BIOCHEMISTRY

Unit 1: AMINO ACIDS

Definition:
Amino acids are organic compounds containing amine (-NH₂) and carboxyl (-COOH) functional groups, along with side chain (R group) specific to each amino acids. They are the monomeric unit or building blocks of proteins.

General structure of amino acid

Twenty different amino acids are commonly found in proteins. The first to be discovered was Asparagine in 1806 by French scientists Vauquelin and Robiquet from asparagus juice hence the name asparagine. The last of the 20 to be found was threonine. It was not identified until 1938.

Nomenclature:
All amino acids have trivial or common names. These names were derived based either on the history of discovery, source of isolation, characteristics or taste. Glutamate was found in wheat gluten; tyrosine was first isolated from cheese (its name is derived from the Greek; tyros means cheese), glycine (Greek; glykos means sweet) was so named because of its sweet taste and Histidine was isolated from tissues (Greek; histos means tissue).

The common amino acids of proteins have been assigned three-letter abbreviations and one-letter symbols which are used as shorthand to indicate the composition and sequence of amino acids polymerized in proteins

All 20 of the common amino acids are α-amino acids. They have a carboxyl group and an amino group bonded to the same carbon atom (α-carbon). They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water. In amino acids that have a carbon chain attached to the α-carbon (as in lysine below) the carbons are labelled in order of α, β, γ, δ, ε and so on. In some amino acids the amine group is attached to the β or γ-carbon and these are therefore referred to as beta or gamma amino acids.
Structures of 20 Amino acids

Classification of Amino acids

- **Classification based on the structure**

1. **Aliphatic amino acid**: Those amino acids whose ‘R’ group contains a chain of carbon atoms
   a. Mono amino mono carboxylic
      i. Simple amino acids: Gly, Ala
      ii. Branched chain: Val, Leu, Ile
      iii. Hydroxyl group containing amino acids: Ser, Thr
      iv. Sulphur containing amino acids: Cys, Met
      v. Amide group containing amino acids: Asn, Gln
   b. Mono amino dicarboxylic acid: Asp, Glu
   c. Poly amino mono carboxylic acid: Arg, Lys
2. **Aromatic amino acids**: Those amino acids whose ‘R’ group has a benzene ring Phe, Tyr, Trp
3. **Heterocyclic amino acids**: The “R” group has a heterocyclic ring, i.e., any of the ring structures which contain different atoms: Trp, His
4. **Iminoacid**: Pro
Classification based on the polarity of the R group

1. Non polar amino acids: these are not soluble in water and thus hydrophobic in nature.
   They are Gly, Ala, Pro, Val, Leu, Ile, Met, Phe, Tyr, Trp
2. Polar amino acids: these are soluble in water and thus hydrophilic in nature.
   They are Ser, Thr, Cys, Asp, Glu, Lys, His, Arg, Asn, Gln

Classification based on the charge of the R group

1. Acidic or negatively charged: Those amino acids that contain a negative charge or an acidic group-Asp, Glu.
2. Basic or positively charged: Those amino acids that contain a positive charge or a basic group-Arg, Lys and His.
3. Neutral Those amino acids that do not contain any charge on the ‘R’ group

Classification based on nutritional value

1. Essential or indispensable amino acids: are those which cannot be synthesized by the body and must be included in the diet. They are essential to the body for proper growth and maintenance of the individual.
   They are Phe, Ile, Leu, Lys, Met, Thr, Trp, Val, His
2. Non-essential: are those which can be synthesized by the body and may not be included in the diet.
   They are Ala, Asn, Asp, Glu, Ser
3. Semi essential: are those which can be synthesized but not in sufficient quantity therefore has to be included. They are particularly essential in growing children, during pregnancy and for lactating mothers.
   They are Arg, His, Cys, Gly, Gln, Pro, Tyr

Classification based on metabolic fate

1. Glucogenic amino acids: these amino acids are used to synthesize glucose.
   They are Ala, Val, Ser, Thr, Gly, Met, Cys, Asn, Gln, Asp, Glu, His, Arg
2. Ketogenic amino acids: these amino acids are used to synthesize ketone bodies.
   They are Leu, Lys
3. Both glucogenic and ketogenic: these amino acids are used to synthesize both glucose and ketone bodies.
   They are Ile, Tyr, Phe, Trp

