Introduction

Amino acids combine many of the properties and reactions of both amines and carboxylic acids.

The proximity of a basic amino group to an acidic carboxyl group in the same molecule also results in some unique properties and reactions.

The side chains of some amino acids also have functional groups that influence the properties and reactions of the specific amino acid.
Properties of α-Amino Acids

Unique Behaviour of α-Amino Acids

Though amino acids are commonly represented as covalently bonded structures (H₂NCHRCO₂H) containing an amino group and a carboxyl group, they have properties that are not consistent with these structures:

(a) In contrast to amines and carboxylic acids, the amino acids are non-volatile crystalline solids which melt with decomposition at fairly high temperatures; > 200 °C (glycine decomposes at 233 °C). This implies greater intermolecular attraction. Contrast this with non-ionic derivatives of amino acids, which melt around 100 °C.

(b) Amino acids are insoluble in non-polar solvents like diethyl ether, while they are appreciably soluble in water. Since like dissolves like, this implies greater polarity or ionic character in the amino acids.

(c) Aqueous solutions of amino acids behave like solutions of high dipole moment. Amino acids have much larger dipole moments (μ) than simple amines or acids. This implies greater charge separation in amino acid molecules.

(d) Amino acids are less acidic than most carboxylic acids and are less basic than most amines. Their Kₐ and Kₐ are ridiculously low for –COOH and –NH₂ groups. Glycine, for example, has Kₐ = 1.6 x 10⁻¹⁰ and Kₐ = 2.5 x 10⁻¹², whereas most carboxylic acids have Kₐ values of about 10⁻⁵ and most aliphatic amines have Kₐ values of about 10⁻⁴.
Properties of α-Amino Acids
The True Structure of α-Amino Acids

• All these properties are attributed to the fact that the amino acids exist in the form of stable ionic salts as dipolar ions or zwitterions. This is as a result of internal salt formation by a proton transfer from the acidic carboxyl group to the basic amino group.

• Note that as much as the amino acids exist as dipolar ions, by and large, the charges cancel and the whole molecule is neutral (overall zero charge).

• Molecules that bear charged groups of opposite polarity are known as zwitterions or dipolar ions.

Reactive form

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{C} \quad \text{C} \quad \text{OH} \\
\text{R} & \\
\end{align*}
\]

Zwitterion form of amino acids

\[
\begin{align*}
\text{H}_3\text{N} & \quad \text{C} \quad \text{C} \quad \text{O}^@ \\
\text{R} & \\
\end{align*}
\]

Dipolar ionic salt

Resting form
Acid-Base Behaviour of $\alpha$-Amino Acids

• Although the neutral amino acid exits in the solid state mainly as the zwitterionic form, there are other species in which amino acids may exist in aqueous solution.
• The predominant form at any particular point is dependent on the pH of the solution in which it is dissolved.
• For example, in an aqueous solution of a strong acid, the zwitterionic form would be protonated to form a cationic carboxylic acid. The zwitterion thus acts as a base.

In acidic solution

\[ +\text{H}_3\text{NCHRCOO}^- + \text{H}^+ \rightarrow +\text{H}_3\text{NCHRCOOH} \]

Zwitterion (Basic)  Acidic
Acid-Base Behaviour of $\alpha$-Amino Acids

- In a strongly basic aqueous solution, however, the zwitterion donates a proton to a strong base to form an anionic amino carboxylate, thus acting as an acid.

In basic solution

\[ ^{+}\text{H}_3\text{NCHRCOO}^- + \text{OH}^- \rightarrow \text{H}_2\text{NCHRCOO}^- \]

- Thus, amino acids can act as bases and as acids and are therefore amphoteric compounds.
- Since they exist in the zwitterionic form, amino acids act as acids through the ammonium ion and as bases through the carboxylate anion.
Acid-Base Behaviour of α-Amino Acids: Isoelectric Point

• As one sweeps the entire pH range starting from low to high pH, there exists an intermediate pH whereby the amino acid exists exclusively in the zwitterionic form.

• This intermediate pH is called the isoelectric pH or isoelectric point (pI).

