PROPERTIES OF Amino Acids

![Diagram of pK values for glycine](image)
Acid-Base Properties of Amino Acids

Alanine

\[
\begin{align*}
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\end{align*}
\]

\(pK_a = 2.4\)  \(pK_a = 9.6\)

Aspartic acid

\[
\begin{align*}
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\text{C} & \text{H}_2 \\
\text{C} & \text{H}_3 \\
\end{align*}
\]

\(pK_a = 2.2\)  \(pK_a = 4.0\)  \(pK_a = 10\)
Acid-Base Properties of **Amino Acids**

- **Lysine**
  - $\text{O} - \text{C} - \text{NH}_3$
  - $\text{H} - \text{C} - \text{NH}_3$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{NH}_3$
  - (+2)
  - $\text{pK}_{a1} = 2.2$

- **Aspartic Acid**
  - $\text{O} - \text{C} - \text{O}^-$
  - $\text{H} - \text{C} - \text{NH}_3$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{NH}_3$
  - (+1)
  - $\text{pK}_{a2} = 9.0$

- **Glutamic Acid**
  - $\text{O} - \text{C} - \text{O}^-$
  - $\text{H} - \text{C} - \text{NH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{CH}_2$
  - $\text{NH}_3$
  - (0)
  - $\text{pK}_{a3} = 10.6$

- **Isoelectric point (pI)**
  - The pH at which the sum of all charges on the amino acid equals the value of zero (charge).

- **Acid-Base Properties**
  - If the pH is **above** the pI, the overall charge is negative.

- If the pH is **below** the pI, the overall charge is positive.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pI</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Asp</td>
<td>3.1</td>
<td>+</td>
</tr>
<tr>
<td>Lys</td>
<td>9.8</td>
<td>-</td>
</tr>
</tbody>
</table>

1/21/2002
Separating Amino Acids - Ion Exchange Chromatography

- The discovery of the difference in charge of amino acids occurred many years ago.
- Scientist at the Rockefeller Institute in New York, used various types of ion exchange chromatography columns to separate amino acids.
- Ion exchange was first used by a Russian botanist named Tswett. He used it to separate plant pigments.
- Chromatography by definition: chromo = color, graphy = writing. “Color writing”.
- All chromatography depend upon the concept that individual components within a mixture will react differently with the environment.

Separating Amino Acids - Ion Exchange Chromatography

- All chromatography is a process of separating compounds using a mobile (moving) phase and a stationary (nonmoving) phase.
- The researchers at Rockefeller Institute utilized polystyrene resin with the ion exchange chromatography principle.
- The ion exchange resin serves as the stationary phase.
- A solution of the various amino acids is the mobile phase.
- The type of resin used was polystyrene crosslinked with divinyl benzene.
Separating Amino Acids - Ion Exchange Chromatography

Crosslinking serves a physical benefit by controlling the amount of shrinkage or expansion the resin can go through.

Chemically place functional group on the polystyrene resins.
- Quaternary amine with a positive charge.
- Resin, chemically inert, solid support, with a positive charge
- Anion exchanger-can combine with negative ions (anions).
Separating Amino Acids - Ion Exchange Chromatography

- Chemically place sulfonic acid functional group on the polystyrene resin.
- Cation exchanger-can combine with positive ions (cation).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pI</th>
<th>pH= 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>6.0</td>
<td>0</td>
</tr>
<tr>
<td>Asp</td>
<td>3.1</td>
<td>-</td>
</tr>
<tr>
<td>Lys</td>
<td>9.8</td>
<td>+</td>
</tr>
</tbody>
</table>

Negatively charged resin

Have a mixture of the three amino acids all at a pH of 11.0. What will happen?

What will happen at a pH of 1.0?

What will happen if a solution at a pH of 3.1 is passed through the column?

What will happen if a solution at a pH of 6.0 is passed through the column?

What will happen if a solution at a pH of 9.8 is passed through the column?
Separating Amino Acids - Ninhydrin (triketohydrindene hydrate)

Rubemann's compound

\[
\text{ninthydrin} \quad \text{X} \quad 2
\]

\[
\text{Rubemann's purple}
\]

Protein mixture is added to column containing cross-linked polymer.

Protein molecules separate by size; larger molecules pass more freely, appearing in the earlier fractions.

Rubemann's compound

Heat

Amino acid

\[
\text{R-C} = \text{O} + \text{H}_2\text{O}
\]

\[
\text{Rubemann's purple}
\]
Separating Amino Acids - Ninhydrin (triketohydrindene hydrate)

Separating Amino Acids - Paper Chromatography

Aspartic acid pH 7.0 hydrophilic
Leucine pH 7.0 hydrophobic
Separating *Amino Acids - Paper Chromatography*

Stationary phase - water binds to the cellulose.
Mobile phase - butanol covers over the paper, taking amino acids along at different solubility.
Aspartic acid is more hydrophilic (polar) and more distributed in aqueous water.
Leucine is more nonpolar and will be more distributed in the butanol solvent.

- Chromatography paper after being sprayed with ninhydrin.
- The spot at the point of origin is where the amino acid mixture was applied.

Separating *Amino Acids - Thin Layer Chromatography*

- Thin layer of cellulose on a solid support usually glass or plastic.
- Paper is quite porous and fibrous and tends to overlap the amino acids as they migrate up the paper.
- Advantages to using thin layer is better separation of individual amino acids and can use smaller quantities of sample.
Separating **Amino Acids by Electrophoresis**

- The movement of a charged particle in an electrical field.
- The negative electrode is the cathode, attracts cations.
- The positive electrode is the anode, attracts anions.

If the pH is **above** the pl the overall charge is negative.
If the pH is **below** the pl the overall charge is positive.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pl</th>
<th>pH = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>6.0</td>
<td>+</td>
</tr>
<tr>
<td>Asp</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Lys</td>
<td>9.8</td>
<td>++</td>
</tr>
</tbody>
</table>