Protein Structure and Function

Introduction

Proteins are indispensable macromolecules, rightly regarded as the workhorses of the cell, executing nearly every function necessary for life, from catalyzing reactions to providing movement and structural support. They achieve this incredible versatility through their precise architecture.

- Proteins are essential macromolecules known as the workhorses of the cell.
- They are linear polymers constructed from amino acids.
- These polymers fold into unique three-dimensional structures.
- The final structure is determined by the specific sequence of amino acids and the chemical properties of their side chains.
- This structure ultimately dictates the protein's specific biological function (e.g., acting as an enzyme, a transporter, or a structural scaffold).

Learning Objectives

By the end of this module, you will be able to:

- Identify amino acids in proteins; describe amino acid structure, properties and classification.
- Describe the peptide bond and the formation of peptides.
- Describe levels of protein structure, identify the force/s that stabilize each level and the nature/shape of the protein.
- Describe the biological functions of proteins (including the comparison of Hemoglobin and Myoglobin).
- Describe protein separation, purification and sequencing; and denaturation of proteins.
- Explain enzyme kinetics and identify key coenzymes in metabolism.

Key Concepts and Definitions

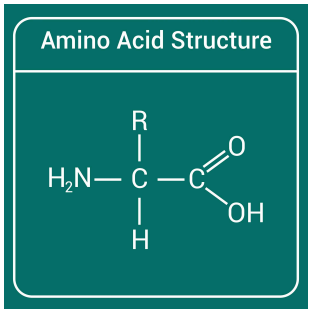
Term	Definition
Amino Acid	The monomer unit of a protein, featuring an α-carbon attached to an NH_2 group, a COOH group, an H atom, and a variable R group (side chain).
Peptide Bond	The covalent amide bond that links two amino acids, formed by a condensation reaction.
Primary Structure	The unique, linear sequence of amino acids, stabilized solely by peptide bonds.
K_m (Michaelis Constant)	The substrate concentration [S] at which the reaction velocity (V_0) is exactly half of the maximum velocity (V_{max}).
Holoenzyme	A complete, catalytically active enzyme, consisting of the protein component (apoenzyme) and any necessary non-protein components (cofactors/coenzymes).

Detailed Discussion

Amino Acid Structure, Properties, and Classification

1. Structure: All 20 standard amino acids share a common fundamental structure:

- A central alpha-carbon (Cα).
- An amino group (NH_3^+ at pH 7.4).
- A carboxyl group (COO^- at pH 7.4).
- A hydrogen atom (H).
- A unique R group (side chain).



2. Properties and Classification (by R Group): The R group determines the amino acid's chemical behavior:

- Nonpolar (Hydrophobic): (e.g., Valine, Leucine). Found clustered in the protein's interior.

- Polar Uncharged: (e.g., Serine, Glutamine). Can form hydrogen bonds.
- Acidic (Negatively Charged): (Aspartate, Glutamate). Participate in ionic bonds (salt bridges).
- Basic (Positively Charged): (Lysine, Arginine). Participate in ionic bonds (salt bridges).

Peptide Bond and Levels of Protein Structure

1. Formation of Peptides: Amino acids link via a condensation (dehydration) reaction, releasing water and forming a covalent bond.

2. Peptide Bond: This bond links the carboxyl carbon (C) of one amino acid to the amino nitrogen (N) of the next.

3. Rigidity: The peptide bond has partial double-bond character, making it rigid and planar. A polypeptide chain is directional, running from the N-terminus (free amino group) to the C-terminus (free carboxyl group).

Levels of Protein Structure

Protein structure is built hierarchically:

Structure Level	Definition	Stabilizing Force/s	Nature/Shape
Primary (1°)	The linear sequence of amino acids, determined by the gene.	Peptide Bonds (Covalent)	Linear chain
Secondary (2°)	Regular, local folding patterns of the polypeptide backbone.	Hydrogen Bonds between backbone C=O and N-H groups.	α-Helix (rod-like) and β-Pleated Sheet (flat, extended).
Tertiary (3°)	The overall three-dimensional	R-Group Interactions: Hydrophobic Effect (primary driver), Ionic bonds,	Globular (compact, soluble) or Fibrous (elongated, structural).

	shape of a single polypeptide chain	Hydrogen bonds, Van der Waals forces, Disulfide Bridges (Covalent).	
Quaternary (4°)	The arrangement of multiple polypeptide subunits (monomers).	Non-covalent R-Group interactions (same as 3°), Disulfide Bridges.	Oligomeric (e.g., Tetramer).

