ENZYMOLOGY

Topic: Protein Sequencing

BY

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References

1. Lehninger Principles Of Biochemistry Fourth Edition: David L. Nelson And Michael M. Cox
2. Biochemistry; Donald Voet And Judith G. Voet

PRIMARY STRUCTURE DETERMINATION OF PROTEINS

Protein sequence determination

The first determination of the complete amino acid sequence of a protein, that of the bovine polypeptide hormone insulin by Frederick Sanger in 1953

1. The knowledge of a protein's amino acid sequence is essential for an understanding of its molecular mechanism of action as well as being prerequisite for the elucidation of its three-dimensional structure by both X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.
2. Sequence comparisons among analogous proteins from the same individual, from members of the same species, and from members of related species have yielded important insights into how proteins function and have indicated the evolutionary relationships among the proteins and the organisms that produce them.
3. Amino acid sequence analyses have important clinical applications because many inherited diseases are caused by mutations leading to an amino acid change in a protein. Recognition of this fact has led to the development of valuable diagnostic tests for many such diseases and, in many cases, to symptom-relieving therapy.

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1. Lehninger Principles Of Biochemistry Fourth Edition: David L. Nelson And Michael M. Cox
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Basic Steps Protein Sequence Determination By Sanger's Method

The basic procedure for primary structure determination is developed by Sanger.

The procedure consists of three conceptual parts, each of which requires several laboratory steps:

1. **Prepare the protein for sequencing**: a. Determine the number of chemically different polypeptide chains (subunits) in the protein. b. Cleave the protein's disulfide bonds. c. Separate and purify the unique subunits.
2. **Sequence the polypeptide chains**: a. Fragment the individual subunits at specific points to yield peptides small enough to be sequenced directly. b. Separate and purify the fragments. c. Determine the amino acid sequence of each peptide fragment. d. Repeat Step 2a with a fragmentation process of different specificity so that the subunit is cleaved at peptide bonds different from before.
3. **Organize the completed structure**: a. Span the cleavage points between one set of peptide fragments by the other. By comparison, the sequences of these sets of polypeptides can be arranged in the order that they occur in the subunit, thereby establishing its amino acid sequence. b. Elucidate the positions of the disulfide bonds, if any, between and within the subunits

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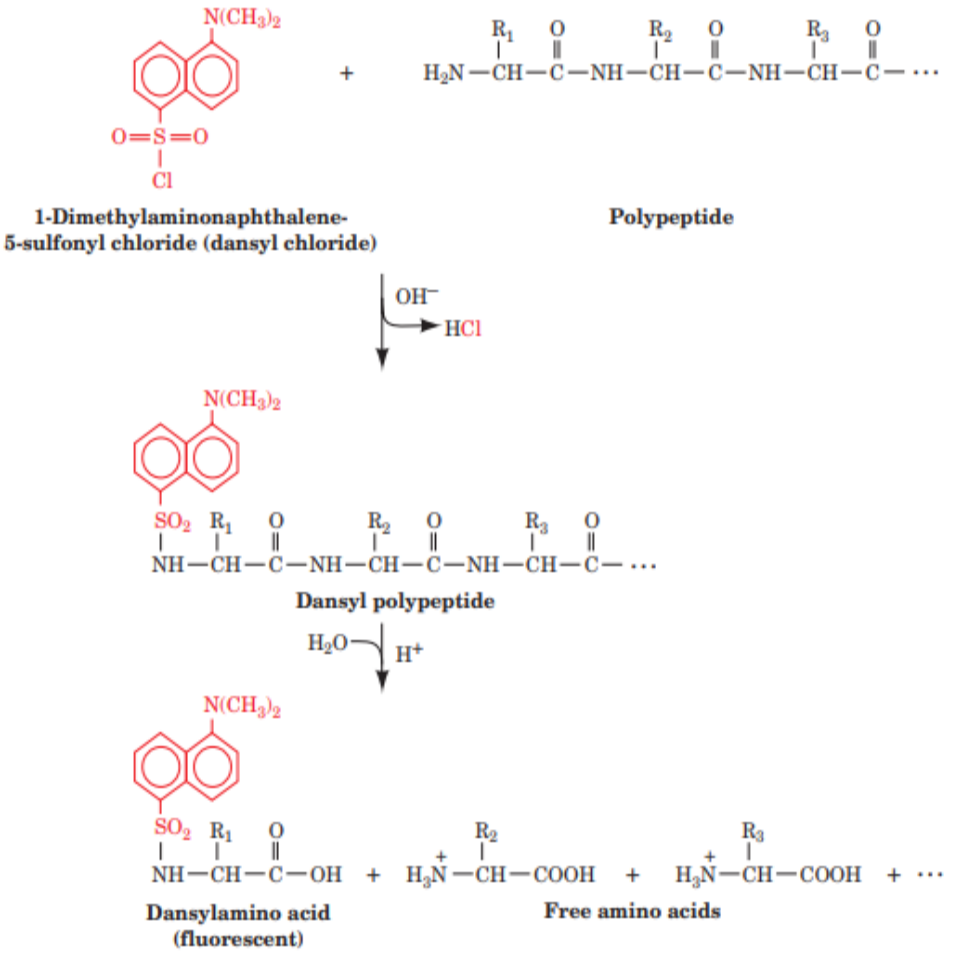
Each polypeptide chain has an N-terminal residue and a C-terminal residue

