BIOMOLECULES (AMINO ACIDS AND PROTEINS)

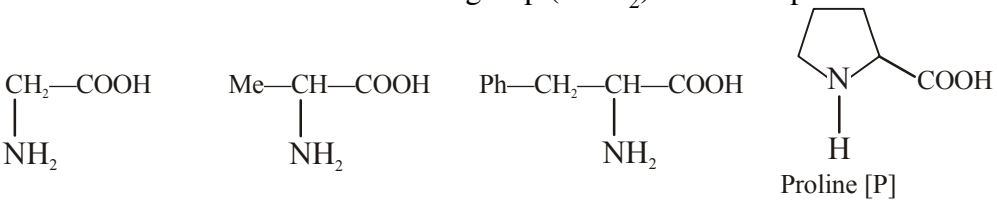
Proteins: The name protein is taken from the Greek word "proteios", which means "first". Of all chemical compounds, proteins must almost certainly be ranked first, for they are the substance of life.

Proteins make up a large part of the animal body, they hold it together and they run it. They are found in all living cells.

Chemically, proteins are high polymers. They are polyamides and the monomers from which they are derived are the α - amino carboxylic acids. A single protein molecule contains hundreds or even thousands of amino acid units. These units can be of twenty-odd different kinds. The number of different combinations, i.e., the number of different protein molecules that are possible, is almost infinite.

Bifunctional compounds having an acidic corboxylic group & a basic amino group are known as amino acid.

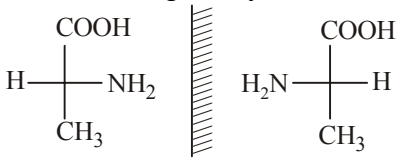
There are 20 amino acids commonly found is proteins and are standard amino acids. All are α amino acids. Most of them have 1° amino group.($-NH_2$). However proline is a 2° amino.



Proline [P]

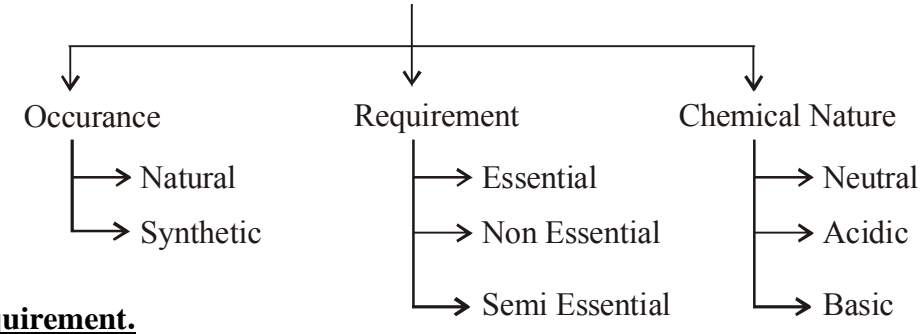
(Gly) Glycine [G] Alanine [A] Phenyl alanine [F] Proline [P] (α-imino acid)

All amino acids are chiral molecules with atleast one chiral carbon (except glycine, $H_3N^{\oplus}CH_2COO^{-}$). Except Glycine all other amino acids are optically active & can be assigned D & L configuration.



D-Alanine L-Alanine

Classification of Amino acids



Based on requirement.

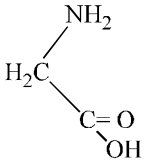
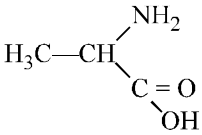
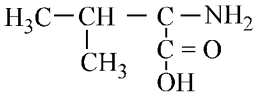
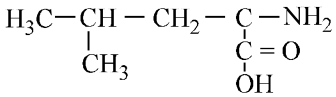
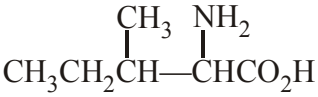
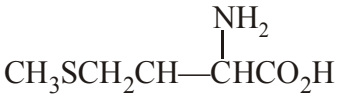
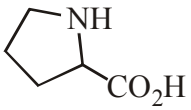
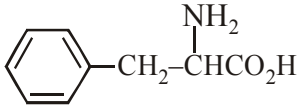
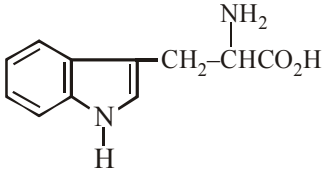
1. Essential amino acids can not be synthesized in human body so dietary intake is required. For any human being 1 gm a day is required.
2. Semi essential amino acids can be synthesized in human body but dietary intake is required during growing stages (when more of cell division is required).
   For example : Early childhood, pregrancy and lactating mother.
3. Non essential amino acid - Body can synthesize them.

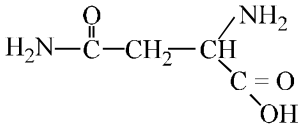
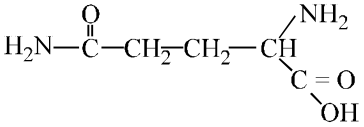
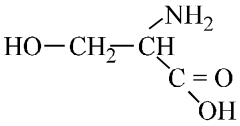
Based on chemical nature

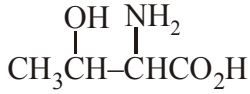
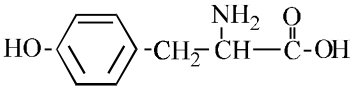
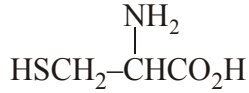
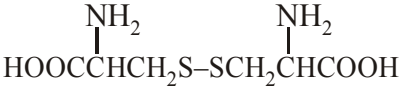
Neutral - Amino acid having equal number of NH_2 and COOH.

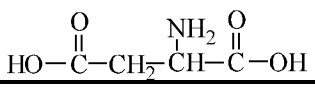
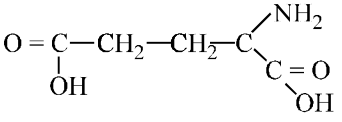
Neutral amino acids are further classified as polar and nonpolar depending on whether their side chains have polar substituents (for example, asparagine with an NH_2CO group) or are completely hydrocarbon in nature (for example alanine, valine etc.).

