

**Faculty Science
Everest Shiwach**

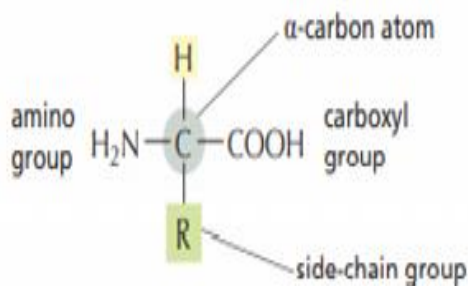
Department: Botany

M.Sc. III Sem Paper- H3002 (Phytochemistry and Metabolism),

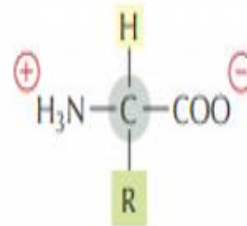
Topic- Protein Part-I Amino acids

Proteins are the most abundant and functionally diverse molecules in living systems. A protein is a linear sequence of amino acids linked together by peptide bonds. Thus, the fundamental units of protein polymers are **α -amino acid**. More than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins. These are only amino acids that are coded for by DNA. **Each amino acid (except proline which has imino group) has a carboxylic group, an amino group and a side chain (R group) bonded to the α - carbon atom.**

The general formula of an amino acid is



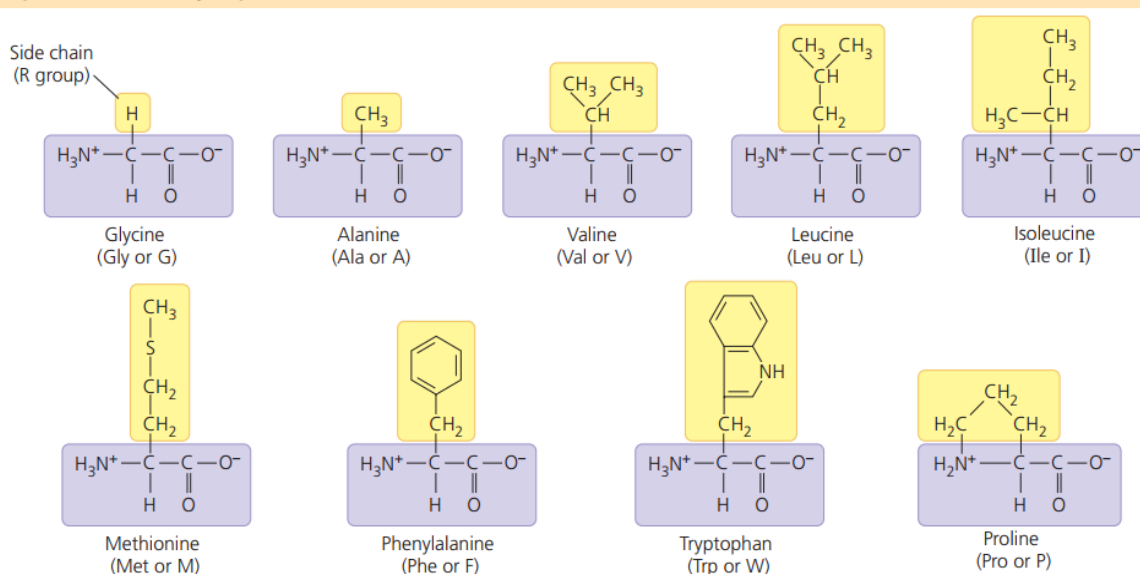
R is commonly one of 20 different side chains. At pH 7 both the amino and carboxyl groups are ionized.



The R group or side chain is different in each amino acid. Amino acids are classified on the basis of properties of their side chains.

A. Amino acids with nonpolar side chains: They have nonpolar side chain that does not bind or give off protons or participate in hydrogen or ionic bonds. They promote hydrophobic interaction.

