CHEMISTRY OF NATURAL PRODUCTS

Amino Acids and Proteins

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Keywords
Proteins, polypeptides, amino acids,, zwitter ions, isoelectric point, amphoteric

Introduction

There are mainly three groups of biological polymers:

1) Polysaccharides: Functions primarily as energy reserves and in plants as structural materials.

2) Nucleic acids: Serve two major purposes; storage and transmission of information.

3) Proteins: They are substances of life.

Of all chemical compounds, proteins must be ranked first. Proteins make up a large part of animal body they hold it together and they run it. Only nucleic acids which control heredity can challenge the position of protein. And nucleic acids are important because they direct synthesis of proteins.

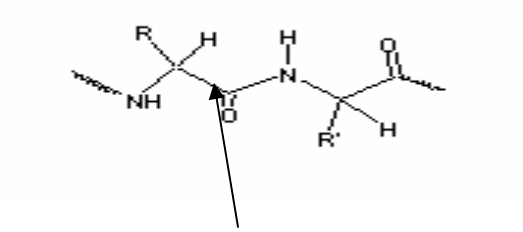
Proteins can be broken down by various chemical and enzymatic methods into smaller and smaller fragments until the final products are the amino acids.

Protein ⟶ Polypeptides ⟶ Peptides ⟶ Amino acids

There is no sharp dividing link between peptides and proteins.

Mol.Wt > 10,000 – proteins
Mol.wt <10,000 – poly peptides

In proteins, amino acids are joined in a linear fashion by peptide linkage. Carboxyl groups of one amino acid forms an amide by combination with the amino group of the next amino acid. Thus protein molecule may be represented as a linear polymer of amino acid molecule.



Peptide Linkage

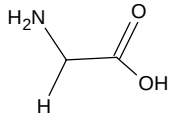
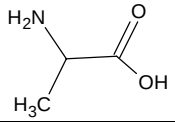
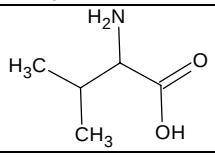
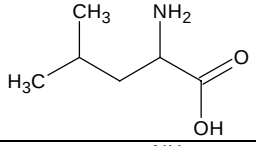
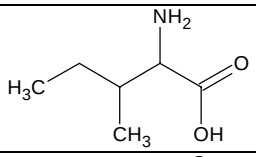
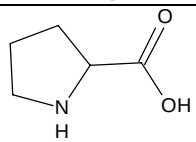
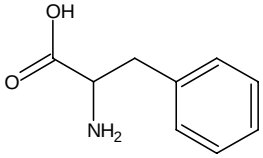
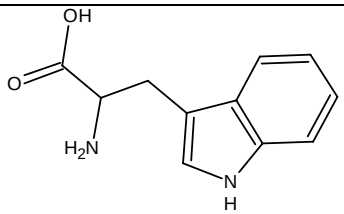
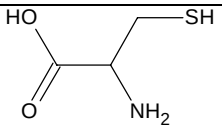
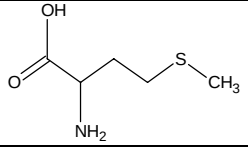
Amino Acids

Amino acids are building blocks of proteins. More than100 amino acids have been isolated and identified but only 25 are obtained upon hydrolysis of typical proteins. All 25 except 2 are α-amino acids; the two exceptions are proline and hydroxy proline, which are imino acids. Only 20 amino acids are of general occurrence because they are usually found in all proteins. Ten of the amino acids are essential amino acids i.e. a deficiency in any one prevent growth in young animals, and may even cause death. They must be furnished in the diet as they are not synthesized in the body.

Classification of Amino Acids

i) **Neutral A.A**: They contains one $–NH_2$ and one –COOH group
ii) **Acidic A.A**: They contains one $–NH_2$ and two –COOH group
iii) **Basic A.A**: They contains two $–NH_2$ and one –COOH group

Table 1: Neutral Amino Acids

Amino acid (ab)	Systematic name	Structure
Glycine[gly (G)]	Aminoacetic acid	
Alanine [Ala(A)]	α –amino propionic acid	
Valine [Val(V)]	α –aminovaleric acid.	
Leucine[Leu (L)]	α –aminoisocaproic acid	
Iso leucine[ILe(I)]	α -amino-β-methyl (-n-Valeric acid.	
Proline[Pro (P)]	Pyrrolidine – α-carboxylic acid	
Phyenylalanine[Phe(F)]	α -Amino- β-phenyl propionic acid	
Tryptophan[Trp(W)]	α -Amino- β-indolepropionic acid	
Cysteine[Cys(C)]	α –Amino- β-mercapto propionic acid	
Methionine[Met(M)]	α-Amino-γ-mehylthio-n-butyric acid	

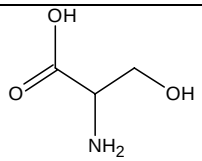
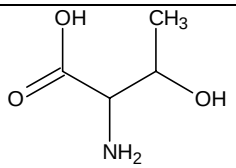
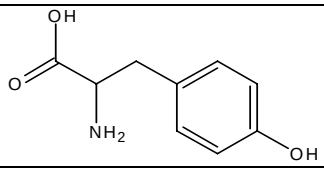
Serine[Ser(S)]	α –Amino- β-hydroxypropionic acid	
Threonine[Thr(T)]	α –Amino- β-hydroxy-n-butyric acid	
Tyrosine[Try(Y)]	α –Amino- β-[p-hydroxyphenyl]propionic acid	

Table 2: Acidic Amino Acids

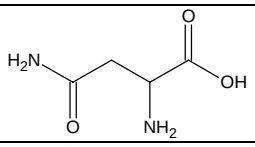
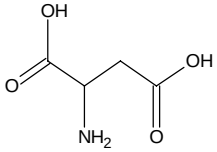
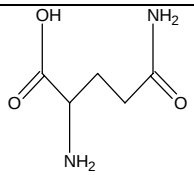
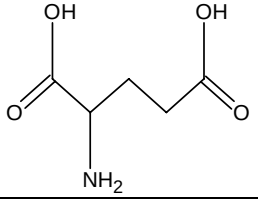
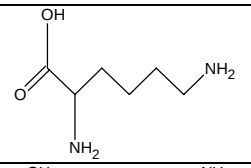
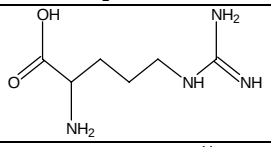
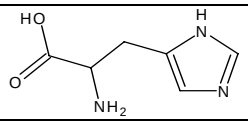
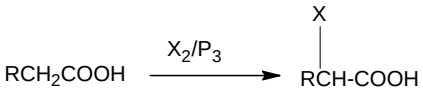
Asparagine [Asp(N)]	α –Aminosuccinamic acid	
Aspartic Acid[Asp(D)]	α –Aminosuccinic acid	
Glutamine[Glu(Q)]	α –Aminoglutaramic acid	
Glutamicacid[Gla(E)]	α –Aminoglutaric acid	

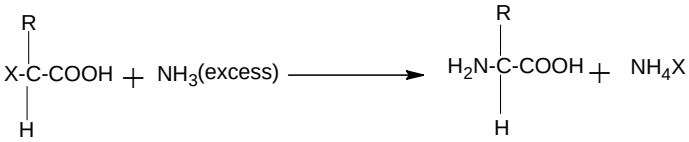
Table 3: Basic Amino Acids

Lysine[Lys(K)	α ,δ-Diamino-n-caproic acid	
Arginine[Arg(R)]	α –Amino- δ-guanidino-n-valeric acid	
Histidine[His(H)]	α –Amino- β-imidazole propionic acid	

Synthetic Methods of preparation of Amino Acids

1) Amination of α-halogen substituted Acids (Perkins et al, 1958): Amination of α –halo (chloro or bromo) acids, obtained by direct halogenation of carboxylic acids, with aqueous or liquid ammonia gives the respective amino acid.

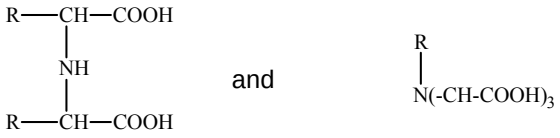




(±) α – Amino acid

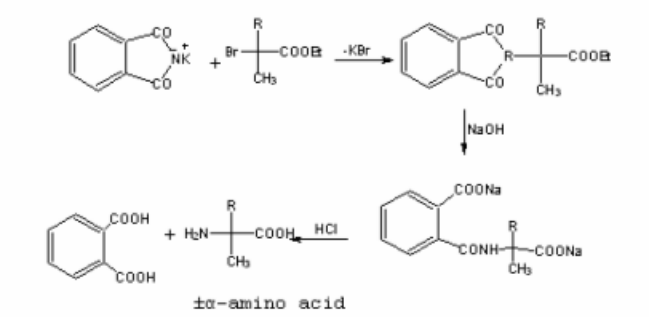
This amination may, however, be applied for the synthesis of alanine, glycine, serine, threonine, valine, leucine and norleucine. All amino acids obtained in this method, are in the form of (±) racemic mixture.