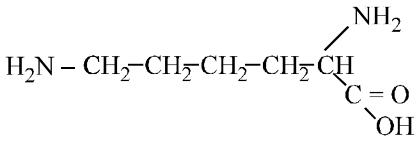
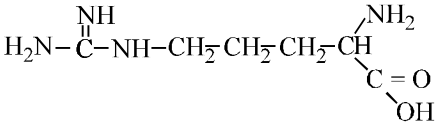
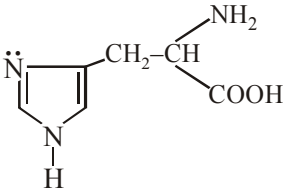
Acidic - Amino acid having more COOH than NH_2 group.
For example : Aspartic acid and glutamic acids, each with a second CO_2H in their side chain are acidic amino acids.
Basic - Amino acid having more NH_2 than COOH group.
For example : Lysine, arginine and histidine)

A -1. Neutral amino acids (with nonpolar side chains)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
@Glycine**	Gly(G)		6.0
Alanine**	Ala(A)		6.0
Valine*	Val(V)		6.0
Leucine*	Leu(L)		6.0
Isoleucine*	Ile(I)		6.0
Methionine*	Met(M)		5.7
@@Proline	Pro(P)		6.3
Phenylalanine*	Phe(F)		5.5
Tryptophan*	Trp(W)		5.9

A -2. Neutral amino acids (with polar, but nonionized side chains)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Asparagine**	Asn(N)		5.4
Glutamine**	Gln(Q)		5.7
Serine**	Ser(S)		5.7

A -3. Neutral amino acids (with polar, but nonionized side chains)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Threonine*	Thr		5.6
Tyrosine**	Tyr(Y)		5.7
Cysteine	Cys		5.1
‡ Cystine	Cys–Cys		

B - Acidic amino acids (side chain with carboxylic acid group)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Aspartic acid**	Asp(D)		2.8
Glutamic Acid	Glu(E)		3.2

C - Basic amino acids (side chain with nitrogenous basic group)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Lysine*	Lys(K)		9.7
Arginine*	Arg(R)		10.8
Histidine*	His(H)		7.6

Note:

* Amino acids with an asterisk are essential amino acids.

** Amino acids with an asterisk are non essential amino acids.

† At pH = 7, Asp and Glu have a net negative charge and exist as anions. At pH = 7, Lys and Arg have a net positive charge and exist as cations. Rest of the amino acids at this pH exist in the neutral form.

‡ Structurally, in cystine, the two cysteine molecules are joined through sulfur (disulfide linkage).

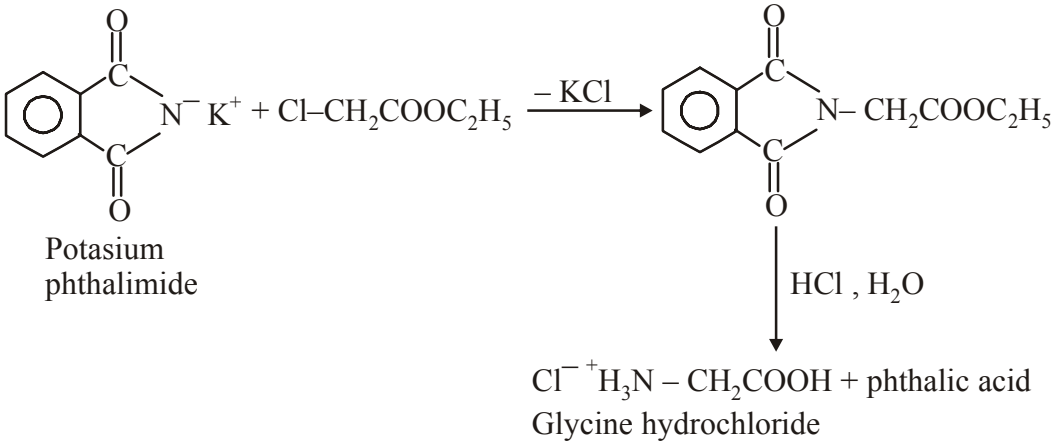
@@ Proline is an α-imino acid, all amino acids are primary amines except proline and 4-hydroxyproline, which are 2° amines.

@ Except Glycine all other amino acids are optically active.

Preparation of amino acids

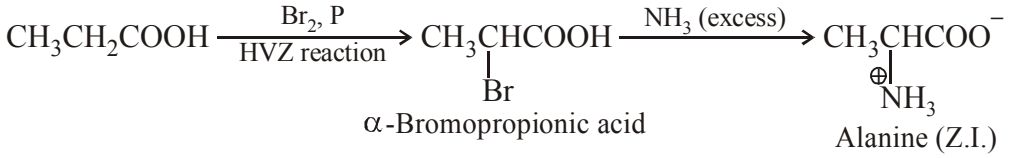
(a) **Gabriel Phthalimide synthesis**

Good yields of amino acids are generally obtained by the Gabriel phthalimide synthesis ; In this method α - halo esters are used instead of α - halo acids .

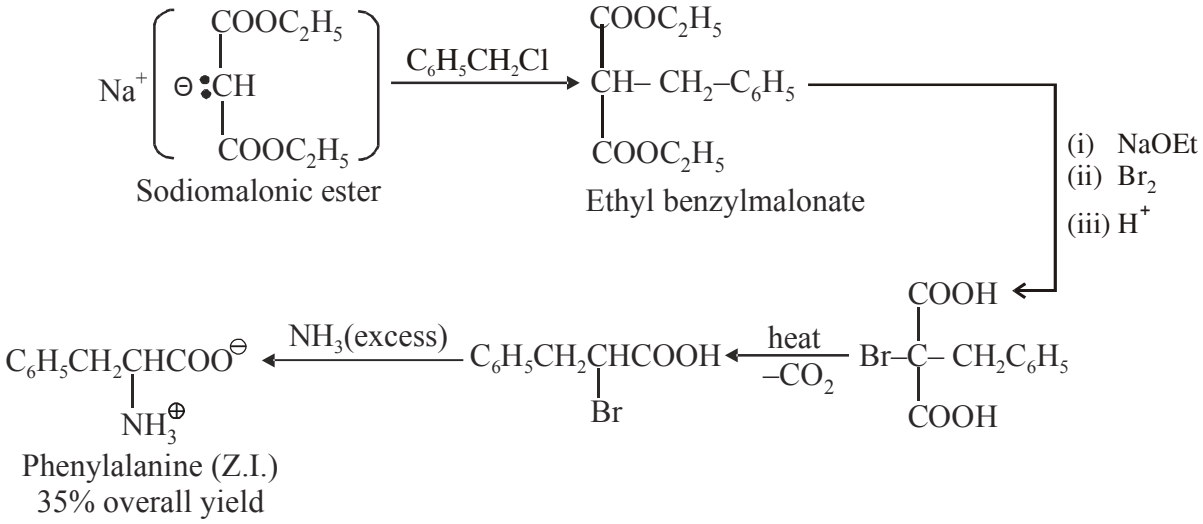


(b) **Amination of α - Halo acids**

Sometimes an α - chloro or α - bromo acid is subjected to direct ammonolysis with excess of concentrated ammonia.

