

Proteins: Structure and Function

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Primary Structure

- The **linear sequence** of amino acids in a polypeptide.
 - Determined by **peptide bonds** ($-\text{CO}-\text{NH}-$) which are strong, planar, and mostly **trans**.
 - Sequence dictates higher-order folding and final protein function.
 - Even a **single amino-acid mutation** (e.g., Val for Glu in sickle cell anemia) drastically changes structure and function.
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Secondary Structure

Local folding patterns stabilized by **hydrogen bonds** between backbone $-\text{C}=\text{O}$ and $-\text{NH}$ groups.

1. α -Helix

- Right-handed spiral with **3.6 residues per turn**.
- Stabilized by **intrachain hydrogen bonds** every 4th residue.
- Side chains project outward.
- Disrupted by **proline**, **glycine**, and charged residues.

2. β -Pleated Sheets

- Polypeptide chains arranged side-by-side (parallel or antiparallel).
- Stabilized by **interchain hydrogen bonds**.
- More extended than α -helix.

3. Turns / Loops

- Sharp bends connecting helices and sheets.
- Often contain **glycine** (flexible) or **proline** (kink-forming).

Description of Secondary Structure

One region forms a helical coil stabilized by hydrogen bonds; another forms zig-zag strands lying side by side, connected by hydrogen bonds.

Tertiary Structure

The **overall 3-dimensional shape** of a single polypeptide chain.

Stabilizing Forces

- **Hydrophobic interactions** – bury nonpolar residues inward.
- **Hydrogen bonds** – stabilize external and internal interactions.
- **Ionic bonds (salt bridges)** – between charged side chains.
- **Disulfide bonds** – covalent S–S links between cysteines.
- **Van der Waals forces** – tight packing in the protein core.

Domains

- Independently folded functional units (e.g., catalytic domains, binding domains).

Description of Tertiary Structure

Hydrophobic residues cluster inward while polar and charged residues orient outward, creating a compact, functional 3D structure.

Quaternary Structure

Association of **two or more polypeptide chains (subunits)** into a functional protein.

Key Features

- Subunits held together by **noncovalent interactions** (H-bonds, ionic, hydrophobic)
- Sometimes by **interchain disulfide bonds**.
- Allows **cooperativity, regulation, and structural stability**.

Examples

- **Hemoglobin** – ??? tetramer
- **Immunoglobulins** – multiple polypeptide chains linked by disulfide bonds
- **Lactate dehydrogenase** – tetrameric enzyme

Description of Quaternary Structure

Multiple folded subunits assemble into a larger complex, each occupying a precise position and contributing to overall function.

Primary Structure of Insulin

- Human insulin is composed of **two polypeptide chains**:

A-Chain

- **21 amino acids**
- Contains an **intra-chain disulfide bond** between **A6–A11**
- N-terminus begins with **Glycine**, C-terminus ends with **Asparagine**

B-Chain

- **30 amino acids**
- N-terminus begins with **Phenylalanine**, C-terminus ends with **Threonine**

Disulfide Bonds

Insulin contains **three disulfide bridges**:

1. **A7 – B7** (inter-chain)
2. **A20 – B19** (inter-chain)
3. **A6 – A11** (intra-chain)

Proinsulin Processing

- Insulin is synthesized as **preproinsulin** → **proinsulin** → **insulin**.
- **C-peptide** connects A- and B-chains in proinsulin and is removed during maturation.
- C-peptide is **biologically inactive** but a marker of insulin secretion.

Important Point

- The **primary structure** (specific amino-acid sequence + disulfide pattern) is essential for insulin's ability to bind its receptor.

Structure–Function Relationship of Insulin

1. Disulfide Bonds Are Essential for Activity

- Correct folding of A- and B-chains requires the precise disulfide pattern.
- Any alteration (mutation, reduction, or improper re-oxidation) → **loss of biological activity**.
- These bonds hold insulin in its functional conformation, enabling receptor binding.

2. A-Chain Provides Stability

- Intra-chain disulfide that stabilizes α -helical structure.
- Mutations in A-chain regions that stabilize the helix → **rapid degradation** or poor receptor binding.

3. B-Chain Determines Receptor Binding

- The **N-terminal residues of the B-chain (Phe1, Val2, Asn3)** are critical for insulin receptor recognition.
- B-chain C-terminal residues also participate in the interaction with the receptor.
- Mutations in the B-chain reduce **potency, binding, and half-life**.

