Overview of Chapter 3

I. Amino Acids
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   3.2 Structures of the 20 Common Amino Acids
   3.4 Ionization of Amino Acids

II. Peptides and Proteins
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   3.8 Amino Acid Composition of Proteins
   3.9 Determining the Sequence of Amino Acids Residues
   3.10 Protein Sequencing Strategies
   3.11 Comparison of the Primary Structures of Proteins
       Reveal Evolutionary Relationships
3.1 General Structure of Amino Acids

Twenty common \( \alpha \)-amino acids have carboxyl and amino groups bonded to the \( \alpha \)-carbon atom.

A hydrogen atom and a side chain (R) are also attached to the \( \alpha \)-carbon atom.

\[
\begin{align*}
\text{H}_3\text{N} & \text{CH} \text{COOH} \\
\text{H}_3\text{N} & \text{CH} \text{COOH}
\end{align*}
\]

Diprotic Acids but Zwitterions \((-\text{NH}_3^+ -\text{COO}^-)\) at pH 7 in aqueous solution.

Stereochemistry of amino acids

19 of the 20 common amino acids are chiral.

Mirror image pairs of amino acids are L (levo) and D (dextro).

Proteins use L-amino acids.
3.2 Structures of the 20 Common Amino Acids

- **Fischer projections** - horizontal bonds from a chiral center extend *toward* the viewer, vertical bonds extend *away* from the viewer

- Abbreviations can be one letter or three letters

- Amino acids are grouped by the properties of their side chains (R groups)

- Classes: Aliphatic, Aromatic, Sulfur-containing, Alcohols, Bases, Acids and Amides

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A. Aliphatic R Groups


---Hydrophobicity increases

Unnumbered figure pg 57 Principles of Biochemistry, 6/e
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Fig 3.3 Stereoisomers of Isoleucine

- Ile has 2 chiral carbons, 4 possible stereoisomers

![Chemical Structures of Stereoisomers of Isoleucine](image)

Proline has a nitrogen in the aliphatic ring system

- **Proline** (Pro, P) - has a three carbon side chain bonded to the \( \text{\textsuperscript{\#}} \text{-amino nitrogen}
- The heterocyclic pyrrolidine ring restricts the geometry of polypeptides
B. Aromatic R Groups

- Phenylalanine [F] (Phe)
- Tyrosine [Y] (Tyr)
- Tryptophan [W] (Trp)

C. Sulfur-Containing R Groups

- Methionine [M] (Met)
- Cysteine [C] (Cys)
Fig 3.4 Formation of cystine (disulfide bonds in proteins)

\[
\begin{align*}
\text{OOC—CH—CH}_2—\text{SH} + \text{HS—CH}_2—\text{CH—COO}^- & \\
\text{OOC—CH—CH}_2—\text{S—S—CH}_2—\text{CH—COO}^- & \\
\end{align*}
\]

Disulfide bridges stabilize the 3D structures of proteins

D. Side Chains with Alcohol Groups

\[
\begin{align*}
\text{H}_3\text{N—C—H} & \\
\text{CH}_2 & \\
\text{OH} & \\
\text{Serine [S] (Ser)} & \\
\end{align*}
\]  
\[
\begin{align*}
\text{H}_3\text{N—C—H} & \\
\text{H—C—OH} & \\
\text{CH}_3 & \\
\text{Threonine [T] (Thr)} & \\
\end{align*}
\]
E. Basic R Groups

- Histidine [H] (His)
- Lysine [K] (Lys)
- Arginine [R] (Arg)

F. Acidic R Groups and Amide Derivatives

- Aspartate [D] (Asp)
- Glutamate [E] (Glu)
- Asparagine [N] (Asn)
- Glutamine [Q] (Gln)

Carboxylic Acidic

Carboxyamides
G. The Hydrophobicity of Amino Acid Side Chains

**Hydropathy**: the relative hydrophobicity of each amino acid (larger the hydropathy indicates greater tendency of an amino acid to prefer a hydrophobic environment)

Hydropathy affects protein folding: hydrophobic side chains tend to be in the interior; hydrophilic residues tend to be on the surface.

