27.3
Acid-Base Behavior of Amino Acids
While their name implies that amino acids are compounds that contain an $\text{--NH}_2$ group and a $\text{--CO}_2\text{H}$ group, these groups are actually present as $\text{--NH}_3^+$ and $\text{--CO}_2^-$ respectively.

How do we know this?
Properties of Glycine

The properties of glycine:

- High melting point (when heated to 233°C it decomposes before it melts)
- Solubility: soluble in water; not soluble in nonpolar solvent

more consistent with this than this

\[
\begin{align*}
\text{H}_3\text{NCH}_2\text{C}&\text{-}\text{O}\text{:} \\
\text{H}_2\text{NCH}_2\text{C}&\text{-}\text{O}\text{H}
\end{align*}
\]
Properties of Glycine

The properties of glycine:

- high melting point (when heated to 233°C it decomposes before it melts)
- solubility: soluble in water; not soluble in nonpolar solvent

more consistent with this

\[
\text{H}_3\text{NCH}_2\text{C}=-\text{O}^+ \quad \text{or} \quad \text{H}_2\text{NCH}_2\text{C}=\text{O}^-\]

called a \textit{zwitterion} or \textit{dipolar ion}
Acid-Base Properties of Glycine

The zwitterionic structure of glycine also follows from considering its acid-base properties. A good way to think about this is to start with the structure of glycine in strongly acidic solution, say pH = 1. At pH = 1, glycine exists in its protonated form (a monocation).
Acid-Base Properties of Glycine

The zwitterionic structure of glycine also follows from considering its acid-base properties.

A good way to think about this is to start with the structure of glycine in strongly acidic solution, say pH = 1.

At pH = 1, glycine exists in its protonated form (a monocation).

\[
\begin{align*}
\text{H}_3\text{NCH}_2\text{C} & \quad \text{OH} \\
\end{align*}
\]
Acid-Base Properties of Glycine

Now ask yourself "As the pH is raised, which is the first proton to be removed? Is it the proton attached to the positively charged nitrogen, or is it the proton of the carboxyl group?"

You can choose between them by estimating their respective pKₐs.

\[
\text{H}_3\text{NCH}_2\text{C}--\text{OH}
\]
Now ask yourself "As the pH is raised, which is the first proton to be removed? Is it the proton attached to the positively charged nitrogen, or is it the proton of the carboxyl group?"

You can choose between them by estimating their respective $pK_a$s.

**Typical ammonium ion:** $pK_a \approx 9$

**Typical carboxylic acid:** $pK_a \approx 5$
Acid-Base Properties of Glycine

The more acidic proton belongs to the CO$_2$H group. It is the first one removed as the pH is raised.

H$_3$NCH$_2$C—OH

typical carboxylic acid: $pK_a \sim 5$
Therefore, the more stable neutral form of glycine is the zwitterion.

\[
\begin{align*}
\text{H}_3\text{NCH}_2\text{C} & \quad \overset{+}{\text{O}} \quad \overset{-}{\text{O}} \\
\text{H}_3\text{NCH}_2\text{C} & \quad \overset{+}{\text{O}} \quad \overset{-}{\text{OH}}
\end{align*}
\]
The measured $pK_a$ of glycine is 2.34.

Glycine is stronger than a typical carboxylic acid because the positively charged N acts as an electron-withdrawing, acid-strengthening substituent on the $\alpha$ carbon.
Acid-Base Properties of Glycine

A proton attached to N in the zwitterionic form of nitrogen can be removed as the pH is increased further.

\[ \text{H}_3\text{NCH}_2\text{C}--\overset{\text{O}^-}{\text{O}}\longrightarrow \text{H}_2\dot{\text{N}}\text{CH}_2\text{C}--\overset{\text{O}^-}{\text{O}} \]

The pK\textsubscript{a} for removal of this proton is 9.60. This value is about the same as that for NH\textsubscript{4}\textsuperscript{+} (9.3).
Isoelectric Point $p_I$

The pH at which the concentration of the zwitterion is a maximum is called the isoelectric point. Its numerical value is the average of the two $pK_a$s.

The $p_I$ of glycine is 5.97.
One way in which amino acids differ is in respect to their acid-base properties. This is the basis for certain experimental methods for separating and identifying them.

Just as important, the difference in acid-base properties among various side chains affects the properties of the proteins that contain them.