Since the method requires an excess of ammonia and also forms side products like



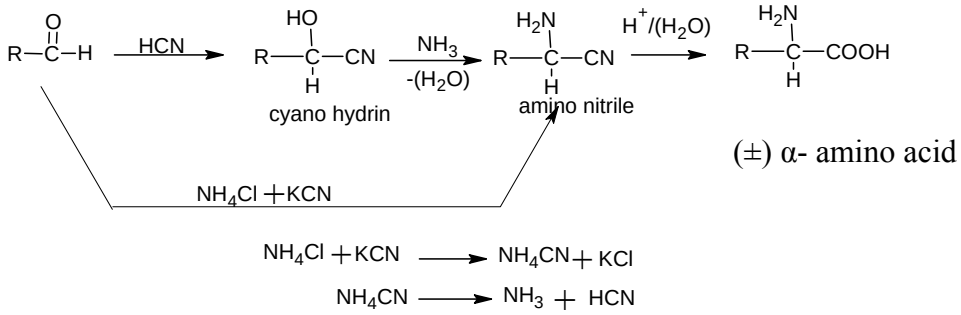
In order to prevent the formation of primary and secondary amines ammonia may be replaced by hexamethylene-tetramine.

2) Gabriel's Pthalimide Sybnthesis (1889): This synthesis involves the treatment of α–halogenated ester with potassium salt of phthalimide and subsequent hydrolysis of the product.



Better yield is obtained in this method than the above .It may also be used for acidic aminoacids e.g. synthesis of aspartic acid.

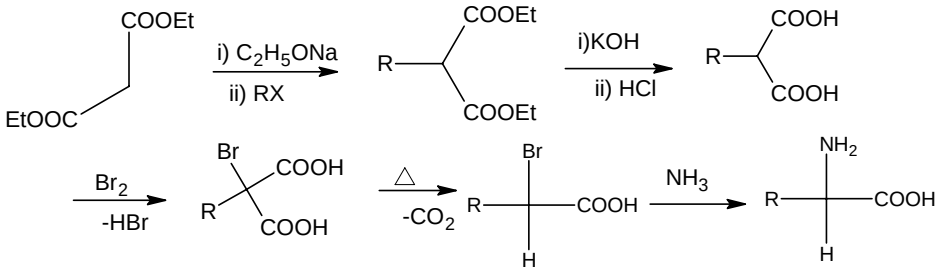
3)Strecker's synthesis(1850): In this synthesis , a cyanohydrin, obtained from aldehyde and hydrogen cyanide, is treated with concentrated ammonia and the resulting aminonitrile is then hydrolysed with an acid. Aminonitrile may also be prepared in one step by taking a mixture of ammonium chloride and potassium cyanide and treating it with oxo compound.



This method is useful for the preparation of glycine, alanine, serine, valine, methionine, leucine,isoleucine, norleucine, phenylalanine and glutamic acid.

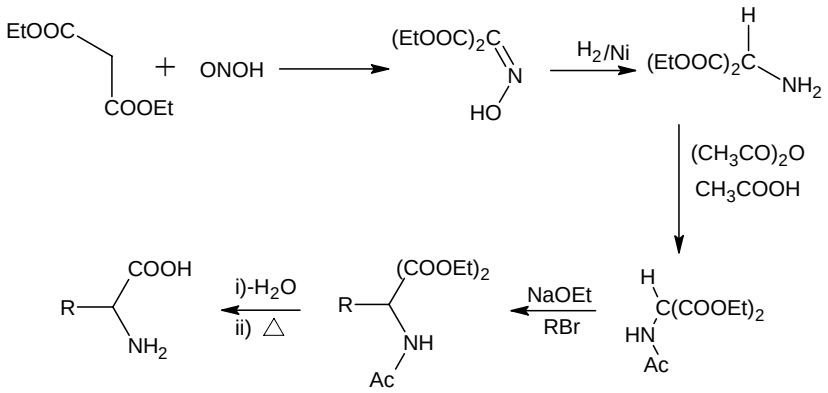
4) Malonic ester synthesis:

i) In this methode α –halogen substituted acid is first prepared from malonic ester and then amination of α –halogenated acid gives the respective amino acid.



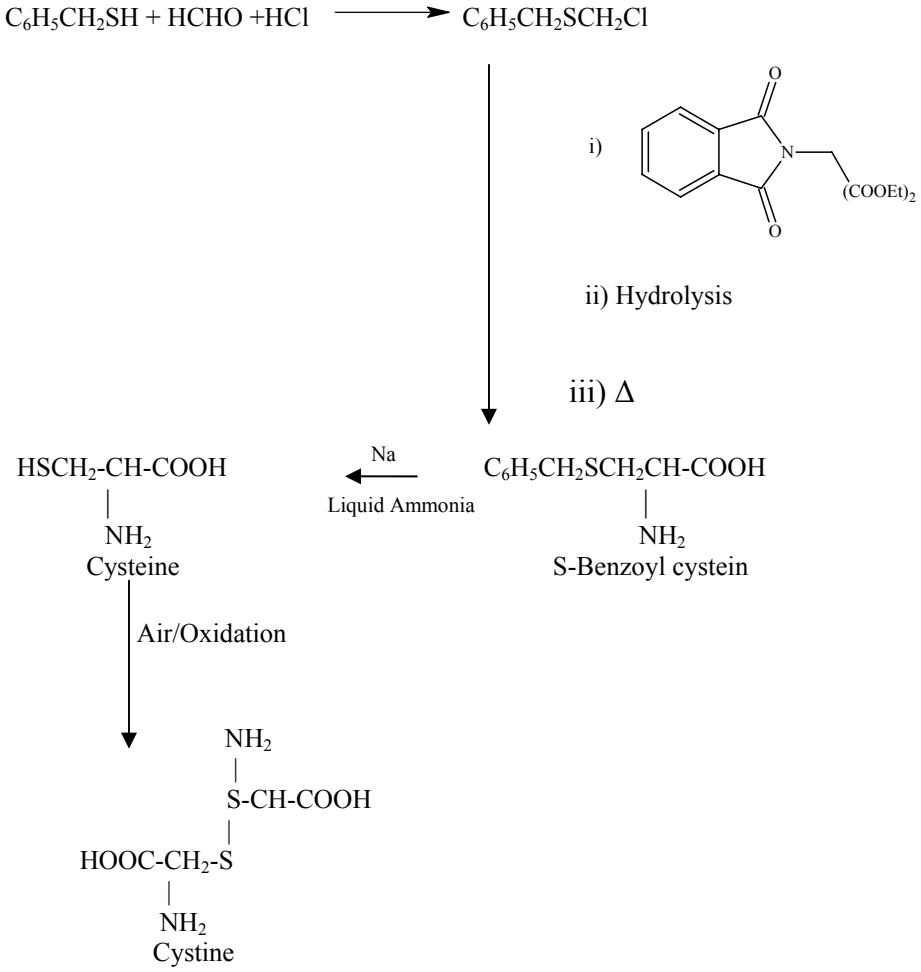
By this method phenylalanine, proline, leucine group and mehionine can be prepared.

ii) This is the slightly modified method of synthesis of amino acid from malonic ester.

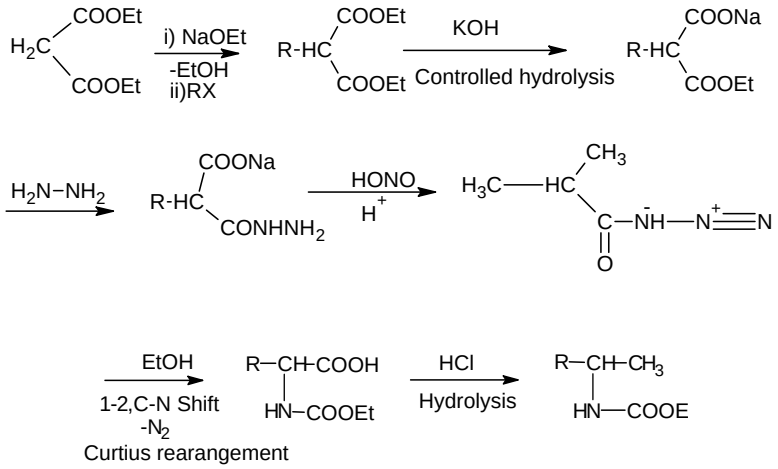


Serine, leucine, valine, methionine, lysine, glutamic acid and ornethine are prepared by this method.

iii) The malonic ester synthesis may also be combined with the Gabriel Phthalimide synthesis to prepare phenylalanine, tyrosine, proline, cystine, serine, methionine, lysine and aspartic acid. For example cystine is prepared from benzylthiol by combining these two methods.