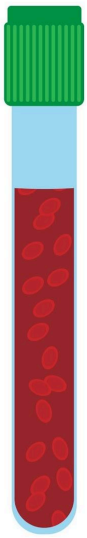
Biological Functions, Hemoglobin vs. Myoglobin, and Denaturation

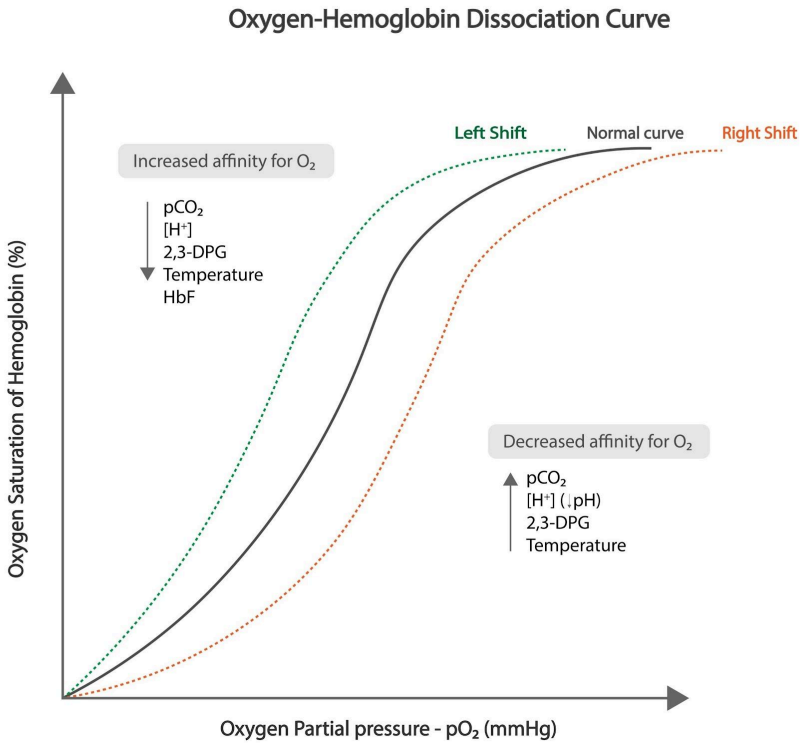
Protein Function Category	Specific Protein Example	Function
Catalysis	Trypsin	Hydrolyzes peptide bonds during digestion.
Structure	Collagen	Provides tensile strength to skin, tendons, and cartilage.
Movement	Myosin	Interacts with Actin to facilitate muscle contraction.
Transport	Hemoglobin	Carries oxygen in the blood.

Hemoglobin (Hb) and Myoglobin (Mb) both bind oxygen using a Heme prosthetic group, but their structures dictate their specialized functions:

Feature	Myoglobin (Mb)	Hemoglobin (Hb)
Function	Oxygen Storage in muscle cells.	Oxygen Transport in red blood cells.
Structure	Monomer (Single polypeptide chain), 3° structure.	Tetramer (Four subunits, $\alpha_2\beta_2$), 4° structure.
Oxygen Binding	Binds 1 O_2 molecule. High affinity for O_2.	Binds 4 O_2 molecules. Shows cooperativity (allosteric).

Binding Curve Shape	Hyperbolic (Releases O_2 only when O_2 concentration is very low).	Sigmoidal (S-shaped) (Efficiently loads O_2 in lungs and releases it in tissues).





Protein Separation, Denaturation, and Sequencing

- **Protein Separation and Purification:** Techniques used to isolate a specific protein based on a unique property:
    - **Gel Filtration Chromatography (Size Exclusion**): Separates proteins based on size.
    - **Ion-Exchange Chromatography**: Separates proteins based on net electric charge.Affinity Chromatography: Separates proteins based on their specific binding affinity for a ligand.
    - **SDS-PAGE**: Separates denatured proteins based on their mass/size.
- **Protein Sequencing**: Determining the precise Primary Structure (amino acid sequence). This can be done directly by chemical methods like Edman degradation

or, more commonly, inferred from the DNA sequence of the gene that encodes the protein.

- **Denaturation of Proteins**: The process of disrupting a protein's 2°, 3°, and 4° structures, causing it to unfold and lose its biological function. The Primary Structure (peptide bonds) remains intact. Denaturing agents include:
    - **Heat** (disrupts weak non-covalent bonds).
    - **Extreme pH** (disrupts ionic bonds/salt bridges).
    - **Urea/Guanidinium** (disrupts H-bonds and hydrophobic interactions).

Enzymes and Coenzymes

a. Describe naming and classification of enzymes

Naming: Generally, the suffix -ase is added to the substrate (e.g., Urease) or the type of reaction (e.g., Dehydrogenase).

