

Methods of Protein Sequencing

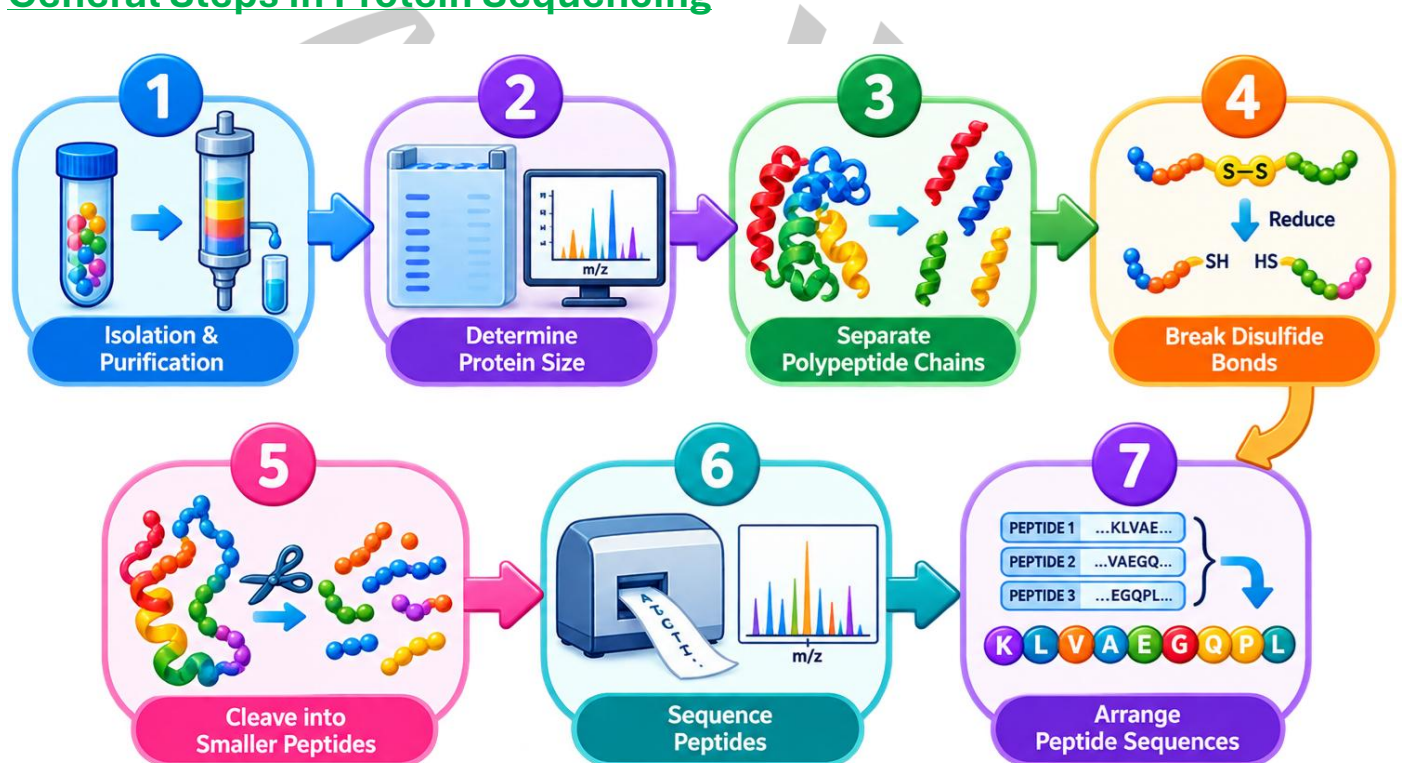
Methods of Protein Sequencing

- Protein sequencing is the process of determining the exact order of amino acids in a protein.
- The sequence of amino acids is very important because it determines:
 - Protein structure
 - Biological function
 - Interaction with other molecules

Importance of Protein Sequencing

- Helps understand protein function
- Used in drug design and biotechnology
- Identifies mutations and structural changes
- Important for quality control in protein formulations

General Steps in Protein Sequencing



1. Isolation and Purification

- The protein of interest is first separated from other proteins and biological materials.
- Techniques such as chromatography and electrophoresis can be used for purification.

2. Determination of Protein Size

- The approximate molecular mass of the protein can be determined using SDS-PAGE or mass spectrometry.

- This information helps in further characterization of the protein.

3. Separation of Polypeptide Chains

- A protein containing more than one polypeptide chain must be separated into its individual chains.
- Each chain can then be studied separately.