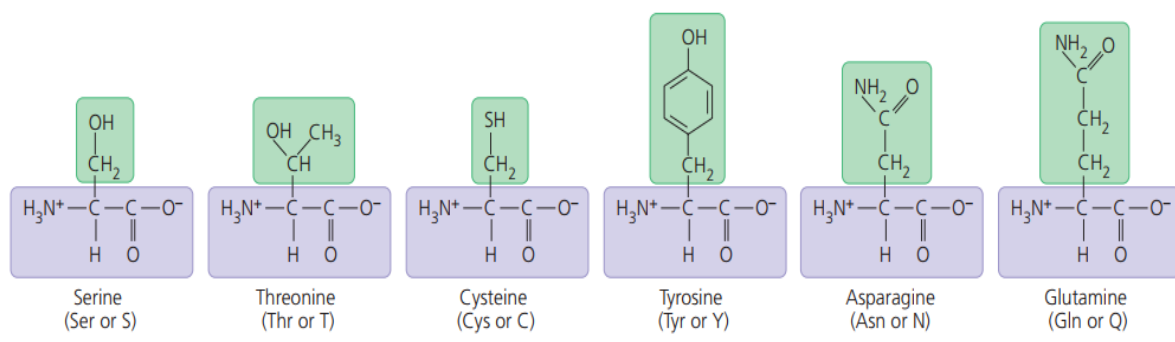
Nonpolar side chains; hydrophobic



S.No.	Amino acids	Characteristics features
1	Glycine	The smallest amino acid having H atom as a side chain, does not exist as a pair of enantiomers.
2	Alanine	The aliphatic side chain is CH ₃ .
3	Valine	Having branched aliphatic side chain.
4	Leucine	Having branched aliphatic side chain.
5	Isoleucine	Having branched aliphatic side chain, it is structural isomer of leucine.
6	Methionine	Having methyl thioether group in side chain (i.e. contain sulfur atom), initiation codon AUG codes for it, precursor of ethylene (gaseous plant hormone) and SAM (S-Adenosyl methionine is a donor of methyl group).
7	Proline	Having imino group (secondary amino group), stress amino acid in plants and heterocyclic pyrrolidine ring of proline restricts the geometry of polypeptides.
8	Phenylalanine	Having aromatic benzene ring in side chain.
9	Tryptophan	contains an aromatic bicyclic indole group in side chain, precursor of serotonin (neurotransmitter), melatonin hormone, auxin plant hormone and vitamin B3 (niacin)

B. Amino acids with uncharged polar side chains: They have zero net charge at neutral pH. Serine, threonine and tyrosine each contain polar hydroxyl group that can participate in hydrogen bond formation.