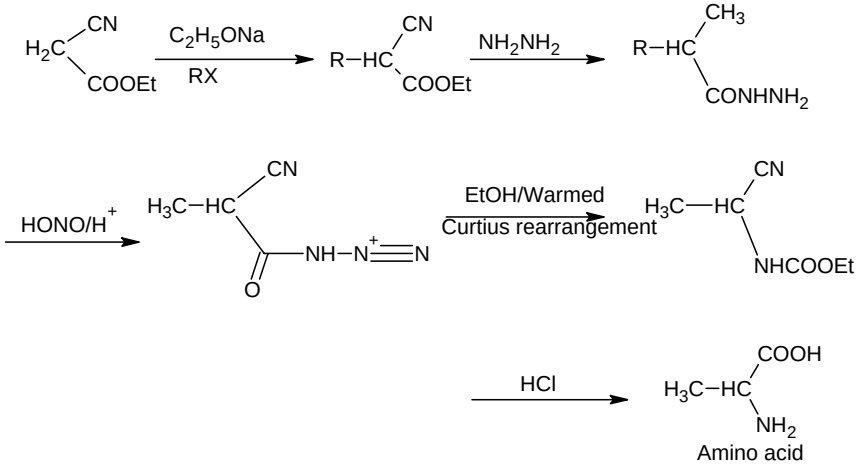


iv) **Curtius reaction:** In this reaction in addition to malonic ester, cyanoacetic ester and ester amides also are used for preparing amino acids.

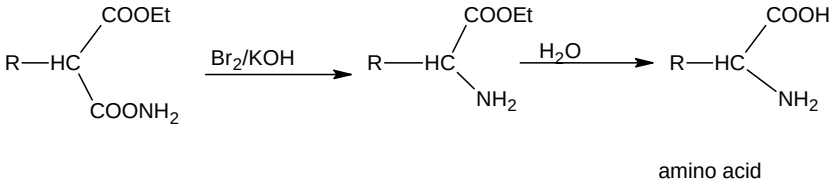


(±) α-amino acid.

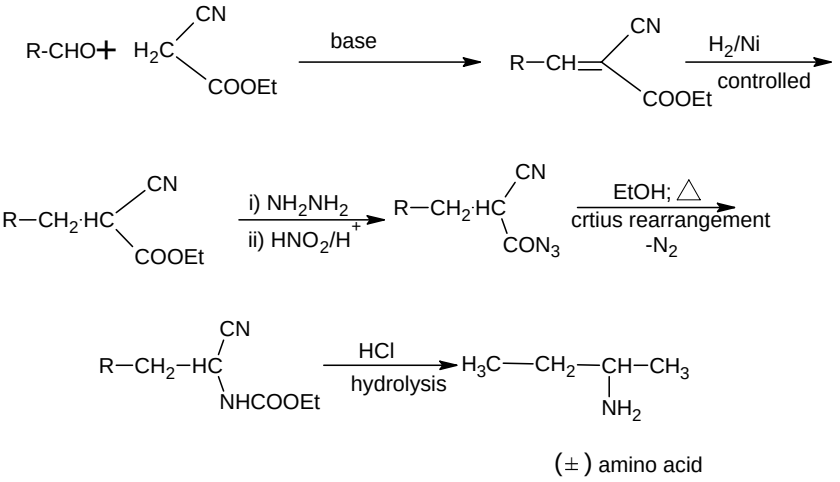
Glycine, alanine, phenylalanine and valine can be prepared by this method. By using cyanoacetic ester phenylalanine and tyrosine can be conveniently prepared.



v) Hofmann's degradation method: This is another variation of malonic ester synthesis and involves the degradation of ester amides.

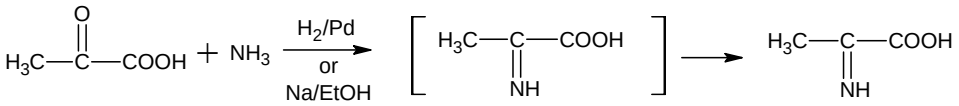


vi) Darapsky(1936): This method involves the condensation of cyanoacetic ester with an aldehyde.



(±) amino acid

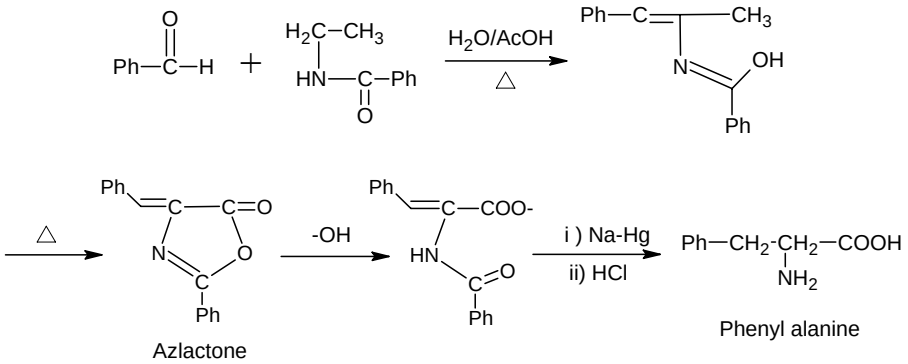
5. Reductive Ammonolysis of α –keto acids :[Koop synthesis]: The treatment of α –keto acids with ammonia forms an imine, which on catalytic reduction gives amino acid.



α - aminoacid

Alanine and glutamic acid are easily prepared by this method.

6. Erlenmeyer Azlactone synthesis: Azlactones are prepared by heating an aromatic aldehyde with benzoyl glycine (hippuric acid) in presence of acetic anhydride and sodium acetate.



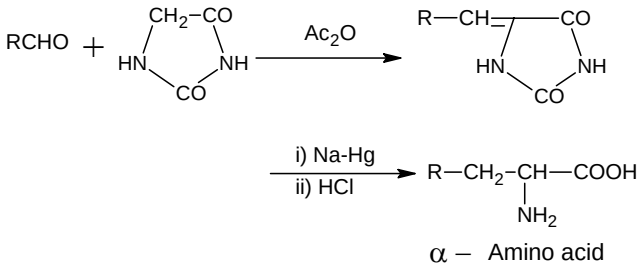
Azlactone

Phenyl alanine

Benzoyl glycine may be replaced by acetyl or any other N-acyl glycine. An aliphatic aldehyde also condenses with benzoyl glycine if lead acetate is used in place of sodium acetate.

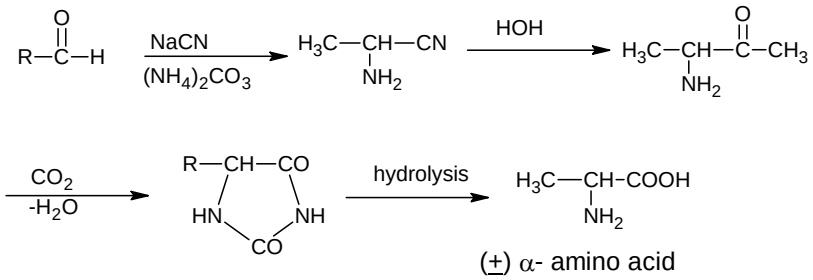
Azlactone synthesis offers a convenient means of preparing phenyl alanine, tyrosine group and tryptophan.

7) Hydantoin Synthesis: In this method aromatic aldehydes condense with hydantoin, and the product is then reduced and hydrated in the usual way to give α –amino acid.

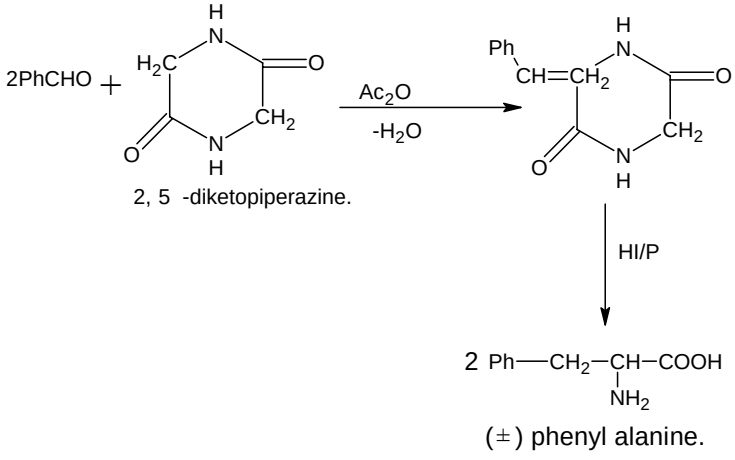


By this method we can prepare tryptophan, phenylalanine tyrosine and methionine.

8) Bucherer's Hydantoin synthesis: In this method oxo compound is first converted into 5-substituted hydantoin by means of ammonium carbonate and sodium cyanide in aqueous ethanol solution.



