A Handbook of Biological Macromolecules

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Volume 4 Issue 1

Received Date: June 04, 2024

Published Date: July 05, 2024

Published by

Academic Strive

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Chapter-1

Structure of Cell

Different living matters are made up of six primary components- carbon, oxygen, hydrogen, phosphorus, sulfur and nitrogen that constitute approximately 90% of the human body's dry weight. In addition, the cells contain Ca, K, Fe, Cu, Zn, F, Na, Se and Cl [1].

Predominant Element of Cell-Carbon

Carbon is the most unique and versatile element present in the cell because of its one of the property to form abundant number of biomolecules. This is due to formation of stable covalent bonds, therefore, the infinitely long C-C chains. Majority of compounds in living organisms possess carbon as one of the element [1].

Chemical Biomolecules (Macromolecules)

Cell possesses lifeless biomolecules which are organic in nature including monosaccharides, amino acids and nucleotides that are monomeric units for the formation of carbohydrates, proteins and nucleic acids (DNA and RNA), respectively. These macromolecules upon organization lead to the formation of supramolecular assemblies that form organelles, cells, tissues, organs and eventually, the whole organism on further arrangement. Protein forms the structural and functional entity (including both static and dynamic) of cell. Deoxyribonucleic acid (DNA) is the primary repository for genetic information. Ribonucleic acid (RNA) is required for protein synthesis. Carbohydrates are the stored form of energy (glycogen) and additionally, form the structural entity of the cell. Lipids form biomembrane part as well as remain store in body for meeting the long term energy demand [1].

Cell: Structural and Functional Unit

Water is the life solvent that constituents even greater than 60% of the weight. Followed by this, protein, primarily in muscles and fat or lipids, mainly of adipose tissue. Rather carbohydrate content is lowest that too is in form of glycogen. It may vary according to physiological state of the person. However, cell is the fundamental, structural and functional unit of the life. Cell concept is mainly provided by Schleiden and Schwann (1838). But, it was about 100 years later, cell structure complexities were exposed. Cell contains different subcellular organelles inside it which can be possibly segregated by differential centrifugation and wrapped by plasma membrane. Despite this, various enzymes are present within these organelles. Diagrammatic representation of eukaryotic cells is given in Figure 1.1 [1].

Cells are Characterized into Two Divisions:

1. Prokaryotes (pro-before, karyon-nucleus; Greek): these can be defined without nucleus with simple structure, e.g., bacteria
2. Eukaryotes (eu-true, karyon-nucleus; Greek): these can be defined with nucleus and are highly complicated in structure and function, e.g., plants and animals.

The human body is comprised of approximately 10^{14} cells with about 250 types of specific different cells, e.g., nerve cells, β-cells of pancreas, muscle cells and erythrocytes. The peculiar difference between animal and plant cell is of cell wall, particularly cellulose and chloroplasts in plant. The rest other points for comparison between plant and animal are mentioned in the Table 1.1 [1].

Property	Prokaryotic Cell	Eukaryotic Cell
Size	Small (1-10 μm)	Large (10-100 μm)
Cellular membrane	Rigid cell wall	Flexible plasma membrane
Sub-cellular organelles	Not present	Distinct types (Golgi bodies, lysosomes, etc.)
Nucleus	Undefined; present as nucleoid	Well defined with surrounding membrane
Histones	Absent	DNA remain attached with histones
Metabolism	Enzymes remain bind to membrane	Enzymes are found in mitochondria
Cell division	Fission	Mitosis
Cytoplasm	Organelles and cytoskeleton are absent	Contain tubules and filaments network

Table 1.1: Difference between prokaryotic and eukaryotic cells [1].

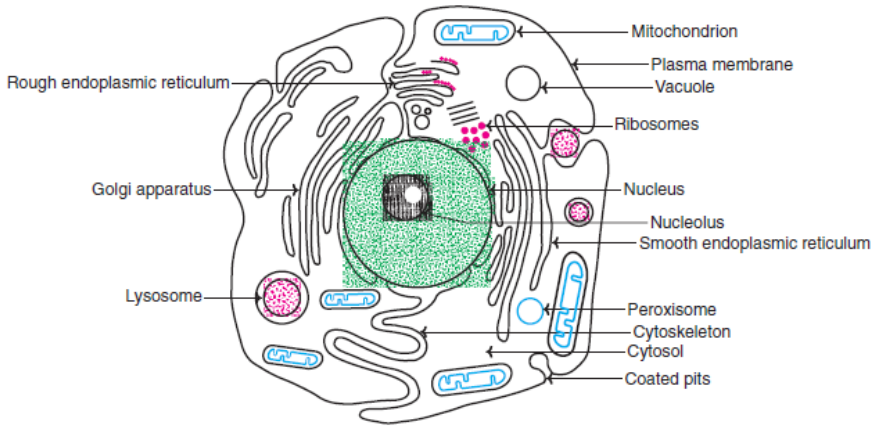


Figure 1.1: Structure of eukaryotic cell [1].

Nucleus

It is the largest organelle with a double membrane called as nuclear envelope. The both nuclear membranes possess pores (approximately 90 nm in diameter) at particular intervals, in fact, the outer layer involve in continuity with the membranous structure of endoplasmic reticulum (Figure 1.1). With the help of these pores, different types of products from nuclear origin can be transported into the cytoplasm. Nucleus possesses the most critical genetic information repository, the DNA. In case of eukaryotes, DNA remains associated with histones; basic proteins (1:1) for the formation of nucleosomes. These nucleosomes collaborate further to form chromatin fibres of chromosomes. Therefore, a human chromosome contains around a million nucleosomes. Different species possess different number of chromosomes that specify a species. Human possesses 23 pairs of chromosomes tightly arranged in the nucleus. Eukaryotic cell nucleus possesses a dense structure called as nucleolus. It contains enrich amount of RNA, mainly ribosomal RNA which passes through nuclear pores in to the cytosol. The ground substance inside nucleus is defined as nucleoplasm which is rich in DNA and RNA polymerases. Hutchinson-Gilford progeria syndrome is an unusual situation of aging that concept at birth. This is due to deformation of nuclear envelope by the assimilation of abnormal lamina A protein [1].

Mitochondria

Mitochondria are the primary site for cell respiration and metabolism, therefore, it is considered as cellular power house. These are of various size and shape, e.g., filamentous bodies or rod-like. One cell possesses approximately 2,000 mitochondria that occupy nearly 1/5th of the whole cellular volume. The mitochondria are double membrane structure with smooth outer membrane and folded inner membranous structure known as cristae to provide greater surface area (Figure 1.1). The internal material of mitochondria is called as mitosol or matrix. Different constituents of oxidative phosphorylation and electron transport chain, i.e. cytochromes b, c_1, c, a and a_3, flavoprotein and coupling factors are embedded in to the inner mitochondrial membrane. The inner matrix possesses different enzymes for carbohydrates, amino acids and lipid catabolism as well as anabolic enzymes for heme and urea. ATPs are primarily produced in mitochondria which are energy currency of the aerobic cells. Despite this, matrix possesses single circular double stranded DNA, RNA and protein synthesizing machinery known as ribosomes with the assistance of which 10% of the proteins are synthesized inside the mitochondria. Prokaryotic cells display identity with mitochondria with respect to its structure and function. Hence, eukaryotic mitochondria have been evolved from aerobic bacteria during the process of evolution [1].

Endoplasmic Reticulum

The membrane encased spaces that form a network which remains elaborated through cytoplasm composes endoplasmic reticulum (ER). Few of these structures that are thread like stretch from nuclear pores to the plasma membrane. Most of the part of ER which remains studded with ribosomes to provide a granular appearance that is known as rough endoplasmic reticulum. However, while performing cell fractionation, rough ER gets deteriorated to form tiny vesicles called as microsomes which are not found usually in the cell. Other than this, smooth endoplasmic reticulum lacks ribosomes because that is involved in the lipids synthesis involving triacylglycerols, sterols and phospholipids and besides this, drugs metabolism and supplying Ca^{2+} [1].

Golgi Apparatus

Eukaryotic cells possess a specific membrane vesicles' cluster called as dictyosomes that further assemble to

form Golgi complex or Golgi apparatus. The primary work is that newly synthesized proteins are pushed towards the Golgi complex for the addition of different moieties to the proteins such as carbohydrates, sulfate and lipids. Such types of changes are required for the proteins transport across the plasma membrane. Many enzymes and proteins are encased inside the vesicles of Golgi complex which are later secreted from cell on receiving appropriate signals, e.g., pancreatic digestive enzymes. It is also needed for synthesis of membrane, especially, for the intracellular organelles formation (e.g. lysosomes, peroxisomes) [1].

Lysosomes

Lysosomes are single membrane enveloped spherical vesicles. These are actively involved in cellular substances digestion such as carbohydrates, lipids, nucleic acids and proteins, therefore, these are considered as cellular digestive tract. This is because of presence of hydrolases' enzymes involving lipases (lipids), cathepsins (proteins), D-glucosidase (glycogen) and ribonucleases (RNA). The cells' compounds remain in dynamic state due to lysosomal enzymes via recycling and degradation. Lysosomes' degraded products undergo diffusion in to surrounding for the further utility for the cell. Some residual components enrich in proteins and lipids, collaborately called as lipofuscin get accumulated in the cell. This is wear and tear or ageing pigment involved in ageing process. With cell death, hydrolytic enzymes get released from lysosomes that lead to post-morteum autolysis. Therefore, for the protection of cells, digestive enzymes remain inside the lysosomes. As a consequence of enzymatic release, the different diseases such as arthritis, allergic disorders and muscle diseases may emerge out in the body. Inclusion cell desease is one of the rare situations that occur due to the absence of certain lysosomal hydrolases. This is due to protein targeting defect since enzymes unable to reach lysosomes [1].

Peroxisomes

These are single membrane bound bodies, also called as microbodies. These are oval or spherical shape bodies that possess catalase enzyme. This enzyme is required by the cell to safeguard it from the toxic, H_2O_2 by catalyzing it to H_2O and O_2. These are also required for long chain fatty acids (> C_{18}) oxidation and glycolipids and plasmalogens synthesis. Plants' specialized peroxisomes also called glyoxysomes undergo glyoxylate pathway. Peroxisome biogenesis disorders, which involve enhanced concentration of very long chain fatty acids (C_{24} and C_{26}) and reduced levels of plasmalogens. Its most severe type is Zellweger syndrome which is characterized by functional peroxisomes absence. The sufferers may meet death within one year of birth [1].

Cytosol and Cytoskeleton

Cell matrix is defined as cytosol and it contains different types of enzymes, salts and metabolites in an aqueous medium which is gel like. Additionally, it possesses the protein filaments network which is spread all around and is defined as cytoskeleton. Cytoplasmic filaments mainly categorized in to three types- actin filaments, microtubules and intermediate filaments. These filaments are required for shape, structure and organization inside the cell [1].

Cellular Functions Integration

Abundant complicated functions or reactions are continuously performed inside the eukaryotic cells to maintain tissues and eventually, the whole organism well-being. Therefore, the different biochemical reactions or processes are regulated greatly. For instance, during digestion, release of different enzymes or cellular division. However, natural cell death is defined as apoptosis which is programmed and tightly regulated process. On the other side, necrosis, e.g. cellular death by radiation and anoxia injury [1].

Chapter 2

Macromolecule in Cell: Carbohydrates

Carbohydrates

These are the most surplus molecules and organic in nature. Sugars mainly comprise of three different elements; carbon, hydrogen and oxygen. Carbohydrates are defined as hydrates of carbon. Few carbohydrates have empirical formula of $(C.H_2O)_n$ where n≤3 that clearly indicates these as carbon hydrates. Exception to this, some non-carbohydrate compounds, e.g. lactic acid, $C_3H_6O_3$ and acetic acid, $C_2H_4O_2$ that appears as carbon hydrates. Amazingly, some of the carbohydrates are not fulfilling to this criterion, e.g., deoxyribose, $C_5H_{10}O_4$; rhamnohexose, $C_6H_{12}O_5$. Therefore, to be more specified, these are polyhydroxyaldehydes or ketones or are compounds which lead to these on hydrolysis. In addition to this, these are water soluble and sweet in taste [1].

Carbohydrates Classification

These are also known as saccharides (sakcharon-sugar; Greek). These are categorized in to three main types: monosaccharides, oligosaccharides and polysaccharides [1].

Monosaccharides: These are the simplest type of carbohydrates and are known as simple sugars. These possess the general formula $C_n(H_2O)n$ and therefore, these cannot be hydrolysed further. The monosaccharides are categorized in to various groups depending on the number of carbon atoms and functional group.

Based on functional group:

- **Aldoses :** It possess the functional group; an aldehyde (-CH=O), e.g., glyceraldehyde, glucose.
- **Ketoses:** It possess the functional group; a keto (C=O group), e.g., dihydroxyacetone, fructose.

Based on the number of carbon atoms: Trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C). The naming of monosaccharides is given based on these terms and functional groups. For example, fructose is called as ketohexose and glucose is referred as aldohexose [1].

Oligosaccharides (Oligo-Few; Greek): It possesses the 2-10 monosaccharide molecules. These are subcategorized further in to disaccharides, trisaccharides and so on [1].

Polysaccharides (Poly-Many; Greek): These are polymers of high molecular weight (about a million), monosaccharide units. These are tasteless and form colloids on exposure to water. These are further of two types: homopolysaccharides and heteropolysaccharides [1].

Stereoisomerism

It is an essential property of monosaccharides. These are the compounds with identical structural formulae, however these differ in spatial configuration. A carbon is defined as asymmetric when it is found to be attached with four various atoms or groups. The number of isomers can be calculated using the formulae, 2^n where n is the number of asymmetric carbon atoms, e.g., Glucose possesses 4 asymmetric carbon atoms, and hence, possesses 16 isomers [1].

D- and L-Isomers

Spatial orientation of OH and H groups on the C5 (5th carbon atom for glucose) that is near to terminal alcoholic carbon determines the D- or L-isomer of sugar. D-series possess the OH group on the right side and L-series on the left side. D- and L-glucose structures depend on the reference, D- and L-glyceraldehyde monosaccharide as shown in Figure 2.1 [1].