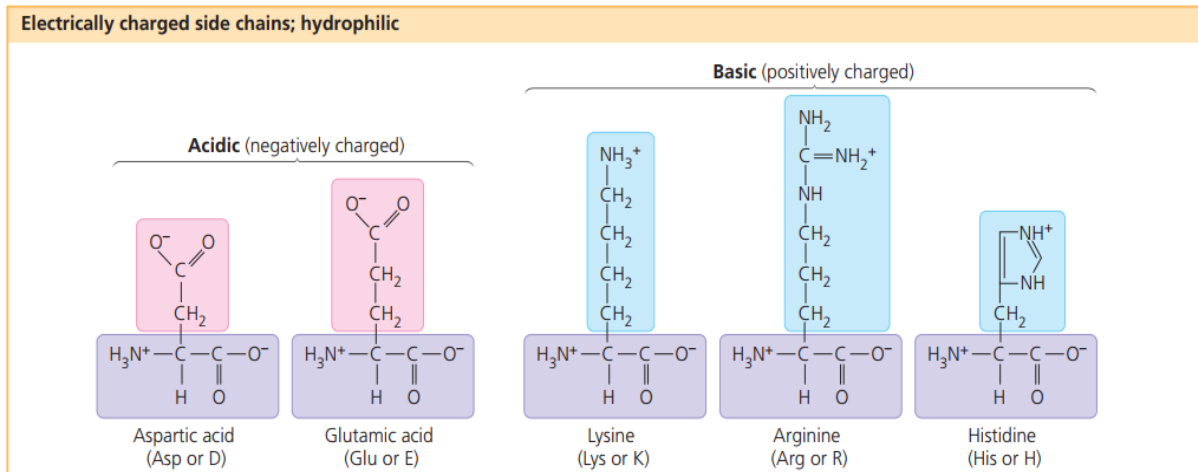
Polar side chains; hydrophilic



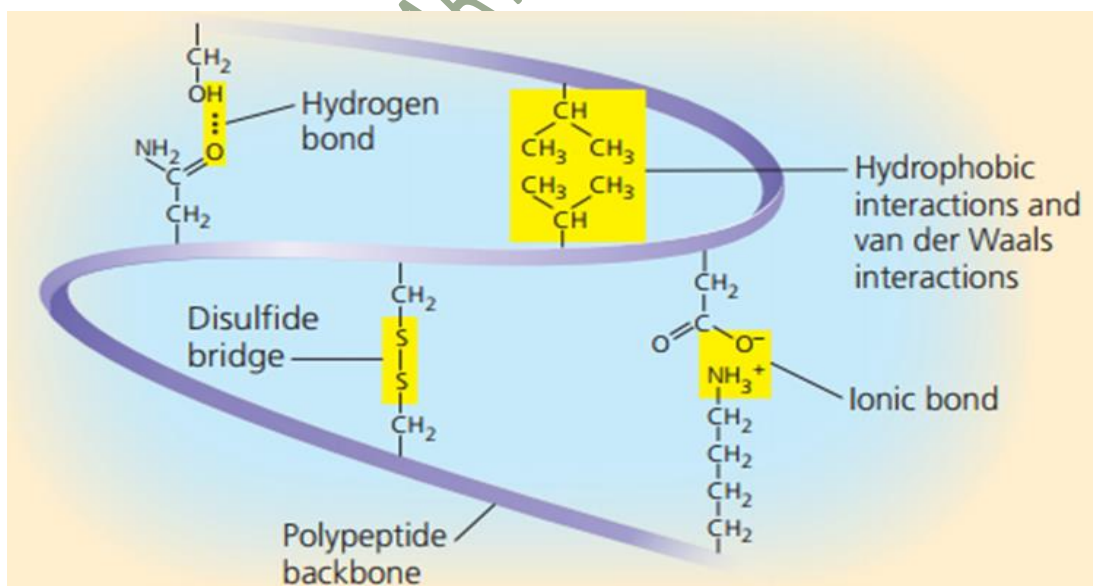
S.No.	Amino acids	Characteristics features
1 2	Serine Threonine	Serine and threonine both contain a polar hydroxyl group which serve as a site to combine with sugars for O-linked glycosylation and also for phosphorylation of protein by kinases . -OH group of serine is an important component of active site of many enzymes e. g. serine proteases . Phosphatidyl serine gives negative charge to inner lipid mono-layer of plasma membrane.
3	Asparagine	Contains amide group in side chain, amide nitrogen serves as a site for N-linked glycosylation .
4	Glutamine	Contains amide group in side chain.
5	Tyrosine	Contains phenol group in side chain which serve as a site for phosphorylation of protein kinases
6	Cysteine	Contains sulfhydryl group, cystine is formed from two oxidized cysteine residues linked by disulfide bond which stabilizes the 3D structures of proteins (e.g. antibodies)

C. Amino acids with acidic side chains: They have negatively charged side chain. At physiological pH, the side chain of these amino acids are fully ionized, containing a negatively charged carboxyl group.

D. Amino acid with basic side chains: They have positively charged side chain. At physiological pH the side Chains of these amino acids are fully ionized and containing a positively charged amino group.

