Amino Acid Sequencing

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1. Introduction

Amino acid sequencing is the process of determining the exact order of amino acids in a polypeptide chain. The primary structure of a protein determines its three-dimensional structure and biological function.

Understanding amino acid sequence is essential for:

- Determining protein structure–function relationships
- Studying genetic mutations
- Protein engineering
- Drug development
- Evolutionary studies

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2. General Strategy for Protein Sequencing

Protein sequencing involves the following steps:

1. Determination of number of polypeptide chains
2. Breaking disulfide bonds
3. Identification of N-terminal and C-terminal residues
4. Fragmentation of protein into smaller peptides
5. Sequencing of individual fragments
6. Reconstruction of full sequence

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3. Determination of Polypeptide Chains

- Many proteins consist of multiple subunits.
- Subunits are separated using:
    - SDS-PAGE

    - Gel filtration chromatography
- Molecular weight is determined before sequencing.

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4. Cleavage of Disulfide Bonds

Disulfide bonds (–S–S–) must be broken before sequencing.

Methods:

1.Oxidation

- Using performic acid
- Converts cysteine → cysteic acid

2. Reduction

- Using β-mercaptoethanol or dithiothreitol (DTT)
- Followed by alkylation with iodoacetate to prevent reformation

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5. Determination of Terminal Amino Acids

A. N-terminal Analysis

(1) Sanger's Method (FDNB Method)

- Reagent: 1-fluoro-2,4-dinitrobenzene (FDNB)
- Reacts with free amino group of N-terminal residue.
- After acid hydrolysis, labeled amino acid is identified.
- Forms DNP-amino acid.

Limitation:

- Only identifies first amino acid.
- Protein is destroyed.

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(2) Dansyl Chloride Method

- Reagent: Dansyl chloride

- Forms fluorescent derivative.
- More sensitive than Sanger's method.

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(3) Edman Degradation (Most Important)

- Reagent: Phenyl isothiocyanate (PITC)
- Reacts with N-terminal amino acid.
- Cleaves first amino acid without destroying rest of peptide.
- Forms PTH-amino acid (phenylthiohydantoin derivative).

Advantages:

- Sequential removal of amino acids.
- Can sequence up to 50 residues.

Limitation:

- Inefficient for long proteins (>50 residues).
- Blocked N-terminus cannot be analyzed**.**

B. C-terminal Analysis

(1) Carboxypeptidase Method

- Enzyme: Carboxypeptidase
- Removes amino acids one by one from C-terminal.
- Identified chromatographically.

Limitation:

- Slow and sometimes non-specific.

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6. Challenges in Amino Acid Sequencing

- Blocked N-terminus

- Post-translational modifications
- Glycosylation
- Very long polypeptide chains
- Low abundance proteins

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7. Applications

- Determining primary structure
- Detecting mutations
- Studying genetic diseases
- Protein engineering
- Evolutionary comparison
- Drug target identification

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8. Comparison: Edman vs Mass Spectrometry

Feature	Edman Degradation	Mass Spectrometry
Sensitivity	Moderate	Very high
Speed	Slow	Fast
Protein size	Small peptides	Any size
PTM detection	Limited	Excellent

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9. Importance of Amino Acid Sequencing

- Establishes primary structure
- Basis of secondary and tertiary structure
- Essential for understanding enzyme mechanism
- Fundamental in biotechnology