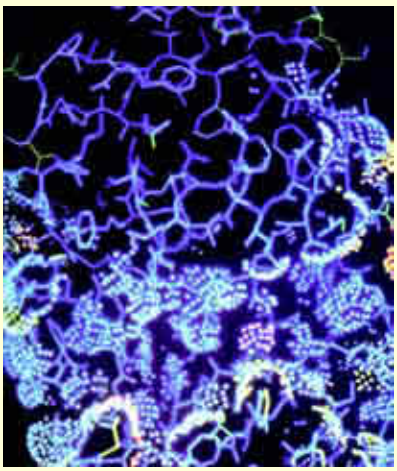
CONTENTS



Computer-generated model of the protein pepsin

This enzyme protein helps in the digestion of food ingested by living beings.

CHAPTER

11

Proteins–III

General Properties

The general properties of proteins are reminiscent of those of the amino acids.

PHYSICAL PROPERTIES

1. Colour and Taste. Proteins are colourless and usually tasteless. These are homogeneous and crystalline.

2. Shape and Size. As already discussed, the proteins range in *shape* from simple crystalloid spherical structures to long fibrillar structures. Two distinct patterns of shape have been recognized :

A. Globular proteins—These are spherical in shape and occur mainly in plants, esp., in seeds and in leaf cells. These are bundles formed by folding and crumpling of protein chains.

e.g., pepsin, edestin, insulin, ribonuclease etc.

B. Fibrillar proteins—These are thread-like or ellipsoidal in shape and occur generally in animal muscles. Most of the studies regarding protein structure have been conducted using these proteins.

e.g., fibrinogen, myosin etc.

Each protein molecule is characterized for its specific size (Fig. 11–1). For example :

(*a*) Hemoglobin has a diameter of 55 Å.

(*b*) Edestin has a diameter of 80 Å.

(*c*) Catalase has dimensions of $80 \times 64 \times 54$ Å (of the axes).

(*d*) Human fibrinogen has a diameter of 38 Å and a length of 700 Å.

(*e*) Collagen is one of the longest proteins, with a length of 3,000 Å.

In general, the protein molecules are always very large, as can be seen in the following examples :

(*a*) Gliadin (from wheat)—$C_{685}H_{1068}N_{196}O_{211}S_5$

(*b*) Zein (from corn)—$C_{736}H_{1161}N_{184}O_{208}S_3$

(*c*) Casein (from milk)—$C_{708}H_{1130}N_{180}O_{224}S_4P_4$

(*d*) Beta-lactoglobulin (from milk)—$C_{1642}H_{2652}O_{492}N_{420}S_{18}$

3. Molecular Weight. The extraordinary size, poor stability, specific solubility conditions and high reactivity have rendered the determination of molecular weight of proteins as a difficult task (Edsall, 1953). However, the proteins generally have large molecular weights ranging between 5×10^3 and 1×10^6 (see Table 11–1). It might be noted that the values of molecular weights of many proteins lie close to or multiples of 35,000 and 70,000. Previously, this was interpreted as a regularity under the name *Svedberg's rule*. Also, it was then assumed that proteins are composed of units of molecular weight 17,500. This corresponds to about 145–150 amino acid residues, since the average molecular weight of an amino acid residue amounts to about 115–120. The discovery in recent times of too many exceptions to this rule, however, finally forced its abandonment.

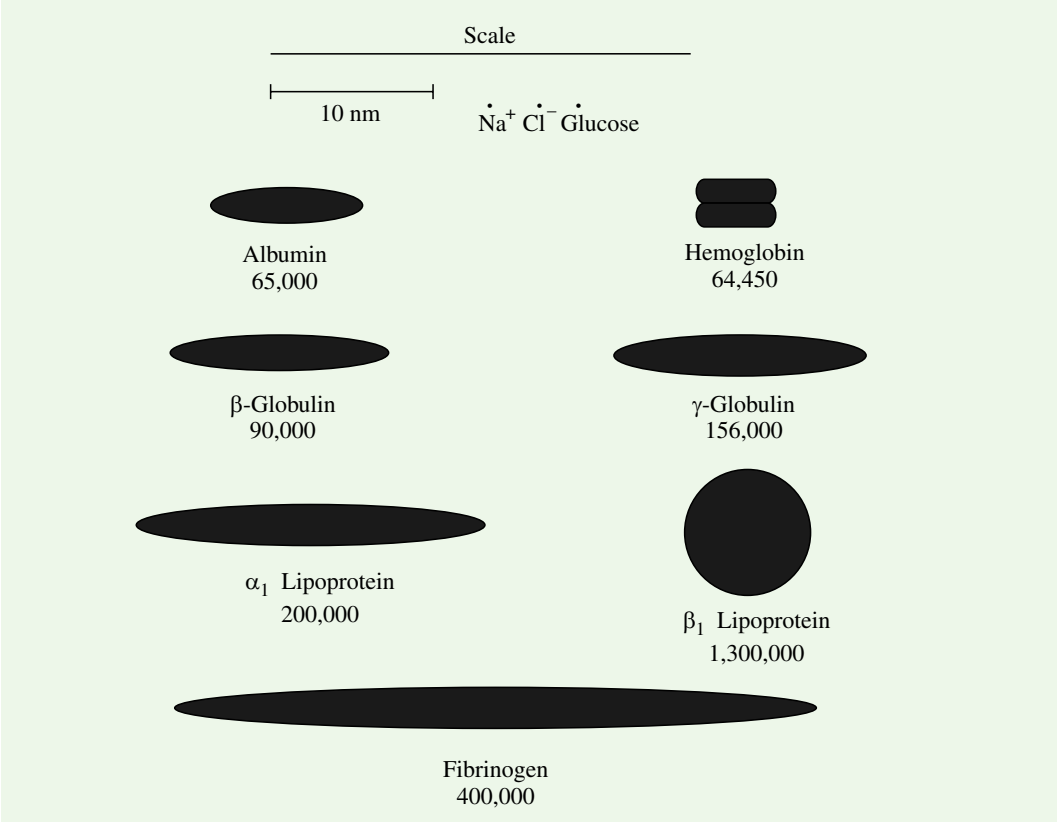


Fig. 11–1. Relative dimensions and molecular weights of some of the protein molecules in the blood (*After Oncley JL, 1949*)

The approximate number of amino acid residues in a simple protein having no prosthetic group can be calculated by dividing its molecular weight by 110. The average molecular weight of the 20 amino acids is about 138. But as the smaller amino acids predominate in most proteins, hence the average molecular weight of an amino acid is nearer 128. Since a molecule of water (MW = 18) is eliminated to produce each peptide bond, the average molecular weight of the amino acid residue is about 128 – 18 = 110. Table 11–1 also gives the number of amino acid residues present in different proteins.

