PROTEINS

CHAPTER
11

LEARNING OBJECTIVES

After the completion of this chapter, you should be able to,

- *Define and classify proteins*
- *Outline the properties, identifying tests and methods of analysis of proteins*
- *Explain the biological significance of proteins*
- *Elaborate the pharmacognostic study of drugs containing proteins.*

1. INTRODUCTION

Definition

Proteins are the complex organic nitrogenous substances found in plants and animals. The term protein is derived from Greek word '*proteios*' meaning 'first'. It was proposed by Mulder in 1838.

Proteins are one of the most abundant biomolecule found in every cell. They constitute 20% of the animal body on the fresh weight basis. Chemically proteins are polymers of amino acids. They contain carbon, nitrogen, hydrogen, sulphur and some amount of phosphorous also.

Proteins are an important component of every living being. These are the body's second most abundant substance next only to water. The hair and finger nails are made up of α-keratin, a structural protein. Our bones and connective tissue have collagen. Our muscles, skin and eyes are made of protein. The internal parts of the body like genes, hormones, enzymes, immunoglobulins, thrombin, fibrin and haemoglobin in blood are some of the many different kinds of proteins.

Proteins are of great importance for the growth and maintenance of the human body. The requirements of protein increases during periods of rapid growth such as in infancy, childhood, teen-age years, pregnant and lactating mothers. Various foods rich in proteins are given in Table. It is observed that some protein molecules are broken down and some are being synthesized as replacement, which means proteins are always in a state of exchange. This constant turnover of protein explains why our diet should comprise of adequate proteins even when there is no growth in the body. Proteins are the only raw material or "building" nutrient from which muscles and other body tissues are built. They supply the same amount of energy as the carbohydrates.

Table: Some Foodstuffs Rich in Protein

Source of protein	Examples
Cereals	Wheat germ, oat meal, bajra or pearl millet, barley, Italian millet, maize, wheat flour etc.
Pulses	Khesari dal (*Lathyrus* pea), green gram, horse gram, cow gram, black gram, Bengal gram, lentil, peas, red gram, soya bean.
Nuts and oil seeds	Ground nut, cashew nut, almond, sesame seeds,
Condiments and Spices	Fenugreek seeds, mustard, dry chillies, coriander seeds, cumin seeds, cardamom seeds, pepper.
Miscellaneous foodstuffs	Dried yeast, rajkeera seeds, papads,
Milk and milk products	Skimmed milk powder, milk powder (whole), cheese, khoa
Fish	Sardine, prawns, mackerel, white bait.
Meat	Beef muscle, duck meat, fowl meat, sheep liver, mutton, pork muscles, pigeon meat, duck's egg, hen's egg.

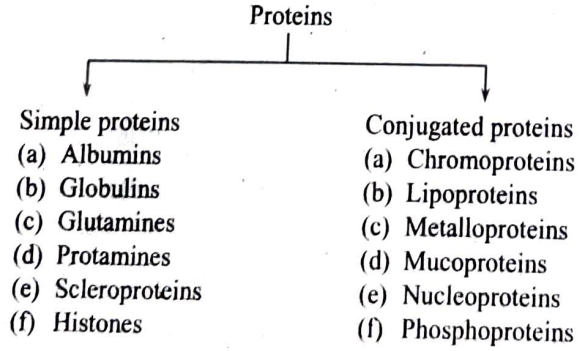
Apart from being important sources of food (milk products, legumes etc.,) proteins also provide therapeutically active compounds like enzymes, hormones, sera, antibiotics etc.

Characteristics

1. Proteins consist of carbon, hydrogen, oxygen, nitrogen and rarely sulphur.
2. Complete hydrolysis of proteins gives amino acids.
3. Exposure to heat, unstable pH, organic solvents or UV radiation causes denaturation of proteins.
4. Few proteins of plant and animal origin are poisonous to human beings. E.gs: Ricin from castor oil, Toxalbumin.

Classification

Proteins are classified as simple and conjugated proteins, depending on the complexity of structure.



