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Application News

Protein Sequencer PPSQ™-50A Series

Analyzing Trace Quantities of Amino Acid Sequences Using a Protein Sequencer —Gradient System—

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User Benefits

- Amino acid sequences from N-terminals can be reliably identified even in trace sample quantities.
- Amino acid sequences can be easily predicted automatically using the software.
- Amino acid sequences of proteins not registered in genome databases can also be easily identified.

■ Introduction

A wide range of proteins with functions in the body are converted from precursor proteins to mature proteins and are discharged from cells through a variety of processes. However, interactions between such proteins can cause diseases. For that reason, protein research is becoming increasingly important for identifying the causes of diseases and for preventing, diagnosing, and treating diseases, and discovering new drugs. Furthermore, due to advances in sample pretreatment technology, which enables purification of even trace quantities of unknown proteins, there is a need for protein sequencers with higher sensitivity that can be used to identify amino acid sequences in trace sample quantities. This article describes an example of using the PPSQ-50 protein sequencer gradient system to analyze trace sample quantities.

■ Analyzing Protein Structures

Proteins in the body have structures made of amino acid sequences translated from genetic information, and those proteins have specific functions of their own. Consequently, they exist as mature proteins with a wide range of modifications. Despite the singular importance of analyzing the structures of mature proteins to identify protein functions, most proteins registered in genome databases are precursor proteins. Proteins consist of polypeptide chains of amino acids connected by peptide bonds, which means they can have a variety of structures, depending on how the amino acids are arranged, and these structures can be classified hierarchically. Structures with amino acids arranged in a single chain are referred to as primary structures. Secondary structures are the portions stabilized by amino acids that are hydrogen bonded to each other. They have three-dimensional structures, such as a helical alpha-helix structure or a beta-sheet structure resembling a folding screen that has been folded. Next, the term tertiary structure refers to the overall three-dimensional structure of proteins, including side chains. The constituent polypeptide chains are three-dimensional structures with multiple folds determined by hydrogen, ion, disulfide, or other bonds. Lastly, quaternary structures are formed from combining multiple sub-units of proteins that contain tertiary structures. Such proteins can have a wide variety of structures, depending on the amino acid sequences they include, and they can have different functions, depending on their structural differences. Amino acid sequences, which are the primary structures in proteins, are either analyzed using a mass spectrometer and a database search engine or by a protein sequencer, based on the conventional Edman degradation technique. The advantages of using a protein sequencer include the high reliability of amino acid sequencing results that are obtained from N-terminals and its easy operability, as well as the ability to: analyze sequences directly without enzymatic digestion, distinguish between Ile and Leu with the same mass number, and determine the presence/absence and position of disulfide bonds (S-S bonds). Consequently, protein sequencers have now become an essential tool for N-terminal protein sequencing.

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

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

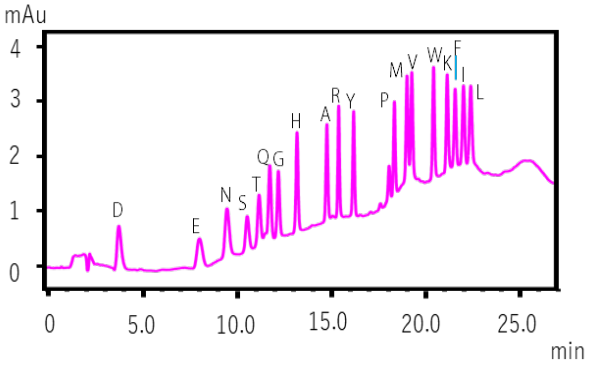

Fig. 2 Analysis of Standard PTH-Amino Acid Mixture (500 fmol Each)

■ Analysis of Amino Acid Sequences in Calmodulin

The results from using a PPSQ-50A protein sequencer gradient system (Fig. 1) with the analytical conditions listed in Table 1 to analyze 500 fmol of a PTH-amino acid standard sample are shown in Fig. 2. Despite the tiny quantity of the PTH-amino acid mixture solution analyzed, the system was more than capable of detecting the amino acids. Next, calmodulin (Merck cat # C4874) was dissolved in a 0.1 % trifluoroacetic acid solution to prepare a sample solution with a 1 pmol/µL calmodulin concentration. 2 µL of the sample solution was added to a glass fiber disc treated with polybrene, and the amino acid sequences were then analyzed. The resulting chromatograms from each cycle are shown in Fig. 3. The raw chromatogram data is shown for cycle 1 and difference chromatograms for cycles thereafter.

