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Section I

PROTEINS AND THEIR COMPONENT AMINO ACID GROUPS

INTRODUCTION

Amino acids are organic acids in which one or more of the hydrogen atoms has been replaced by an amino group, e.g.:

acetic acid   amino acetic acid (glycine)

to quote the simplest example.

Twenty-two amino acids can be obtained from the dietary proteins, and although others may exist, all those essential to the diet have been isolated.

The majority are monocarboxylic acids, a few are dicarboxylic and a number are diamino compounds, etc. The amino acids in general give the reactions characteristic of the carboxyl group and under proper conditions will show all the reactions of primary amines; they act as bases towards acids, and as acids towards bases, due to their acidic (carboxyl) groups and their basic (amino) groups.

Twenty of the amino acids obtained from dietary proteins have an amino group attached to the carboxyl group and are known as α-amino acids.

Amino acids have not been found in the free state in animal tissues after histological processing, although they do exist uncombined in certain plant tissues. Natural proteins consist of a large number of molecules of the same or different amino acids united through condensation with the loss of the elements of water.

The first stage of such condensations may be represented simply by the general equation:

$$\mathrm{COOH{\cdot}CRR'{\cdot}NH_2 + COOH{\cdot}CRR'{\cdot}NH_2 =}$$
$$\mathrm{COOH{\cdot}CRR'{\cdot}NH{\cdot}CO{\cdot}CRR'{\cdot}NH_2 + H_2O}$$

where R and R′ may represent either hydrogen or alkyl radicles; or a great variety of different groups.

The products formed by the condensation of two amino acids as illustrated above are known as *Dipeptides*; by condensation with another molecule of the same or a different amino acid, dipeptides are converted into *Tripeptides*, and further condensation converts them into *Tetrapeptides*, and so on.

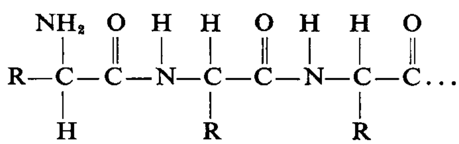
Members of the group of nitrogenous substances known as proteins are synthesized by plants from simple substances such as carbon dioxide, water, inorganic nitrogen, phosphates, sulphates, etc. Animals, however, rely on plants, either directly or indirectly, for their protein supplies which are utilized to provide the main organic structures of the protoplasmic machinery; protein taken in excess of these requirements is utilized, like the carbohydrates, and the lipids to a smaller extent, as sources of energy for driving the body machinery.

The proteins, which are the most important biological substances, constitute a vast group composed of carbon, hydrogen, oxygen and nitrogen, and usually sulphur; many contain phosphorus in addition, and some contain small amounts of iron, iodine, copper, zinc, etc. Proteins are intimately associated, in living cells, with nucleic acids, carbohydrates and their derivatives, lipids, hormones, enzymes, vitamins, etc. Hormones and enzymes themselves have been found to be protein in character, and certain viruses causing diseases of plants and animals are now known to consist of very complex proteins. The chemistry of proteins is exceedingly complex, as there is a greater degree of diversity in their molecular structure and chemical composition than in any other group of biological substances. Due to the basic (amino) and acidic (carboxyl) groups possessed by the amino acid constituents of proteins, which enables them to combine with both acidic and basic groups, which in turn can combine with other groups, the possibilities of building up and otherwise modifying protein molecules by processes of nature are enormous, and the number of types of protein molecules varying in structure and chemical composition is almost infinite.

Due to the fact that protein molecules contain chemical groups which interact reversibly with H^+ ions over a wide pH range, solutions of proteins act as buffers in body tissues. Proteins exist as cations on the acid side and as anions on the alkaline side of their isoelectric point, which is the pH at which the protein does not migrate in an electric field. Proteins may be precipitated by electro-positive ions such as Zn^{++} and Hg^{++} from solutions that are alkaline to the isoelectric point of the protein, since at this pH the protein exists as an electro-negative ion, protein$^-$, which combines with the electro-positive ions to form insoluble metal proteinates. The addition of acid serves to remove the metallic ions from the metal proteinates, converting protein$^-$ to protein$^+$ from which it will be readily understood that the pH of the protein solution is a matter of no small importance if complete precipitation by heavy metallic ions, such as are present in certain fixatives, is to be attained. Electro-negative ions combine with proteins when the latter are in the form of protein$^+$; that is to say, when the proteins are in solutions acid to the protein isoelectric point. These protein salts, e.g. protein phosphotungstate, protein trichloracetate, protein tungstate, protein picrate, protein tannate, protein phosphomolydate, etc. are broken down to form protein$^+$ on addition of alkalies.

Acid and basic dyes also combine with proteins to form salts and proteinates; the acid dye eosin, for instance, combines with proteins that are on the acid side of their isoelectric points (protein$^+$ eosin$^-$), whereas the basic dye, methylene blue, combines with proteins that are in solution alkaline to their isoelectric points (protein$^-$ methylene blue$^+$).