Table 11–1. Exact / Approximate molecular weights and the isoelectric points of some important proteins

Protein	*Molecular weight (MW)*	*Number of residues*	*Number of chains*	*Isoelectric point (pI)*
1. Insulin (bovine)	5,733	51	2	5.4
2. Cytochrome C	12,500	104	—	9.8
3. Ribonuclease (bovine)	14,000	124	1	7.8/9.5
4. Lysozyme (eggwhite)	14,600	129	1	11.0
5. Myoglobin (horse)	16,700	153	1	7.0
6. Chymotrypsin (bovine)	22,600	241	3	—
7. Pepsin	35,500	—	—	2.7
8. Ovalbumin (hen)	40,000	—	—	4.6
9. Zein	40,000	—	—	—
10. Hemoglobin (human)	64,500	574	4	—
11. Serum albumin (human)	68,500	~ 550	1	4.9
12. Hexokinase (yeast)	96,000	~ 800	4	—
13. γ-globulin (horse)	149,900	~ 1,250	4	6.6
14. Catalase	250,000	—	—	5.6
15. Edestin	300,000	—	—	6.9
16. Fibrinogen	450,000	—	—	5.5
17. Urease	480,000	—	—	5.0
18. Glutamate dehydrogenase (bovine)	1,000,000	~ 8,300	~ 40	—
19. Virus protein of TMV	60,000,000	—	—	—

4. Colloidal Nature. Because of their giant size, the proteins exhibit many colloidal properties, such as :

I. Their diffusion rates are extremely slow.

II. They may produce considerable light-scattering in solution, thus resulting in visible turbidity (Tyndall effect).

5. Denaturation. *Denaturation refers to the changes in the properties of a protein.* In other words, it is the loss of biologic activity. In many instances the process of denaturation is followed by **coagulation**— a process where denatured protein molecules tend to form large aggregates and to precipitate from solution.

Denaturation may be brought about by a variety of agents, both physical and chemical. The **physical agents** include mechanical action (like shaking), heat treatment cooling and freezing

operations, rubbing, high hydrostatic pressures, (5,000 to 10,000 atm.), ultraviolet rays, etc. The **chemical agents**, that cause denaturation, are many ionizing radiations (like X-rays, radioactive and ultrasonic radiations), organic solvents (acetone, alcohol), aromatic anions (salicylates), some anionic detergents (like sodium dodecyl sulfate), etc. A common example of protein easily denatured by shaking or heat is the albumin of eggwhite.

$$H_3C—(CH_2)_{10}—CH_2OSO^-Na^+$$

Sodium dodecyl sulfate, SDS

It was suggested by Wu (1931) that denaturation leads mainly to the unfolding of the peptide chain, thus causing disorganization of the internal structure of protein (Fig. 11–2). This is evidenced by the fact that the denatured proteins are more easily hydrolyzed (Mirsky, 1935).

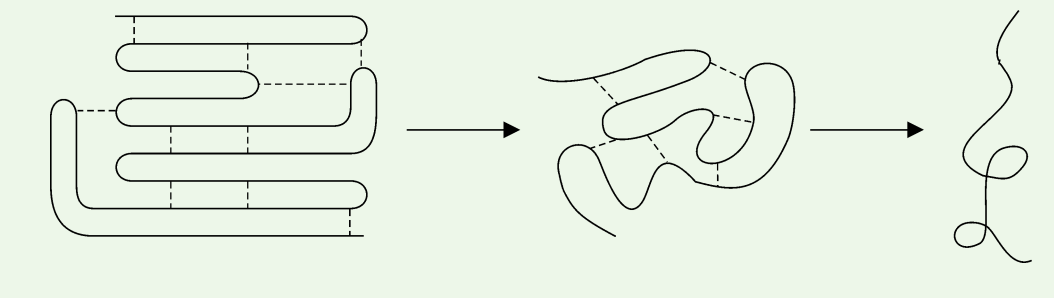


Fig. 11–2. Representation of denaturation of a protein

The process of denaturation breaks bonds.

When the peptide chains or the protein molecules are unrolled, certain bonds split and new sites of bundles are exposed to the action of certain proteolytic enzymes causing hydrolysis. Thus, the H-bonds linking the 2 peptide chains are partly freed and the disulfide (—S—S—) bonds also linking the two peptide chains split open to yield the free sulfhydryl (—SH) groups.

According to Putnam (1953), the proteins, on denaturation, undergo following changes :

1. Decrease in their solubility.
2. Cessation of their biochemical activity as enzymes or hormones.
3. Decrease in size and shape of the molecule.
4. Increased activity of some radicals present in the molecule such as —SH group of cysteine, —S—S— bond of cystine and phenolic group of tyrosine.

Further, on denaturation, new ionizable groups become available for acid-base titration (Steinhardt and Zaiser, 1955) and also there occurs a change in optical rotation in the direction of increased levorotation (Simpson and Kauzmann, 1953). Denaturation also leads to alteration in surface tension and loss of antigenecity.

Some proteins, when denatured, cannot be brought back to their original state. In that case denaturation is described as of '*irreversible*' type. On the other hand, denaturation in other proteins is of '*reversible*' type. For example, if trypsin is exposed to a temperature of 80–90°C, it denatures and when this solution is cooled at 37°C, the solubility and the activity of this protein-enzyme is regained. The process of regaining normal protein properties by a denatured protein is called **renaturation** or **refolding**. During renaturation, certain antibodies may cause a re-rolling of the protein bundles so that most of the original bonds are recovered (Pauling, 1940). The recovery of the renatured protein is, however, never complete.

6. Amphoteric Nature. Like amino acids, the proteins are *amphoteric, i.e.*, they act as acids and alkalies both (Fig. 11–3). These migrate in an electric field and the direction of migration depends upon the net charge possessed by the molecule. The net charge is influenced by the pH value. Each protein has a fixed value of isoelectric point (*pI*) at which it will move in

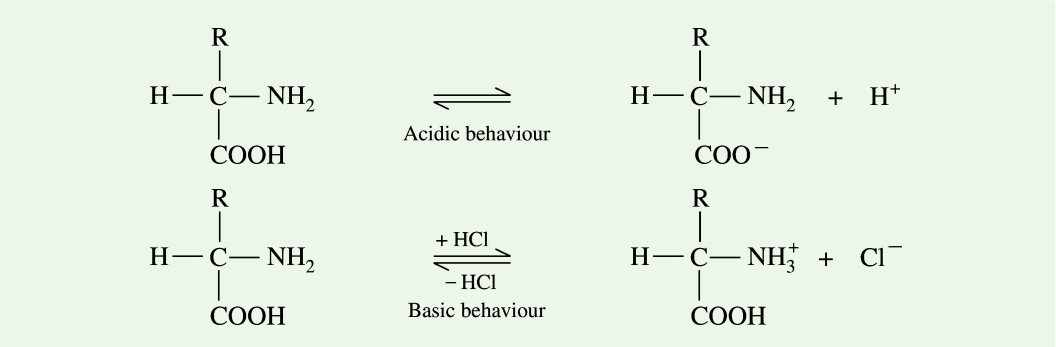


Fig. 11–3. Amphoteric nature of amino acids (and of proteins)

an electric field (refer Table 11–1). **Isoelectric point** (or **isoionic point**) is the pH value at which the number of cations is equal to that of anions. Thus, at isoelectric point, the net electric charge of a protein is always zero. But the total charge on the protein molecule (sum of positive and negative charges) at this point is always maximum. Thus, the proteins are **dipolar ions** or **internal salts** or **zwitterions** (German for 'ion of both kinds' ; amphoteric ions) at *pl* and exist in solution as :

$$(H_3N^+)_m—R—(COO^-)_n$$

This general structure of amino acids, represented as an inner salt, was originally proposed by N. Bjerrum in 1923. The formula depicts the carboxyl group as being dissociated while the amino group is protonated.

At pH values lower than *pl*, the protein will have a net positive charge and, as a cation, will migrate towards negative pole (cathode). Similarly, at pH values higher than *pl*, the protein will have a net negative charge and, as an anion, will move towards positive pole (anode).

