UNIT 2 LIPIDS AND PROTEINS

Structure

2.1 INTRODUCTION

In the last unit you were provided with a broad introduction to metabolic and nutritional aspects of biochemistry. The chemistry of carbohydrates, one of the physiologically important molecule, was focussed next. In this unit, we continue with our study of important molecules by focussing on lipids and proteins. Major topics include structure, function and metabolism of amino acids and lipids. We would like to remind you once again that this unit is the basis for understanding the concepts related to lipids and amino acids/proteins as discussed in the Advance Nutrition and Food Science Courses. Hence, it would be a good idea to go through the units on Lipids and Proteins in these Courses together, as the information in each course would supplement each other.

Objectives

After studying this unit, you will be able to:

- describe the structure of lipids and classify them,
- explain the chemical properties of fatty acids, neutral fats, phospholipids, steroids and eicosanoids,
- illustrate the structure of amino acids, peptides, proteins and nucleic acids,
- classify amino acids, peptides, proteins and nucleic acids, and
- describe the chemistry of proteins and nucleic acids viz. amino acids, peptides, proteins, nucleotides and nucleic acids.

2.2 CHEMISTRY OF LIPIDS – INTRODUCTION

Lipids are another important group of nutrients that should be looked into with interest by a learner of dietetics very seriously. This sub-section intends to focus on certain important aspects of lipid chemistry. These are as follows:

- Chemical nature of lipids
- Major classes of lipids
- Important lipids of each class
- Properties of fatty acids and neutral fats

At the outset, let us get introduced to lipids. Lipids are *heterogeneous group of compounds occurring in both plants and animals*. These are insoluble in water, but soluble in other solvents such as ether, chloroform and benzene. Because of heterogeneous nature, it is difficult to define these compounds in certain terms. However, neutral fats and oils derived from animal and plant sources are called *neutral lipids*, which on hydrolysis yield glycerol and fatty acid. We shall learn about the classification of lipids next.

2.3 LIPIDS – STRUCTURE AND CLASSIFICATION

Lipids are classified on the basis of their chemical structure as presented in Figure 2.1.

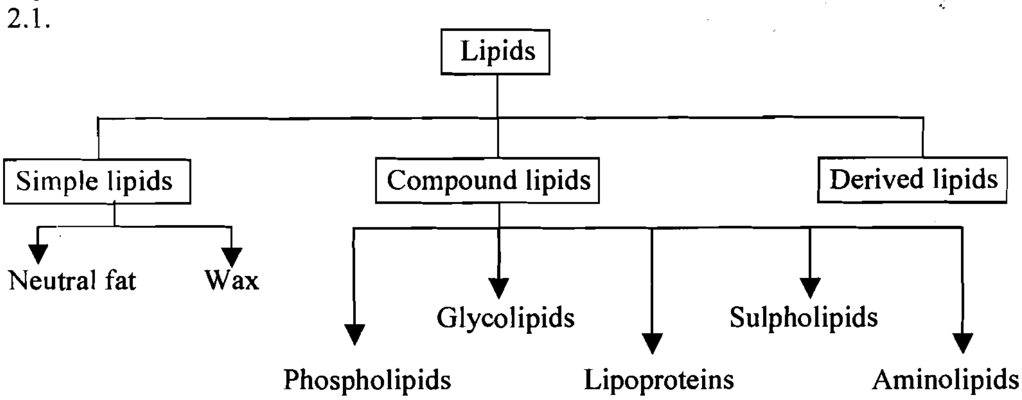


Figure 2.1: Classification of lipids

Simple lipids are esters of fatty acids with various alcohols. They include neutral fats, (which you learnt earlier, are esters of fatty acid with glycerol) and waxes (which are fatty acid with alcohol other than glycerol). *Compound lipids* are esters of fatty acids containing groups in addition to an alcohol and a fatty acid. Examples include phospholipids (containing in addition to fatty acids and an alcohol, a phosphoric acid residue), glycolipids (compounds of fatty acids with carbohydrate, containing nitrogen but no phosphoric acid) etc. *Derived lipids* are substances derived from above groups by hydrolysis. This group includes fatty acids, glycerol, steroids, alcohols, sterols, fatty aldehydes and ketone bodies, vitamin A, D, E and K etc.

In the following section, we shall get to know a bit more about these lipids. A detailed discussion on all lipids is not included herewith, however, some important lipids are focussed. We start our discussion with the chemistry of fatty acids.

2.3.1 Fatty Acids (Saturated and Unsaturated)

We saw earlier that fatty acids are obtained by the hydrolysis of neutral fats. Many different fatty acids occur naturally in foods. In their free form, the fatty acids have the configuration shown herewith.

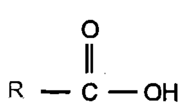
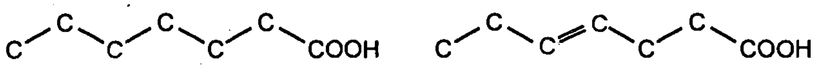


Figure 2.2: Fatty acids

where, R is a hydrocarbon chain (CH_3–CH_2–CH_2……)

Fatty acids are grouped into two classes, *saturated* and *unsaturated* fatty acids. The terms 'saturated' and 'unsaturated' fatty acid may not be new to you. Every other day there is one report or another, highlighting the useful and/or harmful effects of these fatty acids on health.

What are these fatty acids? When a fatty acid contains one or more double bonds (–C=C–), it is said to be unsaturated, otherwise it is a saturated fatty acid (as highlighted in Figure 2.3).



Saturated Unsaturated

Figure 2.3: Saturated and unsaturated fatty acid

Presence of double bond in a fatty acid makes it relatively unstable to air and suitable reagents. Physical properties of the fat are also affected due to the presence of double bonds in the fatty acids. Fats rich in unsaturated fatty acids usually exist as liquid i.e. oil at room temperature while fats rich in saturated fatty acids exist in solid form. All food fats contain both saturated and unsaturated fatty acids in varying proportions. A detailed discussion on saturated and unsaturated fatty acids follows:

- *Saturated fatty acids*

General formula of saturated fatty acids is $CH_3(CH_2)_nCOOH$. It has a polar end with a free –COOH group and a non-polar end with a hydrocarbon chain, as can be seen in the structure of fatty acid illustrated in Figure 2.2. Fatty acids with less carbon atoms are readily miscible with water and the solubility decreases with an increase in carbon atoms. Saturated fatty acids with less than 10 carbon atoms are liquid at room temperature and those having more are solids. Melting point of these fatty acids increases with increasing number of carbon atoms. Table 2.1 lists the common saturated fatty acids of food fats.

Table 2.1: Naturally occurring common saturated fatty acids

Common name	Molecular formula	Occurrence
Butyric acid	$C_3H_7.COOH$	Butter
Caproic acid	$C_5H_{11}.COOH$	Butter
Caprylic acid	$C_7H_{15}.COOH$	Fats of plant origin
Capric acid	$C_9H_{19}.COOH$	Fats of plant origin
Lauric acid	$C_{11}H_{23}.COOH$	Palm kernel, cinnamon, coconut oil
Myristic acid	$C_{13}H_{27}.COOH$	Nutmeg, palm kernel, coconut oil
Palmitic acid	$C_{15}H_{31}.COOH$	Palm oil, animal and plant fats
Stearic acid	$C_{17}H_{35}.COOH$	Animal fat and plant oil
Arachidic acid	$C_{19}H_{39}.COOH$	Groundnut oil
Lignoceric acid	$C_{23}H_{47}.COOH$	Groundnut oil

- *Unsaturated fatty acids*

Unsaturated fatty acids are liquid at room temperature and are characterized by having one or more double bonds. Fatty acids having two or more double bonds are called *poly unsaturated fatty acids* (PUFA) while those with a single double bond are called as *mono unsaturated fatty acids* (MUFA).

Unsaturated fatty acids show different types of isomerisms. We have already learnt about the concept of isomerism in the last unit. You would realize that fatty acids with same molecular formula, as well as, same number of double bonds may differ in the location of double bonds and exhibit positional isomerism. Oleic acid has 15 different positional isomers. Again, the orientation of the two hydrogen atoms attached to the two carbon atoms joined by the double bonds may differ (geometric isomerism).

If both hydrogen atoms are placed on the same side of the double bond, *cis-isomer* results but if these are placed on either side of the double bond, it is a *trans-isomer* as shown in Figure 2.4 ("*cis*" comes from Latin word meaning "same" or same side; "*trans*" comes from Latin word meaning "across" or opposite sides). Examples of *cis-trans* isomer is oleic acid and elaidic acid. Nearly all naturally occurring unsaturated fatty acids are of *cis* configuration.

Cis Configuration   *Trans* Configuration

Figure 2.4: ***cis* and *trans* isomers**

Carbon atoms of fatty acids are numbered from carboxyl carbon. Various conventions are used to indicate the position of double bonds in the fatty acid molecules. Like, Δ^9 indicates a double bond between carbon atoms 9 and 10 of the fatty acid.

The Greek alphabets ($\alpha,\beta,\gamma,\ldots,\omega$) are used to identify the location of the double bonds. The "alpha" carbon is the carbon next to the carboxyl group, and the "omega" is the last carbon of the chain because omega is the last letter of the Greek alphabet. Linoleic acid is an omega-6 fatty acid because it has a double bond six carbons away from the "omega" carbon. Similarly, alpha-linolenic acid is an omega-3 fatty acid because it has a double bond three carbons away from the "omega" carbon. In other words, fatty acids known as $\omega 3$ or n3 fatty acids have one double bond between carbon atoms 3 and 4, from the ω carbon but no double bond between 6 and 7 carbon atoms. On the other hand, $\omega 6$ or n6 fatty acids have double bonds between carbon atoms 6 and 7 from omega carbon. Table 2.2 presents the common unsaturated fatty acids found in nature.

Table 2.2: Naturally-occurring common unsaturated fatty acids

Common name	Chemical structure	Occurrence
Palmitoleic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	All fats
Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	All fats, abundant in olive oil
Elaidic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Hydrogenated fat, margarine
Linoleic acid (LA)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7$ $COOH$	Mainly vegetable oils, also in some animal fats
Linolenic acid (LNA)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}$ $CH\text{-}(CH_2)_7COOH$	Mainly vegetable oils, particularly linseed oil
Arachidonic acid	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2\text{-}$ $CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$	Peanut oil, groundnut oil, traces in some animal fats

While on the topic of fatty acids, we must also talk about essential fatty acids (EFA). Surely you must have heard about these important fatty acids. What are they? As the name suggests, EFA are essential to be provided in the diet. We must have them to live and to be healthy. Our bodies cannot make them from other substances. We must obtain an adequate supply from external sources – from food or from supplements. Two fatty acids are essential to human health. Can you name them? The first is the omega 6 EFA, which is called *linoleic acid (LA)*. LA is abundant in polyunsaturated safflower, sunflower and corn oils. The second, known as the *omega 3 EFA, is called alpha-linolenic acid (LNA) or ALNA*. Sometimes referred to as super-unsaturated, LNA is found abundantly in flex and hemp seeds. Look at Table 2.2, for the structure of these two fatty acids.

LA and its derivatives belong to the omega 6 family of polyunsaturates. In addition to linoleic acid (LA), this family includes gamma-linoleic acid (GLA), dihomogamma-linolenic acid (DGLA) and arachidonic acid (AA).

LNA and its derivatives belong to an omega 3 family of superstaurates. Besides alpha-linolenic acid (LNA), this family includes stearidonic acid (SDA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). If LNA is provided by foods, our cells make SDA, EPA and DHA. You will learn more about the EFA in the Advance Nutrition Course in Unit 5.

Next, we shall focus on neutral fats in this unit but before that, let us review what we have learnt till now.

Check Your Progress Exercise 1

1) How are lipids classified? Give examples.

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2) Differentiate between saturated and unsaturated fatty acids. Give one example each of saturated, monounsaturated and poly unsaturated fatty acid.

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3) What is the difference between the following:

a) *cis* and *trans* fatty acids

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b) n-3 and n-6 fatty acids

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4) What are essential fatty acids? Name 2 EFAs and where these are obtained from?

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2.3.2 Neutral fats

Neutral fats are *esters of fatty acids with glycerol* and found abundantly in nature. These are insoluble in water but readily soluble in ether, chloroform, benzene and carbon tetrachloride. They are bland, odourless substances and neutral in reaction. Neutral fats are good solvents themselves for other fats, fatty acids etc.