1. ***Simple Proteins***

Proteins which give only amino acids upon hydrolysis are simple proteins. These are of following types.

(a) *Albumins*

These are water soluble and are coagulated by heat.

E.gs: Egg albumin, lactalbumin.

(b) *Globulins*

They are water insoluble but soluble in dilute alkaline solution, and upon half saturation with ammonium sulphate they undergo precipitation.

E.gs: Arachin, serum globulin.

(c) *Glutamines*

Glutamines are soluble in dilute alkalis and acids.

E.gs: Glutenin of wheat and oryzenin of rice.

(d) *Protamines*

These are insoluble in water, dilute alkaline solution or absolute alcohol but soluble in 70-80% alcohol.

(e) *Scleroproteins*

They are insoluble in water or alkaline solutions, but soluble in strong acids or alkalis.

E.gs: Keratin of hair, collagen of bones.

(f) *Histones*

Histones are soluble in water, dilute alkalis and acids and insoluble in dilute ammonia.

E.gs: Globins and Gadus histones of codfish sperm.

2. ***Conjugated Proteins***

Conjugated protein is formed when a simple protein is combined with a non-protein group (prosthetic group). These are further divided as follows,

(a) *Chromoproteins*

These are simple proteins which are combined with coloured prosthetic groups.

E.gs: Chlorophyll, haemoglobin.

(b) *Lipoproteins*

These are a combination of proteins and lipids.

E.gs: Blood, milk, egg yolk.

(c) *Metalloproteins*

They are proteins containing heavy metals like Fe, Co, Mn, Zn, Cu, Mg. Many enzymes come under this sub-type.

(d) *Mucoproteins*

Mucoproteins are a combination of proteins and mucopolysaccharides.

E.gs: Found in serum, urine, albumin.

(e) *Nucleoproteins*

These are a combination of proteins and nucleic acids.

E.g: Tobacco mosaic virus.

(f) *Phosphoproteins*

They are phosphoric acid containing proteins.

E.gs: Casein, Egg yolk.

Occurrence

Proteins are found in structural materials of plants and animals.

Protein containing drug	Biological source	Uses
1. ***Drugs of Animal Origin***		
(a) Casein	Milk	As dietary supplement
(b) Collagen	White fibres of connective tissue	Preparation of sutures
(c) Microfibrillar collagen	Bones, skin and connective tissue of animals	To check bleeding during surgery
(d) Gelatin	Skin, tendons, ligaments and bones of animals	In the manufacturing of capsule shells
(e) Heparin sodium	Lungs and intestinal mucosa of mammals	As an anticoagulant in vascular surgery and blood transfusion
2. ***Drugs of Plant Origin***		
(a) Kavach	Seeds of *Mucuna puriens*	Treatment of parkinsonism
(b) Lectins	Obtained from plants, bacteria and fungal growth	In micro-chemical studies
(c) Malt extract	Barley grains, *Hordeum vulgare*	As a nutritional supplement
(d) Thaumatic	*Thaumatococcus denielli* fruits	As a sweetener
(e) Yeast	*Saccharomyces cereviseae* fungus	In brewing industry and as a nutritive.

Properties

Physicochemical properties of proteins are as follows.

Physical Properties

1. Proteins are colourless and amorphous in nature.
2. They have indefinite melting and boiling points.
3. When subjected to heat they undergo decomposition.
4. They are optically active. Most of the proteins are laevorotatory (–) in nature.
5. Like amino acids, proteins are dipolar in nature i.e. they exist as zwitter ions.
6. Proteins are amphoteric in nature. They show both acidic and basic properties owing to the presence of both positive and negative charges (i.e., COO^- and - NH_3^+), depending upon the pH of the amino acid solution.
7. Different proteins show different isoelectric points depending upon the functional group. Most of the proteins are slightly soluble and show minimum stability at their respective isoelectric points..

