative nitrogen of one peptide bond and the carbonyl oxygen of the third amino acid beyond it.

B**. β-Pleated sheet :-**

Pauling and Corey (1953) identified a second type of repetitive, minimum energy or stable conformation, which they named β sheet. The formation of β- pleated sheets depends on intermolecular (Inter chain) hydrogen bonding, although sheet structure is formed by the parallel alignment of a number of polypeptde chains in a plane, with hydrogen bonds between the >C=0 and –N-H groups of adjacent chains. The R groups of the constituent amino amino acids in one polypeptide chain alternately project above and below the plane of the sheet, leading to a two residue repeat unit. Glycine and Alanine are the two amino acids found commonly in β-pleated structure.

e.g. **Silk** and certain synthetic fibres s/a **Nylon** and **Orlon.**

Silk fibroin is one example of a protein that has the anti parallel beta pleated sheet structure. It is a member of a class of fibrillar proteins C/d α **-keratins**. ***Silk fibrion*** composed mainly of glycine , alanine, and serine units linked together by the peptide bonds. Glycine constitutes approximately 45% of the total amino acid residues, and alanine plus serine compose another 42% Hence, the R Group extending above and below the plane of the bleated sheet are small and allow the pleated sheets to stack. The two chains which run in opposite direction are linked by hydrogen bonds.



Tertiary Structure of proteins :-

Definition:

The tertiary structure (3^0 Structure) of a protein is its three dimensional arrangement that is the folding of its structural elements, together with the spatial dispositions of its side chain.

e.g. most of the globular proteins show tertiary conformation of proteins.

(i) Myoglobin :

Taking example of myoglobin globular protein we can understand the tertiary structure of proteins.

It is relatively small, oxygen binding, heme protein, found in muscle cells, it is k/n to be the first globular protein whose 3-D structure elaborated by X-ray diffraction studies. This was done by John C. Kendrew.

Myoglobin molecule contains a single polypeptide chain of **153 amino acid** residues and a single prosthetic iron porphyryin (or heme) group, identical with that of hemoglobin, is responsible for the deep red-brown colour of myoglobin and also of hemoglobin. The function of myoglobin to bind oxygen in the muscles and to enhance its transport to the

mitochondria which consume oxygen during respiration, myoglobin is an extremely compact macromolecule with oblate, spheroid shape and leaves little empty space in its interior. Its overall molecular dimensions are 45 x 35 x 25A^{o}. The backbone of the molecule is made up of 8 almost straight ∫ helical segments, designated from N-terminus as A through H.

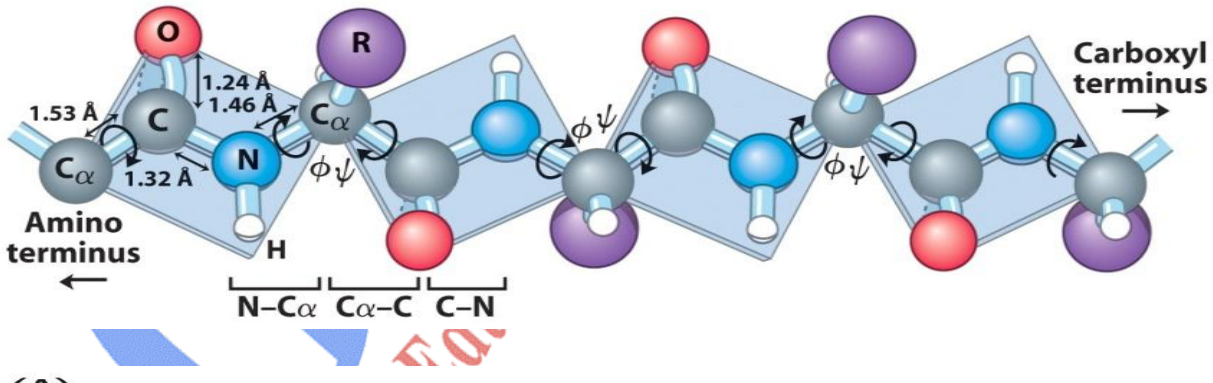
Q.2 Write short notes on:

(a) Ramchandran Plot
(b) X-ray crystallography

Ans **(a) Ramchandran Plot**: A Ramachandran plot also known as a Ramachandran diagram or a [φ,ψ] plot), was developed in 1963 by G. N. Ramachandran. This contour diagram was suggested for defining the Secondary structure of proteins. It is one of way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. The peptide backbone conformation can be described in terms of two dihedral angles, Phi and Psi. Where

P**h**i is the dihedral angle for the N-C_α bond (**h**etero)

P**s**i is the dihedral angle for the C_α-C bond (**s**ame)

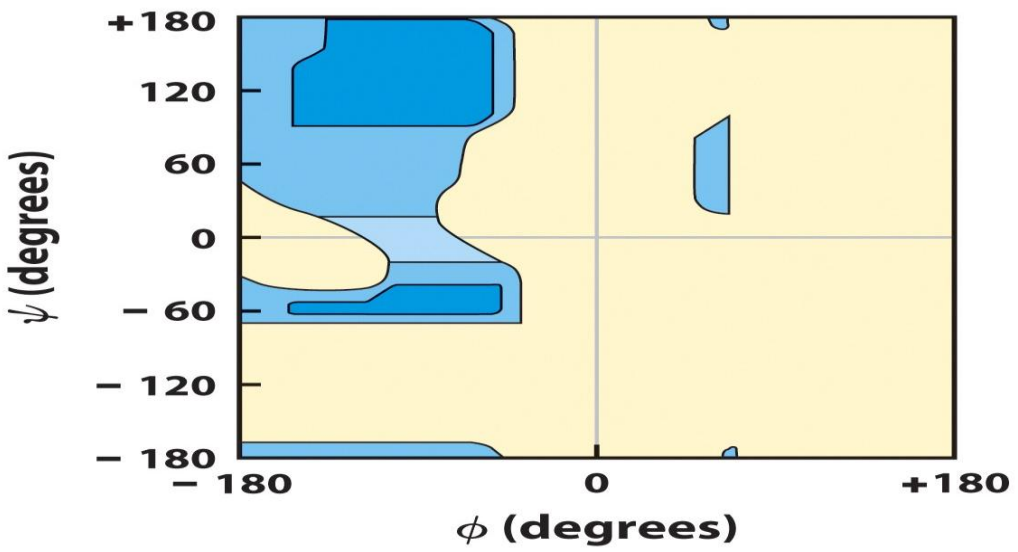


(A)

Pauling and **Corey** described the constraints on the secondary structure of proteins that the peptide bond formed between the C of carboxyl group and N of $–NH_2$ holds a partial double bond character due to the resonance of pi electrons of carbon and oxygen of

carboxyl group providing rigidity to the peptide bond and limiting the conformations of proteins.

This was further elaborated by Ramchandran by giving a plot consisting of different allowed and non allowed regions varying according to amino acids. It can be seen here in the diagram for L-Alanine.



Ramachandran plot fo rL-Ala. "Allowed regions" of conformational space are in blue.

Two main allowed regions:ϕ = -57º; ψ = -47º (α_R region) = -125º; ψ = +125º (β region)

The mirror image of α_R is α_L and is only permitted for Gly.

