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AMINO ACIDSANDPROTEINS: STUDY IN ORGANIC CHEMISTY

DR. MANOJ KUMAR MISHRA,ASSISTANT PROFESSOR
S. L.B.S.DEGREE COLLEGE U. P

Abstract

Both animal and plant proteins are made up of about 20 common amino acids. The proportion of these amino acids varies as a characteristic of a given protein, but all food proteins—with the exception of gelatin—contain some of each. Amino nitrogen accounts for approximately 16% of the weight of proteins. Amino acids are required for the synthesis of body protein and other important nitrogen-containing compounds, such as creatine, peptide hormones, and some neurotransmitters. Although allowances are expressed as protein,the biological requirement is for amino acids.Proteins and other nitrogenous compounds are being degraded and resynthesized continuously. Several times more protein is turned over daily within the body than is ordinarily consumed, indicating that reutilization of amino acids is a major feature of the economy of protein metabolism. This process of recapture is not completely efficient, and some amino acids are lost by oxidative catabolism. Metabolic products of amino acids (urea, creatinine, uric acid, and other nitrogenous products) are excreted in the urine; nitrogen is also lost in feces, sweat, and other body secretions and in sloughed skin, hair, and nails. A continuous supply of dietary amino acids is required to replace these losses, even after growth has ceased.

Keywords: Amino acids, Proteins, Supplementing Diets, Histidine, Leucine, Plant proteins,Threonine

Introduction

Amino acids consumed in excess of the amounts needed for the synthesis of nitrogenous tissue constituents are not stored but are degraded; the nitrogen is excreted as urea, and the keto acids left after removal of the amino groups are either utilized directly as sources of energy or are converted to carbohydrate or fat.Nine amino acids—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine—are not synthesized by mammals and are therefore dietarily essential or indispensable nutrients. These are commonly called the essential amino acids. Histidine is an essential amino acid for infants, but was not demonstrated to be required by adults until recently (Cho et al., 1984; Kopple and Swendseid, 1981). Under special circumstances (e.g., in premature infants or in people with liver damage), amino acids such as cystine and tyrosine, not normally essential, may become so because of impaired conversion from their precursors (Horowitz et al., 1981). Arginine is synthesized by mammals but not in amounts sufficient to meet the needs of the young of most species. Although it is not believed to be required by the human infant for normal growth, the need for arginine by the premature infant is unknown. When arginine is present in small amounts relative to other amino acids (such as in intravenous solutions or amino acid mixtures), or when liver function is compromised, arginine synthesis may be insufficient for adequate function of the urea cycle.rotein deficiency rarely occurs as an isolated condition. It usually accompanies a deficiency of dietary energy and other nutrients resulting from insufficient food intake. The symptoms are most commonly seen in deprived children in poor countries. Where protein intake is exceptionally low, there are physical signs—stunting, poor musculature, edema, thin and fragile hair, skin lesions—and biochemical changes that include low serum albumin and hormonal imbalances. Edema and loss of muscle mass and hair are the prominent signs in adults. Deficiency of this severity is very rare in the United States, except as a consequence of pathologic conditions and poor medical management of the acutely ill.

Protein

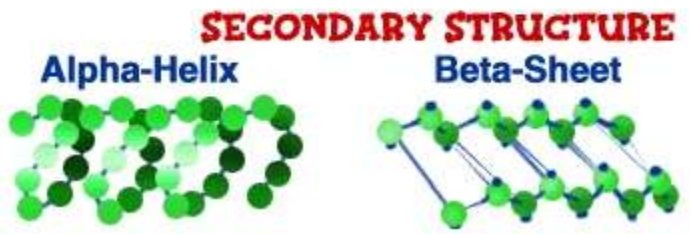
The word protein was coined by Berzelius in 1838 and was used by G. J. Mulder first time 1840. 15% of protoplasm is made up of protein.Average proteins contain 16% nitrogen, 50–55% carbon, oxygen 20–24%, hydrogen 7% and sulphur 0.3 – 0.5%. Iron, phosphorous, copper, calcium, and iodine are also present in small quantity.

