PROTEINS

A protein is a complex, high molecular weight organic compound that consists of amino acids joined by peptide bonds. The word protein is derived from greek '*protos'* meaning '*of primary importance*'*.* Proteins are essential to the structure and function of all living cells. Many proteins are enzymes or subunits of enzymes. Other proteins play structural or mechanical roles, such as those that form the struts and joints of the cytoskeleton, serving as biological scaffolds for the mechanical integrity and tissue signalling functions.

They are obtained from both plant and animal sources.

In plants they are stored in the form of aleurone grains.

In animals they are present in structural material in the form of collagen (connective tissue), keratin (hair, wool, hairs, feathers, and horns), elastin (epithelial connective tissue), casein (milk), and plasma proteins. Casein, gelatin, heparin, and hemoglobin are pharmaceutically important proteins of animal origin.

Proteins are generally large molecules, having molecular masses of up to 3,000,000 (the muscle protein titin has a single amino-acid chain 27,000 subunits long). However, protein masses are generally measured in kiloDaltons (kDa). Such long chains of amino acids are almost universally referred to as proteins, but shorter strings of amino acids are referred to as 'polypeptides', 'peptides', or rarely, 'oligo-peptides'. The dividing line is undefined, though 'polypeptide' usually refers to an amino-acid chain lacking tertiary structure which may be more likely to act as a hormone (like insulin), rather than as an enzyme (which depends on its defined tertiary structure for functionality).

There are about 20 different amino acids, eight of which must be present in the diet. The eight essential amino acids required by humans are: leucine, isoleucine, valine, threonine, methionine, phenylalanine, tryptophan, and lysine.

For children, histidine is also considered to be an essential amino acid. Unlike animal proteins, plant proteins may not contain all the essential amino acids in the necessary proportions, and so the proteins derived from plants are grouped as incomplete and from animals are grouped as complete. However, a varied vegetarian diet means a mixture of proteins are consumed, the amino acids in one protein compensating for the deficiencies of another.

The structure of protein could be differentiated into four types:

1. Primary structure: the amino-acid sequence
2. Secondary structure: highly patterned substructures– alpha helix and beta sheet– or segments of chain that assume no stable shape. Secondary structures are locally defined, meaning that there can be many different secondary motifs present in one single protein molecule.
3. Tertiary structure: the overall shape of a single protein molecule; the spatial relationship of the secondary structural motifs to one another
4. Quaternary structure: the shape or structure that results from the union of more than one protein molecule, usually called protein subunits in this context, which function as part of the larger assembly or protein complex.

Proteins are sensitive to their environment. They may only be active in their native state, over a small pH range, and under solution conditions with a minimum quantity of electrolytes. A protein in its native state is described as folded and that is not in its native state is said to be denatured. Denatured proteins generally have no well-defined secondary structure. Many proteins denature and will not remain in solution in distilled water also they are denatured due to heat, changes in pH, treatment of organic solvents or by ultra violet radiation.

Proteins are essential for growth and repair. They play a crucial role in virtually all biological processes in the body. All enzymes are proteins and are vital for the body's metabolism. Muscle contraction, immune protection and the transmission of nerve impulses are all dependent on proteins. Proteins in skin and bone provide structural support. Many hormones are proteins. Protein can also provide a source of energy. Generally the body uses carbohydrate and fat for energy but when there is excess dietary protein or inadequate dietary fat and carbohydrate, protein is used. Excess protein may also be converted to fat and stored.

GELATIN

Synonyms

Gelfoam; puragel; gelatinum.

Biological Source

Gelatin is a protein derivative obtained by evaporating an aqueous extract made from bones, skins, and tendons of various domestic animals. Some important sources are: Ox, *Bos taurus*, and Sheep, *Ovis aries* belonging to family Bovidae

Preparation

The process of manufacture of gelatin vary from factory to factory. However, the general outline of the process is given below.

Raw material

Bones, skins, and tendons of Bovideans is collected and subjected to liming operation.

Liming Process

The raw material is first subjected to the treatment known as 'liming'. In this process, the skins and tendons are steeped for fifteen to twenty and sometimes for

40 days in a dilute milk of lime. During this, fleshy matter gets dis-solved, chondroproteins of connective tissues gets removed and fatty matter is saponified. The animal skin is further thoroughly washed in running water.

Defattying

In case of bones, the material is properly ground and defatted in close iron cylinders by treatment with organic solvents such as benzene. The mineral and inorganic part of the bone is removed by treatment with hydrochloric acid.

Extraction

The treated material from bones, skins and tendons is boiled with water in open pans with perforated false bottom. This process can also be carried out under reduced pressure. The clear liquid runs of again and again and is evaporated until it reaches to above 45 per cent gelatin content.

Setting

The concentrated gelatin extract is transferred to shallow metal trays or trays with glass bottom. It is allowed to set as a semisolid jelly.

Drying

The jelly is transferred to trays with a perforated wire netting bottom and passed through series of drying compartments of 30–60°C increasing each time with 10°C. About a month is taken for complete drying.

Bleaching

In case of darker colour, finished product is subjected to bleaching by sulphur dioxide. Bleaching affords a light coloured gelatin.

Characteristics

Gelatin occurs as a colourless or slightly yellow, transparent, brittle, practically odourless, tasteless sheet, flakes or course granular powder. In water it swells and absorbs 5–10 times its weight of water to form a gel in solutions below 35–40°C. It is insoluble in cold water and organic solvents, soluble in hot water, glycerol, acetic acid; and is amphoteric. In dry condition it is stable in air, but when moist or in solution, it is attacked by bacteria. The gelatinizing property of Gelatin is reduced by boiling for long time. The quality of gelatin is determined on the basis of its jelly strength (Bloom strength) with the help of a Bloom gelometer. Jelly strength is used in the preparation of suppositories and pessaries.