(c) **X-ray crystallography:**

History:

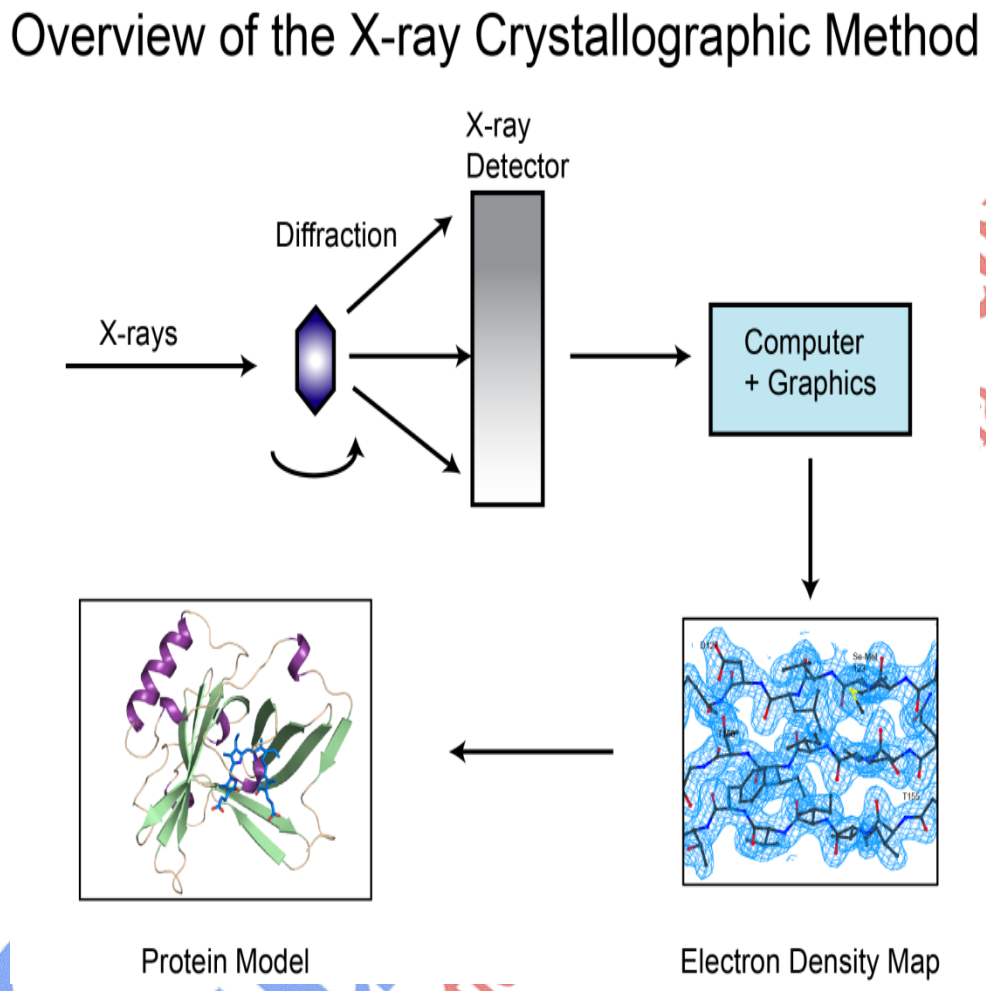
In 1895 Wilhelm Röntgen, a German physicist, discovered X-rays and demonstrated that this type of radiation could pass directly through human tissues leaving behind images of bones. He won the Nobel Prize in Physics in 1901. Another German physicist, Max von Laue proved in 1912 that crystals could diffract X-rays. The first X-ray structure solved was that of table salt in 1913 by William Henry Bragg and William Lawrence Bragg.

Introduction:

It is a method used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the

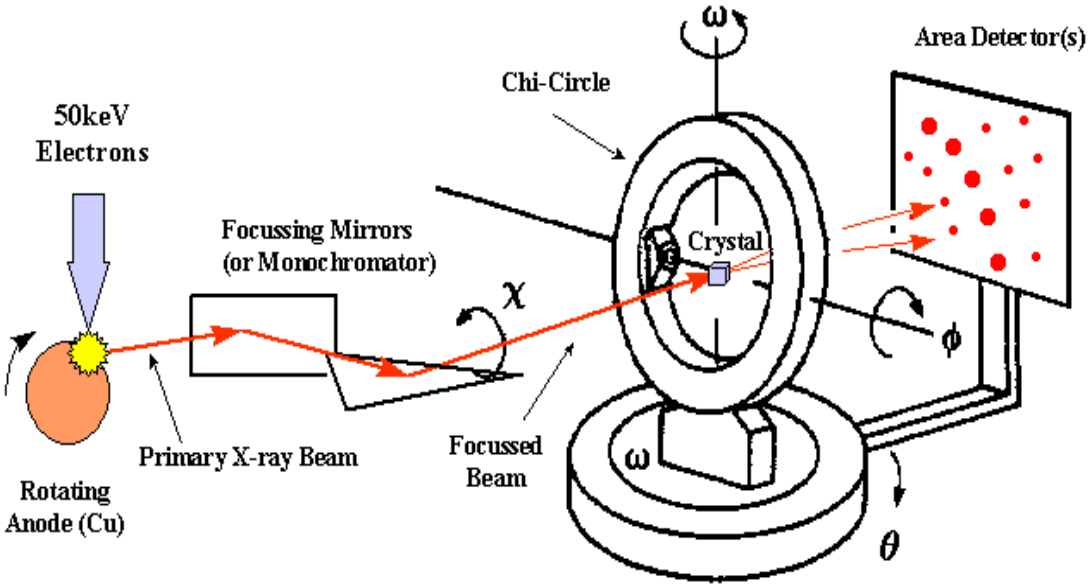
crystal can be determined, as well as their chemical bonds, their disorder and various other information can also be obtained.

Principle and method:



The technique is based on the principle of **Diffraction and scattering of light.**
The method involves following steps:

- Crystallization of the molecule whose structure has to be determined.
- Put the crystals in a special X-ray beam.
- The crystal scatters the X-rays onto an electronic detector, which functions as a recorder.
- With specialized computer programs it is possible to use the information gathered on the detector to construct a so-called "electron density map," which is basically a roadmap that tells us what the molecule looks like in three-dimensions. From the map, a model of the molecule is constructed using specialized computer graphics programs.



4-Circle Goniometer (Eulerian or Kappa Geometry)

Importance of X- ray Crystallography:

Proteins are one of the most important of of all the biomolecules that are not just the building blocks of our body but form an important part of the metabolism machinery as enzymes or the intermediates. Any malfunctioning in them may lead to great disease. In such cases where Protein dysfunction result into a disease determining the 3-d structure it is possible to design a drug that will stop the protein from malfunctioning. This process of determining the structure of a protein and designing molecules that will bind to it to affect its function is known as "structure-based" drug design. Pharmaceutical companies use this very technique for making new drugs to treat all sorts of things such as high blood pressure or high cholesterol.

This technique played a key role in the development of inhibitors to fight the human immunodeficiency virus (HIV), which is the causative agent of acquired immunodeficiency syndrome or AIDS.

Limitations:

(a) Crystallizing Protein is a tough task as proteins are Fragile and requires a crystal with shortest side 0.2 mm

(b) Flaws of Crystallization: may result into

(c) Disorder in Unit Cell

(d) Vibrations of molecules

(d) Distortion in Crystallization

Section- C

Structure of Nucleic Acid

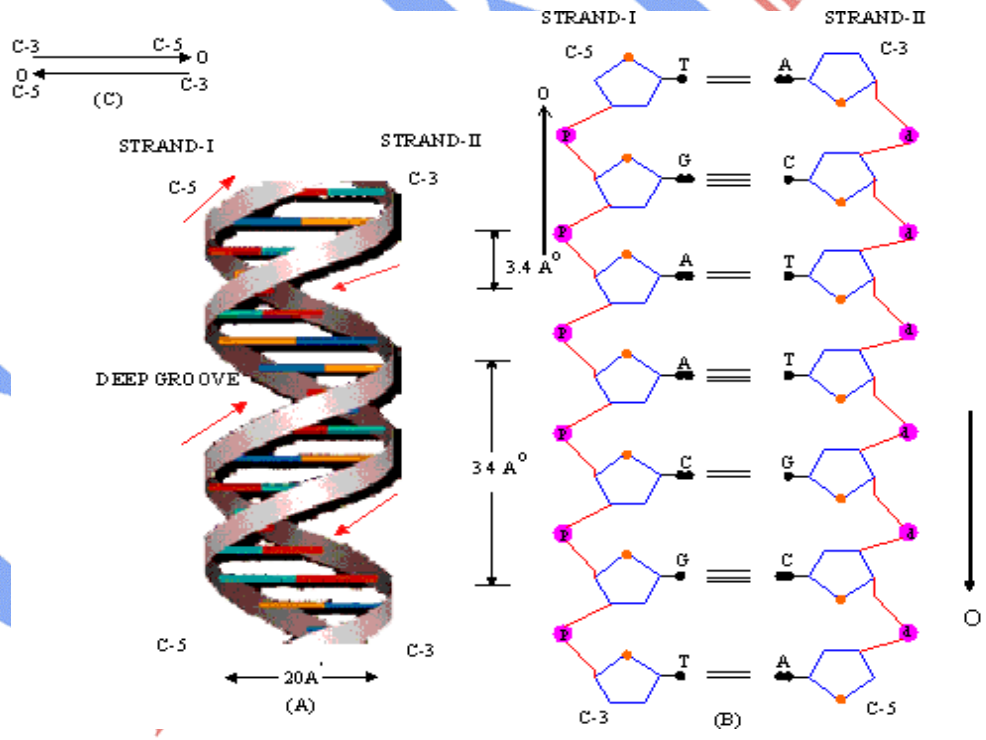
Q.1 Describe the structure of DNA and add a note on types of DNA ?