Structure of Proteins

It is due to different rearrangement of amino acids. When carboxyl group (-COOH) of one amino acid bonded with amino group ($– NH_2$) of another amino acid the bond is called peptide bond. A peptide may be dipeptide, tripeptide and polypeptide. The simplest protein is Insulin. According to Sanger (1953) insulin consists of 51 amino acids. A protein can have up to four level of conformation.

(i) **Primary structure :**The primary structure is the covalent connections of a protein. It refers to linear sequence, number and nature of amino acids bonded together with peptide bonds only. e.g. ribonuclease, insulin, haemoglobin, etc.

(ii) **Secondary structure :** The folding of a linear polypeptide chain into specific coiled structure (α - helix) is called secondary structure and if it is with intermolecular hydrogen bonds the structure is known as ß -pleated sheet. α - helical structure is found in protein of fur, keratin of hair claws, and feathers. ß - pleated structure is found in silk fibres.



(iii) **Tertiary structure :** The arrangement and interconnection of proteins into specific loops and bends is called tertiary structure of proteins.

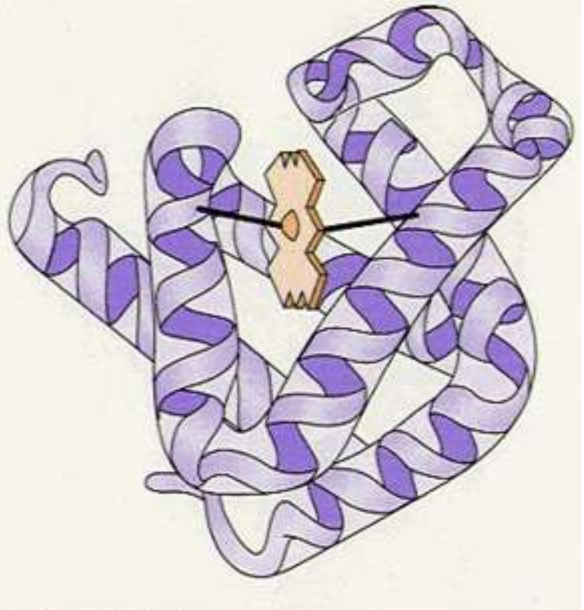
It is stabilized by hydrogen bond, ionic bond, hydrophobic bond and disulphide bonds. It is found in myoglobin (globular proteins).

(iv) **Quaternary structure :** It is shown by protein containing more than one peptide chain. The protein consists of identical units. It is known as homologous quaternary structure e.g. lactic dehydrogenase. If the units are dissimilar, it is called as heterogeneous quaternary structure e.g. hemoglobin which consists of two α -chains and two ß -chains.

Classification of Proteins

Proteins are classified on the basis of their shape, constitution and function.

On the basis of shape



Myoglobin, a globular protein

(i) **Fibrous protein/Scleroprotein :**Insoluble in water. Animal protein resistant to proteolytic enzyme is spirally coiled thread like structure form fibres. e.g. collagen (in connective tissue), actin and myosin, keratin in hairs, claws, feathers, etc.

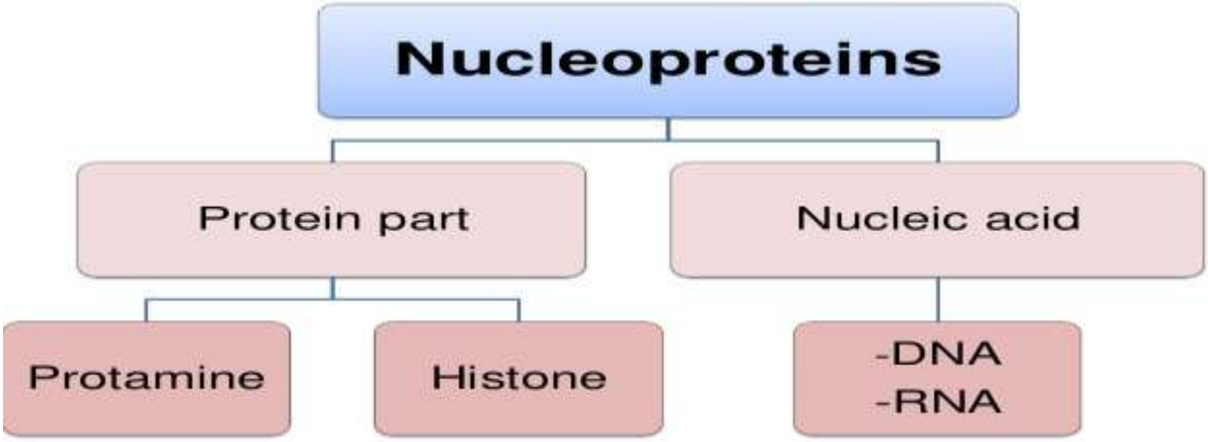
(ii) **Globular proteins :**Soluble in water. Polypeptides coiled about themselves to form oval or spherical molecules e.g. albumin insulin hormones like ACTH, oxytosin, etc.