As glycerol has three hydroxyl groups, three molecules of fatty acids may get attached with it at the maximum. When one molecule of fatty acid binds with the glycerol, a monoacylglycerol (monoglyceride-old name) forms. Diacylglycerol and triacylglycerol form when two or three molecules of fatty acids combines with one molecule of glycerol, as indicated in Figure 2.5. Fatty acids reacting with the glycerol may be same or different.

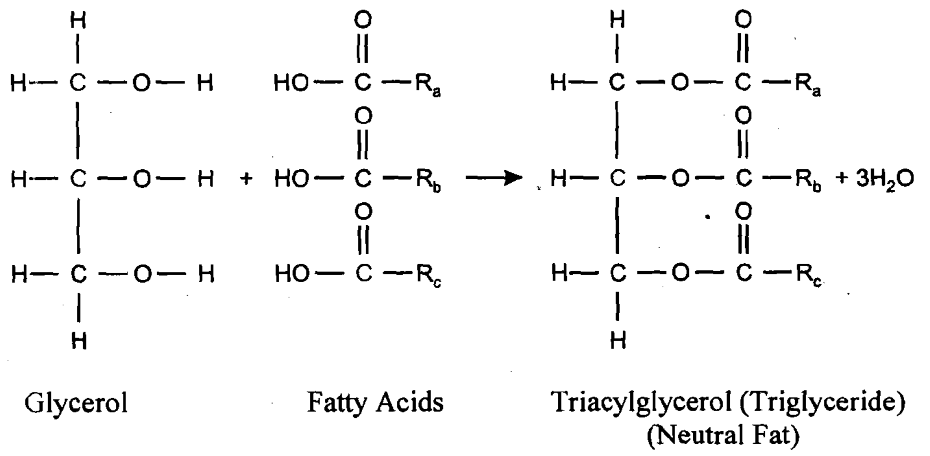


Figure 2.5: Triacylglycerol

From simple fats, we move on to compound lipids, wherein, we shall focus on phospholipids.

2.3.3 Phospholipids

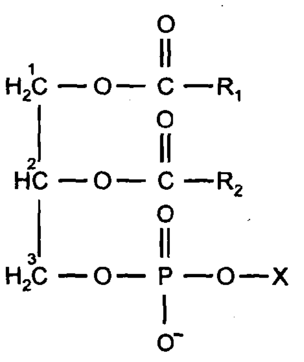
Phospholipids are *a heterogeneous group of compounds widely distributed in animal tissues particularly in the cell membranes.*

Phospholipids are classified on the basis of alcohol present in the molecule. These are classified as:

a) Glycerophosphatides, in which glycerol is the alcohol.
b) Phosphoinositides, in which inositol is the alcohol.
c) Phosphosphingosides, in which sphingosine is the alcohol.

Glycerophosphatides are *esters of fatty acid with glycerol containing an esterified phosphoric acid and a nitrogen base.* Brain and nervous tissues, liver, kidney, pancreas and heart contain large quantity of phospholipids.

In order to produce phospholipids (also called as phosphatides), first a phosphatidic acid is formed. In this compound, a phosphate group is present at the carbon atom 3, while two fatty acids are present in carbon atoms 1 and 2. This is, in fact, the simplest type of phosphoglyceride from which several other types are derived. The basic structure of phospolipids is very similar to that of the triacylglycerides except that C-3 (*sn*3) of the glycerol backbone is esterified to phosphoric acid as highlighted in the Figure 2.6.



X is usually a nitrogenous base.

Figure 2.6: Structure of phospholipids

Phospholipids exhibit important biological functions. They:

a) increase the rate of fatty acid oxidation
b) act as inorganic ion carrier across the membrane
c) help in blood clotting
d) act as prosthetic group for some enzymes, and
e) form the membrane structure.

Some important phospholipids are phosphatidylcholine (lecithin), phosphatidylserine, phosphatidylethanolamine (cephalin), phosphatidalethanolamine (plasmalogen), diphosphatidylglycerol (cardiolipin) and phosphatidylinositol. The chemical structure of phosphatidylcholine (lecithin) illustrated here in Figure 2.7, is typical of the phosphatides found in the brain, lung and spleen.

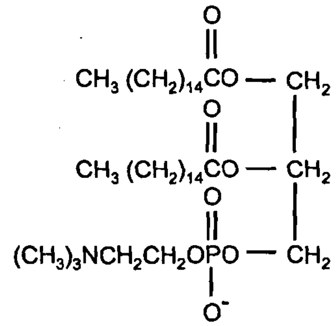


Figure 2.7: Structure of lecithin

We learnt earlier that fatty acids, steroids, hormones, vitamins A and D, etc. are categorized as derived lipids. You will find a detailed review of the chemistry of vitamins in unit 3. Hence, we shall not dwell on them at this stage. Steroids are important compounds. We shall get to know the chemistry of these compounds next, followed by eicosanoids.

2.3.4 Steroids

Steroids form a group of compounds which are often found in association with fats but structurally and functionally these are somewhat unrelated to most other lipids. Steroids are soluble in fat but resistant to sodium hydroxide i.e. these are unsaponifiable.

Basic structure of steroids consist of three cyclohexane rings (1, 2, 3 rings) and one cyclopentane ring (ring 4) as in the Figure 2.8. The parent compound of steroids is called as *cyclopentano-per-hydrophenanthrene* that has two methyl side chains typically at positions 10 and 13 constituting carbon atoms 18 and 19 in most steroids.

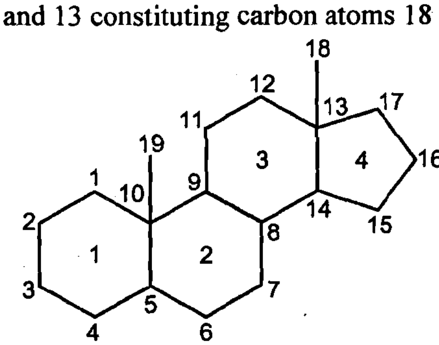
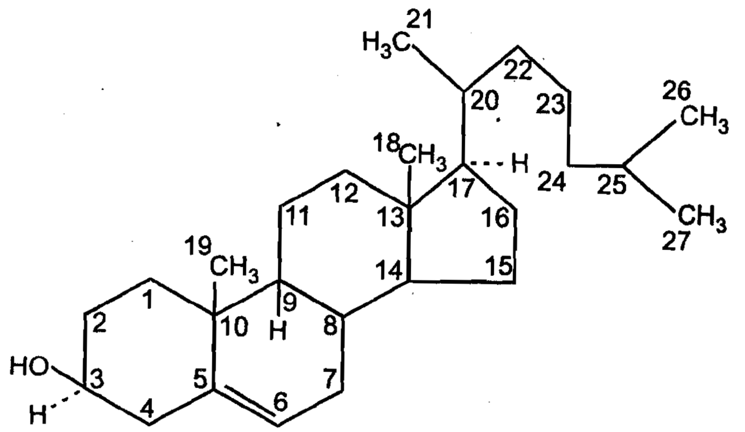


Figure 2.8: Basic structure of steroids

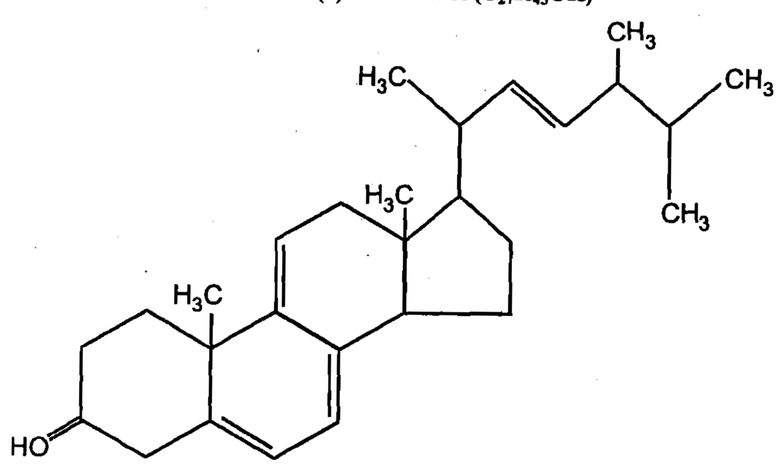
Steroids are of many types. These are given below:

a) *Sterols* : Cholesterol, ergosterol.
b) *Bile acids* : Glycocholic acid, taurocholic acid.
c) *Hormones* : Testosterone, estradiol, corticosterone.
d) *Vitamin* : Vitamin D_2 and D_3
e) *Cardiac glycosides* : Stropanthin
f) *Saponins* : Digitonin

Steroids containing an alcoholic group at C-3 position and a side chain of 8 to 10 carbon atoms at C-17 are called as 'sterols'. The best known examples are cholesterol (yes, the much talked about compound because of its association with atherosclerosis) and ergosterol (which is a precursor of vitamin D) as illustrated in Figure 2.9 (a) and (b). Sterols, such as cholesterol, are alcohols with the cyclopentanophenanthrene ring system (atoms 1 through 17 in the Figure 2.9 (a)). This substructure is also found in steroid hormones such as testosterone (refer to Figure 2.10), progesterone and cortisol. Cholesterol is considered an alcohol because it has a hydroxyl group (–OH) in position 3 of the ring system. While cholesterol is most abundant in the brain, nervous tissue, adrenals and skin, ergosterol is mainly found in yeasts, moulds etc. Sterols of vegetable origin are called "phytosterols". They have the same basic structure as cholesterol, but differ in the side chains attached to carbon 17. Phytosterols, such as *stigmasterol* from soybean oil, are of current interest because they lower blood cholesterol levels.

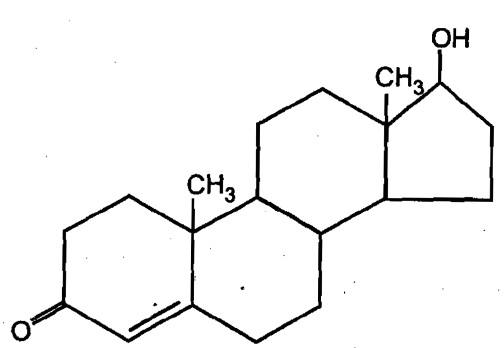


(a) Cholesterol ($C_{27}H_{45}OH$)



(b) Ergosterol

Figure 2.9: Structure of sterols



Testosterone

Figure 2.10: Structure of steroid hormone

After steroids, we shall focus on a family of compounds called eicosanoids derived from polyunsaturated fatty acids. What are eicosanoids? What is their role in the body? Let's find out.

2.3.5 Eicosanoids

Eicosanoids are the local hormones formed by body tissues during self-healing responses to stimuli. Eicosanoids are *a family of compounds derived from poly unsaturated eicosanoic acids*. They are produced from arachidonic acid, a 20-carbon poly unsaturated fatty acid. Eicosanoids comprise the prostanoids, leukotrienes (LTs) and lipoxins (LXs). Prostanoids include prostaglandins (PGs), prostacyclins and thromboxanes as shown in Figure 2.11. You will realize that prostaglandins and related compounds are collectively known as *eicosanoids*. Let us learn about these different eicosanoids.