  ![Diagram showing the pH range and transitions between different charged forms of an amino acid.](image)

• The Isoelectric point is the pH at which the amino acid bears no net charge; it corresponds to the pH at which the concentration of the zwitterion is a maximum.
Isoelectric Point of Amino Acids

- The isoelectric point is the pH of an aqueous solution of an amino acid at which the molecules on average have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged groups.

- The isoelectric point can be found graphically by sweeping the entire pH range of the amino acid.
Classification of $\alpha$-Amino Acids based on their Isoelectric Point

- The standard amino acids can be grouped into three categories: amino acids with acidic, neutral and basic isoelectric points.
- This classification predictably conforms to the acid-base character of the side chains of these amino acids.
- Neutral amino acids have near neutral isoelectric points around 6: (5.0 to 6.3). They have no strongly acidic or basic side chains. Their isoelectric points are slightly acidic (from about 5 to 6), because the $\text{–NH}_3^+$ group is slightly more acidic than the $\text{–COO}^-$ group is basic.
Classification of $\alpha$-Amino Acids based on their Isoelectric Point

- **Acidic amino acids** have acidic isoelectric points around 3: aspartic acid (2.8) and glutamic acid (3.2).

\[
\begin{align*}
\text{Aspartic anion} & : & \text{HOOC} - & \text{COO}^- & +\text{NH}_3 \\
\text{Glutamic acid} & : & \text{HOOC} - & \text{COO}^- & +\text{NH}_3
\end{align*}
\]

- **Basic amino acids** have basic isoelectric points: lysine (9.7), arginine (10.8) and histidine (7.6)

\[
\begin{align*}
\text{Histidine} & : & \text{H}_2\text{N} - & \text{NH} & \text{COO}^- & +\text{NH}_3 \\
\text{Arginine} & : & \text{HN} & \text{N} & \text{NH}_2 & \text{COO}^- & +\text{NH}_3 \\
\text{Lysine} & : & \text{H}_2\text{N} & \text{COO}^- & +\text{NH}_3
\end{align*}
\]
pH-Dependent Molecular Charge Changes of α-Amino Acids vs Isoelectric Point

• By varying the pH of an aqueous solution of an amino acid, the charge on an amino acid can be controlled. Glycine bears a negative charge in basic solution (high pH), a positive charge in acidic solution (low pH), and at near neutrality the ion is dipolar with a net charge of zero.

\[
\begin{align*}
\text{Acidic solution} & \quad \text{Basic solution} \\
\text{Low pH} & \quad \text{Sweep the entire pH range by adding a base} \quad \text{High pH}
\end{align*}
\]

Overall charge

Electric Field

- **Beginning**
  - Migrates to Cathode
  - Cationic in acid
  - Doesn't Migrate
  - Neutral

- **End**
  - Migrates to Anode
  - Anionic in base
Electrophoresis: pH-Dependent Charge Changes of α-Amino Acids

• One of the most practical applications of amino acids exploits this pH-dependent variation of charge. This ability to control the charge on an amino acid has been exploited in the separation and identification of amino acids in mixtures using the technique of electrophoresis.
• Electrophoresis is a separation or purification technique that relies on the movement of charged particles in an electric field.
• Electrophoresis can be applied in the separation of amino acids by taking advantage of the pH-dependent charge differences on amino acids. These charge differences arise out of the differences in isoelectric points of the various amino acids. These have been taken advantage of in electrophoresis to separate mixtures of amino acids.
Electrophoresis
Separation of Mixtures of $\alpha$-Amino Acids

- In electrophoresis, a streak of the amino acid mixture is placed at the centre of a piece of filter paper wet with a buffer solution.
- The opposite ends of the strip are placed in separate compartments containing the buffer and each compartment is connected to a source of direct electric current by an electrode.