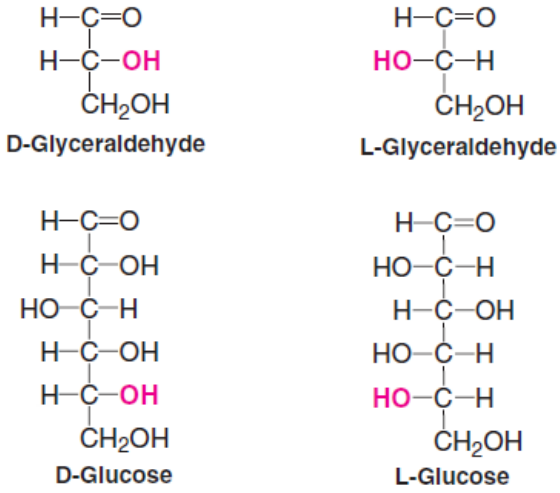


Figure 2.1: Structure of D- and L-Glucose and comparable position of –OH group [1].

Sugars' optical activity

This feature is mainly possessed by compounds containing asymmetric carbon atom. When polarized light beam is passed through optical isomer solution, the isomer gets rotated either to the left or right. The compounds that rotate the plane of polarized light to the right is called as dextrorotatory (d+) and that rotate the plane of polarized light to the left is called as levorotatory (l–) [1].

Racemic Mixture

Equal concentration of d- and l-isomers makes dl mixture or racemic mixture. It does not possess any optical activity because dextro- and levorotatory forms cross cancel their activity. The term dextrose is known for glucose solution, especially due to dextrorotatory activity of glucose [1].

Epimers

Two monosaccharides differ with respect to configuration about a single specific carbon (other than anomeric atom) are known as epimers to each other. For example, glucose and galactose differs with respect to carbon 4, therefore, are known as C4-epimers. Glucose and mannose differs with respect to carbon 2, hence, are known as C2-epimers [1].

Enantiomers

These are special group of stereoisomers that are mirror images for each other. Glucose enantiomers are shown in Figure 2.2. D- and L-sugars structures are depicted in Figure 2.3. Most of the carbohydrates present in the higher animals including man are D-isomers. In contrast to this, diastereomers are the stereoisomers that are not mirror forms of each other [1].

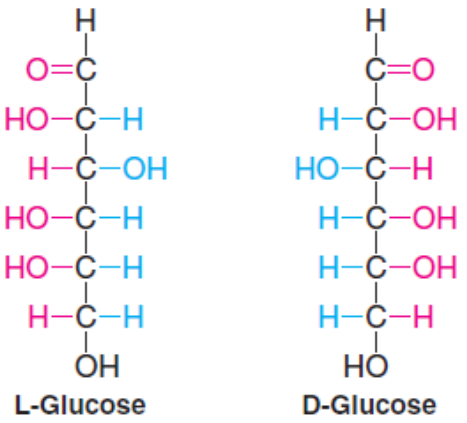


Figure 2.2: Structure of D- and L-Glucose [1].

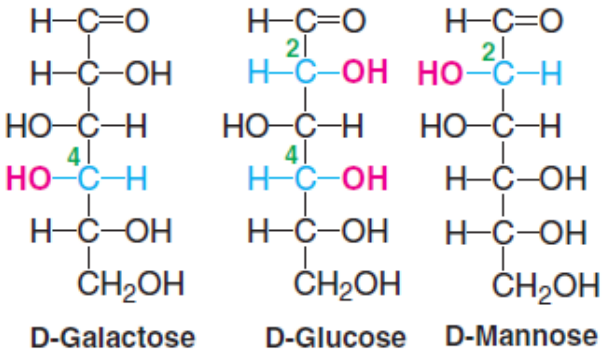


Figure 2.3: Epimers of Glucose; Galactose and Mannose [1].

Anomers

The cyclic α and β forms for D-glucose are referred to as anomers. Both forms differ at the level of C1 are called as anomeric carbon or hemiacetal carbon. For D-anomer, the OH group is present opposite to the CH_2OH of sugar ring that is bound on the anomeric carbon. This is reverse in case of β-anomer. In case of anomers, difference also exists in case of physical and chemical properties [1].

Mutarotation

The α- and β- glucose anomers possess the different optical rotations. The specific optical rotation for glucose freshly prepared solution is +112.2° which changes steadily and achieves an equilibrium of +52.7°. However, in the presence of alkali, optical rotation reduces rapidly. β-glucose optical rotation is+18.7°. It is defined as specific optical rotation change that shows the interconversion of α and β form to the

equilibrium [1].

Diasscharides

These contain two monosaccharide units, dissimilar or similar which are bound together via a glycosidic bond. Such types of sugars are water-soluble, crystalline and sweet in taste. Structures of few disaccharides are shown in Figure 2.4. These are further subdivided in to two groups:

1. Reducing sugars are those that possess the free keto or aldehyde group, e.g., lactose, maltose.
2. Non-reducing disaccharides do not possess the free keto or aldehyde group e.g. trehalose, sucrose [1].

Maltose

Maltose contains two α-D-glucose units bind by α (1→ 4) glycosidic bond. The presence of free aldehyde group on first carbon of second glucose is responsible for reducing reactions and osazone formations (sunflower-shaped). It can be hydrolysed using dilute acid or maltase enzyme to form 2 molecules of α-D- glucose. Whereas, in case of isomaltose, the glucose units bind by α (1 → 6) glycosidic linkage. Similar to maltose in structure, cellobiose, an another disaccharide, however, it possesses the β (1 → 4) glycosidic linkage. On hydrolysis of cellulose, cellobiose is formed [1].

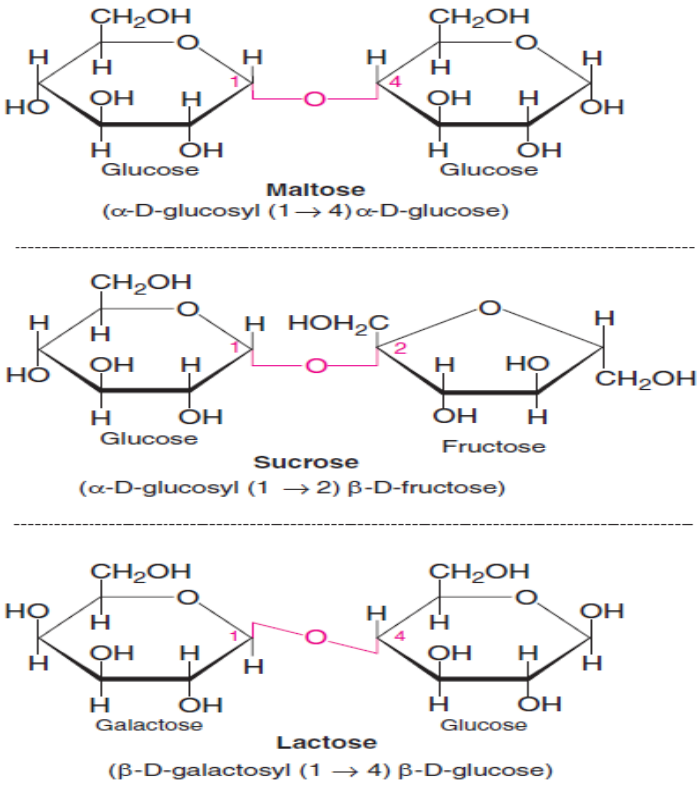


Figure 2.4: Structure maltose, sucrose and lactose [1].

Sucrose

It is a commercial sugar which is produced by sugar beets and sugar cane. It consists of α-D-glucose and β-D-fructose. Both units are bind by a glycosidic bond (α1→β2) between first carbon of D-glucose and second carbon of β-fructose. Since the reducing groups of both units are involved for glycosidic bond, therefore, it is a non-reducing sugar and do not form osazones. It is sweeter to glucose, maltose and lactose, except fructose and hence, it is used as sweetening agent in food industry. Enzyme, sucrase hydrolyses sucrose to glucose and fructose so that it can be absorbed easily [1].

Sucrose Inversion

Sucrose is dextrorotatory (+66.5°), however, on hydrolysis it turns to levorotatory (-28.2°). This process of optical rotation change from dextrorotatory (+) to levorotatory (-) is known as inversion. The invert sugar possesses glucose and fructose that is obtained after the hydrolysis of sucrose (either by invertase (sucrase) or dilute acid). Sucrose primarily breakdown into α-D-glucopyranose (+52.5°) and β-D-fructofuranose which are dextrorotatory. But, due to less stability of β-D-fructofuranose, it get converted to strongly levorotatory, β-D-fructopyranose (-92°). Eventually, the

dextro sucrose (+66.5°) form levo sucrose (-28.2°) [1].

Lactose

It is commonly known as milk sugar because it is found in milk. It is comprised of β-D-galactose and β-D-glucose bind by β (1→4) glycosidic bond. However, the anomeric first carbon is free; therefore, it possesses the reducing characteristics and forms osazones (hedgehog or powder-puff structure) [1].

Polysaccharides

Polysaccharides are composed of monosaccharides or their derivatives repeat units bind by glycosidic bonds. These are primarily known for two essential functions-storage of energy and structure formation. These are both linear as well as branched polymers. In contrast to this, proteins and nucleic acids possess only linear polymers. The branches can be formed at any of the hydroxyl groups in a monosaccharide [1].

These are of two types:

1. Homopolysaccharides: these form only one type of monosaccharide. Their naming is based on the monosaccharide nature. Hence, glucose polymers are called as glucans, whereas, fructose polymers are called as fructosans.
2. Heteropolysaccharides: these on hydrolysis provide few monosaccharides or their derivatives mixture [1].

Homopolysaccharides

Starch: It is the reserve carbohydrate of plants and is required by higher animals including man. Starch is found in great content in roots, tubers, vegetables, cereals etc. It is a homopolymer of D-glucose units bind by α-glycosidic bonds. It is called as glucan or glucosan. Starch is composed of two polysaccharide units- amylose (15-20%) which is water soluble, while amylopectin (80-85%) which is water insoluble. Amylose is a long chain which is unbranched with about 200-1,000 α-glucose molecules bind by α (1→ 4) glycosidic linkages. In contrast to this, amylopectin possesses the branched chain with branches containing α (1→ 6) glycosidic bonds and α (1→ 4) linkages in straight chains. Structure of amylose and amylopectin is shown in Figure 2.5. Starch on hydrolysis by pancreatic or salivary amylases release dextrins and eventually, glucose and maltose units. During hydrolysis process, various intermediates such as soluble starch which is blue in color, erythrodextrin displays red color, amylodextrin shows violet color and achrodextrin do not display any colour [1].

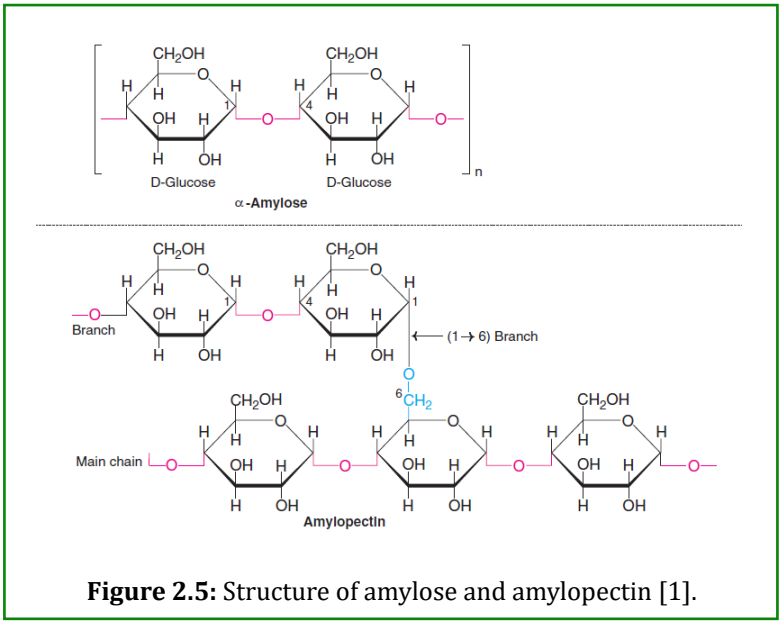


Figure 2.5: Structure of amylose and amylopectin [1].

Dextrans: These are glucose polymers which are produced by the help of microorganisms. These are plasma volume expanders during chromatography (e.g. gel filtration) and transfusion [1].

Inulin: It is a fructose polymer i.e., fructosan. It is mainly found in onion, garlic, dahlia bulbs etc. it is water soluble and possesses low molecular weight (about 5,000). Body cannot utilize this. It is required for measuring kidney functioning via the analysis of glomerular filtration rate (GFR) [1].

Glycogen: It is reserved carbohydrate food in animals; therefore, it is commonly named as animal starch. Its highest concentration is found in liver, thereafter, muscle, brain etc. It is present in plants lacking chlorophyll, e.g., fungi, yeast. Glycogen structure is identical to amylopectin except the presence of multiple branches. Glucose molecules are the key entity for glycogen bind together by α (1→ 4) glycosidic

bonds and at branching possesses α (1→ 6) glycosidic bonds. However, the variability exists at the level of glucose units' number and molecular weight based on source [1].

Cellulose: It is found particularly in plants and most abundantly as an organic material. It is predominant part of cell wall. However, it is absent in animals. It is comprised of β-D-glucose units joined by β (1→ 4) glycosidic bonds. Amazingly, it cannot be digested by man and rest mammals because of absence of β-glycosidic bonds' cleaving enzymes. However, few herbivorous and ruminants animals possess gut microorganisms for producing β-glycosidic bonds cleaving enzymes. Cellulose on hydrolysis provides cellobiose, a disaccharide, thereafter, β-D-glucose. In addition to this, it is a main component present in fibers which is non-digestable. Fibers reduce the intestinal cholesterol and glucose absorption and enhance feces bulk [1].

Chitin: It is comprised of N-acetyl D-glucosamine bind by β (1→ 4) glycosidic bonds. It is predominant in some invertebrates' exoskeleton, therefore, it is structural polysaccharide, e.g., crustaceans and insects [1].

Heteropolysaccharides

These polysaccharides are comprised of different sugars or their derivatives, hence, these are called as heteroglycans or heteropolysaccharides [1].