It may be stated in general that those proteins having an excess of carbonyl groups will tend to have a lower *pl* while those having an excess of amino groups will tend to have a higher *pl*.

The osmotic pressure and viscosity of the protein solution are a minimum at the isoelectric point. Also at the isoelectric point, proteins are found to be least soluble and can be precipitated most easily.

7. Ion Binding Capacity. Being amphoteric in nature, the proteins can form salts with both cations and anions based on their net charge. In fact, a mixture of different proteins at a given pH (except at *pl*) will include cations and anions both and the salts of protein-protein combinations will be formed. This occurs in tissues since both acidic and basic proteins are present.

Many ions form insoluble salts with proteins and serve as excellent precipitating agents for proteins. For example, anions of some acids like phosphotungstic, trichloroacetic, picric etc., form insoluble salts with proteins when the latter behave **as cations** (acid side of their *pl*).

Heavy metals are used for precipitating proteins on the alkaline side of their *pl*, the proteins behaving **as anions**. Ions of Hg, Cu, Ag, Zn etc., are frequently used for this purpose. Many acid dyes find practical use for colouring the insoluble proteins like silk and wool.

8. Solubility. The solubility of proteins is markedly influenced by pH. *Solubility is lowest at isoelectric point and increases with increasing acidity or alkalinity*. This is because when the protein molecules exist as either cations or anions, repulsive forces between ions are high, since all the molecules possess excess charges of the same sign. Thus, they will be more soluble than in the isoelectric state.

A. '*Salting-in*' *effect*. Globulins are sparingly soluble in water but their solubility is greatly increased by the addition of neutral salts like NaCl. This phenomenon is commonly described as

'salting-in' effect.

B. '*Salting-out*' *effect*. Proteins are precipitated from aqueous solution by high concentrations of neutral salts. This is the 'salting-out' process. Divalent and trivalent ions are more effective than univalent ions. The salts commonly used for this purpose are $Na_2.SO_4$, $(NH_4)_2.SO_4$, magnesium salts and phosphates.

C. *Isoelectric precipitation*. Some proteins like casein of milk, however, are readily precipitated at or near their isoelectric point. This process is, therefore, described as isoelectric precipitation.

9. Optical Activity. All protein solutions rotate the plane of polarized light to the left, *i.e.*, these are *levoratotory*. For example, the specific rotation $[\alpha]_D$ for ovalbumin is near —30° over the *p*H range between 3.5 and 11. However, at lower or higher pH values the rotation becomes more negative, *e.g.*, at pH 13, the $[\alpha]_D$ is about —60°. The rotation is further increased by subjecting proteins to high temperatures.

CHEMICAL PROPERTIES

A. HYDROLYSIS

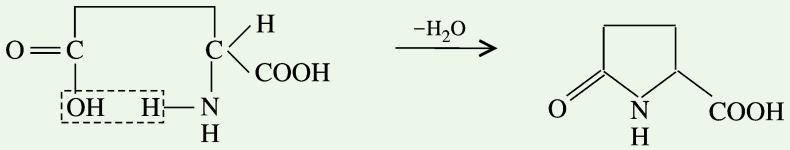
Proteins are hydrolyzed by a variety of hydrolytic agents.

1. By acidic agents. Proteins, upon hydrolysis with conc. HCl (6–12N) at 100–110°C for 6 to 20 hrs, yield amino acids in the form of their hydrochlorides. Undesirable side-effects of acid hydrolysis include the following:

(*a*) Tryptophan, serine and threonine are destroyed during acid hydrolysis and as such this reaction is not used for their isolation.

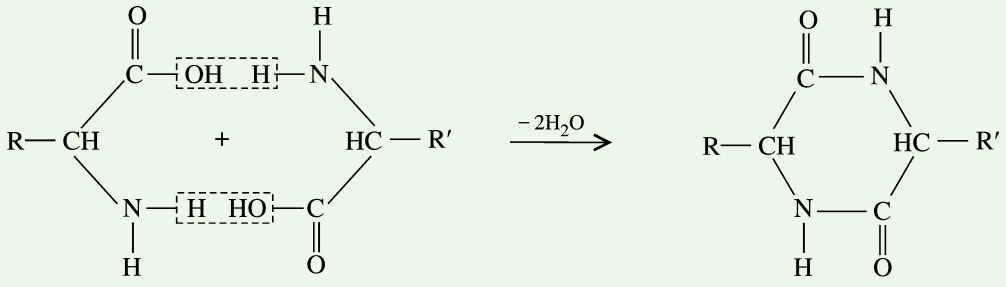
(*b*) Asparagine and glutamine are deamidated to aspartate and glutamate respectively.

(*c*) Glutamic acid undergoes *intramolecular dehydration* to pyrollidone 5-carboxylic acid.



Glutamic acid — Pyrollidone 5-carboxylic acid

(*d*) Other amino acids may undergo *intermolecular dehydration* forming cyclic anhydrides or diketopiperazines.



Amino acid — Amino acid — a diketopiperazine

2. By alkaline agents. Proteins may also be hydrolyzed with 2N NaOH. Alkaline hydrolysis is, however, less used as it is highly disadvantageous:

(*a*) It leads to the destruction of certain amino acids like arginine, cysteine, cystine, serine, threonine etc.

(*b*) It also causes loss of optical activity (or racemization) of the amino acids.

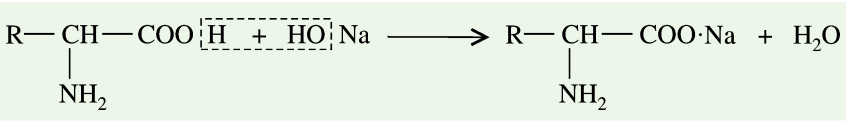
3. By proteolytic enzymes. Under relatively mild conditions of temperature and acidity, certain proteolytic enzymes like pepsin and trypsin hydrolyze the proteins. Enzyme hydrolysis is used for the isolation of certain amino acids like tryptophan. Two important drawbacks with this type of hydrolysis are:

(*a*) It requires prolonged incubation.

(*b*) Hydrolysis is incomplete.

B. REACTIONS INVOLVING COOH GROUP

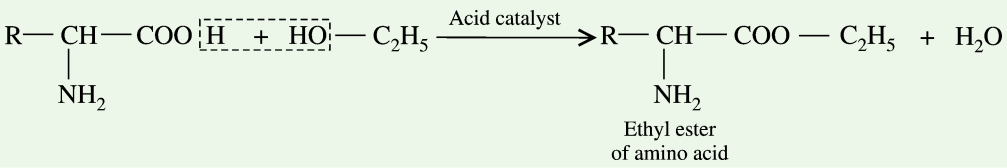
1. Reaction with alkalies (Salt formation). The carboxylic group of amino acids can release a H^+ ion with the formation of carboxylate (COO^-) ions. These may be neutralised by cations like Na^+ and Ca^{2+} to form salts. Thus, amino acids react with alkalies to form *salts*.



Sodium salt of glutamic acid (monosodium glutamate) is used commercially as a flavouring agent. It imparts a meat-like flavour to soups, for example.