### Partitioning of AAs between Organic and Aqueous Phases

\[ \text{AA}_{\text{org}} \downarrow \text{AA}_{\text{aq}} \]

\[ K_{eq} = \frac{[\text{AA}_{aq}]}{[\text{AA}_{org}]} \]

\[ G = -RT \ln K_{eq} \]

? \( G < 0 \) for favorable transfer of an amino acid from an organic phase to an aqueous phase

#### Table 3.1: Hydrophathy scale for amino acid residues

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Free-energy change for transfer (~kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly hydrophobic</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.5</td>
</tr>
<tr>
<td>Valine</td>
<td>2.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.1</td>
</tr>
<tr>
<td>Less hydrophobic</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.17</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.08</td>
</tr>
<tr>
<td>Proline</td>
<td>-0.29</td>
</tr>
<tr>
<td>Threonine</td>
<td>-0.35</td>
</tr>
<tr>
<td>Serine</td>
<td>-1.1</td>
</tr>
<tr>
<td>Highly hydrophilic</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>-1.7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>-7.6</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>-7.7</td>
</tr>
<tr>
<td>Glutamate</td>
<td>-7.8</td>
</tr>
<tr>
<td>Asparagine</td>
<td>-5.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>-4.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>-7.5</td>
</tr>
</tbody>
</table>

*The free-energy change is for transfer of an exocyclic side chain from the interior of a lipid bilayer to water.
On other scales, hydrophilicity is lower hydrophobicity value.

Table 3.1 Principles of Biochemistry, 4/e © 2006 Pearson Prentice Hall, Inc.
### 3.4 Ionization of Amino Acids

- Ionizable groups in amino acids: (1) -carboxyl, (2) -amino, (3) some side chains
- Each ionizable group has a specific $pK_a$

$$\text{AH}^+ \quad \text{B} + \text{H}^+$$
- For a solution pH below the $pK_a$, the protonated form predominates (AH)
- For a solution pH above the $pK_a$, the unprotonated form predominates (B)

---

**Fig 3.6  Titration curve for alanine**

- $pK_1 = 2.4$
- $pK_2 = 9.9$
- $pI_{\text{Ala}} = \text{isoelectric point}$
- $pI = \{2.4 + 9.9\}/2$
Fig 3.7 Ionization of Histidine

\[
\begin{align*}
pK_1 &= 1.8 \\
pK_2 &= 6.0 \\
pK_3 &= 9.3
\end{align*}
\]

Why is the pI of His between 6.0 and 9.3?

Fig 3.7 (b) Deprotonation of imidazolium ring

Imidazolium ion (protonated form) of histidine side chain

\[
\begin{align*}
&\text{Imidazole (deprotonated form) of histidine side chain}
\end{align*}
\]
Table 3.2

\[ \text{Henderson-Hasselbach equation} \]
Calculating ratio of proton acceptor to proton donor at any pH

\[ \text{pH} = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]} \]

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#### pK\textsubscript{a} values of amino acid ionizable groups

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>pK\textsubscript{a} value</th>
<th>α-Carboxyl group</th>
<th>α-Amino group</th>
<th>Side chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leucine</td>
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<td></td>
<td></td>
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<td>Isoleucine</td>
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<td></td>
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<td>Methionine</td>
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<td></td>
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<tr>
<td>Tryptophan</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.9</td>
<td></td>
<td>10.1</td>
<td>8.4</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.2</td>
<td></td>
<td>10.2</td>
<td>10.9</td>
</tr>
<tr>
<td>Asparagine</td>
<td>2.1</td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.2</td>
<td></td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2.0</td>
<td></td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.1</td>
<td></td>
<td></td>
<td>4.1</td>
</tr>
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<td>Lysine</td>
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<td></td>
<td>9.1</td>
<td>10.5</td>
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<tr>
<td>Arginine</td>
<td>1.9</td>
<td></td>
<td>9.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.8</td>
<td></td>
<td>9.3</td>
<td>6.0</td>
</tr>
</tbody>
</table>
Fig 3.8 (a) Ionization of the protonated \( \gamma \)-carboxyl of glutamate

\[
\begin{align*}
\text{Carboxylic acid (protonated form) of glutamate side chain} & \quad \text{Carboxylate ion (deprotonated form) of glutamate side chain} \\
\text{Prentice Hall c2002 Chapter 3} & \quad 23
\end{align*}
\]

