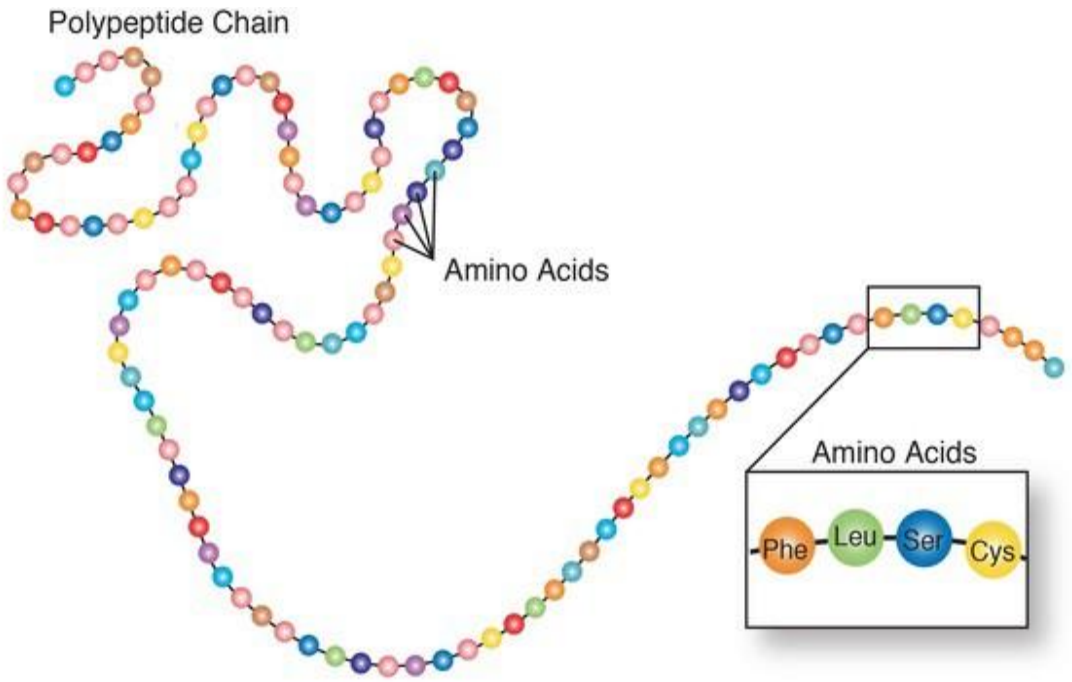
Peptide Sequencing by Edman Degradation



Protein Sequencing

- Protein sequencing is a technique to determine the amino acid sequence of a protein.
- It is a method to understand the structure and function of proteins in living organism.
- Amino acid sequence determines the eventual 3-D structure of protein.
- Protein sequencing is useful in identification of unknown proteins, determination of fragment sequences following degradation or some modifications, or to check glycosylation patterns, effects or changes.

Why need Sequencing?

- By the middle of the twentieth century it was known that proteins were composed of amino acids, but not how these were joined together. Were they arranged in blocks of similar residues? or randomly mixed together? or in repeated patterns?

Determination of Amino Acid Composition

- Amino acid composition and purity must be known before starting sequencing.
- The polypeptide chains of multi meric proteins should be separated and mol.wt. of each chain should be measured.
- The determination of amino acid is done by hydrolysis, separation and quantitative analysis.

Methods used for Sequencing

- Fredrick Sanger and colleagues for the sequencing of insulin was to characterize series of small overlapping peptides produced by cleavage of the parent molecule. Determination of the overall amino acid content and the identity of the amino- (N-)terminal residue for each peptide allowed deduction of the sequence of the whole molecule **(Sanger, 1959).**
- An alternative approach was that described by **Pehr Edman (1950).** This allowed determination of extended sequences of peptides or whole proteins, and has been used widely up to the present day.

Basic Steps of Sequencing

The usual strategy for determining the amino acid sequence of a protein involves eight basic steps.

- If the protein contains more than one polypeptide chain, the chains are separated and purified.
- Intrachain S--S (disulfide) cross-bridges between cysteine residues in the polypeptide chain are cleaved. If these disulfides are interchain linkages, then step 2 precedes step1.
- The amino acid composition of each polypeptide chain is determined.
- The N-terminal and C-terminal residues are identified.
- Each polypeptide chain is cleaved into smaller fragments.
- Sequence determination of peptide fragments.
- The overall amino acid sequence of the protein is reconstructed from the sequences in overlapping fragments.
- The positions of S--S cross-bridges formed between cysteine residues are located.

Steps Contd.....