Ans: Structure of DNA :

Introduction :

The double helical structure of DNA was suggested by Watson and Crick for commonly found DNA k/a B-type of DNA.

DNA molecule consists of two helical polynucleotide chain which are coiled around a common axis in the form of a right double helix.



Various features of DNA:

- DNA consist of various components that add to its structure but before describing these a brief about the basic aspects of DNA structure.

- The two helices of DNA are wound such that it produces two inter-chain spacing's or grooves, a major and a minor grove. The major or wide groove has width 12 A^0 depth 8.5 A^0 and minor or narrow groove having width 6 A^0 and depth 7.5A^0 The two groove arise because the glycoside bonds of a base bair are not diametrically opposite each other. The minor groove contain the pyrimidine O-2 and the purine N-3 of the base pair, and the major groove is on the opposite side of the pair, each groove is lined by potential hydrogen bond donor and acceptor atoms. The major groove displays more distinctive features than does the minor groove.

- The two helices wind along the molecules parallel to the phosphodiester back bones. In these grooves, specific protein interact with DNA molecules. Such double helices cannot be pulled apart and can be separated only by an unwinding process. As both the chain are anti parallel , in one strand the ring oxygen of sugar moieties face upwards while in the other strand, the ring oxygen faces downwards.

- The phosphate and deoxyribose units are found on the periphery on the helix, whereas the purine & pyrimidine bases occur in the centre. The Planes of the bases are perpendicular to the helix axis. The planes of the sugars are almost right angle to the bases.

- The diameter of the helix is 20 A^0 The bases are 3.4 A^0 apart along the helix axis and are related by a rotation of 36 degrees. Therefore, the helical structure repeats after 10 residues on each chain i.e. at interval of 34 A^0.

- The two chains are held together by hydrogen bond between pairs of bases.

  Adenine always pairs with thymine by 2 H bonds and guanine with cytosine with

  3 hydrogen bonds.

Components of DNA :-

DNA is a biopolymers of high molecular weight with mononucleotide as their repeating units.

Elemental Composition:

DNA consist of carbon, Hydrogen, oxygen, Nitrogen and phosphorus, Out of these elements.

Nitrogen and phosphorus contribute 15% and 10% respectively.

Other components:

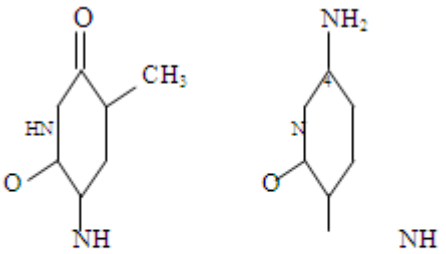
(a) **Nitrogenous bases :** The two types of nitrogenous bases found in DNA and these are the derivatives of pyrimidine and purines.

(b) Pyrimidine derivatives

Thymine and Cytosine are the two pyrimidines found in DNA.

Thymine : Isolated from thymus and named after.

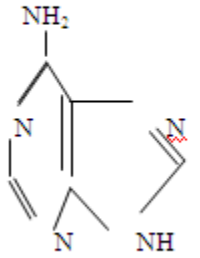
Cytosine: It is a white crystalline substance.



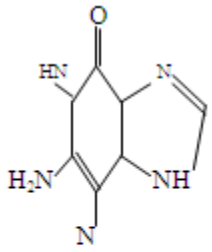
Thymine $C_5H_6O_2N$

Cytosine $C_4H_5ON_3$

Purines Derivatives These are two ring compounds which includes adenine and guanine.



Adenine (6 aminopurine $C_5H_5N_5$)

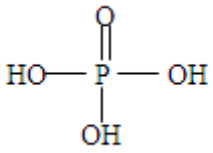


Guanine(2-) amino - 6 - oxypurine $C_5H_5ON_5$)

(c) Phosphoric acid :

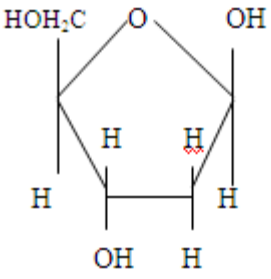
DNA consist of phosphoric acid that gives a-ve charge. The molecular formula phosphoric of H3Po4. It contains

3 Monovalent hydroxyl groups and a divalent oxygen atom, all linked to the bentavalent phosphorus atom



(c) Pentose Sugar :

DNA consist of D-2 deoxyribose, sugar, hence the name deoxyribose nucleic acid. Pentose sugar in DNA is present in the furanose form and are of β configuration.



D-2 Deoxyribose

(β-D-2 Deoxyribofuranose)

Base composition of DNA:

- In 1950 Erwin charge off first analyzed & gave the pattern of base composition of DNA:
- The sum of purines (Pu) is equal to the sum of pyrimidine (py) i.e. pulpy = 1.In other words, A+G = T+C (+ MC-methyl cytosine)
- The ratio of adenine to thymine is also one i.e. A/T=1
- The ratio of guanine to cytosine is also one i.e. G/C=1.
- Bases with 6 amino groups are equal to bases with 6-keto (-OH) groups i.e. A+C = G+T

The ratio of A+T/G+C known as dissymmetry ratio varies greatly from one species of DNA to the other and is characteristic of that species.

When this ratio exceeds one, such a DNA is called at type while if the value is len than one, such a DNA is designated as GC type.

In animals this ratio ranges from 1.3 – 2.2 while in higher plants 1.1 to 1.7.

Q .2 Write a note on the types of DNA?

Ans. DNA is a biopolymers of high molecular weight with mononucleotide as their repeating units. On the basis of variations in the dimensions and conditions in which they occur are of various types.

Type of DNA :-

DNA is found in different form there are about 5 types of DNA known :-

1. A DNA
2. B DNA
3. C DNA
4. D DNA
5. Z DNA

Out of these types type of DNA is an extremely rare variant with only 8 base pairs per turn. This type of DNA is found in some DNA molecules devoid in guanine. There is an

axial rise of 3.03 A^0 per base pair, with a tilting of 16.7^0 from the axis of the helix of form of DNA

The characteristic features of rest of the DNA molecules are described below :

Characteristics	A-DNA	B-DNA	C-DNA	Z-DNA
1. Condition	70%Relative humidity, Na+, K+, Cs+ Ions	92% relative humidity, lowion strength	60% relative humidity Li+iou	Very high saltion one entrain
2. Shape	Broadest	Intermediate	Narrow	Narrowest
3.Helix-sense	Right+handed	Right+=handed	Right handed	Left handeld
4. Rise per base pair	2.3 A^0	3.4A^0	3.32 A^0	3.8 A^0
5.Helix diameter	25.5A^0	23.7 A^0	19.0A^0	18.4 A^0
6.Base pairs per turn of helix	11	10.4^0	9.33	12 (= 6 Dimers)
7 Helix Pitch	25.30 A^0	35.36 A^0	30.97 A^0	45.60A^0
8 Base Pair tilt	19^0	1^0	7.8^0	9^0
9Glycosidic Bond	Anti	Anti	-	Anti for C,T syn for A,G, flat
10 Major Groove	Narrows	Wide & Quite deep	-	-
11Minor Groove	Very broad and Shallow	Narrow & quite deep	-	Very Narrow and deep

Q. 3 Give an account on the structure of different types of RNA?

Ans. Introduction-

RNA stands for Ribonucleic acid is one of the type of nucleic acid known for performing various functions like the coding, decoding, regulation, and expression of genes.

