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Application News

Protein Sequencer PPSQ™-50A Series

Improving the Yield of Basic Amino Acids in a Protein Sequencer

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User Benefits

- Enables the use of simple pretreatment steps to improve the yield of basic amino acids from Edman degradation.
- Improves yield of basic amino acids and simplifies subsequent automated software-based estimations.
- Enhances yield of PTH-amino acids to improve the reliability of amino acid sequencing.

■ Introduction

Biopharmaceuticals are drugs developed and manufactured using biotechnological techniques, including protein and antibody drug products. Biopharmaceutical production involves multiple processes, including manufacture, purification, formulation development, and storage. Quality assurance of biopharmaceuticals requires not only quality tests on the final product but must also address the impact of raw materials and the manufacturing process. As a result, manufacturing and quality controls for biopharmaceuticals differ from those for small-molecule pharmaceuticals produced by chemical synthesis. Guidelines established for evaluating the quality of biopharmaceuticals call for the characterization of various aspects of production, including N-terminal amino acid sequencing of the protein's primary structure. This characterization is used to compare the N-terminal amino acid sequence of the manufactured biopharmaceutical against the N-terminal amino acid sequence encoded in the gene sequence. Edman degradation is a process that sequentially removes single amino acids from the N-terminus of proteins and is used to determine amino acid sequences with high reliability. The PPSQ-50A system is a protein sequencer that automates this Edman degradation method and provides a straightforward means of determining the N-terminal amino acid sequence of proteins and peptides (Fig. 1). The derivatives of amino acids removed by Edman degradation are called PTH-amino acids, and yields of these PTH-amino acids differ depending on the amino acid removed. Yields from basic amino acids (arginine, histidine, lysine) can be particularly low depending on the amino acid sequence and the sample, and these low yields can impact the estimation of the amino acid sequence. This article describes the development of a method that improves the yield of basic amino acids from Edman degradation.

Fig. 1 PPSQ™-50A Protein Sequencer Gradient System

Table 1 Analytical Conditions

Column:	Wakopak Wakosil PTH-GR (S-PSQ) (250 mm × 2.0 mm I.D.)
Mobile Phase A:	PTH-Amino Acids Mobile Phase A (for Gradient Elution)
Mobile Phase B:	PTH-Amino Acids Mobile Phase B (for Gradient Elution)
Flowrate	0.3 mL/min
Time Program:	B Conc. 0 % (0 min)- 0 % (4 min)- 100 % (17 - 30 min)- 0 % (30.01 – 45 min)
Column Temp.:	35 °C
Detection:	UV 269 nm (SPD-M30A) High Sensitivity Flow Cell

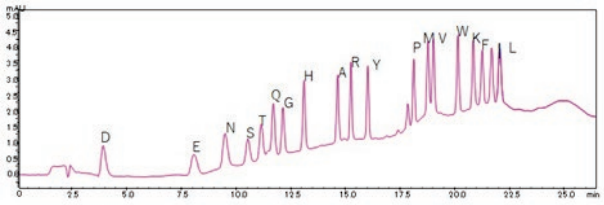


Fig. 2 Analysis of PTH-Amino Acids Mixture Standard (Containing 500 fmol of Each Amino Acid)

■ Detecting PTH-Amino Acids

The PPSQ-51A/53A Gradient System was used to perform gradient elution and identify the PTH-amino acids obtained from Edman degradation. The analytical conditions used are shown in Table 1. This gradient elution method separated and detected PTH-amino acids in a mixture containing 500 fmol of each PTH-amino acid (Fig. 2).

■ Improved Protocol

Bovine serum albumin (BSA) (A2153, Merck) was used for evaluation. The N-terminal amino acid sequence of BSA is Asp-Thr-His-Lys-Ser-Glu-Ile-Ala-His-Arg-Phe-Lys-Asp-Leu-. Low yields of the basic amino acids in this sequence, specifically the fourth (Lysine), ninth (Histidine), tenth (Arginine), and twelfth (Lysine) residues, can make amino acid sequencing difficult when the amount of a sample is small.