Classification (IUBMB System): Enzymes are grouped into six classes based on the type of reaction catalyzed:

1. Oxidoreductases: Redox reactions (transfer of electrons).
2. Transferases: Transfer of functional groups.
3. Hydrolases: $\mathrm{Hydrolysis}$ reactions (bond cleavage with H_2O.
4. Lyases: Cleavage of C-C, C-O, C-N bonds without hydrolysis.
5. Isomerases: Transfer of groups within a molecule to form isomers.
6. Ligases: Formation of bonds coupled with ATP cleavage.

b. Classify chemical reactions based on reaction rate

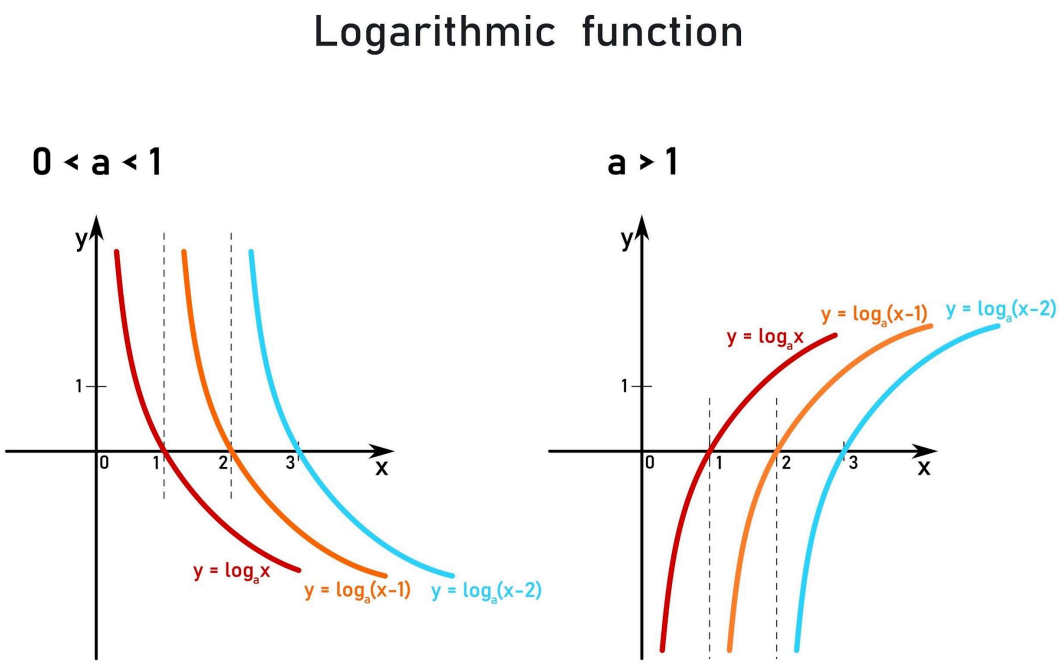
- The rate of a reaction is defined by its reaction order, which describes how the reaction rate depends on the concentration of reactants.
    - Zero-order: Rate is independent of substrate concentration (Rate = k).
    - First-order: Rate is proportional to a single reactant concentration (Rate = k[A]).
- Enzyme-catalyzed reactions are complex: they follow first-order kinetics at low substrate concentrations and become zero-order at very high (saturating) substrate concentrations.

c. Explain principles of enzyme-catalyzed reaction, kinetics and describe enzyme inhibition through Michaelis-Menten and Lineweaver-Burk plots

- **Principles**: Enzymes are highly specific catalysts that accelerate reactions by lowering the activation energy (E_a). They form a temporary Enzyme-Substrate Complex (ES) at the active site, stabilizing the transition state.
- **Michaelis-Menten Equation**: Describes the velocity *(V_0)* as a function of substrate concentration ([S]):

$$V_0 = \frac{V_{max}[\mathrm{S}]}{K_m + [\mathrm{S}]}$$

- **Lineweaver-Burk Plot (Double Reciprocal Plot):** Plots $1/V_0$ against $1/[\mathrm{S}]$, yielding a straight line, which is useful for determining V_{max} and K_m.
- **Inhibition Patterns:**
  - **Competitive Inhibition:** Inhibitor binds to the $\mathrm{active\ site}$. K_m **increases** (lowers affinity); V_{max} is **unchanged**.
  - **Non-Competitive Inhibition:** Inhibitor binds to an allosteric site. V_{max} **decreases**; K_m is **unchanged**.



d. Identify the coenzymes used in metabolism, the reactions in which they are involved and their precursors

Coenzymes are often organic molecules derived from vitamins that assist enzymes by carrying chemical groups or electrons.

Coenzyme	Reactions Involved	Group Carried	Vitamin Precursor
NAD+ / NADP+	Oxidation/Reduction	Hydride ion (H^-) / Electrons	Niacin (Vitamin B_3)
FAD / FMN	Oxidation/Reduction	Two Hydrogen atoms (H) / Electrons	Riboflavin (Vitamin B_2)
Coenzyme A (CoA)	Acyl-group transfer (e.g., in TCA cycle)	Acyl groups (e.g., Acetyl)	Pantothenic Acid (Vitamin B_5)
PLP (Pyridoxal Phosphate)	Amino group transfer (Transamination)	Amino groups ($-NH_2$)	Pyridoxine (Vitamin B_6)

References

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