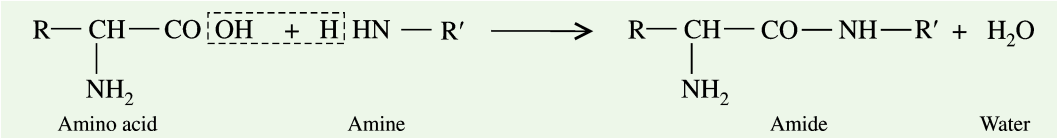
Monosodium glutamate (MSG) or 'ajinomoto' in common porlance, or the 'taste powder' as some people call it, has a long history of use in China. Like sugar or salt, it is regarded as a fairly standard part of cuisine. It makes a great difference to the way the food tastes. MSG actually represents a new taste - the meaty, broth-like taste, common to foods as diverse as steak, lobster and tomato. *Virtually, all proteins contain glutamates, including mother's milk*. Most glutamates are `bound' and have no effect on the flavour but 'free' glutamates increase food's palatibility. Every 100 g of Parmesan cheese, for instance, contains 9,847 mg of bound and 1,200 mg of free glutamates. Tomatoes, peas, meat stock and canned foods and snacks also have it. Human body carries about 2 kg of glutamate in muscles, brain, organs and tissues. Oriental cooks have long added a large seaweed, *Laminaria japonica*, to their soup stocks to enhance that brothy flavour. The seaweed contains glutamate. MSG is used as a flavour enhancer not only by food processors but also by consumers as well as culinary experts in restaurants and hotels. In fact, MSG is one of the most ubiquitous of all food additives and is a popular ingredient of Chinese cuisine. Consuming food containing too much MSG has precipitated attacks of sweating, headaches and gastrointestinal disorders in individuals, sensitive to the chemical. The disease characterized by the above symptoms is called **Kwok's disease** or the **'Chinese Restaurant Syndrome (CRS)**. Kwok, a pattern of eateries, described people experiencing numbness of the back of the neck, general weakness and palpitations after eating in Chinese restaurants (hence, its nomenclature). People with high blood pressure should abstain foods added with MSG.

2. Reaction with alcohols (Esterification). With alcohols, corresponding *esters* are produced. The esters, so obtained, are volatile in contrast to the free amino acids.



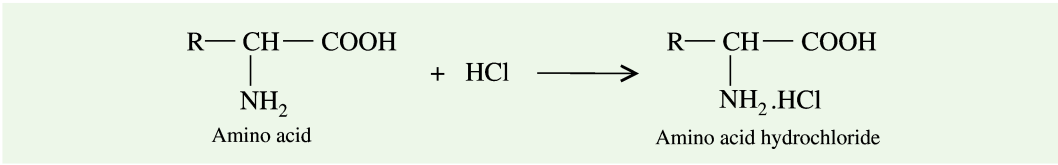
The reaction was, for the first time, used by Emil Fischer for the isolation of amino acids in pure form from protein hydrolysates by the fractional distillation in vacuum of their ethyl esters.

3. Reaction with amines. Amino acids react with amines to form *amides*.



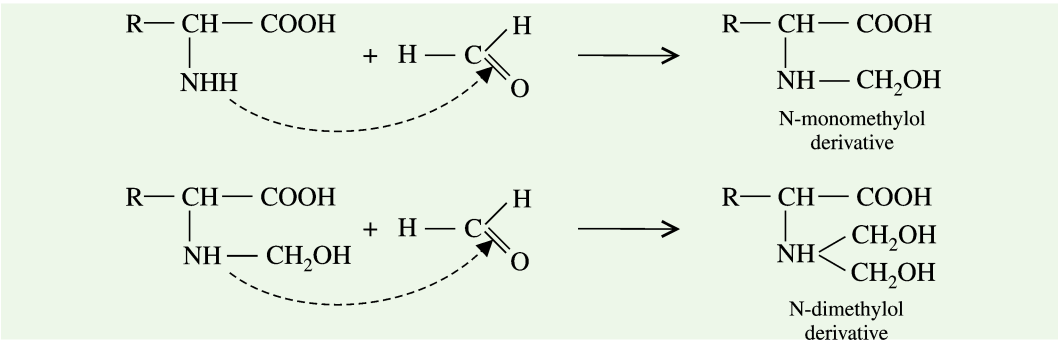
C. REACTIONS INVOLVING NH_2 GROUP

1. Reaction with mineral acids (Salt formation). When either free amino acids or proteins are treated with mineral acids like HCl, the *acid salts* are formed.



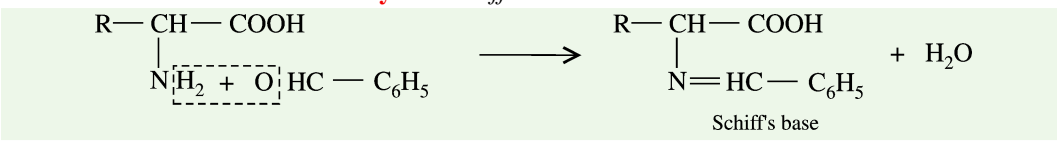
The basic amino acids, arginine and lysine react with CO_2 in the presence of air to form carbonate salts. Because of this property, these are usually stored and also sold in the form of their monochlorides.

2. Reaction with formaldehyde. With formaldehyde, the *hydroxy-methyl* derivatives are formed. These derivatives are insoluble in water and resistant to attack by microorganisms. Because of this action, formaldehyde is the principal reagent in embalming fluids and is used to harden and preserve certain fibres (Aralac, Vicara) obtained from globular proteins.

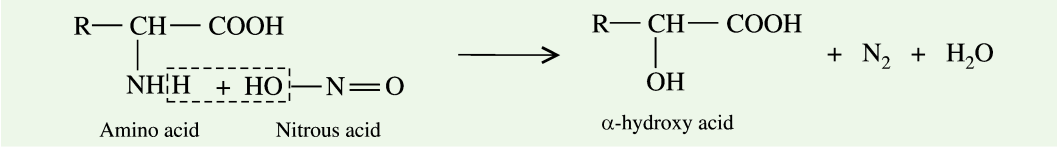


This reaction is the basis of the Sorensen titration method for determining the purity of the individual amino acids.

3. Reaction with benzaldehyde. *Schiff's* bases are formed.

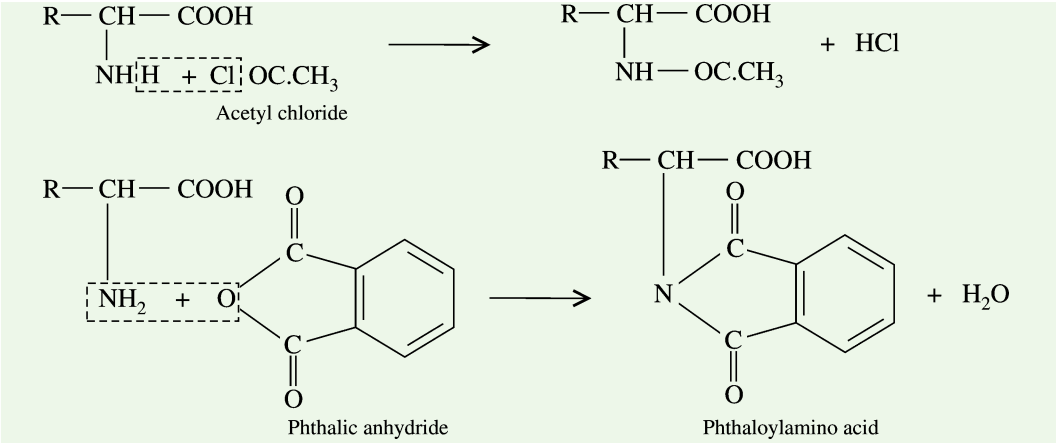


4. Reaction with nitrous acid (Van Slyke reaction). The amino acids react with HNO_2 to liberate N_2 gas and to produce the corresponding α-*hydroxy acids*.