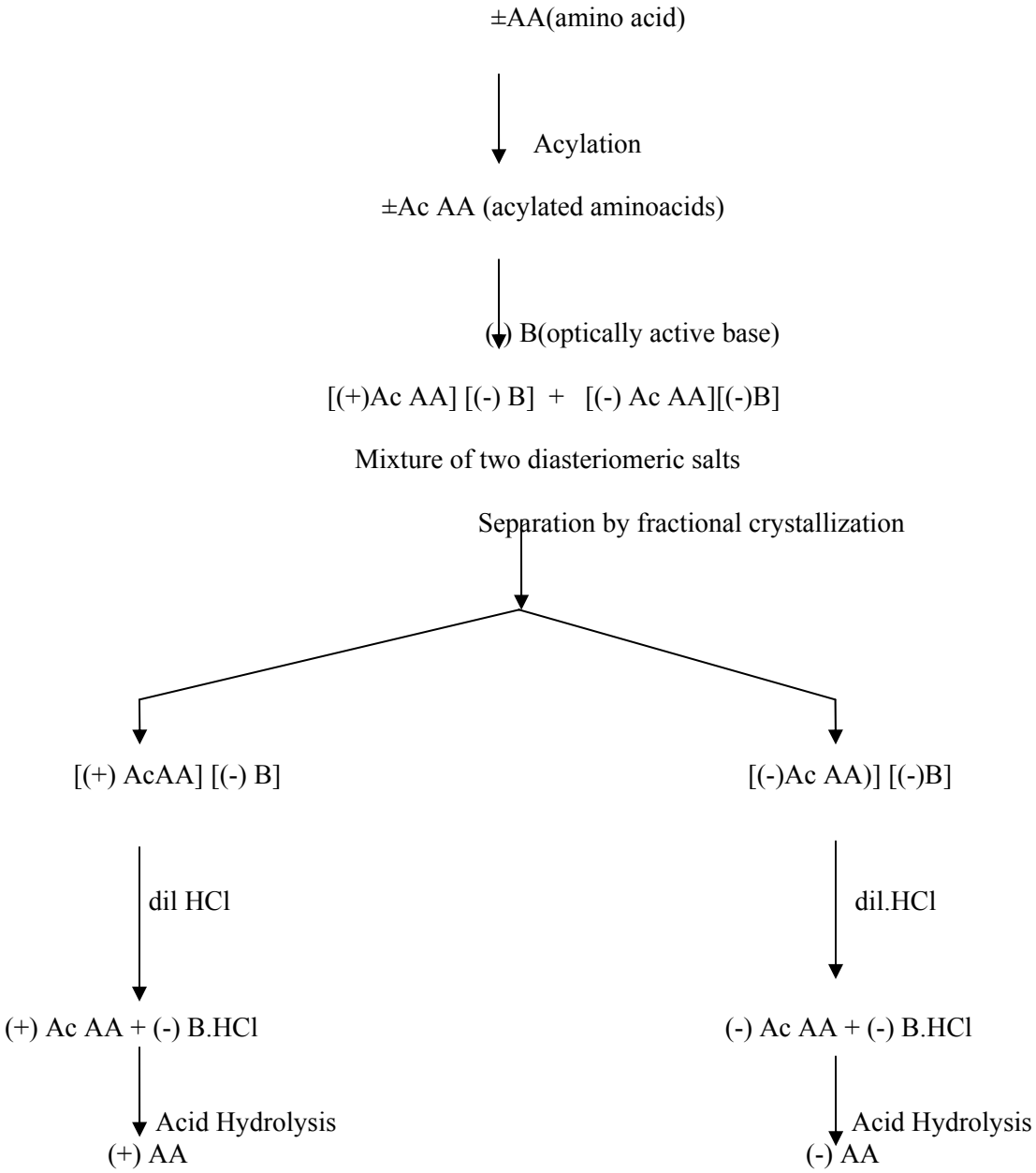
9) Synthesis via Diketo Piperazine: This involves the condensation of aromatic aldehyde with diketo piperazine and product formed is heated with red phosphorous and hydroiodic acid to get amino acid.



Phenhylalanine, tyrosine and methionine may be prepared by this method.

Resolution of synthetic Racemic mixture of Amino acids: Generally in all synthetic methods we get racemic mixture of α –aminocacid. This reaction mixture has to be resolved into enantiomers when required.

In resolution first acylation is carried out to block the amino group. After acylation they readily form salts with optically active bases and diasterioisomeric salts formed are of different solubilities hence they are separated by fractional crystallization. After that isolated salts are hydrolyzed and then acylated amino acids are deacylated.



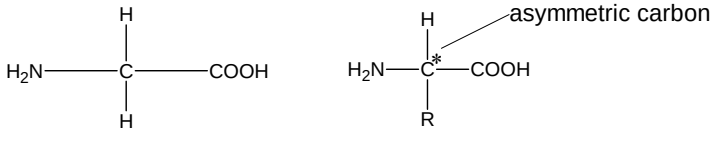
A more recent method is the elective destruction of one enentoiomer by a specific D-or L-Oxidase.

General properties of Amino acids

1) Physical properties:

i) Amino acids are colourless, crystalline, stable, high melting solids having sweet taste. They melt with decomposition at high temperature, but a few have tendency to sublime.

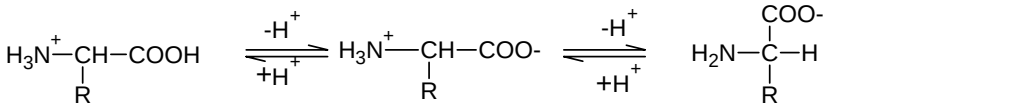
ii) They are generally soluble in water but sparingly soluble in organic solvents such as petroleum ether, ethanol and benzene.
iii) All α–amino acids contain at least one asymmetric carbon (except glycine) and are optically active.



optically inactive amino acid | optically active amino acid

iv) Zwitter ion: When the dipole moment of glycine is measured in aqueous solution, this value is found to be very large. To account for this large value it has been suggested that glycine consists, in solution, as an inner salt, as it has both basic –NH2 group and acidic –COOH group, it exists as double charged ion which is known as Zwitter ion or dipolar ion. Finally X-ray analysis has shown that all amino acids exist as dipolar ions.

In neutral solution an aminoacid will be present in the following species, which are in equilibrium

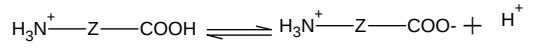


Cation form [In strong acidic at pH=0] | Zwitter ion form | Anionic form [In strong bascic condition (pH =14)]

v) Isoelectric point: The position of above equilibrium depends on the pH of the solution, in acid solutions the conjugated acid predominates and in alkaline solution the conjugate base predominates. For each amino acid there is a particular pH value at which the concentration of the dipolar ion is maximum since the net charge is zero, the dipolar ion is electrically neutral and consequently , in this condition the amino acid does not migrate when placed in an electric field. This pH at which migration does not occur is called the isoelectric point of that amino acid.

We can calculate the iso electric point of an amino acid as follows:

If we represent the isoelectric aminoacid as , $H_3N^+—Z—CO_2^-$,we have the following equilibrium.



C.A | D.I

D.I | C.B

$$K_1 = \frac{[D.I]\,[H^+]}{[CA]} \quad ; \quad K_2 = \frac{[C\,B]\,[H^+]}{[D.I]}$$

$$C\,A = \frac{[DI]\,[H^+]}{K_1} \quad ; \quad C\,B = \frac{K_2\ [D\,I]}{[H^+]}$$

At the isoelectric point (pHi), [D.I] is a maximum and since the net charge is zero.

$$[CA] = [CB]$$

$$\frac{[D.I]\,[Hi^+]}{K_1} = \frac{K_2\,[D.I]}{[Hi^+]}$$

$$[Hi+]^2 = K_1 K_2$$

$$2\,pHi = pK_1 + PK_2$$

$$pHi = \frac{(pK_1 + pK_2)}{2}$$

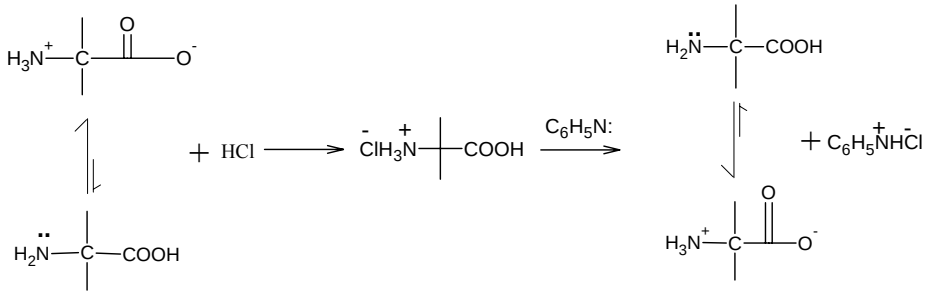
e.g. For glycine; pK_1=2.4,pK_2 = 9.6

pHi = 2.4=9.6/2 =6.0

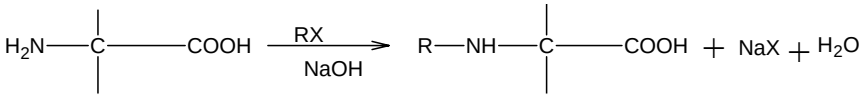
2) Chemical properties:

a) Reaction due to $-NH_2$ group:

i) Salt formation with strong acids: Amino acids form salt with strong mineral acids. These salts are usually less soluble in water. But solution becomes strongly acidic. Free amino aids may be liberated from these salts by means of strong bases like pyridine.

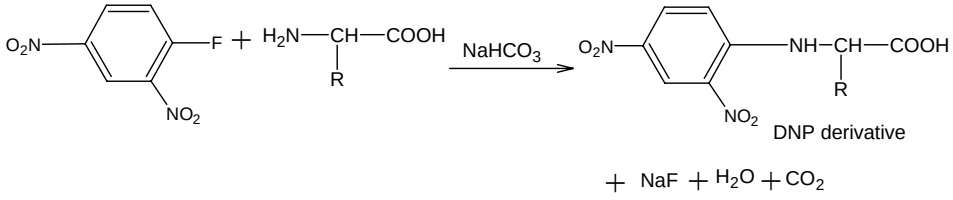


ii) Alkylation: In basic solution amino group can displace the halogen of alkyl halids.