Types of RNA:

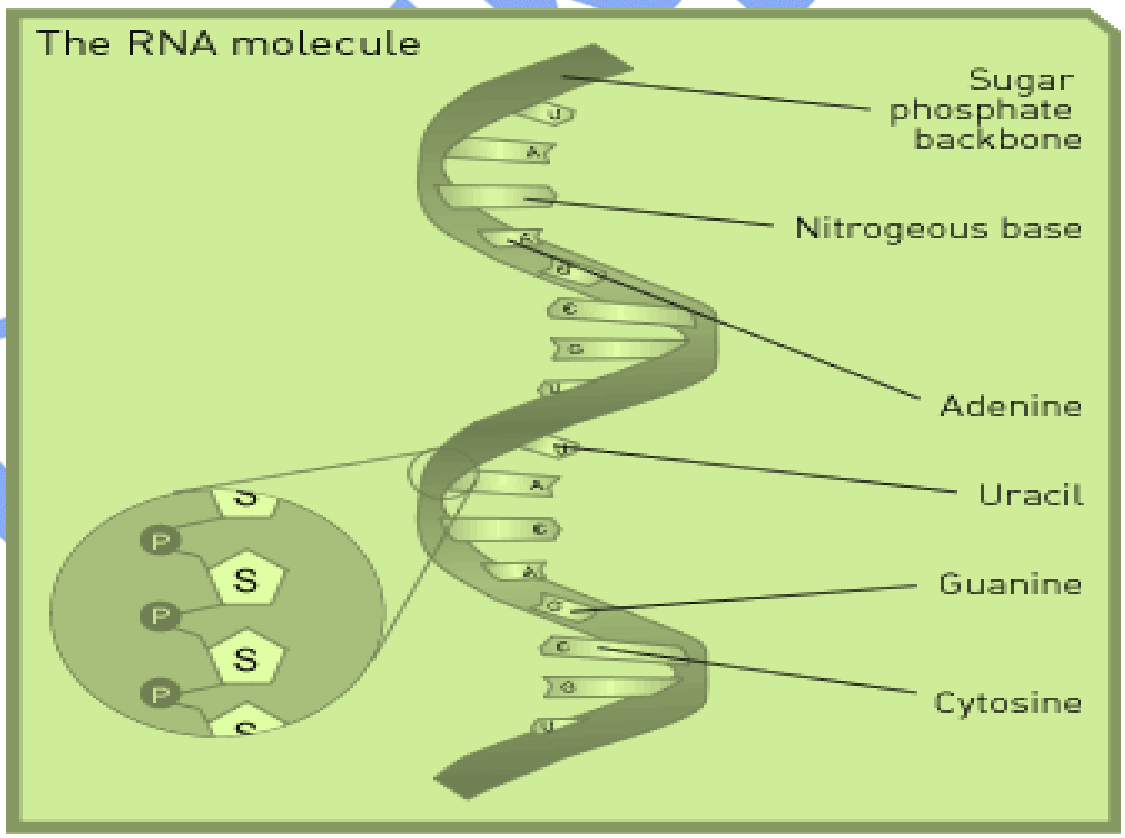
The Major types of RNA include:

(i) rRNA : transcribed by RNA Polymerase -I

(ii) mRNA transcribed by RNA Polymerase -II

(iii)tRNA transcribed by RNA Polymerase –III

Structure of RNA:



Compared to DNA, RNA is a single stranded molecule consisting of major components as:

(i) Pentose sugar- The sugar in RNA is a Ribose sugar.

(ii) Phosphate group similar to DNA

(iii)Nitrogen bases –Adenine, Guanine, Cytosine, Uracil.

Structure of different types of RNA:

(i)rRNA:

This is the major form of RNA as it constitutes 80% of total RNA.The **secondary** structure of **rRNA** is a complex pattern of short **double-stranded stems**, [red blocks] interspersed with unpaired **single-stranded loops** and **bubbles** [black lines]. **T**he **rRNA** structure below is a single, continuous strand **H**-bonded back on itself, with a **5'** beginning and **3'** end. The **tertiary** structure of **rRNA** will fold this structure up amongst a complex of proteins.

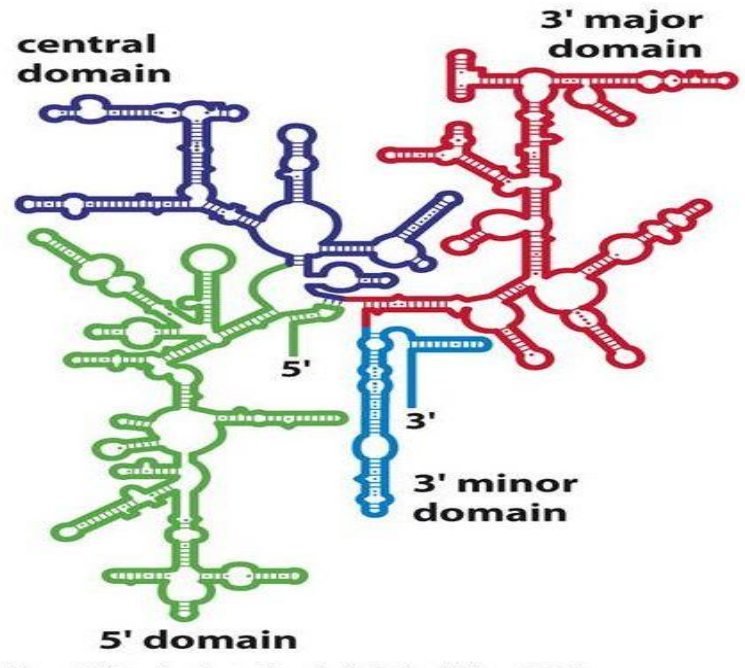


Figure 6.5 Introduction to Genetics (© Garland Science 2012)

(ii) tRNA

Transfer RNA also called as **sRNA** or **soluble** RNA or **adaptor RNA** composed of 73 to 94 nucleotides in length. The structure of tRNA can be divided into its primary structure, its secondary structure (**cloverleaf structure**), and its tertiary structure (**L-shaped 3D structure**)that allows them to fit into the P and A sites of the ribosome). The cloverleaf structure becomes the 3D L-shaped structure through coaxial stacking of the helices.

The structure of tRNA composed of following:

1. The **5'-terminal** phosphate group.
2. The **acceptor stem** which is 7-base pair (bp) stem made by the base pairing of the 5'-terminal nucleotide with the 3'-terminal nucleotide which contains the CCA 3'-terminal group used to attach the amino acid. The acceptor stem may contain non-Watson-Crick base pairs.
3. The **CCA tail** is a cytosine-cytosine-adenine sequence at the 3' end of the tRNA molecule. This sequence is important for the recognition of tRNA by enzymes and critical in translation. In prokaryotes, the CCA sequence is transcribed in some tRNA sequences. In most prokaryotic tRNAs and eukaryotic tRNAs, the CCA sequence is added during processing and therefore does not appear in the tRNA gene.
4. The **D arm** is a 4 bp stem ending in a loop that often contains **dihydrouridine** termed **DHU loop.**
5. The **anticodon arm** is a 5-bp stem whose loop contains the anticodon.
6. The **T arm** is a 5 bp stem containing the sequence **TΨC** where Ψ is a **pseudouridine.**
7. Bases that have been modified, especially by methylation, occur in several positions throughout the tRNA. The first anticodon base, or wobble-position, is sometimes modified to **inosine** (derived from adenine), **pseudouridine** (derived from uracil) or **lysidine** (derived from cytosine).

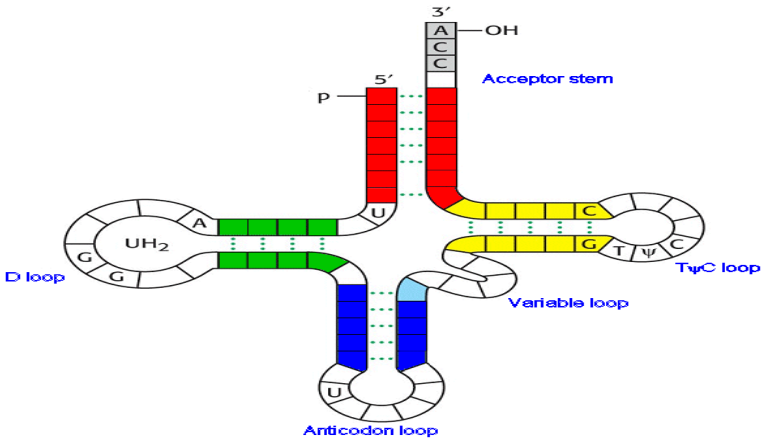


Fig- Cloverleaf structure of tRNA.