4. Zinc Stabilizes Storage, Not Action

- In pancreatic β -cells, insulin forms **hexamers** with zinc.
- Hexamer → storage form
- Monomer → biologically active form
- Rapid-acting insulin analogs are designed to **prevent hexamer formation**, improving

absorption.

5. C-Peptide Does Not Affect Function

- Removed before secretion.
 - Its presence prevents early folding and aggregation during insulin synthesis.
 - Used as a **clinical marker** for endogenous insulin production.
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6. Mutations Affect Structure and Diabetes

- Point mutations in the insulin gene can disturb folding ? “**insulinopathies**.”
- Examples include improper disulfide pairing or disrupted receptor domains leading to early-onset diabetes.

Sequence Analysis

Definition

- Sequence analysis determines the **exact order of amino acids** in a protein.
- Essential for understanding protein structure, function, and genetic mutations.

Methods

1. Edman Degradation

- Sequential removal of **N-terminal amino acids** one at a time.
- Each residue is identified ? gives **primary structure**.
- Useful for peptides up to ~50 residues.

2. Enzymatic Cleavage

- Proteases like **trypsin**, **chymotrypsin**, **pepsin** cut proteins at specific residues.
- Fragments are analyzed and assembled into the full sequence.

3. Chemical Cleavage

- Cyanogen bromide cleaves at **methionine** residues.

4. Mass Spectrometry

- Modern method for rapid **peptide sequencing**.
- Identifies amino acids based on mass/charge ratios.
- Detects post-translational modifications (phosphorylation, acetylation, disulfide patterns).

Applications

- Identification of mutations (e.g., sickle cell anemia: Glu→Val).
- Protein engineering & recombinant insulin design.
- Quality control in biotech proteins.

Iso-electric pH (Iso-electric Point, pI)

Definition

- pI is the **pH at which an amino acid or protein has zero net charge**.
- Exists predominantly as a **zwitterion**.

Importance

- **Minimum solubility** at pI → proteins precipitate easily.
- No movement in an electric field → basis of **isoelectric focusing**.
- Proteins with a high content of acidic residues → low pI.
- Proteins rich in basic residues → high pI.

Determination

- For neutral amino acids:
$$pI = (pK_1 + pK_2) / 2$$
- For acidic and basic amino acids: all three pKs considered.

Clinical Relevance

- Abnormal pI helps detect protein variants in diseases (e.g., HbS, HbC).
- Used in separation of serum proteins (albumin, globulins).

Precipitation Reactions

Proteins precipitate when their **solubility decreases**, caused by pH, salts, organic solvents, or heat.

1. Precipitation at Iso-electric Point (pI)

- Proteins have **minimum solubility** at pI.
 - Used to isolate casein from milk at pH 4.6.
 - Used in laboratory protein purification.
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2. Salting Out

- High salt concentration (ammonium sulfate) removes water from proteins.
 - Protein–protein interactions increase ? precipitation.
 - Common in protein purification and enzyme isolation.
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3. Organic Solvent Precipitation

- Alcohols (ethanol, acetone) reduce dielectric constant ? protein denaturation ? precipitation.
 - Used in **plasma protein fractionation**.
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4. Heavy Metal Precipitation

- Hg^{2+} , Pb^{2+} , Ag^+ bind $-\text{COO}^-$ and $-\text{SH}$ groups ? protein precipitation.
 - Used in poisoning treatment (egg white protects gut mucosa).
 - Basis of some diagnostic tests.
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5. Heat Coagulation

- High temperature disrupts hydrogen bonds and hydrophobic interactions ? coagulation.
 - Example: egg white becoming solid on heating.
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6. Acid and Alkali Precipitation

- Extreme pH denatures proteins.
 - Used in **gastric digestion** (HCl).
 - Used to precipitate casein or remove proteins from solutions.
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7. Denaturation-Induced Precipitation

- Mild denaturing agents (urea, guanidinium chloride) disrupt tertiary structure.
- Leads to exposure of hydrophobic groups and aggregation.

Denaturation of Proteins

Definition

- Denaturation is the **loss of secondary, tertiary, or quaternary structure** of a protein **without breaking peptide bonds**.
- The primary structure **remains intact**, but the protein loses shape and function.

What Happens During Denaturation?

