

Protein Sequencing

During the Edman degradation **phenylisothiocyanate (PITC)** reacts with the amino acid residue (figure 1) at the amino terminus under basic conditions (provided by *n*-methylpiperidine/methanol/water) to form a **phenylthiocarbamyl derivative (PTC-protein)**. Trifluoroacetic acid then cleaves off the first amino acid (figure 3) as its **anilinothialinone derivative (ATZ-amino acid)** and leaves the new amino terminus for the next degradation cycle. The ATZ amino acid is then removed by extraction with ethyl acetate and converted to a **phenylthiohydantoin derivative (PTH-amino acid)** with 25% TFA/water (figure 4). Several by-products are also formed during the Edman degradation chemistry and are shown in figure 2.

The **PTH-amino acid** is transferred to a reversed-phase C-18 column for detection at 270nm. A standard mixture of 19 PTH-amino acids (figure 5) is also injected onto the column for separation (usually as the first cycle of the sequencing run). This chromatogram provides standard retention times of the amino acids for comparison with each Edman degradation cycle chromatogram. The HPLC chromatograms are collected using a computer data analysis system. To determine the amino acid present at a particular residue number, the chromatogram from the residues of interest is compared with the chromatogram from the previous residue by overlaying one on top of the other. From this, the amino acid for the particular residue can be determined. This process is repeated sequentially to provide the N-terminal sequence of the protein/peptide. Thereby up to 30 amino acid residues can be determined in average during one sequencing run.