Fig 3.8 (b) Deprotonation of the guanidinium group of Arg

\[
\begin{align*}
\text{Guanidinium ion (protonated form) of arginine side chain} & \quad \text{Guanidine group (deprotonated form) of arginine side chain} \\
\text{Prentice Hall c2002 Chapter 3} & \quad 24
\end{align*}
\]
3.5 Peptide Bonds Link Amino Acids in Proteins

- **Peptide bond** - linkage between amino acids is a secondary amide bond

- Formed by condensation of the \(\text{-carboxyl}\) of one amino acid with the \(\text{-amino}\) of another amino acid (loss of \(\text{H}_2\text{O}\) molecule)

- Coupling in cells requires use of a **high energy** intermediate to active carboxylic acid group

- **Primary structure** - linear sequence of amino acids in a polypeptide or protein

---

![Peptide bond between two amino acids](image)

**Fig 3.9 Peptide bond between two amino acids**

\[
\text{CH}_3
\]
\[
\text{H}_3\text{N} \rightarrow \text{CH} \rightarrow \text{COO}^\ominus + \text{CH}_2\text{OH}
\]

\[
\text{H}_2\text{O} \downarrow
\]

\[
\text{N-terminus} \quad \text{CH}_3 \quad \text{O} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{C-terminus}
\]

Peptide bond

Condensation Reaction not spontaneous as shown (\(\downarrow \text{G} > 0\))
Polypeptide chain nomenclature

Amino acid “residues” compose peptide chains

Peptide chains are numbered starting from the N (amino) terminus to the C (carboxyl) terminus

Amino-end Gly-Arg-Phe-Ala-Lys Carboxyl-end GRFAK

Peptide bond formation eliminates the ionizable \(-\)carboxyl and \(-\)amino groups of the free amino acids (only one titratable amino and carboxyl group remain from the alpha amino and carboxylic acid groups)

3.6 Protein Purification Techniques

Stages of Protein Purification (Water Soluble)

1) Disrupt Cells (or Homogenize Tissue)

2) Isolate crude mixture of proteins (from organelles, membranes, DNA)

3) Fractionate proteins according to solubility, size, charge or affinity (e.g., ammonium sulfate/centrifugation; column chromatography)

4) Analyze Purity at each stage and calc yield
Common types of Column Chromatography

**Ion-exchange chromatography** - separation based upon the **overall charge** of molecules

**Gel-filtration chromatography** - separation based upon **molecular size**

**Affinity chromatography** - separation by **specific binding** interactions between column matrix and target proteins

**Hydrophobic Interaction chromatography** – separation by **hydropathic index** of surface residues

---

**Fig 3.11 Column Chromatography**

(a) Separation of a protein mixture

(b) Detection of eluting protein peaks
3.7 Analytical Techniques

- **Electrophoresis** separation of molecules using a gel and an electric field

**Polyacrylamide gel electrophoresis (PAGE)**
polyacrylamide gel matrix is used

**SDS-PAGE.** Sodium dodecyl sulfate (SDS) coats proteins with negative charges. Separation determines relative molecular mass and purity.

**Native–Gel PAGE.** SDS not used. Separation determines relative mass and aggregation state.

---

**Fig 3.12 (a) SDS-PAGE Electrophoresis**
(b) Protein banding pattern after run

![Diagram of SDS-PAGE](image)
Analytical Techniques: **Mass Spectroscopy**

Accurately determines $m/z$ of a protein or mixture

**Electrospray mass spectroscopy** (ESI) uses high voltage to create charged proteins for $m/z$ separation

**Matrix-Assisted Desorption Ionization** (MALDI) uses a matrix and laser to create charged proteins for separation

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**MALDI-TOF Mass Spectrometer**

![Diagram of MALDI-TOF Mass Spectrometer](image_url)

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3.8 Amino Acid Composition of Proteins

Amino acid composition – determines the number and type of amino acids in a protein

- Peptide bonds are cleaved by acid hydrolysis (6M HCl, 110°, 16-72 hours)
- Amino acids are separated chromatographically and quantitated
- Phenylisothiocyanate (PITC) used to derivatize the amino acids prior to HPLC analysis

Fig 3.16 Amino acid treated with PITC yield a Phenylisothiocarbamoyl-AA
Fig 3.15 Chromatogram from HPLC-separated PTC-amino acids

Mixture of PTC-AA from peptide with unknown composition is compared to a std mixture ↓ Type and Number of AA