- **Hydrogen bonds, ionic bonds, hydrophobic interactions, and disulfide bonds** are disrupted.
- Protein unfolds ? **loss of biological activity**.
- Hydrophobic groups become exposed ? aggregation or precipitation.

Causes of Denaturation

- **Heat**
- **Strong acids or alkalis**
- **Organic solvents** (alcohol, acetone)
- **Heavy metals** (Hg^{2+} , Pb^{2+})
- **Detergents** (SDS)
- **Reducing agents** (β -mercaptoethanol)
- **Radiation or ultrasonic vibration**

Consequences

- Loss of **enzymatic activity**
- Loss of **solubility** ? precipitation
- Change in **physical properties** (viscosity, optical rotation)
- Sometimes reversible, but usually **irreversible** in biological systems

Examples

- Cooking an egg (albumin coagulation)
- Denaturation of enzymes during fever
- Alcohol-based hand sanitizers denature viral proteins
- HCl in the stomach denatures dietary proteins

Importance

- Essential in **digestion, diagnostics, food processing**, and understanding protein folding diseases.

Heat Coagulation

Definition

- Coagulation is **precipitation of proteins due to heat**, caused by irreversible denaturation and aggregation.

Mechanism

- Heat breaks **weak non-covalent bonds** (hydrogen bonds, hydrophobic interactions).
- Protein unfolds ? exposed hydrophobic regions stick together ? **insoluble coagulum** forms.

Examples

- Egg white (albumin) turning solid when boiled
- Coagulation of milk proteins when heated
- Clotting of serum proteins during diagnostic heat tests
- Heat-labile enzymes losing activity at high temperatures

Clinical Relevance

- **Heat coagulation test** for detecting proteins in urine or cerebrospinal fluid.
- Fever can partially denature heat-sensitive enzymes, affecting metabolism.
- Heat instability in certain genetic enzyme deficiencies.

Difference from Simple Denaturation

- Denaturation = unfolding
- Coagulation = **unfolding + aggregation + precipitation**

Description of Heat Coagulation Process

A protein heated above its stability range begins to unfold, exposing hydrophobic inner residues. These residues stick to one another, forming large aggregates that become insoluble and appear as a solid mass or cloudy precipitate.

Classification of Proteins

Proteins can be classified based on **composition**, **shape**, and **function**.

A. Based on Composition

1. Simple Proteins

- Yield **only amino acids** on hydrolysis.
- Examples:
 - **Albumins** (serum albumin)

- **Globulins** (IgG)
- **Histones**

2. Conjugated Proteins

- Protein + **non-protein (prosthetic) group**.

Examples:

- **Glycoproteins** ? carbohydrate
- **Lipoproteins** ? lipids
- **Metalloproteins** ? metal ions (hemoglobin, cytochromes)
- **Phosphoproteins** ? phosphate (casein)
- **Nucleoproteins** ? nucleic acids

3. Derived Proteins

- Formed by partial hydrolysis or denaturation.
 - Examples: **metaproteins, proteoses, peptones, polypeptides**.
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B. Based on Shape

1. Fibrous Proteins

- Long, thread-like
- Structural role
- Low solubility
- Examples: **Collagen, Keratin, Elastin**

2. Globular Proteins

- Spherical, compact
 - Functional role (enzymes, hormones)
 - Soluble in water
 - Examples: **Enzymes, Hemoglobin, Albumin**
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C. Based on Function

- Enzymatic (lipase, trypsin)
- Structural (collagen, keratin)
- Transport (hemoglobin)
- Storage (ferritin)
- Regulatory (insulin)
- Protective (antibodies)

Quantitative Estimation of Proteins

Common biochemical methods used to measure protein concentration:

1. Biuret Method

- Based on **violet complex** formed between Cu^{2+} and **peptide bonds** in alkaline medium.
- Requires **two or more peptide bonds** ? minimum tripeptide.
- Used for plasma proteins.

2. Lowry Method

- More sensitive than Biuret.
- Combines Biuret reaction + reduction of **Folin–Ciocalteu reagent** ? blue color.
- Sensitive to aromatic amino acids.

3. Bradford Method

- Uses **Coomassie Brilliant Blue dye**.
- Dye binds to basic and aromatic residues ? blue color.
- Very sensitive, widely used for micro-assays.

4. UV Absorption (280 nm)

- Aromatic residues (Phe, Tyr, Trp) absorb UV at 280 nm.
- Rapid method for pure proteins.