Classification based on whether used in protein synthesis or not

1. Standard amino acids: these are used in the protein synthesis. Also called as proteinogenic. E.g., all 20 amino acids
2. Non-standard amino acids: these occurs naturally in cells but not used in the protein synthesis. They are generated by modification of standard amino acids in the peptide molecule E.g., L-DOPA, GABA
Uncommon acids

- γ-carboxy glutamate: found in the blood clotting protein prothrombin.
- 4- hydroxyl proline: a derivative of proline found in plant cell wall proteins, collagen
- 5- hydroxyl lysine: derived from lysine found in collagen
- 6-N-Methyllysine: a constituent of myosin
- Desmosine: derivative of four lys residues found in elastin
- Thyroxine: iodinized derivative of Tyr found in thyroglobulin (thyroid gland)
In addition to 20 standard amino acids, two more amino acids were identified that take part in the protein synthesis. They are Selenocysteine and Pyrrolysine.

Selenocysteine (Sec, U) is a cysteine analogue with a selenium containing selenol group in place of sulfur containing thiol group. It is present in several enzymes for example glutathione peroxidase, glycine reductase.

\[
\begin{align*}
\text{Cysteine} & : \quad \text{H}_2\text{N}^+\text{C}==\text{COOH} \\
\text{Seleno cysteine} & : \quad \text{H}_3\text{N}^+\text{C}==\text{CO}_\text{SeH}
\end{align*}
\]

Pyrrolysine (Pyl, O) is an alpha amino acid that is used in the biosynthesis of proteins in some methanogenic archa and bacteria. It is not present in humans. Its pyrroline sidechain is similar to that of lysine.

\[
\begin{align*}
\text{Lysine} & : \quad \text{H}_2\text{N}^+\text{C}==\text{COOH} \\
\text{Pyrrolysine} & : \quad \text{H}_3\text{N}^+\text{CH}\text{C}==\text{NH}_2
\end{align*}
\]

Properties of Amino acids

General properties

- Amino acids are colourless, non-volatile crystalline solids melting and decomposing at temperature above 200°C.
- Most amino acids are soluble in water and insoluble in organic solvents.
- All amino acids except glycine are optically active.
- Amino acids (especially aromatic acids like phenyl alanine, tryptophan, and tyrosine) absorb light at characteristic wavelength. This spectroscopic properties can be used to measure its concentration in the unknown sample.
- All amino acids are amphoteric in nature. In the physiological pH, both carboxylic acid and amino group of α-aminoacids are completely iononized and therefore can act as an acid or base. Substances with this properties are said to be amphoteric and may also referred to as ampholytes.
Optical properties

For all the common amino acids except glycine, α-carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom (in case of glycine, the R group is another hydrogen atom). The α-carbon atom is thus a chiral center. Because of the tetrahedral arrangement of the bonding orbitals around the α-carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers. Since they are non-superimposable mirror images of each other the two forms represent a class of stereoisomers called enantiomers. All molecules with a chiral center are also optically active that is, they rotate plane-polarized light.

Special nomenclature has been developed to specify the absolute configuration of the four substituents of asymmetric carbon atoms. The absolute configurations of simple sugars and amino acids are specified by the D, L system. Based on the absolute configuration of the three-carbon sugar glyceraldehyde, a convention proposed by Emil Fischer in 1891. (Fischer knew what groups surrounded the asymmetric carbon of glyceraldehyde but had to guess at their absolute configuration; his guess was later confirmed by x-ray diffraction analysis.)

For all chiral compounds, stereoisomers having a configuration related to that of L-glyceraldehyde are designated L, and stereoisomers related to D-glyceraldehyde are designated D.

Nearly all biological compounds with a chiral center occur naturally in only one stereoisomeric form, either D or L. The amino acid residues in protein molecules are exclusively L stereoisomers. D-Amino acid residues have been found only in a few, generally small peptides, including some peptides of bacterial cell walls and certain peptide antibiotics.

Acid base properties

When an amino acid is dissolved in water, it exists in solution as the dipolar ion, or zwitterion (German for “hybrid ion”). A zwitterion can act as either an acid or proton donor (reaction a) or a base or proton acceptor (reaction b). Substances having this dual nature are amphoteric and are often called ampholytes.
Amino Acids Have Characteristic Titration Curves. Acid-base titration involves the gradual addition or removal of protons.

The above figure shows the titration curve of the diprotic form of glycine. The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine. Each of the two stages resembles in shape the titration curve of a monoprotic acid, such as acetic acid and can be analyzed in the same way.