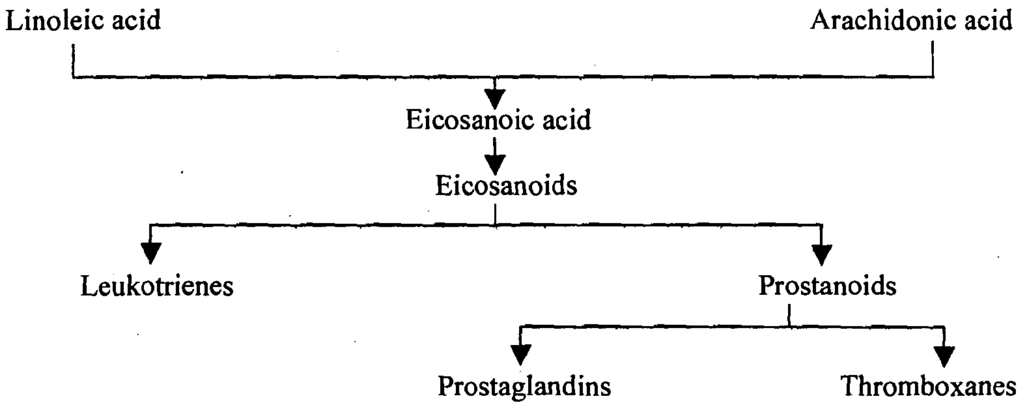


Figure 2.11: Classification of eicosanoids

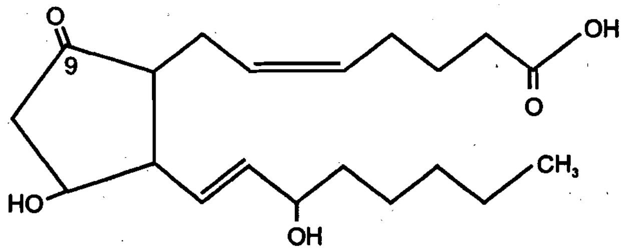
- *Prostaglandins*

Prostaglandins belong to a subclass of lipids known as the *eicosanoids* because of their structural similarities to the C-20 polyunsaturated fatty acids, the eicosanoic acids. Prostaglandins are known to occur in almost all tissues in very small quantities and have important physiologic and pharmacologic activities. These are synthesized *in vivo* by cyclization of arachidonic acid, which is either derived from dietary linoleic acid or ingested as such, to form a cyclopentane ring. There are three fatty acids known as eicosanoic acids (characterized by the number of double bonds present in the structure), which by joining with the cyclopentane ring, gives rise to three groups of prostaglandins viz. PG1, PG2, PG3. These are listed in Table 2.3.

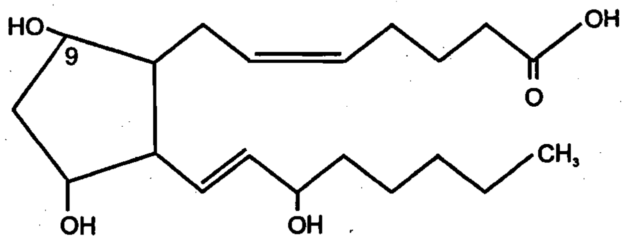
Table 2.3: Eicosanoic acids

Name	Molecular formula	Position of unsaturation	Food source
Timnoionic acid	$C_{19}H_{33}.COOH$	5,8,11,14,17	Fish oil
Clupanodonic acid	$C_{12}H_{32}.COOH$	7,10,13,16,19	Fish oil
Cervonic acid	$C_{21}H_{31}.COOH$	4,7,10,13,16,19	Fish oil

Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins. Of the six types of prostaglandins known, the primary types are prostaglandins E and F. All prostaglandin E types have a keto group in position 9 of the structure but all prostaglandin F types have a hydroxyl group in that position as highlighted in Figure 2.12.



Prostaglandin E_2



Prostaglandin F_2

Figure 2.12: Prostaglandins E and F type

- *Thromboxanes*

Thromboxanes are the second group of eicosanoids, first discovered in platelets and found to have a cyclopentane ring in the structure that is interrupted with an oxygen atom, as highlighted in the Figure 2.13.

Different types of thromboxanes (A, B, etc.) in each series (1,2,3) occur due to the variation of the substituent groups attached to the ring.

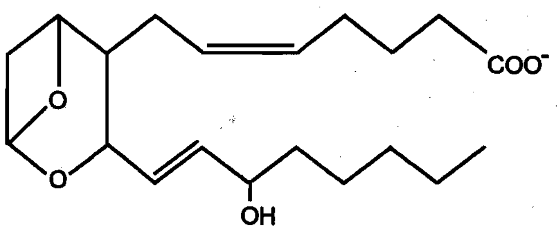


Figure 2.13: Thromboxane A_2

- *Leukotrienes*

Leukotrienes are the third group of eicosanoids, first discovered in leukocytes. These are characterized by the presence of three conjugated double bonds. Figure 2.14 presents the structure of leukotriene.

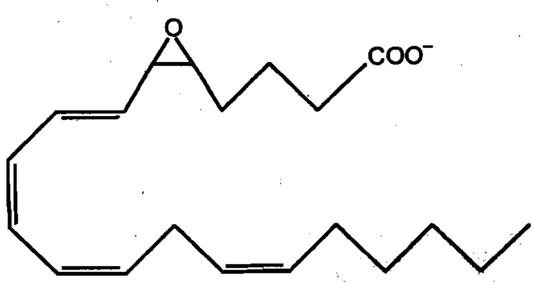


Figure 2.14: Leukotriene A_4

In the section above, we learnt about the lipids, their classification and structure. This basic knowledge, you would realize, will be useful to us in understanding the chemical properties of lipids, particularly the fatty acids and neutral fats, which is the focus of the next section.

2.4 CHEMICAL PROPERTIES OF FATTY ACIDS AND NEUTRAL FATS

Fatty acids and neutral fats respond to different chemical reagents in different ways depending on the nature of these substances. Some of the important reactions of both of these groups of compounds are discussed briefly here. We start with the chemical properties of fatty acids.

2.4.1 Chemical Properties of Fatty Acids

This section is a review of the chemical properties of fatty acids, which include esterification, hydrogenation, halogenation etc. Recognition of these properties is useful in understanding fatty acids. Let us learn about these properties then.

- *Esterification*

Like any other organic acid, fatty acids also form esters with various alcohols. An alcohol such as glycerol is reacted with fat or oil to produce esters such as mono- and di-acylglycerols. Using the esterification process, edible acids, fats and oils can be reacted with edible alcohols to produce useful food ingredients that include many of the emulsifiers such as mono and diglycerides, lecithin etc.

- *Soap formation*

When fatty acids react with alkalies, metallic salts of fatty acids commonly called as 'soaps' are formed. Potassium soap of fatty acids is more water soluble than sodium soap.

- *Hydrogenation*

When exposed to hydrogen at high pressure and temperature in presence of Ni or Pt catalyst, an unsaturated fatty acid (containing a double bond) accepts the hydrogen at the double bonds and is converted to a saturated fatty acid as shown herewith.

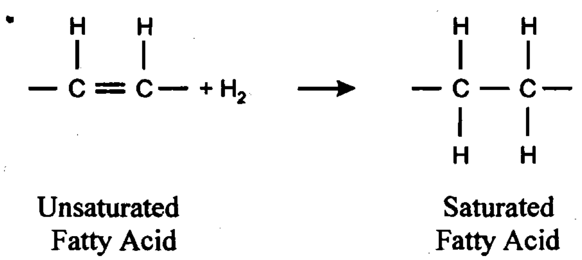


Figure 2.15: Hydrogenation reaction

Generally speaking, hydrogenation is used to change liquid oil into a semisolid or solid fat at ambient temperatures to enhance oxidative stability.

- *Halogenation*

Fatty acids accept chlorine and iodine at the double bonds when treated with reagents such as iodine monochloride and a fatty halide results.

2.4.2 Chemical Properties of Neutral Fats

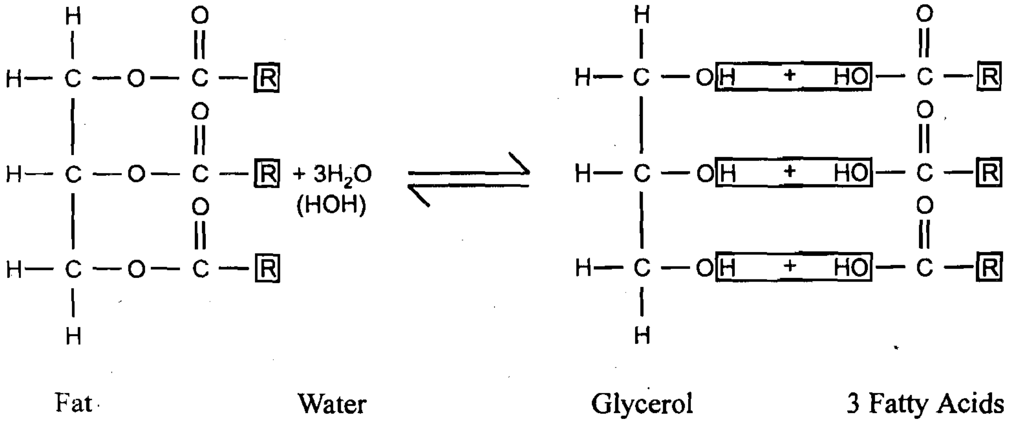
The chemical properties of neutral fats are highlighted in this section. These include:

- *Saponification*

Fats when boiled with alcoholic solution of NaOH or KOH undergo hydrolysis into glycerol and fatty acids and the latter form soaps with Na or K. The reaction is known as 'saponification'.

- *Hydrolysis*

Fats when boiled with water at 220°C under pressure in an autoclave, undergo hydrolysis to first form a diglyceride and then ultimately glycerol and fatty acids are formed as illustrated in the Figure 2.16.



Side chains are represented by R

Figure 2.16: Hydrolysis of a fat molecule to yield glycerol and fatty acids

- *Hydrogenation*

We have already talked about the hydrogenation property of fats in sub-section 2.4.1. Hydrogenation, you learnt, is *the process of turning liquid oil into solid fat.* In presence of finely ground Ni or Pt catalyst and at 150-220°C, glycerides of unsaturated fatty acids can be saturated by the action of hydrogen.

Partial hydrogenation produces margarines, shortenings, shortening oils, and partially hydrogenated vegetable oils. These products contain large quantities of *trans*-fatty acids and other altered fat substances, some of which are known to be detrimental to health because they interfere with the normal biochemical processes. *Trans* fatty acids are considered even more harmful than saturated fatty acids.

- *Rancidity*

This results from the formation of aldehyde due to the oxidation of unsaturated glycerides or by the liberation of fatty acids due to hydrolysis.

In autoxidation, oxygen reacts with unsaturated fatty acids. Initially, peroxides are formed, which in turn, breakdown to hydrocarbons, ketones, aldehydes and smaller amounts of epoxides and alcohols.

The result of the autoxidation of fats and oils is the development of objectionable flavours and odours characteristic of the condition known as *oxidative rancidity.*

Check Your Progress Exercise 2

1) What are neutral fats? Give its any two important properties.

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2) Discuss briefly the chemistry and functions of phospholipids.

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3) What are sterols? Give the structure of an important sterol of animal origin.

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4) What are the precursors of prostaglandins in the body?

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5) Mention the significance/usefulness of the esterification property of fatty acids.

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6) What is hydrogenation? What are the harmful health effects of partial hydrogenation of fats?

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7) What do you understand by the following terms:

a) Oxidative rancidity

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b) Saponification

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2.5 CHEMISTRY OF PROTEINS AND NUCLEIC ACIDS

Proteins are the third group of macronutrients and much attention should be given to these extremely important compounds. Nucleic acids are the main constituents of gene and are responsible for protein synthesis. You being a student of dietetics should have some idea of such an important group of compounds that controls our activities. This sub-section will focus on the following aspects of protein and nucleic acid chemistry:

- Definition of amino acids, peptides, proteins and nucleic acids
- Classification of amino acids, peptides and proteins
- Structure of proteins
- Physico-chemical properties of amino acids and proteins
- Biologically important peptides
- Structure and classification of nucleic acids

We begin our study on this topic by first getting introduced to proteins and nucleic acids.

Introduction to proteins and nucleic acids

Protein is derived from a Greek word "PROTOS" which means *"Pre eminent", "first or foremost"* or primary matter. This name was suggested by *Berzilius* to Dutch Scientist, *Gerard Johanan Mulder,* who coined the term *protein* in 1838. Earlier, protein was described as albuminous material, in that, it resembled albumin or the white of egg.

Proteins are compounds of carbon, hydrogen, oxygen and nitrogen. On an average, proteins contain 16% nitrogen. Most proteins also contain sulphur and some proteins contain iron, copper, phosphorus and zinc. Proteins are, in fact, *polymers consisting of chains of monomeric units*. The chains are essentially linear and contain no branches. The monomeric units of proteins are *amino acids* (which you may already know are the building blocks of the body) and the linkage between two amino acids is called a *peptide bond*. We learn about the amino acids and the peptide bond in a little while from now.

A typical protein contains about 300 amino acids and proteins have a molecular weight ranging from 5,000 to several million daltons. Proteins are essential constituents of living organisms and found in all plant and animal foods.

