Chapt. 6-7 Amino Acids and Proteins

Student Learning Outcomes:
• Explain basic structure of amino acids, and classification by side chain
• Explain the structure of peptide bond
• Describe the different levels of structure of proteins
  • 1st product is polypeptide, from gene sequence
• Explain relationship of primary structure of protein to its final function, and modifications of amino acids
• Describe effects of amino acids substitutions in the primary sequence

Proteins are most diverse macromolecules. Thousands of proteins direct most activities of cell:
• Structural components
• Transport, storage of small molecules (e.g. O₂)
• Transmit information between cells (protein hormones),
• Defense against infection (antibodies)
• Enzymes

Proteins (polypeptides) are linear polymers formed from 20 different amino acids
- translated from mRNA, transcribed from DNA

Amino acids

Amino acid structure:
• NH₂ end, -COOH end: 2 pKₐ
  (Some side chains 3rd pKₐ)
• Zwitterion at physiological pH: both + and – charges

Amino acids can be D and L configuration:
• Defined by glyceraldehyde
  • Asymmetric α-carbon
  • R = side chain
• Only L-form in proteins of humans, other organisms
• (bacteria have D-aa in cell walls, some antibiotics)
The Peptide bond

Peptide bond:
- Joins 2 amino acids
- Condensation reaction
- **Protein primary sequence:** linear order of aa from gene
- Note: N- and C- terminus
- **R** = Side chains of the aa
  - confer functional properties, dictate folding

Fig. 6.3

Amino acids side chains

Side chains of Amino acids dictate function and reactivities:
- **pK_a** for functional groups (Table 1):
  - -COOH, -NH_2,
  - Side chains
- **Hydropathic index**:
  - + = hydrophobic
  - - = hydrophilic

Fig. 6.8

Side chains interact in structure of protein

Side chains of amino acids interact for structure:
Hydrophobic, H-bonds, oxidation, electrostatic

A. Hydrophobic interaction

B. Hydrogen bonds

Basic amino acid Histidine has 3 pK_a

Basic amino acid Histidine has 3 pK_a

Fig. 6.8
pH affects dissociation of side chains of amino acids

pK_a is pH at which half molecules have charged side chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Form below pH_{k_a}</th>
<th>Form above pH_{k_a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
<tr>
<td>Glutamate</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
<tr>
<td>Histidine</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
<tr>
<td>Cysteine</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
<tr>
<td>Lysine</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
<tr>
<td>Arginine</td>
<td>CH_3C=O-COOH</td>
<td>CH_3C=O-COOC^- + H^-</td>
</tr>
</tbody>
</table>

Levels of protein structure

3 levels of structure for a polypeptide:
- Primary sequence dictates folding:
- Quaternary structure involves multiple polypeptides

Protein structure

1° structure is the linear polypeptide
Peptide backbone is rather rigid:
Often trans-configuration - alternating side chains

Secondary structure – alpha helix

Secondary structures are localized interactions:
- Alpha helix is rigid, stable, compact
- Alpha helix from H-bonds of peptide bond
  - C=O to N-H
  - Between aa 4 residues away

Fig. 7.1

Fig. 7.2

Fig. 7.3-7.4
Secondary structures are localized interactions: 

**β-pleated sheet** from H bonds C=O to N-H of adjacent strands.

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**Tertiary Structure**

Longer range interactions involving 2D structures
- Results in **domains**, basic units
- Hydrophobic aa localize in interior
- Hydrophilic aa localize on surface

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**Post-translational amino acid modifications**

**Modified amino acids in proteins**
- Often post-translational
- Target or anchor proteins,
- Regulate activity (PO₄⁻²)

**Transmembrane proteins**
- Transport proteins, hormone receptors, ion channels
- α-helical regions: hydrophobic aa expose to lipid bilayer
- Hydrophilic in aqueous; often post-translational modified aa
Quaternary structure

**Quaternary structure**: interactions between multiple polypeptides to form the active protein
- Ex. hemoglobin $2\alpha$ chains, 2 $\beta$ chains
- Myoglobin only 1 chain

**Species conservation - insulin**

**Species conservation of insulin primary structure**: High conservation: porcine and bovine insulin used by diabetics before recombinant human insulin

**Fig. 6.12 Insulin**

**Gel electrophoresis separates proteins**

Gel electrophoresis at neutral pH can separate proteins based on overall charge:
- Distinguish normal, and person lacking IgG, or myeloma
- Distinguish normal and mutant forms of hemoglobin: HbA vs. HbS (Glu6 $\rightarrow$ Val); determine genotypes: AA, AS, SS

**Denaturing gel electrophoresis**

Denaturing gel electrophoresis (SDS-PAGE) separates proteins based on size (aa length)
- Ex. Normal serum proteins, and after depletion of albumin, IgG and transferrin with little columns

**Good Biotech Co.**

Lane 1 serum proteins
Lane 1 depleted serum
Lane 3 eluted proteins
(1) IgG; (2) transferrin
(3) Albumin (4) IgG H-chain
(5) IgG L-chain
Enzyme isoforms in different tissues

**Enzyme isoforms** (isoenzymes) are present in different tissues.

- Same function, slightly different size, charge, tissue, developmental stage.
- Ex. Creatine Kinase (CK) has B and M forms: BB (brain), MB (heart), MM (skeletal muscle).
- After heart attack: diagnosis by increased MB isoform in blood.