Figure 21 - Apparatus for paper electrophoresis.
Electrophoresis: Separation of Mixtures of α-Amino Acids

• Consider the electrophoresis of a mixture containing alanine, aspartic acid and lysine in a buffer that matches the isoelectric point of alanine (pH 6.0).
• Alanine is at its isoelectric point, in its polar zwitterionic form with a net charge of zero.
• A pH of 6 is more acidic than the isoelectric point for lysine (9.7), so lysine is in the cationic form. Aspartic acid has an isoelectric pH of 2.8, so it is in the anionic form.
Electrophoresis: Separation of Mixtures of $\alpha$-Amino Acids

- When a voltage is applied to a mixture of alanine, lysine, and aspartic acid at pH 6, alanine does not move. Lysine migrates towards the cathode, and aspartic acid migrates towards the anode.
Synthesis of Enantiopure α-Amino Acids
Techniques for Accessing Enantiomerically Pure Amino Acids

- The proteinogenic L-α-amino acids are produced industrially by:
  
  (i) Biotechnology through fermentation methods using genetically engineered strains of micro-organisms (bacteria),
  
  (ii) Extraction from hydrosylates of natural proteins (animal and plant proteins),
  
  (iii) Enantioselective synthesis on a large scale and
  
  (iv) Enzymatic (aminoacylase) resolution of racemic mixtures of α-amino acids.
Production of Amino Acids through Fermentation

- Biotechnological processes that produce L-α-amino acids through fermentation require the development of the appropriate strains of bacteria to ferment a mixture of sugar (glucose or sucrose), ammonia, growth factors and minerals to the L-α-amino acids.
- To manufacture high quality L-α-amino acids, it is of prime importance to employ a strain of a micro-organism with good production efficiency and minimum by-products.
- The fermentation medium consists of glucose as a carbon source, ammonia as a nitrogen source, a small amount of minerals and vitamins as growth factors.
- The principal application of L-α-amino acids is as food additives.
Production of Amino Acids through Hydrolysis of Natural Proteins

• Naturally occurring L-α-amino acids can also be obtained by hydrolysis of natural peptides or proteins, followed by the chromatographic separation of the individual amino acids on ion-exchange resins.
• Considering the complexity of many of the proteins and the diversity of amino acid residues that comprise them, separation of the component amino acids in the hydrosylate can be quite tedious.

Reasons why the isolation of α-amino acids from nature is considered uneconomical over their direct synthesis:
• There are no true natural sources of free amino acids. Indeed, most natural amino acids exist in nature linked either to other amino acids as peptides or to other non-peptide units in conjugate proteins.
Isolation of $\alpha$-Amino Acids from Nature vs Direct Synthesis

- To isolate any specific amino acid from nature, a natural peptide in which the targeted amino acid is present has to be identified. The identification and isolation of such a peptide source can be onerous task.
- Considering that peptides occur in plant and animal cells, isolation of measurable quantities of the target amino acid will require mass destruction of the plant or animals species leading to an ecological imbalance.
- There are a variety of enantioselective methods for synthesizing amino acids on a large scale that provide enantiomerically pure amino acids.
- Since natural peptides are composed of L-amino acids only, in the event D-amino acids are desired for specific applications, they can only be obtained through synthesis.
Synthesis of Enantiopure α-Amino Acids

The chiral pool synthesis of D-alanine from L-lactic acid can be achieved via conversion to p-toluenesulphonate.

\[
\begin{align*}
\text{L-Lactic acid} & \xrightarrow{\text{PhCH}_2\text{OH}, \text{DCC}} \text{tosylate} & \xrightarrow{\text{Et}_3\text{N}} \text{D-Alanine}
\end{align*}
\]

SN₂ substitution on the tosylate with NaN₃ leads to inversion of configuration at the chiral centre.

Any synthetic success dictates for a judicious introduction of protecting groups in the starting material to assist subsequent functionalization to the target molecule.
The chiral pool synthesis of L-alanine from L-lactic acid can be achieved via double inversion through an iodide.

Note that successive SN$_2$ substitutions lead to retention of configuration at the chiral centre.

Note that the iodination with PPh$_3$/I$_2$ follows an SN$_2$ pathway leading to inversion of configuration during the formation of the iodide from benzyl lactate.
Synthesis of Enantiopure \(\alpha\)-Amino Acids

Synthesis of Amino Acids through Enolate Alkylation

- A chiral auxiliary physically blocks one of two possible trajectories of attack, leaving only one trajectory open.
- The enolate is alkylated from the least sterically hindered face.
- Illustrated below are examples of chiral auxiliary approaches in the synthesis of D-valine