At very low pH, the predominant ionic species of glycine is the fully protonated form, \(^{\text{NH}_2} \text{H}_3 \text{N-CH}_2-\text{COOH}\). At the midpoint in the first stage of the titration, in which the -COOH group of glycine loses its proton, equimolar concentrations of the proton-donor (\(^{\text{NH}_2} \text{H}_3 \text{N-CH}_2-\text{COOH}\)) and proton-acceptor (\(^{\text{NH}_2} \text{H}_3 \text{N-CH}_2-\text{COO}^\cdot\)) species are present.

At the midpoint of any titration, a point of inflection is reached where the pH is equal to the pKa of the protonated group being titrated. For glycine, the pH at the midpoint is 2.34, thus its -COOH group has a pKa of 2.34 (labeled pK1 in Fig.). pH and pKa are simply convenient notations for proton concentration and the equilibrium constant for ionization, respectively. The pKa is a measure of the...
tendency of a group to give up a proton, with that tendency decreasing tenfold as the pKa increases by one unit.)

As the titration proceeds, another important point is reached at pH 5.97. Here there is another point of inflection, at which removal of the first proton is essentially complete and removal of the second has just begun. At this pH glycine is present largely as the dipolar ion \( \text{H}_2\text{N-CH}_2\text{-COO}^- \).

The second stage of the titration corresponds to the removal of a proton from the -NH_3 group of glycine. The pH at the midpoint of this stage is 9.60, equal to the pKa for the -NH_3 group (labeled pK2 in Fig.). The titration is essentially complete at a pH of about 12, at which point the predominant form of glycine is \( \text{H}_2\text{N-CH}_2\text{-COO}^- \).

**Keypoints:**

From the titration curve of glycine we can derive several important pieces of information.

First, it gives a quantitative measure of the pKa of each of the two ionizing groups: (in case of Gly, 2.34 for the \(-\text{COO}^-\) group and 9.60 for the \(-\text{NH}_3^+\) group.)

The second piece of information provided by the titration curve of glycine is that this amino acid has two regions of buffering power. In acidic solution, the \( \text{COO}^- \) ion acquires a proton and the amino acid becomes an ammonium salt of the acid. In alkaline solutions the \( \text{NH}_3^+ \) loses a proton and the amino acid becomes the anion of the salt.

This is observed in the relatively flat portion of the curve, extending for approximately 1 pH unit on either side of the first pKa of 2.34, indicating that glycine is a good buffer near this pH. The other buffering zone is centered around pH 9.60. (Note that glycine is not a good buffer at the pH of intracellular fluid or blood, about 7.4.) Within the buffering ranges of glycine, the Henderson-Hasselbalch equation can be used to calculate the proportions of proton-donor and proton-acceptor species of glycine required to make a buffer at a given pH.

Another important piece of information derived from the titration curve of an amino acid is the relationship between its net electric charge and the pH of the solution. At pH 5.97, the point of inflection between the two stages in its titration curve, glycine is present predominantly as its dipolar form, fully ionized but with no net electric charge. The characteristic pH at which the net electric charge is zero is called the isoelectric point or isoelectric pH, designated pI. It can be calculated using the following formula,

\[
\text{pI} = \frac{1}{2} (\text{pK}_1 + \text{pK}_2)
\]

At any pH above its pI it has a net negative charge and will move toward the positive electrode (the anode) when placed in an electric field. At any pH below its pI, glycine has a net positive charge and will move toward the negative electrode (the cathode).

(The farther the pH of a glycine solution is from its isoelectric point, the greater the net electric charge of the population of glycine molecules. At pH 1.0, for example, glycine exists almost entirely as the form \( \text{H}_3\text{NOCH}_2\text{COOH} \), with a net positive charge of 1.0. At pH 2.34, where there is an equal mixture of \( \text{H}_3\text{NOCH}_2\text{COOH} \) and \( \text{H}_3\text{NOCH}_2\text{COO}^- \), the average or net positive charge is 0.5. The sign and the magnitude of the net charge of any amino acid at any pH can be predicted in the same way).
Chemical Reactions

Reactions due to –COOH group

1. Amino acid react with alkali like sodium hydroxide to give salt and water.

\[
\text{Amino acid} + \text{NaOH} \rightarrow \text{Amino acid salt} + \text{H}_2\text{O}
\]

2. Esterification with alcohol, to give an amino ester.

\[
\text{Amino acid} + \text{Ethanol} \rightarrow \text{Amino ester} + \text{H}_2\text{O}
\]

3. Amino acids can be reduced to amino alcohol in the presence of lithium borotetrahydride or lithium aluminumhydride.
4. Decarboxylation: Amino acids are decarboxylated to form corresponding amines. Trp is decarboxylated to form tryptamine. Similarly, Phe becomes phe ethylamine; histidine becomes histamine (this is involved in the local immune response; neurotransmitter); tyrosine becomes tyramine; glutamic acid becomes gamma amino butyric acid. (GABA). It is an important inhibitory neurotransmitter in central nervous system.