Mucopolysaccharides

These are heteroglycans of different repeating units such as uronic acids and amino sugars. These are commonly called as glycosaminoglycans (GAG). In this structure, acetylated amino groups other than carboxyl and sulfate group are present. Carboxyl and sulfate group are responsible for the molecules acidity, therefore, these are known as acid mucopolysaccharides. Few mucopolysaccharides are present with proteins' combination that leads to the formation of proteoglycans or mucoids or mucoproteins. Mucoproteins may possess up to 5% protein and 95% carbohydrate. Tissue contains mucopolysaccharides as a critical part. Tissue extracellular spaces that include skin, cartilage, tendons and blood vessels contain elastin fibers and collagen rooted in ground matrix substance. Ground matrix specifically possesses GAG. For instance, chondroitin 4-sulfate, hyaluronic acid, heparin, keratan sulfate and dermatan sulfate [1].

Hyaluronic Acid: It is predominantly present in synovial fluid's ground substance of joints and eyes' vitreous humor. It is also connective tissues' ground substance and builds a gelly layer of ovum. It is a lubricant and joints' shock absorbant. It is comprised of alternate N-acetyl D-glucosamine and D-glucuronic acid bind by β (1→ 3) glycosidic bond. Hyaluronidase enzyme break downs β 1→ 4 linkages of GAGs and hyaluronic acid. It is available naturally in seminal fluid, testes and in few insect and snake venoms. This enzyme in semen plays a critical role in fertilization by clearing ovum's hyaluronic acid and sperm can easily penetrate the ovum. In case of bacteria, this assists for their penetration inside tissue [1].

Chondroitin Sulfates: It is also known as Chondroitin 4-sulfate and is mainly found in different mammalian tissues such as valves, bone, tendons, skin, cartilage, heart etc. it is comprised of N-acetyl D-galactosamine 4-sulfate and D-glucuronic acid. It is identical in structure to that of hyaluronic acid [1].

Heparin: It is an anticoagulant present in blood, kidney, lung, spleen and liver etc. It assists in the lipoprotein lipase release that clears the lipemic plasma turbidity. It is comprised of glucuronate 2-sulfate and N-sulfo D-glucosamine 6-sulfate present alternatively [1].

Dermatan sulphate: It is related to chondroitin 4-sulfate structurally except the configuration inversion around the 5th carbon of D-glucuronic acid to eventually form L-iduronic acid. It is present in skin [1].

Keratan Sulfate: It is comprised of N-acetylglucosamine 6-sulfate and D-galactosamine alternatively. This is heterogeneous with varied sulfate content along with sialic acid, mannose, fructose etc [1].

Chapter 3

Macromolecule in Cell: Lipids

Lipids are the primary storage and concentrated energy form, despite their function in cellular structure and different biochemical roles. These are heterogenous, organic in nature, water insoluble and soluble in ether, alcohol etc. which are organic in nature. However, the disimiliarity between proteins, polysaccharides and nucleic acids is that these are not polymers, rather these are small molecules [1].

Classification of Lipids

These are four of types: simple, complex, derived and miscellaneous lipids. Further, these are subdivided into various types:

Simple Lipids: these are defined as esters of fatty acids with alcohols. These are of two subtypes:

1. Fats and oils: these are triacylglycerols. Oils are liquid, while fats are solid and can be defined as esters of fatty acids with glycerol.
2. Waxes: these are esters of long chain fatty acids with alcohols. Alcohols can be aliphatic or alicyclic. For instance, cetyl alcohol is the most common. These are utilized for preparing lubricants, candles, polishes etc.

Compound or Complex Lipids: these are esters of fatty acids with alcohols, however possess the additional groups including carbohydrate, phosphate, nitrogenous base, protein and so on. These are further of three types:

1. **Phospholipids:** These possess phosphoric acid and oftenly, a nitrogenous base as additional group.
   - Glycerophospholipids: these possess the glycerol as an alcohol e.g., cephalin, lecithin.
   - Sphingophospholipids : these possess the sphingosine as an alcohol e.g., sphingomyelin.
2. **Glycolipids:** These possess carbohydrate, fatty acid and nitrogenous base. Sphingosine is present as alcoholic group, therefore, these are known as glycosphingolipids. e.g., gangliosides, cerebrosides.
3. **Lipoproteins:** it is complexes of proteins with lipids.
4. **Other complex lipids:** Aminolipids, sulfolipids, and lipopolysaccharides.

Derived lipids: these are obtained on hydrolysis of simple and compound lipids. Therefore, these possess the lipids characteristics. e.g., fatty acids, fat soluble vitamins, hydrocarbons, steroid hormones and ketone bodies.

Miscellaneous lipids: these are compounds possess the lipid properties. e.g., hydrocarbons such as pentacosane present in bees wax, carotenoids etc.

Note: Neutral Lipids: These lipids are uncharged and hence, are named so. e.g., cholesterol, mono-, di-, and triacylglycerols and cholesteryl esters [1].

Lipids' Functions

1. Fuel reserve of the body.
2. Component of membrane structure and responsible for membrane permeability due to cholesterol and phospholipids.
3. Remain attached with fat soluble vitamins (A, D, E and K) and thus, act as potential source of these vitamins.
4. Metabolic regulators such as prostaglandins and steroid hormones.
5. Provide protection to internal organs including insulating materials and providing smooth appearance and shape [1].

Fatty Acids

These are carboxylic acids with the side chain of hydrocarbon. These are simplest form and mainly occur in esterified form. These also remain as free or unesterified. Those are originated from animals possess simple structure as compare to plant origin that contains hydroxyl group, epoxy group, cyclopentane rings and keto group [1].

Even and Odd Carbon Fatty Acids

Natural lipids fatty acids mainly are even carbons, mostly 14C-20C. Biosynthesis mainly occurs by addition of 2 carbon units sequentially. e.g., Palmitic acid (16C) and stearic acid (18C). Propionic acid (3C) and valeric acid (5C) are common odd chain fatty acids [1].

Saturated and Unsaturated Fatty Acids

Saturated fatty acids are without double bonds, whereas unsaturated fatty acids are with one or more double bonds. Both of these are almost in equal proportion in case of natural lipids. Unsaturated fatty acids can be monounsaturated that possesses one double bond or polyunsaturated fatty acids (PUFA) possess two or sometimes more double bonds [1].

Fatty Acids Nomenclature

Naming is given based on the number of carbon atoms. Generally, saturated fatty acids contain anoic as suffix, e.g., octanoic acid, whereas unsaturated fatty acids contain enoic in the end, e.g., octadecanoic acid. Numbering starts from carboxyl carbon as the first carbon (carboxyl C), followed by adjacent carbons as 2, 3, 4 and so on or α, β, γ and so on and

terminal carbon with methyl group is named as omega (ω) carbon. If starts from methyl end, numbering to carbons is given as omega 1, 2, 3 and so on [1].

Fatty Acids Hydrocarbon Chain Length: Based on chain length, fatty acids are of three types: Short chain (less than 6 carbons), medium chain (8 to 14 carbons) and long chain (16 to 24 carbons) [1].

Fatty Acids Shorthand Representation: Firstly, total numbers of carbon atoms are written, followed by double bonds' number and lastly, the double bonds' position, beginning from carboxyl group. e.g., palmitic acid is named as 16 : 0, oleic acid is named as 18 : 1; 9, arachidonic acid is named as 20 : 4; 5, 8, 11, 14 which are saturated fatty acids. In case of oleic acid, the number 9 represents the presence of double bond between 9 and 10 carbon [1].

Essential Fatty Acids

The synthesis of such type of fatty acids is not possible by the body and hence, these should be supplied via the food, hence are called as essential fatty acids (EFA). For instance, polyunsaturated fatty acids, linolenic acid (18 : 3; 9, 12, 15) and linoleic acid (18 : 2; 9, 12) are EFAs and sometimes, arachidonic acid (20 : 4; 5, 8, 11, 14) should be provided in diet if linoleic acid which is precursor for it is not supplied by diet in adequate amount. Essentiality of linoleic and linolenic acid is because of lack of enzymes that can incorporate double bonds after 9 to 10 carbons [1].

EFA Functions

These are essential for cholesterol transport, lipoproteins formation, fatty liver prevention, structure and function of membrane and eicosanoids synthesis. EFA deficiency leads to toad skin or phrynoderma that includes the symptoms such as horny eruptions on the back and buttocks, on the lateral and posterior parts of limbs, poor wound healing and hair loss [1].

Unsaturated Fatty Acids' Isomerism

These possess the geometric isomerism based on the groups' orientation around the double bond axis. If atoms are present on the similar side, then it is cis isomer, otherwise, if atoms are present on the opposite side, then it is trans isomer. Trans isomers exhibit more stability compare to cis isomer. Naturally found unsaturated fatty acids remain in cis state. In this state, there is molecular binding at the bond. Due to this, oleic acid is found in an L-shape, whereas elaidic acid is found in straight chain form. More number of double bonds are responsible for increase in kinks or bends, therefore, arachidonic acid possesses 4 double bonds, hence it delivers U-shape appearance. Cis isomers pack membrane structure compactly. Structure of cis-trans isomerism is shown in Figure 3.1 [1].

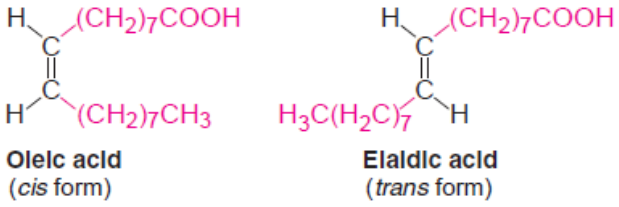


Figure 3.1: Unsaturated fatty acids' cis-trans isomerism [1].

Hydroxy Fatty Acids

β-Hydroxybutyric acid is one of the ketone bodies which is produced during metabolism is a hydroxy fatty acids. While, cerebronic acid and recinoleic acid are hydroxy fatty acids with long chain [1].

Cyclic Fatty Acids: Although these are rare, chaulmoogric acid of chaulmoogra oil possesses cyclopentenyl ring which is used in leprosy treatment [1].

Eicosanoids: these are correlated with eicosapolyenoic fatty acids, e.g., prostacyclins, prostaglandins, thromboxanes and leukotrienes [1].

Triacylglycerols: these are esters of glycerol with fatty acids, commonly called as triglycerides. e.g., triacylglycerols. These are water insoluble along with non-polar property, hence commonly called as neutral fats. It is the reserve animal fuel, in fact fuel reserve of normal humans is adequate for 2-3 months for survival [1].

Storage: Fat is found in adipose tissue, especially in adipocytes that resides in abdominal cavity and subcutaneous layer. Globules of fat are dispersed throughout the cytoplasm. Triacylglycerols are not found in membranes [1].

Acylglycerols' Structures

Simple triacylglycerols possess the similar type of fatty acids at three carbons of glycerol, e.g., tristearoyl glycerol or

tristearin. In contrast to this, mixed triacylglycerols possess 2 or 3 different fatty acids. Generally, C1 fatty acid is saturated, C2 fatty acid is unsaturated, while C3 can be any one of these. Structures of some acylglycerols are shown in Figure 3.2 [1].

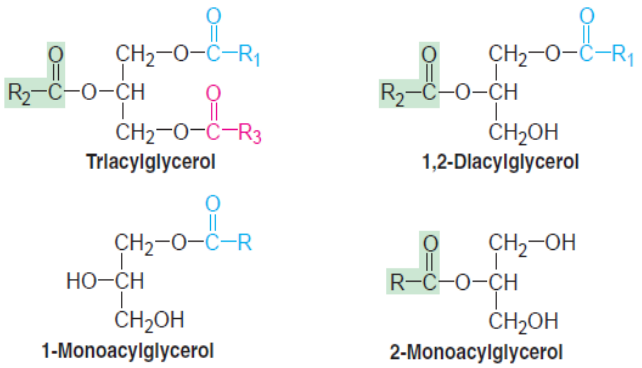


Figure 3.2: Structure of some acylglycerols [1].

Triacylglycerols' Properties [1]

Hydrolysis: Triacylglycerol, on enzymatic hydrolysis leads to free fatty acids and glycerol release. Lipases are essential for fat digestion and mobilization of fat from adipose tissues [1].
Saponification: Triacylglycerols hydrolysis by alkali provides glycerol and soaps. Therefore, this process is known as saponification.

Triacylglycerol + 3 NaOH→Glycerol + 3 R-COONa (soaps)

Rancidity: It is the process of fats and oils deterioration that leads to unpleasant taste. Fats with unsaturated fatty acids are more liable towards rancidity. It is due to fats and oils exposure to the moisture, air, bacteria, light, etc. However, hydrolytic rancidity is triacylglycerols' partial hydrolysis by bacterial enzymes. Oxidative rancidity is unsaturated fatty acids oxidation that leads to unpleasant byproducts including aldehydes, ketones, dicarboxylic acids etc. Therefore, rancid fats and oils are not suitable for human consumption. Antioxidants are the components that can prevent oxidative rancidity are called as antioxidants. For instance, antioxidants including α-naphthol, hydroquinone, tocopherols (vitamin E) and gallic acid is used for the fats and oils' commercial preparations to stop rancidity. In addition, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate are used for food preservation.
Lipid Peroxidation: Lipids on oxidation produce free radicals and peroxides that damages tissue. For instance, free radicals are responsible for inflammatory diseases, atherosclerosis, cancer, ageing etc., however, cell contains enzyme; superoxide dismutase, vitamin E and urate to stop lipid peroxidation in vivo [1].

Tests for the Fats and Oils' Purity

Iodine number: it is commonly presented as number or grams of iodine shocked by 100 g fat or oil. It is required to know the level of fat unsaturation and is proportional to the unsaturated fatty acids content. Hence, less is the iodine number, lower is the unsaturation degree. It is useful to find the adulteration degree for provided oil [1].
Saponification Number: it is commonly presented as number or mg of KOH needed to saponify or hydrolyse one gram of fat or oil. It is a measurement of fatty acids' average molecular size that is present. This value is greater for short chain fatty acids containing fats. For instance, saponification numbers for human fat ranges from 195 to 200, for butter from 230 to 240 and for coconut oil from 250 to 260
Reichert-Meissl (RM) Number: it is commonly presented as the number of ml of 0.1 N KOH needed to neutralize completely soluble volatile fatty acids that are distilled from 5 g fat. This is useful for testing butter purity because it possesses volatile fatty acids such as caproic acid, butyric acid and caprylic acid in good concentration. Butter possesses RM of the range, 25-30, whereas it is lower than 1 for other edible oils.
Acid Number: it is presented as number of KOH mg needed to totally neutralize free fatty acids that occurs in one gram fat or oil. In normal conditions, refined oils are free of free fatty acids. However, over decomposition with chemical or bacterial contamination results in free fatty acids production. Hence, oils with the enhanced acid number are unsafe for consumption by human [1].