Types of protein	Isoelectric point value
Casein	4.60
Gelatin	4.80 – 4.85
Serum albumin	4.88
Insulin	5.30 – 5.35
Serum globulin	5.50
Haemoglobin (Hb)	6.79 – 6.83

8. Colloidal property : When dissolved in water, proteins form colloidal dispersion.

This colloidal dispersion shows two phases: Dispersed phase/internal phase, containing the protein particles and continuous phase/external phase, containing the water molecules.

Such colloidal dispersions are incapable of penetrating the biological membranes. This property of proteins is very much useful for diagnostic purpose.

Example: Presence of proteins (albumin) in urine is indicative of a damage to the blood vessels in kidney.

Chemical Properties

1. *Protein-protein Interactions*

Protein molecules, when dispersed in solution, are always in constant motion and collide with other protein molecules. When the shapes of two protein molecules match closely, they form stable association. This concept can be explained with the help of an example, representing hypothetical model to show interactions between proteins. Protein P has a shape that closely matches the surface on protein X and therefore greater number of covalent bonds can be formed between protein P and protein X. Protein P may not show stable association with protein Y and protein Z.

2. *Denaturation of Proteins*

The term denaturation refers to reversible or an irreversible (except in some cases) change in the structure of the protein molecule resulting in the reduction or loss of its biological properties.

Example: Albumin of egg white solidifies on boiling. This is because the secondary structure of the egg white protein is destroyed as a result of the breakage of hydrogen bonds.

A denatured protein may show changes in their solubility, optical rotation, chemical, physical and biological properties.

The process of denaturation can be attained by action of heat, alcohol, salts of heavy metals, exposure to radiations usage of detergents, high pressure etc.

The enzyme trypsin denatures when exposed to high temperature of 80° to 90° C. When the temperatures are brought back to normal (37° C), it regains its native form and activity. This phenomenon of getting back to native form and activity by a denatured protein is referred to as *renaturation*, *refolding*, *reversible denaturation* or *annealing of protein*.

Coagulation of proteins is of biological significance as most of the reactions in the body are of this type.

Examples:

(i) Blood clotting

(ii) Casein precipitation at the time of digestion of milk.

(iii) Bacteria being proteinaceous in nature, get precipitated when come in contact with the suitable precipitating agents. This is the principle behind many poisons and disinfectants.

3. *Hydrolysis of Proteins*

When proteins are treated with strong acids such as HCl and H_2SO_4 or alkalies (NaOH) and enzymes (like proteases), they get hydrolyzed to various amino acids. Hydrolysis in the presence of strong alkali is undesirable, as it may lead to racemization of the amino acid solution. Enzymes such as proteases can also be used for hydrolysis of proteins, but the reaction is very slow.

$$\text{Protein} \xrightarrow[H^+/H_2O]{\text{Hydrolysis}} \text{Amino acids} + \text{Prosthetic groups}$$

4. *Oxidation Reaction*

When proteins are burnt in the presence of air, they get oxidized and liberate products such as NH_3, N_2, CO_2 and H_2O and emanate a foul smell. This phenomenon is known as *putrefaction*.

Identifying Tests

For topic refer Chapter-13.

Method of Analysis

Some of the important methods for quantitative analysis of proteins in organic samples are,

1. *Kjeldahl's Method*

This method was first developed by Johann Kjeldahl in the year 1883. Even today it is considered to be the standard method for quantitative determination of proteins, although several modifications have been made in the method to decrease the time required and to increase its accuracy. This method involves the digestion of organic samples (containing proteins) with a strong acid which would enable in the liberation of nitrogen, that is estimated by a suitable titration technique. The amount of proteins present in the original sample is determined from the nitrogen concentration of the same. However since this method does not directly indicate the amount of proteins present in the sample, it requires a conversion factor (F) which enables in converting the nitrogen concentration into protein concentration. In most cases, the value of conversion factor is taken as 6.25 (equivalent to 0.16 g nitrogen per gram of protein). However, this is only an average value as it differs among proteins depending upon their amino acid sequence.

Kjeldahl's method can be divided into the following 3 steps.