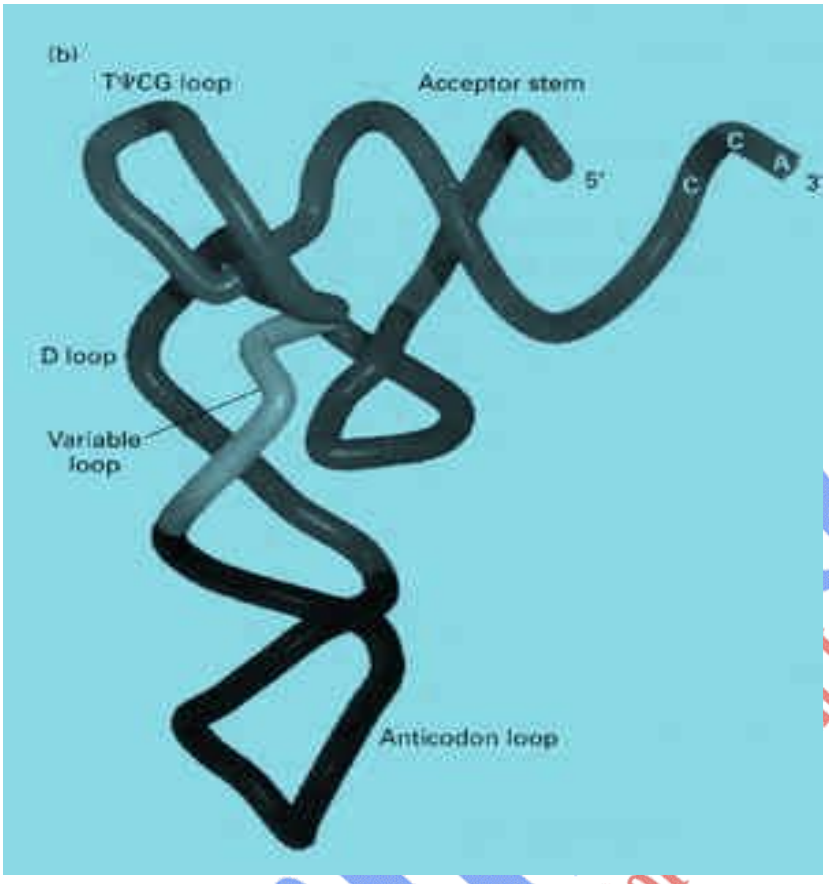


Fig- L shaped three dimensional structure of tRNA.

(d) mRNA

messenger RNA (**mRNA**) is a large family of RNA molecules that convey genetic information from DNA to the ribosome, where they specify the amino acid sequence of the protein products of gene expression.

As in DNA, mRNA genetic information is encoded in the sequence of nucleotides, which are arranged into codons consisting of three bases each. Each codon encodes for a specific amino acid, except the stop codons, which terminate protein synthesis.

Structure

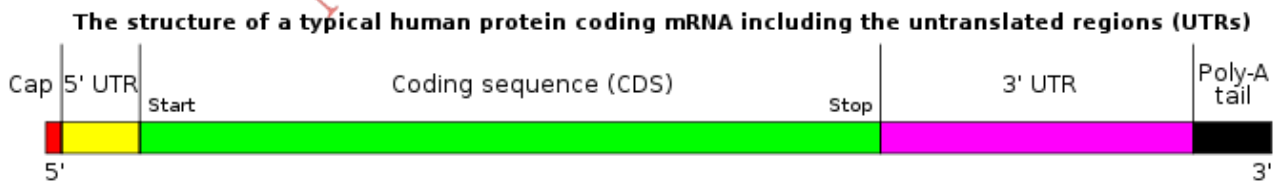


Fig: The structure of a mature eukaryotic mRNA.

Components of mRNA:

A fully processed mRNA includes a **5' cap, 5' UTR**, coding region, 3' UTR, and poly(A) tail.

5' cap

The *5' cap* is a modified guanine nucleotide added to the "front" (5' end) of the pre-mRNA using a 5'-5'-triphosphate linkage. This modification is critical for recognition and proper attachment of mRNA to the ribosome, as well as protection from 5' exonucleases. It may also be important for other essential processes, such as splicing and transport.

Coding regions

Coding regions are composed of codons, which are decoded and translated (in eukaryotes usually into one and in prokaryotes usually into several) into proteins by the ribosome. Coding regions begin with the start codon and end with a stop codon. In general, the start codon is an AUG triplet and the stop codon is UAA, UAG, or UGA. The coding regions tend to be stabilised by internal base pairs, this impedes degradation. In addition to being protein-coding, portions of coding regions may serve as regulatory sequences in the pre-mRNA as exonic splicing enhancers or exonic splicing silencers.

Untranslated regions

Untranslated regions (UTRs) are sections of the mRNA before the start codon and after the stop codon that are not translated, termed the five prime untranslated region (5' UTR) and three prime untranslated region (3' UTR), respectively. These regions are transcribed with the coding region and thus are exonic as they are present in the mature mRNA. They are responsible for mRNA stability, mRNA localization, and translational efficiency. The ability of a UTR to perform these functions depends on the sequence of the UTR and can differ between mRNAs.

Poly(A) tail

The 3' poly(A) tail is a long sequence of adenine nucleotides (often several hundred) added to the 3' end of the pre-mRNA. This tail promotes export from the nucleus and translation, and protects the mRNA from degradation.

Heterogeneity of mRNA- It refers to the two types of mRNA:

(a) Monocistronic -An mRNA molecule is said to be monocistronic when it contains the genetic information to translate only a single protein chain (polypeptide). This is the case for most of the eukaryotic mRNAs.

(b)Polycistronic mRNA- It carries several open reading frames (ORFs), each of which is translated into a polypeptide. These polypeptides usually have a related function (they often are the subunits composing a final complex protein) and their coding sequence is grouped and regulated together in a regulatory region, containing a promoter and an operator. Most of the mRNA found in bacteria and archea is polycistronic. Dicistronic or bicistronic is an mRNA that encodes only two proteins.

Section D

Photoreception

Q.1 Give a detailed account on the photoreception.

Ans **Photoreception refers** to the the biological responses of animals to stimulation by light.

In animals photoreception refers to mechanisms of light detection that lead to vision and depends on specialized light-sensitive cells called photoreceptors, which are located in the eye. The quality of vision provided by photoreceptors varies enormously among animals. For example, some simple eyes such as those of flatworms have few photoreceptors and are capable of determining only the approximate direction of a light source. In contrast, the human eye has 100 million photoreceptors and can resolve one minute of arc (one-sixtieth of a degree), which is about 4,000 times better than the resolution achieved by the flatworm eye.

Photoreceptor cells:

These are specialized type of neurons found in the retina that is capable of phototransduction. These cells can convert light (visible electromagnetic radiation) into signals that can stimulate biological processes.

These cells are of two types which provide vision in light and dark respectively:

(i) **Cones**

Cones provide vision in light (i.e. it requires a larger numbers of photons) in order to produce a signal. In humans, there are three different types of cone cell, distinguished by their pattern of response to different wavelengths of light. The three types of cone cell respond (roughly) to light of short, medium, and long wavelengths and are also responsible for the coloured vision. The different responses of the three types of cone cells are determined by the likelihoods that their respective photoreceptor proteins will absorb photons of different wavelengths. So, for example, an L cone cell contains a photoreceptor protein that more readily absorbs long wavelengths of light (i.e., more "red"). Light of a shorter wavelength can also produce the same response, but it must be much brighter to do so.

(ii)Rods

The rods are narrower than the cones and distributed differently across the retina, but the chemical process in each that supports phototransduction is similar. Rods are extremely

sensitive, and can be triggered by as few as 6 photons.These are responsible for the vision atlow light levels.

The human retina contains about 120 million rod cells and 6 million cone cells. The number and ratio of rods to cones varies among species, dependent on whether an animal is primarily diurnal or nocturnal. Certain owls, such as the tawny owl, have a tremendous number of rods in their retina. In addition, there are about 1.5 million ganglion cells in the human visual system, 1 to 2% of them photosensitive.