On the basis of constituents

(i) **Simple proteins :**The proteins which are made up of amino acids only. e.g. albumins, globulins, prolamins, glutelins, histones, etc.

(ii) **Conjugated proteins :**These are complex proteins combined with characterstic non–amino acid substance called as prosthetic group. These are of following types :–

(a) **Nucleoproteins :**Combination of protein and nucleic acids, found in chromosomes and ribosomes. e.g. deoxyribonucleoproteins, ribonucleoproteins, etc.



(b) **Mucoproteins :**These are combined with large amount (more than 4%) of carbohydrates e.g. mucin.

(c) **Glycoproteins :**In this, carbohydrate content is less (about 2 – 3%) e.g. immunoglobulins or antibiotics.

(d) **Chromoproteins :** These are compounds of protein and coloured pigments. e.g. haemoglobin, cytochrome, etc.

(e) **Lipoproteins :** These are water soluble proteins and contain lipids. e.g. cholesterol and serum lipoproteins.

(f) **Metalloprotein :** These are metal binding proteins, AB_1–globin known as transferring is capable of combining with iron, zinc and copper e.g. chlorophyll.

(g) **Phosphoprotein :** They composed of protein and phosphate e.g. casein (milk) and vitellin (egg).

(iii) **Derived proteins :** When proteins are hydrolysed by acids, alkalies or enzymes, the degredation products obtained from them are called derived proteins. On the basis of progressive cleavage, derived proteins are classified as primary proteoses, secondary proteoses, peptones, polypeptides, amino acids, etc.

On the basis of nature of molecules

(i) **Acidic proteins :**They exist as anion and include acidic amino acids. e.g. blood groups.
(ii) **Basic proteins :**They exist as cations and rich in basic amino acids e.g. lysine, arginine etc.

Function of Proteins

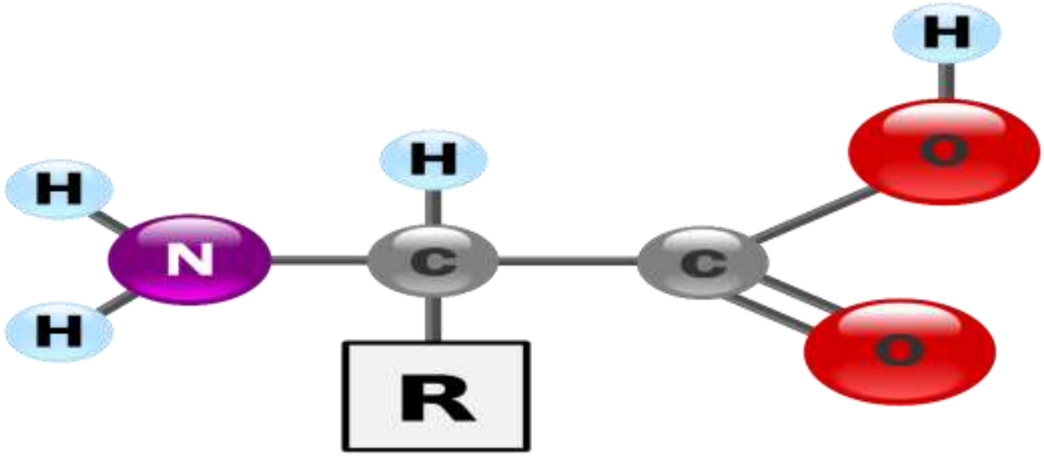
(i) Proteins occur as food reserves as glutelin, globulin casein in milk.
(ii) Proteins are coagulated in solutions, alkaline to the isoelectric pH by positive ions such as Zn^{2+}, Cd^{2+}, Hg^{2+} etc. Casein – pH 4.6, cyt. C – 9.8, resum globulin 5.4, pepsin 2.7, lysozyme 11.0 etc.
(iii) Proteins are the most diverse molecule on the earth.
(iv) Proteins work as hormone as insulin and glucagon.
(v) Antibiotics as gramicidin, tyrocidin and penicillin are peptides.
(vi) They are structural component of cell.
(vii) They are biological buffers.
(viii) Monellin is the sweetest substance obtained from African berry (2000 time sweeter than sucrose).
(ix) Proteins helps in defence, movement activity of muscles, visual pigments receptor molecules, etc.
(x) Natural silk is a polyamide and artificial silk is a polysaccharide. Nitrogen is the basic constituent.