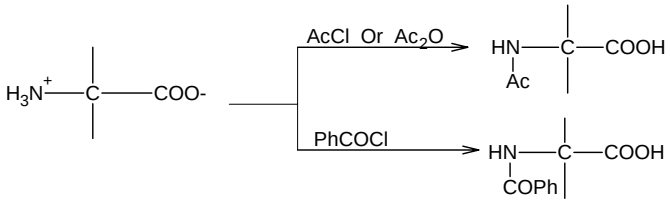
N-Alkyl amino acid

iii) Arylation : In solution the amino group can also displace halogen of acyl halide. For example amino acids form DNP derivative with 2,4- Dinintro fluorobenzene. This reaction is useful for the determination of N- terminal amino acid.

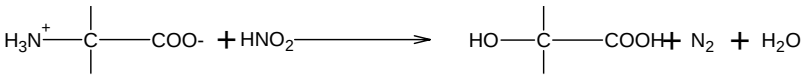


iv) Acylation and Benzoylation: Amino acids may be acylated with acid chloride or anhydride which blocks the amino group and acylated amino group behaves as typical organic acid.

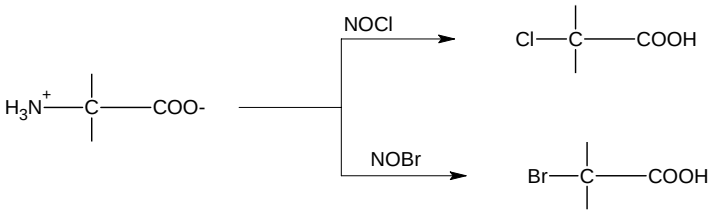
Similarly with benzoyl chloride amino acids yield benzoyl derivatives.



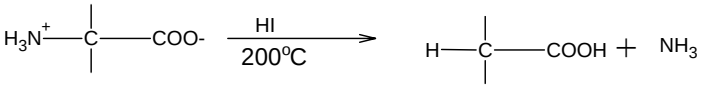
v) Reaction with Nitrous Acid: By the action of nitrous acid amino acid is converted to hydroxyl acid with the liberation of nitrogen. Measurement of the nitrogen evolved is the basis of Van slyke method of estimation of amino acids.



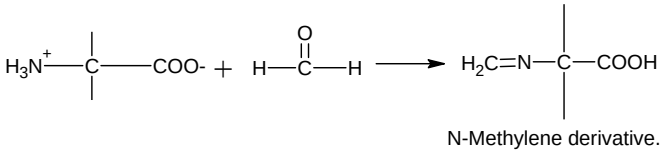
vi) Reaction with Nitrosyl Chloride or Bromide: Amino acids with this reagent give chloro or bromo acids.



viii) Reaction with hydroiodic acid: When heated with hydroiodic acid at about 200^{o}C the amino group is eliminated to produce the corresponding fatty acid.



viii) Condensation with Formaldehyde: They form N- methylene derivative with frormaldehyde.

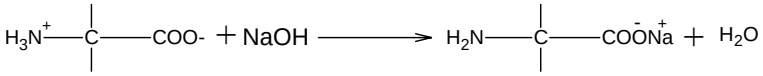


N-Methylene derivative.

Since N- methylene derivative thus formed containing a free COOH group can be titrated against standard alkali. This reaction is used for the estimation of amino acids and known as Sorenson formal titration method.

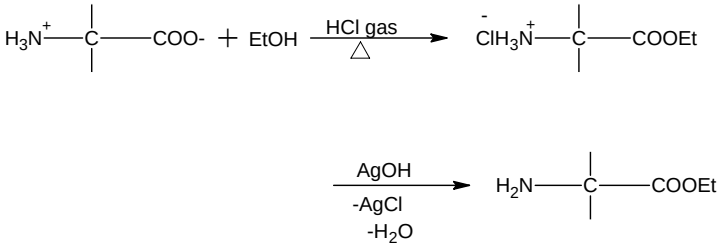
B) Reaction due to –COOH group:

i) Formation of salt with bases: Amino acids forms salts with strong bases.



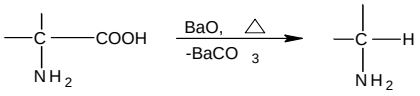
sod. salt of amino acid

ii) Ester formation: When heated with an alcohol in the presence of dry hydrogen chloride they form their ester hydrochloride. Free ester is obtained by the action of silver hydroxide or aqueous sodium carbonate solution on them.

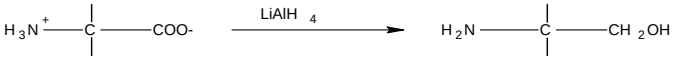


iii) Decarboxylation : Amino acids may be decraboxylated by dry distillation with acids, bases , barium oxide or specific enzymes to give corresponding amine.

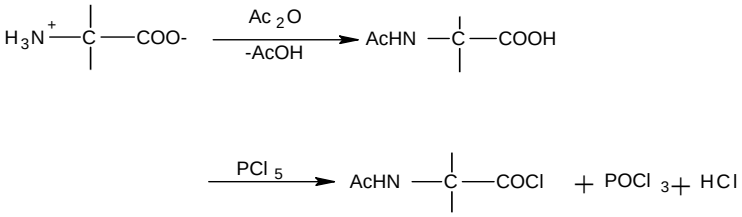
e.g:



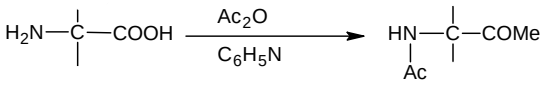
iv) Reduction: Amino acids on reduction with $LiAlH_4$ give corresponding amino alcohols.



v) Formation of Acid Chloride: In this reaction amino group of amino acid is treated with phosphorous penta chloride to give corresponding acid chloride.



vi) Dakin – West reaction: When acids are treated with acid anhydride in pyridine solution, they are converted to methyl α- acetamidoketone. The reaction is referred to as the Dakin-West reaction.

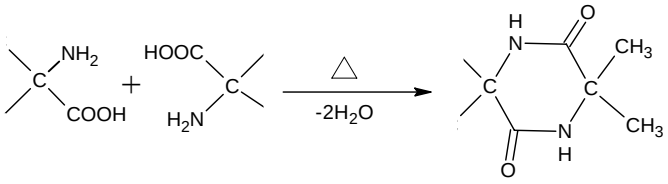


Methyl α -acetamidoketone

3) Reactions due to both Amino and Carboxyl groups:

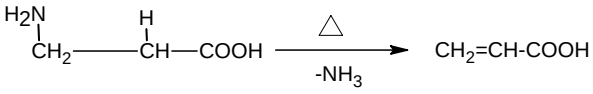
i) Action of heat: On heating amino acids behaves as hydroxyl acids..

a) α-Amino acids lose two molecules of water between two molecules of amino acids and give cyclic amides known as Diketo piperzine.

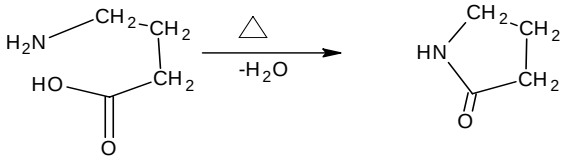


2, 5 -Diketopeiperazine

b) β- Amino acids eliminate a molecule of ammonia and yield α ,β-unsaturated acids.

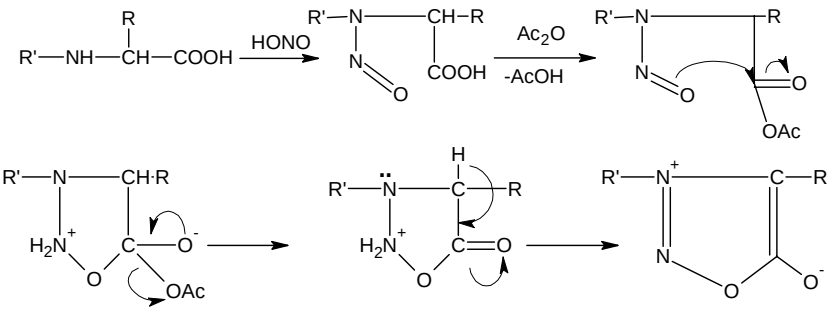


c) γ and δ- amino acids by losing one molecule of water within a molecule form cyclic amides called lactams.

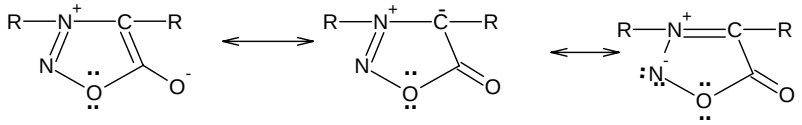


Lactams

ii) Action of Nitrous acid: N-alkyl or N-aryl amino acids form N-nitroso derivatives with nitrous acid and these derivatives dehydrate in presence of acetic anhydride to give 'sydnones"

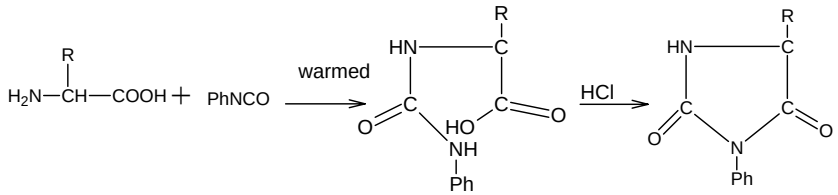


Although sydnones look like β- lactones, they are very stable because of having aromatic sextet. Sydnones are best represented as resonance hybrid of following three structures.