Next, let us get to know what are nucleic acids? *Nucleic acids*, like proteins, *are high molecular weight polymers present in all living cells.* The two major classes of nucleic acids are *DNA* and *RNA*. Surely, you are aware of these nucleic acids. The monomeric units of nucleic acids are called as *nucleotides*. Complete hydrolysis of nucleic acids yields heterocyclic nitrogenous bases, five carbon sugars and phosphoric acid molecules. Their main function is storage and transmission of genetic information.

With a basic introduction to proteins and nucleic acids, let us get to know more about amino acids, proteins and nucleic acid with respect to their structure, classification and properties. We shall begin with amino acids.

2.6 AMINO ACIDS – STRUCTURE, CLASSIFICATION AND PROPERTIES

Amino acids are the building blocks of proteins. All proteins consist of a sequence of amino acids, which are compounds containing an *amino group* ($-NH_2$) and a *carboxyl group* ($-COOH$) as indicated in the general structural formula in the Figure 2.17. Normally 20 different 2-amino acids or α-amino acids (amino acids having $-NH_2$ group at carbon 2) are found in proteins, though very recently two more amino acids are also found to be present in some proteins. All the amino acids can be represented by a general structural formula,

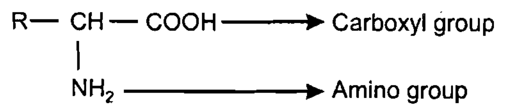


Figure 2.17: General structural formula of amino acids

in which R (the side chain) is a variable factor that changes from amino acid to amino acid, giving a character specific to a particular amino acid.

2.6.1 Classification of Amino Acids

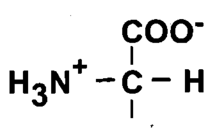
You would realize that amino acids are classified into four broad groups according to the nature of the side chain (R group). These are:

a) amino acids with non polar side chain
b) amino acids with polar but uncharged side chain
c) amino acids with positively charged side chain, and
d) amino acids with negatively charged side chain.

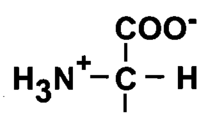
Let us get to know about them.

1. *Amino acids with non polar or hydrophobic side chain*

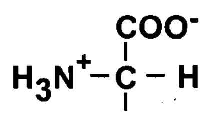
These are eight in number, as indicated in Figure 2.18, alanine, leucine, isoleucine, methionine, phenylalanine, proline, tryptophan and valine (Ala, Ile, Leu, Met, Phe, Pro, Trp and Val). These are sparingly or less soluble in water than polar amino acids. Hydrophobicity increases with an increasing side chain length.



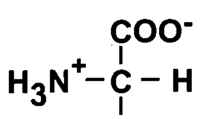
Alanine
Ala, A, (α-amino propionic acid)



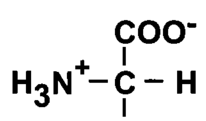
Leucine
Leu, L, (α-amino isocaproic acid)



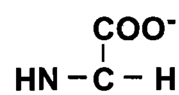
Isoleucine
Ile, I, (α-amino-methyl valeric acid)



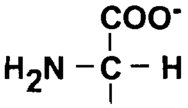
Methionine
Met, M, (α-amino-γ methylthiol-n-butyric acid) **Thioether group non polar**

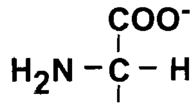


Phenylalanine
Phe, F, (α-amino-β-phenyl propionic acid)



Proline
Pro, P (Pyrrolidone-2-carboxylic acid)





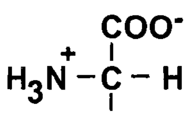
Tryptophan
Trp, W, (α-amino-β or
3- indole propionic acid)

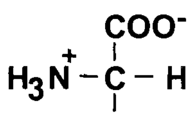
Valine
Val,V,(α-amino
isovaleric acid)

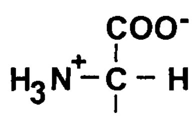
Figure 2.18: Structures of amino acids with hydrophobic side chain

2. *Amino acids with uncharged polar (hydrophilic) side chain*

These have neutral polar functional groups and are able to establish hydrogen bonding with appropriate molecules, such as water. Polarity of serine and threonine is due to –OH group and of asparagine (Asn) and glutamine (Gln) is due to $-CONH_2$ (amide) groups. Sometimes glycine is also included in this group. Cysteine and tyrosine possess most polar functional groups of this category, since both thiol and phenol groups may undergo partial ionization at pH values close to neutrality. In proteins, cysteine is often present in oxidized forms (i.e. cystine). Asparagine (Asn) and glutamine (Gln) also readily hydrolyze to form asparatic acid (Asp) and glutamic acid (Glu) in presence of an acid or alkali.



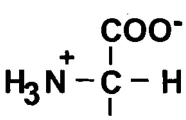


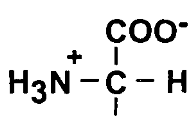


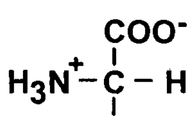
Serine
Ser, S, (α-amino
β-hydroxy propionic
acid)

Threonine
Thr, T, (α-amino-β
hydroxy Butyric acid)

Glutamine
Gln, Q, (Amide
of Glutamic acid)



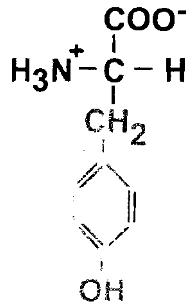




Asparagine
Asn, N, (Amide of
aspartic acid)

Glycine
Gly, C, (α-amino
acetic acid)

Cysteine
Cys, C, (α-amino
β-mercapto
propionic acid)

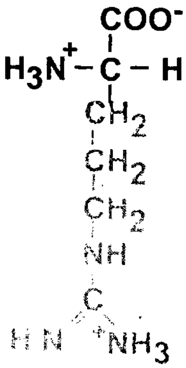


Tyrosine
Tyr, Y, [α-amino-β (p-hydroxyphenyl) propionic acid]

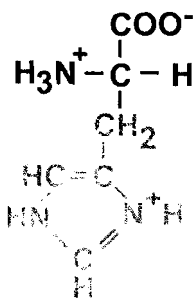
Figure 2.19: Structures of amino acids with hydrophilic side chains

3. *Amino acids with positively charged side chains (at pH close to neutral)*

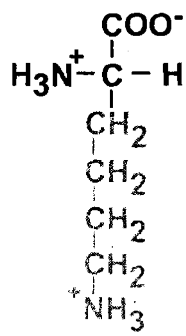
Lysine, histidine and arginine are the examples of this group. Amino group of lysine and guanidino group of arginine are responsible for +ve charges. Imidazole group of histidine is 10% protonated at pH 7.0 and 50% at pH 6.0.



Arginine
Arg, R (α-amino-δ-guanidino valeric acid)



Histidine
His, H (α-amino-β-imidazole propionic acid)

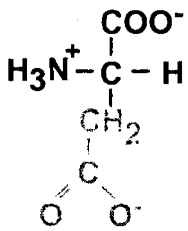


Lysine
Lys, K (α, ε diamino caproic acid)

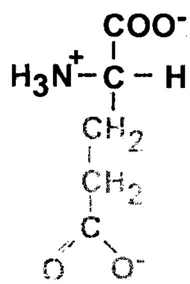
Figure 2.20: Structures of amino acids with positively charged side chains

4. *Amino acids with negatively charged side chain*

Aspartic acid (Asp) and glutamic acid (Glu) are the examples of this class.



Aspartic acid
Asp, D (α-amino succinic acid)



Glutamic acid
Glu, E (α-amino glutaric acid)

Figure 2.21: Structures of amino acids with negatively charged side chains

For your convenience, a complete list of these 20 amino acids is presented in Table 2.4.

Table 2.4: Amino acids commonly found in proteins

Amino acids with non polar side chain	Amino acids with polar but uncharged side chain
Alanine	Glycine
Valine	Serine
Leucine	Threonine
Isoleucine	Cysteine
Proline	Tyrosine
Methionine	Asparagine
Phenylalanine	Glutamine
Tryptophan	
Amino acids both positively charged side chain	**Amino acids with negatively charged side chain**
Lysine	Aspartic acid
Arginine	Glutamic acid
Histidine	

With this, we come to an end of our discussion on classification of amino acids. Next, let us learn about the chemical reactions of amino acids. But, first let us recapitulate what we have learnt so far about amino acids.

Check Your Progress Exercise 3

1) What do you understand by the following terms? Explain giving examples.

a) Proteins

....

....

b) Nucleic acids

....

....

c) Nucleotides

....

....

2) How many amino acids are found in proteins? Name any five of them.

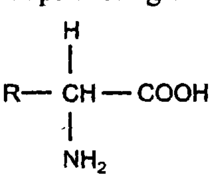
....

....

....

3) Indicate:

a) Carboxyl and amino groups in the given structure of amino acid.



b) polar, non-polar, basic or acidic amino acids from the following:

(i) Glycine
(ii) Proline
(iii) Threonine
(iv) Aspartate
(v) Lysine

2.6.2 Properties and Chemical Reactions of Amino Acids

In this section we shall look at the physical and chemical properties of amino acids. We shall first study about the physical properties of amino acids.

A) *Physical Properties*

Solubility: Amino acids are readily soluble in water, slightly soluble to insoluble in ethanol and insoluble in ether. Tyrosine is soluble in hot water but only sparingly soluble in cold water. Cysteine with difficulty is soluble in only hot water. Proline and hydroxy proline are soluble in alcohol and ether.

Amino acids are generally soluble in acids and bases and form salts. Tyrosine is moderately soluble in acids and bases. Cysteine is soluble in strong mineral acids (HCl) but slightly soluble in acetic acid and dilute ammonia.

Melting Points: Amino acids have a high melting points in the range of 200 - 300°C.

Taste: Amino acids are usually sweet, tasteless or bitter. Eg. Gly, Ala, Val, Pro, hydroxy-Pro, Ser, Try and His are sweet; Leu is tasteless, whereas, Ile and Arg are bitter in taste.

Sodium salt of glutamic acid (Monosodium glutamate, MSG, also known as Ajinomoto) is valuable as a flavouring agent for certain foods and sauces since it imparts and enhances the flavour (also for meat and meat products). MSG is being used since 1908 when a Japanese Chemist *Kikunae Ikeda* discovered that MSG could improve the flavour of the food products. However, tests on animals have shown that it can cause brain damage and cancer. It has not been proved to be dangerous to humans. However, food experts do not approve its use for infants and pregnant women. The acceptable daily intake of MSG for a person weighing about 60 Kg is approximately 7.2 gms.

Acidobasic Properties: Depending upon the pH of the solution, amino acids ionize and act as weak Bronsted acid or base (i.e. proton donor or acceptor). This ionization facilitates their quantitative analysis. Thus, all the amino acids may be an acid, or a base.

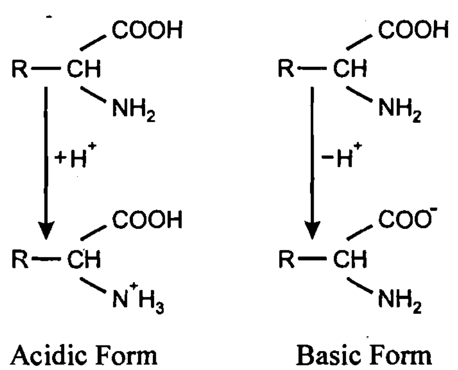


Figure 2.22: Acidobasic property of amino acids

Let us understand this concept better. Look at the structure of amino acid given in Figure 2.22. It is evident that amino acids contain both acidic (–COOH) and basic (–NH_2) groups. Because of this unique structure, amino acids have the characteristics of an acid and a base and are capable of reacting chemically either as an acid or a base. Hence they are said to be *amphoteric*. They can donate or accept protons (and thus can serve as an acid or a base). However, when both the groups are ionized, the amino acid is said to be a *zwitterion* and behaves as a neutral compound. Among amino acids, neutral amino acids have a net zero charge because their structure is as illustrated in Figure 2.23. The positive and negative charge neutralizes each other. Any compound which has a net zero charge is called a 'zwitterion'.

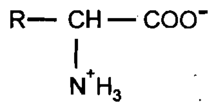


Figure 2.23: Zwitterion

Zwitterions are *amphoteric molecules* (no net charge on the molecule), *which behave both as acid and base* and the pH at which this form exists, is known as *isoelectric point* (pl).