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Protein families

**Protein families - evolution from ancestral gene**
- Sequence from proteins, or from DNA of genomes
- Shared features – conserved residues, conservative changes
- Different forms at different times
  - (HbF fetal = α,γ; HbA adult = α,β)

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Adenylyl cyclase (3',5'-cAMP synthesis)
- 9 different isoforms, different genes, different tissues (differential response to hormones);
- invariant C1 and C2 regions for synthesis of cAMP

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Structure-function of myoglobin; hemoglobin
- Ex. Hemoglobin
  - 2 α chains, 2 β chains
- **Myoglobin** 1 chain
Oxygen saturation of hemoglobin, myoglobin

- **Hyperbolic myoglobin**
- **Sigmoidal hemoglobin:**
  - Tetramer inhibits $O_2$ binding at low $pO_2$
  - Facilitates release of $O_2$ in tissues by HbA, where bound by Myo and stored.
- $O_2$ binds Fe$^{+2}$ of Heme
- Changes shape of Hb

*Fig. 7.11*

Cooperativity in $O_2$ binding of hemoglobin

(\textit{model of Monod, Wyman, Changeux}:)

T (tense) state low affinity for $O_2$;
R (relaxed) state higher affinity.

Binding of $O_2$ to one subunit can change shape of all subunits

*Fig. 7.14*

Clinical comments

**Will Sichel – Sickle cell anemia:**
- autosomal recessive
- (Glu-6$\rightarrow$Val in $HBB$ gene)
- Hb $\beta$ chain ($HbS = \alpha_2\beta^s_2$)
- In low oxygen, HbS polymerizes, distorts rbc, occludes in capillaries $\rightarrow$ ischemia, pain
- Hydroxyurea treatment to increase expression of fetal HbF ($\alpha_2\gamma_2$).

Clinical comments

**Di Abietes – IDDM diabetes**
- Measurement of HbA$_{1c}$ indicates hyperglycemia (the hemoglobin gets glycosylated)
- other proteins also glycosylated, interfere function
- Insulin is Humulin (rDNA human insulin from \textit{E. coli})
- Newer Humalog lispro ultrafast insulin version:
  - lys29pro28$\rightarrow$ lys28pro29
  - Acts as monomer vs. the hexamer-Zn form of humulin; absorbed faster
Antibodies are immunoglobulins

Immunoglobulins (Antibodies) bind specific molecules as the adaptive immune response:
- 2 light, 2 heavy chains
- IgG circulating form
- Constant domains,
- Variable domains

Figs. 7.15, 16

Chaperonins

Chaperonins
- Heat shock proteins help other proteins fold
- Induced after stress.
- hsp70 keep nascent polypeptides unfolded
- Hsp60 helps proteins refold
- Uses ATP

Fig. 7.17

Prions are misfolded, infectious proteins

Prions are misfolded, toxic, infectious proteins
Neurodegenerative diseases: Creutzfeld-Jakob (CJD)
Scrapie, Mad Cow, Elk Wasting
Misfolded has more β-sheet; aggregates
Genetic mutations increase chance of misfolding

Fig. 7.19
Normal vs disease form

Key concepts

Key concepts of amino acids and proteins:
- Linear sequence (1°) from translation dictates the unique features of proteins, including 3-D shape
- All aa have α-C and –NH2, -COOH
  - and H and a side chain (R = H for glycine)
- aa can be modified after translation (as PO4)
- Proteins have 4 levels of structure: 1°, 2°, 3°
  - Quaternary only for multiple subunits
- Proteins have domains, including ligand binding
- Proteins can be denatured by various agents
### Chapt. 6 Review questions

**Review question:**

4. Protein kinases phosphorylate proteins only at certain –OH groups on aa side chains. Which of the groups of aa all contain side-chain –OH groups?
   a. Aspartate, glutamate, serine
   b. Serine, threonine, tyrosine
   c. Threonine, phenylalanine, arginine
   d. Lysine, arginine, proline
   e. Alanine, asparagine, serine

### Chapt. 7 Review question

Which of the following is a characteristic of globular proteins?
   a. Hydrophilic amino acids tend to be on the inside
   b. Hydrophobic amino acids tend to be on the outside
   c. Tertiary structure is formed by hydrophobic and electrostatic interactions between amino acids, and by hydrogen bonds between amino acids and water
   d. Secondary structures are formed principally by hydrophobic interactions between amino acids
   e. Covalent disulfide bonds are necessary to hold the protein in a rigid conformation