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Application News

No. B93

Protein Sequencer

N-terminal Amino Acid Sequencing of Mouse IgG Using the PPSQ™-51A/53A Gradient System

Introduction

As genome analysis technology has evolved, the genome database has become more comprehensive, and proteome analysis which is one form of post-genome analysis using the database - has grown by leaps and bounds. As a result of technical developments in mass spectrometers, we were able to achieve high-throughput analysis in the amino acid sequencing of proteins by using a mass spectrometer and its database. Although many proteins in the genome database are registered as precursor proteins, the various proteins expressed in cells are transported into the body as mature proteins that result from processing of these precursor proteins. For this reason, when the amino acid sequences of mature proteins are identified using a mass spectrometer and its database, the mass numbers may differ from the theoretical ones, leading to lower scores as the searching results. In addition, identification of the amino acid sequence using a mass spectrometer may be troublesome for species of living organism whose genome database has not yet been completed, and the sequences obtained may be unreliable. On the other hand, the conventional method for protein sequencing using the Edman degradation technique takes time, but the sequencing results are highly reliable and it is extremely effective for analysis of the amino acid sequences of samples for which no database has been established yet.
This article introduces amino acid sequencing using a trace protein of IgG (derived from mouse serum). The PPSQ-51A/53A Gradient System is an indispensable instrument for proteome analysis for its reliable and easy identification of amino acid sequences.

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Analysis of PTH-Amino Acids

With the PPSQ-51A/53A Gradient System, the PTH-amino acids obtained in the Edman reaction are analyzed by gradient elution. The analysis conditions are shown in Table 1. The order in which the PTH-amino acids are eluted is different from that in isocratic elution, but the peaks for the detected PTH amino acids obtained by gradient elution are generally three to five times higher than those by isocratic elution. Gradient elution also enables the separation and detection of PTH-amino acids at 500 fmol (Fig. 1).

Table 1 Analysis Conditions

Column	:	Wakopak™ Wakosil® PTH-GR (S-PSQ) (250 mm L, 2.0 mm I.D.)
Mobile phase	:	A: PTH-amino Acids Mobile Phase A (for Gradient Elution) B: PTH-amino Acids Mobile Phase B (for Gradient Elution)
Flow rate of mobile phase	:	0.3 mL/min
Column temp.	:	35 °C
Detection	:	SPD-M30A (269 nm) with High Sensitivity Flow cell

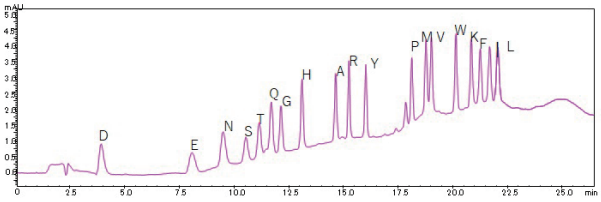

Fig. 1 Analysis of PTH-Amino Acid Standard Mixture at 500 fmol

Method

In terms of basic structure, IgG consists of two H-chains (heavy chains; larger molecular weight) and two L-chains (light chains; smaller molecular weight). Since intact antibodies have a large molecular weight (approximately 150 kDa), it is very difficult for a protein sequencing system to perform N-terminal amino acid sequencing on them without pretreatment. In this example, 2 pmol of IgG derived from mouse serum (SIGMA-ALDRICH cat#I5381) was reduced and SDS-PAGE (Sodium Dodecyl Sulfate PolyAcrylamyde Gel Electrophoresis) was carried out, then electroblotting was performed on a PVDF membrane. After staining the PVDF membrane with CBB, the bands of L-chains and H chains were excised and analyzed with the PPSQ-53A Gradient System.

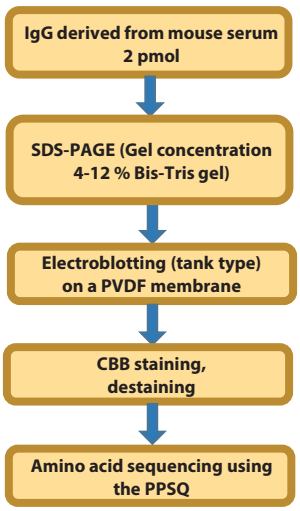

Fig. 2 N-terminal Amino Acid Sequencing Protocol

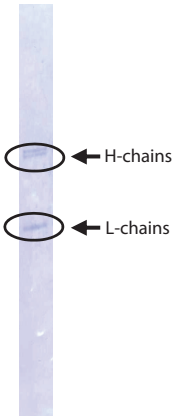

Fig. 3 PVDF Membrane After Electroblotting

Results of N-Terminal Amino Acid Sequencing

Fig. 4 and Fig. 5 show the chromatograms of N-terminal amino acid sequencing of L-chains and H-chains from 2 pmol of IgG (Cycle 1 is the raw chromatogram and others are subtracted chromatograms). In Fig. 4, the amino acid residues of N-terminus and the 2nd cycle in the L-chain were identified as asparagine (Asp) and isoleucine(Ile), respectively. After performing 15 cycles, the sequence from the N-terminus up to the 13th residue was identified as Asp-Ile-Gln-Met-Thr-Gln-Ser-Pro-Ala-Ser-Leu-Ser-Ala (Val). A database search revealed this to be immunoglobulin kappa light chain.