Commercially two types of gelatin, A and B, are avail-able. Type A has an isoelectric point between pH 7 and 9. It is incompatible with anionic compounds such as Acacia, Agar and Tragacanth. Type B has an isoelectric point between 4.7 and 5, and it is used with anionic mixtures. Gelatin is coloured with a certified colour for manufacturing capsules or for coating of tablets. It may contain various additives.

Chemical Constituents

Gelatin consists of the protein glutin which on hydrolysis gives a mixture of amino acids. The approximate amino-acid contents are: glycine (25.5%), alanine (8.7%), valine (2.5%), leucine (3.2%), isoleucine (1.4%), cystine and cysteine (0.1%), methionine (1.0%), tyrosine (0.5%), aspartic acid (6.6%), glutamic acid (11.4%), arginine (8.1%), lysine (4.1%), and histidine (0.8%). Nutritionally, gelatin is an incomplete protein lacking tryptophan. The gelatinizing compound is known as chondrin and the adhesive nature of gelatin is due to the presence of glutin.

Chemical Tests

1. ***Biuret reaction:*** To alkaline solution of a protein (2 ml), a dilute solution of copper sulphate is added. A red or violet colour is formed with peptides containing at least two peptide linkages. A dipeptide does not give this test.

2. ***Xanthoproteic reaction:*** Proteins usually form a yellow colour when warmed with concentrated nitric acid. This colour becomes orange when the solution is made alkaline.

3. ***Millon's reaction:*** Millon's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

4. *Ninhydrin test:* To an aqueous solution of a protein an alcoholic solution of ninhydrin is added and then heated. Red to violet colour is formed.

5. On heating gelatin (1 g) with soda lime, smell of ammonia is produced.

6. A solution of gelatin (0.5 g) in water (10 ml) is precipitated to white buff coloured precipitate on addition of few drops of tannic acid (10%).

7. With picric acid gelatin forms yellow precipitate.

Uses

Gelatin is used to prepare pastilles, pastes, suppositories, capsules, pill-coatings, gelatin sponge; as suspending agent, tablet binder, coating agent, as stabilizer, thickener and texturizer in food; for manufacturing rubber substitutes, adhesives, cements, lithographic and printing inks, plastic compounds, artificial silk, photographic plates and films, light filters for mercury lamps, clarifying agent, in hectographic matters, sizing paper and textiles, for inhibiting crystallization in bacteriology, for preparing cultures and as a nutrient.

It forms glycerinated gelatin with glycerin which is used as vehicle and for manufacture of suppositories. Combined with zinc, it forms zinc gelatin which is employed as a topical protectant. As a nutrient, Gelatin is used as commercial food products and bacteriologic culture media.

CASEIN

Biological Source

Casein is a proteolytic enzyme obtained from the stomachs of calves. It is extracted from the proteins of the milk; in the milk, casein is structured in voluminous globules. These globules are mainly responsible for the white colour of the milk. According to various species, the casein amount within the total proteins of the milk varies.

The casein content of milk represents about 80% of milk proteins. The principal casein fractions are alpha (s1) and alpha (s2)-caseins, β-casein and κ-casein. The distinguishing property of all casein is their low solubility at pH 4.6. The common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues. These phosphate groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content.

Within the group of caseins, there are several distinguishing features based on their charge distribution and sensitivity to calcium precipitation:

Alpha (s1)-casein: (molecular weight 23,000; 199 residues, 17 proline residues).

Two hydrophobia regions, containing all the proline residues, separated by a polar region, which contains all but one of eight phosphate groups. It can be precipitated at very low levels of calcium.

Alpha (s2)-casein: (molecular weight 25,000; 207 residues, 10 prolines).

Concentrated negative charges near N-terminus and positive charges near C-terminus. It can also be precipitated at very low levels of calcium.

β-*casein:* (molecular weight 24,000; 209 residues, 35 prolines).

Highly charged N-terminal region and a hydrophobia C-terminal region. Very amphiphilic protein acts like a detergent molecule. Self association is temperature-dependent; will form a large polymer at 20°C but not at 4°C. Less sensitive to calcium precipitation.

κ-*casein:* (molecular weight 19,000; 169 residues, 20 prolines).

Very resistant to calcium precipitation, stabilizing other caseins. Rennet cleavage at the Phe l05 – Met l06 bond eliminates the stabilizing ability, leaving a hydrophobia portion, para- κ-casein and a hydrophilic portion called κ-casein glycomacropeptide (GMP), or more accurately, caseinomacropeptide (CMP).

Characteristics

The isoelectric point of casein is 4.6. The purified protein is water insoluble. While it is also insoluble in neutral salt solutions, it is readily dispersible in dilute alkalis and in salt solutions such as sodium oxalate and sodium acetate. Casein does not coagulate on heating. It is precipitated by acids and by a proteolytic enzyme (rennet).

Chemical Constituents

Milk consists of 80% of milk proteins (casein). The major constituents of casein are alpha (s1) and alpha (s2)-caseins, β-casein and kappa-casein. These caseins are conjugated proteins with phosphate group(s) which are esterified into serine residues they have a low solubility at pH 4.6.

Uses

It is used in the manufacture of binders, adhesives, protective coatings, plastics (such as for knife handles and knitting needles), fabrics, food additives, and many other products. It is commonly used by bodybuilders as a slow-digesting source of amino acids. There is growing evidence that casein may be addictive for some individuals, particularly those on the autism spectrum or having schizophrenia.