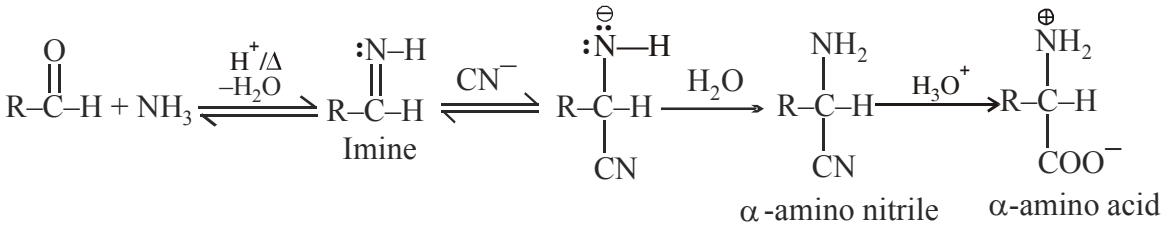


(c) **From diethyl malonate**

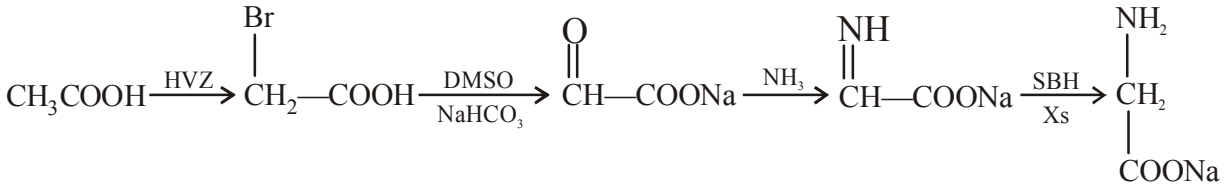


(d) **Strecker's synthesis**

Strecker's synthesis is also used for preparing α - amino acids



(e) **Using KOOP synthesis**



Properties of Amino acids

Although the amino acids are commonly shown as containing an amino group and a carboxyl group, $H_2NCHRCOOH$, certain properties, (both physical and chemical) are not consistent with this structure

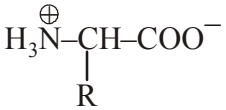
I. Physical properties

In contrast to amines and carboxylic acids, the amino acids are nonvolatile crystalline solids, which melt with decomposition at fairly high temperatures. They are insoluble in non-polar solvents like petroleum ether, benzene or ether and are appreciably soluble in water. Their aqueous solutions behave like solutions of substances of high dipole moment due to existance.

Amino acids as dipolar ions as zwitter ion

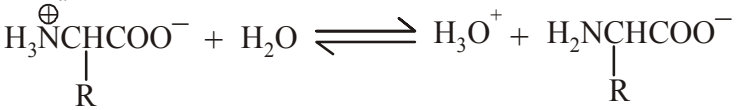
Acidity and basicity constant are ridiculously low for –COOH and $–NH_2$ groups. Glycine, for example, has $K_a = 1.6 \times 10^{-10}$ and $K_b = 2.5 \times 10^{-12}$, whereas most carboxylic acids have K_a values of about 10^{-5} and most aliphatic amines have K_b values of about 10^{-4}.

All these properties are quite consistent with a dipolar ion structure for the amino acids (I)



Amino acids : dipolar ions (Zwitter ion)

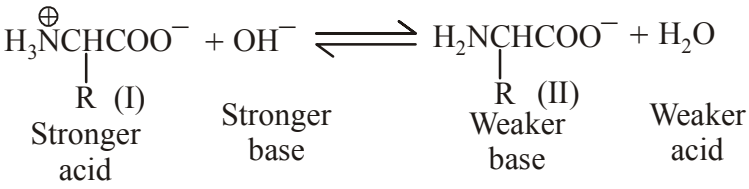
Physical properties - melting point, solubility, high dipole moment - are just what would be expected of such a salt. The acid-base properties also become understandable when it is realized that the measured K_a actually refers to the acidity of an ammonium ion, RNH_3^+,



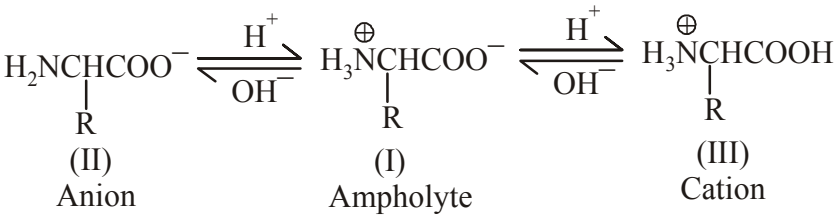
Acid

$$K_a = \frac{[H_3O^{\oplus}][H_2NCHRCOO^{\ominus}]}{[H_3\overset{\oplus}{N}CHRCOO^{\ominus}]}$$

When the solution of an amino acid is made alkaline, the dipolar ion (I) is converted into the anion(II). The stronger base, hydroxide ion, removes a proton from the ammonium ion and displaces the weaker base, the amine.



