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Application News

Protein Sequencer PPSQ™-50A Series

# Analysis of Long-Chain Amino Acid Sequences Using a Protein Sequencer — Gradient System —

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## User Benefits

- Amino acid sequences from the N-terminal can be reliably identified.
- Amino acid sequences can be automatically and easily predicted using software.
- Even protein amino acid sequences not registered in a genome database can be easily identified.

## ■ Introduction

The PPSQ-50A gradient system protein sequencer identifies amino acids based on Edman degradation. It enables easy protein sequencing by preparing and analyzing highly purified protein samples. The system is also easy to operate and can provide highly reliable amino acid sequencing results by using a high-sensitivity cell to detect PTH-amino acids based on Edman degradation.

This article describes an example of analyzing a long-chain amino acid sequence from the N-terminal.

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

## ■ PPSQ-50A Gradient System

The PPSQ-50A gradient system (Fig. 1) uses gradient elution to analyze the PTH-amino acids obtained from Edman degradation. The analytical conditions used to analyze the PTH-amino acids are indicated in Table 1 and the corresponding chromatogram is shown in Fig. 2. The PPSQ-50A gradient system offers the advantages of an isocratic system described in Application News 01-00521 and also the following benefits:

- Improved PTH-amino acid peak shapes
- Lower background levels

Gradient systems elute PTH-amino acids in a slightly different order than isocratic systems, but they can detect PTH-amino acids with three to five times higher intensity levels. Also, the concentration of mobile phase B, which has a greater ability to elute PTH-amino acids as the analysis progresses, improves the separation of highly hydrophobic PTH-amino acids. The chromatogram peaks are not from PTH-amino acids but are mainly from by-products of Edman degradation, such as dimethylphenylthiourea (DMPTU), diphenylthiourea (DPTU), diphenylurea (DPU), and substances contained in the Edman reagent. Edman degradation by-products are generated from the reaction reagent phenylisothiocyanate and trimethylamine (TMA), which are used to create a basic atmosphere. To reduce the generation of by-products and lower the overall chromatogram baseline, the reagent used to create a basic atmosphere was changed from TMA to N-methylpiperidine, and a 25 % trifluoroacetic acid solution with less dithiothreitol was used. The protein sequencer compared the increase and decrease in PTH-amino acid peaks in the cycles before and after to identify uniquely detected PTH-amino acids. The gradient system enabled peak changes to be clearly determined based on the height of detected peaks and by decreasing the chromatogram background level. It was even able to identify amino acid sequences present in trace quantities.

Table 1 Analytical Conditions (Gradient System)

| | |
|---|---|
| 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 |

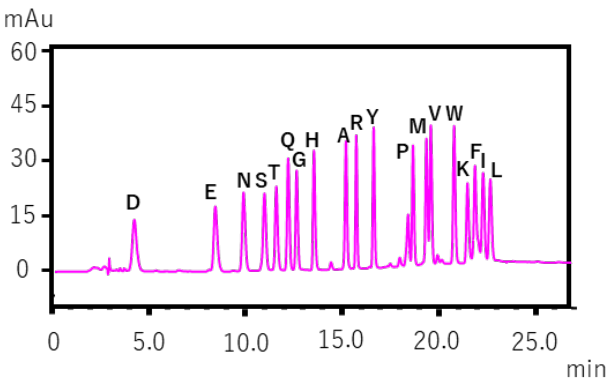


Fig. 2 Analysis of Standard PTH-Amino Acid Mixture (10 pmol each)

## ■ Analysis of Amino Acid Sequence in Mouse IgG H-Chains

Samples were prepared by electroblotting mouse IgG H-chains on a PVDF membrane. The pretreatment process for IgG mouse blood serum (Sigma-Aldrich cat. # I5381) is shown in Fig. 3. A total of 30 pmol IgG was applied to each well of electrophoresis gel. After separating samples into L-chains and H-chains, they were electroblotted onto a PVDF membrane and dyed with CBB (Fig. 4). Two bands were cleaved from the dyed H-chains and analyzed with the PPSQ-53A gradient system. The results are shown in Fig. 5. Amino acid sequences from 46 residues were identified. However, the protein sequencer also has some disadvantages, such as poor throughput, and it requires larger sample quantities for analysis than mass spectrometer-based amino acid sequencing methods.

## ■ Conclusion

Once samples are applied to the membrane, analysis using the protein sequencer is almost completely automatic and requires no additional time or trouble. By using protein sequencer data to supplement information obtained with a mass spectrometer, which is currently the most common method, even more reliable information can be obtained about amino acid sequences in proteins and peptides.