- **Separation of Polypeptide Chains:**

  Subunit associations in multimeric proteins are typically maintained solely by noncovalent forces, and therefore most multimeric proteins can usually be dissociated by exposure to pH extremes, 8 M urea, 6 M guanidinium hydrochloride, or high salt concentrations.
- **Cleavage of Disulfide Bridges:**

  Oxidation of a disulfide by performic acid results in the formation of two equivalents of cysteic acid.
- **Identification of the N- and C-Terminal Residues**

N-Terminal analysis:

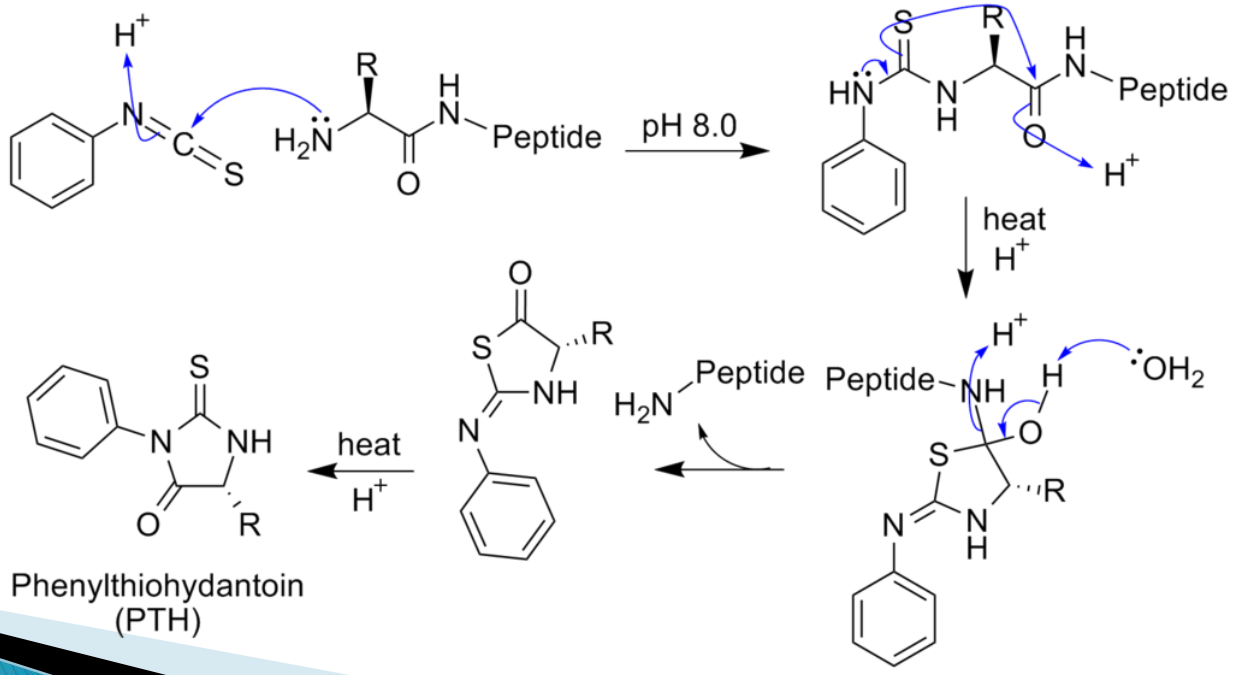
Amino terminal end of polypeptide chain determined by either of three methods namely sangar method, **Edmann's method** and dansyl chloride method.

Edman degradation:

In Edman degradation method, Phenyl isothio cyanate (PIT) is used as reagent.

First the polypeptide is rected with phenyl isothio cyanate to form a polypeptidyl phenylthiocarbamyl derivative. Gentle hydrolysis releases the amino terminal amino acid as a phenylthiohydantoin (PTH), which can be separated and detected spectrophotometrically.

Upto this stage, this method is used to determine N-terminal aminoacid.