Stronger acid — Stronger base — Weaker base — Weaker acid

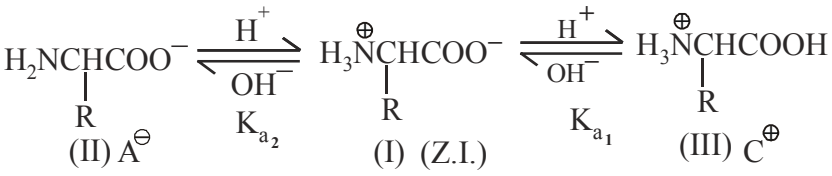


(II) Anion — (I) Ampholyte — (III) Cation

Wherever feasible, we can speed up a desired reaction by adjusting the acidity or basicity of the solution in such a way as to increase the concentration of the reactive species.

Isoelectric point of amino acids

What happens when a solution of an amino acid is placed in an electric field depends upon the of the solution.



(II) $A^{\ominus}$ — K_{a_2} — (I) (Z.I.) — K_{a_1} — (III) $C^{\oplus}$

In quite alkaline solution, anions (II) exceed cations (III), and there is a net migration of amino acid toward the anode. In quite acidic solution, cations (III) are in excess, and there is a net migration of amino acid toward the cathode. If (II) and (III) are exactly balanced, there is no net migration ; under such conditions any one molecule exists as a positive ion and as a negative ion for exactly the same amount of time, and any small movement in the direction of one electrode is subsequently cancelled by an equal movement back towards the other electrode. **The hydrogen ion concentration of the solution in which a particular amino acid does not migrate under the influence of an electric field is called the isoelectric point (pI) of that amino acid. The isoelectric point (pI) is the pH at which the amino acid exists only as a dipolar ion with net charge zero.**

For glycine, for example, the isoelectric point is at pH 6.1.

An amino acid usually shows its lower solubility in a solution at the isoelectric point, since here there is the highest concentration of the dipolar ion. As the solution is made more alkaline or more acidic, the concentration of one of the more soluble ions, (II) or (III) increases.

$$Ka_1 = \frac{[Z.I.]\ [H^{\oplus}]}{[C^{\oplus}]} \qquad Ka_2 = \frac{[A^{-}]\,[H^{\oplus}]}{[DI]} \text{ at pI } [A^{\ominus}] = [C^{\oplus}]$$

$$\frac{[Z.I.]\ [H^{\oplus}]}{Ka_1} = \frac{Ka_2[DI]}{[H^{\oplus}]} \qquad [H^{\oplus}]^2 = Ka_1 \ \& \ Ka_2$$

$$\text{on taking antilog pI} = \frac{P^{Ka_1} + P^{Ka_2}}{2}$$

An amino acid having –COOH group more than NH_2 group or such amino acid have pI less than 7.

An amino acid having more – NH_2 more than COOH group such amino acid have pI more than 7.

Q. Write the structure of alanine at pH 2.5, 10.5 and 6.

Electrophoresis

The movement of charged molecules (like amino acid) under the influence of an electric field is called electrophoresis. Electrophoresis separates amino acids on the basis of their pI values.

Amino acid is positively charged (moves towards cathode) if pH of the solution < pI

Amino acid is negatively charged (moves towards anode) if pH of the solution > pI

Q. How will you separate a ternary mixture of arginine, alanine & aspartic acid?

Ans. A few drops of a solution of an amino acid mixture are applied to the middle of a piece of filter paper. When the paper is placed in a buffer solution (pH = 5) between the two electrodes and an electric field is applied then arginine & alanine with pI > pH move towards the cathode and aspartic acid with pI < pH moves towards the anode. Out of arginine & alanine, alanine will move slowly towards the cathode due to lesser positive charge.

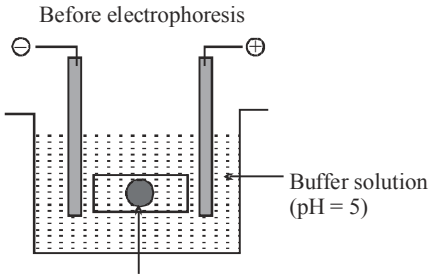


Figure (i)

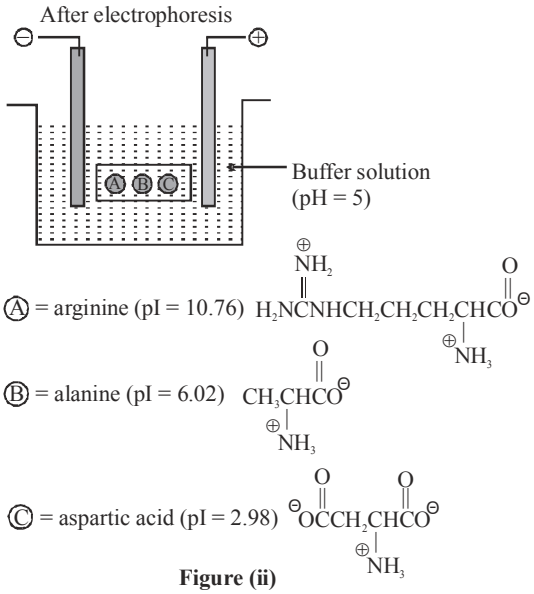
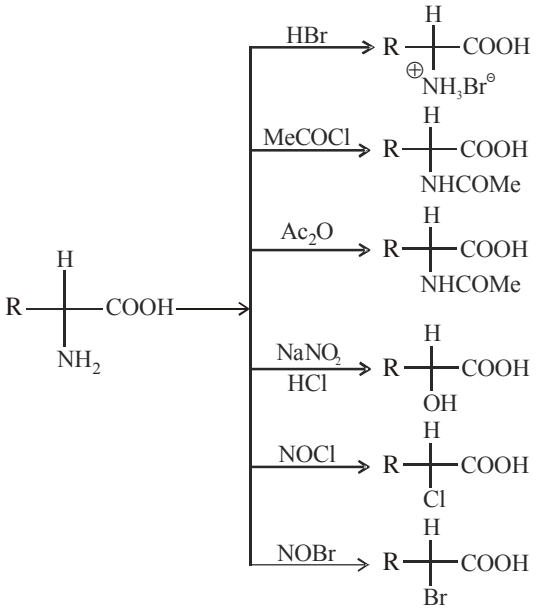
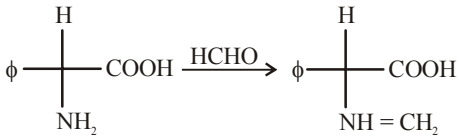