Amino Acids

Amino acids are normal components of cell proteins (called amino acid). They are 20 in number specified in genetic code and universal in viruses, prokaryotes and eukaryotes. Otherwise amino acids may be termed rare amino acids, which take part in protein synthesis e.g. hydroxyproline and non- protein amino acids do not take part in protein synthesis e.g. Ornithin, citrullin, gama-aminobutyric acid (GABA) a neurotransmitter, etc.

Structure and Composition

Amino acids are basic units of protein and made up of C, H, O, N and sometimes S. Amino acids are organic acids with a carboxyl group (–COOH) and one amino group ($-NH_2$) on the a -carbon atom. Carboxyl group attributes acidic properties and amino group gives basic ones. In solution, they serve as buffers and help to maintain pH. General formula is $R-CHNH_2.COOH$.Amino acids are amphoteric or bipolar ions or Zueitter ions. Amino acids link with each other by peptide bond and long chains are called polypeptide chains.



Classification

Based on R-group of amino acids.

(a) Simple amino acids **: These have no functional group in the side chain. e.g. glycine, alanine , leucine, valine etc.**

(b) Hydroxy amino acids **: They have alcohol group in side chain. e.g. threonine, serine, etc.**

(c) **Sulphur containing amino acids** : They have sulphur atom in side chain. e.g. methionine, cystenine.

(d) **Basic amino acids** : They have basic group ($-NH_2$) in side chain. e.g. lysine, arginine.

(e) **Acidic amino acids** : They have carboxyl group in side chain. e.g. aspartic acid, glutamic acid.

(f) **Acid amide amino acids :**These are the derivatives of acidic amino acids. In this group, one of the carboxyl group has been converted to amide ($-CONH_2$). e.g. asparagine, glutamine.

(g) **Heterocyclic amino acids :**These are the amino acids in which the side chain includes a ring involving at least one atom other than carbon. e.g. tryptophan, histidine.

(h) **Aromatic amino acids :**They have aromatic group (benzene ring) in the side chain. e.g. phenylalanine, tyrosine, etc.

On the basis of requirements : On the basis of the synthesis amino acids in body and their requirement, they are categorized as :–

(a) **Essential amino acids :**These are not synthesized in body hence to be provided in diet e.g. valine, leucine, isoleucine, theronine ,lysine, etc.

(b) **Semi-essential amino acids :**Synthesized partially in the body but not at the rate to meet the requirement of individual. e.g., arginine and histidine.

(c) **Non-essential amino acids :**These amino acids are derived from carbon skeleton of lipids and carbohydrate metabolism. In humans there are 12 non-essential amino acids e.g. alanine, aspartic acid, cysteine, glutamic acid etc. Proline and hydroxyproline have, NH (imino group) instead of NH_2 hence are called imino acids. Tyrosine can be converted into hormone thyroxine and adrenaline and skin pigment melanin. Glycine is necessary for production of heme.

Tryptophan is the precursor of vitamin nicotinamide and auxins. If amino group is removed from amino acid it can form glucose and if COOH group is removed, it forms amines e.g. histamine.