The amino acids of proteins are united through an acid amine type of bond which is known as a *peptide linkage*:



In analytical histology, proteolytic enzymes such as trypsin, are used for the hydrolysis and consequent breaking of these bonds; the process is usually slow and incomplete, because the linkage is resistant to hydrolysis; however, the use of proteolytic enzymes for the purpose does not cause alteration in the physical and chemical properties of the amino acids, as acid or alkaline hydrolysis does.

CLASSIFICATION OF THE PROTEINS

Proteins may be classified into three major groups, i.e. (1) Simple proteins; (2) Conjugated proteins; and (3) Derived proteins. These three classes may be sub-divided as follows:

SIMPLE PROTEINS

The members of this group, which is further sub-divided as below, are defined as those proteins which yield on hydrolysis only amino acids or their derivatives.

(A) ALBUMINS

These are water soluble proteins present in animals and plants. They are coagulated by heat, and are precipitated from their solutions by saturation with ammonium sulphate. Albumins usually lack glycine, and some members of the class contain carbohydrates in their molecules and should, therefore, be grouped with the glycoproteins.

Among the albumins are: myogen occurring in muscle, egg albumin, serum albumin, lactalbumin of milk, and leucosin occurring in wheat.

(B) GLOBULINS

These are insoluble in water, but soluble in dilute solutions of neutral salts. They are precipitated from solution by complete saturation with magnesium sulphate or half saturation with ammonium sulphate. Globulins generally contain glycine, and they are widely distributed in animals and plants. Examples of this class of protein are: myosin of muscle, serum globulin, ovoglobulin occurring in egg yolk; edestins from hemp, cotton and sunflower seeds; amandin of almonds, legumin from beans, arachin from peanuts; fibrinogen, which occurs in blood plasma, and is converted into fibrin as the blood clots.

(C) GLUTELINS

These are present in cereals and are insoluble in neutral solvents, but soluble in very dilute acids and alkalies. Examples are: glutenin from wheat and oryzenin from rice.

(D) PROLAMINS

These are plant proteins which occur mainly in seeds. They are soluble in 70 to 80 per cent ethyl alcohol, but insoluble in absolute alcohol, water and neutral solvents.

(E) SCLEROPROTEINS

These substances, which are also known as **Albuminoids**, are insoluble in alcohol, water, salt solutions, dilute acids and alkalis, and are the least soluble of all the proteins. The scleroproteins occur only in animals, and they constitute a very diverse class of proteins which exhibit widely different chemical and physical properties from each other. Examples of scleroproteins are: keratins from hair, epithelium, horn, hooves, nails; elastin of connective tissues and ligaments; collagen of bones, cartilage and tendons; fibroin and sericin from silk, and spongin from sponges.

(F) HISTONES

The histones are basic proteins, soluble in water, dilute acids and in dilute alkalies, but insoluble in dilute ammonia solution. On hydrolysis, they yield a large proportion of basic amino acids. The histones, owing to their basic nature, generally occur in tissues in salt combinations with acidic substances such as nucleic acids and haeme of haemoglobin, and such combinations are classed as conjugated proteins.

(G) PROTAMINS

These strongly basic proteins on hydrolysis yield mainly basic amino acids, of which arginine is the most abundant. The protamins are soluble in water, dilute acids and dilute alkalies including ammonia, and they precipitate other proteins from solution. They are the simplest of proteins and may be regarded as large polypeptides. The protamins generally occur in salt combinations with acids, such as nucleic acids with which they form the nucleoproteins. Protamins are usually of relatively low molecular weight (around 2,000).

Protamins do not contain sulphur, and they have been divided into four divisions, according to their basic amino acid contents, i.e.: (*a*) Monoprotamins, these contain only arginine; (*b*) Lysine Diprotamins, containing lysine and arginine; (*c*) Histidine Diprotamins, containing histidine and arginine; (*d*) Triprotamins, containing arginine, lysine and histidine.

Examples of the protamins are: salmine, which contains about 88 per cent of arginine and occurs in salmon sperm; scombrine of mackerel sperm; sturine from sturgeon sperm, and cyprinine of carp sperm.

CONJUGATED PROTEINS

These contain a simple protein combined with non-protein substances which are called prosthetic groups (i.e. addition groups). The Conjugated Proteins are subdivided into the following classes:

(A) NUCLEOPROTEINS

These are the proteins of cell nuclei and appear to be the chief constituents of chromatin. The Nucleoproteins are most abundant in those tissues of animals and plants which possess a large proportion of nuclear material, e.g. thymus and other glandular organs; in sperm; in yeast, in asparagus tips, etc. They are composed of simple basic proteins (protamins or histones) in combination with nucleic acids.

