Determination of Primary Structure of Peptides :-

Structure determination of peptides means arriving at the sequence of amino acids in a polypeptide chain. When the polypeptide chain is a protein, this sequence is refered to as its primary structure. In order to determine the structure of a peptide, we must know:

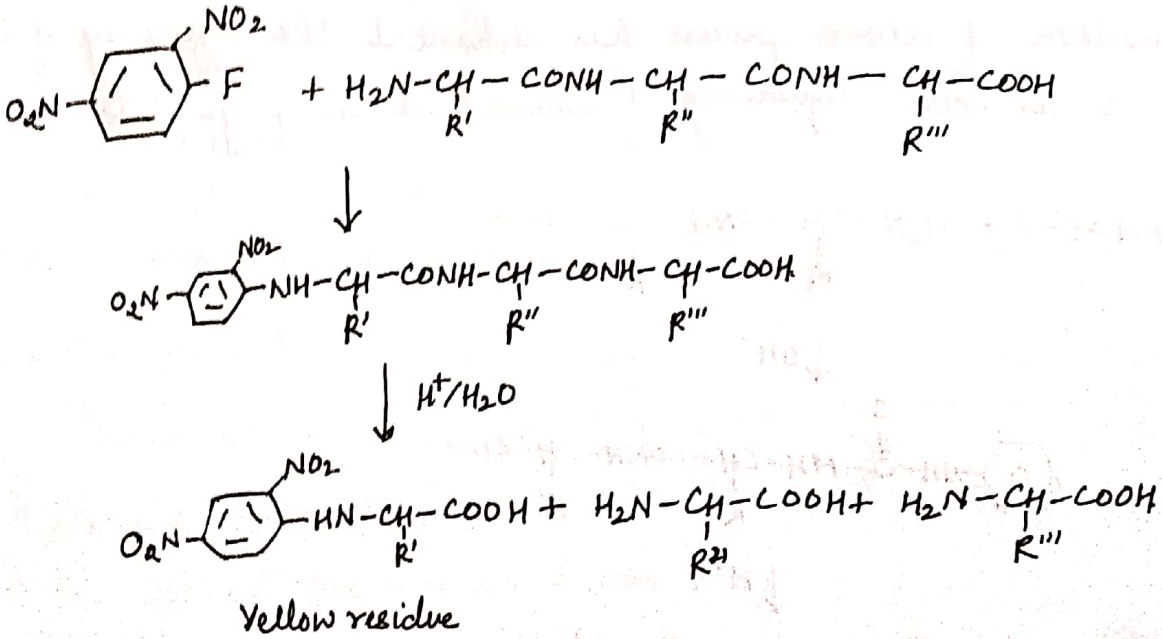
1. The kind of amino acid that make up the peptide.
2. The number of amino acids of each type.
3. The order in which these amino acids are aranged in the peptide chain.

Therefore, it is necessary to have a qualitative and quantitative analysis of the amino acids present. In the first step, the peptide chain is converted into its amino acid components by complete hydrolysis. In the second step, the hydrolysate that contains the mixture of amino acid from first step is analysed to determine the amino acid composition. In order to calculate the number of residues of each amino acid present, we must know the molecular weight of the polypeptide, which is determined by suitable physical methods. Finally, in the third step, the sequence of amino acid is determined.

Identification of the N-Terminal Amino Acid :-

1) DNP Method (Sanger Method):

This method was introduced by F. Sanger. In this peptide chain is treated with (DNFB) 2,4-dinitrofluorobenzene which undergoes nucleophilic substitution by the free amino group to give an N-dinitrophenyl derivative (DNP). Complete hydrolysis of the DNP-peptide with acid gives yellow DNP-derivative of the N-terminal residue together with free amino acids, which can be identified by chromatography.



Yellow residue

The DNP derivatives of most of the amino acids have been prepared and characterised. The DNP method can not be used repeatedly since its use requires complete hydrolysis of the DNP derivative.

