Protein sequencing by Edman degradation:

The sequence of amino acids in a protein or peptide can be identified by Edman degradation, which was developed by Pehr Edman.

This method can label and cleave the peptide from N-terminal without disrupting the peptide bonds between other amino acid residues.

The Edman degradation reaction was automated in 1967 by Edman and Beggs.

Nowadays, the automated Edman degradation (the protein sequenator) is used widely, and it can sequence peptides up to 50 amino acids i.e. small peptides can be sequenced. For larger peptides, we need to break them into small peptides and then sequence them separately. These separate sequences are then used to reconstruct the sequence of the larger protein but discussion of these methods for larger proteins is out of scope here.

This process is used to confirm the identity of the recombinant proteins after their production, isolation and purification. This is done by sequencing the N-terminal amino acid residues.

Stepwise mechanism of Edman's degradation:

1. Peptide is made to react with phenylisothiocyanate (PITC) at the amino terminus under mildly alkaline conditions to give a phenylthiocarbamoyl derivative (PTC-peptide).
2. Then, conditions are changed to acidic by adding anhydrous trifluoroacetic acid (CF_3COOH).
3. This leads to the thiocarbonylsulfur of the PTC-derivative attacking the carbonyl carbon of the N-terminal amino acid. Thus, peptide bond linking the N-terminal amino acid to the rest of the peptide is attacked for cleavage.
4. As a result of this reaction, first amino acid is cleaved as anilinothiazolinone derivative (ATZ-amino acid) and the remaining peptide is isolated and subjected to the next degradation cycle.
5. Once formed, the ATZ- derivative (also called as thiazolinone derivative) is more stable than phenylthiocarbamoyl derivative.
6. The ATZ amino acid is now separated by extraction with ethyl acetate and converted to a phenylthiohydantoin derivative (PTH-amino acid) by treatment with aqueous acid.
7. Chromatography is then used to identify the PTH residue generated by each cycle.