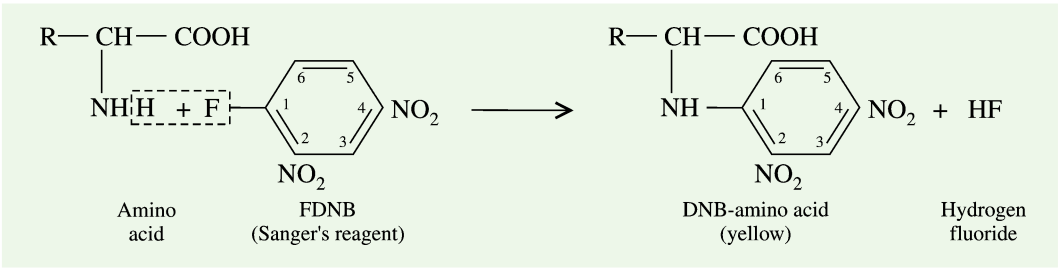
This reaction is characteristic of aliphatic primary amines and has been utilized by Van Slyke (1912) as the basis for his `*nitrous acid' method* for the estimation of amino acids by measuring the volume of N_2 gas liberated. The imino acids proline and hydroxyproline, however, do not respond to this reaction.

5. Reaction with acylating agents (Acylation). Acylation is brought about by many acid chlorides ($CH_3.COCl$, $C_6H_5.COCl$) and acid anhydrides ($CH_3.CO—O—OC.CH_3$, phthalic anhydride), when amino acids in alkaline medium react with them.



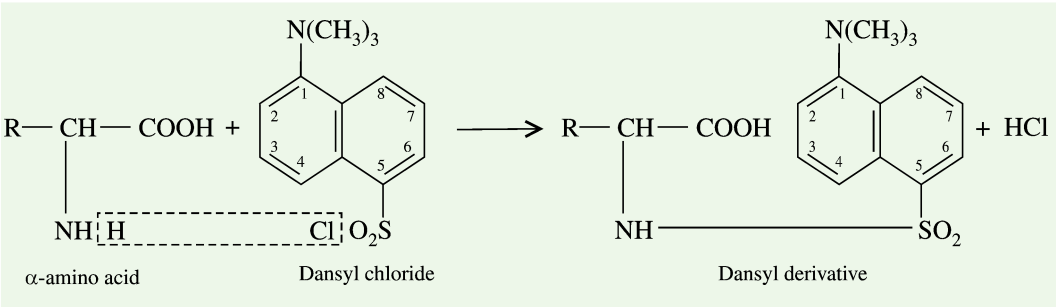
6. Reaction with FDNB* or Sanger's reagent. In mildly alkaline solution, FDNB (1-fluoro- 2, 4-dinitrobenzene) reacts with α-amino acids to produce yellow coloured derivative, *DNB-amino acid.*

FDNB is also called as **DNFB** (2, 4-dinitrofluorobenzene).



This reaction is valuable in elucidation of protein structure and has been successfully uitlized by Sanger in England in determining the sequence of amino acids in insulin.

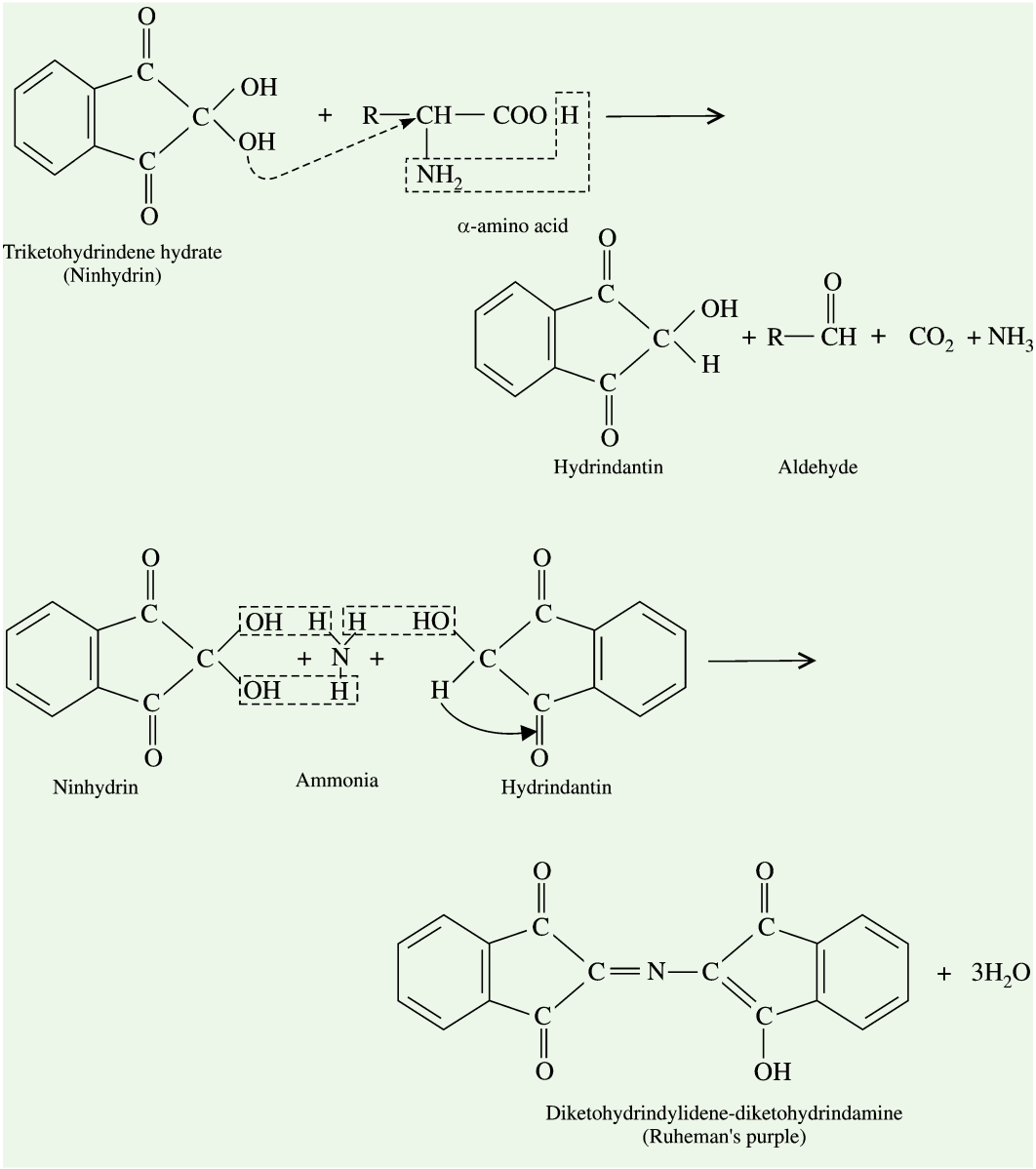
7. Reaction with dansyl chloride. The N-terminal amino acid of the protein also combines with 1-dimethylaminonaphthalene-5-sulfonyl chloride (or dansyl chloride) to form a fluorescent *dansyl derivative*.