Although the peaks were small, the system detected a characteristic increase in PTH-amino acids that continued through to cycle 30, which enabled identification of the amino acids.

■ Conclusion

N-terminal amino acid sequencing of proteins using a protein sequencer can identify amino acid sequences reliably even from tiny sample quantities. Protein sequencers can also be used to analyze the structures of proteins with few expressions that are not included in databases. Consequently, they are considered an indispensable tool for analyzing amino acid sequences in proteins.

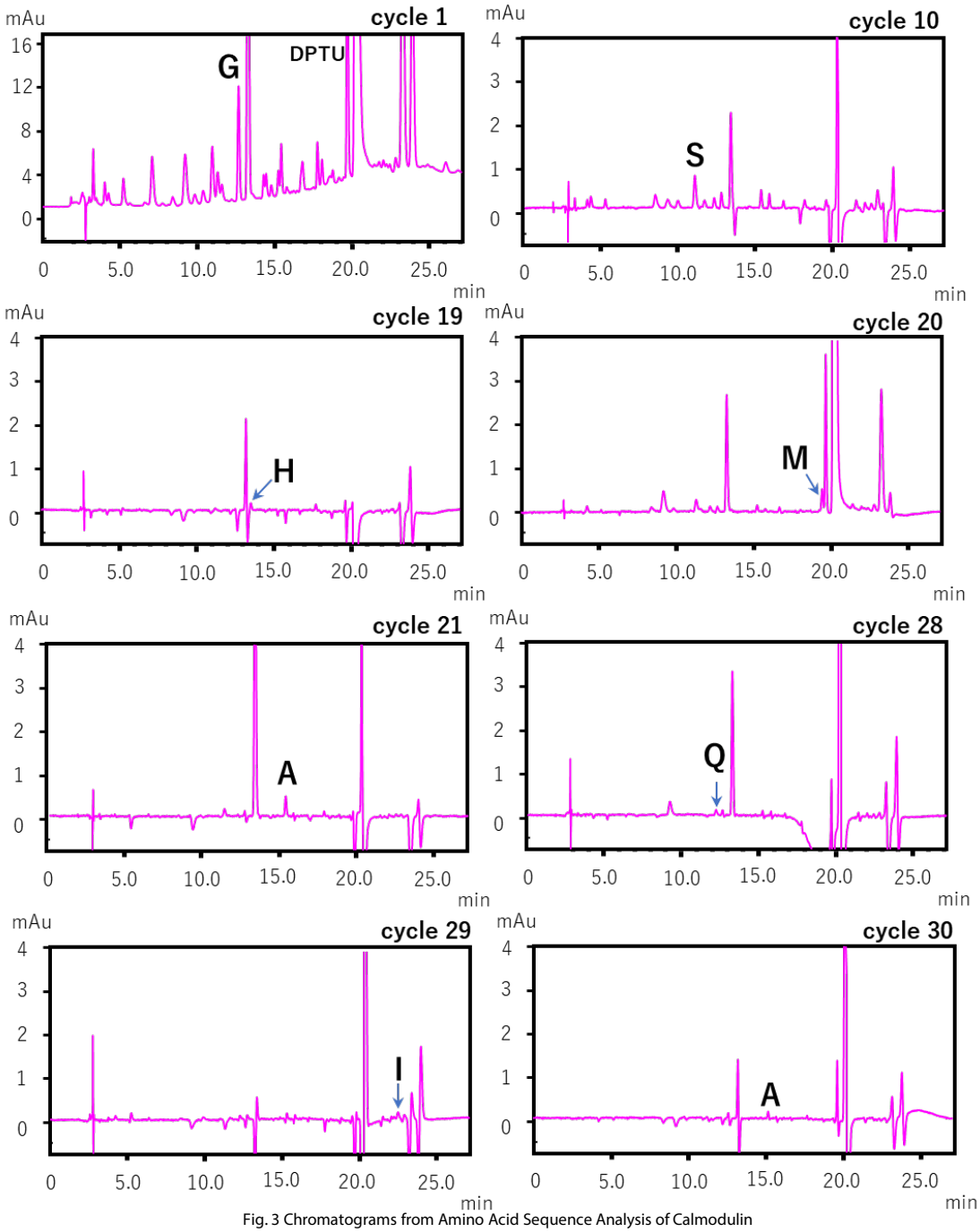

Fig. 3 Chromatograms from Amino Acid Sequence Analysis of Calmodulin

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