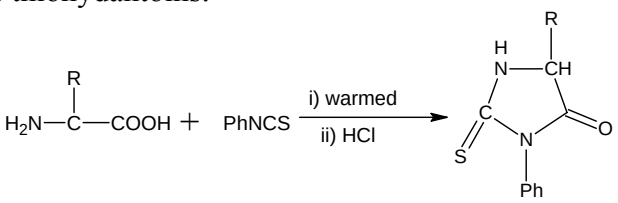


I II III

3) Reaction with phenyl isocyanate and phenyl thiocyanate: Amino acids with phenyl isocyanate form phenyl hydantoic acids which on treatment with hydrochloric acid easily form hydantoins.



Phenyl isocyanate yields thiohydantoins.



Protein

General Nature of Protein

The term protein was introduced by Mulder (1839). Derived from the greek word proteious meaning first. Proteins are nitrogeneous substances occuring in the protoplasm of all animal and plant cells. Their composition varies with the source; carbon, 45-46%;hydrogen, 6-9% ; oxygen, 12-30 %; nitrogen, 10-32%; sulphur, 0.2-0.3%. Other elements may also be present, e.g. Phosphorous (nucleoproteins), Iron (heamoglobin).

There is no sharp dividing line between peptide, poly peptides and proteins. In general they differ in physical and chemical properties which can be correlated with the difference in molecular size. Both groups often exhibit physiological activity, behaving as e.g., enzymes, hormones growth factor etc.

Proteins are amphoteric, they behave as an anion or a cation depending on the pH of the solution. If some definite pH, characteristic for each protein, the positive and negative charge is exactly balanced, i.e no net change on the protein molecule, and the molecules will not migrate in an electric field. In this condition the protein is said to be at its iso electric point and at this pH the protein has its least solubility i.e it is most readily precipitated. The amphoteric nature of the proteins is due to the presence of a large number of free acidic and basic groups arising from the

amino acid units in the molecule. The osmotic pressure and viscosity of the protein solution are also a minimum at the isoelectric point.

All proteins are optically active, may be coagulated and precipitated from aqueous solution by heat, addition of acids, alkali salts, organic solvents etc. Protein in this precipitated state are said to be denatured, and the process of reaching this state is known as Denaturation. Denaturation occurs most readily near the isoelectric point. Denaturation is the result of changes in conformation or unfolding of the protein molecule. After denaturation loss of optical rotation and biological activity occurs e.g enzymes becomes inactive when denatured.

Denaturation is generally irreversible, but in many cases the process has been reversed. This reversal of denaturation has been called renaturation or refolding. When denaturation is effected by heat, renaturation does not usually result on rapid cooling. If, however, cooling is carried out very slowly, renaturation often occurs. In these circumstances the process of renaturation has been refereed to as annealing.

Colour reaction

Proteins exhibit a variety of colour reactions:

i) Biuret reaction: Alkaline solution of protein and dilute copper sulphate solution gives red or violet colour. (Due to coordination of Cu^{2+} with –CONH- group at least two peptide linkages must be present (polypeptides do not give the test)

ii) Xanthoproteic reaction: Protein solution and concentrate nitric acid on warming gives a yellow precipitate which changes colour to orange at alkaline conditions due to nitration of aromatic groups present.

iii) Ninhydrin test: Protein on boiling with dilute ninhydrin solution gives violet colouration.

iv) Millon's test: Millon's reagent [mercuric nitrate in concentrated nitric acid having traces of nitrous acid] on adding to protein solution gives a white precipitate which on further heating produces a red precipitate due to the presence of phenolic group.

Classification of Proteins

Several arbitrary classifications of the proteins are in use.

I: According to solubility:

a) Fibrous proteins: These are insoluble in common solvents but are soluble in concentrated acids and alkalies. These are highly resistant to digestion by proteolytic enzymes. These are proteins appearing as fibers made of linear molecules that are arranged roughly parallel to the fiber axis. The long linear molecules of proteins are held together by inter molecular hydrogen bonds. Examples: silk, wool, skin, hair, horn, nails, quills, connective tissue and bone.

b) Globular proteins: These are soluble in water and in dilute acids, alkali and salts. These proteins are more highly branched and cross linked condensation products of basic or acidic amino acids. The polypeptide chains in this type of proteins are held together by cross linked groups. Example: enzymes, oxygen carrying proteins, protein hormones etc.

II: On the basis of increasing complexity into their structures: This is a more common method of classification according to which proteins may be divided into three main groups.

1) Simple proteins: These give amino acids or their derivatives on hydrolysis. These are including the following groups:

a) Albumins: Soluble in water, acids and alkalies, coagulated by heat and precipitated by saturated salt solution like ammonium sulphate and low in glycine. Some albumins are serum albumin, egg albumin and lactalbumin.

b) Globulins: These are insoluble in water, but are soluble in dilute salt solution and in dilute solutions of strong inorganic acids and alkalies and precipitated by ammonia solution, coagulated by heat. Some globnulins are serum globulin, tissue globulins and vegetable globulin.

c) Prolamins: Insoluble in water, soluble in dilute acids and alkalies and contain large amount of proline. Example: zein from maize, gliadine from wheat, hordein from barley.

d) Glutelins: insoluble in water, soluble in dilute acid and alkalies coagulated by heat, rich in a arginine, proline and glutamic acid. Example: glutenin from wheat, oyrzenin from rice.

e) Scleroproteins(albuminoids): Insoluble in water , soluble in concentrated acids and alkalies. Not attacked by enzyme. Example: Keratin from hair, hoof, fibroin from silk.

Sub members of albumanoids are:
i) Collagens: Present in skin, tendons and bones, they form gelatins which is water soluble.
ii)Elastins: Present in tendons and arteries, not converted to gelatin.

f) Basic protein: They are strongly basic and fall into two groups:
i) Histones: These are soluble in water or dilute acid, not coagulated by heat, contain large amount of histidine and arginine, low in cystine or methionine. These are proteins of nuclic acid and haemogloblin etc.
ii) Protamins: Less basic than histones, soluble in water, dilute acids and dilute ammonia, not coagulated by heat, precipitated from ethanol. They contain large amount of arginine and occur in various nucleic acids.

2) Conjugated protein: They contain non-protein group attached to protein part, called prosthetic group. These groups may be separated from protein part by careful hydrolysis. **Some sub members are:**

i) Nucleoprotein: The prosthetic group is nucleic acid.

ii) Chromoproteins: The prosthetic group is chromophoric group called prosthetic group. E.g. chlorophyll and haemoglobin.

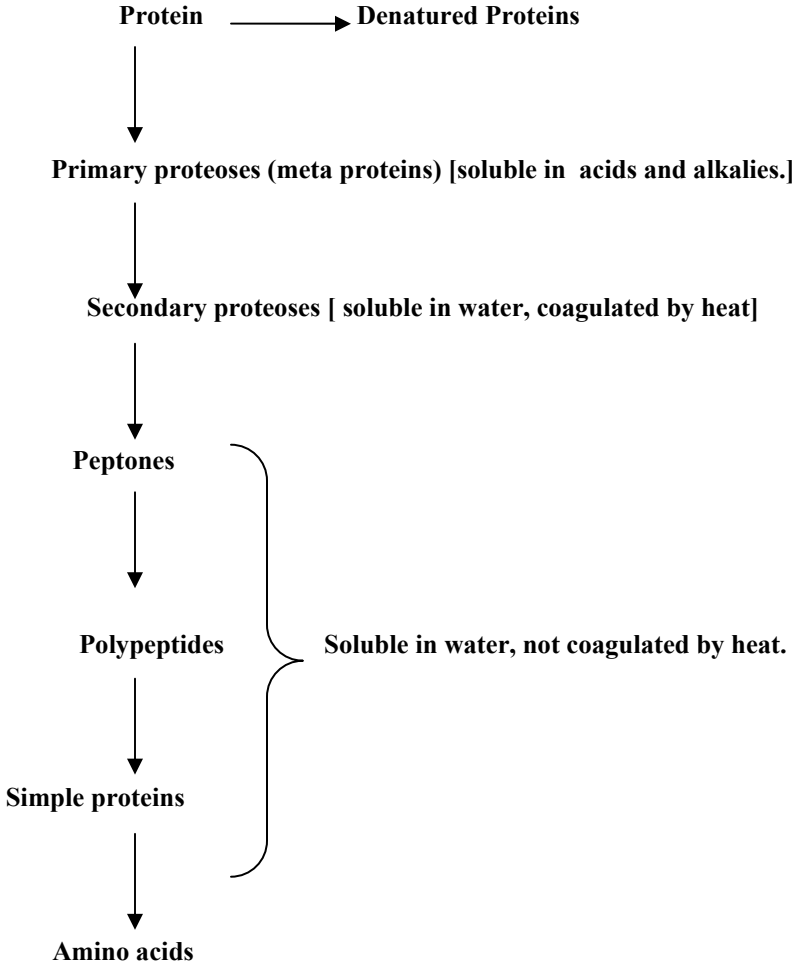
iii) Glycoproteins: They contain carbohydrate prosthetic group. They are also known as muco proteins, e.g. egg albumin, serum albumin and certain serum globulin.

iv) Phosphoproteins: In these, prosthetic group possesses phosphoric acid in some form other than in nucleic acids.

v) Lipoproteins: Prosthetic groups are phospholipids and cholesterol.

vi)Metalloproteins: In these proteins, metal is an integral part of the structure. Generally iron, magnesium, copper and manganese. e.g. haemoglobin and chlorophyll.