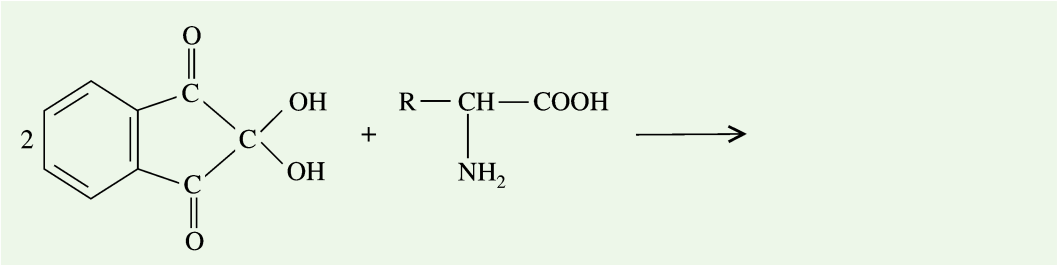
D. REACTIONS INVOLVING BOTH COOH AND NH_2 GROUPS

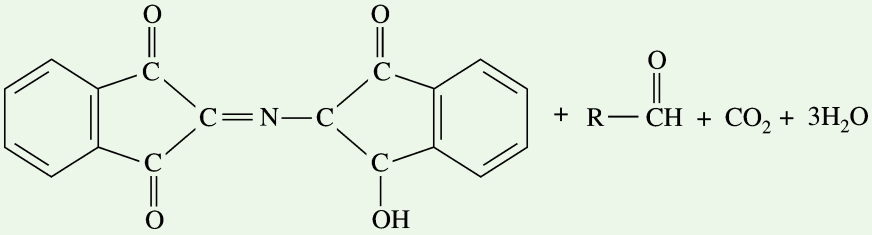
1. Reaction with triketohydrindene hydrate (Ninhydrin reaction). Ninhydrin (= indane-1, 2, 3, -trione hydrate) is a powerful oxidizing agent and causes oxidative decarboxylation of α-amino acids producing CO_2, NH_3 and an aldehyde with one less carbon atom than the parent

amino acid. The reduced ninhydrin (or hydrindantin) then reacts with the liberated NH_3 and a mole of ninhydrin, forming blue-coloured Ruheman's complex.



The net equation may, thus, be written as follows:

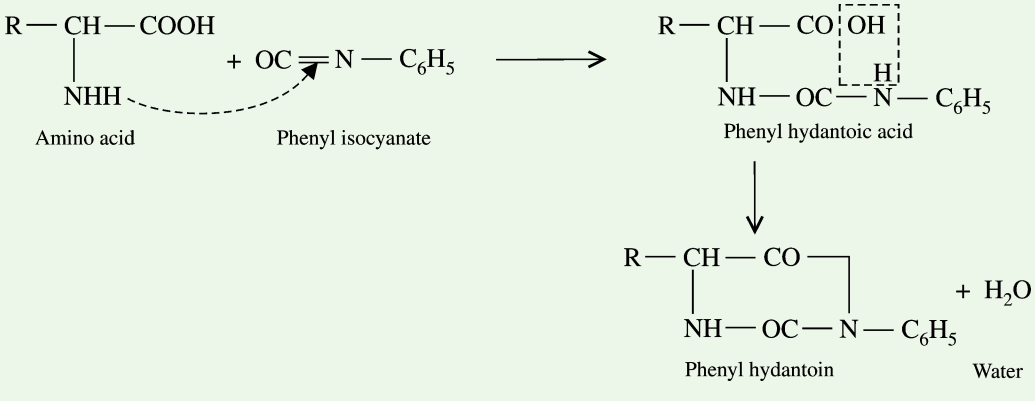




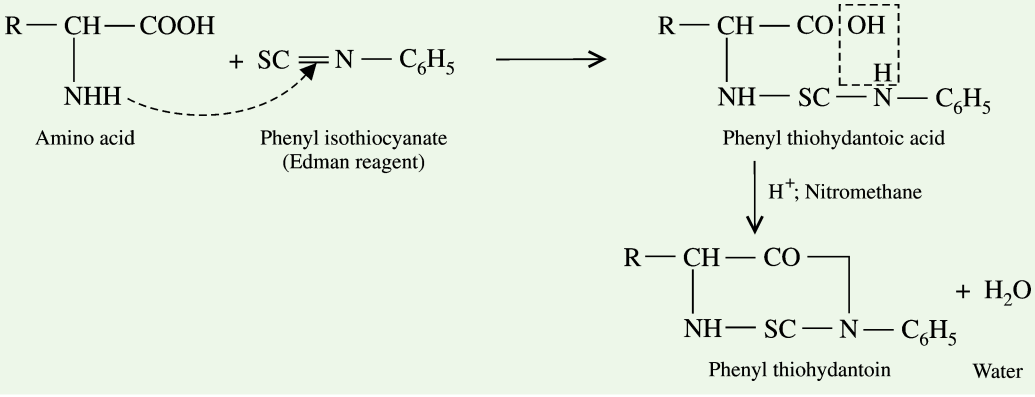
This reaction has been used by Van Slyke as a basis for quantitative estimation of α-amino acids. Here the CO_2 produced may be measured manometrically. It is more specific than the 'nitrous acid' method. This reaction is extremely sensitive and gives reliable results with small amounts of material.

Amines, other than α-amino acids, also react with ninhydrin forming a blue complex but without evolving CO_2. The evolution of CO_2 is, thus, indicative of the presence of an α-amino acid. Proline and hydroxyproline, however, produce yellow complexes rather than blue with ninhydrin.

2. Reaction with phenyl isocyanate. With phenyl isocyanate, *hydantoic acid* is formed which in turn can be converted to *hydantoin*.

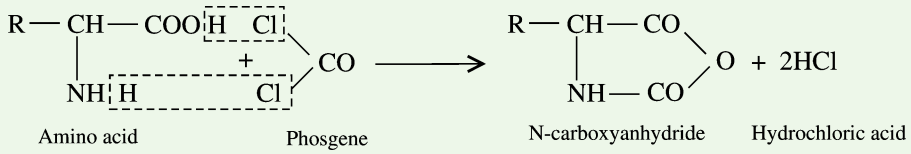


3. Reaction with phenyl isothiocyanate or Edman reagent. Phenyl isothiocyanate also reacts similarly with amino acids to produce *thiohydantoic acid*. On treatment with acids in nonhydroxylic solvents, the latter cyclize to *thiohydantoin*.



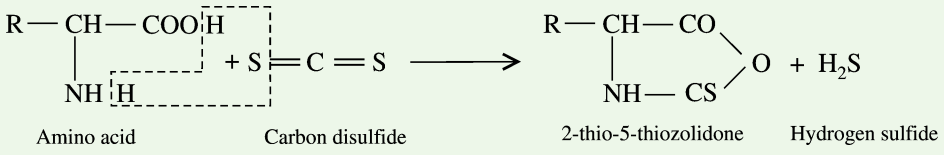
This reaction has proved useful in the studies of protein structure.

4. Reaction with phosgene. With phosgene, *N-carboxyanhydride is formed.*



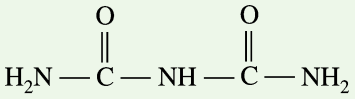
Phosgene (synonyms: carbonyl chloride, carbon oxychloride, carbonic dichloride), an acidic chloride, is a colourless gas and has a musty odour, resembling fresh mown hay or green corn. It liquefies at 8°C. Since it is heavier (3.43 times) than air, it was used extensively in gas warfare in World War I, and caused 80% of the deaths by gas in that conflict. Luckily, gas warfare was sparingly used in World War II, thus reducing the number of casualties. Phosgene does not occur in nature. It was first synthesized by Sir Humphrey Davy in 1812 by passing CO and chlorine through charcoal; *it is deadlier than both CO and* Cl_2 as it kills rapidly in as low a concentration as 550 ppm. Phosgene was also implicated in the Bhopal tragedy that occurred in India in December, 1984. However, phosgene is also an important industrial chemical being used in the synthesis or manufacture of isocyanates, polyurethane, polycarbonate resins, aniline dyes, pharmaceuticals, plastics and insecticides.