Figure (ii)

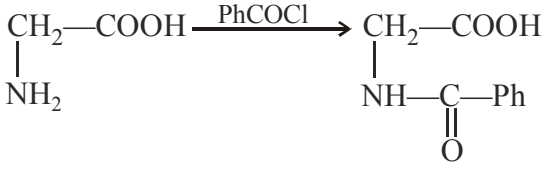
General reactions of amino acids

(1) **Reactions due to – NH_2 group**





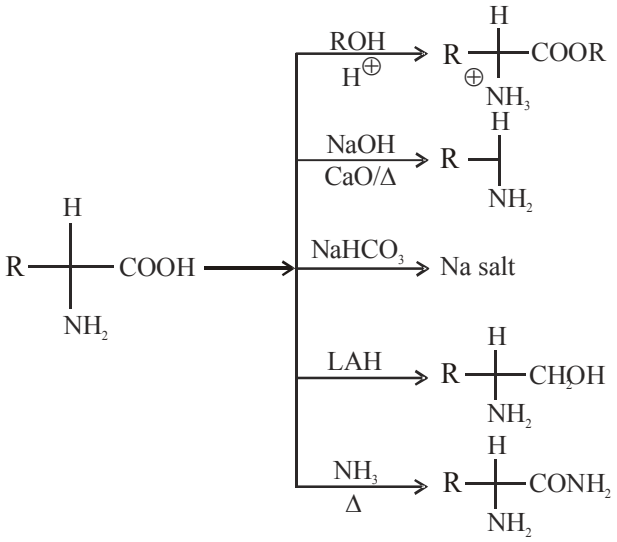
Sorison's titration method.



Hipuric acid

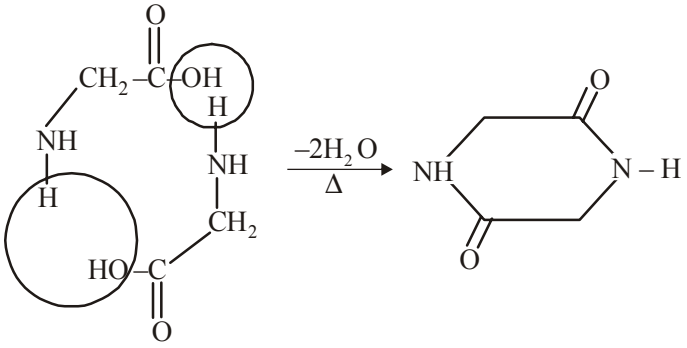
Reactions is used to block – NH_2 group during volumetric analysis in.

(2) **Reactions due to – COOH group.**

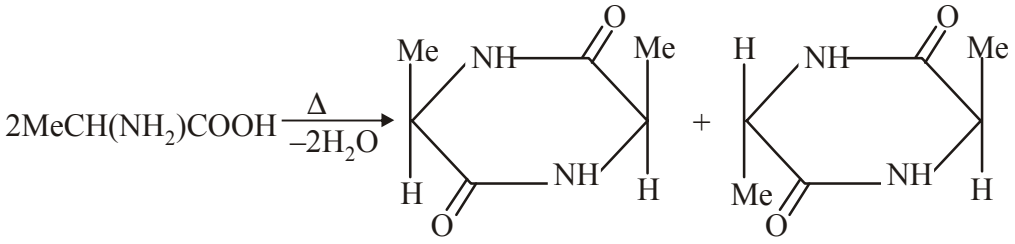


(3) **<u>Heating Effect</u>**

(i) Heating of amino acids leads to intermolecular dehydration to form cyclic diamides.

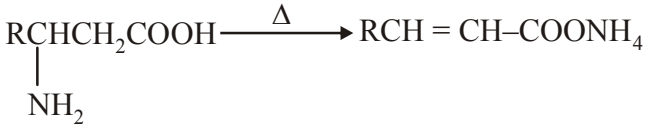


(ii) When alanine is heated, then two diastereomers are obtained. One of them (trans) is not resolvable.

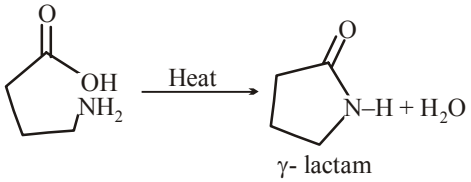


Cis (Racemic) Trans (Meso)

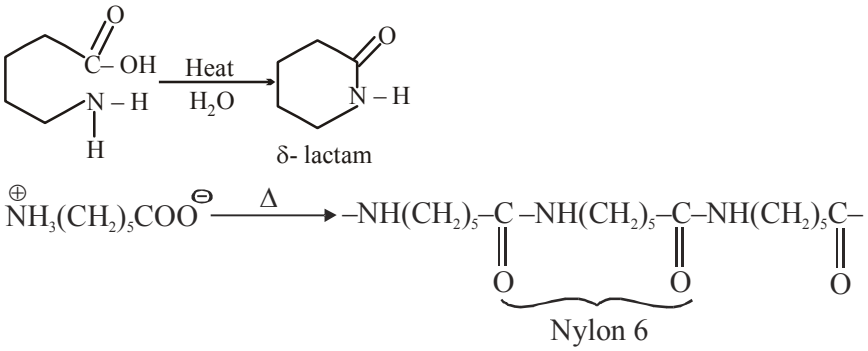
(iii) When β - amino acids are heated, α, β - unsaturated salt are formed.