Phospholipids [1]

These contain phosphoric acid, fatty acids, alcohol and nitrogenous base; therefore, these are compound or complex lipids. These are of two types [1]:

1. **Glycerophospholipids:** These possess glycerol as the

alcohol. These are called as phosphoglycerides. These are primarily found in biological membranes. These contain glycerol 3-phosphate that is esterified at carbon 1 and carbon 2 with fatty acids. Generally, carbon 1 possesses saturated fatty acid, while carbon 2 possesses unsaturated fatty acid. These are of following types:

a. **Phosphatidic Acid:** It is the simplest one and an intermediate for the triacylglycerols and phospholipids synthesis.
b. **Lecithins:** This is the most abundant one and present in cell membranes. This is also called as phosphatidylcholine. It contains choline as the base. It is the body's choline storage form.

I. **Dipalmitoyl lecithin:** It is present in lungs and is a surface active agent. It prevents the inner surface adherence in lungs because of surface tension. Additionally, respiratory distress syndrome is due to absence of dipalmitoyl lecithin in infants.
II. **Lysolecithin:** It is due to removal of the fatty acid either at C1 or C2.

c. **Cephalins:** It is also known as phosphatidylethanolamine. It contains ethanolamine as the nitrogenous base.
d. **Phosphatidylinositol:** This forms an essential part of cell membranes. The myo-inositol in its steroisomer form on attachment with phosphatidic acid provides phosphatidylinositol (PI). It is also responsible for the action of some hormones, e.g. oxytocin, vasopressin.
e. **Phosphatidylserine:** The amino acid in this group is serine. However, in some tissues, phosphatidylthreonine is also present.
f. **Plasmalogens:** In this, C1 of glycerol possesses a fatty acid via an ether linkage. The most indispensable one is phosphatidalethanolamine which is identical to phosphatidylethanolamine in its structure except presence of ether linkage rather than ester. The ethanolamine may be substituted by choline, inositol and serine to provide another plasmalogens.
g. **Cardiolipin:** It is named upon the source of isolation, heart muscle. It consists of two molecules of phosphatidic acid bind by one more glycerol via phosphate groups. It forms component of inner mitochondrial membrane and required for mitrochondrial function. It possesses antigenic properties out of all phosphoglyceride. Barth syndrome is one of the disorders that occurs due to reduced cardiolipin levels that results in aging, hypothyroidism, mitochondrial dysfunction and cardioskeletal myopathy [1]. Structures of few phospholipids are shown in Figure 3.3.

Sphingophospholipids: These possess sphingosine as the alcohol. These are called as sphingomyelins.

1. **Sphingosine and Ceramide:** It is an amino alcohol and is found in sphingomyelins. Sphingomyelins are critical component of myelin and these are present in nervous tissues and brain in good quantity. These do not possess glycerol. It is attached with the fatty acid via amide bond to form ceramide. It is a signaling molecule or second messenger. It regulates the apoptosis or programmed cell death, cell differentiation and cell cycle. Skin possesses ceramide that contain 30-carbon fatty acid so that it controls the skin's water permeability [1].

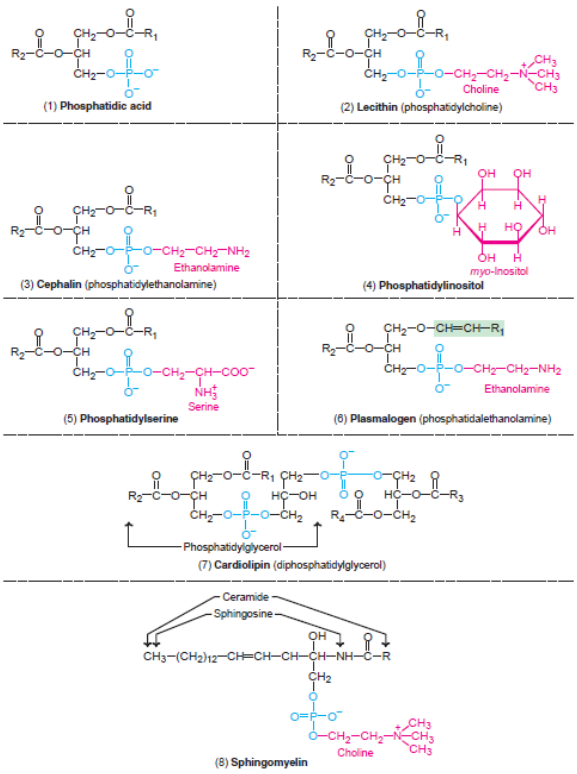


Figure 3.3: Structures of few phospholipids [1].

Functions of phospholipids [1]

These perform wide variety of essential functions:

1. In co-ordination with proteins, it forms the membranes' structural components to control the membrane permeability.
2. It maintains the electron transport chain components' conformation and hence, respiration process. e. g., cephalin, cardiolipin and lecithin.
3. It is involved in the fat absorption from the intestine.
4. These are required for various lipoproteins synthesis and therefore, require for lipids' transport.
5. Fat accumulation in liver that is known as fatty liver can be prohibited by phospholipids, hence, these are considered as lipotropic factors.
6. An unsaturated fatty acid, arachidonic acid that are released from phospholipids to act as precursor for the

eicosanoids' synthesis (prostacyclins, thromboxanes, prostaglandins etc).

7. These are involved in reverse cholesterol transport and helps in the cholesterol removal from the body.
8. These are surfactants, therefore lowers the surface tension. For example, dipalmitoyl phosphatidylcholine is a critical lung surfactant. Respiratory distress syndrome is related to inadequate synthesis of this surfactant in infants.
9. Cephalins is involved in blood clotting.
10. Phosphatidylinositol leads to the production of inositol triphosphate and diacylglyceol which are second messengers and are required for function of some horomones [1].

Glycolipids: These are essential nervous tissues (specifically the brain) and cell membrane's constituents. Cerebrosides are the simplest one known glycolipids. These possess a ceramide that contain sphingosine binds to a fatty acid and one or more carbohydrates. For instance, glucocerebroside and galactocerebroside that is also known as galactosylceramide are the most vital known glycolipids. Sulfagalactosylceramide is derived from galactosylceramide. Gangliosides: These are mostly present in ganglions and are cerebrosides' derivatives. It is the most complex glycosphingolipids. It possesses one or more molecules of sialic acid that are known as N-acetylneuraminic acid (NANA). The most critical gangliosides are GM1, GM2, GD, and GT that are present in brain where G indicates ganglioside, whereas, M, D and T represents mono-, di- or tri- sialic acid residues and number shows the carbohydrate sequence bind to ceramide. Tay-Sachs disease occurs due to accumulation of ganglioside, GM2 [1].

Lipoproteins: These are lipids and proteins' molecular complexes. These are required for transport of vehicles in the circulation. These are mainly of five types: very low density lipoproteins (VLDL), chylomicrons, low density lipoproteins (LDL), free fatty acid-albumin complexes and high density lipoproteins (HDL) [1].

Steroids: Steroids contain a cyclic steroid ring or nucleus which is called as cyclopentanoperhydrophenanthrene (CPPP). It contains phenanthrene nucleus (rings A, B and C) that is bound with a cyclopentane ring (D). The structure of CPPP is shown in Figure 3.4 [1].

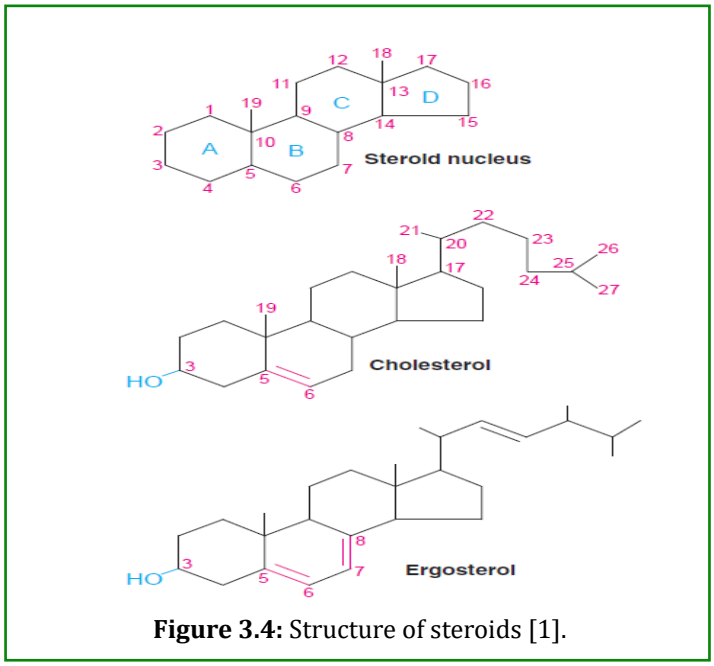


Figure 3.4: Structure of steroids [1].

The steroid nucleus displays saturated carbons except double bonds as shown in Figure 3.4. The methyl side chains (19 & 18) are bound to carbons 10 & 13 by single bonds and a side chain is present at carbon 17. Many different steroids are present in the biological system. These include bile acids, sex hormones, cholesterol, vitamin D, cardiac glycosides, alkaloids, sitosterols and adrenocortical hormones. In addition, steroid containing one or more hydroxyl groups is named as sterol or solid alcohol [1].

Cholesterol: It is only present in animals and is spread in all types of cells. It is a main constituent of lipoproteins and cell membranes. It is the most abundant sterol present in animals. It was segregated firstly from bile. It means solid alcohol from bile [1].

Structure and Occurrence: The chemical formula of cholesterol is $C_{27}H_{46}O$. It possesses a hydroxyl group at the carbon 3 and one double bond between carbon 5 and carbon 6. An 8 carbon side chain which is aliphatic in nature is bound to the C_{17}. Cholesterol possesses 5 methyl groups in total. It is weakly amphiphilic due to the presence of an –OH group.

Cholesterol determines the membrane permeability since it forms the plasma membranes' structural component. It is present in much higher quantity in sub-cellular organelles' membranes. Cholesterol in association with fatty acids forms cholesteryl esters and esterification happens at the C_3 OH group [1].

Properties: Cholesterol is yellow crystalline solid and possesses a notched appearance. It is water insoluble and soluble in solvents which are organic in nature including chloroform, ether, benzene etc. Many reactions such as Salkowski's test, Zak's test and Liebermann-Burchard reaction which are required for quantitative estimation and qualitative identification of cholesterol [1].

Cholesterol Functions

It is poor conductor of electricity and heat due to its high dielectric constant. It is present in abundance especially in nervous tissues. It is an insulating cover in the nervous tissue for electrical impulses transmission. Cholesterol is involved in bile acids, vitamin D and hormones (cortical and sex) synthesis and performs many other biochemical role including its function in membrane structure [1].

Ergosterol: It is present in plants. It is structural constituent of yeast and fungi membranes. It is also an essential vitamin D precursor. On exposure to light, ergosterol ring B opens and eventually, it gets converted to ergocalciferol which possesses vitamin D activity. In addition, plants contain stigmasterol and β-sitosterol [1].

Amphipathic Lipids: Lipids are hydrophobic in nature due to the presence of hydrocarbon groups, therefore, insoluble in nature. But, few lipids contain hydrophilic or polar groups, thus are soluble in water. In addition, lipids' molecules possess hydrophilic and hydrophobic groups both are called as amphipathic (amphi-both, pathos-passion; Greek) lipids. For instance, phospholipids, fatty acids, sphingolipids, cholesterol and bile salts (to some extent). Phospholipids possess a hydrophilic head (phosphate group bind to ethanolamine, choline, inositol etc.) along with one long hydrophobic tail. The structure of an amphipathic lipid is shown in Figure 3.5. Fatty acids possess a carboxyl (COO^-) group on a hydrocarbon chain at physiological pH. Fatty acid hydrocarbon chain is hydrophobic and carboxyl group is hydrophilic or polar in nature, therefore, it possesses affinity towards water [1].

Amphipathic Lipids' Orientation: On mixing amphipathic lipids in aqueous phase such as water, non-polar or tails orient in opposite direction to water and polar or head group orient towards water phase that result in micelles' formation. Micelles are mainly amphipathic lipids' molecular aggregates (Figure 3.5). Formation of micelles is facilitated by bile salts that are required for lipid digestion and absorption [1].

Membrane Bilayers: Lipids' bilayer in biological membranes is formed by orienting the hydrophilic heads on either side towards outer aqueous phase and the nonpolar tails inside (Figure 3.5). This lipid bilayer forms the membrane structure basis [1].

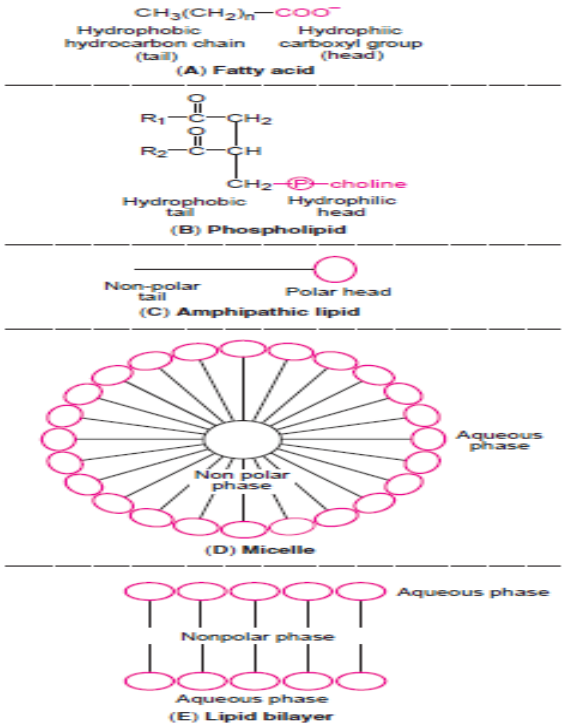


Figure 3.5: Different intermittent structures during micelle formation and lipid bilayer [1].

Liposomes: Amphipathic lipids on sonification in aqueous medium produce liposomes. In this process, many intermittent aqueous phases are involved. Liposomes along with tissue specific antigens are utilized as drugs' carriers to target tissues [1].

Emulsions: When nonpolar lipids such as triacylglycerols are dissolved in water, emulsions are formed. These are of larger size and ultimately, stabilized due to emulsifying agents such as amphipathic lipids including phospholipids and bile salts [1].