Table 27.2 gives $pK_a$ and $pI$ values for amino acids with neutral side chains.
Table 27.2
Amino Acids with Neutral Side Chains

Glycine

\[
\begin{array}{c}
\text{H}_3\text{N} & \text{C} & \text{C} & \text{O}^- \\
\text{H} & + & \text{O} & \text{H} \\
\end{array}
\]

\[
pK_{a1} = 2.34, \quad pK_{a2} = 9.60, \quad pI = 5.97
\]
Table 27.2
Amino Acids with Neutral Side Chains

Alanine

\[ \text{H}_3\text{N}^+\text{C} \equiv \text{C} - \text{O}^- \]

\[ \text{CH}_3 \]

\( pK_{a1} = 2.34 \)

\( pK_{a2} = 9.69 \)

\( p/ = 6.00 \)
Table 27.2
Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pK&lt;sub&gt;a1&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a2&lt;/sub&gt;</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>2.32</td>
<td>9.62</td>
<td>5.96</td>
</tr>
</tbody>
</table>

Valine: \[
\begin{array}{c}
\text{H} \quad \text{N} \\
\text{C} \quad \text{C} \\
\text{O} \\
\text{CH(CH}_3\text{)}_2
\end{array}
\]

\[\text{pK}_{a1} = 2.32, \quad \text{pK}_{a2} = 9.62, \quad \text{pI} = 5.96\]
Table 27.2
Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pK&lt;sub&gt;a1&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a2&lt;/sub&gt;</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>2.36</td>
<td>9.60</td>
<td>5.98</td>
</tr>
</tbody>
</table>

Chemical structure of Leucine:

\[
\text{H}_3\text{N}+\text{C}==\text{C}==\text{O}\text{O}^-\text{CH}_2\text{CH(\text{CH}_3)_2}
\]

- pK<sub>a1</sub> = 2.36
- pK<sub>a2</sub> = 9.60
- pI = 5.98
### Table 27.2
Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pK&lt;sub&gt;a1&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a2&lt;/sub&gt;</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine</td>
<td>2.36</td>
<td>9.60</td>
<td>5.98</td>
</tr>
</tbody>
</table>

![Isoleucine structure](image)

**Isoleucine**

\[
\begin{array}{c}
\text{H}_3\text{N} \quad \text{C} \quad \text{C} \quad \text{O}^- \\
\text{CH}_3\text{CHCH}_2\text{CH}_3
\end{array}
\]

- \( pK_{a1} = 2.36 \)
- \( pK_{a2} = 9.60 \)
- \( pI = 5.98 \)
### Table 27.2

Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>( pK_a ) 1</th>
<th>( pK_a ) 2</th>
<th>( pI )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>2.28</td>
<td>9.21</td>
<td>5.74</td>
</tr>
</tbody>
</table>

Methionine: \( \text{CH}_3\text{SCH}_2\text{CH}_2 \)

\[
\text{H}_3\text{N}^-\text{C}^-\text{C}^-\text{O}^- + \text{H}_2\text{O} \rightarrow \text{H}_3\text{N}^-\text{C}^-\text{C}^-\text{O}^- + \text{H}_2\text{O}^+ 
\]

\[
pK_{a1} = 2.28 \quad pK_{a2} = 9.21 \quad pI = 5.74
\]
Table 27.2
Amino Acids with Neutral Side Chains

Proline

\[
\begin{array}{c}
\text{H}_2\text{N} + \text{C} = \text{C} = \text{C} - \text{O}^{-} \\
\text{H}_2\text{C} \quad \text{C} \quad \text{CH}_2 \\
\text{H}_2
\end{array}
\]

\[pK_{a1} = 1.99\]
\[pK_{a2} = 10.60\]
\[pI = 6.30\]
### Table 27.2
Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pK&lt;sub&gt;a1&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a2&lt;/sub&gt;</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>1.83</td>
<td>9.13</td>
<td>5.48</td>
</tr>
</tbody>
</table>
Table 27.2
Amino Acids with Neutral Side Chains

Tryptophan

\[
\begin{align*}
\text{H}_3\text{N} & \quad \text{C} & \quad \text{C} & \quad \text{O}^- \\
\text{CH}_2 & & & & \\
\text{N} & & & & \\
\text{H} & & & &
\end{align*}
\]

\[pK_{a1} = 2.83\]
\[pK_{a2} = 9.39\]
\[pI = 5.89\]
Table 27.2
Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>$pK_{a1}$</th>
<th>$pK_{a2}$</th>
<th>$pI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>2.02</td>
<td>8.80</td>
<td>5.41</td>
</tr>
</tbody>
</table>
Table 27.2
Amino Acids with Neutral Side Chains