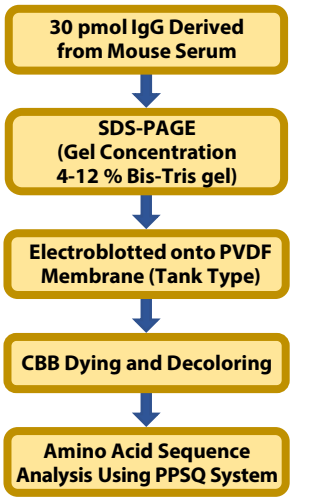


Fig. 3 IgG Pretreatment

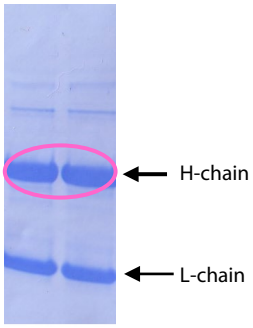


Fig. 4 PVDF Membrane after Electroblotting

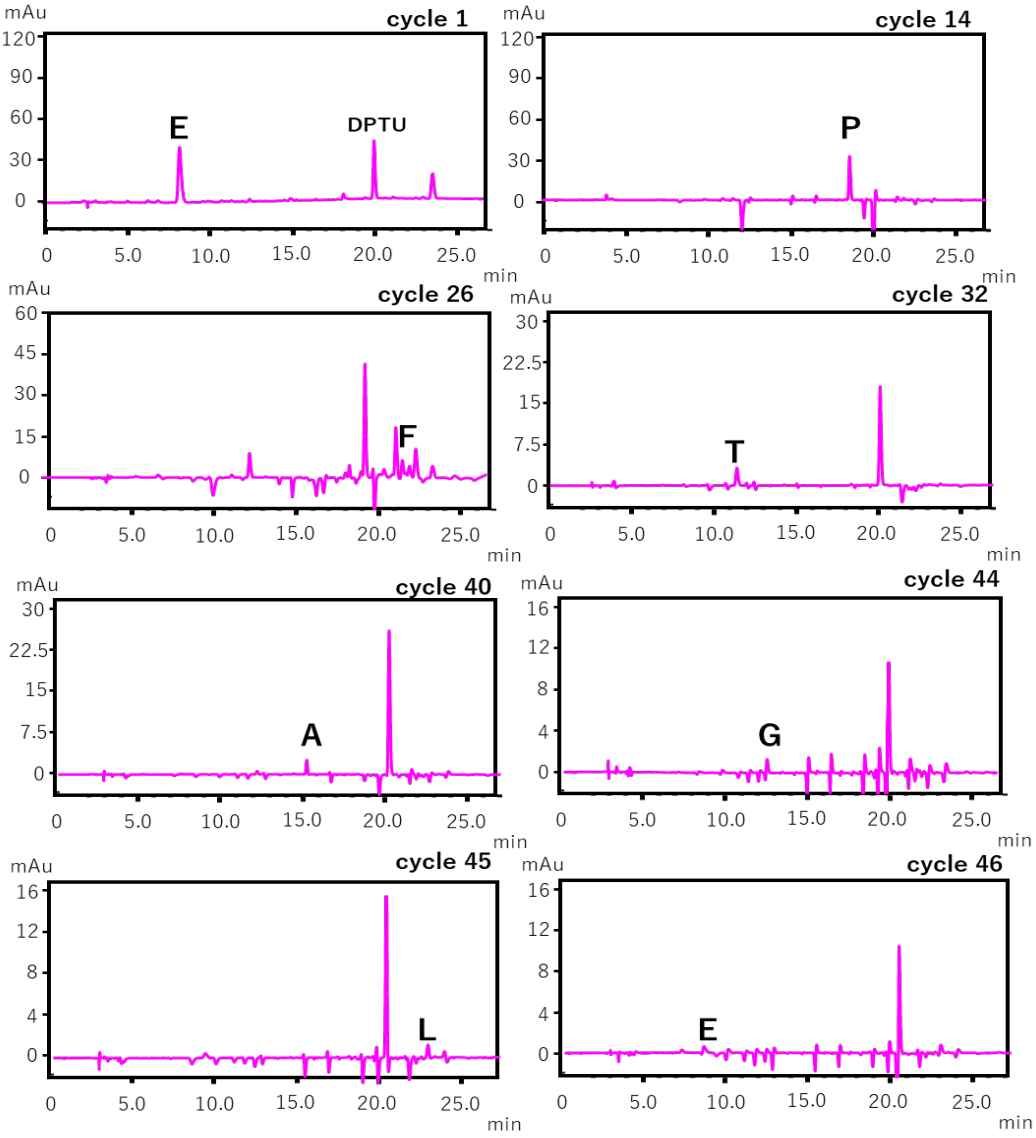


Fig. 5 Chromatogram for Amino Acid Sequence Analysis of IgG H-Chain from Monoclonal Antibody



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