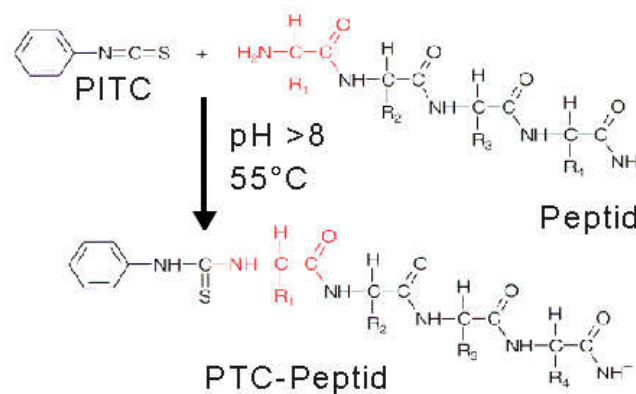


Fig.1: Coupling-reaction

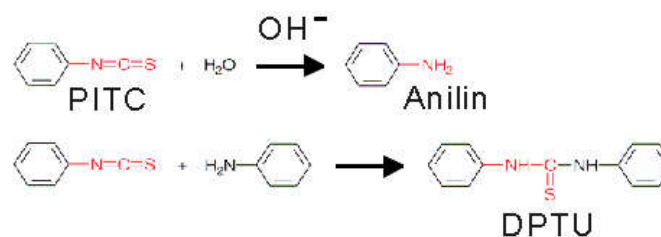


Fig.2: Side-reaction

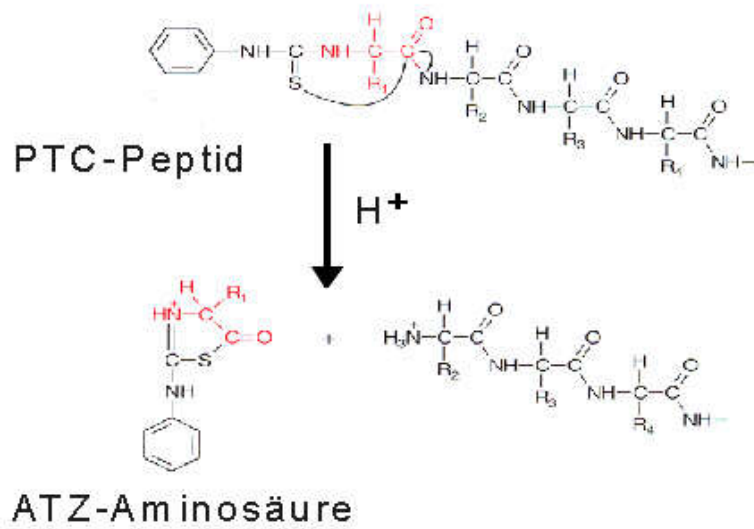


Fig.3: Cleavage-reaction

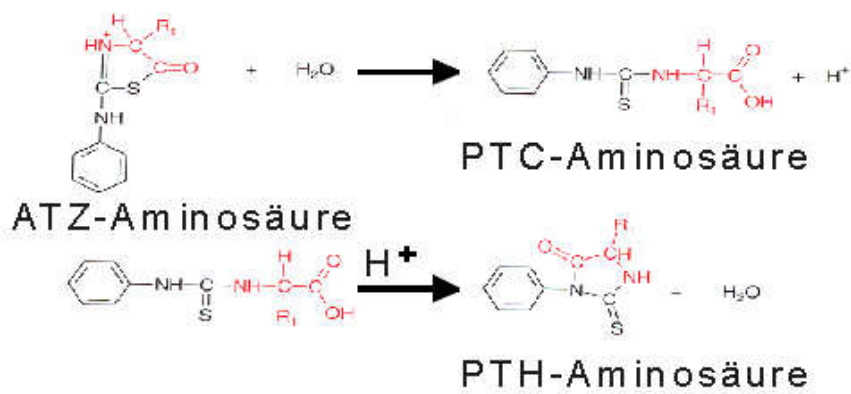


Fig. 4: Conversion-reaction

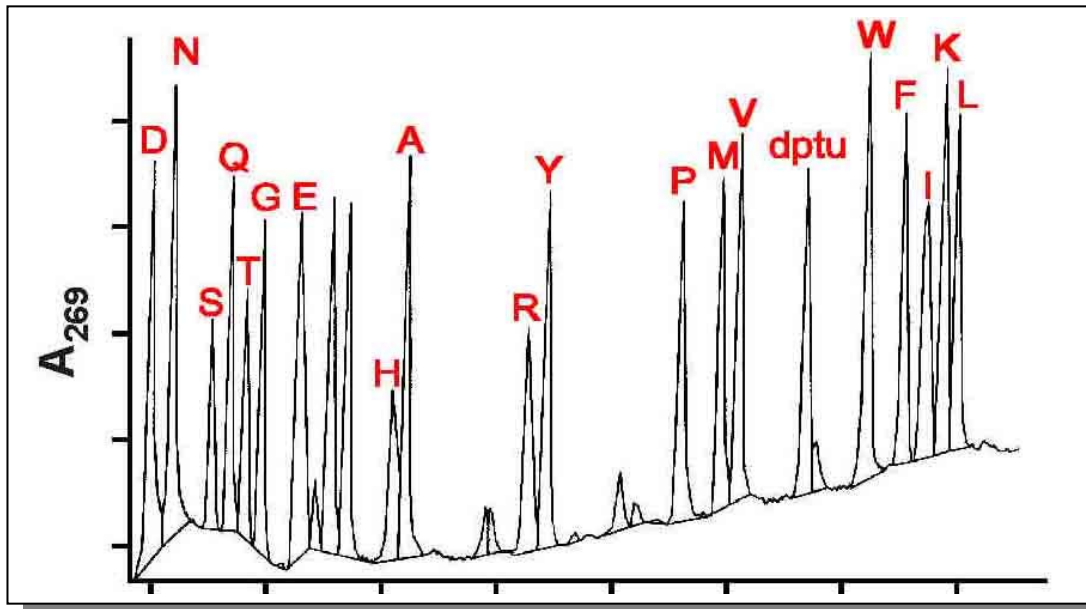


Fig. 5: HPLC separation of PTH amino acids

Sample Requirements

Sample Amount	1-50 pmols is preferred, although a lower amount can be acceptable.
Sample Form	<p>I. In 5-30 microliters of volatile solvents such as water, acetonitrile, propanol, acetic acid, or formic acid. Pure lyophilized peptide/protein is also acceptable.</p> <p>II. On a PVDF membrane. The sample should be as concentrated as possible on the PVDF membrane (e.g. 1 µg/lane). Several bands can be used. The bands should be stained with Coomassie Blue, Ponceau S, or Amido Black. After staining/destaining, a blotted membrane must be rinsed thoroughly with deionised water.</p>
Purity	Liquid samples should contain only one protein or peptide. Buffers, SDS, salts, amino acids, primary amines, and other contaminants must be removed from your sample. These contaminants affect Edman degradation reaction on the instrument, contaminate the instrument or affect PTH amino acid detection. Samples submitted on PVDF (blotted from SDS-PAGE gels) should have well separated bands to minimize contamination.
Cysteine Modification	Since unmodified Cys residues cannot be detected, Cys should be modified before sample submission if you wish to identify Cys.
N-terminal blockage	If the amino terminus is blocked, the protein or peptide cannot be sequenced using Edman degradation.
Glycosylation and other Modifications	Glycosylated amino acids and phosphorylated amino acids may result in blank cycles, reduced peaks or altered retention times.