In determining the requirement for protein, the subcommittee first considered requirements for the essential amino acids. The required amounts of the nine essential amino acids must be provided in the diet, but because cystine can replace approximately 30% of the requirement for methionine, and tyrosine about 50% of the requirement for phenylalanine, these amino acids must also be considered. The essential amino acid requirements of infants, children, men, and women were studied extensively from 1950 to 1970. Except for infants, where the criterion was growth and nitrogen accretion, the requirement was accepted to be the amount of intake needed to achieve nitrogen equilibrium in short-term studies of adults or positive balance in children (see review by FAO/WHO, 1973; NRC, 1974; WHO, 1985). Estimates of amino acid requirements for various age groups are listed in Table

TABLE Estimates of Amino Acid Requirements

	Requirements, mg/kg per day, by age group			
Amino Acid	**Infants, Age 3–4 mo**[b]	**Children, Age ~2 yr**[c]	**Children, Age 10–12 yr**[d]	**Adults**[e]
Histidine	28	?	?	8–12
Isoleucine	70	31	28	10

Requirements, mg/kg per day, by age group

Amino Acid	Infants, Age 3–4 mo[b]	Children, Age ~2 yr[c]	Children, Age 10–12 yr[d]	Adults[e]
Leucine	161	73	42	14
Lysine	103	64	44	12
Methionine plus cystine	58	27	22	13
Phenylalanine plus tyrosine	125	69	22	14
Threonine	87	37	28	7
Tryptophan	17	12.5	3.3	3.5
Valine	93	38	25	10
Total without histidine	714	352	214	84

In a novel approach to examining these requirements, the need for four amino acids was examined in children whose diets were strictly controlled because of inborn errors of metabolism and who were developing normally (Kindt and Halvorsen, 1980). Requirements determined in this way during the first 3 years of life are in good agreement with the values for isoleucine, leucine, phenylalanine plus tyrosine, and valine given in Table for infants and 2-year-old children.

The requirement for histidine has not been quantified beyond infancy. Requirement values are difficult to establish because deficiency symptoms occur only after long periods of low intake. Kopple and Swendseid (1981) demonstrated that nitrogen balance diminished when histidine intake was less than 2 mg/kg per day, and increased when intake was increased to 4 mg/kg per day. WHO (1985) estimated the probable adult histidine requirement to be between 8 and 12 mg/kg per day by extrapolation from the infant requirement; this estimate is likely to be high, but safe.The relatively low requirements estimated for adults have been confirmed by Inoue et al. (1988) using the nitrogen balance method. Studies of whole body lysine, leucine, valine, and threonine oxidation rates suggest that adult requirements for these essential amino acids have been underestimated. Approximations of average requirements according to the ^{13}C tracer studies are leucine, 40 mg/kg (Meguid et al., 1986a); lysine, 35 mg/kg (Meredith et al., 1986); threonine, 15 mg/kg (Zhao et al., 1986); and valine, 16 mg/kg (Meguid et al., 1986b). These new estimates have been challenged on methodologic and theoretical grounds (Millward and Rivers, 1986) and require further confirmation.Studies on requirements for individual essential amino acids in the elderly are inconsistent.

Some suggest that requirements are increased in the elderly; others indicate that they are decreased (Munro, 1983). In the one study in which the same methodology and design were applied to the elderly as in a study of young men, no differences in requirements between age groups were found (Watts et al., 1964). The pattern of requirement for essential amino acids in the elderly is accepted to be the same as for younger adults.