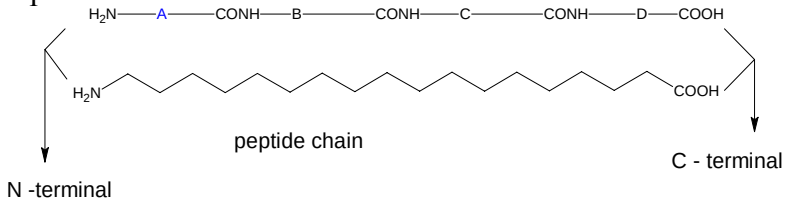
3) Derived proteins: These are the degradated products obtained by the action of acids, alkalies or enzymes on proteins.



Structure of Proteins

Primary structure of proteins: It is concerned with the sequence of amino acids in peptide chain. In every poly peptide, there is a specific sequence of amino acids. Biological activity of poly peptides depends on the sequence of amino acid. If only one amino acid in the sequence is changed, then total biological activity of amino acid may be changed. Bio synthesis of proteins is regulated by nucleic acids (DNA, RNA). In haemoglobin there is a specific sequence of 574 amino acids. If only one amino acid is replaced it becomes defective haemoglobin which produces sickle cell anemia disease.

The amino end is said to be N-terminal and the 'carboxyl end' is said to be 'C'-terminal. The general method of writing the sequence of amino acids in a peptide is with the terminal amino group on the left. Fro example



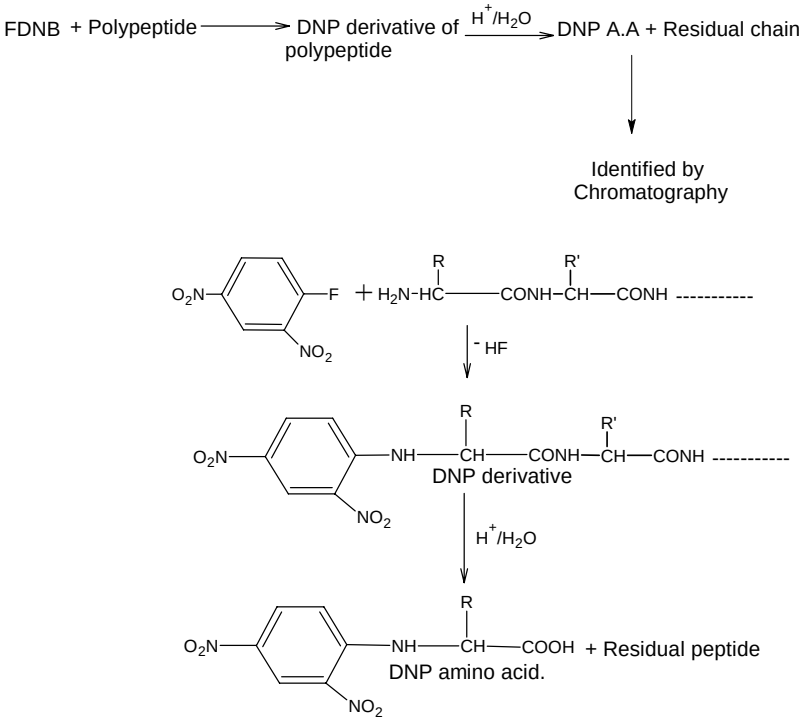
Determination of 1^{o} structure of proteins: Following steps are involved in the determination of structure of protein.

1. Protein must be isolated in pure state.
2. Find out that whether the protein consists of only one peptide chain or composed of sub units.
3. Complete hydrolysis of protein into their constituent amino acid.
4. Minimum mol.wt determination from percentage composition of amino acid.
5. End group analysis to determine sequence.

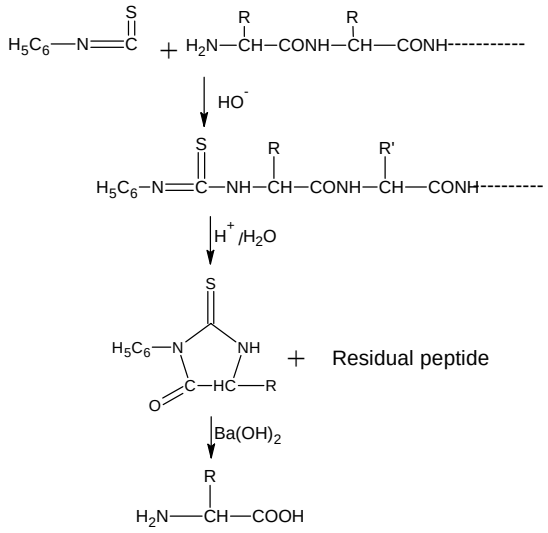
Terminal group analysis:

A) N-terminal analysis:

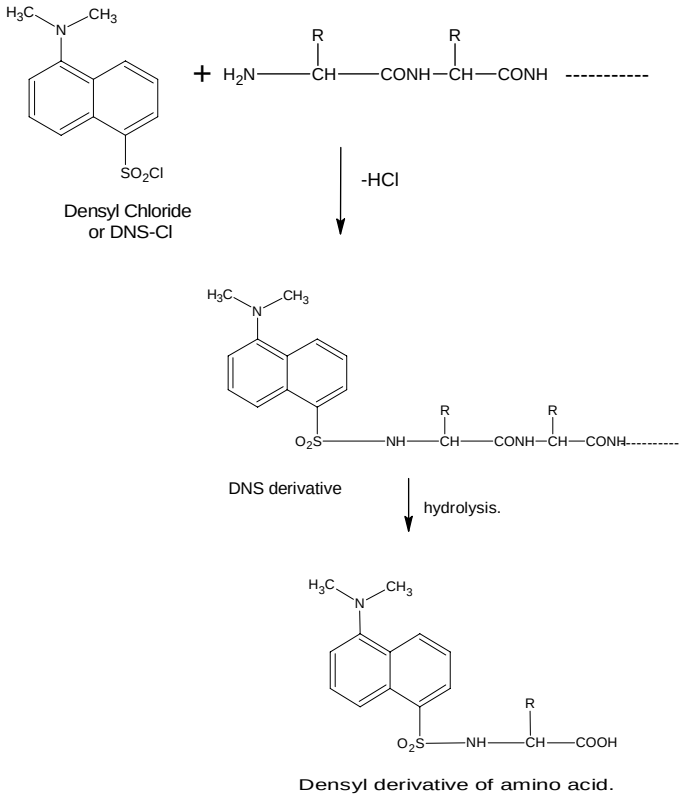
I) Sangers method:



II) Edman's method:



III) Densyl method:

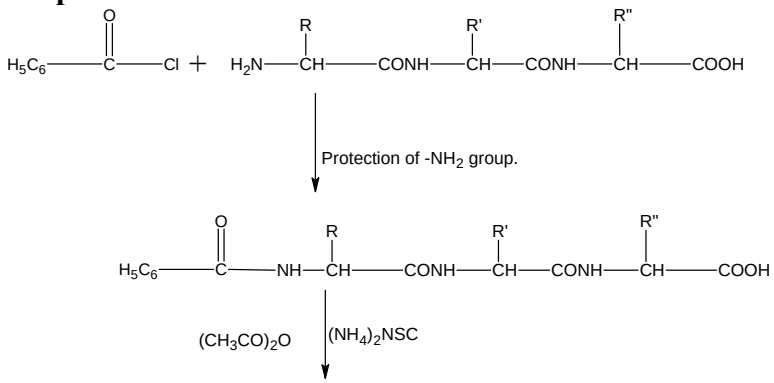


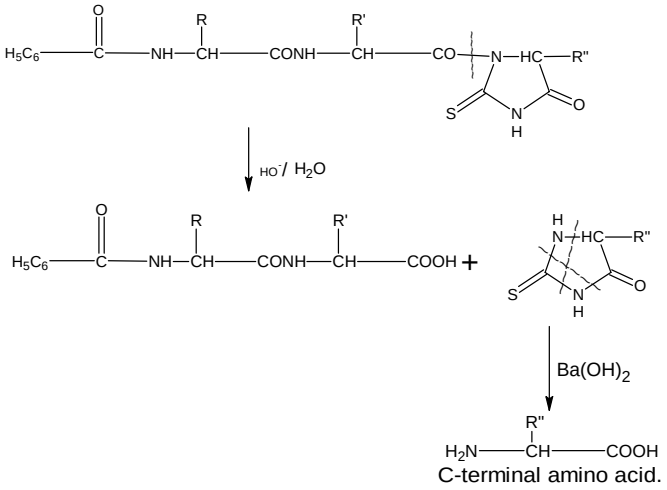
This densyl method is now widely used because the densyl group being highly fluorescent permits the detection and estimation of densyl amino acids in mixture amounts by flouroimetric methods.