5. Kjeldahl Method

- Measures **total nitrogen content**; indirectly measures total protein.
 - Used in food analysis.
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Protein Folding & Chaperones

Protein Folding

- Folding converts a linear polypeptide into its **functional 3D conformation**.
- Driven by:
 - Hydrophobic interactions
 - Hydrogen bonds
 - Ionic interactions
 - Disulfide bonds
 - Van der Waals forces

Energy Landscape

- Folding follows a path toward a **stable low-energy state** (native structure).
 - Incorrect folding ? aggregation ? diseases (Alzheimer's, Parkinson's).
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Molecular Chaperones

- Specialized proteins that **assist in proper folding** without becoming part of the final protein.

Types

- **Hsp60 (Chaperonins)** — barrel-shaped; provide isolated environment for folding.
- **Hsp70** — binds hydrophobic regions and prevents aggregation.
- **Protein disulfide isomerase (PDI)** — rearranges disulfide bonds.
- **Peptidyl-prolyl isomerase** — converts cis/trans proline bonds.

Functions

- Prevent misfolding
 - Promote refolding
 - Assist in transport across membranes
 - Prevent aggregation during stress
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Renaturation

Definition

- Some denatured proteins can **regain their native structure and function** when denaturing agents are removed.

Features

- Works only if the **primary structure is intact**.
- Demonstrates that **all information for proper folding lies in the amino-acid sequence**.

Classic Example

- **Ribonuclease** regains full activity after denaturation by urea and β -mercaptoethanol when reagents are removed.

Limitations

- Not all proteins renature easily.
- Aggregated proteins generally **cannot** renature.
- In cells, chaperones are required to guide renaturation.

Protein Functions

Proteins perform diverse biological roles essential for life.

1. Enzymatic Function

- Proteins act as **biocatalysts** (enzymes).
- Increase reaction rate without being consumed.
- Examples: amylase, lipase, DNA polymerase.

2. Structural Function

- Provide strength, shape, and support.
- Examples: **collagen, keratin, elastin, actin, tubulin.**

3. Transport Function

- Carry molecules across membranes or in blood.
- Examples: hemoglobin (O₂), transferrin (iron), albumin (fatty acids & drugs).

4. Regulatory Function

- Hormonal and signaling proteins regulate metabolism.
- Examples: insulin, glucagon, growth hormone.

5. Protective / Immune Function

- Immunoglobulins defend against infection.
- Complement proteins help destroy pathogens.
- Fibrinogen involved in clot formation.

6. Storage Function

- Ferritin stores iron.
- Casein stores amino acids in milk.

7. Contractile / Motor Function

- Actin and myosin enable muscle contraction.
- Dynein and kinesin move organelles inside cells.

8. Buffering Function

- Proteins help maintain acid–base balance due to amphoteric nature.

9. Receptor Function

- Membrane-bound receptors bind hormones, neurotransmitters, and antigens.

Plasma Proteins

Plasma proteins include **albumin, globulins, and fibrinogen.**

1. Albumin

- Major plasma protein (~60%).
- Maintains **oncotic pressure** ? prevents edema.
- Transports fatty acids, bilirubin, calcium, drugs.
- Reduced in malnutrition, liver disease, nephrotic syndrome.

2. Globulins

• ? and ? globulins

– Transport proteins (transferrin, ceruloplasmin, lipoproteins).

• ?-globulins (Immunoglobulins)

– Antibodies: IgG, IgA, IgM, IgE, IgD.

3. Fibrinogen

- Precursor of **fibrin** for blood clot formation.
- Synthesized in liver.

A/G Ratio

- Normal: **1.2–1.8**.
- Low ratio ? increased globulins (infection), low albumin (liver disease).

Clinical Significance

- Used in diagnosing liver disease, kidney disease, malnutrition, inflammation.
- Electrophoresis helps detect multiple myeloma.

Collagen & Elastin

Collagen

Structure

- Most abundant protein in the body.
- Triple-helical structure: **three polypeptide chains** wound into a rope-like helix.
- Rich in **glycine**, **proline**, and **hydroxyproline**.

Types

- **Type I** – bone, skin, tendon
- **Type II** – cartilage
- **Type III** – blood vessels
- **Type IV** – basement membrane (network-forming)

Synthesis Highlights

- Requires **vitamin C** for hydroxylation of proline and lysine.
- Cross-linking via lysyl oxidase requires **copper**.