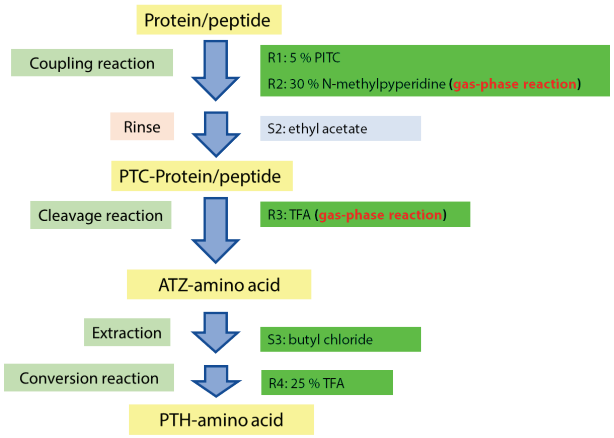


Fig. 3 Edman Degradation Protocol

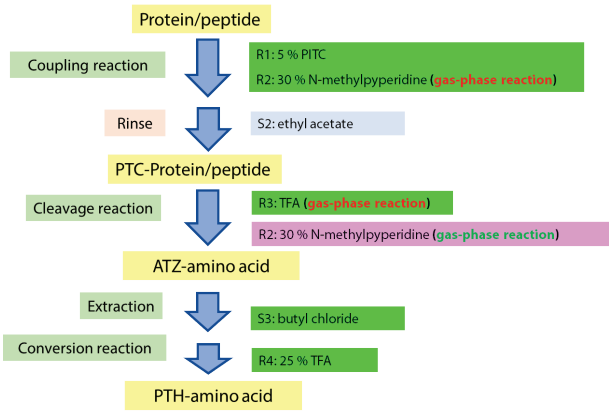


Fig. 5 Improved Edman Degradation Protocol

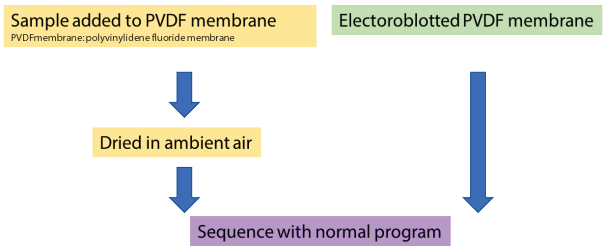


Fig. 4 Normal Sample Application Method

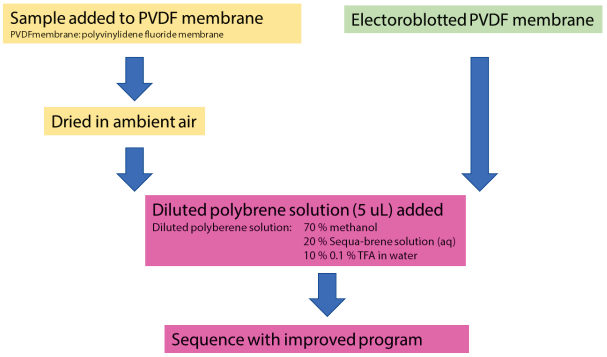


Fig. 6 Improved Sample Application Method (Compared to Normal Method)

Fig. 3 shows a schematic flow diagram of the Edman degradation process. Potential causes of reduced yields include:

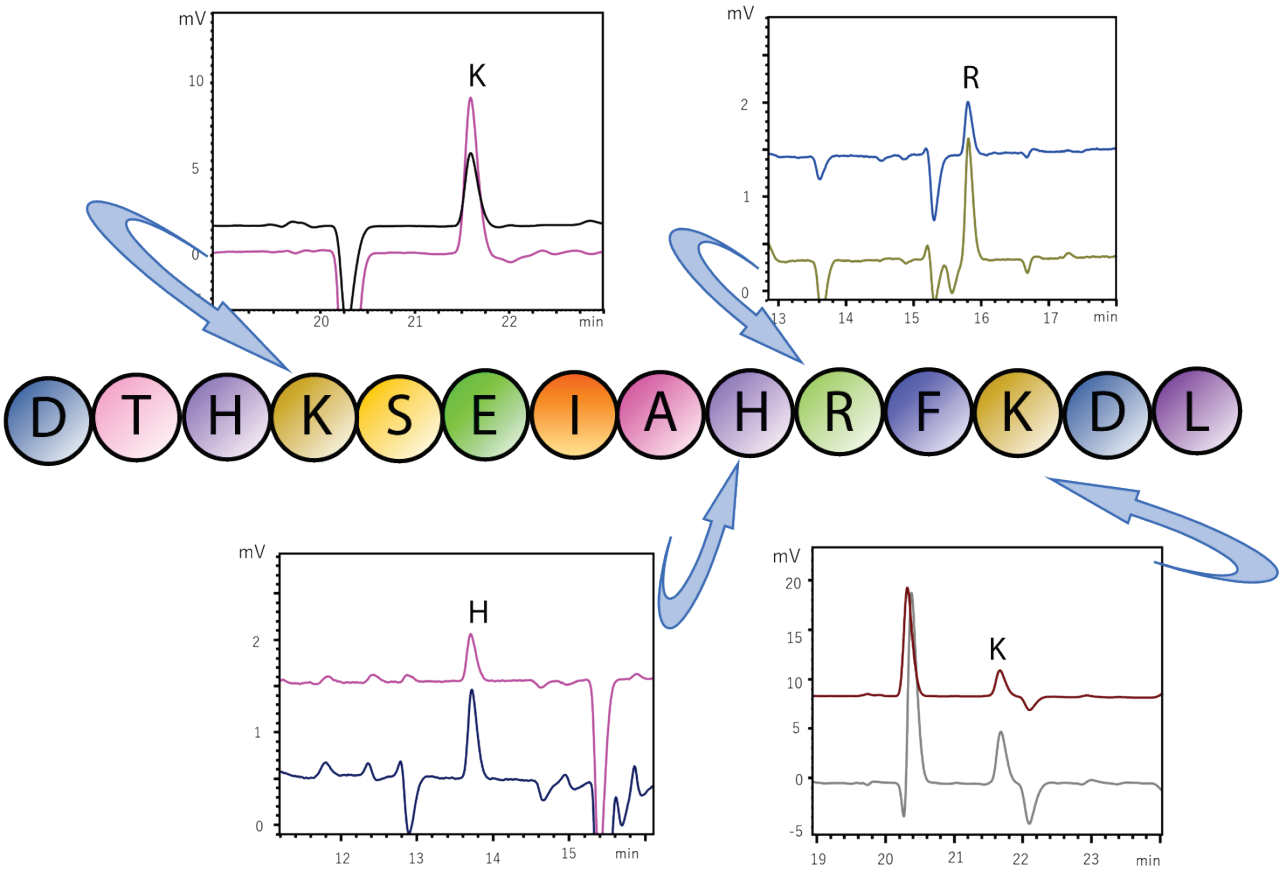
- Poor coupling reaction between phenylisothiocyanate (PITC) and N-terminal amino acid residue
- Sample washed out of sample carrier during the washing process
- Poor cleavage of peptide bonds in the cleavage reaction
- Poor extraction after peptide bond cleavage
- Poor conversion reaction

Since yield and percent yield are only reduced during reaction cycles that sequence basic amino acids, an attempt was made to improve the extraction process by focusing on characteristics specific to basic amino acids. After cleavage, the conversion reaction uses 1-chlorobutane solution (S3) to extract ATZ-amino acids from the sample carrier in the conversion flask. To increase the yield of this extraction step, the following two improvements were made to the sequencing protocol and the sample application method.

- ATZ-amino acids were extracted in a basic atmosphere instead of an acidic atmosphere.
- Polybrene solution was added during sample application to reduce the impact of basic amino acid side chains on Edman degradation.

Fig. 3 shows the normal Edman degradation protocol, and Fig. 4 shows the normal sequencing protocol. The Edman degradation protocol was altered to recover ATZ-amino acids in a basic atmosphere (Fig. 5). The method used to apply the sample to the support membrane was also improved to reduce the impact of basic amino acid side chains on Edman degradation (Fig. 6). The Seekabren solution used in Fig. 6 is the polybrene solution used during protein sequencing with glass fiber discs.

10 pmol of BSA was sequenced using the improved Edman degradation protocol shown in Fig. 5 and the improved sample application method shown in Fig. 6. Fig. 7 shows the resulting differential chromatograms with an enlarged view of the sequencing cycles that correspond to basic amino acid residues from the N-terminus of BSA. Comparing the differential chromatograms obtained using the normal and improved protocols showed the yield of PTH-amino acids derived from basic amino acids was increased by the improved protocol.



Upper: Normal protocol
Lower: Improved protocol

Fig. 7 Comparison of Sequencing Results (Showing Subtracted Chromatograms)

■ Conclusion

Although the improved sequencing protocol caused a slight increase in background noise in chromatograms due to the products of Edman degradation, this increase had no impact on identification of PTH-amino acids, and the results confirmed an improved percent yield of basic amino acids.

Based on the above findings, the improved protocol seems effective at improving basic amino acid yields from Edman degradation.

01-00518-EN First Edition: Apr. 2023

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