N-Terminus Identification

1. There are several effective methods by which a polypeptide's N-terminal residue may be identified.
2. 1-Dimethyl-aminonaphthalene-5-sulfonyl chloride (dansyl chloride) reacts with primary amines (including the ε-amino group of Lys) to yield dansylated polypeptides.
3. Acid hydrolysis liberates the N-terminal residue as a dansylamino acid, which exhibits such intense yellow fluorescence that it can be chromatographically identified from as little as 100 picomoles of material



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1. Lehninger Principles Of Biochemistry Fourth Edition: David L. Nelson And Michael M. Cox
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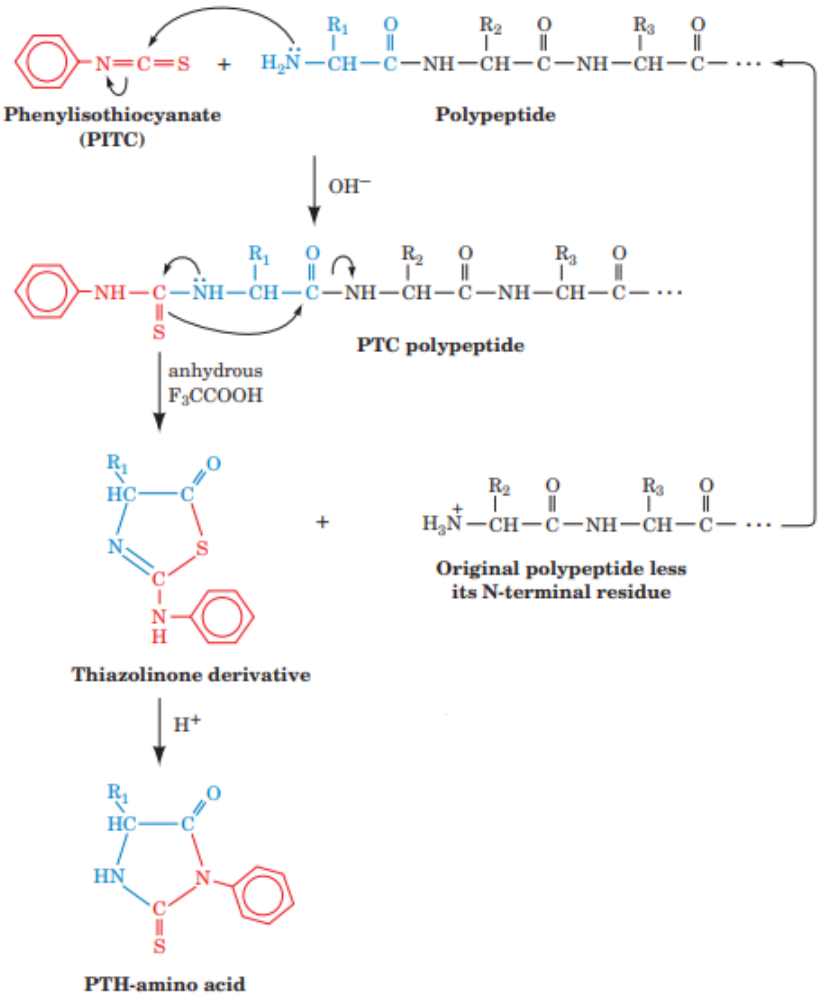
Edman degradation (inventor, Pehr Edman),

1. phenylisothiocyanate (PITC, Edman's reagent) reacts with the N-terminal amino groups of proteins under mildly alkaline conditions to form their phenylthiocarbamyl (PTC) adduct.
2. This product is treated with an anhydrous strong acid such as trifluoroacetic acid, which cleaves the N-terminal residue as its thiazolinone derivative but does not hydrolyze other peptide bonds.
3. The Edman degradation therefore releases the N-terminal amino acid residue but leaves intact the rest of the polypeptide chain.
4. The thiazolinone-amino acid is selectively extracted into an organic solvent and is converted to the more stable phenylthiohydantoin (PTH) derivative by treatment with aqueous acid.
5. This PTH-amino acid is most commonly identified by comparing its retention time on HPLC with those of known PTH-amino acids.



- The most important difference between the Edman degradation and other methods of N-terminal residue identification is that we can determine the amino acid sequence of a polypeptide chain from the N-terminus inward by subjecting the polypeptide to repeated cycles of the Edman degradation and, after every cycle, identifying the newly liberated PTH-amino acid.
- This technique has been automated, resulting in great savings of time and materials

References
1. Biochemistry; Donald Voet And Judith G. Voet

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1. Lehninger PRINCIPLES OF BIOCHEMISTRY Fourth Edition: David L. Nelson and Michael M. Cox
2. BIOCHEMISTRY; DONALD VOET and JUDITH G. VOET