![Decarboxylation diagram](image)

5. Amino acids undergoes condensation to form its amides, this is important in the transport of NH₃ in the body.

![Condensation reaction diagram](image)

6. Amino acids react with hydrazine to form hydrazide. This is used to detect the C-terminal amino acid in proteins. It cleaves all peptide bonds and convert to their corresponding hydrazide except C-Terminal amino acid.

![Hydrazine reaction diagram](image)
Reactions due to –NH₂ group

1. Ninhydrin reaction: This is used as a color test to detect amino acids, also used in paper chromatography to visualise the separated amino acids. In forensics Ninhydrin is used to detect fingerprints because it reacts with amino acids from the proteins in skin cells transferred to the surface by the individual leaving the fingerprint.
2. Van Slyke's reaction: it's a method by which amino acids are measured upon the amount of N₂ released.

\[
\text{Amino acid} + \text{Nitrous acid} \rightarrow \alpha\text{-hydroxy acid} + \text{N}_2 + \text{H}_2\text{O}
\]

3. Carboxylation: amino acids can be carboxylated to form its carboxy acids.

\[
\text{Amino acid} + \text{CO}_2 \rightarrow \text{Carboxy acid} + \text{H}_2\text{O}
\]

4. With formaldehyde: amino acid react with formaldehyde to give monohydroxy (or N-hydroxy) methyl derivative. Further addition of formaldehyde yields dihydroxy (or N,N-Bishydroxy) methyl derivative.
5. Amino acids combine with hydrochloric acid to give salt like NH₃Cl

6. Sanger reaction: amino acid react with 2,4 dinitro fluorobenzene (DNFB) or 1 fluoro 2,4 Dinitrobenzene (FDNB) or Sanger’s reagent to give 2,4 dinitrophenyl derivative of corresponding amino acid. This was developed by Fredrick Sanger in 1945. It is used to detect N-terminal amino acid residue in protein sequencing.

7. Edman reaction: amino acids react with phenyl isothiocyanate (PITC) under mildly alkaline condition to give phenyl thiocarbamoyl derivative. This is less stable, so it forms a cyclic structure called phenyl thiohydantoin derivative of corresponding amino acids. This reaction is used to detect N-terminal amino acids in protein sequencing.
8. Oxidative deamination: an amino group is removed and corresponding α-keto acids are formed. This is converted to glucose or ketone bodies or completely oxidised.

**Reaction due to –SH group**

Cysteine is readily oxidized to form a covalently linked dimeric amino acid called cystine, in which two cysteine molecules or residues are joined by a disulfide bond. The disulfide-linked residues are strongly hydrophobic (nonpolar). Disulfide bonds play a special role in the structures of many proteins by forming covalent links between parts of a protein molecule or between two different polypeptide chains.
## Qualitative tests for amino acids