Edman Degradation

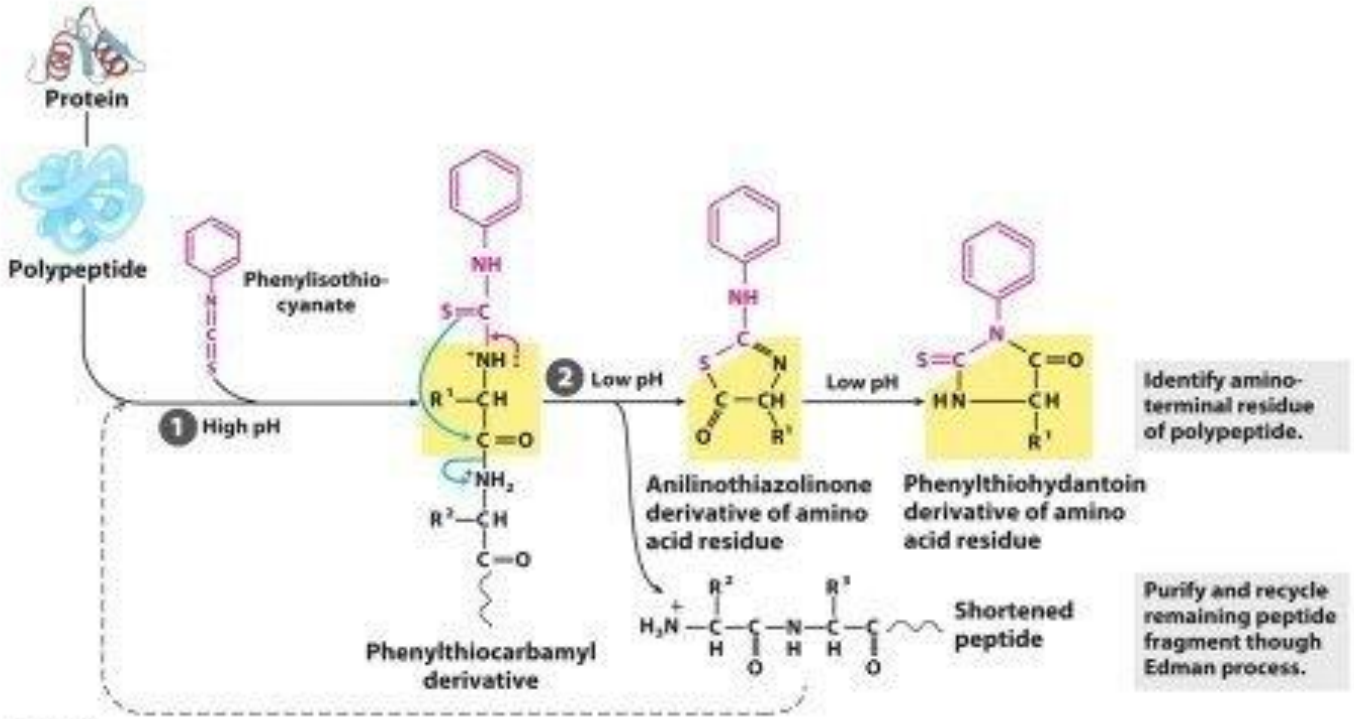


Figure 3-27
Lehninger Principles of Biochemistry, Sixth Edition

Steps Contd.....

C- Terminal Analysis:

There are two methods used for C-terminal determination. They are Hydrazine method and carboxy peptidase method.

Carboxypeptidases are enzymes that cleave amino acid residues from the C-termini of polypeptides in a successive fashion. Four carboxypeptidases are in general use: A, B, C, and Y.

- **Fragmentation of the Polypeptide Chain**

  The aim at this step is to produce fragments useful for sequence analysis. The cleavage methods employed are usually enzymatic, but proteins can also be fragmented by specific or nonspecific chemical means (such as partial acid hydrolysis).

Steps Contd...

- **Reconstruction of the Overall Amino Acid Sequence**

  The sequences obtained for the sets of fragments derived from two or more cleavage procedures are now compared, with the objective being to find overlaps that establish continuity of the overall amino acid sequence of the polypeptide chain. Peptides generated from specific hydrolysis of the polypeptide can be aligned to reveal the overall amino acid sequence.

Steps contd..

- **Locating Disulfide Bonds**

  If the primary structure includes disulfide bonds, their locations are determined in an additional step after sequencing is completed. A sample of the protein is again cleaved with aa reagent such as trypsin, this time without first breaking the disulfide bonds. The resulting peptides are separated by electrophoresis and compared with the original set of peptides generated by trypsin. For each disulfide bond, two of the original peptides will be missing and a new, larger peptide will appear. The two missing peptides represent the regions of the intact polypeptide that are linked by the disulfide bond.

Applications of Protein Sequencing

- Knowledge of the sequence of Amino acid in a protein can offer insights into its three dimentional structure and its function in cellular location.
- Certain amino acid sequences serve as signals that determine the cellular location chemical modification on an half life of a protein
- Protein sequence can elucidate the history of life on earth.

Institutes doing Protein Sequencing

- India
  - IIT, Bombay
  - TCGA, Kolkata
  - IGIB,New Delhi
  - IISC, Banglore
- Abroad
  - Novo Nordisk foundation centre for protein research, University of Copenhagen.
  - Institute of protein research of the Ras, Pushchino, Moscow.

References

- Peptide Sequencing by Edman Degradation John Bryan Smith, Celltech Chiroscience plc, Slough, UK.
- www.creative-proteomics.com
- https://en.wikipedia.org/wiki/Edman_degradation