Structure :

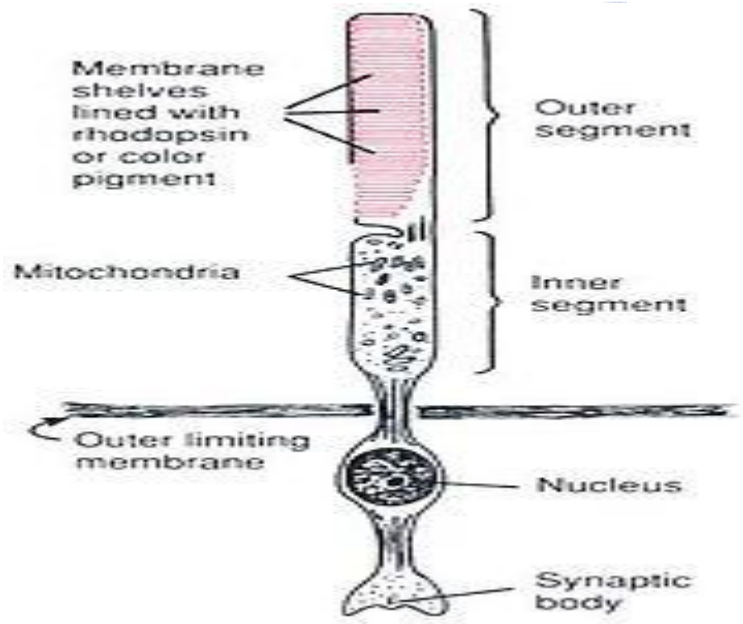


Fig: Structure of a rod cell.

Rod and cone photoreceptors are found on the outermost layer of the retina; they both have the same basic structure. Closest to the visual field (and farthest from the brain) is the axon terminal, which releases a neurotransmitter called glutamate to bipolar cells. Farther back is the cell body, which contains the cell's organelles. Farther back still is the inner segment, a specialized part of the cell full of mitochondria. The chief function of the inner segment is to provide ATP (energy) for the sodium-potassium pump. Finally, closest to the brain is the outer segment, the part of the photoreceptor that absorbs light. Outer segments are modified cilia that contain disks filled with opsin, the molecule that absorbs photons, as well as voltage-gated sodium channels.

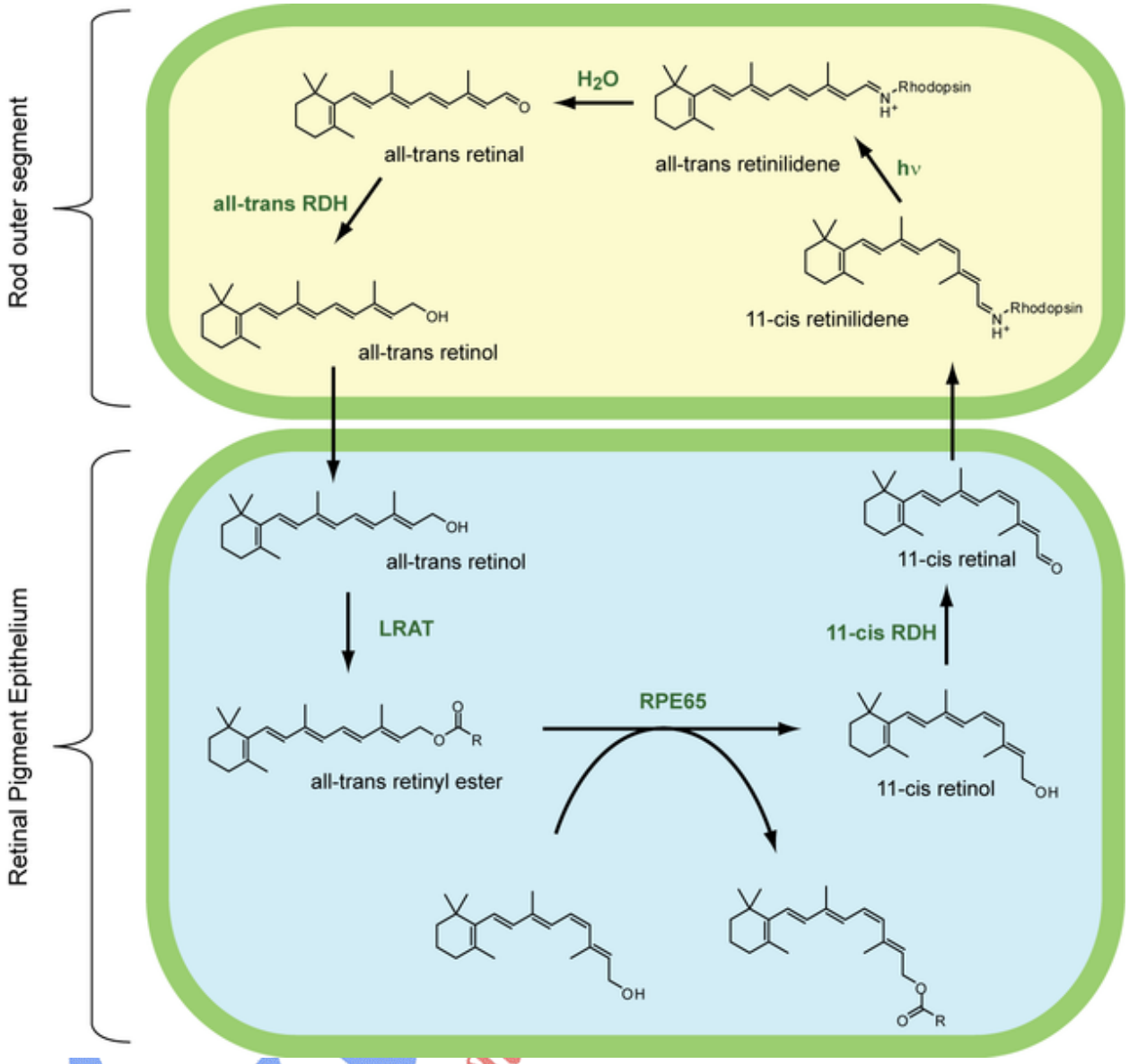
Q .2 Explain the visual cycle?

Ans. Visual phototransduction is a process by which light is converted into electrical signals in the rod cells, cone cells and photosensitive ganglion cells of the retina of the eye.

There are certain cells called photoreceptor cells that receives the photon and covert them into light. These cells consist of certain pigments that are responsible for photo transduction.The membranous photoreceptor protein *opsin* contains a pigment molecule called *retinal*. In rod cells, these together are called **rhodopsin.** In cone cells, there are different types of opsins that combine with retinal to form pigments called **photopsins**. Three different classes of photopsins in the cones react to different ranges of light frequency, a differentiation that allows the visual system to calculate color. The function of the photoreceptor cell is to convert the light energy of the photon into a form of energy communicable to the nervous system and readily usable to the organism: This conversion is called signal transduction.

The visual cycle is the biological conversion of a photon into an electrical signal in the retina. This process occurs via G-protein coupled receptors called opsins which contain the chromophore 11-cis retinal. 11-cis retinal is covalently linked to the opsin receptor via Schiff base forming retinylidene protein. When struck by a photon, 11-cis retinal undergoes photoisomerization to all-trans retinal which changes the conformation of the opsin GPCR leading to signal transduction cascades which causes closure of cyclic GMP-gated cation channel, and hyperpolarization of the photoreceptor cell.

Followed by isomerization and release from the opsin protein, all-trans retinal is reduced to all-trans retinol and travels back to the retinal pigment epithelium to be "recharged". It is first esterified by lecithin retinol acyltransferase (LRAT) and then converted to 11-cis retinol by the isomerohydrolase RPE65. The isomerase activity of RPE65 has been shown; it is still uncertain whether it also acts as hydrolase. Finally, it is oxidized to 11-cis retinal before traveling back to the rod outer segment where it is again conjugated to an opsin to form new, functional visual pigment (rhodopsin).


Rod outer segment
Retinal Pigment Epithelium
H_2O
Rhodopsin
all-trans retinilidene
all-trans retinal
all-trans RDH
all-trans retinol
hν
11-cis retinilidene
Rhodopsin
all-trans retinol
LRAT
all-trans retinyl ester
RPE65
11-cis retinal
11-cis RDH
11-cis retinol

Q.3 Define Photosynthesis. Add a note on the Z-scheme of Photosynthesis.

Ans Definition:

Photosynthesis is a process used by plants and other organisms to convert light energy, from the sun, into chemical energy that can be used to fuel the organisms' activities.

Carbohydrates, such as sugars, are synthesized from carbon dioxide and water (hence the name photosynthesis, from the Greek **phos** "light", and **synthesis.**

The general equation for photosynthesis is:

$2n\ CO_2 + 2n\ DH_2 + photons \rightarrow 2(CH_2O)_n + 2n\ DO$

Carbon dioxide + electron donor + light energy → carbohydrate + oxidized electron donor

This is one of the mode of photoreception in most plants, most algae, and cyanobacteria which perform the process of photosynthesis, and are called photoautotrophs. Photosynthesis maintains atmospheric oxygen levels and supplies all of the organic compounds and most of the energy necessary for all life on Earth.