Soaps and Detergents

1. **Soaps:** These are fatty acids' sodium or potassium salts. These are formed by fats' saponification. Sodium soaps results in bar soaps due to hardness. These act as cleansing agents because these can emulsify oils and therefore, removes the dirt [1].
2. **Detergents:** These are cleansing agents which are synthetic in nature e.g. sodium lauryl sulfate. These are better than soaps in their cleansing properties and are utilized in tooth paste and washing clothes [1].

Chapter 4

Macromolecule in Cell: Proteins

Proteins are of utmost value due to their role not only for structural aspects, but also functionally. Proteins are made up of amino acids which are linked by peptide bonds. There are about 300 amino acids that exist in nature, out of which 20 are found in human. All amino acids are alpha means amino and carboxyl group are attached to same carbon except proline as shown in Figure 4.1 [2].

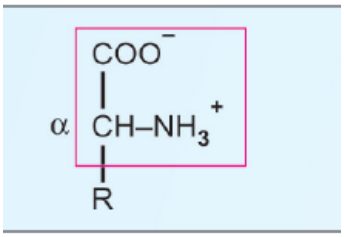


Figure 4.1: General structure of amino acid [2].

A. Amino Acids' Classification [2]

On basis of structure

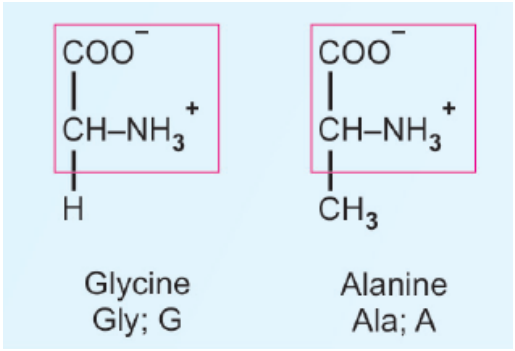
Aliphatic amino acids: These can be further divided based on number of amino and carboxylic acids [2].

Monoamino Monocarboxylic Acids:

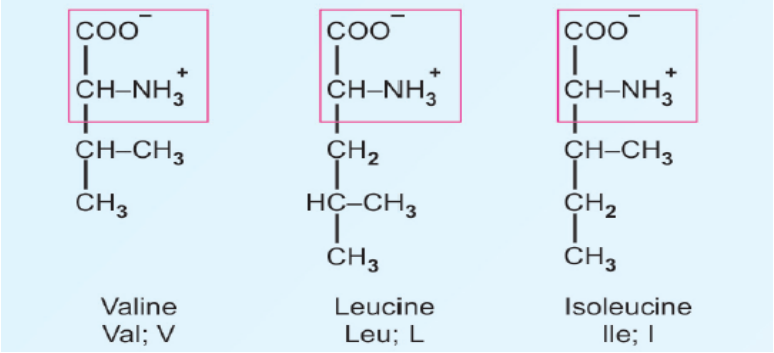
- Simple amino acids: Glycine, Alanine
- Branched chain amino acids: Valine, Leucine, Isoleucine
- Hydroxyamino acids: Serine, Threonine
- Sulfur-containing amino acids: Cysteine, Methionine
- Amino acids with amide group: Asparagine, Glutamine

1. **Monoamino dicarboxylic acids**: Aspartic acid, Glutamic acid
2. **Dibasic monocarboxylic acids:** Lysine, Arginine
3. **Aromatic amino acids:** Phenylalanine, Tyrosine
4. **Heterocyclic amino acids:** Tryptophan, Histidine
5. **Imino acid:** Proline
6. **Derived amino acids:** These are of three different types:

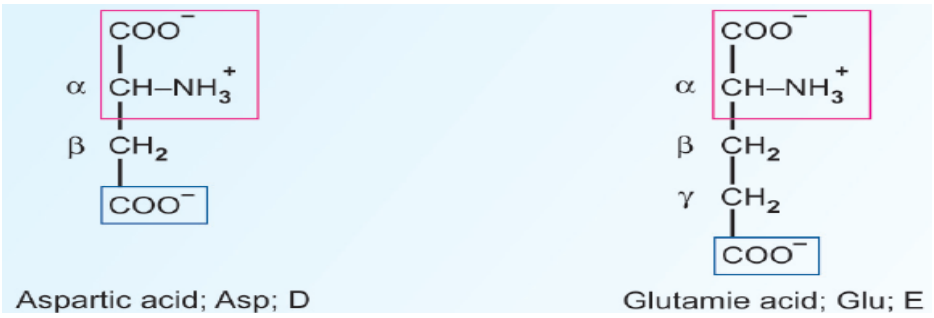
After completion of proteins synthesis, few amino acids are modified, e.g. hydroxy proline and hydroxy lysine (present in collagen). Glutamic acid residues' gamma carboxylation is required for clotting process. In ribosomes and histones, amino acids are extremely acetylated and methylated [2].



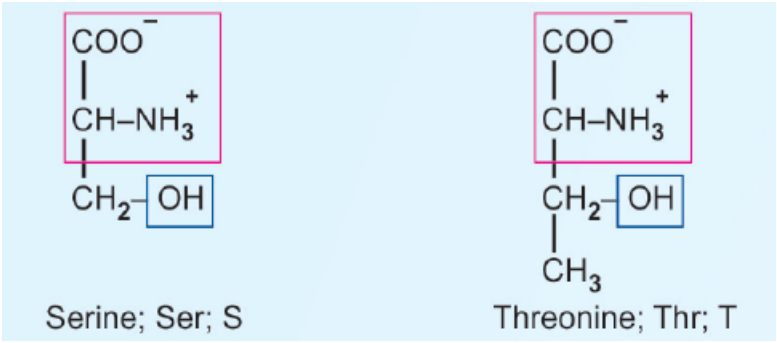
Structure of simple amino acids [2].



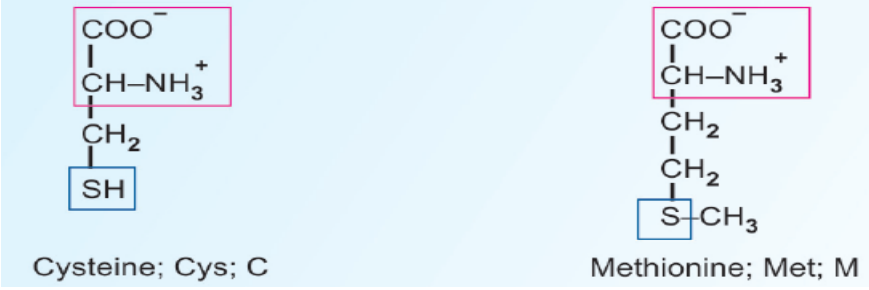
Structure of branched chain amino acids [2].



Structure of dicarboxylic amino acids [2].

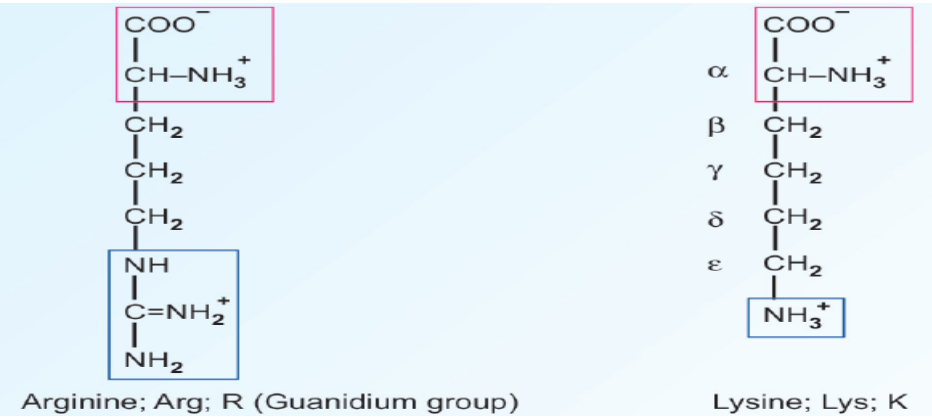


Structure of hydroxyamino acids [2].

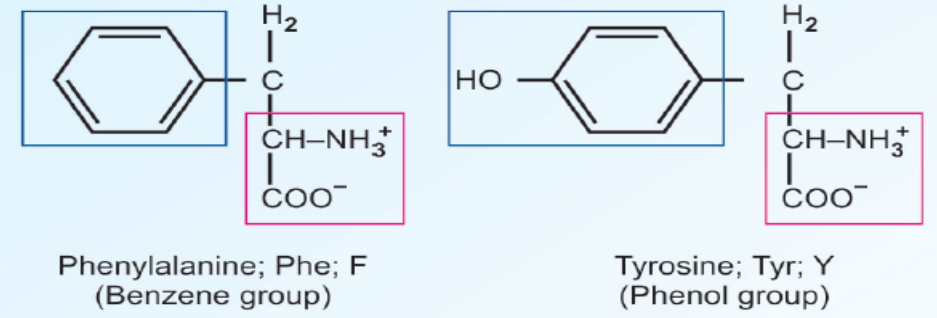


Structure of sulfur containing amino acids [2].

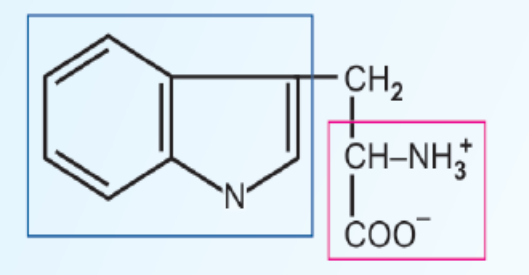
Structure of amino acids with amide groups [2].



Structure of dibasic amino acids [2].



Structure of aromatic amino acids [2].



Tryptophan [2].

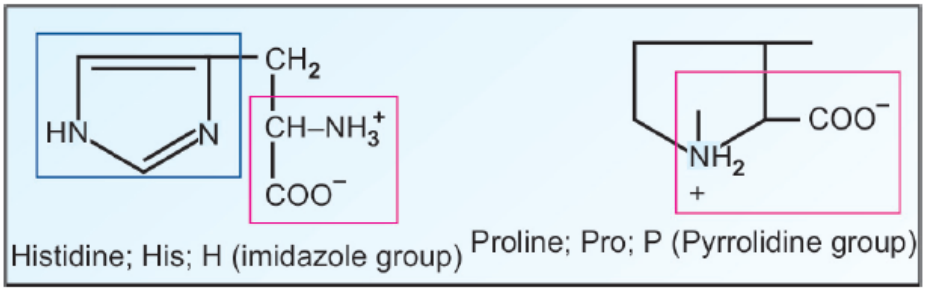


Figure 4.2: Structure of different types of amino acids [2].

i. Derived amino acids which are free in cell: these are called as nonprotein amino acids. e.g. Ornithine, Homocysteine, Citrulline. Tyrosine leads to thyroxine [2].

ii. Non-alpha amino acids: Glutamic acid is derivatized to gamma amino butyric acid (GABA). Beta alanine is a component of coenzyme A and pantothenic acid (vitamin) [2].

Note: Few amino acids possess special groups such as arginine possesses guanidinium group, Phenylalanine has benzene, Tyrosine contains phenol, Tryptophan with indole, Histidine with imidazole and Proline contains pyrrolidine. Proline contains secondary amino group and thus, it is called as an imino acid [2].

Structure of different amino acids is shown in Figure 4.2 [2].

On the Basis of Side Chain [2]

A. Amino acids with non-polar side chains: It involves lipophilic and water repellant or hydrophobic nature of groups. For instance, Alanine, Isoleucine, Methionine, Valine, Leucine, Tryptophan, Phenylalanine and Proline [2].

B. Amino Acids with Non-Ionic Polar and Uncharged Side Chains: It involves hydrophilic amino acids. For instance, Tyrosine, Glutamine, Threonine, Cysteine, Glycine, Asparagine and Serine. In addition, Cysteine and Tyrosine possess hydrophobic nature when in the interior of the protein [2].

C. Amino Acids with Ionic Polar or Charged Side Chains: These are hydrophilic in nature.

a. **Acidic amino acids:** These possess negative charge above the R group. For instance, Aspartic acid and Glutamic acid. However, Tyrosine is moderately acidic.

b. Basic amino acids: These possess positive charge above the R group. For instance, Arginine, Histidine and Lysine [2].

On the Basis of Nutritional Requirements [2]

1. **Essential or Indispensable:** These are the amino acids whose carbon skeleton cannot be synthesized by human body, however, these are indispensable for growth. Hence, these should be taken in diet. For instance, Leucine, Threonine, Isoleucine, Methionine, Phenylalanine, Lysine, Valine and Tryptophan. This is the main reason for growth restriction in case of deficiency of these amino acids.
2. **Partially Essential or Semiessential:** Arginine and Histidine are partially essential amino acids. This is because growing children need these amino acids, however, not the adults.
3. **Non-Essential or Dispensable:** The rest 10 amino acids are not considered as essential because their carbon skeleton can be manufactured by human being. However, these are necessary for protein synthesis normally. For instance, Asparagine, Aspartic acid, Glycine, Proline, Alanine, Cysteine, Glutamine, Glutamic Acid, Tyrosine and serine. All of the proteins possess non-essential amino acids.
4. **Conditionally Essential Amino Acids:** Little to life threatening chronic illnesses makes a person to lose the potential to synthesize sufficient non-essential amino acids and hence, need supplementation. If problems persist with digestive system and in physiological stress conditions, then it is critical to provide non-essential amino acids as a supplementation in diet. For instance, Cysteine, Tyrosine, Arginine, Glycine, Taurine, Proline and Glutamine [2].