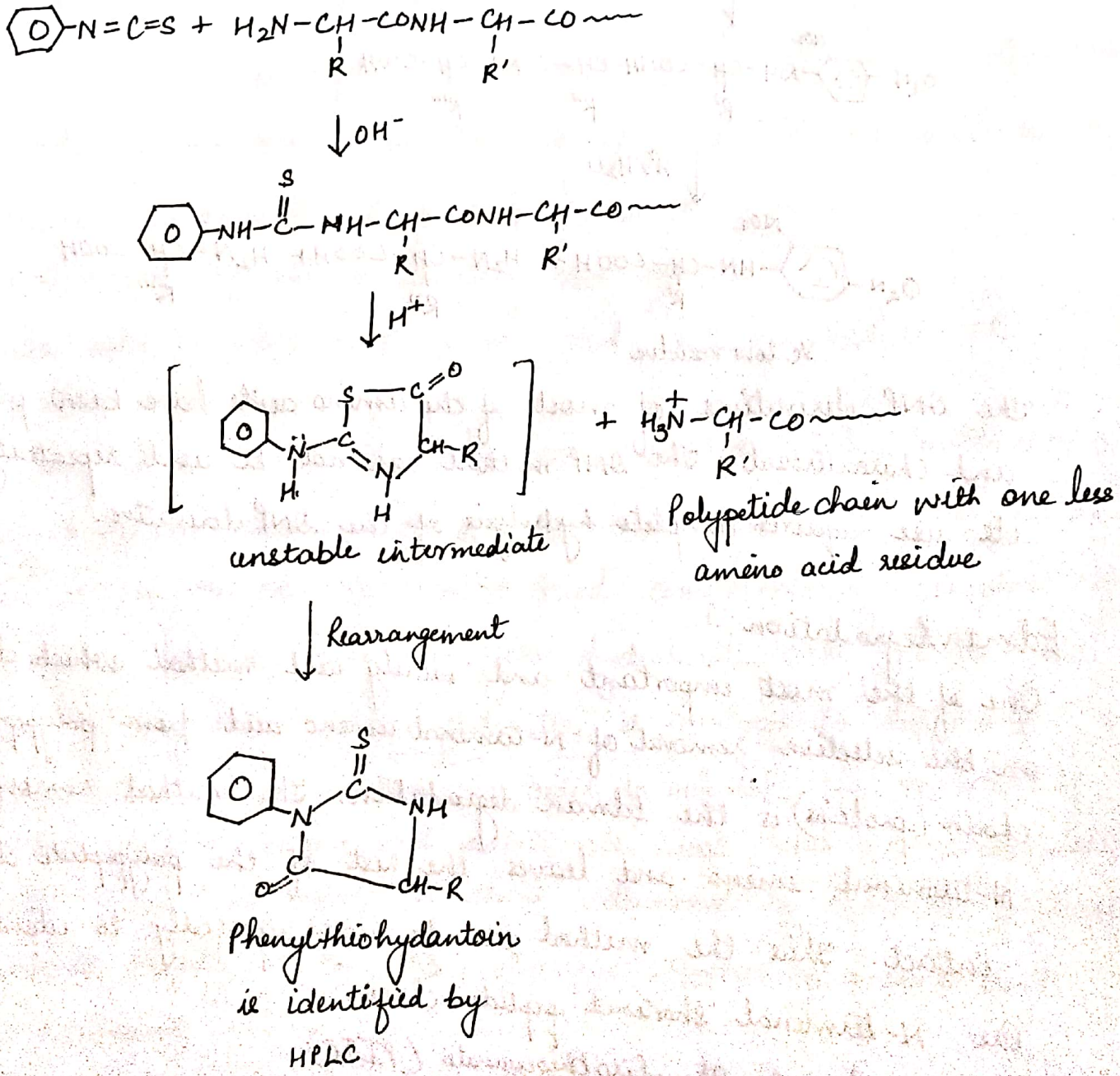
Edman Degradation :-

One of the most important and widely used method which depend on the selective removal of N-terminal amino acid from polypeptide chain (protein) is the Edman degradation. The method removes N-terminal amino and leaves the rest of the polypeptide chain intact. Thus the method can be used repeatedly to identify the N-terminal shortened peptide chain.