In H-chains the sequence from the N-terminus was identified as Glu-Val-Gln-Leu-Gln-Glu-Ser-Gly-Pro-Glu-Leu-Val, and a database search revealed this to be immunoglobulin heavy chain.

Conclusion

These results indicate that the Protein Sequencer PPSQ-51A/53A Gradient System enables easy and accurate identification of N-terminus sequences, and is an effective system for high-sensitivity analysis of trace samples.

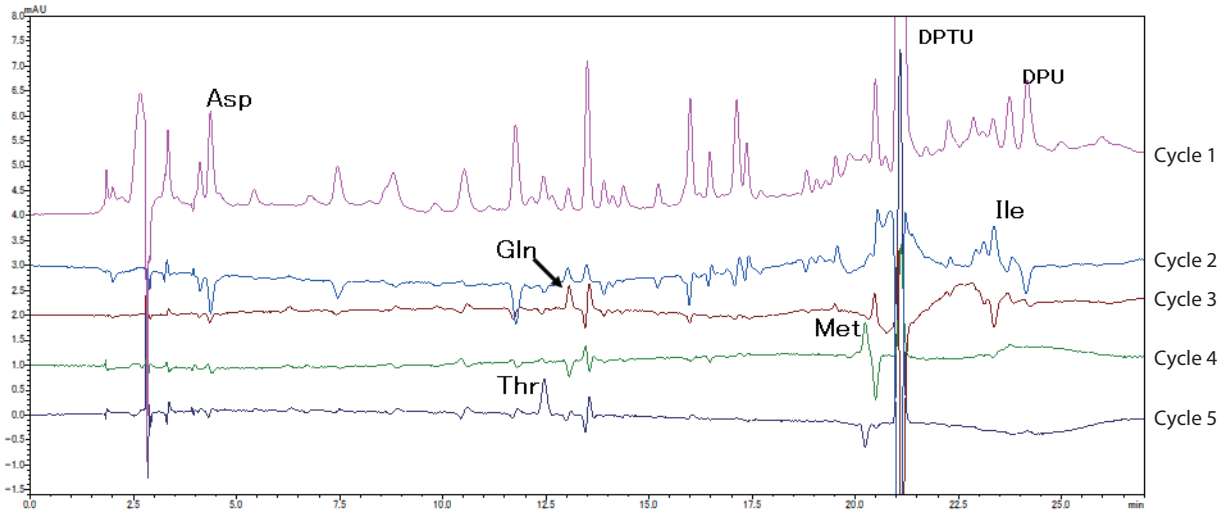

Fig. 4 Chromatograms (Cycles 1 to 5) of L-chains

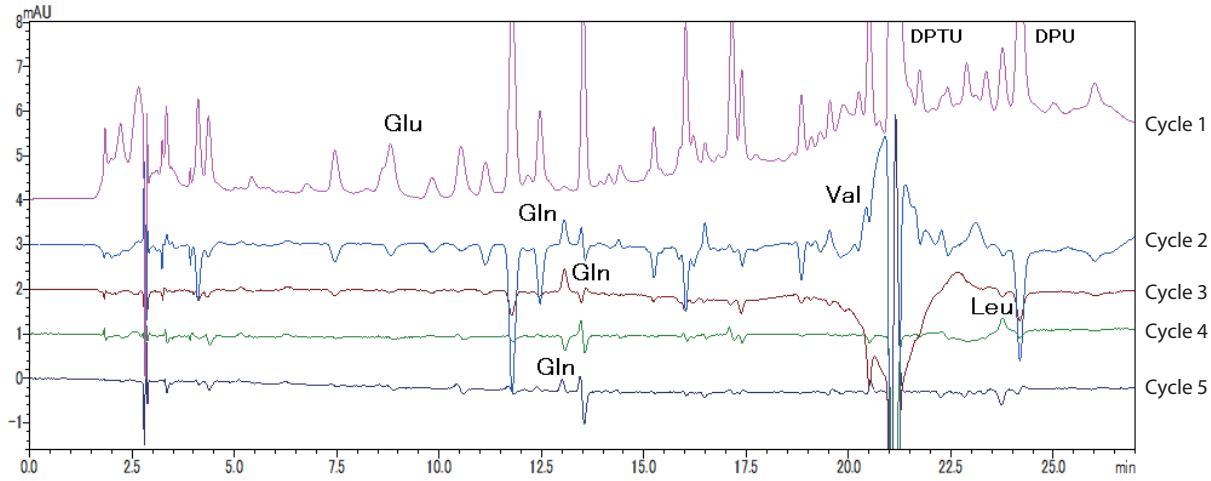

Fig. 5 Chromatograms (Cycles 1 to 5) of H-chains

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