Stereochemistry of Amino Acids: Optical Properties

All amino acids (except glycine) rotate the plane of polarized light because of the presence of an asymmetric center at C-2. Both L and D enantiomers are possible for the amino acids. What do we mean by enantiomers? Do you recall reading about them in the last unit under the topic of isomerism? Like carbohydrates, amino acids too exist as L and D isomers. The definition of D and L depends on the position of $-NH_2$ group on C-2. When the amino group is on the left of C2, it is called *L form.* Essentially, all the amino acids in the diet and in the body occur as the L isomer as shown in Figure 2.24.

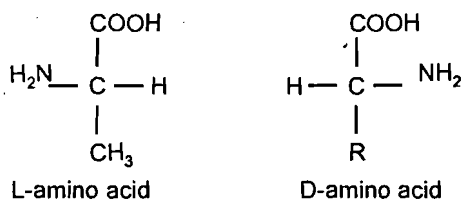


Figure 2.24: D– and L– forms of a typical amino acid

Ile, Thr, hydroxyproline and hydroxylysine have 2nd asymmetric carbon also in their structure, thus four stereoisomers are possible for these amino acids. Optically active amino acids can be converted into racemic mixture by heating them with alkali.

Hydrophobicity of Amino Acids

Hydrophobicity of amino acids and also of peptides and proteins may be determined by relative solubility of amino acid in water and in a less polar solvent (eg. ethanol) respectively. Amino acids are grouped as hydrophilic and hydrophobic depending on whether the side chains (R) like water (hydrophilic) or hate water (hydrophobic).

Ala, Arg, Asn (Asparagine), Asp, Cys, Glu, Gln (Glutamine), Gly, His, Lys, Pro, Ser, Thr are classified as hydrophilic. Sometimes, Tyr is included in this group though Tyr is more hydrophobic than hydrophilic. Amino acid side chains other than above are hydrophobic.

Absorption Spectra and Fluorescence Properties

Of all the amino acids, Try, Tyr and Phe absorb UV light and have a maximum absorption at 280, 274 and 260 nm, respectively. Cysteine shows a slight absorption at 238 nm. All the amino acids absorb at wavelength near 210 nm.

Try, Tyr and Phe are the only amino acids, which show natural fluorescence. Try fluorescence remains even when it is bound in protein. Next, let us get to know about the chemical properties of amino acids.

B) ***Chemical Reactions of Amino Acids***

The chemical reactions of amino acids can be divided into three classes:

i) Reactions of carboxylic group
ii) Reactions of amino group
iii) Reactions of the side chain

Here in this unit, we shall not go into each and every reaction specific to the three classes mentioned above, but few important reactions are described herewith.

i) *Reactions of Carboxylic Group*

a) *Salt formation:* In alkaline medium, –COOH group reacts with metal hydroxide to form amino acid salts as shown in Figure 2.25.

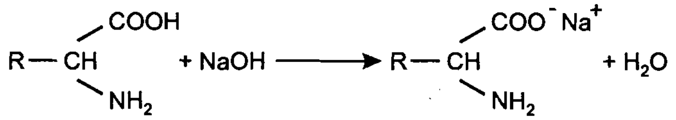


Figure 2.25: Salt formation

b) *Ester formation:* In presence of dry HCl, amino acids react with alcohol to form esters. This is one of the ways of blocking –COOH group in the chemical synthesis of proteins. Figure 2.26 illustrates the ester formation.

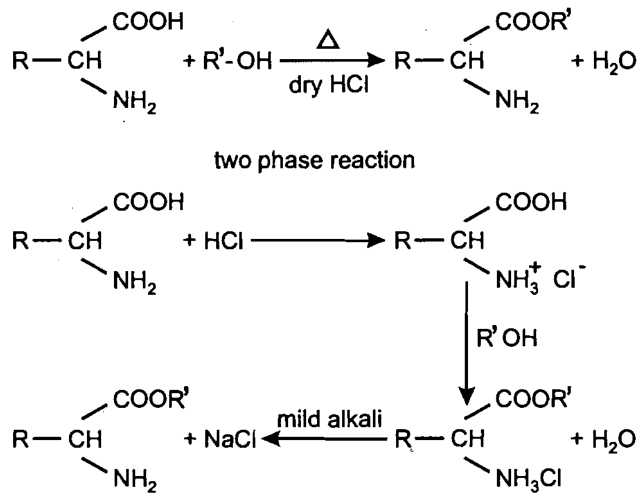
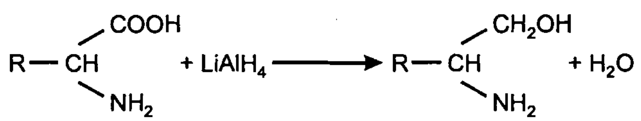


Figure 2.26: Two step reaction showing ester formation with alcohol

This ester as well as free amino acid can be reduced to corresponding alcohol with lithium aluminium hydride ($LiAlH_4$).



This is one of the ways of identifying C-terminal amino acid in the form of amino alcohol.

c) *Decarboxylation:* Amino acids undergo decarboxylation reaction, enzymatically or by treatment with heat, acid or alkali (barium hydroxide) to form the corresponding amines as shown in Figure 2.27.

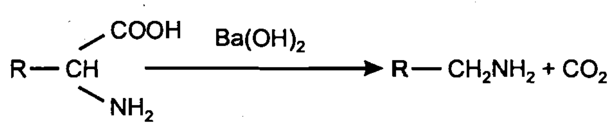


Figure 2.27: Decarboxylation reaction

In biological systems, a number of amines generated by this way have biological activity. For eg. histamine (decarboxylation product of histidine) mediates allergic reactions, shock and stress. Similarly, histamine and tyramine (decarboxylation product of tyrosine), both possess pharmacological properties.

ii) *Reactions of Amino Group*

There are many reactions of amino group, namely methylation, reaction with nitrous acid, oxidative deamination of amino acids, reaction with aldehydes etc. We shall not discuss each one of them. Some important reactions are described here.

Ninhydrin reaction: Ninhydrin is a powerful oxidizing agent. When it reacts with amino acids, oxidative decarboxylation results in the formation of CO_2, NH_3 and an aldehyde. The reduced ninhydrin subsequently reacts with liberated NH_3 forming a blue/purple complex which has maximum absorption at 570 nm. Proline forms a yellow colour in this reaction. This reaction is very useful for quantitative estimation of amino acids.

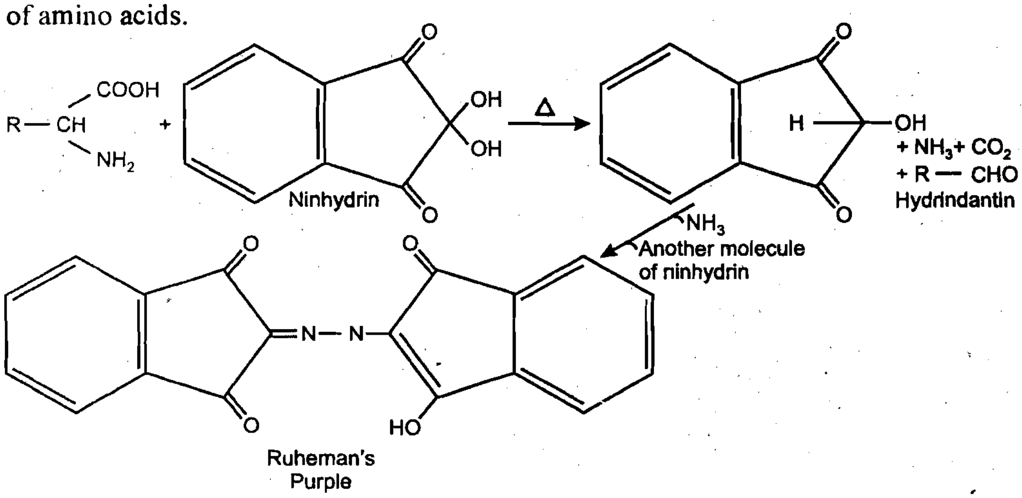


Figure 2.28: Ninhydrin reaction

Sanger's reaction: This reaction is specific for the amino group of the amino acids or peptides. 1-fluro-2,4-dinitrobenzene (FDNB) reacts with the sample resulting in the formation of DNP- derivative, which is a yellow crystalline compound.

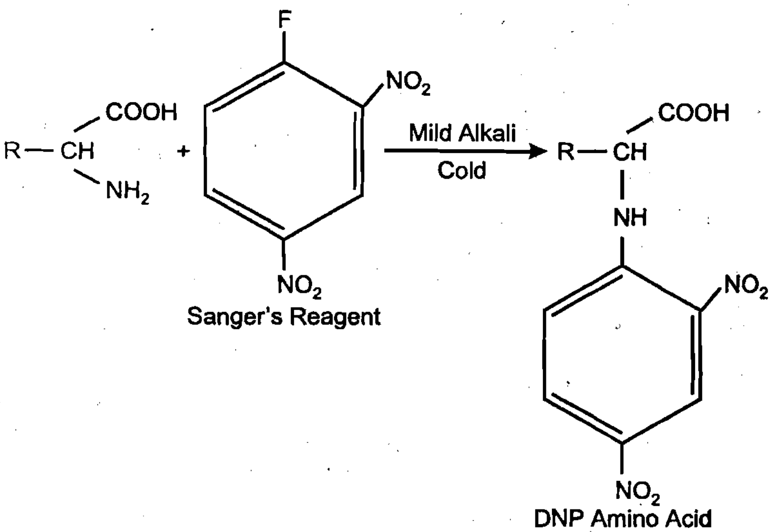


Figure 2.29: Sanger's reaction

Edman's reaction: This is an important reaction for the identification of the amino acid at the N-terminal of the peptide. Phenylisothiocyanate reacts with the amino acid to yield a cyclic product, phenylthiohydantoin.

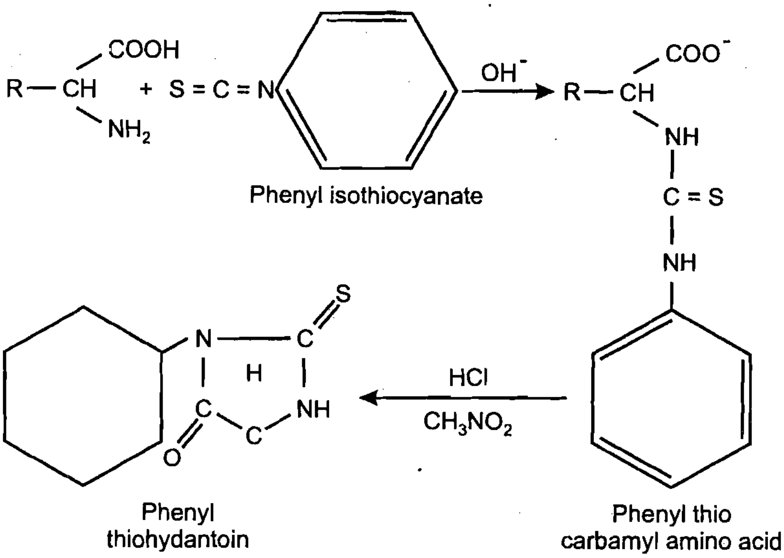


Figure 2.30: Edman's reaction

iii) *Reactions of side chain*

The reactions of the side chain include, reaction of thiol group with iodoacetic acid, reaction of thioether group with formic acid (used for the determination of methionine as methionine sulphone) with p-chloro (p-hydroxy) mercuric benzoate (useful tool for the determination of cysteine), with Ellman's reagent (reaction is used for the determination of cysteine) etc. These reactions are given in Figures 2.31 a,b,c,d.

$$R—SH + ICH_2COOH \rightarrow R—S—CH_2OH + HI$$

Figure 2.31 (a): Reaction with iodoacetic acid

$$R—SH + HCOOH \rightarrow R—SO_3H$$

Figure 2.31 (b): Reaction with formic acid

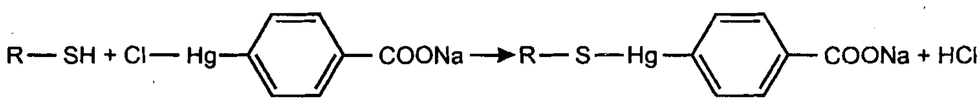


Figure 2.31 (c): Reaction with p-chloromercuric benzoate

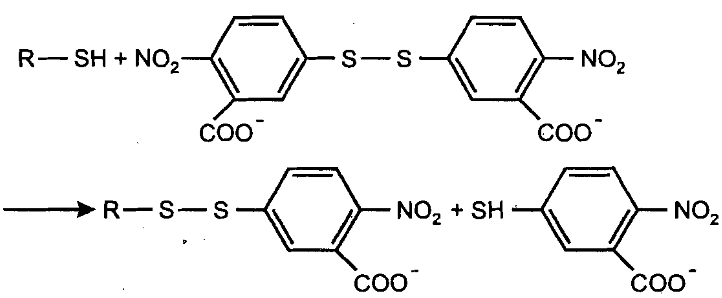


Figure 2.31 (d): Reaction with Ellman's reagent

You may find the information provided in this section a bit technical and tough. Do not panic. We do not expect you to memorize the reactions etc. Our interest in giving you this information is to make you aware of a few of these reactions which will help you identify an amino acid or understand its functioning. You will learn more about this in the Practical Manual. With this, we come to an end to our study about amino acids.