5. Reaction with carbon disulfide. With carbon disulfide, *2-thio-5-thiozolidone* is produced.



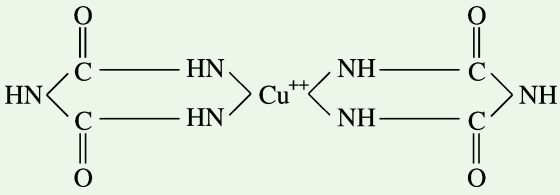
E. REACTIONS INVOLVING R GROUP OR SIDE CHAIN

1. Biuret test. Compounds containing peptide bonds produce a characteristic purple colour when treated with an alkaline 0.2% copper sulfate solution (or biuret reagent). This reaction is termed as 'biuret reaction' since it is also given by the substance biuret.

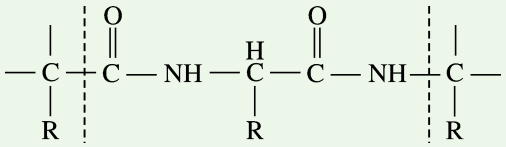


Biuret

The colour deepens as the number of peptide bonds is increased and the proteins produce a deep blue-violet colour due to the probable formation of a coordination complex whose structure is given below:



The test is, in fact, given by biuret as well as any similar structure having 2 amide or peptide bonds linked directly or through an intermediate carbon atom. The required unit is shown below between the two broken lines:



All proteins except dipeptides, therefore, respond to this reaction. This reaction is widely used both as a qualitative test for the detection of proteins and as a quantitative measure of protein concentration.

2. Xanthoproteic test. Yellow colour develops on boiling proteins with conc. HNO_3 due to the presence of benzene ring. This reaction is due to the nitration of the phenyl rings (of *tyrosine, tryptophan* and *phenylalanine*) to yield yellow substitution products, which turn orange upon addition of alkali.

$$\text{Protein} + HNO_3 \xrightarrow{\text{Xanthoproteic Test}} \underset{\text{(= Picric acid)}}{\text{Trinitrophenol}} + NO\uparrow$$

3. Millon's test. Red colour develops when proteins are heated with $Hg.NO_3$ in HNO_2. The reaction is specific for *tyrosine* and takes place between mercuric and mercurous nitrates and tyrosine residues of the protein. *Tryptophan* also responds to this reaction.

4. Hopkins–Cole test or Glyoxylic acid test. Violet ring develops on addition of conc. H_2SO_4 (36 N) at the junction of protein and glyoxylic acid solutions. The test is specific for *tryptophan*.

5. Folin's test. Blue colour develops with phosphomolybdotungstic acid in alkaline solution due to the presence of phenol group. The test is specific for *tyrosine*.

6. Sakaguchi test. Red colour develops with a-naphthol and sodium hypochlorite. The test is applied for the detection of *arginine*.

7. Pauly test. Red colour develops with diazotized sulfanilic acid in alkaline solution. The reaction is specific for *tyrosine* and *histidine*.

8. Ehrlich test. With *p*-dimethylaminobenzaldehyde in 12 N HCl, *tryptophan* develops a blue colour.

F. REACTIONS INVOLVING SH GROUP

1. Nitroprusside test. Red colour develops with sodium nitroprusside in dilute $NH_4.OH$. The test is specific for *cysteine*.

2. Sullivan test. *Cysteine* develops red colour in the presence of sodium 1, 2-naphthoquinone-4-sulfonate and sodium hydrosulfite.

The various colour reactions for different amino acids are summarized in Table 11–2.

Table 11–2. Colour reactions for specific amino acids

S.N.	*Test*	*Reagent*	*Colour*	*Amino acid(s)*
1.	Biuret test	Alkaline $Cu.SO_4$ soln.	Violet	Peptide bonds
2.	Xanthoproteic test	Conc. HNO_3	Yellow	Tyrosine Tryptophan Phenylalanine
3.	Millon's test	$Hg.NO_3$ in HNO_2; heat	Red	Tyrosine Tryptophan

4.	Hopkins–Cole test	Glyoxylic acid + conc. H_2SO_4	Violet	Tryptophan
5.	Folin's test	Alkaline phosphomolybdotungstic acid	Blue	Tyrosine
6.	Sakaguchi test	α-naphthol + sodium hypochlorite	Red	Arginine
7.	Pauly test	Diazotized sulfanilic acid in alkaline soln.	Red	Tyrosine Histidine
8.	Ehrlich test	*p*-dimethylaminobenzaldehyde in 12 N HCl	Blue	Tryptophan
9.	Nitroprusside test	Sodium nitroprusside + dil. NH_4OH	Red	Cysteine
10.	Sulivan test	Sodium 1,2-naphthoquinone-4-sulfonate + sodium hydrosulfite	Red	Cysteine

REFERENCES

1. **Agard DA:** To fold or not to fold. *Science*. **260:** *1903-1904, 1993.*
2. **Anfinsen CB (editor) :** Aspects of Protein Biosynthesis. A*cademic Press, Inc., New York. 1970.*
3. **Anfinsen CB:** Principles that govern the folding of polypeptide chains. *Science.* **181:** *223-230, 1973.*
4. **Annual Review :** Advances in Protein Chemistry. *Academic Pres, Inc., New York. 1944-current.*
5. **Barrett GC (editor) :** Chemistry and Biochemistry of the Amino Acids. *Chapman and Hall, New York. 1985.*
6. **Bell JE, Bell ET:** Proteins and Enzymes. *Prentice-Hall Inc., New Jersey. 1988.*
7. **Blake CCF et al:** Structure of the Hen Egg-white Lysozyme. A Three Dimensional Fourier Synthesis at 2 Å Resolution. *Nature* **206** : *760, 1965.*
8. **Bosch L :** Mechanism of Protein Synthesis and its Regulation. *Elsevier Publishing Co., New York. 1972.*
9. **Boulter D :** Protein synthesis in plants. *Ann. Rev. Plant Physiol.* **21** *: 1, 1970.*
10. **Branden C, Tooze J:** Introduction to Protein Structure *Garland, New York. 1991.*
11. **Bunn HF, Forget BG:** Hemoglobin: Molecular, Genetic and Clinical Aspects. *W.B. Saunders, Philadelphia, Pa. 1986.*
12. **Burke DC:** The status of interferon. *Sci. Amer.* **236** *(April) : 42, 1977.*
13. **Cantor CR, Schimmel PR :** Biophysical Chemistry. Pt 1. The Conformation of Biological Molecules. *Freeman, San Francisco. 1980.*
14. **Clark BFC, Marcker KA :** How Proteins Start. *Sci. Amer.,* **218 (1) :** *36-42, 1968.*
15. **Creighton TE:** Disulfide bonds and protein stability. *Bioessays*. **8 :** *57-63, 1988.*
16. **Creighton TE:** Proteins: Structure and Molecular Properties . *2nd ed., W.H. Freeman, New York. 1993.*
17. **Croft LR:** Handbook of Protein Sequences. *2nd ed., John Wiley, New York. 1980.*
18. **Dayhoff MO (editor) :** Atlas of Protein Structure and Sequence. *Vol. 5. Supple. 1Ä3. National Biomedical Research Foundation, Washington D.C. 1972-79.*