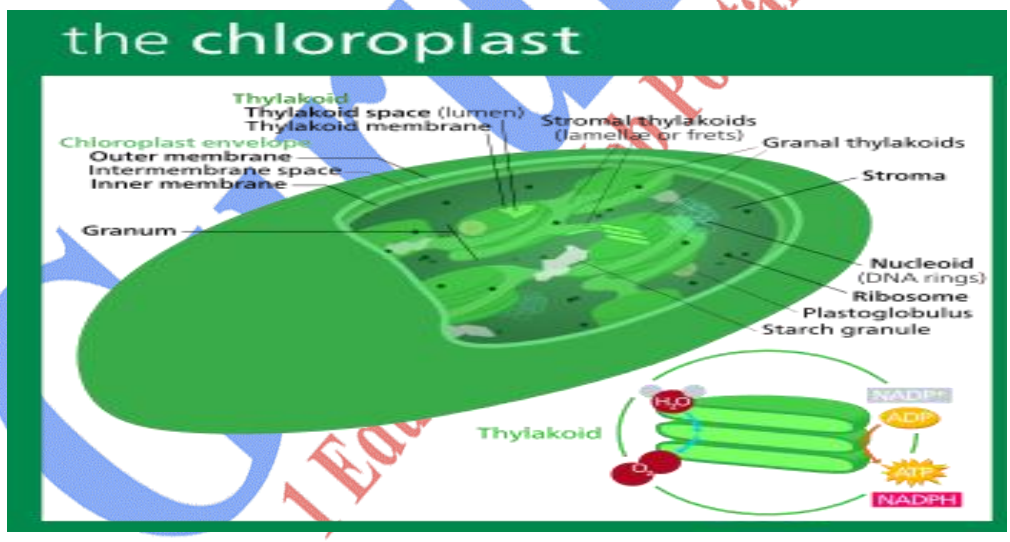
Although photosynthesis is performed differently by different species, the process always begins when energy from light is absorbed by proteins called reaction centres that contain green chlorophyll pigments. In plants, these proteins are held inside organelles called chloroplasts, which are most abundant in leaf cells, while in bacteria they are embedded in the plasma membrane. In these light-dependent reactions, some energy is used to strip electrons from suitable substances such as water, producing oxygen gas. Furthermore, two further compounds are generated: reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), the "energy currency" of cells.

In plants, algae and cyanobacteria, sugars are produced by a subsequent sequence of light-independent reactions called the Calvin cycle, but some bacteria use different mechanisms, such as the reverse Krebs cycle. In the Calvin cycle, atmospheric carbon dioxide is incorporated into already existing organic carbon compounds, such as ribulose bisphosphate (RuBP). Using the ATP and NADPH produced by the light-dependent

reactions, the resulting compounds are then reduced and removed to form further carbohydrates such as glucose.

Site and pigments for photosynthesis

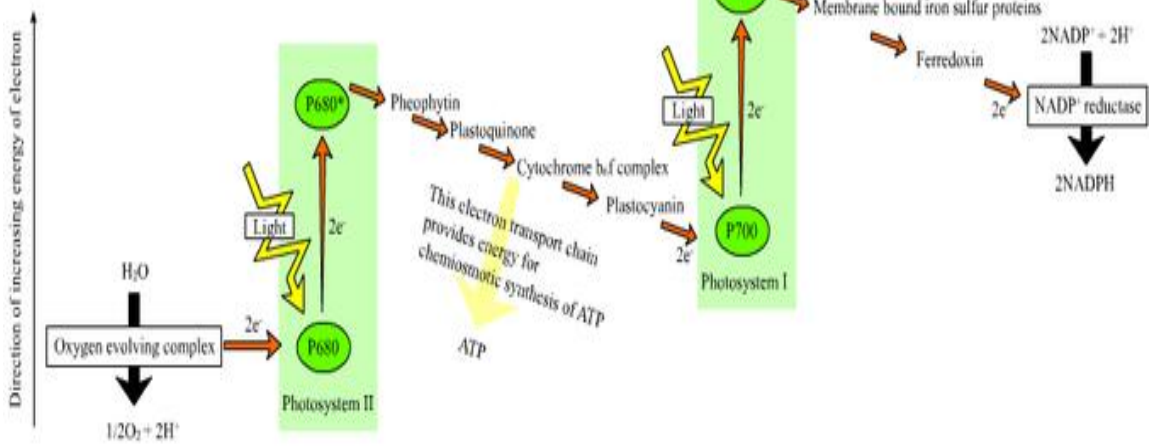
In plants and algae, photosynthesis takes place in organelles called chloroplasts. A typical plant cell contains about **10 to 100 chloroplasts**. The chloroplast is enclosed by a membrane. This membrane is composed of a phospholipid inner membrane, a phospholipid outer membrane, and an intermembrane space between them. Within the membrane is an aqueous fluid called the stroma. The stroma contains stacks (grana) of thylakoids, which are the site of photosynthesis. The thylakoids are flattened disks, bounded by a membrane with a lumen or thylakoid space within it. The site of photosynthesis is the thylakoid membrane, which contains integral and peripheral membrane protein complexes, including the pigments that absorb light energy, which form the photosystems.



Plants absorb light primarily using the pigment chlorophyll, which is the reason that most plants have a green color. Besides chlorophyll, plants also use pigments such as carotenes and xanthophylls. Algae also use chlorophyll, but various other pigments are present as phycocyanin, carotenes, and xanthophylls in green algae, phycoerythrin in red algae (rhodophytes) and fucoxanthin in brown algae and diatoms resulting in a wide variety of colors.

These pigments are embedded in plants and algae in special antenna-proteins. In such proteins all the pigments are ordered to work well together. Such a protein is also called a **light-harvesting complex.**

Z-scheme of photosynthesis:



Multiple Choice Question

Section A and Section B

Q1. Which vitamin is known as sunshine vitamin?

(a)Vitamin D (b) Vitamin B

(c) Vitamin E (d) Vitamin C

Q2. Deficiency of which vitamin causes Scurvy?

(a) Vitamin A (b) Vitamin B

(c) Vitamin E (d) *Vitamin C*

Q3. Fat soluble vitamins are

(a)*A,D,E,K* (b) A,B,C,K

(c) A,C,E,B (d) B and C

Q4. Water soluble vitamins are

(a)A,D,E,K (b) A,B,C,K

(c) A,C,E,B (d) *B and C*

Q5. Edman's reagent is

(a)PITC (b) PTH

(c)PTCA (d) None of these

Q6. Which type of bonding stabilizes the secondary structure of proteins?

(a)Hydrogen bonding (b) vander vaals interactions

(c) ionic bonding (d) covalent bonding

Q7. Wich type of bonding interactions stabilizes tertiary structure of proteins.

(a Hydrophobic interactions (b) vander vaals interactions

(c) ionic bonding (d) covalent bonding

Q8. How many amino acids are involved in formation of proteins

(a) 30 (b) *20*

(b) 25 (d) 360

Q9. Peptide bond is formed between

(a) Carboxyl group of one amino acid and amino group of another amino acid

(b) *Bet*ween two carboxyl groups

(c) Between carboxyl group of one amino acid and the side chain

(d) Between two amino groups

Q10. Beta pleated sheets are examples of

(a) primary structure (b)secondary strucure

(c) tertiary structure (d)quaternary structure

Q11. Which part of amino acid differentiate it from the other amino acid

(a) amino group (b) Carboxyl group

(c) *Side chain* (d) All of these

Q12. In a folded protein the hydrophobic amino acid lies at

(a) *inner part of the protein* (b) exposed outside

(b) distributed equally (d) distributed randomly

Q13. Which of the following specify the information about the 3d- shape of the protein