(iv) γ,δ,ε - amino acids when heated alone gives γ, δ - lactam and polymer respectively. The reason for the formation of polymer is that when ε - amino cyclises intramolecularly, it leads to large angle strain within the compound



γ- lactam



(4) **Peptide**

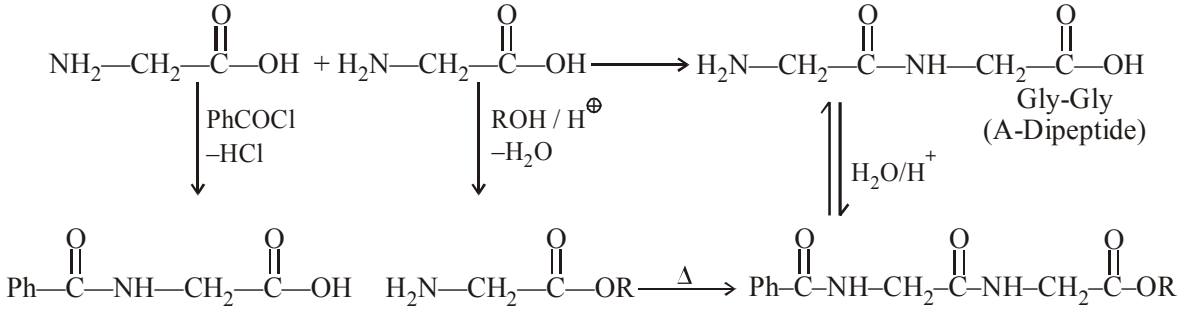
A peptide is a kind of amide formed by intermolecular reaction of the amino group of one amino acid and the carboxyl group of a second amino acid. Dipeptides are made from two amino acids, tripeptides from three amino acids, etc, which may be the same or different. If there are three to ten amino acid residues, the peptide is also called an oligopeptide.

If they give 3 to 10 amino acid they are oligopeptide

If they give 11 to 100 amino acid they are Polypeptide

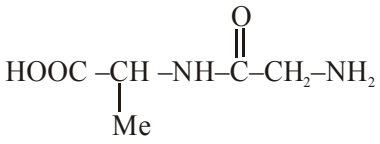
For more than 100 it is Macropeptide

- Peptides can be prepared by blocking technique



Firstly, the amino and carboxyl groups that are not to be linked in peptide bonds must be blocked to make unreactive.

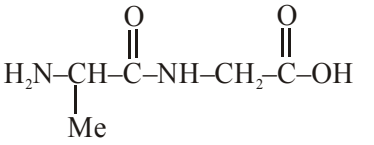
Abbreviated name of amion acid with free NH_2 is written first.



Dipeptide

Gly–Ala [Glycine Alanine]

By convention, the amino acid with the free amino group (N-terminal) is written at the left end and the one with the unreacted carboxyl group (C-terminal) at the right end.



Ala - Gly [Alanine-Glycine]

- When different amino acids are involved in pepetide formation.

Then total number of polypeptide possible = X^n

[X = type of amino acid interacting,

n = number of amino acid molecule are interacting.]

Q. Glycine can form how many Dipeptide ? [Ans. One]
Q. Glycine can form how many Tripeptide ? [Ans. One]
Q. Glycine and Ala can form how many Dipeptide ? [Ans. Four]
Q. Gly, Ala, and Phenyl Ala can form how many Dipeptide ? [Ans. Nine]
Q. Gly, Ala, can form how many Tripeptide ? [Ans. Eight]

A polypeptide with more than hundred amino acid residues, having molecular mass higher than 10,000 is called a protein. However, the distinction between a polypeptide and a protein is not very sharp. Polypeptides with fewer amino acids are likely to be called proteins they ordinarily have a well defined conformation of a protein such as insulin which contains 51 amino acids.

Structure of Proteins

Proteins can be classified into two types on the basis of their molecular shape.

(a) Fibrous proteins

When the polypeptide chains run parallel and are held together by hydrogen and disulphide bonds, then fibre-like structure is formed. Such proteins are generally insoluble in water. Some common examples are keratin (present in hair, wool, silk) and myosin (present in muscles), etc.

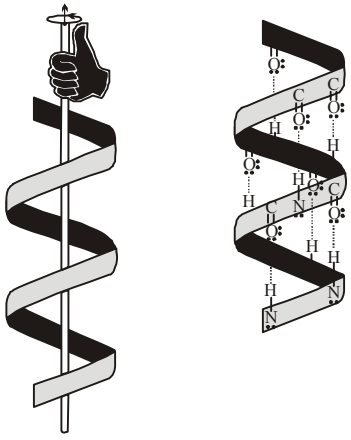
(b) Globular proteins

This structure results when the chains of polypeptides coil around to give a spherical shape. These are usually soluble in water. Insulin and albumins are the common examples of globular proteins.

Structure and shape of proteins can be studied at four different levels, .i.e., primary, secondary, tertiary and quaternary, each level being more complex than the previous one.

(i) Primary structure of proteins : Proteins may have one or more polypeptide chains. Each polypeptide in a protein has amino acids linked with each other in a specific sequence and it is this sequence of amino acids that is said to be the primary structure of the protein. Any change in this primary structure i.e., the sequence of amino acids creates a different protein.

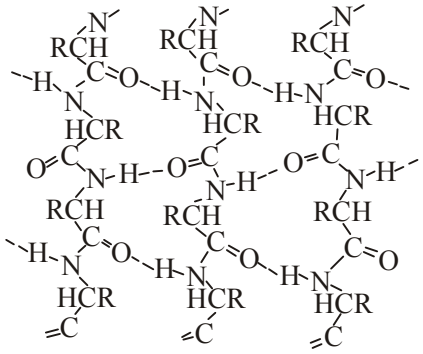
(ii) Secondary structure of proteins : The secondary structure of protein refers to the shape in which a long polypeptide chain can exist. They are found to exist in two different types of structures viz. α-helix and β-pleated sheet structure. These structures arise due to the regular folding of the backbone of the polypeptide chain due to hydrogen bonding between $-\overset{\overset{O}{\|}}{C}-$ and — NH — groups of the peptide bond.



α-Helix structure of proteins

α-Helix is one of the most common ways in which a polypeptide chain forms all possible hydrogen bonds by twisting into a right handed screw (helix) with the — NH group of each amino acid residue hydrogen bonded to the $\diagdown\!\!\diagup C = O$ of an adjacent turn of the helix as shown in figure.

In β-structure all peptide chains are stretched out to nearly maximum extension and then laid side by side which are held together, by intermolecular hydrogen bonds. The structure resembles the pleated folds of drapery and therefore is known as β-pleated sheet.



β-Pleated sheet structure of proteins

(i) Ionic bonding : between COO^- and NH_3^+ at different sites.

(ii) H-bonding : mainly between side-chain NH_2 and COOH, also involving OH's (Of serine, for example) and the N–H of tryptophan.

(iii) Weakly hydrophobic Van der Waal's attractive forces engendered by side-chain R groups and

(iv) Disulfide cross linkages between loops of the polypeptide chain.