When a nucleoprotein is subjected to gastric digestion, a large proportion of the protein is converted into peptones or proteoses, which dissolve, leaving an insoluble residue. This precipitate, which still contains a protein group, is referred to as *nuclein*, and this can be further broken down by the enzyme, trypsin to yield nucleic acid and a protein substance which is usually a histone or a protamin. Examples of Nucleoproteins are: nucleohistone and nucleoprotamin. The Nucleoproteins receive further mention later (*see* index).

(B) GLYCOPROTEINS

These are composed of simple proteins combined with carbohydrate groups, other than those of nucleic acids, and these are dealt with later in the section on Carbohydrates.

Examples of Glycoproteins are: proteins of gastric and other mucins, vitreous humor, umbilical cord, serum globulins, thyroglobulin, tendomucoid from tendons, chondroproteins of cartilage, pregnancy urine hormone, osseomucoid of bone, ovomucoid of eggs and certain bacterial proteins.

(C) CHROMOPROTEINS

These are simple proteins united with a coloured prosthetic group, and the most important members of this group are the Haemoglobins, which are respiratory proteins in which the prosthetic group is the iron-containing porphyrin complex haeme. Among the Chromoproteins are the following:

Cytochromes: cellular oxidation-reduction proteins containing haeme as the prosthetic group;

Flavoproteins: cellular oxidation-reduction proteins with riboflavine as the prosthetic group.

(D) PHOSPHOPROTEINS

These are proteins in combination with phosphoric acid as the prosthetic group, and casein from milk, as well as vitellin of egg yolk, are members of this group.

(E) LIPOPROTEINS

These are formed by the combination of protein with a lipid, or lipine, such as lecithin; cephalin; fatty acids, etc. Phospholipid protein complexes, also known as lecithoproteins, are widely distributed in animal and plant materials, and they also occur in bacterial antigens and viruses.

DERIVED PROTEINS

These consist of combinations of simple and conjugated proteins. This group, which is not well defined, may be divided into two classes, i.e. Primary Derived

Proteins and Secondary Derived Proteins, and each class is further sub-divided as below:

(A) PRIMARY DERIVED PROTEINS

These are also known as *denatured proteins* and they are sub-divided into three classes, i.e. (*a*) Proteans, (*b*) Metaproteins, and (*c*) Coagulated Proteins.

Denatured proteins are protein derivatives produced by changes within the molecules of the parent proteins which cause them to become insoluble in solvents in which they were originally soluble. Denaturation of proteins is brought about by various agents and processes, such as the incipient action of water, very dilute acids and enzymes. This conversion of proteins into primary derived or denatured proteins takes place in several phases. Denaturation is also caused by physical agencies such as heat, X-rays, ultra-violet light, and up to the present time it has been found possible to bring about the complete reversal of only a limited number of denatured proteins to the parent proteins. Denatured proteins possess a fibrous keratin-like structure, and their chemical characteristics are different from the original protein. The primary derived proteins, or denatured proteins, possess a greater number of reactive SH and SS groups than the original proteins from which they are derived.

(i) *Proteans*

These are insoluble products generally derived from globulins by the action of enzymes, very dilute acids, etc. Examples are: myosan from myosin, fibrin from fibrinogen, and edestan from edestin.

(ii) *Metaproteins*

These are produced by further action of acids, alkalis, etc. on proteins. Most members of this class are insoluble in neutral solvents, but soluble in very dilute acids and alkalis. Examples of the Metaproteins are: acid and alkali albuminates.

(iii) *Coagulated Proteins*

These are produced by the action of heat or alcohol, X-rays, ultra-violet light and other physical and chemical agents, on natural proteins. Examples are: alcohol-precipitated proteins, cooked egg albumin, etc.

(B) SECONDARY DERIVED PROTEINS

These substances represent a great diversity of molecules of amino acid composition. They are produced by the progressive cleavage, through hydrolysis, of the peptide linkages of protein molecules. Secondary derived proteins are divided into three main classes: (i) Proteoses, (ii) Peptones, and (iii) Peptides, and each class represents very many different substances:

(i) *Proteoses*

These substances, which are also known as *Albuminoses*, are produced by the hydrolysis of proteins. They are soluble in water, and are precipitated when their solutions are saturated with ammonium sulphate.

(ii) *Peptones*

These also are produced by the hydrolysis of proteins. They are soluble in water and are not precipitated by ammonium sulphate, as the proteoses are. Peptones are, however, precipitated by phosphotungstic acid, and they are simpler in molecular structure than the proteoses.

(iii) *Peptides*

These also are produced as hydrolysis products of proteins, and they are simpler in structure than proteoses or peptones, being composed, usually, of only a few amino acid groups. They are soluble in water, are not precipitated by salts such as ammonium sulphate, but many peptides are precipitated by phosphotungstic acid.

They are referred to as di-, tri-, tetrapeptides, according to the number of amino acid groups present in their molecules.

* * *

Proteins are characterized by their amino acid components, but unfortunately most of the known biochemical tests are too destructive to be employed for the detection and localization of these in microscopic preparations, and accordingly only a few amino acid components of proteins can be demonstrated, by colour reactions, and these are described in the following pages.