Next, let us learn about peptides and their classification.

2.6.3 Peptides – Classification and Biologically Important Peptides

What are peptides? Peptides, as you may already know, are formed by linking amino acids by peptide bond formation that involves a carboxylic group and an amino group with the elimination of water molecule as shown in Figure 2.32 (a). Figure 2.32 (b) shows the reaction of two amino acids, where **R** and **R'** are the side chains. The blue circle shows the water **(H_2O)** that is released, and the red circle shows the resulting peptide bond **(–C (=O) NH–)**.

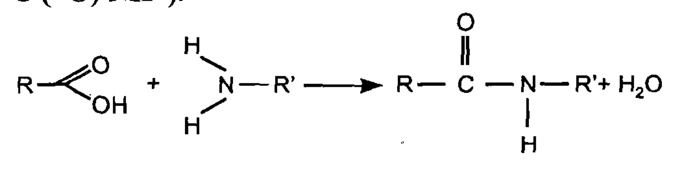


Figure 2.32(a): Peptide bond formation

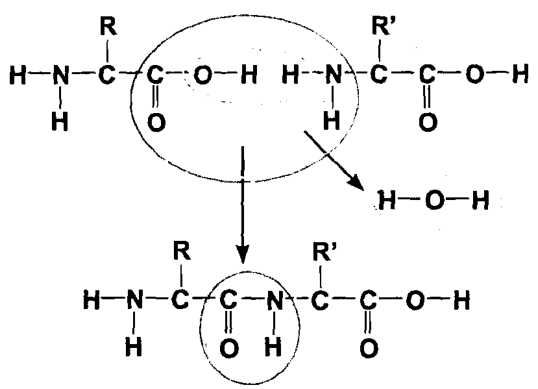


Figure 2.32(b): Peptide bond formation

Polypeptides and proteins are chains of amino acids held together by peptide bonds. Two amino acids link to form a dipeptide, three a tripeptide and so on. The peptide is called an oligopeptide when the number of amino acids in it is not more than 10. Beyond that, it is a polypeptide. Each peptide begins with an amino terminal and ends with a free carboxyl terminal.

Among the various peptides existing, some are especially important from the physiological point of view. These are:

Glutathione: It is a tripeptide made up of glutamic acid, cysteine and glycine. The compound is involved in oxidation-reduction reactions.

Oxytocin and *vasopressin:* These cyclic peptide hormones of the pituitary gland consist of 8 amino acids. These aid in the ejection of milk and re-absorption in the kidney, respectively, besides other functions.

Angiotensin: Angiotensin I consisting of 10 amino acids, has slight effect on blood parameters. Angiotensin II, consisting of 8 amino acids, has significant effect on blood pressure.

Insulin: It consists of 51 amino acids, contains two polypeptide chains linked together by two –S–S– bridges. It helps in the utilization of sugar by the cells.

Having gone through section 2.6, we are now ready to embark on our journey to the world of proteins. Let's explore. But first recapitulate what you have learnt so far.

Check Your Progress Exercise 4

1) Discuss the 'solubility' property of amino acids, giving examples.

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2) What are amphoteric amino acids?

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3) What is a zwitterion? Explain why neutral amino acids exist in the zwitterion form.

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4) Of the two isomers of amino acids, which one occurs in our diet and body?

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5) Define peptides and polypeptides. Name any two polypeptides that are important from physiological point of view.

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6) What is a peptide bond? Explain how it is formed?

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2.7 PROTEINS – STRUCTURE, CLASSIFICATION AND PROPERTIES

Proteins, as you may have realized by now, are the *dehydration products of amino acids with each amino acid residue joined to its neighbour by a specific covalent bond.* In this section, we shall unravel the mystery related to its structure, classification and properties.

2.7.1 Classification of Proteins

Proteins may be classified broadly into three groups: a) Simple proteins, b) Conjugated proteins, and c) Derived proteins. Let us get to know them better.

Simple proteins contain only amino acids and do not have any non-protein part. Conjugated proteins have a non-protein part. Derived proteins are formed from either simple or conjugated proteins due to the unfolding of the tertiary structure or due to the cleavage of peptide bonds producing primary and secondary derivatives, respectively. These three main classes of proteins can further be sub-classified according to their structure and properties. Table 2.4 depicts in detail the various classes of protein, their main characteristics and few examples of such proteins.

Table 2.4: Classification of proteins

Class	Characteristics	Examples
Simple proteins		
a) Albumins	Soluble in water, coagulated by heat	Ovalbumin (egg), lactalbumin (milk)
b) Globulins	Insoluble in water, soluble in dilute salt solution	Vitellin (egg yolk), tuberin (potato)
c) Glutelins	Insoluble in water, soluble in dilute acids and alkalis	Glutenin (wheat), oryzenin (rice)
d) Prolamins	Insoluble in water, soluble in 70% alcohol	Gliadin (wheat), Zein (maize)
e) Protamins	Soluble in water, dilute acids and alkalis	Salmine (salmon sperm)
f) Histones	Soluble in water	Globin of haemoglobin
Conjugated proteins		
a) Nucleoproteins	Consisting of simple proteins and nucleic acids, soluble in water	Nucleohistones
b) Lipoproteins	Consisting of proteins and lipids	Lipoproteins of egg yolk, milk
c) Phosphoproteins	Protein containing phosphate	Caseinogen (milk), Ovovitellin (egg yolk)
d) Chromoproteins	Consisting of protein and the porphyrin	Haemoglobin, myoglobin
e) Flavoproteins	Protein containing riboflavin	Flavoproteins of liver and kidney
f) Metalloproteins	Proteins containing different metal ions	Ceruloplasmin (Cu), Ferritin (Fe)
g) Glycoproteins	Combination of proteins with carbohydrates	Ovomucoid (egg white), Mucin (saliva)
Derived proteins		
a) Primary derivatives		
i) Proteins	Derived by the action of dilute acids, alkalis or enzymes	Fibrin from fibrinogen
ii) Metaproteins	Derived by the action of slightly stronger acids and alkalis	Acid and alkali metaproteins
iii) Coagulated proteins	Due to the action of heat, x-rays, UV rays, etc.	Coagulated albumin
b) Secondary derivatives		
i) Proteoses	By the action of pepsin or trypsin, incoagulable by heat	Albumose from albumin
ii) Peptones	Due to further cleavage of proteoses. Soluble in water, incoagulable by heat	–
iii) Peptides	Compounds containing two or more amino acids	Glycyl-alanine

2.7.2 Structure of Proteins

We have already seen that each molecule of protein is composed of many molecules of amino acids joined by peptide bonds. But, protein is a complicated macromolecule and the complexity of its structure depends on its molecular size and shape. Considering the long peptide chains and variation in the structure of twenty different amino acids, biochemists have assigned four basic structural levels to proteins – primary, secondary, tertiary and quaternary – as illustrated in Figure 2.33. Let us get to know a bit more about these structures.

i) *Primary structure:* It refers to *the linear arrangement of amino acid residues in a given polypeptide chain linked through peptide bonds,* as shown in Figure 2.33. The shortest peptide studied may have 20-100 amino acids (secretin and glucagons). Most of the proteins have 100-150 amino acids. Some rare chains may have up to 1000 amino acids. The number and sequence of amino acids determine the specificity of the protein and any disturbance in these would create a different protein. A good example is haemoglobin. A single replacement of glutamic acid with valine in position 6 of haemoglobin molecule changes the characteristics of haemoglobin resulting in the disease sickle cell anaemia.

ii) *Secondary structure:* It refers *to a three dimensional arrangement of various atoms of the protein molecules.* The polypeptide is folded systematically and the secondary conformation is stabilized due to binding forces between different segments of the peptide chain. The bonds that generally formed are hydrogen bonds, disulphide bonds, ionic bonds and hydrophobic bonds. Some proteins are found to be in a coiled form (α-helix) e.g. α-keratin and some possess sheet like structure (β -pleated sheet) e.g. β-keratin, collagen as illustrated in Figure 2.33.

iii) *Tertiary structure:* The term refers to *the tendency of the polypeptide chain* {containing well defined (α-helix, β-bends or sheets) or ill defined (random coil) secondary structure} *to undergo extensive coiling or folding and produce a complex, somewhat rigid structure.* Most native proteins have this kind of structure. The structure is stabilized by different types of intermolecular bonds such as hydrogen bonds, ionic bonds and hydrophobic bonds. Due to the bonding, the distant regions of the chain are brought closer and the protein assumes a spherical, globular or ellipsoidal conformation.

iv) *Quaternary structure:* Many proteins, particularly enzymes, consist of *several peptide chains linked by disulphide bonds.* Such proteins are said to possess a quaternary structure. The most studied protein of this class is *haemoglobin* that consists four polypeptide chains, 2 α-chains and 2 β-chains bound together.

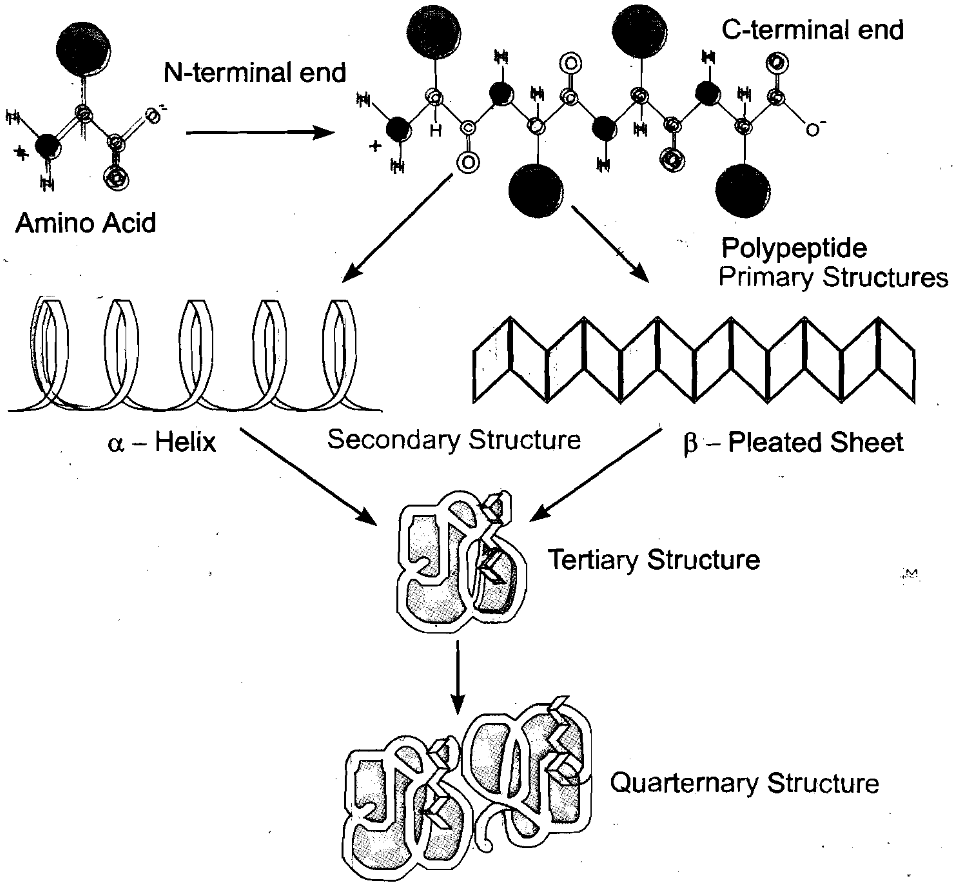

Figure 2.33: Structure of proteins

Having learnt about the structure, classification of proteins, let us finally study the properties of proteins.