Glutamine

\[
\begin{align*}
\text{H}_3\text{N} & \quad \text{C} \quad \text{C} \quad \text{O}^- \\
\text{H}_2\text{NCCCH}_2\text{CH}_2 & \\
\text{O} &
\end{align*}
\]

\begin{align*}
pK_{a1} &= 2.17 \\
pK_{a2} &= 9.13 \\
\rho_l &= 5.65
\end{align*}
Table 27.2
Amino Acids with Neutral Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pK&lt;sub&gt;a1&lt;/sub&gt;</th>
<th>pK&lt;sub&gt;a2&lt;/sub&gt;</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>2.21</td>
<td>9.15</td>
<td>5.68</td>
</tr>
</tbody>
</table>

![Structural diagram of Serine]

\[
\text{Serine: } H_3N \text{C} = \text{C} = \text{O}^- + \text{CH}_2\text{OH}
\]

\[
pK_{a1} = 2.21, \quad pK_{a2} = 9.15, \quad pI = 5.68
\]
Table 27.2
Amino Acids with Neutral Side Chains

Threonine

\[
\begin{array}{c}
\text{H}_3\text{N} \\
\text{C} \\
\text{C} \\
\text{O} \\
\text{CH}_3\text{CHOH}
\end{array}
\]

\[
pK_{a1} = 2.09 \quad pK_{a2} = 9.10 \quad p/ = 5.60
\]
**Table 27.3**

Amino Acids with Ionizable Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>$pK_{a1}$</th>
<th>$pK_{a2}$</th>
<th>$pK_{a3}$</th>
<th>$pI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1.88</td>
<td>3.65</td>
<td>9.60</td>
<td>2.77</td>
</tr>
</tbody>
</table>

For amino acids with acidic side chains, $pI$ is the average of $pK_{a1}$ and $pK_{a2}$.
Table 27.3
Amino Acids with Ionizable Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt; Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>pK&lt;sub&gt;a1&lt;/sub&gt; = 2.19, pK&lt;sub&gt;a2&lt;/sub&gt; = 4.25, pK&lt;sub&gt;a3&lt;/sub&gt; = 9.67, pI = 3.22</td>
</tr>
</tbody>
</table>

[Diagram of Glutamic acid structure]
Table 27.3
Amino Acids with Ionizable Side Chains

Tyrosine

\[
\text{H}_3\text{N}^+\text{C}
\begin{array}{c}
\text{C} \\
\text{C} \\
\text{O}^-
\end{array}
\text{CH}_2
\text{O}
\text{OH}
\]

\[\text{pK}_{a1} = 2.20\]
\[\text{pK}_{a2} = 9.11\]
\[\text{pK}_{a3} = 10.07\]
\[\text{pI} = 5.66\]
Table 27.3
Amino Acids with Ionizable Side Chains

Cysteine:  
\[
\begin{array}{c}
\text{H}_3\text{N} + \text{C} - \text{C} - \text{O}^- \\
\text{CH}_2\text{SH}
\end{array}
\]

- \( pK_{a1} = 1.96 \)
- \( pK_{a2} = 8.18 \)
- \( pK_{a3} = 10.28 \)
- \( pI = 5.07 \)
Table 27.3
Amino Acids with Ionizable Side Chains

For amino acids with basic side chains, pI is the average of pK\textsubscript{a2} and pK\textsubscript{a3}.
### Table 27.3
Amino Acids with Ionizable Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>$pK_{a1} = 2.17$</td>
</tr>
<tr>
<td></td>
<td>$pK_{a2} = 9.04$</td>
</tr>
<tr>
<td></td>
<td>$pK_{a3} = 12.48$</td>
</tr>
<tr>
<td></td>
<td>$p_l = 10.76$</td>
</tr>
</tbody>
</table>

![Structure of Arginine](image)
### Table 27.3
Amino Acids with Ionizable Side Chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>$pK_a$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>$pK_{a1} = 1.82$</td>
</tr>
<tr>
<td></td>
<td>$pK_{a2} = 6.00$</td>
</tr>
<tr>
<td></td>
<td>$pK_{a3} = 9.17$</td>
</tr>
<tr>
<td></td>
<td>$pI = 7.59$</td>
</tr>
</tbody>
</table>

**Histidine**

\[
\text{H}_3\text{N} \quad \text{C} \quad \text{C} \quad \text{O}^- \\
\text{CH}_2
\]

\[
\text{N} \quad \equiv \quad \text{NH}
\]