(a) peptide linkage (b) interaction with other polypeptides

(c) interaction with chaperonin (d) *proteins amino acid sequence*

Q14. What is the function of insulin

(a) agonistic to glucagon

(b) decreases storage of glycogen in the liver

(c) *reduces hyperglycemia*

(d) all the above

Q15. What is the target of ACTH

(a) *Adrenal cortex* (b) mammary glands

(b) thymus (d) all of these

Q16.Adrenaline is

(a) produced by adrenal cortex

(b) *also called as epinephrine*

(c) released when the parasympathetic nervous system gets stimulated

(d) all of these

Q17 Technique that provides information about 3-d structure of molecules

(a) *X-ray crystallographyy* (b) Agarose gel electrophoresis

(c) Scanning electron microscopy (d) confocal laser technology

Q18. The technique of X-ray crystallography is based on the principle of

(a) *Diffraction* (b) Wave optics

(b) Trasmission (d) None of the following

Q19. The source of light in the technique of X-ray crystallography is

(a) Monochrmatic (b) Multichromatic

(c)Dichromatic (d) All of these

Q20. Which of the following act as a cofactor

(a) Iron- sulfur cluster (b) Mg^{2+}

(c) Cu^{2+} (d) *All of these*

Section C

Q 1. The physiological form of DNA is

(a) A (b) *B* (c) C (d) D

Q 2. Which form of DNA is left handed

a) C (b) D (c) B (d) *Z*

Q 3. The constituent of nucleic acid include

(a) *Phosphate; nitrogen base; pentose sugar*

(b) Phosphate; nitrogen bases; hexose sugar

(c) Phosphorus; nitrogen bases; pentose sugar

(d) Phosphorus ; nitrogen bases; hexose sugar

Q4.The difference between RNA and DNA is

(a) Instead of Uracil DNA have Thymine

(b) RNA is single stranded while DNA is double stranded

(c) The sugar in DNA is deoxyribose while in RNA is Ribose

(d) All of these

Q5. DNA consist of following bases

(a) U;T;A;G

(b) A;T;G;C

(c) A;U;G;C

(d) None of these

Q6. Which of the following type of RNA carries information for protein formation

(a) rRNA (b) tRNA (c) *mRNA* (d) SnRNA

Q7. The double helical structure of DNA was proposed by

(a) *Watson and Crick* (b) Friedrich Miescher

(c) Griffith (d) None of these

Q8. DNA is stabilized by which type of bonding

(a) Hydrogen bonding (b) Phosphodiester linkage

(c) Both (a) and (b) (d) None of these

Q9. Highest number of bases are found in which type of DNA

(a) A (b)B

(c)C (d) *Z*

Q10. DNA stands for

(a) Deoxyribose nucleic acid (b) *2'D- deoxyribose nucleic acid*

(c) 3'deoxyribose nucleic acid (d) None of these

Q11. Which of the following can exist in cytoplasm in free state

(a) DNA (b)*RNA*

(c) DNA and RNA both (d) Noe of these

Q12. Which of the following RNA is also called as insoluble RNA

(a) mRNA (b) *Rrna*

(c) tRNA (d) hnRNA

Q13. Which type of RNA is most stable

(a) mRNA (b) tRNA

(c) *rRNA* (d) All are equally stable

Q14. The secondary structure of tRNA is

(a) *Clover Leaf shaped* (b) L-shaped

(c) U-shaped (d)None of these

Q15. How many loops are there in tRNA

(a) 2 (b) *3* (c) 5 (d) 6

Q16. The base sequence of acceptor arm is

(a) ACC (b) *CCA*

(c) AGC (d) GACC

Q17. Which type of RNA carries anticodon

(a) mRNA (b) rRNA

(c) *tRNA* (d) hnRNA

Q18. Which type of RNA carries codon

(a) *mRNA* (b) rRNA

(c) tRNA (d) hnRNA

Q19. mRNA should be

(a) Heterogeneous (b) Non-heterogeneous

(c) Mixed (d) Homogeneous

Q20 Which of the following is a type of active membrane transport system

(a) Osmosis (b) Diffusion

(c) *Na^+ / K^+ pump* (d) Facilitated diffusion

Q21. Which of the following type of membrane transportation process requires ATP

(a)*Active* (b) Passive

(c) Cotransport (d) All of these

Q22. The concentration of sodium is higher in

(a)ECF (b)ICF

(c) Equal in both (d) depending upon the cell type

Q23. Which type of membrane transport requires carrier poteins

(a) osmosis (b) diffusion

(c) facilitated diffusion (d) All of these

Q24. The value of resting membrane potential is

(a)-70mV (b) -75mV

(c) -80mV (d)- 60mV

Section-D

Q1. Photosynthesis occurs in which of the following

- Plants, bacteria, fungi, algae(b) Plants, bacteria, fungi (c) *Plants, bacteria, algae*(d) Plants and bacteria

Q2. Photosynthetic organisms are called as

- *Photoautotrophs* (b) Heterotrophs (c)Saprotrophs(d) All of these

Q3. Which of the following is a photosynthetic pigment

(a) Chlorophyll (b) Xanthophyll (c) Carotenes (d) All of these

Q4. Which of the follwing pigment out of these is universal in its occurrence?

(a) Chlorophyll a (b) Chlorophyll b (c) Pheophytin (d) none of these

Q5. The cells that are responsible for vision in dark?

(a) *Rhods* (b) Cones (c) Both (d) None

Q6. The cells that are responsible for vision in vision?

(a) Rhods (b) *Cones* (c) Both (d) None

Q7.Which pigment is found in rhods

(a) Photopsin (b)Scotopsin (c) *Rhodopsin* (d) All

.Q8. 11-cis retinal photoisomerisation to

(a) *all trans retinal* (b) 11-trans retinal (c) 5-cis retinal(d)None

Q9. Which vitamin helps in the process of vision

(a)*Vit A*(b)Vit B (c) Vit C(d) VitD

Q10. In fluorescence the wavelength and energy of light absorbed is

(a) Energy higher and wavelength less in radiations emitted

(b) *Energy lesser and wavelength higher in radiations emitted*

(c) Remains unchanged

(d) none of these

Glossary-molecular biophysics

Adenine (A): A base; one of the molecular components of DNA and RNA. Bonds with thymine (A-T) in DNA, and with uracil (A-U) in RNA.

Amino acids: The basic building blocks of proteins. There are 20 different amino acid types. Each protein consists of a different sequence of amino acids linked together according to the genetic information encoded in DNA.

Bases: Nitrogenous compounds that interact to form the molecular building blocks of DNA and RNA: adenine, cytosine, guanine, thymine, and (in RNA only) uracil.

Base pair: Two of the building blocks of DNA held together by hydrogen bonds. In a DNA molecule, adenine always bonds with thymine (A-T), and cytosine always bonds with guanine (C-G). In RNA, A binds to uracil (A-U).

Base sequence: The order of bases in a DNA molecule.

DNA (deoxyribonucleic acid): The long, spiralling molecule that orchestrates the cell's daily operations and provides the genetic blueprint for the physical characteristics of all living organisms. When made up of two complementary strands, the strands intertwine like a spiral staircase to form a structure called a double helix. Subunits, called bases, are the rungs of the staircase. See also RNA.

DNA replication: The use of existing DNA as a template for the synthesis of new DNA strands.

DNA sequence: The relative order of base pairs in any sample of DNA. See base sequence analysis.

Double helix: A common name for DNA; it refers to the molecule's double-stranded, spiraling structure.

Enzyme: A protein that acts as a catalyst, speeding the rate at which a biochemical reaction proceeds but not altering the direction or nature of the reaction. All cellular metablolism is controlled by enzymes.

Guanine (G): A base; one of the molecular components of DNA and RNA. Always bonds with cytosine (G-C).

Messenger RNA (mRNA): A single-stranded molecule of ribonucleic acid that directs protein production.

Mutation: A spontaneous or induced change in the DNA of a cell.

Nucleotide: A molecular subunit of DNA or RNA consisting of a base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA). Thousands of nucleotides are linked to form a DNA or RNA molecule.

Polymerase: Polymerase is an enzyme that acts like a molecular assembly line to build new strands of DNA.

Polypeptide: A molecule made up of a string of amino acids. A protein is an example of a polypeptide.

Proteins: The active molecules in all cells. Proteins control biochemical reactions and determine the physical structure of organisms.

Ribonucleic acid (RNA): A chemical cousin of DNA, RNA (ribonucleic acid) is responsible for translating the genetic code of DNA into proteins.

Ribosomal RNA (rRNA): A class of RNA found in the ribosomes of cells.

Ribosome: The small cellular structure in which RNA translates the genetic code into proteins.

Template: a single DNA strand that serves as pattern for building a new second strand.

Thymine (T): A base; one of the molecular components of DNA and RNA. Always bonds with adenine (T-A).

Transcription: The process by which DNA passes genetic information to RNA. Transcription is the first step in producing proteins.

Transfer RNA (tRNA): A class of RNA that carries amino acids into ribosomes and bonds with mRNA for protein production.

Translation: The process by which RNA makes proteins.

Uracil (U): A base; one of the molecular components of RNA. Bonds only with adenine (U-A).