2.7.3 Physico-Chemical Properties of Proteins

Proteins, you learnt earlier, are high molecular compounds and exhibit characteristic properties in different environmental conditions. Some of these properties are described here.

a) *Isoelectric pH:* Many ionizable groups are present in a protein molecule. Depending on the pH of the medium, some of these groups act as proton donors and some others act as proton acceptors. Thus, proteins are amphoteric compounds. At a specific pH, the protein exists as a dipolar ion (one positive and one negative ion) or zwitterion. So, at this pH the net charge of the protein becomes zero. This pH is known as *isoelectric pH* or *pI* of the protein. Having no net charge, protein does not move to either electrode in an electric field.

b) *Solubility:* Proteins behave differently in solution. Globular proteins are generally more soluble in aqueous medium in comparison to elongated fibrous proteins such as keratins. Solubility behaviour of proteins, however, is influenced by the nature of solvent, pH, temperature, etc.

c) *Precipitation:* Proteins may be precipitated in different ways,

   i) Isolectric precipitation: At isoelectric pH (p^1), you already know, a protein does not have any net charge. So, they easily aggregate and precipitate without denaturation because of having minimum electrostatic repulsion.
   ii) Salting out: Proteins in aqueous medium can be precipitated by adding trichloroacetic acid (TCA) or perchloric acid (PCA). Salts of heavy metals, phosphomolybdic acid or phosphotungstic acid are commonly used for protein precipitation. This is known as *salting out.* Concentrated solutions of neutral mineral salts such as $MgSO_4$, Na_2SO_4 and $(NH_4)_2SO_4$ are also commonly used for precipitation of proteins.
   iii) Action of non-polar organic solvents: A non-polar solvent like chloroform enhances the electrostatic attraction between the ions of proteins and thus facilitates their aggregation and precipitation.

The chemical properties of proteins are largely those of the side chains of the constituent amino acids. The arginine side chains, each containing a guanidine group can react with α-naphthol in the presence of an oxidizing agent such as sodium hypochlorite to produce red colour (Sakaguchi reaction). Similarly, tryptophan side chains, being indoles, can react with glyoxylic acid in the presence of concentrated sulphuric acid to produce a purple colour (Hopkins-Cole reaction). Tyrosine side chains, each possessing a phenolic group can undergo a variety of reactions. If treated with mercuric sulphate and sodium nitrate and then heated, a red complex is produced by the Millon reaction. They also undergo the Folin-Ciocalteu reaction, if treated with tungstate and molybdate, a blue colour being formed. All of these procedures, particularly the last mentioned, can be used for the quantitative estimation of proteins, the intensity of the colour produced being dependent on the number of reacting groups present. The functional groups in amino acid side chains play an important role in the catalytic activity of enzymes. Many agents can inactivate enzymes by binding to these functional groups, for e.g. heavy metal ions bind strongly to the sulphydryl groups of cysteine residues and thus may act as poisons to a great many enzymes.

With the properties of proteins we come to the end of our journey through the world of proteins. Hope you enjoyed the trip. Next, we move on to nucleic acids.

Check Your Progress Exercise 5

1) Classify proteins into three broad groups, with one example from each group.

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2) Explain the four structural levels of proteins.

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3) Define pI.

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4) What do you mean by the term 'salting out'? Give examples.

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2.8 STRUCTURE AND CLASSIFICATION OF NUCLEIC ACIDS

Nucleic acids are *polymers of nucleotides*. Nucleotides are *nucleoside phosphates*. Nucleosides are formed from a nitrogenous base and a pentose sugar. Thus, the nucleotides, as illustrated in the Figure 2.34, consist of a *nitrogenous base* which may be either a purine (adenine or guanine) or a pyrimidine (cytosine or uracil or thymine) base, *a pentose sugar* (containing a five carbon atoms) which may be either ribose or deoxyribose and a *phosphate*. When several neuclotides join, they form a polynucleotide.

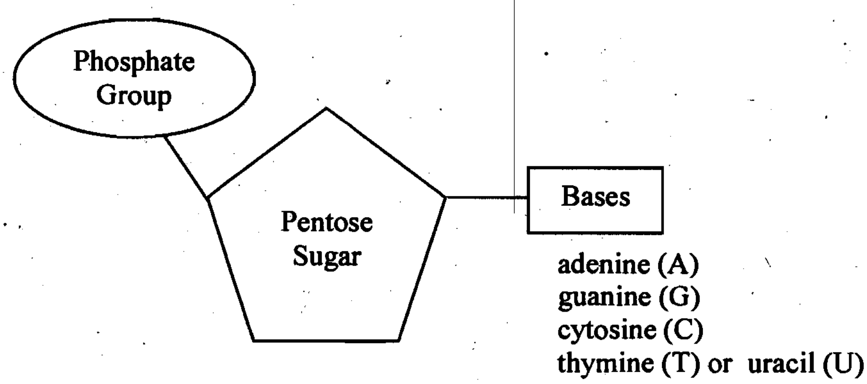

Figure 2.34: Nucleotide structure

According to the presence of ribose or deoxyribose, they are called as ribonucleotides or deoxyribonucleotides. One of the most important naturally occurring nucloeotides is adenosine-5'-monophosphate (AMP). This compound together with two of its derivatives, adenosine-5'-diphosphate (ADP) and adenosine-5'-triphosphate (ATP), plays an extremely important role in the conservation and utilization of energy released during cellular metabolism. Other common nucleotides are guanosine-5'-monophosphate (GMP), cytidine-5'-monophosphate (CMP) and uridine-5'-monophosphate etc.

Thus ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) have ribose and deoxyribose as the pentose sugar, respectively. They also differ, to some extent, in possession of nitrogenous bases. DNA contains four types of nitrogenous bases – adenine, thymine, cytosine and guanine, while RNA contains uracil in place of thymine and the three other bases as in DNA. Figure 2.35 illustrates the chemical structure of these nitrogenous bases.

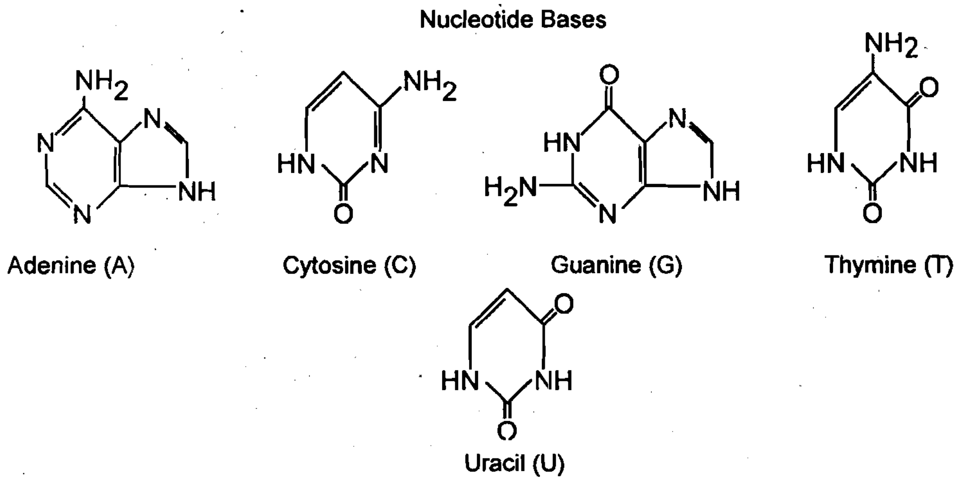

Figure 2.35: Chemical structure of nitrogenous bases

DNA is composed of two polynucleotide chains wound into a right handed helix that coil around a central axis. The chains are held together by hydrogen bonds between the bases of two opposite chains. A critical feature of the double helix is the base-pairing relationship; adenine pairs with thymine and guanine pairs with cytosine. Thus, the base sequence of one chain matches with the sequence of the other. In simple terms, DNA forms a double helix (see Figure 2.36 (b) in which the nucleotide bases are attached to deoxyribose units linked through phosphate groups. The bases in the center of the DNA helix always occur in complementary matched pairs, with cytosine linking to guanine and thymine linking to adenine through hydrogen bonding (shown as dotted lines in Figure 2.36 a).

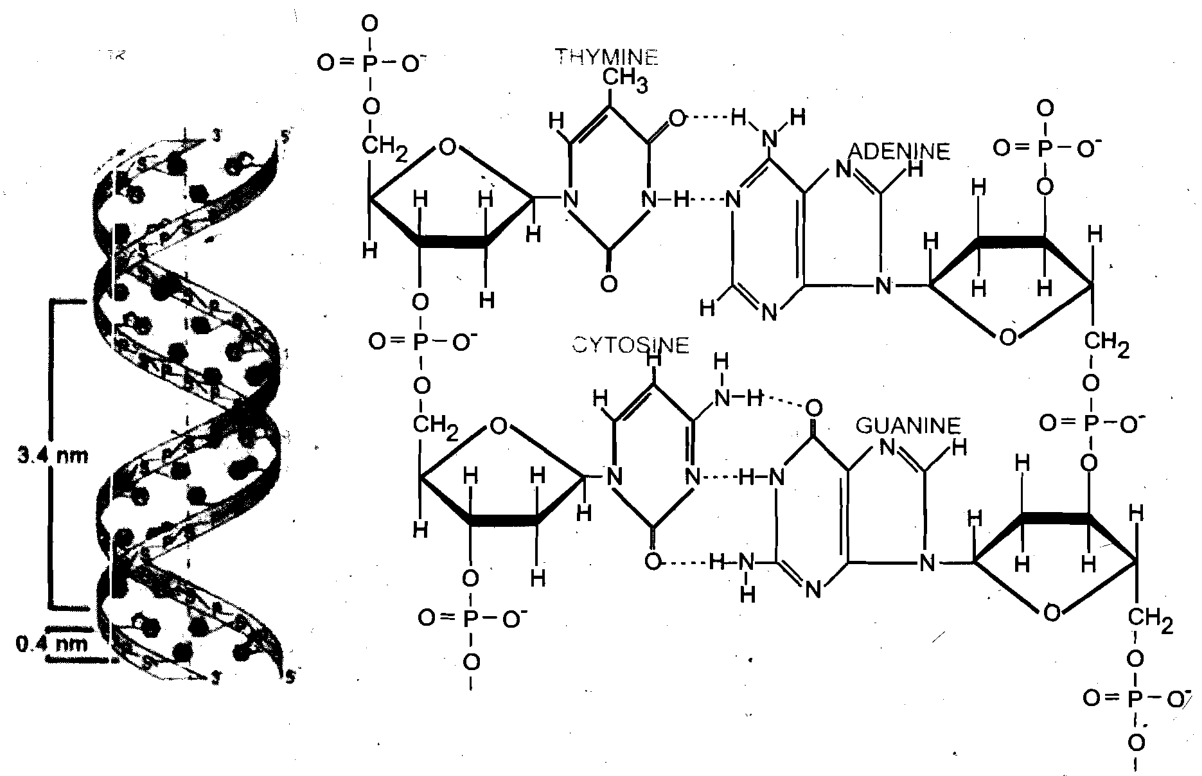

Figure 2.36: (a) and (b) DNA Structure

Structurally RNA differs significantly from DNA. Each molecule of RNA is single stranded and is made as a complementary strand of one of the two chains of DNA. RNA molecules are smaller than DNA molecules. There are three forms of RNA found in the cell, messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) having specific functions during protein synthesis. You will learn about their functions in the Advance Nutrition course.

Check Your Progress Exercise 6

1) What is meant by the term 'nucleotide'? Name one of the nucleotides mentioning its bases.

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2) Structurally, how is DNA different from RNA? Explain.

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3) What is the critical feature of the double helix of DNA?

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2.9 LET US SUM UP

In this unit, we studied about chemistry of lipids, proteins and nucleic acids. We learnt the structure and classification of lipids – one of the physiologically important compounds. Then we had a look at the chemical properties of both fatty acids and neutral fats.

In the consequent sections, we learnt about proteins and amino acids, where we saw the structure and properties of amino acids and proteins. Finally, we dealt with nucleic acids and polynucleotides such as DNA and RNA, where we saw how these essential polynucleotides are relevant to us and differ from each other structurally.