Disorders

- Scurvy (impaired hydroxylation)
 - Osteogenesis imperfecta (defective Type I collagen)
 - Ehlers–Danlos syndrome (defective cross-linking)
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Elastin

Structure

- Highly elastic protein allowing tissues to stretch and recoil.
- Rich in **glycine, alanine, valine**.
- Contains unique amino acids **desmosine** and **isodesmosine** ? cross-links.

Location

- Found in ligaments, lungs, skin, arterial walls.

Properties

- Random coil structure ? elasticity.
- Cross-linking provides resilience and recoil.

Disorders

- **Marfan syndrome** — defective fibrillin (scaffolding protein for elastin).
- Leads to hyperelastic joints, lens dislocation, aortic aneurysm.

Frequently Asked Questions (FAQs)

1. What is the primary structure of a protein?

It is the **linear sequence of amino acids** linked by peptide bonds.

2. Why is the peptide bond planar?

Because it has **partial double-bond character** due to resonance, restricting rotation.

3. What forces stabilize secondary structure?

Hydrogen bonds between the carbonyl oxygen and amide hydrogen of the peptide backbone.

4. What is an α -helix?

A right-handed helical structure with **3.6 residues per turn**, stabilized by intrachain hydrogen bonds.

5. What breaks α -helices?

Proline, glycine, and clusters of charged residues.

6. What is a β -pleated sheet?

A stretched peptide arrangement stabilized by **interchain hydrogen bonds**; can be **parallel or antiparallel**.

7. What stabilizes tertiary structure?

Hydrophobic interactions, hydrogen bonds, ionic bonds, van der Waals forces, and **disulfide bonds**.

8. What is a protein domain?

A compact, independently folded, functional region within a protein.

9. What is quaternary structure?

Association of **two or more polypeptide subunits** into a functional protein.

10. Give examples of quaternary proteins.

Hemoglobin, immunoglobulins, LDH (tetramer).

11. What are simple proteins?

Proteins that yield **only amino acids** on hydrolysis (e.g., albumin, globulin).

12. What are conjugated proteins?

Proteins with a **prosthetic group** (e.g., hemoglobin, lipoproteins, glycoproteins).

13. What is the main function of albumin?

Maintains **oncotic pressure** and transports fatty acids, bilirubin, and drugs.

14. What is the A/G ratio?

Albumin–globulin ratio; normal is **1.2–1.8**.

15. What amino acids are abundant in collagen?

Glycine, proline, and hydroxyproline.

16. What does vitamin C do in collagen synthesis?

Required for **hydroxylation** of proline and lysine.

17. What is the major defect in scurvy?

Impaired hydroxylation ? unstable collagen.

18. What is unique about elastin?

Contains **desmosine** and **isodesmosine**, which provide elasticity.

19. What is the main defect in Marfan syndrome?

Defective **fibrillin**, which is required for elastin scaffolding.

20. What is heat coagulation?

Denaturation and precipitation of proteins on heating (e.g., cooking an egg).

21. How is protein concentration estimated most commonly?

Biuret method (violet complex with peptide bonds).

22. What is the basis of Bradford assay?

Binding of **Coomassie Blue dye** to basic and aromatic residues.

23. What is renaturation?

Recovery of native protein structure after removal of denaturing agents—possible only if the **primary structure remains intact**.

24. What proteins assist in folding?

Chaperones, including Hsp70, Hsp60 (chaperonins), and PDI.

25. What happens if a protein misfolds?

It may aggregate and cause diseases like **Alzheimer's, Parkinson's**, and prion diseases.

MCQs

1. The peptide bond is planar due to:

- A. Ionic bonding
- B. Hydrogen bonding
- C. Resonance causing partial double-bond character
- D. Hydrophobic interactions

Answer: C

2. Which amino acid disrupts α -helix formation?

- A. Alanine
- B. Proline
- C. Valine
- D. Cysteine

Answer: B

3. β -pleated sheets are stabilized mainly by:

- A. Disulfide bonds
- B. Hydrogen bonds between different segments
- C. Hydrophobic interactions
- D. Van der Waals forces

Answer: B

4. Which is an example of quaternary structure?

- A. Single chain of myoglobin
- B. Triple helix of collagen
- C. $\alpha\beta\gamma\delta$ arrangement of hemoglobin
- D. α -helix in keratin