Amino Acids' Properties [2]

1. **Taste:** Tryptophan, histidine, alanine, valine, serine, glycine and proline are sweet, whereas, arginine and isoleucine are bitter; in addition, leucine is tasteless. Aspartame consists of phenylalanine and aspartic acid which is an artificial sweetener. Sodium glutamate is a flavoring agent.
2. **Melting points:** These all possess high melting points, about greater than 200°C.
3. **Solubility:** These possess solubility in polar solvents, e.g., water and alcohol, however, these are insoluble in benzene or non-polar solvents.
4. **Ampholytes and Isoelectric Point:** Amino acids can be found as zwitter ions or ampholytes based on the pH of the surrounding. However, at the isoelectric point stage, amino acids carry no charge since all groups' charges cancel out each other, hence possess no mobility. The pH where molecules exist without any net charge is called as isoelectric pH or isoelectric point (pI). Moreover, at isoelectric pH, the buffering capacity and solubility is minimum.
5. In acidic conditions, these are cationic and in alkaline conditions, these are anionic.
6. **Optical Activity:** Amino acids with asymmetric carbon possess optical activity. Asymmetry exists when four different groups are bound to single carbon. Glycine does not possess optical activity since it is simplest and without any asymmetric carbon atom.
7. The mirror images are produced with respect to the alpha carbon atom are known as D and L isomers.
8. The L-amino acids are natural amino acids since it is found in nature. D-amino acids are components of some antibiotics including Polymyxin, Actinomycin-D, Gramicidin-S and Valinomycin and also, of peptidoglycans present in bacterial cell wall. These assist in forming cross-links.

9. Isoleucine and Threonine possess 2 optically active centers and hence, these have 4 diastereoisomers.

Proteins [2]: Protein word means Primary which is taken from word, "proteios" which is a Greek word. Proteins are of great importance biologically since out of total body weight, proteins consists approximately 3/4ths. Proteins contribute main structural and functional body aspects which are utilized by body. Protein structure abnormality leads to molecular diseases with metabolic functions' alterations. Carbon, Hydrogen, Oxygen and Nitrogen are the main component of protein with Sulfur and Phosphorus as lesser constituents. Nitrogen component is approximately 16% by weight in ordinary weight. Proteins are amino acids polymers. Amino acids are bound by CO-NH bridge or peptide bond which is formed by alpha carboxyl group of one amino acid that reacts with alpha amino group of another amino acid. Dipeptide are composed of two amino acids, tripeptide are of three amino acids and tetrapeptide are of four amino acids. Moreover, oligopeptide are made up of few amino acids and polypeptide is of 10 to 50 amino acids. Proteins are long polypeptide chains with more than 50 amino acids. For instance, in case of tripeptide, there are 3 amino acids, thus, 203 = 8000 different combinations and permutations are possible. Therefore, even there are 20 amino acids, with the sequences changes, there will be abundant different proteins which are possible [2].

Proteins' Structure (Proteins' organization) [2]

Proteins possess different structural organization; primary, secondary, tertiary and quaternary [2]. The structure of different level of organization is given in Figure 4.5.

Definitions of Organizational Level [2]

1. **Primary structure:** It displays the amino acids' order of the polypeptide chain and disulfide bonds' location.
2. **Secondary structure:** It displays the amino acids' steric relationship.
3. Tertiary structure: It displays the overall arrangement and inter-relationship of different domains or regions of a single polypeptide chain.
4. **Quaternary structure:** These possess two or more polypeptide chains which are bound by noncovalent forces [2].

Primary Structure: It represents the number and sequence of amino acids. Rest other higher level organizations are determined by the primary structure. Polypeptide chain sequence is coded from a gene sequence. It possesses the covalent bonds among peptide linkages for its maintenance. For example: Gly- Ala-Val is the first sequence and Gly-Val-Ala is the second one. Therefore, due to sequence difference, different peptides are synthesized [2].

Peptide Bond Characteristics

1. It is a partial double bond [2].
2. The C-N bond contains 'trans' nature and hence, it is without rotational freedom due to its partial double bond character.
3. The length is 1.32Å which is intermittent between double bond (1.27Å) and single bond (1.49Å).
4. Moreover, the side chains are rotable on either side.
5. **Note:** The rotatory angles are called as Ramachandran angles since Dr GN Ramachandran performed pioneering work for the structure related aspects of the proteins [2].

Amino Acids Numbering in Proteins

1. At one end of protein, there is a free alpha amino group, it is called as N-terminal or amino terminal end. This amino acid with the contribution of alpha-amino group is called as first amino acid.
2. The amino terminal end is present on the left side and also, the protein biosynthesis starts at the same end. The other terminal end is known as C-terminal or carboxy terminal end because it possesses free alpha carboxyl group of last amino acid. Rest alpha amino and alpha carboxyl groups are engaged in peptide bond formation.
3. In case of amino acid residues, ine suffix should be changed with yl, e.g. peptide of Glycine, Alanine and Valine is named as glycyl-alanyl-valine [2]

Branched and Circular Proteins

1. Most of the time, polypeptide are linear. However, branches exist with the interchain disulphide bridges. The interchain covalent disulphide bonds that are between different polypeptide chains or intrachain bonds that occur within same polypeptide chain.
2. Rather than alpha COOH group, it is gamma group that is involved in the formation of peptide bond, e.g. Glutathione (gammaglutamyl-cysteinyl-glycine). The term for this is 'pseudopeptide' that represents the involvement of carboxyl group for peptide bond formation.
3. Protein may exist in circular structure, e.g. Gramicidin [2].

Insulin Primary Structure

The best example of primary structure is insulin. This was explained by Sanger in the year 1955. The structure of insulin is shown in Figure 4.3.

1. Insulin possesses two polypeptide chains. The A chain contains 21 amino acids (Glycine chain) and B chain consists of 30 amino acids (Phenylalanine).
2. These are bound by two disulfide bonds which are interchain in nature. Seventh cysteine of A chain and 7th cysteine of B chain are bound. Similarly, 20th cysteine of A chain and 19th cysteine of B chain are connected. In

addition, one intrachain disulfide bond exists between 6th and 11th cysteine residues in a chain.

3. The amino acids at position 8, 9 and 10 vary in A chain and also, in B chain at its C-terminal. Rest other sites are conserved during the evolution [2].

For example, human and porcine insulin are similar structure wise except (Thr → Ala) at C-terminal of B chain. However, porcine insulin after de-alanination possess no antigenic difference and hence, no antibodies production after long term use. Human insulin as replacement therapy is now synthesized as recombinant DNA technology [2].

"Pro-insulin"

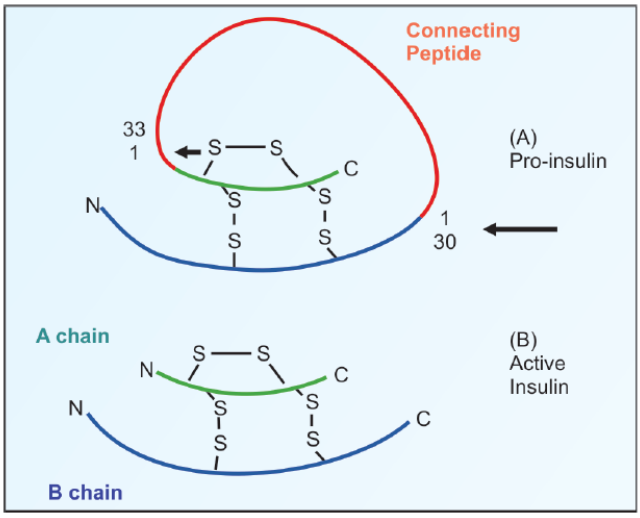


Figure 4.3: Structure of insulin [2].

Beta islets pancreatic cells synthesized insulin as prohormone. It is single polypeptide with 86 amino acids. However, activated insulin is synthesized after the removal of central portion. This connecting peptide is also secreted in the circulation [2].

Primary Structure and Biological Activity

Specific primary shape naturally form a three dimensional structure. Therefore, it is the primary structure that determines the different higher organization levels of proteins. A single change of amino acid in the primary structure leads to difference in entire function, therefore, it is deleterious. For instance, at the 6th amino acid location, change of glutamic acid with valine in case of normal hemoglobin results in sickle cell anemia (HbA to HbS). Structure of different levels of proteins' organization is shown in Figure 4.5 [2].

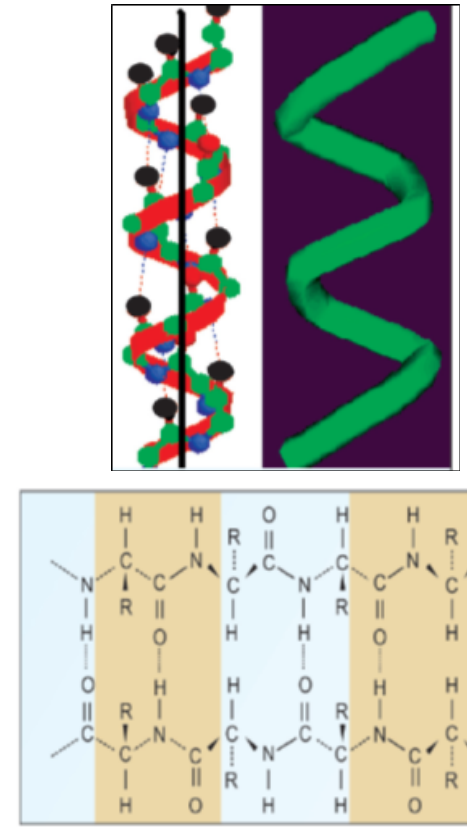


Figure 4.4: Structure of Alpha-helix and β-pleated sheet [2].

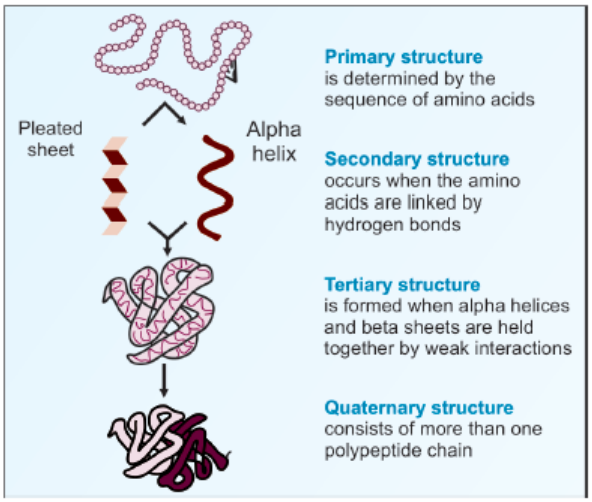


Figure 4.5: Structure of different level of proteins' organization [2].

Secondary Structure

It describes the relationship between residues at the level of configuration especially which are around at the distance of

3-4 amino acids in primary sequence. Secondary and tertiary protein structure exist due to noncovalent bonds or forces, e.g. electrostatic bonds, hydrogen bonds, van der Waals forces and hydrophobic interactions. The different types of secondary structure, alpha and beta-pleated sheet is shown in Figure 4.4. The definition of different bonds is described below [2]:

1. **Hydrogen Bond:** It is weak electrostatic bond between an electronegative atom such as O or N and one hydrogen atom associated covalently with second electronegative atom. Hydrogen atoms is derived from -NH_2 (lysine, arginine), -NH (indole, imidazole, peptide) and-OH (threonine, serine). There are many groups including COO- (glutamic, aspartic), S-S (disulfide) and C = O (peptide).
2. **Electrostatic Bonds (Ionic Bonds):** -ve charges are denoted by beta and gamma level carboxyl groups of glutamic and aspartic acids. +ve charges are provided by epsilon group of lysine, imidazolium group of histidine and guanidinium group of arginine.
3. **Hydrophobic bonds:** These are formed by the bonding of nonpolar hydrophobic side chains with the water molecules elimination. This is required to bind lipophilic side chains together.
4. **The Van Der Waals Forces:** These are very weak forces, however collaborately contribute highest for the protein structure stability.

Pauling and Corey provided the alpha helix and beta-pleated sheet structures of proteins in 1951 [2].

Alpha Helix

1. It is most stable and common structure of proteins. For instance, for myoglobin and hemoglobin, alpha helix is abundant, while chymotrypsin does not possess it.
2. It possesses spiral structure as shown in Figure 4.4. In this, the side chains extend outward from polypeptide bonds back-bone.
3. The hydrogen bonds is stabilized between NH and C=O groups. Each turn possesses 3.6 residues and the distance is 1.5 Å between different amino acid residues.
4. It is right handed generally, whereas, left-handed structures are rare, because amino acids are of L-type in protein, therefore, it removes the left handedness. In addition, Proline and hydroxy proline does not permit alpha helix formation [2].

Beta-Pleated Sheet

1. The polypeptide chains are almost fully extended in proteins. The distance between amino acids that are adjacent is 3.5Å.
2. These structures are stabilized by hydrogen bonding between NH and C=O groups of adjacent polypeptide chains.
3. Adjacent chains exist either in parallel (similar) or anti-parallel (opposite) beta sheet structure. (Figure 4.4). This is found for Flavodoxin (parallel), silk Fibroin (anti-parallel) and Carbonic anhydrase (that possesses both) [2].
4. Beta bends are also present that are formed by abrupt U-turn folding in chains of some proteins which are stabilized by intrachain disulfide bridges. For instance, collagen helix has triple helical structure that is commonly found in collagen [2].

Tertiary Structure

1. Just like secondary structures describe the configurational relationship of residues that are at the distance of 3-4 amino acids. Actually, secondary structure exists due to organization at amino acids level. Whereas, tertiary structure describes whole protein three dimensional shape. It exists due to steric relationship of faraway amino acids in the linear sequence, however it exists in close relationship three-dimensionally. The structure is shown in Figure 4.5.
2. The tertiary structure is created by noncovalent bonds including electrostatic bonds, hydrophobic bonds and van der Waals forces. It is most stable thermodynamically.
3. Domain is a compact, relatively independent and globular functional structure of protein. Different domains are connected with the flexible areas. For instance, Phenyl alanine hydroxylase enzyme consists of three domains; a catalytic, a regulatory and a protein-protein interaction domains.
4. A fold is the tertiary structure element which on arrangements control intracellular calcium level. For example, calmodulin, the calcium binding regulatory protein that controls intracellular calcium level [2].

Quaternary Structure (Figure 4.5)

1. Few polypeptides on aggregation form a single functional protein. This is known as quaternary structure.
2. However, subunits on dissociation lose its function.
3. The bonds involve hydrophobic bonds, hydrogen bonds, electrostatic bonds and van der Waals forces.

Protein may be defined as monomer that possesses 1 chain, dimer consists of 2 chains, tetramer contains 4 chains, etc. Every polypeptide chain is called as monomer or subunit. Homodimer consists of the same polypeptide chains. Heterodimer consists of different types of polypeptides., e.g. a hemoglobin contains 2 alpha and 2 beta-chains, immunoglobulin G consists of 2 heavy and 2 light chains. Lactate dehydrogenase is a tetramer and creatine kinase is a dimer [2].