(a) *Digestion*

This step involves the addition of a weight quantity of the sample to a specially designed flask called as Kjeldahl's flask which is made up of pyrex glass. About 25 ml of H_2SO_4 along with potassium sulphate and copper sulphate are added. Acid serves to digest the sample, potassium sulphate increases the speed of reaction by increasing the boiling point while $CuSO_4$ acts as a catalyst.

Due to digestion, nitrogen in the sample is converted into ammonia, which is in the form of ammonium sulphate (that remains in solution).

$$\underset{\text{Sample}}{N_2} \longrightarrow (NH_4)_2SO_4$$

(b) *Neutralization*

This step initiates with transferring the cooled contents from Kjeldahl's flask into a round bottom flask which is then connected to a receiving flask by a tube. In this, excess alkali (NaOH) is added to convert the so formed ammonium sulphate into ammonia. The gas so liberated is led into the receiving flask containing excess boric acid. pH of the solution in the receiving flask is low due to which the gas is converted to NH_4^+ and boric acid is converted to borate ion.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + 2H_2O + Na_2SO_4$$
$$NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$$

(c) *Titration*

Ammonium borate so formed is titrated with standard H_2SO_4 or HCl using a suitable indicator, to determine the nitrogen content of the sample.

$$H_2BO_3^- + H^+ \longrightarrow H_3BO_3$$

The concentration of H^+ ions (in moles) required to attain the end-point is equivalent to the concentration of nitrogen in the sample.

$$\% \text{ of } N_2 = \frac{1.4\,VN}{w}$$

Where,

V = Volume of acid

N = Normality of acid

w = Weight of the sample

Blank titration is also performed and the % of nitrogen obtained from the above equation is converted into protein content by utilizing an appropriate conversion factor.

Merits

1. It has good precision, accuracy, reproducibility and hence is universally used for estimating the content of proteins in samples.
2. It is a highly reliable technique.

Demerits

1. It does not give the actual measure of true protein because all the nitrogen may not be present in proteinaceous form in the proteins.
2. Different correction factors have to be utilized for different proteins since they differ in the amino acid sequence.
3. It is a time-consuming method.

2. ***Modified Dumas Method***

This method is a more advanced form of the method proposed by Dumas which was based on the principle of heating nitrogen containing compounds at high temperatures to yield free nitrogen. Presently it has transformed into an automated instrumental technique which helps in rapid determination of proteins in the sample.

Procedure

1. Weighed quantity of sample is subjected to very high temperature (about 900°C) along with oxygen in the heating chamber. Such combustion yields N_2, CO_2 and H_2O.
2. The gases so liberated are then allowed to pass over specialized columns which function to absorb CO_2 and water.
3. Remaining gases are passed through a column which is connected to a thermal conductivity detector. This column functions in selective absorption of nitrogen gas from the residual mixture of CO_2 and water.
4. Instrument is calibrated by utilizing a known pure standard, whose nitrogen concentration is already known.
5. Sample is also analyzed and nitrogen content is determined by suitable conversion of the signal from thermal conductivity detector.
6. Similar to Kjeldahl method, Dumas method also requires appropriate conversion factors for converting the concentration of nitrogen in the sample to protein content.

Merits

1. Less time-consuming when compared to Kjeldahl method.
2. It is easy to use.
3. Avoids the need of catalyst or other toxic chemicals.
4. Enables automatic measuring of sample.

Demerits

Apart from being associated with high initial costs, all the other demerits are similar to Kjeldahl's method.

3. ***Methods Utilizing UV-Visible Spectroscopy***

These methods utilise UV-visible spectroscopy for determining the concentration of proteins in the sample. The basic principle underlying these methods is that they either depend on the light scattering property of protein molecules in the UV-visible region or in case if they are not capable then they are suitably modified to attain that property. Various method that are based on UV-visible spectroscopy are Biuret method, Lowry method, dye binding method and turbidimetric method with direct measurement at 280 nm.

Principle

All the methods that are based on UV-visible spectroscopy are based on same basic principle. A series of protein solutions of known concentrations are analyzed using which a calibration curve is plotted with absorbance (turbidity) vs protein concentration. Absorbance of the sample solution is determined at the same wavelength and the concentration of proteins in the sample is extrapolated from the graph.