The same kind of attractive and repulsive forces responsible for the tertiary structure operate to hold together and stabilize the subunits of the quaternary structure.

(iii) Tertiary structure of proteins : The tertiary structure of proteins represents overall folding of the polypeptide chains i.e., further folding of the secondary structure. It gives rise to two major molecular shapes viz. fibrous and globular. The main forces which stabilise the 2° and 3° structures of proteins are hydrogen bonds, disulphide linkages, van der walls and electrostatic forces of attraction.

(iv) Quaternary structure of proteins : Some of the proteins are composed of two or more polypeptide chains referred to as sub-units. The spatial arrangement of these subunits with respect to each other is known as quaternary structure. Example : Heamoglobin, Chlorophyll.

According the their biological action, they are classified as enzymes, hormones, antibodies, etc.

Protein found in living system with definite configuration and biological activity is termed as native protein. If a native protein is subjected to physical or chemical treatment, which may disrupt its higher structures (conformations) without affecting its primary structure, the protein is said to be denatured. During denaturation, the protein molecule uncoils form an ordered and specific conformation into a more random conformation leading to precipitation. Thus denaturation leads to increase in entropy and loss of biological activity of the protein. The denaturation may be reversible or reversible. Thus, the coagulation of egg white on boiling of egg protein is an example of irreversible protein denaturation. However, in certain cases it is found that if the disruptive agent is removed the protein recovers its original physical and chemical properties and biological activity the reverse of denaturation is known as renaturation.

Test Of Amino acids and Proteins

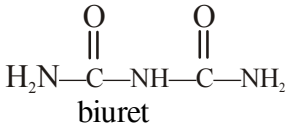
1. Biurate Test **2. Nin hydrin Test** **3. Xanthoproteic test**

4. Sakaguchi test **5. Millon's Test**

1. Biurate Test

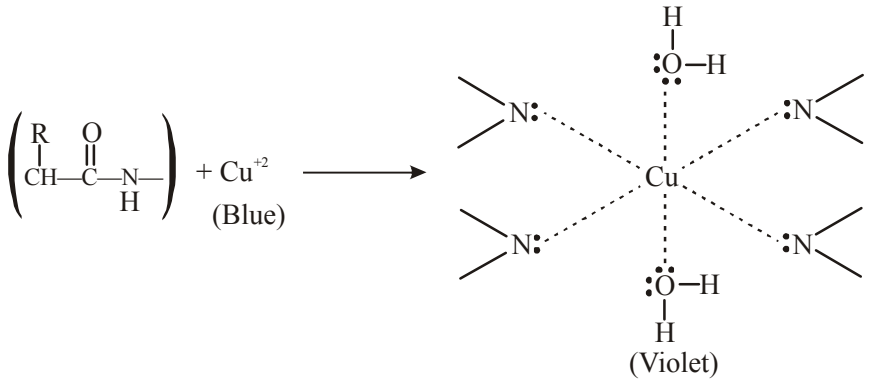
Addition of a very dilute solution of $CuSO_4$ to an alkaline solution of a protein is done. A positive test is indicated by the formation of a pink violet to purple violet color.

The name of test is derived from a specific compound, biuret, which gives a positive test with this reagent

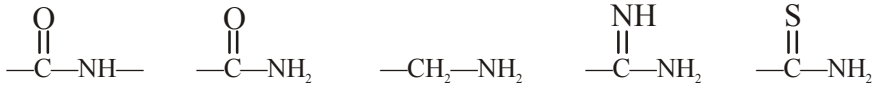


biuret

When a protein reacts with copper (II) sulfate (blue), the positive test is the formation of a violet colored complex.



The biuret test works for any compound containing two or more of the following groups.

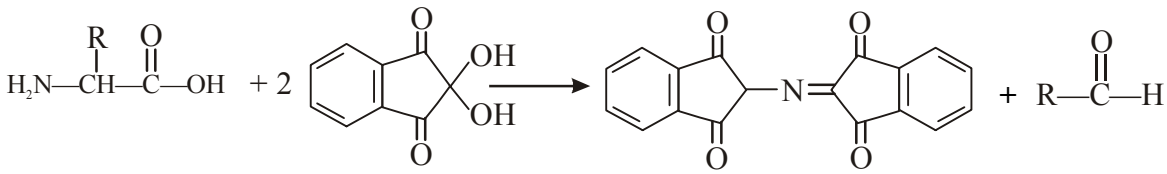


2. Nin hydrin Test

The ninhydrin test is a test for amino acids and proteins with a free $-NH_2$ group.

Amino acids are detected by ninhydrin test. All amino acids give violet - coloured product with ninhydrin (triketo hydroindene hydrate) except proline and 4 - hydroxy proline, which gives yellow colour with it.

When such an $-NH_2$ group reacts with ninhydrin, a purple-blue complex is formed.



(Purple-Blue)