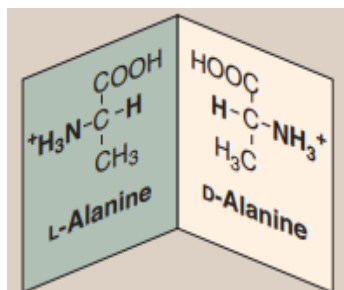


S No.	Amino acids	Characteristics features
1	Lysine	Both contain positively charged side chains. Histone proteins are basic due to these amino acids. Arginine is the most basic of 20 amino acids.
2	Arginine	
3	Histidine	Contains an imidazole ring (protonated form of this ring is called as imidazolium ion, weak basic).
4	Aspartic acid	Both contain negatively charged side chains.
5	Glutamic acid	



A diagram showing different types of interactions between side chains of amino acids

Properties of amino acids: They are crystalline solids, soluble in water. All amino acids except glycine have four different groups at α -carbon atom. They can exist in either of two **enantiomers called L or D amino acids** which are **mirror images** of each other. Only L isomer is found in proteins.



D and L forms of alanine

Aromatic amino acids (tryptophan, tyrosine and phenyl alanine) absorb maximum UV light at 280 nm.

Amphoteric nature and zwitterion formation- All amino acids contain both carboxyl and amino group. They can react with acids and bases to form salts. So that these are called amphoteric compounds. **In aqueous solution, a hydrogen ion is transferred from carboxylic group to amino group of an amino acids so that zwitterion is formed. An amino acid has both positive and negative charge but electrically neutral.**

Acidic and basic properties: Amino acids in aqueous solution contain weakly acidic α -carboxyl groups and weakly basic α -amino groups. In addition, each of the acidic and basic amino acids contains an ionizable group in its side chain. Thus, both free amino acids and some amino acids combined in peptide linkages can act as buffers. Recall that acids may be defined as proton donors and bases as proton acceptors. Acids (or bases) described as “weak” ionize to only a limited extent. The concentration of protons in aqueous solution is expressed as pH, where $\text{pH} = \log 1/[\text{H}^+]$ or $-\log [\text{H}^+]$. The quantitative relationship between the pH of the solution and concentration of a weak acid (HA) and its conjugate base (A^-) is described by **the Henderson-Hasselbalch equation**. Consider the release of a proton by a weak acid represented by HA:



Weak acid proton salt form or conjugate base

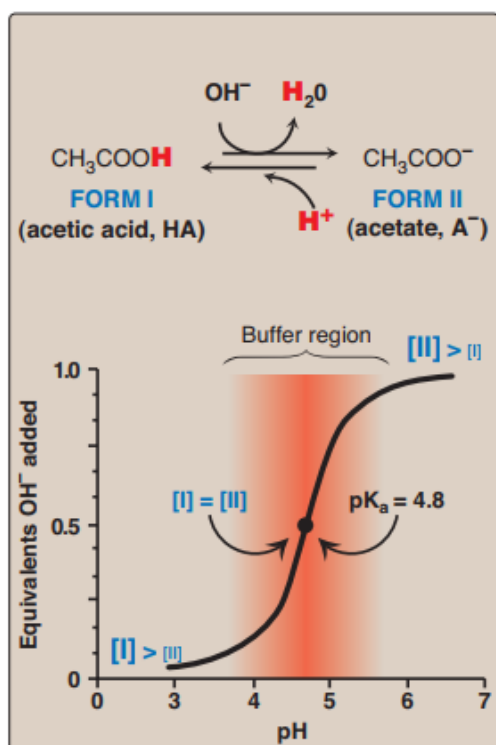
The dissociation constant of the acid K_a is $K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$

The larger the K_a , the stronger the acid, because most of the HA has dissociated into H^+ and A^- . Conversely, the smaller the K_a , the less acid has dissociated and, therefore, the weaker the acid. By solving for the $[\text{H}^+]$ in the above equation, taking the logarithm of

both sides of the equation, multiplying both sides of the equation by -1 , and substituting $\text{pH} = -\log [\text{H}^+]$ and $\text{pK}_a = -\log K_a$, we obtain the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