Essential Amino Acids and Protein Quality

If the essential amino acid requirements of fish are known, it should be possible to meet these needs in culture systems in a number of ways from different food proteins or combinations of food proteins.Phenylalanine is spared by tyrosine. It is not known to be chemically modified nor rendered unavailable by the harsh conditions to which feedstuff proteins are normally subjected during processing. Measurement of phenylalanine in proteins is uncomplicated so that the provision and evaluation of phenylalanine in proteins in practical diets presents little difficulty. Lysine is a basic amino acid. In addition to the □ -amino acid group normally bound in peptide linkage, it also contains a second, □ -amino group. This □ -amino group must be free and reactive, otherwise the lysine, although chemically measurable, will not be biologically available. During the processing of feedstuff proteins the □ -amino group of lysine may react with non-protein molecules present in the feedstuff to form additional compounds that render the lysine biologically unavailable. Methionine is spared by cystine. However, measurement of the methionine content of feed proteins is not easy as the amino acid is subject to oxidation during processing. After processing, methionine may be present as such or as the sulphoxide or as the sulphone. The sulphoxide may be formed from methionine during acid hydrolysis of the feed protein prior to measurement of its any-no acid composition. Acid hydrolysis of proteins before analysis disturbs the original equilibrium between the two compounds so that the composition of the hydrolysate no longer reflects that of the protein. In determining the methionine content of pure proteins, oxidation of the amino acid to methionine sulphone is normally quantitative. In the case of feed proteins, however, this will not reveal how much methionine or methionine sulphoxide was present in the protein prior to performate oxidation and hydrolysis. Methionine sulphoxide may have some biological value for fish which may have some capability of reconverting it to methionine and thus partially make up for some of the methionine oxidized during processing. Methods have recently been reported for measurement of methionine in proteins using an iodoplatinate reagent before and after reduction with titanium trichloride, to give values for both methionine and the sulphoxide in the original protein. A method for measuring methionine specifically by cyanogen bromide cleavage has also been described. Both methods remain to be independently assessed. Microbiological assay of methionine in feed proteins is a valuable tool although there is the danger that oxides of methionine may differ in their activity for micro-organisms and misrepresent values.

Supplementing Diets With Amino Acids

One solution to the use of proteins that are relatively deficient in one or more amino acids is to supplement the protein with appropriate amounts of the amino acid needed in practical diets. Fish appear to utilize free amino acids at various degrees of efficiency. Young carp, Cyprinuscarpio, were shown to be unable to grow on diets in which the protein component (casein, gelatin) was replaced by a mixture of amino acids similar in overall composition. A trypsin hydrolyzate of casein was equally ineffective. However, if a diet containing free amino acids as the protein component is carefully neutralized with NaOH to pH 6.5-6.7 then some growth of young carp does occur. This growth was markedly inferior to that occurring on a comparable casein diet under the same conditions. Channel catfish are also unable to utilize free amino acids given as supplements to deficient proteins. When soybean meal was substituted isonitrogenously for menhaden meal, growth and feed efficiency of channel catfish were substantially reduced. Addition of free methionine, cystine or lysine, the most limiting amino acids, to these soy-substituted diets did not enhance weight gain.

Raising the arginine level of catfish diets from 11 to 17 g/kg by isonitrogenous substitution of gelatin for casein enhanced weight gain significantly but the addition of free arginine, cystine, tryptophan or methionine to casein had little effect on growth or food conversion. Salmonids are able to utilize free amino acids for growth. A zein-gelatin diet supplemented with lysine and trytophan was shown to be markedly superior to an unsupplementedzein-gelatin diet for rainbow trout when weight gain and protein utilization were used as criteria. Several investigators have demonstrated the potential of supplementing amino acid deficient proteins with limiting amino acids in diets for salmonids. Casein supplemented with six amino acids produced feed conversion ratios with Atlantic salmon similar to those obtained when an isolated fish protein was used as the dietary protein source. Soybean meal supplemented with five or more amino acids (including methionine and lysine) was a superior protein source to soybean meal alone for rainbow trout. Single additions of methionine and lysine did not, however, improve the value of soybean meal. These results suggest that the amino acid spectrum of the isolated fish protein they used may possibly approximate the amino acid requirement of rainbow trout. The nutritional value of a soy protein isolate could be enhanced by supplementing it with the first limiting amino acid; i.e., methionine. Diets containing, as protein component, fishmeal, meat and bone meal, and yeast and soybean meal could be improved by supplementing with cystine (10 g/kg) and tryptophan (5 g/kg) together. Fishmeal can be entirely replaced without a reduction in food conversion rate in diets for rainbow trout by a mixture of poultry by-product meal and feather meal together with 17 g lysine HCL/kg, 4.8 g DL-methionine/kg, and 1.44 g

DL-tryptophan/kg.

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