IV) Enzymatic method: The enzyme Leucine amino peptidase attacks peptides only at the end of which contains the free amino group.

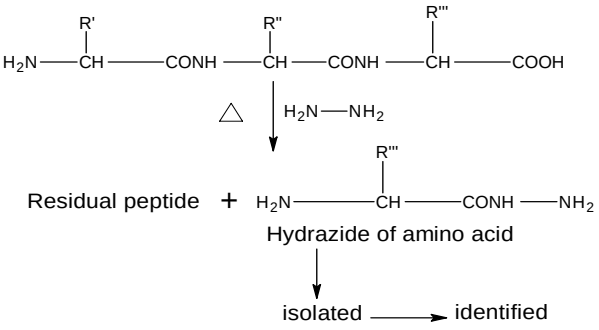
B) C-termianl analysis:

I) Schloack and Kumph's method:

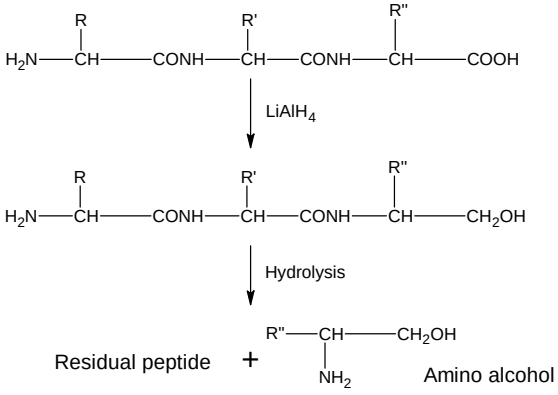




II)Hydrazinolysis:

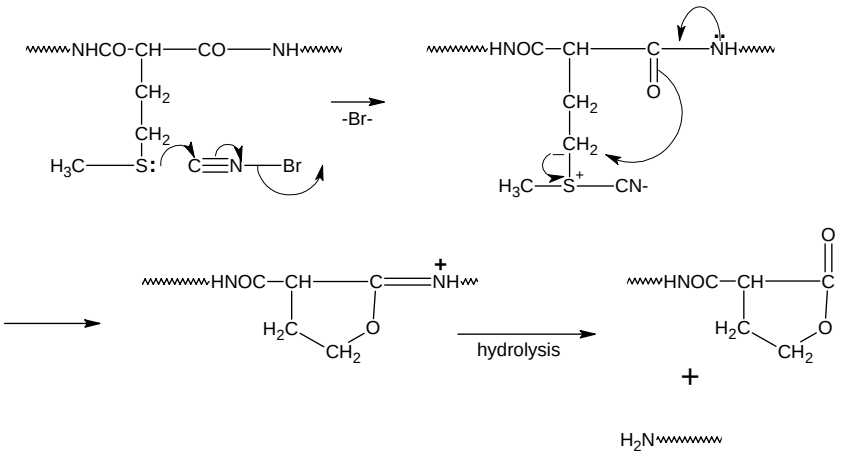


III) Reduction:



IV) Enzymatic method: Carboxypeptidase attacks peptides only at the end which contains the free α-carboxyl group. Suppose there is a peptide …..x,y,z. after attacking by enzyme, a number of successive terminal amino acids will be liberated from this peptide in amounts……Z>Y>X. These amino acids can be identified and quantitatively estimated and sequence can established.

Cyanogen Bromide method: Specific reagent to cleave the peptide chain at the peptide bond formed by –COOH groups of methionine.



Mass spectrometry may also be used to determine the amino acid sequence in the protein or in the various fragments obtained by partial hydrolysis.

Secondary structure of Protein: Concerned with three dimensional arrangement of the polypeptide chain i.e. conformation of poly peptide chains which arise as a result of Hydrogen bonding.

The α-Helix : Proposed by Pauling et al (1951)on the basis of the following arguments:

i) Planarity of peptide bond.

ii) The dihedral angles Ψ and Φ taken about C^{α}-C^{1} and N-C^{α} bonds, respectively, are close to those corresponding to potential minima in the system.

iii) Conformation of the protein is stabilized by hydrogen bonding which is formed between >C=O and N-H group and the strength of this bond is a maximum of the atom concerned (C=O----H-N) are linear.

iv) Maximum number of hydrogen bond.

In this model polypeptide coils together about itself in spiral manner. Helix may be left or right handed. There are 3-4 amino acids of one turn is hydrogen bonded with amino acid of other turn.

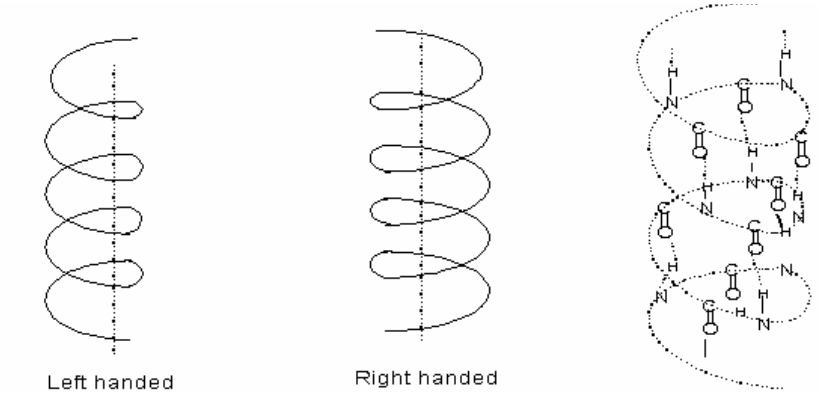


Figure-1: α-Helix structure of Protein

β-Pleated sheet structure: In this arrangement polypeptide chains are extended in a linear or zig-zag manner. Neighboring chains are bonded together by reciprocal inter chain hydrogen bonding. The result is a structure resembling pleated sheet.

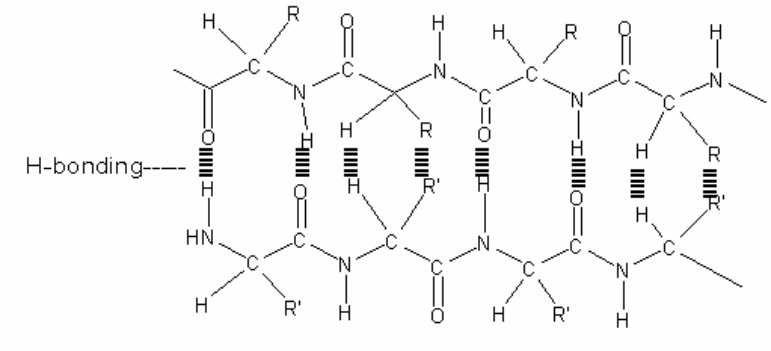
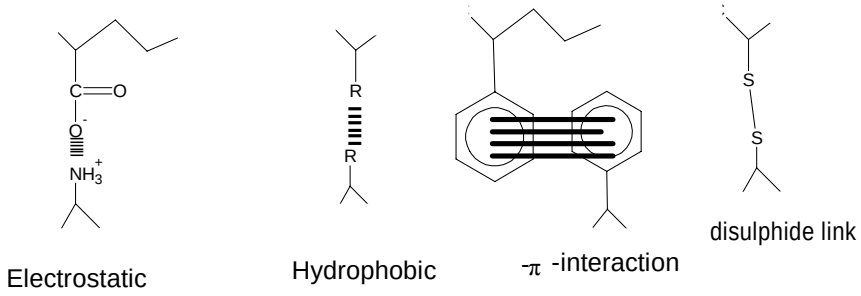


Figure-2: β-Pleated sheet structure of protein

Other non bonding interactions:



In β- conformation, there are two types of pleated sheets in which the alignment of the peptide chains may be parallel to one another or anti parallel.

Parallel: Chains run in the same direction

Anti parallel: Chains run alternatively in opposite direction

Tertiary structure: Dividing line between secondary and tertiary structure is not very clear. It refers to the three dimensional structure of the poly peptide chain that results from interaction between amino acid residue relatively far apart in the sequence. Actually it may be regarded as gross overall folding of peptide chains; this is due to the non bonding interactions. Disulphide linkage plays important role.

Quaternary structure of Proteins: "Gross folding pattern and arrangement of two or more protein chain" this is the relation of one protein fold with other. Quaternary structure also results from the non bonding interactions. Proteins such as haemoglobin, which consists of more than one polypeptide chain, are said to possess quaternary structure. These proteins having this type

of structure are said to be oligomeric and the individual polypeptide chains are known as promoters or sub units.

The unambiguous determination of quaternary structure is possible only by crystallographic methods.

Suggested Readings

i) Organic Chemistry by I. L. Finar, vol. 2, 6thedition.
ii) Organic Chemistry by Robert T. Morrison and Robert Neilson Boyd, 6th edition.
iii) Organic Chemistry by K. Peter C. Vollhardt and Neil E. Schore, 4th editeion.
iv) Chemistry of Natural Products by S. V. Bhat, B. A. Nagasampagi and M. Siva Kumar