Answer:

5. Which protein is most abundant in plasma?

- A. Globulin
- B. Fibrinogen
- C. Albumin
- D. Transferrin

Answer: C

6. Which plasma protein maintains oncotic pressure?

- A. IgG
- B. Albumin
- C. Fibrinogen
- D. Ceruloplasmin

Answer: B

7. Glycine, proline, and hydroxyproline are abundant in:

- A. Elastin
- B. Keratin
- C. Collagen
- D. Albumin

Answer: C

8. Vitamin C is essential for which step in collagen synthesis?

- A. Glycosylation
- B. Hydroxylation
- C. Disulfide bond formation
- D. Cleavage of propeptides

Answer: B

9. Elastin contains which unique amino acid cross-links?

- A. Hydroxylysine
- B. Desmosine and isodesmosine
- C. Selenocysteine
- D. Ornithine

Answer: B

10. Defect in fibrillin is seen in:

- A. Scurvy
- B. Marfan syndrome
- C. Osteogenesis imperfecta
- D. Ehlers–Danlos syndrome

Answer: B

11. The Biuret test detects proteins based on:

- A. Reaction with aromatic rings
- B. Copper binding to peptide bonds
- C. Reaction with sulfhydryl groups
- D. Reduction of Folin reagent

Answer: B

12. Bradford assay is based on:

- A. Ninhydrin binding
- B. Protein absorption at 280 nm
- C. Coomassie Blue dye binding to proteins
- D. Reduction of metal ions

Answer: C

13. Chaperones assist in:

- A. Peptide bond formation
- B. Proper protein folding
- C. DNA replication

D. Protein degradation

Answer: B

14. Renaturation is possible only when:

- A. Disulfide bonds break
- B. Primary structure remains intact
- C. Protein is heat-coagulated
- D. Aggregates are present

Answer: B

15. Which of the following is a simple protein?

- A. Hemoglobin
- B. Lipoprotein
- C. Albumin
- D. Glycoprotein

Answer: C

16. Collagen triple helix contains how many chains?

- A. One
- B. Two
- C. Three
- D. Four

Answer: C

17. Radioimmuno-electrophoresis is mainly used to detect:

- A. Plasma albumin
- B. Immunoglobulins
- C. Fibrinogen
- D. Transferrin

Answer: B

18. Which factor causes heat coagulation of proteins?

- A. Peptide bond hydrolysis
- B. Breaking of weak non-covalent interactions
- C. Increased hydrogen bonding
- D. Enhanced tertiary folding

Answer: B

19. Which enzyme stabilizes disulfide bonds during folding?

- A. Hsp70
- B. PDI (Protein disulfide isomerase)
- C. Peptidyl-prolyl isomerase
- D. Trypsin

Answer: B

20. Which protein fraction increases in chronic infection?

- A. Albumin
- B. Fibrinogen
- C. α -Globulins
- D. α -Globulins only

Answer: C

Viva Voce

1. What is the primary structure of a protein?

The linear sequence of amino acids linked by peptide bonds.

2. Why is the peptide bond rigid?

Because resonance gives the $-\text{CO}-\text{NH}-$ bond **partial double-bond character**, restricting rotation.

3. What stabilizes secondary structure?

Hydrogen bonds between backbone --C=O and --NH groups.

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A right-handed helix stabilized by intrachain hydrogen bonds; 3.6 residues per turn.

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Hemoglobin (????).

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Cross-linking via **desmosine** and **isodesmosine**.

17. What is defective in Marfan syndrome?

Fibrillin, the scaffolding protein for elastin.

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Disruption of weak non-covalent bonds by heat ? unfolding + aggregation.

19. What is the basis of the Biuret test?

Copper ions bind to peptide bonds in alkaline medium ? violet color.

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A denatured protein regains its native structure when the denaturant is removed, provided the primary structure is intact.

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Assist correct protein folding and prevent aggregation.

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Protein disulfide isomerase (PDI).

23. What is a simple protein?

A protein that yields only amino acids on hydrolysis (e.g., albumin).

24. What is a conjugated protein?

Protein + prosthetic group (e.g., hemoglobin, lipoprotein, glycoprotein).

25. What property allows proteins to act as buffers?

They are **amphoteric**, containing both acidic and basic groups.