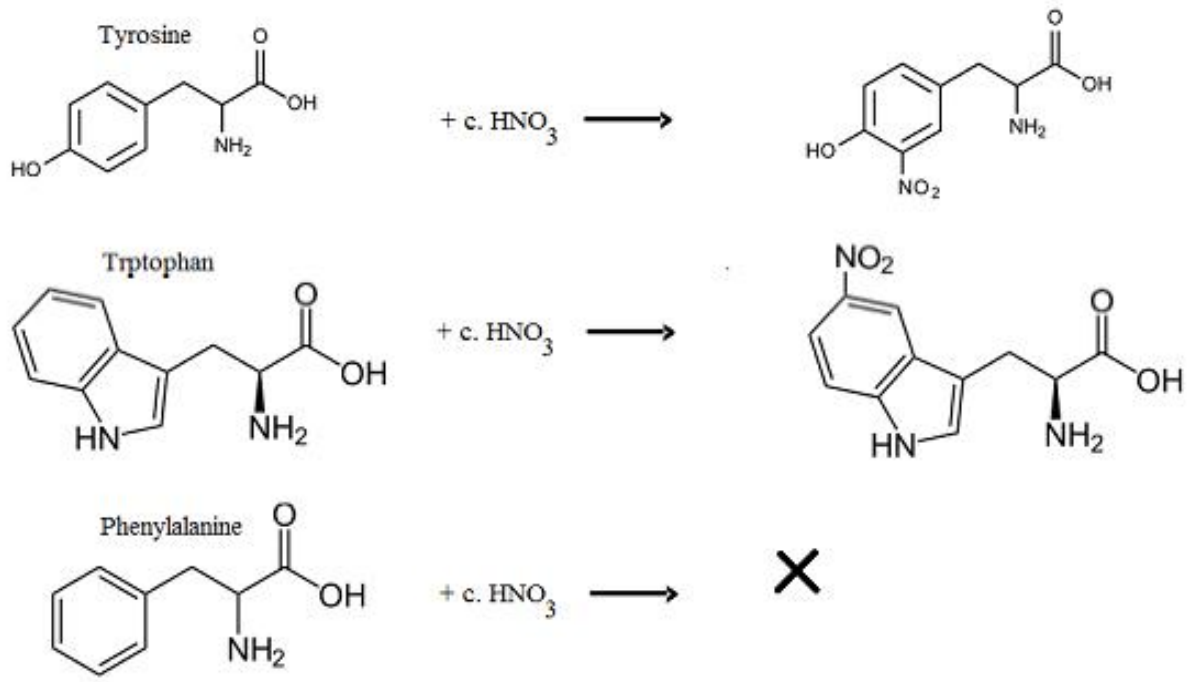
The same violet coloured dye forms from all α - AA's with 1° amino groups because only their nitrogen is incorporated into it. The 2° amines proline and 4 - hydroxyproline give different adducts that absorb light at a different and thus have a different yellow colour.

3. Xanthoproteic test

This test is used for aromatic amino acids which give positive result from other amino acids. Such as tyrosine, and tryptophan gives Xanthoproteic test, phenyl alanine does not respond with this test.

Principle:

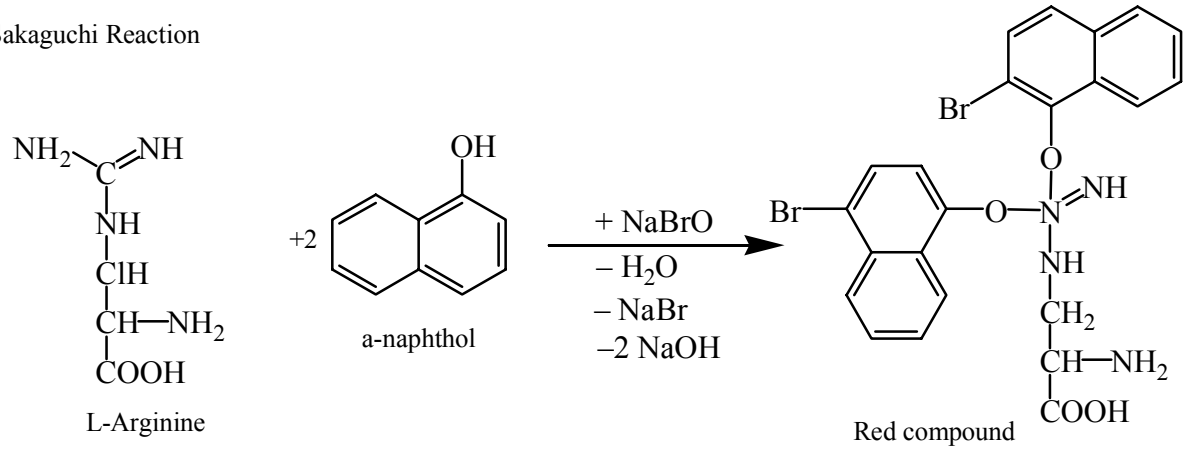
Xanthoproteic test is used to detect amino acids containing an aromatic nucleus (tyrosine, tryptophan and phenylalanine) in a protein solution which gives yellow color nitro derivatives on heating with conc. HNO_3. The aromatic benzene ring undergoes nitration to give yellow colored product. Phenylalanine gives negative or weakly positive reaction though this amino acid contains aromatic nucleus because it is difficult to nitrate under normal condition. On adding alkali to these nitro derivative salts, the color change for yellow to orange.



4. Sakaguchi test

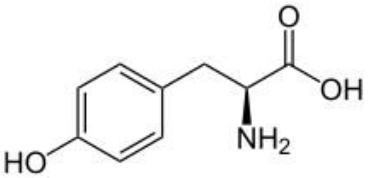
The Sakaguchi test is a chemical test used for detecting the presence of arginine in proteins Sakaguchi reagent consists of α-Naphthol and a drop of sodium hypobromite. The guanidine group in arginine reacts with Sakaguchi reagent to form a red-coloured complex.

Sakaguchi Reaction

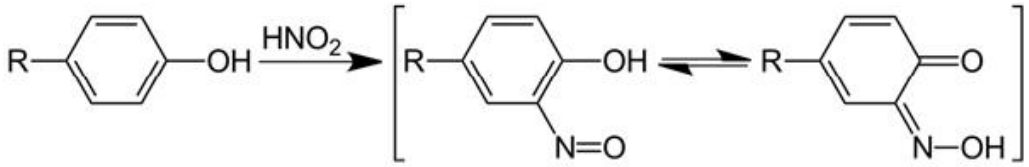


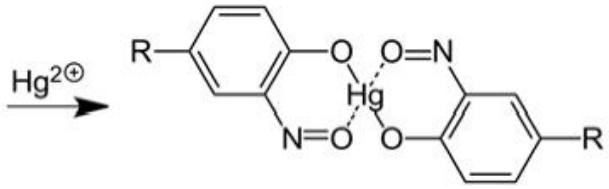
5. Millon's Test

Millon's reagent is an analytical reagent used to detect the presence of soluble proteins. A few drops of the reagent are added to the test solution, which is then heated gently. A reddish-brown coloration or precipitate indicates the presence of tyrosine residue which occur in nearly all proteins



Tyrosine Here R = $-CH_2CH(NH_2)COOH$.





reddish-brown colored complex

Sanger's Method of sequencing of Amino acids of polypeptide chain

It is N-terminal amino acid analysis

Sanger's reagent (1-fluoro-2,4-dinitrobenzene)

1. React the peptide with a reagent that will selectively label the N-terminal amino acid.
2. Hydrolyse the protein.
3. Determine the amino acid by chromatography and comparison with standards.