Merits

1. These methods have high sensitivity meaning that even low concentrations of proteins can be measured easily.
2. These are simple and less time-consuming.

Demerits

1. Dilute or transparent sample solutions are required for analysis hence organic samples require considerable sample preparation prior to their analysis.
2. Sample solution should not contain any contaminating substances which may scatter light at the same wavelength as the sample.
3. Different proteins absorb at different wavelengths as they differ in the sequence of amino acids.

Significance of Proteins

The various functions of dietary proteins in our body are,

1. They replace the daily loss of proteins.
2. They have catalytic function to carry out all the life processes in a cell.
3. Provide amino acids for the formation of tissue proteins.
4. They help in transportation of oxygen (haemoglobin carries oxygen in the blood) and storage of molecules like albumin in egg, casein in milk.
5. They have immune function. Antibodies help us to fight disease and infection.

6. Provide amino acids for the formation of blood proteins, enzymes and certain hormones.
7. Provide amino acids for the growth of foetus during pregnancy and for the production of milk during lactation.
8. They help in motility (movement) of organisms such as contractile proteins in muscles.
9. They are responsible for transmission of nerve impulses.
10. They aid in growth and differentiation.
11. They provide mechanical support. Examples: Elastin gives elasticity to the skin, keratin is the protein of hair, horns, claws, silk of spider webs and cocoons of silk moth.

2. GELATIN

Synonyms

Gel foam, Gelatina, Puragel.

Biological Source

It is extracted from collagenous tissues of animals like skins, ligaments, tendons and bones by partial hydrolysis in boiling water.

Method of Preparation

Bones are defatted and decalcified with organic solvent followed by mineral acid. The resultant material is treated with water maintained at 85°C, as this dissolves the collagen into gelatin. It is then bleached and concentrated under reduced pressure for desired gelatin content and is set in shallow trays. The gelatin so obtained is placed in the drying room to remove moisture.

Types

1. ***Absorbable Gelatin Sponge***

   It is a sterile, tough, white and finely porous spongy material, which is absorbable and water soluble in nature. Preparation involves whisking of warm gelatin solution to form a foam of uniform porosity which is then dried, cut and finally sterilized at 150°C for 1 hour. It can take up not less than 30 times its weight of water and 9 g of gelatin sponge can take up to 45 times its weight of well agitated oxalate whole blood.

2. ***Absorbable Gelatin Film***

   It is obtained from specially prepared gelatin formaldehyde solution by drying and sterilization. Absorbable gelatin film is sterile, light amber in colour, pliable (flexible) and non-antigenic in nature.

Characteristics

Colour	-	Faintly yellow or amber coloured
Odour	-	Characteristic
Solubility	-	It is soluble in hot water and forms a jelly on cooling. It is insoluble in cold water and swells, softens and absorbs 5-10 times its weight of water. It is insoluble in volatile and fixed oils, chloroform, alcohol and ether, whereas it is soluble in a mixture of water and glycerin.
Stability	-	Dry gelatin is stable in air, but moist gelatin tends to degrade upon microbial attack.
Bloom strength	-	It is defined as the weight in gram, when applied to a plunger of 11.7 mm diameter, under controlled conditions shall produce a 4 mm deep depression in a jelly containing 6.66% w/w gelatin in water, and natured at 10°C. Bloom gelometer number is used to designate the jelly strength.
		British pharmacopoeia recommends a jelly strength of NLT 150 bloom for pharmaceutical use, and higher jelly strength are used for making capsules and for microbial culture.

Standards

(a)	Ash	-	NLT 32%
(b)	Gel strength	-	150 - 250
(c)	Loss on drying	-	NLT 15%
(d)	pH of 1% solution	-	3.6 - 7.6
(e)	Microbial limits	-	1 g of gelatin should not contain more than 1000 bacteria.

Chemical Constituents

Gluten protein, lysine (essential amino acid).