Buffers- A buffer is a solution that resists change in pH following the addition of an acid or base. A buffer can be created by mixing a weak acid (HA) with its conjugate base (A^-). If an acid such as HCl is then added to such a solution, A^- can neutralize it, in the process being converted to HA. If a



base is added, HA can neutralize it, in the process being converted to A^- . Maximum buffering capacity occurs at a pH equal to the pK_a, but a conjugate acid/base pair can still serve as an effective buffer when the pH of a solution is within approximately ± 1 pH unit of the pK_a. If the amounts of HA and A^- are equal, the pH is equal to the pK_a. A solution containing acetic acid (HA = $\text{CH}_3\text{-COOH}$) and acetate ($\text{A}^- = \text{CH}_3\text{-COO}^-$) with a pK_a of 4.8 resists a change in pH from pH 3.8 to 5.8, with maximum buffering at pH 4.8. At pH values less than the pK_a, the protonated acid form ($\text{CH}_3\text{-COOH}$) is the predominant species. At pH values greater than the pK_a, the deprotonated base form ($\text{CH}_3\text{-COO}^-$) is the predominant species in solution.

Titration curve of acetic acid

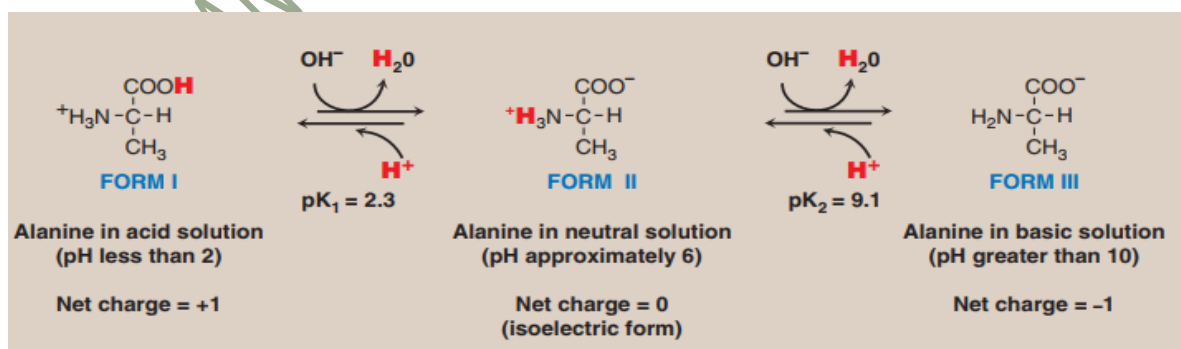


Figure-1. Ionic forms of alanine in acidic, neutral, and basic solutions

Dissociation of the carboxyl group: The titration curve of an amino acid can be analyzed in the same way as described for acetic acid. Consider alanine, for example, which contains both an α -carboxyl and an α -amino group. At a low (acidic) pH, both of these groups are protonated. As the pH of the solution is

raised, the – COOH group of Form I can dissociate by donating a proton to the medium. The release of a proton results in the formation of the carboxylate group, – COO⁻. This structure is shown as Form II, which is the dipolar form of the molecule. This form, also called a zwitterion, is the isoelectric form of alanine, that is, it has an overall (net) charge of zero.

Application of the Henderson-Hasselbalch equation: The dissociation constant of the carboxyl group of an amino acid is called K_1 , rather than K_a , because the molecule contains a second titratable group. The Henderson-Hasselbalch equation can be used to analyze the dissociation of the carboxyl group of alanine in the same way as described for acetic acid:

$K_1 = \frac{[H^+][II]}{[I]}$ where I is the fully protonated form of alanine, and II is the isoelectric form of alanine. This equation can be rearranged and converted to its logarithmic form to yield: $pH = pK_1 + \log \frac{[II]}{[I]}$

Dissociation of the amino group: The second titratable group of alanine is the amino(NH₃⁺) group. This is a much weaker acid than the – COOH group and, therefore, has a much smaller dissociation constant K_2 . Its pK_a is therefore larger. Release of a proton from the protonated amino group of Form II results in the fully deprotonated form of alanine, Form III.

pKs of alanine: The sequential dissociation of protons from the carboxyl and amino groups of alanine is summarized in **figure-1**. Each titratable group has a pK_a that is numerically equal to the pH at which exactly one half of the protons have been removed from that group. The pK_a for the most acidic group (COOH) is pK_1 , whereas the pK_a for the next most acidic group(NH₃⁺).