Chapter 5

Macromolecule in Cell: Nucleic Acids and Nucleotides

History

Friederich Miescher separated nuclein or nucleic acid from pus cells in 1868. Followed by this, in 1882, Albrecht Kossel explained the difference between RNA and DNA. Then, Kossel elaborated 4 different bases in nucleic acids in 1906. Nucleotides are the basic unit for the formation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). These nucleic acids are required for the transfer and storage of genetic information. ATP, the universal energy currency is also a nucleotide derivative. Co-enzymes such as NAD^+ and FAD and metabolic regulators, e.g. cAMP and cGMP also contain nucleotides [2].

Nucleotides' Composition

Each nucleotide contains three constituents:

a. A purine or a pyrimidine: Nitrogenous base

b. Pentose sugar: deoxyribose or ribose

c. Phosphate groups esterified with the either deoxyribose or ribose

A base on joining with a pentose sugar is called a nucleoside and nucleoside on esterification with a phosphate group is called a nucleoside monophosphate or nucleotide. This on esterification with second phosphate is called a nucleoside diphosphate and followed by with 3rd phosphate group leads to nucleoside triphosphate formation. The nucleic acids, e.g. DNA and RNA are nucleoside monophosphates' polymers.

a. Nitrogenous Base: Two Types; Purines and Pyrimidines Bases

Purine Bases: These are further of two types; adenine and guanine that are constituent of RNA and DNA. Adenine is called as 6-amino purine and guanine is called as 2-amino, 6-oxopurine. Structures of pyrimidine bases are shown in Figure 5.1 [2].

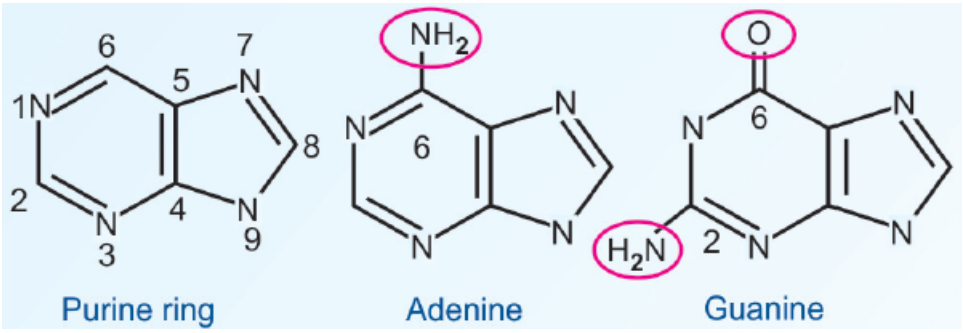


Figure 5.1: Structure of purine ring and bases, adenine and guanine [2].

The bases which are present in small amounts inside the nucleic acids are called as minor purine bases. For instance, hypoxanthine is called as 6-oxopurine and xanthine is called as 2, 6-di-oxopurine. Other purine bases on catabolism form uric acid which is called as 2, 6, 8-tri-oxopurine. This exists in two tautomeric forms, enol and keto. In physiological conditions, keto form is the most predominant form [2].

Pyrimidine Bases: The pyrimidine bases are of three types: thymine, cytosine and uracil. DNA as well as RNA contains cytosine, additionally, DNA possesses thymine and RNA possesses uracil. Structures of pyrimidine bases are shown in Figure 5.2. In addition, some other pyrimidine bases such as 5-methyl cytosine and dihydrouracil are also found in few types of RNA [2].

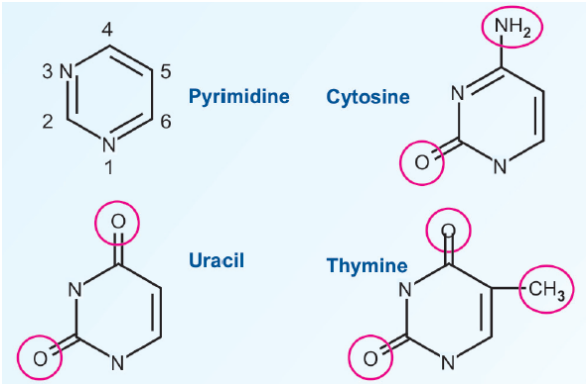


Figure 5.2: Structure of pyrimidine ring and bases, cytosine, uracil and thymine [2].

Nucleosides [2]

- Nucleosides are generated on addition of bases to the pentose sugar such as 2-deoxy-D-ribose or D-ribose.
- The pentose sugar carbon atoms are denoted by prime number to differentiate from pyrimidine or purine rings carbon atoms.
- Bases are bound to the pentose sugar with the beta-N-glycosidic bond between first carbon of pentose sugar and N1 of a pyrimidine or N9 of a purine.
- Pyrimidine nucleosides contain suffix -dine at the end and purine bases contain suffix -sine at the end.
- The deoxy nucleosides are represented by using the prefix d- before the name of nucleoside.
- Thymine associates with deoxy ribose only and Uracil binds with ribose only [2].

Nucleotides [2]

- These are formed by esterification of phosphate group to the nucleosides. Nucleotide contains base, pentose sugar and phosphoric acid.
- This process of esterification happens in pentose sugar at 3rd or 5th hydroxyl group. Although, the most of them are 5'-phosphates, therefore, 3' AMP is written as 3'-AMP and whereas, 5'-AMP is written as AMP.
- Some co-enzymes are derived from adenosine monophosphate. For instance, FAD, NAD+, NADP and Co-enzyme A.
- Nucleic acid and nucleotides absorb at 260nm; hence, this fact is utilized to quantify both. Since nucleic acids absorb UV light, it results in mutation and eventually, carcinogenesis.
- In addition, a base binds with deoxyribose or ribose that can be phosphorylated at either 5' or 3' position [2].

Nucleoside Triphosphates [2]

- Nucleoside on esterification with further phosphate groups leads to formation of nucleoside di- and tri-phosphates. Generally, any nucleoside triphosphate can be written as NTP or d-NTP.
- Cyclic nucleotides such as cAMP or 3', 5'-cyclic AMP contain phosphodiester linkage that is formed between 3' and 5' positions of ribose group. This compound acts as prime metabolic regulator. Cyclic GMP also acts in same manner. Actually, these function as second messengers for mediating the several hormones action.
- Nucleoside diphosphate possesses one high energy bond, while triphosphates contain two high energy bonds. For instance, ATP is known as the universal energy currency and this is formed by oxidative processes with the trap of released energy in the form of high energy phosphate bond.
- Few examples of high energy compounds include amino acid adenylates, methionine and active sulfate which possess adenosine monophosphate.
- DNA is made up of deoxy ribonucleotides, whereas RNA is made up of ribonucleotides. In pseudouridylic acid that is present in tRNA, uridine is bound to ribose phosphate via a C-C bond rather than C-N bond of UMP [2].

Purine Nucleotides' Biosynthesis [2]

1. Most of the tissues biosynthesized purines nucleotides. But, the main site is the liver. This occurs only in cytoplasm.
2. Its major pathway is defined as de novo synthesis because the purine ring is biosynthesized using various small components. In case of human being, the purine and pyrimidine bases can be synthesized de novo, hence they are called as prototrophs.
3. Purine ring is synthesized on a ribose-5-phosphate molecule during de novo synthesis. Therefore, the nucleotides are synthesized de novo synthesis.
4. Mainly, ten steps are involved in the de novo synthesis pathway. The enzymes are multienzyme complex in case of eukaryotic cells for increasing the pathway efficiency [2].

Preparatory Step (Step 0) or PRPP Synthesis [2]

The first few steps involving preparatory phase is shown in Figure 5.3.

1. Phosphoribosyl pyrophosphate (PRPP) contributes ribose-5-phosphate for the de novo synthesis. The reaction is shown below: Ribose-5-phosphate + ATP →ADP + Phosphoribosyl pyrophosphate (PRPP). Later, the purine ring gets assembled on the ribose-5-phosphate.
2. In addition, PRPP is utilized for nucleotide co-enzymes, pyrimidine nucleotides and so on for salvage biosynthetic pathway. Hence, PRPP synthesis is considered as a preliminary or preparatory step.
3. The very first step is rate limiting. Whereas, the conversion of formyl glycinamide ribonucleotide to formyl glycinamidine ribonucleotide is inhibited by anticancer drug, azaserine. The amination process of IMP to AMP is inhibited by 6-mercaptopurine and hence, it is an anticancer drug [2].

The Conversation of IMP to GMP

It mainly involves two important steps, the first one is oxidation of IMP to xanthosine monophosphate (xanthylic acid; XMP) via enzyme; NAD^+ dependent dehydrogenase. Thereafter, NH2 group is transferred from glutamine to XMP for the formation of GMP by an amido transferase. Moreover, AMP and GMP can be converted to further di- and triphosphates. One purine nucleotide molecule needs 6 high energy phosphates for its synthesis (Figure 5.4) [2].

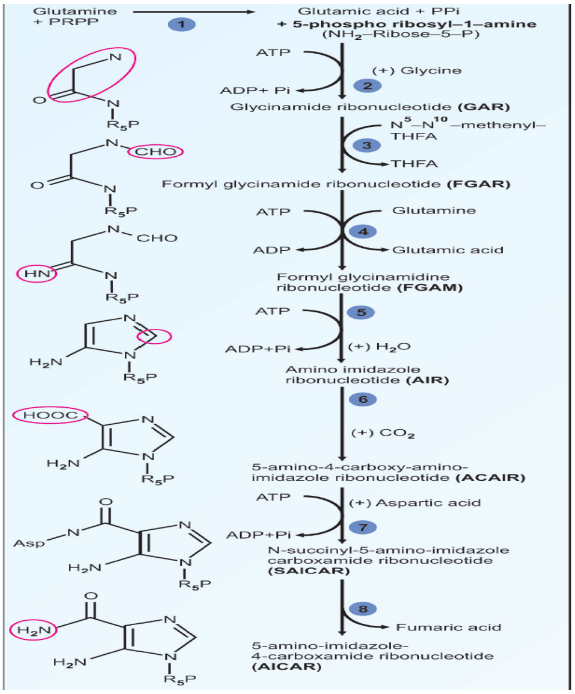


Figure 5.3: First few steps of purine synthesis [2].

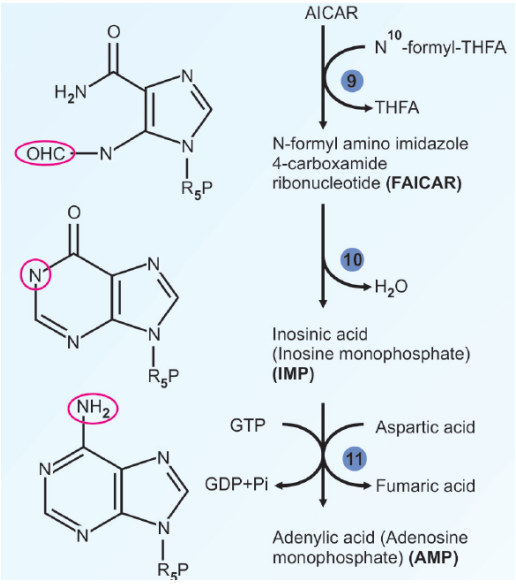


Figure 5.4: The last few steps in the purine synthesis [2].

Salvage Pathway [2]

1. This cycle decides the purines recycling that are generated by nucleotides' degradation. In addition, deoxy-nucleosides and nucleosides may also be salvaged [2].
2. PRPP is the initial stage material for this pathway and also, substrate that is required for de novo synthesis. Therefore, these both are inter-related pathways.
3. The salvation process of free purines involves two different enzymes; hypoxanthine guanine phosphoribosyl transferase (HGPRTase) and adenine phosphoribosyl transferase (APRTase).
4. The salvage pathway is of prime importance for the tissues where de novo pathway is not functional just like brain and RBCs. It is critical for economizing intracellular energy expenditure [2].

The lack of salvage pathway enzymes leads to specific clinical syndromes. The summary of salvage pathway is given below:

Adenine + PRPP $\xrightarrow{\text{APRTase}}$ AMP + PPi

Guanine + PRPP $\xrightarrow{\text{HGPRTase}}$ GMP + PPi

Hypoxanthine + PRPP $\xrightarrow{\text{HGPRTase}}$ IMP + PPi [2]

Purine Synthesis Regulation [2]

1. The de novo synthesis committed step is the reaction that is catalyzed by enzyme, amido-transferase, whereas, it is inhibited by GMP and AMP. These are allosteric modifiers because the AMP and GMP binding on enzyme leads to the conversion of monomeric active form to a dimeric inactive form.
2. Because the AMP and GMP binding occurs on the different sites of same enzyme, these both act in synergistic manner.
3. AMP and GMP inhibit self synthesis via feedback inhibition of enzymes; adenylosuccinate synthetase and IMP dehydrogenase, respectively.
4. IMP to AMP needs GTP and GMP synthesis needs ATP. Thus, ATP and GTP are present in adequate quantities. The conversion of IMP to GMP is shown in Figure 5.5.
5. The another important regulatory factor is PRPP. The synthesis of PRPP by PRPP synthetase is controlled via negative modifiers including purine and pyrimidine nucleotides [2].

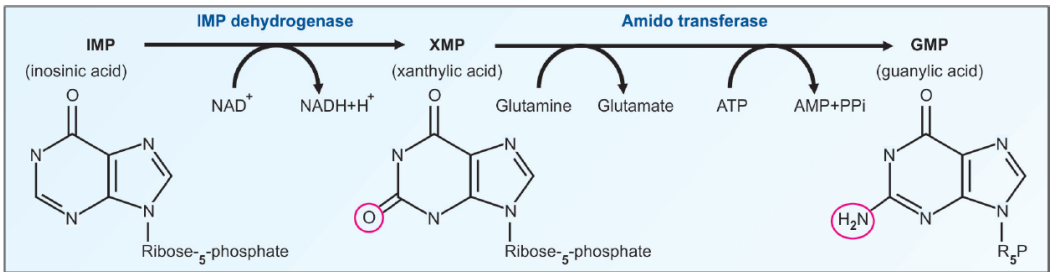


Figure 5.5: The conversion of IMP to GMP [2].