Edman's Reagent - Phenylisothiocyanate (PITC)

PITC reacts with N-terminal of polypeptide under mild alkaline conditions (PH-8) to form phenylthiocarbamyl-peptide. This is then treated with dil. acid (HCl) which cleaves N-terminal residue that on mild hydrolysis results in the selective separation of N-terminal amino acid as phenylthiohydantoin which is identified by HPLC. The rest of the polypeptide chain remains intact. Now shortened polypeptide chain is subjected to second round of edman degradation.

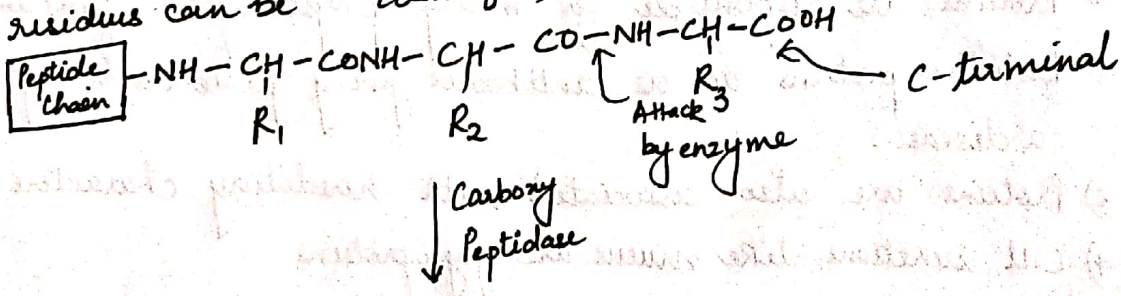
The automation of above process has enhanced the efficiency of this method to continue sequencing of amino acid in polypeptide.

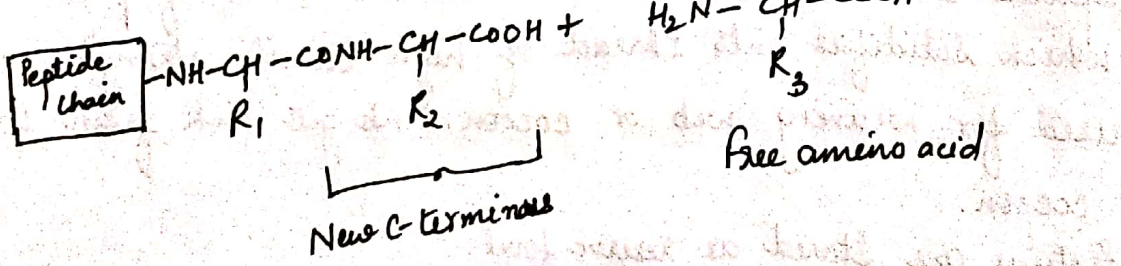


* The repeated Edman degradation can be used for identification polypeptide containing 50 or even more amino acid.

C-terminal Analysis :- (Enzymatically)

C-terminal Analysis is carried out even more efficiently by enzymatic method rather than by chemical method. The enzyme Carboxypeptidase cleaves selectively the peptide linkage adjacent to C-terminal (free Carboxylic group) in polypeptide chain. The removal of C-terminal residue results in the formation of shortened polypetide chain, which can be treated with Carboxypeptidase enzyme to determine new C-terminals. (Under favourable conditions the free three or four amino acid residues can be identified)





C-terminal Analysis :-

Thiohydantin formation:-

This method is analogous to Edman's method for N-terminus amino acids. The C-terminus amino acid is converted into thiohydantoin by treating with ammonium isocyanate in presence of Acetic anhydride. The thiohydantoin is liberated by free Cold alkali and identified. The free amino group, however must be protected first.