<table>
<thead>
<tr>
<th>Sl. NO</th>
<th>TEST</th>
<th>REAGENT</th>
<th>COLOR</th>
<th>AMINO ACIDS RESPONSIBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ninhydrin test</td>
<td>Ninhydrin reagent</td>
<td>purple</td>
<td>All α-amino acids</td>
</tr>
<tr>
<td>2</td>
<td>Xanthoproteic test</td>
<td>Boiling Con. HNO3</td>
<td>Yellow</td>
<td>Tyr, Phe, Trp</td>
</tr>
<tr>
<td>3</td>
<td>Lowry’s reaction</td>
<td>FC reagent</td>
<td>Blue</td>
<td>Tyr</td>
</tr>
<tr>
<td>4</td>
<td>Sakaguchi reaction</td>
<td>Alpha naphthol Sodium hypochlorite</td>
<td>Red</td>
<td>Arg</td>
</tr>
<tr>
<td>5</td>
<td>Millon’s test</td>
<td>Millions reagent (Solution of mercuric sulfate in sulfuric acid)</td>
<td>Red</td>
<td>Tyr, Trp</td>
</tr>
<tr>
<td>6</td>
<td>Folin’s test</td>
<td>Alkaline phosphor molybdo tungstic acid</td>
<td>Blue</td>
<td>Trp</td>
</tr>
<tr>
<td>7</td>
<td>Nitroprusside test</td>
<td>Sodium nitroprusside in the presence of excess NH4OH</td>
<td>Red</td>
<td>Cys</td>
</tr>
<tr>
<td>8</td>
<td>Lead acetate test</td>
<td>Lead acetate</td>
<td>Red</td>
<td>Cysteine and Cystine</td>
</tr>
<tr>
<td>9</td>
<td>Hopkin’s kole test</td>
<td>Glyoxylic acid and con. Sulfuric acid</td>
<td>Violet</td>
<td>Trp</td>
</tr>
<tr>
<td>10</td>
<td>Pauly test</td>
<td>Diazotized sulfanilic acid under alkaline condition</td>
<td>Red</td>
<td>Tyr, His, Arg</td>
</tr>
<tr>
<td>11</td>
<td>Ehrlich test</td>
<td>p-dimethylaminobenzaldehyde (DMAB)</td>
<td>Blue</td>
<td>Trp</td>
</tr>
<tr>
<td>12</td>
<td>Sullivan’s test</td>
<td>Sodium 1,2 naphthoquinone-4-sulfonate and NaOH</td>
<td>Red</td>
<td>Cys</td>
</tr>
<tr>
<td>13</td>
<td>Biuret test</td>
<td>Alkaline coppersulfate</td>
<td>Blue</td>
<td>dipeptide</td>
</tr>
</tbody>
</table>

## Functions (or Importance) of amino acids

1. They are the monomers of proteins. Amino acids are linked to each other by peptide bond to form proteins.
2. Certain derivations of amino acids, especially of glutamate, are used as surfactants in mild soaps and shampoos. D-Phenylglycine and D-hydroxyphenylglycine are intermediates used for the chemical synthesis of β-lactam antibiotics (e.g., synthetic versions of penicillin). Aspartame is a sweetener prepared from the individual component amino acids aspartic acid and phenylalanine.
3. Several α-amino acids (or their derivatives) act as chemical messengers. For example, γ-aminobutyric acid (gamma-aminobutyric acid, or GABA; a derivative of glutamic acid), serotonin and melatonin (derivatives of tryptophan), and histamine (synthesized from histidine) are neurotransmitters. Thyroxine (a tyrosine derivative produced in the thyroid gland of animals) and indole acetic acid (a tryptophan derivative found in plants) are two examples of hormones.
4. Some amino acids (Glucogenic amino acids) are used as precursors to synthesize carbohydrates.
5. They act as buffers and it helps in maintaining the pH of the medium. As the pH increases it donates H+ ions and as the pH decreases it accepts H+ ions.
6. Some amino acids serve as nitrogen storage. (Asn, Gln)
7. Amino acids and their derivative help in nerve transmission, cell growth, biosynthesis of purines and pyrimidines.
8. Some aromatic rings of Phe, Tyr, Trp help in electron transfer (in ATP synthesis).
9. Our body uses the amino acids like Arg to make nitric oxide which helps lower the blood pressure by relaxing muscles in our blood vessels.
10. Under certain conditions amino acids can be metabolized for energy.
11. Amino acids (proteins) function as biological catalyst.
12. Amino acids are used therapeutically for nutritional and pharmaceutical purposes. For example L-dihydroxyphenylalanine (L-dopa) for Parkinson disease; glutamine and histidine to treat peptic ulcers; and arginine, citrulline, and ornithine to treat liver diseases.
13. Several standard and nonstandard amino acids often are vital metabolic intermediates. Important examples of this are the amino acids arginine, citrulline, and ornithine, which are all components of the urea cycle. The synthesis of urea is the principal mechanism for the removal of nitrogenous waste.
14. Amino acids are precursors of a variety of complex nitrogen-containing molecules. Prominent among these are the nitrogenous base components of nucleotides and the nucleic acids (DNA and RNA). Furthermore, there are complex amino-acid derived cofactors such as heme and chlorophyll. Heme is the iron-containing organic group required for the biological activity of vitally important proteins such as the oxygen-carrying hemoglobin and the electron-transporting cytochrome c. Chlorophyll is a pigment required for photosynthesis.