19. **Dickerson RE, Geis I:** Hemoglobin : Structure, Function, Evolution, and Pathology. *Benjamin/Cummings, Menlo Park, California. 1982.*
20. **Dickerson RE, Geis I:** Proteins : Structure, Function, and Evolution. *2nd ed., Benjamin/ Cummings, California. 1983.*
21. **Doolittle RF :** Proteins. *Scientific American.* **253 (4) :** *88-99, 1985.*
22. **Fermi G, Perutz MF:** Atlas of Molecular Structures in Biology. Vol. 2 Hemoglobin and Myoglobin. *Oxford Univ. Press, New York. 1981.*
23. **Fox JL, Deyl Z, Blazej A (editors) :** Protein Structure and Evolution. *Marcel Dekker, New York. 1976.*
24. **Fraser RDB :** Keratins. *Sci. Amer.* **221(2):** *86-96, 1969.*
25. **Goldberg ME :** Protein folding and assembly. *Trends in Biochemical Sciences.* **10 :** *388, 1985.*
26. **Goldenberg D:** Genetic studies of protein stability and mechanisms of folding. *Ann. Rev. Biophys. Chem., 17: 481, 1988.*
27. **Greenstein JP, Winitz M:** Chemistry of the Amino Acids. *Vol. 3. Wiley. 1961.*
28. **Gross J:** Collagen. *Scientific American* **204 (5) :** *120, 1961.*
29. **Haschemeyer RH, Haschemeyer AEV :** Proteins: A Guide to Study by Physical and Chemical Methods. *John Wiley and Sons Inc., New York. 1973.*
30. **Haurowitz F:** The Chemistry and Function of Proteins. *2nd ed., Academic Press, Inc., New York. 1963.*
31. **Hughes RC:** Glycoproteins. *Chapman and Hall, London. 1983.*
32. **Kendrew JC:** The Three-Dimensional Structure of a Protein Molecule. *Scientific American* **205 (6).** *96-110, 1961.*
33. **Kendrew JC:** Myoglobin and the structure of Proteins. *Science.* **139 :** *1259, 1963.*
34. **Kilmartin JV:** The Bohr effect of human hemoglobin. *Trends in Biochemical Sciences. 2: 247, 1977.*
35. **Koshland DE Jr:** Protein Shape and Biological Control. *Sci. Amer.* **229 :** *52-64, 1973.*
36. **Kyte J:** Structure in Protein Chemistry. *Garland. 1994.*
37. **Linderström-Lang K:** Proteins and Enzymes. *Lane Lectures No. 6, p.93. Univ., Press, Stanford, California. 1952.*
38. **Meister A:** Biochemistry of the Amino Acids. *2nd ed., Vols. 1 and 2. Academic Press, Inc., New York. 1965.*
39. **Monod J, Wyman J, Changeaux J:** On the nature of allosteric transitions: A plausible model. *J. Mol. Biol.* **12 :** *88–118, 1965.*
40. **Neurath H, Hill RL (editors):** The Proteins. *3rd ed., Academic Press, Inc., New York. 1975–1977.*
41. **Pauling LC, Corey RB, Hayward R:** The structure of protein molecules. *Scientific American.* **191(1) :** *51, 1954.*
42. **Pauling LC, Itano HA, Singer SJ, Wells IC:** Sickle cell anemia: A molecular disease. *Science.* **110:** *543-548, 1949.*
43. **Perutz MF:** Proteins and Nucleic Acids: Structure and Function. *Elsevier Publishing Co., New York. 1962.*
44. **Perutz MF:** The hemoglobin molecule. *Scientific American.* **211(5) :** *64, 1964.*
45. **Perutz MF:** Hemoglobin Structure and Respiratory Transport. *Sci. Amer.* **239(6) :** *92-125, 1978.*

46. **Perutz MF:** Regulation of oxygen affinity of hemoglobin: Influence of the globin on the heme iron *Ann. Rev. Biochem.* **48:** *327, 1979.*

47. **Perutz MF:** Molecular anatomy, physiology and pathology of hemoglobin. In Stamatayonnopoulos C (editor) : Molecular Basis of Blood Diseases. Saunders. 1987.

48. Perutz MF: Mechanisms of cooperativity and allosteric regulation in proteins. *Quart. Rev. Biophys*. **22:** *139-237, 1989.*

49. **Perutz MF, Fermi G, Luisi B, Shaanan B, Liddington RC:** Stereochemistry of cooperative mechanisms in hemoglobin. *Acc. Chem. Res.* **20:** *309 – 321, 1987.*

50. **Ramachandran GN (editor):** Aspects of Protein Structure. *Academic Press, Inc., New York. 1963.*

51. **Richards FM:** The protein of folding problem. *Sci. Amer.* **264 (1) :** *54–63, 1991.*

52. **Richardson JS:** The Anatomy and Taxonomy of Protein Structure. *Adv. Protein Chem.* **34:** *168, 1981.*

53. **Robson B:** Protein folding. Trends in Biochemical *Sciences*. **1 :** *49, 1976.*

54. **Rossman MG, Argos P:** Protein Folding. *Ann. Rev. Biochem* **50 :** *497, 1981.*

55. **Sanger F:** Sequences, sequences and sequences. *Ann. Rev. Biochem.* **57 :** *1–28, 1988.*

56. **Scheraga HA:** Protein Structure. *Academic Press, Inc., New York. 1963.*

57. **Schroder E, Lubke K:** The Peptides. *Vols.1 and 2. Academic Press, Inc., New York. 1965-66.*

58. **Schrosder WA :** The Primary Structure of Proteins. *Harper and Row, New York. 1968.*

59. **Schultz GE, Schirmer RH :** Principles of Protein Structure. *Springer-Verlag, New York. 1990.*

60. **Scott JE:** Molecules for strength and shape. Trends Biochem. *Sci.* **12 :** *318–321, 1987.*

61. **Srinivasan PR, Fruton JS, Edsall JT (editors):** The origins of modern biochemistry: A retrospective on proteins. *Ann. N.Y. Acad. Sci. 325, 1979.*

62. **Stein WH, Moore S:** The Chemical Structure of Proteins. *Scientific American.* **204(2) :** *81, 1961.*

63. **Tschesche H (editor):** Modern Methods in Protein Chemistry. *de Gruyter, New York. 1983.*

64. **Wilson AC:** The molecular basis of evolution. *Sci. Amer.* **253 :** *164-173, 1985.*

65. **Wyman J, Gill SJ:** Binding and Linkage. Functional Chemistry of Biological Molecules. *University Books. 1990.*

66. **Zuckerlandl E:** The evolution of hemoglobin. *Sci. Amer*. **212(5) :** *110, 1965.*

PROBLEMS

1. After proper purification, the Edman reaction was used to sequence a dodecapeptide. The following data were obtained. The C-terminal amino acid is isoleucine; N-terminal amino acid is methionine; peptide fragments are Ala-Ala-Ile, Leu-Arg-Lys-Lys-Glu-Lys-Glu-Ala, Met-Gly-Leu, and Met-Phe-Pro-Met. What is the sequence of this peptide ?
2. Why is pepsin useful as a digestive aid in precooked foods ?