Identification Tests

1. When gelatin is heated with soda lime it results in the evolution of ammonia.
2. It develops a white precipitate with mercuric nitrate and turns to brick red colour on warming.
3. Trinitrophenol and tannic acid solution precipitate gelatin, whereas alum, lead acetate or acid do not indicating the absence of chondrin.

Uses

1. Gelatin is used in the manufacturing of hard and flexible capsule shells.
2. It is used in the preparation of pessaries, pastes, pastilles and suppositories.
3. Absorbable gelatin sponge is used as a haemostatic, in the treatment of brittle finger nails and non-mycotic defects of the nails.
4. It is employed in the microencapsulation of drugs, perfumes, flavours and industrial materials.
5. It is used in the preparation of Pitkin's menstruum in addition to dextrose, acetic acid and water, which is a vehicle for heparin injection.
6. Gelatin is also used for preparing culture media.
7. Absorbable gelatin film is used in surgical repair of defects in membranes like pleura and dura mater, serving as a replacement matrix, mechanical protective and temporary support structure.

3. COLLAGEN

Synonym

Ossein

Biological Source

It is a protein which is extracted from connective tissues of the animals like bones, teeth, tendons and skin.

Method of Preparation

Collagen is mostly present in skin, tendon, ligament, etc.

1. Collagen obtained from these sources after slaughtering the animal is cleaned and soaked in an acidic solution.
2. It is then squeezed into a coagulating solution and the fibres so obtained are oriented by stretching.
3. Fibres are then treated with formaldehyde or chromium salts or both to delay their absorption time in the body.
4. Filaments are then rolled or spun into strands of required size.

Characteristics

These collagen fibres are about 10-100 μm in diameter. When viewed under a microscope they appear as bands in the extracellular matrix of the connective tissues. The structural unit of collagen is tropocollagen. This tropocollagen consists of 3 left-handed helices that are wound around each other to form a right handed supercoil. It is to be noted that the tensile strength of collagen is greater than steel and it is tough and unextensible. The interchain of tropocollagen triple helices are held by hydrogen bonds between the OH group of 4-hydroxyproline residues.

Chemical Constituents

Collagen is rich in glycine and proline that are found in the central core of the tropocollagen. Apart from glycine and proline, collagen also possess two uncommon amino acid derivatives like hydroxyproline and hydroxylysine along with minute quantities of tyrosine and sulphur. Collagen is of various types which differ in the sequence of amino acids.

Uses

1. Collages fibres are used in the preparation of sutures that are limited to ophthalmic surgery.
2. It is useful in photographic emulsions.

4. KAVACH

Synonyms

Cow harge, cow itch plant

Biological Source

It refers to the dried seeds of the plant *Mucuna pruriens*, family- Fabaceae (Leguminosae).

Location

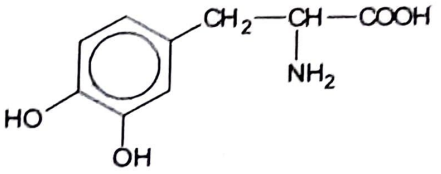
It thrives well throughout the country and is grown commercially in Maharashtra and other tropical regions.

Characteristics

Colour	-	Black
Odour	-	Odourless
Taste	-	Bitter
Shape	-	Reniform or kidney-shaped
Size	-	10-20 mm in length and 7-15 mm in width

Chemical Constituents

1. Seeds are abundant in amino acids such as 15% *l*-dopa (3, 4-dihydroxyphenylalanine) and 11.5% lecithin.
2. Other constituents include alkaloids like mucunine, mucunadine, prurienine, prurieninine as well as fats.



l-dopa

Uses

1. Levodopa (*l*-dopa) extracted from the seeds is the main drug used in the treatment of parkinsonism which is characterized by symptoms like difficulty in speech, postural instability, rigidity, hypokinesia, dysphagia (difficulty in swallowing), excessive secretion of saliva (sialorrhoea) etc.
2. It is also used in the treatment of stress-related infertility as it increases the sperm count.
3. It increases libido as it is an aphrodisiac.