Purine Nucleoside Di- and Triphosphates [2]

The below mentioned reactions are catalyzed by specific kinases and ATP donates the phosphate group. For example,

GMP + ATP $\xrightarrow{\text{(Monophosphate kinase)}}$ GDP + ADP

GDP + ATP $\xrightarrow{\text{(Diphosphate kinase)}}$ GTP + ADP

Purine Nucleotides Degradation

The last product of catabolism of purine nucleotide is uric acid or urate. The site for degradation is liver. The steps for purine nucleotides degradation are shown in Figure 5.6. The xanthine oxidase enzyme is a metalloflavoprotein with molybdenum, iron and FAD. Since xanthine get oxidized to uric acid, firstly electrons are transferred to molybdenum followed by FAD and eventually, get transferred to molecular oxygen and hence, hydrogen peroxide which is one of the reactive oxygen species, is generated [2].

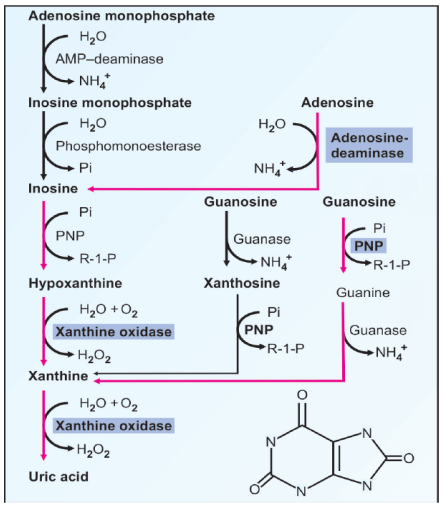


Figure 5.6: The steps of purine nucleotides degradation [2].

De Novo Synthesis of Pyrimidine [2]

The pyrimidine ring is produced as free pyrimidine, not like purine and followed by this, integration into the nucleotide. The synthesis of pyrimidine nucleotides is shown in Figure 5.7 [2].

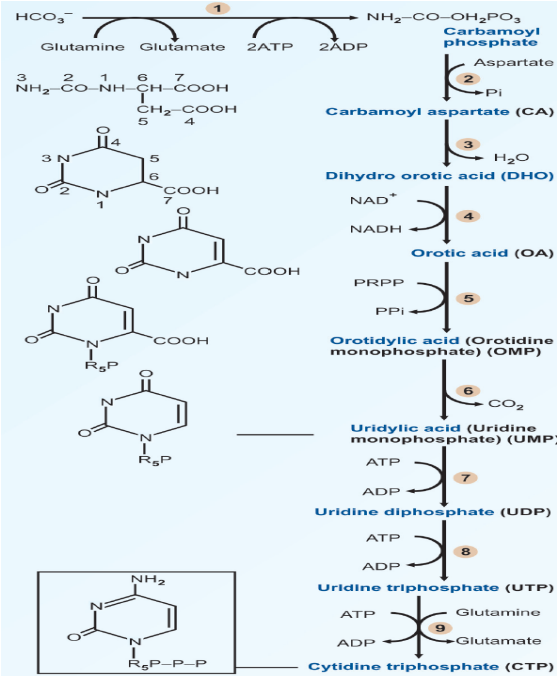


Figure 5.7: Pyrimidine nucleotides' synthesis [2].

Step 1: The synthesis of Carbamoyl Phosphate: This reaction site is cytoplasm, whereas, urea synthesis takes place in mitochondria. Primarily, the nitrogen of bicarbonate and glutamine on reaction forms carbamoyl phosphate. The enzyme that is involved is called as carbamoyl phosphate synthetase II (CPS II).

Step 2: Rate limiting step: Carbamoyl phosphate and aspartate on combination forms carbamoyl aspartate. The enzyme involved is called as aspartyl trans carbamoylase (ATC), the regulation of which takes place allosterically. The atoms; C2 and N3 are derivative of carbamoyl phosphate and the remaining are taken from aspartate.

Step 3: Formation of Pyrimidine Ring: The joining of 3rd nitrogen and 4th carbon occurs by a covalent bond and the cyclization of carbamoyl aspartate takes place. Dihydo orotic acid is synthesized during this step. The enzyme that is involved is dihydro orotase (DHOase) as shown in Figure 5.7.

Step 4: Oxidation reaction: During this step, the enzyme involved is dihydro orotate dehydrogenase (DHODH) and co-enzyme is NAD which removes hydrogen atoms from C5 and C6 locations for the production of orotic acid.

Step 5: OMP formation: Ribose-5-phosphate on addition to orotic acid leads to the production of orotidylic acid or orotidine monophosphate (OMP). PRPP is the donor for ribose-5-P and the enzyme involved is orotate phosphoribosyl transferase (OPRTase).

Step 6: Decarboxylation reaction: The 7th carbon of OMP is removed as carbon dioxide, hence, uridine monophosphate (UMP) is synthesized. UMP is the first pyrimidine that is produced by this process and this step utilizes enzyme; OMP-decarboxylase (OMPDC). 6-aza-uridine acts as inhibitor for this step and hence, it is used as an anticancer drug.

Step 7: Triphosphates synthesis: UMP on phosphorylation forms UDP (uridine diphosphate) by using energy of ATP and enzyme, nucleoside monophosphate [2]

DNA Structure: The structure of DNA double helix is shown in Figure 5.8 [2].

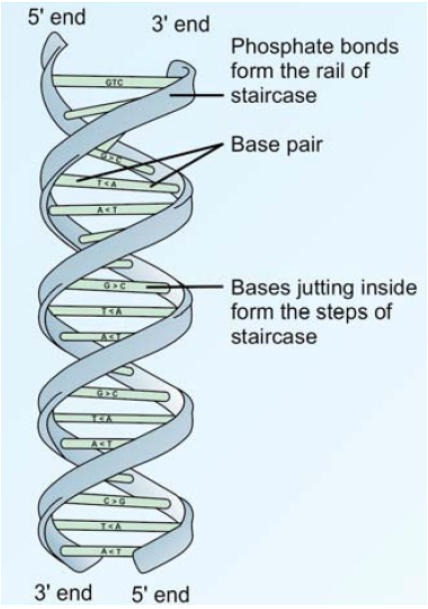


Figure 5.8: DNA double helical structure [2].

Deoxyribonucleic acid (DNA) is comprised of 4 different units or deoxyribonucleotides including deoxyadenylate (A), deoxyguanylate (G), deoxycytidylate (C) and thymidylate (T). These four units are joined via 3′ to 5′ phosphodiester bonds for the formation of a long polypeptide chain. One nucleotide is composed of a base, a sugar and a phosphoric acid. The 3′-hydroxyl of one sugar is joined with the 5′-hydroxyl of another sugar via a phosphate group as shown in Figure 5.8. The DNA base sequence is critically important since the genetic information lies in specific bases' sequence, therefore, on alteration of bases, information also get altered. The deoxyribose and phosphodiester linkages are identical in all the repeating nucleotides [2].

Polarity of DNA molecule: The sequences of bases are written from the 5′ end to the 3′ end which is defined as the DNA chain polarity [2].

DNA Watson-Crick Model [2]**:** The basic characteristics of DNA Watson-Crick model are explained below [2]:

Right Handed Double Helix: DNA is composed of two polydeoxyribonucleotide chains which are twisted about one another. The twisting takes right handed just like spirals. The bases remain inside the spiral structure at perpendicular location to the helix axis and sugars (approximately at right angles) and phosphate groups remain in the handrail [2].

The Base Pairing Rule: The bases in the pairs in two strands complement each other. Therefore, the adenine pair with thymine, whereas the guanine pair with the cytosine. This is called as Chargaff's rule where purines number is equal to the pyrimidines number [2].

Hydrogen Bonding: The purine and pyrimidine bases are bind by hydrogen bond. The two hydrogen bonds exists between A and T, whereas, three hydrogen bonds exists between C and G. The GC bond is more strengthful to the AT bond. The spatial arrangement possesses the type of organization which supports only purine to pyrimidine base pairing. Both A-T and C-G base pairs possess the same shape. However, the mispairing detereoriate the double helical structure stability [2].

Antiparallel Nature: One strand run in antiparallel direction to that of other. For example, if one strand trail in 5′ to 3′ direction, whereas, other one is in 3′ to 5′ direction [2].

Other Characteristics: Each DNA strand is a template for the opposite strand synthesis during the DNA replication process. DNA spiral possess 3.4 nanometers per turn pitch. Each turn contains 10 base pairs. Therefore, the nearby bases are at 0.34 nm distance. The width or diameter is 1.9 to 2.0 nm. A major (1.2nm) and minor (0.6nm) groove remain parallel to the phosphodiester backbone. Different proteins possess interaction with exposed bases in these grooves. The base pairs' stacking is responsible for the double helix stability via the hydrophobic effect and the ring systems conformation. It is the DNA that is the storehouse for the genetic information [2].

DNA Strands' Denaturation: DNA double strand get denatured and separated on heating which is defined as DNA melting. Tm or melting temperature is defined as the temperature where half of the helical structure gets denatured. On lowering the temperature, melted strands get re-associated which is defined as the annealing. On denaturation, bases unstacking results in enhanced absorbance at 260nm which is known as hyperchromicity [2].

DNA higher organization: In the higher organisms, DNA is well organized in to the nucleus. Double stranded DNA in association with histones as it is remain wound round the histones to forms the nucleosomes. Chromatin is DNA long stretch with histones. It get condensed more and more to form chromosomes. In the similar manner, the DNA molecule on folding and compression of 10,000 fold generates the chromosomes [2].

Histones

These proteins possess higher basic amino acids concentration. These are of 5 types: H1, H2A, H2B, H3 and H4. The H1 histone is bound loosly with the DNA, whereas others are core histones since these form nucleosome. One-third amino terminal of H2A and H2B are enriched with lysine, while H3 and H4 are enriched with arginine. The synthesis of histone get stopped on ceasing the DNA synthesis. The histones which are synthesized into the cytoplasm will migrate to the nucleus. Even histones are modified by methylation, ADP-ribosylation, acetylation and phosphorylation. The most common for lysine residues is acetylation and methylation. Histones acetylation leads to transcription activation, while, de-acetylation leads to transcriptional depression. Phosphorylation is related to the chromosomes' condensation, however, ADP-ribosylation is related with the repair of DNA. In addition, gene is suppressed via the methylation. On the other hand, the histones are joined with small ubiquitin related modifier (Sumo) which is known as sumoylation, e.g., during transcription repression. Besides histones, many other unique proteins interact with DNA specific location. The DNA-proteins interactions are hold via three motifs; zinc finger, helix-turn-helix and leucine zipper motifs. It is only the small part of protein which directly interact with DNA, whereas, rest are involved in different functions such as ligand-binding, dimerization, and interaction with corepressors and coactivators, etc. However, transactivation and DNA binding domains for most of the regulatory proteins are noninteractive and separate [2].

Nucleosomes and DNA condensation

A histone octamer is formed of H2A, H2B, H3 and H4 which is wrapped by double stranded DNA twice. This super-twisted helix spherical is of 10 nm diameter which is called as nucleosome. Nucleosomes condense DNA which stabilizes DNA. Nucleosomes group forms DNA fibrils, further 6 such fibrils on supercoiling form chromatin threads or chromatin fibers of 30 nm diameter that result in 100 times folding which is stabilized by histones. Up to100,000 bp loops which remain anchored in nuclear matrix are present in interphase chromosomes. Human genome is made up of about 7 × 109 base pairs and distance between bases is 0.34 nm. The whole length of DNA is condensed to 8,000 to 10,000 fold for the chromosomes generation. The different stages in condensation of DNA are shown in Figure 5.9 [2].

Chromosomes

The DNA fibrils on supercoiling and condensation generate the chromosomes in the cell cycle, M phase during which DNA can be visualized under microscope in superpacked chromosomes form where sister chromatids are joined by centromere. Human possesses 23 pairs of chromosomes. It is the centromere position which determines the chromosome

specificity. A centromere represents a rich region with repeated DNA sequences consist of approximately one million base pairs in case of mammals.in addition, the similar region contain many specific centromere binding proteins which is defined as kinetochore that acts as an anchor for the mitotic spindle [2].

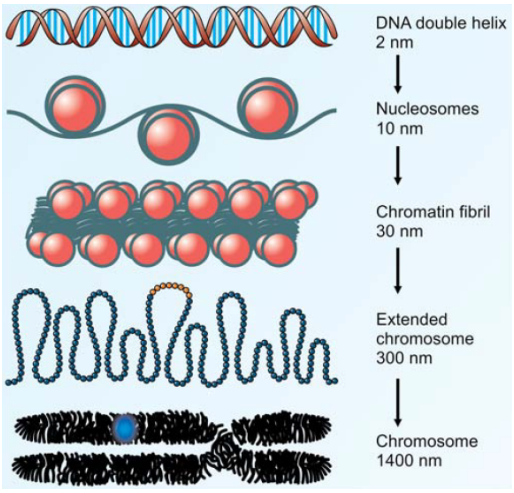


Figure 5.9: DNA on condensation form chromosome [2].

Chromatin activity

Chromatin transcriptionally active regions (100,000 bp length) are prone to digestion with deoxyribonuclease-1 (DNase-1), however, certain hypersensitive sites of 100–200 nucleotides length to DNase which are present in active regions are also there. These are the sites where transcription factor proteins get assembled for the initiation of transcription. Less dense regions are defined as euchromatin, while dense regions which are transcriptionally inactive are called as heterochromatin, however, it is most of the euchromatin which fills nucleus majorily [2].

DNA Inactivation during Differentiation

Zygote is the single cell responsible for the synthesis of all cells of the body. Hence, genetic material is same in all cells, but the structure of one type of cell differs from other. For example, gastrointestinal epithelial cell are different from central nervous system cell. This differentiation exists because 90% DNA remain permanently inactive by the assistance of hostones [2].

Introns, Exons and Cistron

During the evolution process, there has been an increase in DNA content. The protein coding gene segment is called as exon or expressed regions which are interspaced with silent region are called as introns or intervening areas that on removal form mature mRNA. The introns never get translated, but its actual function is not known. It mainly serves the purpose of genetic recombination. Additionally, cistron is the genetic expression unit, it is gene biochemical counterpart. It is one cistron that code one polypeptide chain, it simply means four different protein subunits are formed by 4 cistrons which is known as one cistron-one polypeptide concept [2].

References

1. Satyanarayana U (2013) Biochemistry. 4th (Edn.), Elsevier, West Bengal, New Delhi, India, pp: 812.

2. Vasudevan DM, Sreekumari S, Vaidyanathan K (2013) Textbook of Biochemistry for Medical Students. 7th (Edn.), Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India, pp: 791.