3.9 Determining the Sequence of Amino Acids

**Edman degradation procedure** – Chemical Method for releasing one AA at a time from the N-terminus

1. Treat peptide with PITC which reacts with the N-terminus to form a PTC-peptide
2. Treat with trifluoroacetic acid (TFA) to selectively cleave the N-terminal peptide bond
3. Separate N-terminal derivative from peptide
4. Convert derivative to PTH-amino acid
Fig 3.16 Edman degradation procedure

Phenylisothiocyanate (Edman reagent) + \( \text{\(N\)-terminal residue} \)

\( \text{pH} = 9.0 \)

Phenylthiocarbamoyl-peptide

\( \text{F3CCOOH} \)

Edman degradation procedure (cont)

Anilinothiazolinone + Polypeptide chain with \(n-1\) amino acids

\( \text{Aqueous acid} \) \( \text{Returned to alkaline conditions for reaction with additional phenylisothiocyanate in the next cycle of Edman degradation} \)

Phenylthiohydantoin derivative \( \text{Amino acid identified} \)
3.10 Protein Sequencing Strategies

Large Proteins are selectively cleaved into smaller pieces prior to Edman degradation

Proteases or Chemical Agents (e.g., BrCN)
Protease enzymes selectively cleave peptide bonds containing ‘unique’ amino acid R-groups

**Chymotrypsin** - carbonyl side of aromatic or bulky noncharged aliphatic residues (Phe, Tyr, Trp, Leu)

**Trypsin** - carbonyl side, basic residues (Lys, Arg).

**Staphylococcus aureus V8 protease** - carbonyl side of negatively charged residues (Glu, Asp).

---Ala-Phe-Ala-Gly-Lys-Ser-Ala-Glu-Met---

Fig 3.21 Cleavage, sequencing an oligopeptide

(a) \( \ce{H2N-Gly-Arg-Ala-Ser-Phe-Gly-Asn-Lys- Trp- Glu-Val-COO^-} \) \\
\( \text{Trypsin} \) \\
\( \ce{H2N-Gly-Arg-COO^- + H2N-Ala-Ser-Phe-Gly-Asn-Lys-COO^- + H2N-Trp-Glu-Val-COO^-} \)

(b) \( \ce{H2N-Gly-Arg-Ala-Ser-Phe-Gly-Asn-Lys- Trp- Glu-Val-COO^-} \) \\
\( \text{Trypsin} \) \\
\( \ce{H2N-Gly-Arg-Ala-Ser-Phe-COO^- + H2N-Gly-Asn-Lys-Trp-COO^- + H2N-Glu-Val-COO^-} \)

(c) \( \begin{align*}
\text{Gly-Arg} & \quad \text{Ala-Ser} & \quad \text{Phe-} & \quad \text{Gly-Asn} & \quad \text{Lys-Trp} & \quad \text{Glu-Val} \\
\text{Gly-Arg} & \quad \text{Ala-Ser} & \quad \text{Phe} & \quad \text{Gly-Asn} & \quad \text{Lys-Trp} & \quad \text{Glu-Val}
\end{align*} \)
Other Protein Sequencing Methods

Sequence of DNA \(\rightarrow\) Sequence of protein

Rapid relative to Edman degradation

DNA \(\sim\sim\sim\sim\ A\ A\ G\ A\ G\ T\ G\ A\ A\ C\ C\ T\ G\ T\ C\ \sim\sim\sim\)

Protein \(\sim\sim\sim\sim\ \text{Lys} \sim\text{Ser} \sim\text{Glu} \sim\text{Pro} \sim\text{Val} \sim\sim\sim\)

ESI-Mass Spectroscopy – Very Rapid, but Limited to short peptides (30-40 AAs)

3.11 Comparisons of the Primary Structures of Proteins Reveal Evolutionary Relationships

Amino Acids Sequences are **Homologous** if their sequences are similar

Homology programs align sequences and identify similarities (invariable; conserved vs hypervariable residues) \(\sim\) % **Sequence Homology**

Proteins with sequence homology of 50% usually have nearly identical **3D** structures (Structure Prediction)

Differences reflect evolutionary change from a common ancestral protein sequence and are used to construct a **Phylogenetic tree**
Fig 3.22

Phylogenetic tree for cytochrome c