SHIMADZU
Excellence in Science

Application News

Protein Sequencer PPSQ™-50A Series

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

Tomoko Kuriki

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

Shimadzu PPSQ-50A series protein sequencers determine amino acid sequences based on Edman degradation. They can identify amino acid sequences in pmol levels of purified proteins. Though samples used for analysis must be purified, the process is extremely simple. For example, after proteins are separated by electrophoresis, they are electrically transferred (electroblotted) onto a PVDF membrane and dyed. Then the protein spots are excised, placed directly in the reactor, and analyzed automatically. This article describes using this system to analyze a long-chain amino acid sequence from the N-terminal.

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

■ PPSQ-50A Isocratic System

Shimadzu offers two types of protein sequencers, isocratic and gradient. This article describes using the PPSQ-50A isocratic system, which can analyze PTH-amino acids by isocratic elution based on Edman degradation (Fig. 1). The analytical conditions used to analyze PTH-amino acids are indicated in Table 1 and the corresponding chromatogram is shown in Fig. 2. The PPSQ-50A isocratic system offers the following benefits:

- Easy operability
- Low running costs
- Reliable amino acid sequence identification
- Trace analysis capability using a high-sensitivity cell
- Can discriminate between Ile and Leu amino acids with identical mass

Amino acid sequences are identified using a protein sequencer by comparing chromatograms before and after to identify uniquely characteristic increases in PTH-amino acids. The isocratic system uses isocratic elution, so the elution position of each PTH-amino acid never varies. That means PTH-amino acids that increase during that cycle can be determined easily and reliably. Furthermore, the system recycles the mobile phase to reduce running costs and is more environmentally friendly by not generating liquid waste.

Because leucine (Leu) and isoleucine (Ile) have identical mass, they are difficult to differentiate using mass spectrometer-based amino acid sequencing, but the protein sequencer separates them by HPLC to identify Leu and Ile easily. A high-sensitivity cell can also be used for PTH-amino acid detection to enable identification of amino acid sequences in even pmol-level sample quantities.

Table 1 Analytical Conditions (Isocratic System)

Column:	Wakopak Wakosil PTH-II (S-PSQ) (250 mm × 4.6 mm I.D.)
Mobile Phase:	PTH-Amino Acids Mobile Phase
Flowrate:	1.0 mL/min
Time Program:	T. Flow 1.0 mL/min (0 – 21.25 min)- 0.3 mL/min (21.5 - 45.25 min)- 1.0 mL/min (45.5 – 45.51 min)
Column Temp.:	40 °C
Detection:	UV 269 nm (SPD-M30A) High Sensitivity Flow Cell

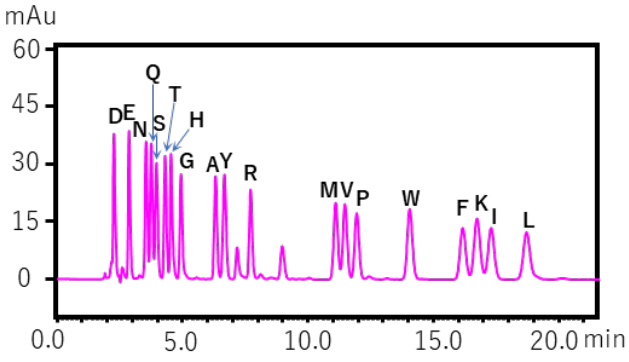

Fig. 2 Analysis of Standard PTH-Amino Acid Mixture (Containing 25 pmol of Each Amino Acid) Using a PPSQ-50A Isocratic System

■ Analyzing Human Erythropoietin

A total of 30 pmol of erythropoietin (Calbiochem No. 329871) was analyzed, which is a well-known biopharmaceutical. The resulting chromatograms are shown in Fig. 3. The 49th amino acid residue from the N-terminal could be reliably identified. Using the same technique, even amino acid residues around the 50th residue could be identified.

■ Conclusion

Biopharmaceuticals are drugs produced using living organisms, such as microbes or cell cultures. Unlike small-molecule drugs manufactured by chemical synthesis, incubation during biopharmaceutical manufacturing can be impacted by a variety of factors, so even slight changes can affect the final products. Therefore, to maintain the safety and efficacy of biopharmaceuticals, the manufacturing processes must be strictly controlled. N-terminal amino acid sequences are typically analyzed for characterization analysis, which is one such quality control technique. Using a protein sequencer for that analysis can ensure highly reliable amino acid sequencing results. Therefore, a protein sequencer is not only useful for research and development applications but can also be very useful for quality control applications.

