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Perspective

Edman Degradation: The Protease Digestion of a Protein

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DESCRIPTION

Edman degradation is a chemical reaction that sequentially removes N-terminal amino acids from a peptide or protein in a series of steps. Edman sequencing, on the other hand, is limited to single protein samples for proper data interpretation and amino acid assignments, and hence lacks high capabilities. We offer a new approach for determining the N-terminal sequences of numerous protein fragments in solution with high throughput. Proteolytic processing can alter the activity of bioactive proteins, disclose cryptic binding sites, and create proteins with novel functionalities (neoproteins) that were not present in the original molecule. Pehr Edman invented the Edman degradation method for sequencing amino acids in peptides. This method tags and cleaves the amino-terminal residue from the peptide without disrupting the peptide bonds between the other amino acid residues. Under gently alkaline circumstances, phenyl isothiocyanate reacts with an uncharged N-terminal amino group to generate a cyclical phenylthiocarbamoyl derivative. This terminal amino acid derivative is then cleaved into a thiazolinone derivative under acidic conditions. The thiazolinone amino acid is then selectively extracted into an organic solvent and acid-treated to create the more stable phenylthiohydantoin (PTH)- amino acid derivative, which can be identified using chromatography or electrophoresis.

Peptide cyclic degradation based on the reaction of phenylisothiocyanate with the free amino group of the N-terminal residue, in which amino acids are eliminated one at a time and their phenylthiohydantoin derivatives are discovered. To create a phenylthiocarbamoyl derivative, an uncharged peptide is reacted with phenylisothiocyanate at the amino terminus under gently alkaline conditions. The thiocarbonyl sulphur of the derivative then attacks the carbonyl carbon of the N-terminal amino acid under acidic circumstances. The first amino acid is cleaved to form an anilinothiazolinone derivative (ATZ-amino acid), and the rest of the peptide can be separated and degraded in the next step. This thiazolone derivative is more stable than the phenylthiocarbamyl derivative once generated. Following that, the ATZ amino acid is extracted using ethyl acerate and transformed to a phenylthiohydantoin derivative (PTH-amino acid). Chromatography can also be used to determine the PTH residue produced by each cycle.

The technique can then be repeated to determine the amino acid that comes next. The peptides sequenced in this way cannot have more than 50 to 60 residues, which is a significant disadvantage of this method. Because cyclical derivatization does not always go to completion, the peptide length is restricted. Large peptides can be cleaved into smaller peptides before starting with the procedure, which solves the derivatization issue. With contemporary equipment capable of over 99 percent efficiency per amino acid, it can sequence up to 30 amino acids with precision. The Edman degradation has the benefit of requiring only 10 to 100 picomoles of peptide for sequencing. Edman and Beggs automated the Edman degradation reaction in 1967 to speed up the process, and by 1973, 100 automated units were in operation globally. Because Edman degradation starts at the protein's N-terminus, it won't work if the N-terminus is chemically altered (e.g. by acetylation or formation of pyroglutamic acid). If a non-amino acid (such as isoaspartic acid) is met, the preferred five-membered ring intermediate cannot be created, and the sequence will be terminated. When it comes to determining the positions of disulfide bridges, Edman degradation is often ineffective. For detectable results, peptide concentrations of 1 picomole or more are required.

Proteins can be transferred to a polyvinylidene difluoride (PVDF) blotting membrane for further analysis after 2D SDS PAGE. A PVDF membrane can be used to execute Edman degradations. Sequencing of the N-terminal residues resulting in five to ten amino acids may be enough to identify a Protein of Interest.

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