2.10 GLOSSARY

Amino acids	:	building blocks of the body.
Amphoteric	:	amino acids having the characteristics of an acid and a base.
***Cis*-isomer**	:	both hydrogen atoms located on the same side of the double bond.
Eicosanoids	:	local hormone formed by body tissues during self-healing responses to stimuli.
Hydrogenation	:	the process of turning liquid oil into solid fat.
Lipids	:	heterogenous group of compounds occurring in both plants and animals.
Monounsaturated fatty acid	:	fatty acids having a single double bond.
Nucleic acids	:	high molecular weight polymers present in all living cells.
Neutral fats	:	esters of fatty acid with glycerol containing an esterified phosphoric acid and a nitrogen base.
Nucleosides	:	compounds formed from a nitrogenousbase and a penstose sugar.

Nucleotides	:	compounds consisting of a nitrogenous base, a pentose sugar and a phosphate group.
Oxidative rancidity	:	the development of objectionable flavours and odours characteristic of the autoxidation of fats and oils.
Peptide bond	:	linkage between carboxyl group and amino group.
Phytosterol	:	sterols of vegetable origin.
Polyunsaturated fatty acid	:	fatty acid having two or more double bonds.
Proteins	:	compounds of carbon hydrogen, oxygen and nitrogen.
Rancidity	:	Formation of aldehyde due to oxidation of unsaturated glycerides.
Saponification	:	hydrolysis of fats when boiled with alcoholic solution of NaOH/KOH into glycerol and salts of fatty acids (soaps).
Steroids	:	a group of compounds which are often found in association with fat.
Sterols	:	steroids containing an alcoholic group at c-3 position and a side chain of 8 to 10 carbon atoms at c-17.
***Trans*-isomer**	:	both hydrogen atoms are placed on either side of the double bond.
Unsaturated fatty acid	:	a fatty acid containing one or more double bond.
Zwitterion	:	any compound which has a net zero charge.
Isoelectric pH	:	pH at which the net charge of the protein becomes zero.

2.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) Lipids are classified into simple, compound and derived lipids.
Simple lipids are esters of fatty acids with various alcohols. They include neutral fats and waxes. Compound lipids are esters of fatty acids containing groups in addition to an alcohol and a fatty acid. Examples include phospholipids (containing, in addition to fatty acids and an alcohol, a phosphoric acid residue), glycolipids (compounds of fatty acids with carbohydrate, containing nitrogen but no phosphoric acid) etc. Derived lipids are substances derived from above groups by hydrolysis. This group includes fatty acids, glycerol, steroids, alcohols, sterols, fatty aldehydes and ketone bodies, vitamin A, D, E, K etc.

2) Fatty acids having a polar end with a free –OH group and a non-polar end with a saturated hydrocarbon chain saturated fatty acids while unsaturated fatty acids are characterized by having one or more double bonds.
Saturated fatty acid - butyric acid
MUFA - palmitoleic acid
PUFA - linoleic acid

3) a) If both hydrogen atoms are placed on the same side of the double bond, cis-isomer results but if theses are placed on either side of the double bond, it is a trans-isomer.

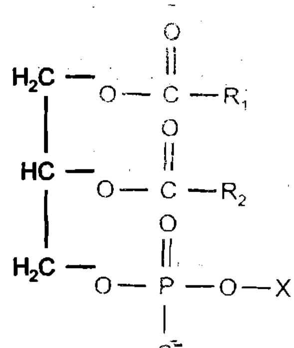
b) n-3 fatty acids have one double bond between carbon atoms 3 and 4, from the omega carbon but no double bond between 6 and 7 carbon atoms. On the other hand, ω6 or n6 fatty acids have double bonds between carbon atoms 6 and 7 from the omega carbon.

4) EFAs are essential for health. We must have them to live and to be healthy. Our bodies cannot make them from other substances. We must obtain an adequate supply from external sources – from food or from supplements. Two fatty acids are essential to human health. The omega 6 EFA is called linoleic acid (LA). LA is abundant in polyunsaturated safflower sunflower, and corn oils. The other one, known as the omega 3 EFA, is called alpha-linolenic acid (LNA). Sometimes referred to as super-unsaturated, LNA is found abundantly in flex and hemp seeds.

Check Your Progress Exercise 2

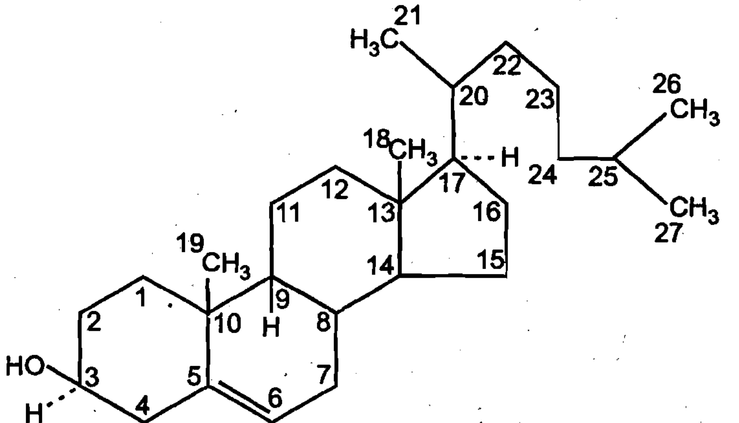
1) Neutral fats are esters of fatty acids with glycerol and found abundantly in nature. These are insoluble in water but readily soluble in ether, chloroform, benzene and carbon tetrachloride. They are bland, odourless substances and neutral in reaction. Neutral fats are good solvents themselves for other fats, fatty acids etc.

2) Phospholipids are esters of fatty acid with glycerol containing an esterified phosphoric acid and a nitrogen base. In order to produce phospholipids, first a phosphatidic acid is formed. In this compound, a phosphate group is present at the carbon atom 3, while two fatty acids are present in carbon atoms 1 and 2 of glycerol. This is the simplest type of phosphoglyceride from which several other types are derived. The basic structure of phospolipids is very similar to that of the triacylglycerols except that C-3 (*sn*3) of the glycerol backbone is esterified to phosphoric acid as highlighted in the figure.



Phospholipids exhibit important biological functions. They increase the rate of fatty acid oxidation, act as inorganic ion carrier across the membrane, help in blood clotting, act as prosthetic group for some enzymes and form the membrane structure.

3) Sterols are the steroids containing an alcoholic group at C-3 position and a side chain of 8 to 10 carbon atoms at C-17, position. An important sterol of animal origin is cholesterol and its structure is:



Cholesterol

4) Arachidonic acid is the precursor of prostaglandins in the body.

5) Using the esterification processes, edible acids, fats, and oils can be reacted with edible alcohols to produce useful food ingredients that include many of the emulsifiers such as mono-diglycerides, lecithin etc.

6) When exposed to hydrogen at high pressure and temperature in presence of Ni or Pt catalyst, an unsaturated fatty acid (containing a double bond) accepts the hydrogen at the double bonds and is converted to a saturated fatty acid. This is referred to as hydrogenation.

   Partial hydrogenation produces margarines, shortenings, shortening oils, and partially hydrogenated vegetable oils. These products contain large quantities of trans-fatty acids and other altered fat substances, some of which are known to be detrimental to health because they interfere with normal biochemical processes.

7) a) The result of the autoxidation of fats and oils is the development of objectionable flavors and odors characteristic of the condition known as oxidative rancidity.

   b) Fats when boiled with alcoholic solution of NaOH or KOH undergo hydrolysis into glycerol and fatty acids and the latter form soaps with Na or K. The reaction is known as saponification.

Check Your Progress Exercise 3

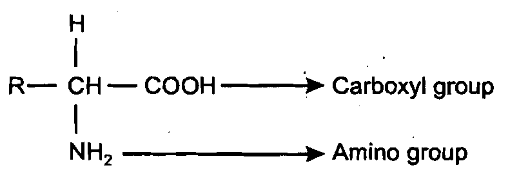
1) a) Proteins are polymers consisting of chains of monomeric units, called amino acids.

   b) Nucleic acids are high molecular weight polymers present in all living cells e.g. DNA and RNA.

   c) Peptides are formed by linking amino acids by peptide bond formation that involves a carboxyl group and an amino group with the elimination of water molecule.

2) 20 amino acids are found in proteins. Alanine, leucine, valine, isoleucine, proline are a few amino acids.

3) a)



   b) (i) Glycine - Polar
   (ii) Proline - Non polar
   (iii) Threonine - Polar
   (iv) Aspartate - Negatively charged side chain.
   (v) Lysine - Positively charged side chain.

Check Your Progress Exercise 4

1) Amino acids are readily soluble in water, slightly soluble to insoluble in ethanol and insoluble in ether. Tyrosine is soluble in hot water but only sparingly soluble in cold water. Cysteine with difficulty only is soluble in hot water.

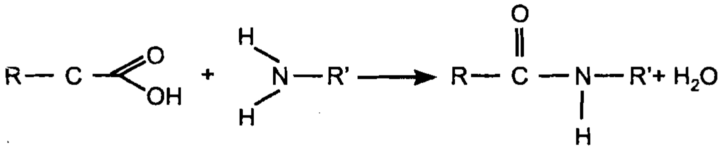
2) Amino acids that have the characteristics of an acid and a base and are capable of reacting chemically either as an acid or a base are amphoteric amino acids.

3) Any compound which has a net zero charge is called a zwitterion. Neutral amino acids behave as zwitterion as it posses net zero charge when its both acidic and basic groups are ionized.

4) L-Amino acid occurs in our diet and body.

5) Peptides are formed by linking amino acids by peptide bond formation that involves a carboxylic group and an amino group with the elimination of water molecule. Polypeptides are chains of more than 10 amino acids held together by peptide bonds. Glutathione, oxytocin, vasopressin, angiotensin and insulin are important polypeptides from physiological point of view.

6) A peptide bond is formed between a carboxylic group and an amino group with elimination of water molecule.



Check Your Progress Exercise 5

1) The three broad groups of proteins are simple, conjugated and derived proteins.

   Simple proteins contain only amino acids and do not have any non-protein part. Conjugated proteins have a non-protein part. Derived proteins are formed from either simple or conjugated proteins due to the unfolding of the tertiary structure or due to the cleavage of peptide bonds producing primary and secondary derivatives, respectively.

2) The four structural levels of proteins are:

   Primary structure: It refers to the linear arrangement of amino acid residues in a given polypeptide chain linked through peptide bonds. The number and sequence of amino acids determine the specificity of the protein and any disturbance in these would create a different protein.

   Secondary structure: It refers to a three dimensional arrangement of various atoms of the protein molecules. The polypeptide is folded systematically and the secondary conformation is stabilized due to binding forces between different segments of the peptide chain

   Tertiary structure: The term refers to the tendency of the polypeptide chain {containing well defined (α-helix, β-bends or sheets) or ill defined (random coil) secondary structure) to undergo extensive coiling or folding and produce a complex, somewhat rigid structure. The structure is stabilized by different types of intermolecular bonds such as hydrogen bonds, ionic bonds and hydrophobic bonds.

   Quaternary structure: Many proteins, particularly enzymes, consist of several peptide chains linked by disulphide bonds. Such proteins are said to possess a quaternary structure.

3) At a specific pH, the protein exists as a dipolar ion (one positive and one negative ion) or zwitterion. So, at this pH the net charge of the protein becomes zero. This pH is known as isoelectric pH or pI of the protein.

4) Proteins in aqueous medium can be precipitated by adding TCA or PCA. Salts of heavy metals, phosphomolybdic acid or phosphotungstic acid are commonly used for protein precipitation. This is known as salting out.

Check Your Progress Exercise 6

1) Nucleotides are nucleoside phosphates. Nucleosides are formed from a nitrogenous base and a pentose sugar. Thus nucleotides consist of a nitrogenous base which may be either a purine (adenine or guanine) or a pyrimidine (cytosine or uracil or thymine) base, a pentose sugar (ribose or deoxyribose) and a phosphate group.

2) Structurally RNA differs significantly from DNA. Each molecule of RNA is single stranded and is made as a complementary strand of one of the two chains of DNA. RNA molecules are smaller than DNA molecules.

3) The base-pairing relationship is the critical feature of the double helix of DNA. Adenine pairs with thymine and guanine pairs with cytosine. Thus, the base sequence of one chain matches with the sequence of the other.