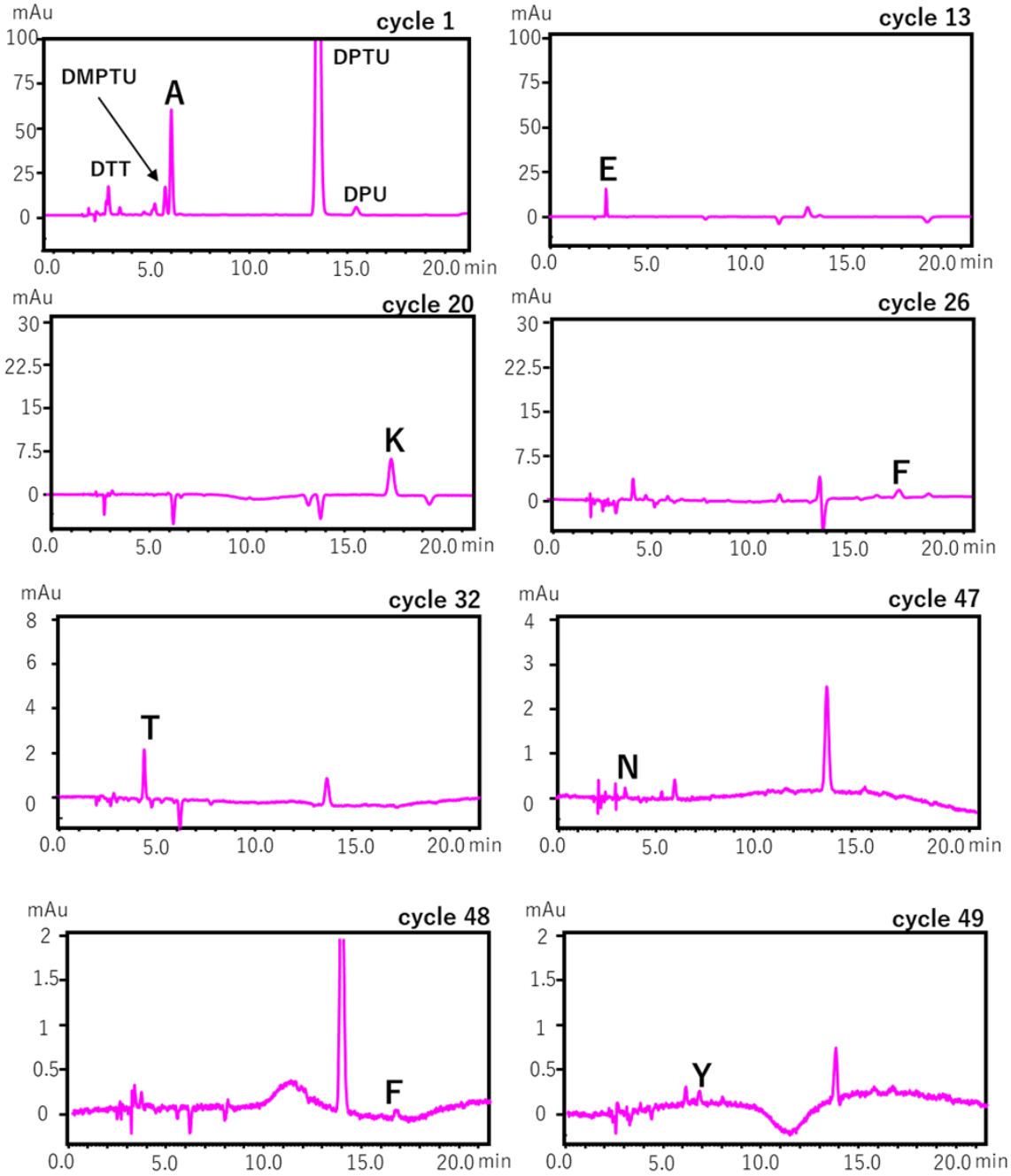

Fig. 3 Chromatograms from Amino Acid Sequencing of 30 pmol Erythropoietin

01-00521-EN First Edition: Apr. 2023

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/