4. Breaking of Disulfide Bonds

- Disulfide bonds between cysteine residues may connect different regions or chains of a protein.
- These bonds can be reduced so that the polypeptide chains can be analyzed more easily.

5. Cleavage into Smaller Peptides

- Large proteins are difficult to sequence directly.
- Proteolytic enzymes or chemical reagents are therefore used to break them into smaller peptides.

6. Sequencing of Peptides

- The amino acid sequence of each peptide is determined.
- Edman degradation or mass spectrometry can be used for this purpose.

7. Arrangement of Peptide Sequences

- The sequences of individual peptides are arranged in the correct order.
- Peptides produced by different cleavage methods can provide overlapping sequences that help reconstruct the complete protein sequence.

Main Approaches

A. Chemical Methods

- Directly determine amino acid sequence using chemical reactions.

B. Enzymatic Methods

- Use enzymes to break proteins into smaller peptides.

C. Mass Spectrometry-Based Methods

- Most advanced and widely used today.

Common Methods of Protein Sequencing

1. Edman Degradation

- Stepwise removal of amino acids from N-terminal
- Identifies sequence one residue at a time

2. Mass Spectrometry

- Determines molecular weight of peptides
- Helps identify amino acid sequence
- Fast and highly sensitive

3. Enzymatic Digestion

- Proteins are cut into smaller fragments using enzymes
- Makes sequencing easier

4. DNA Sequencing (Indirect Method)

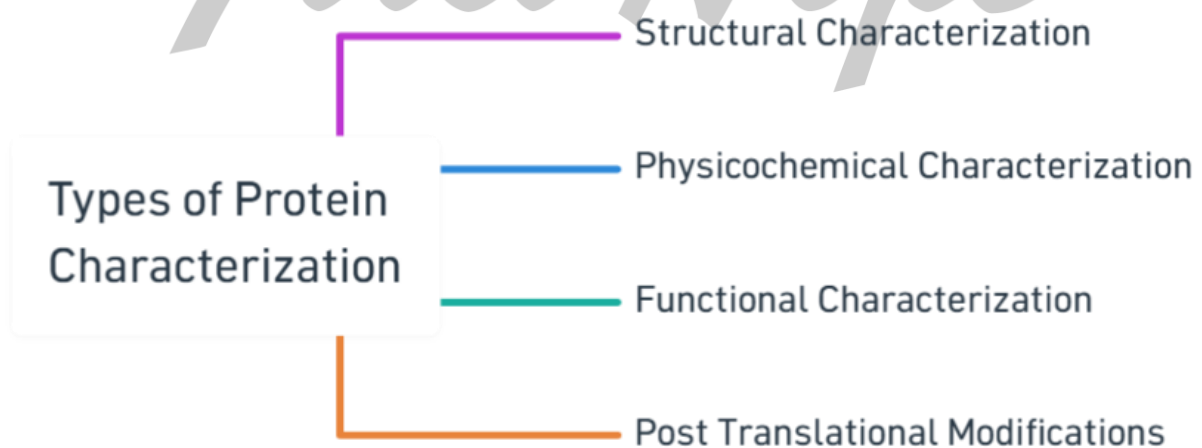
- Gene encoding protein is sequenced
- Amino acid sequence is predicted from DNA

Protein Characterization

Definition

- Protein characterization involves studying the **structural, physicochemical, and functional properties** of proteins.

Types of Protein Characterization



1. Structural Characterization

A. Primary Structure

- Amino acid sequence of protein

B. Secondary Structure

- Alpha-helix and beta-sheet content
- **Technique:**
 - Circular Dichroism (CD)

C. Tertiary Structure

- Three-dimensional (3D) folding of protein
- **Techniques:**
 - X-ray crystallography
 - NMR spectroscopy

D. Quaternary Structure

- Arrangement of multiple protein subunits

2. Physicochemical Characterization

- Molecular weight
- Isoelectric point (pI)
- Solubility
- Stability

3. Functional Characterization

- Enzyme activity assays
- Binding studies
- Measurement of biological activity

4. Post-Translational Modifications

- Chemical modifications after protein synthesis
- **Examples:**
 - Phosphorylation
 - Glycosylation
- Important for protein function and regulation

Importance of Protein Characterization

- Helps in understanding protein function
- Supports drug development
- Ensures quality control in formulations
- Important in biotechnology and research

Edman Degradation

Definition

- Edman degradation is a method used to determine the amino acid sequence of a protein by removing one amino acid at a time from the N-terminal end.



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