Titration curve of alanine: By applying the Henderson Hasselbalch equation to each dissociable acidic group, it is possible to calculate the complete titration curve of a weak acid. **Figure-2** shows the change in pH that occurs during the addition of base to the fully protonated form of alanine (I) to produce the completely deprotonated form (III).

a. Buffer pairs: The – COOH/– COO⁻ pair can serve as a buffer in the pH region around pK_1 , and the – NH₃⁺/– NH₂ pair can buffer in the region around pK_2 .

b. When pH = pK: When the pH is equal to pK_1 (2.3), equal amounts of Forms I and II of alanine exist in solution. When the pH is equal to pK_2 (9.1), equal amounts of Forms II and III are present in solution.

c. Isoelectric point: At neutral pH, alanine exists predominantly as the dipolar Form II in which the amino and carboxyl groups are ionized, but the net charge is zero. **The isoelectric point (pI) is the pH at which an amino acid is electrically neutral, that is, in which the sum of the positive charges equals the sum of the negative charges.**

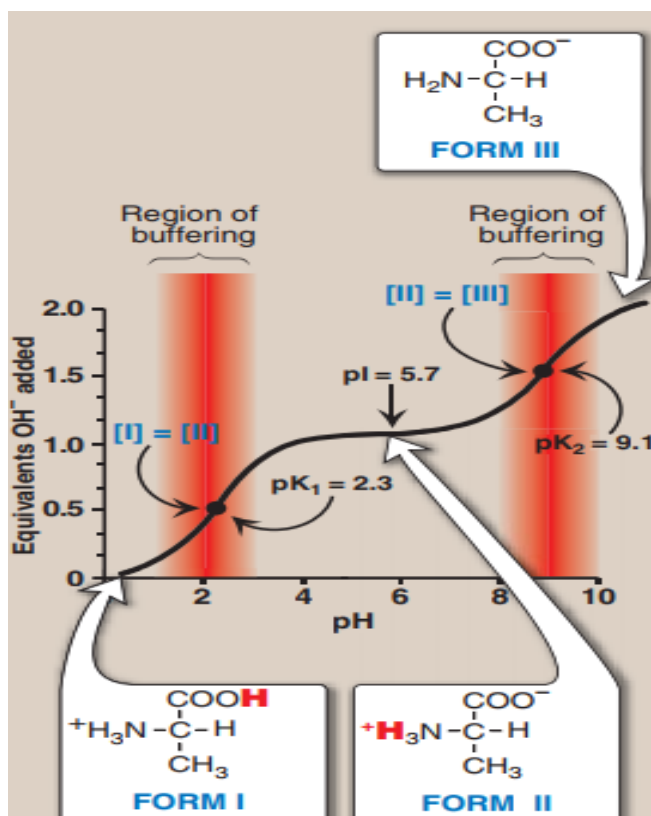


Figure-2. The titration curve of alanine

For an amino acid, such as alanine, that has only two dissociable hydrogens (one from the α -carboxyl and one from the α -amino group), the pI is the average of pK₁ and pK₂ ($pI = [2.3 + 9.1]/2 = 5.7$, see **Figure-1**). The pI is thus midway between pK₁ (2.3) and pK₂ (9.1). pI corresponds to the pH at which the Form II (with a net charge of zero) predominates, and at which there are also equal amounts of Forms I (net charge of +1) and III (net charge of -1).

Reference

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