SHIMADZU
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Application News

No. B111

Protein Sequencer

Detection of Cysteine/Cystine Using Protein Sequencer

Introduction

The main approach in protein analysis at present is proteomic analysis, in which proteins are identified by using a mass spectrometer and search engine employing a genome database. The proteins expressed in a living body have various functions as a result of post-translational modification, and the theoretical mass numbers of the precursor proteins and maturation proteins are different in some cases. Identification of amino acid sequences with a mass spectrometer without using a database is both complex and difficult. In contrast, the conventional protein sequencer method using Edman degradation provides highly reliable amino acid sequence results, while enabling easy identification of amino acid sequences even the database is inadequate. This article introduces an example of an amino acid sequence analysis of a sample containing cysteine and cystine by using the Shimadzu PPSQ™-50A protein sequencer systems (isocratic system and gradient system).

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Detection of Cysteine and Cystine

In proteins, polypeptides fold into distinct 3-dimensional conformations that are unique to that protein. Proteins consist of multiple polypeptide chains that are dehydrated and condensed when amino acids for peptide bonds. The sequence of the amino acids of the polypeptide chains is called the "primary structure" of the protein. The maturation proteins which are expressed in living organisms function by assuming thermodynamically stable preferred 3-dimensional conformations depending on the interactions of their constituent amino acid side chains. This type of high order 3-dimensional structure of a protein is called a high order structure (HOS). Proteins fold successively into secondary, tertiary, and quaternary structures that are distinct from the primary structure. A 3-dimensional structure which is formed by the interaction of the side chains of amino acids while maintaining its secondary structure is termed a tertiary structure, and that protein possesses functional activity as a result of the formation of a thermodynamically stable preferred tertiary structure. The disulfide bond (S-S bond) is one of these interactions between amino acid side chains. Determination of the positions of the cysteines and amino acids that make up the disulfide bond is necessary and indispensable in structural analysis of proteins. Tables 1 and 2 show the conditions of the protein sequencer analysis.

Table 1 Analysis Conditions (Isocratic System)

Column	:	Wakopak™ Wakosil® PTH-II (250 mmL, 4.6 mm I.D.)
Mobile phase	:	PTH-amino Acids Mobile Phase
Flow rate of mobile phase	:	1.0 mL/min
Column temp.	:	40 °C
Detection	:	SPD-M30A (269 nm) with High Sensitivity Flow cell

Table 2 Analysis Conditions (Gradient System)

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

A polybrene-treated glass fiber disk was used in the analysis as a sample support. Figs. 1 through 4 show the analysis results for cysteine and cystine with a standard amino acid mixture.

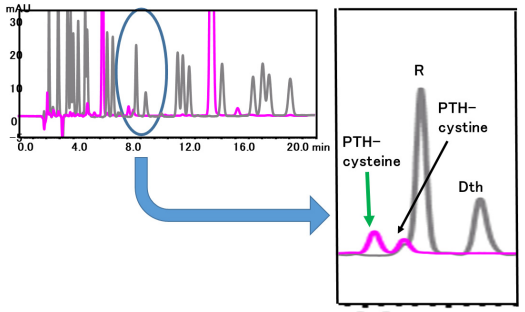

Fig. 1 Chromatograms for Edman Degradation Gray : PTH-Amino Acid Mixture Standard (25 pmol) and Pink : 100 pmol of PTH-Cysteine, and Enlargement of Area Indicated in Blue in Fig. 1 (Isocratic System)

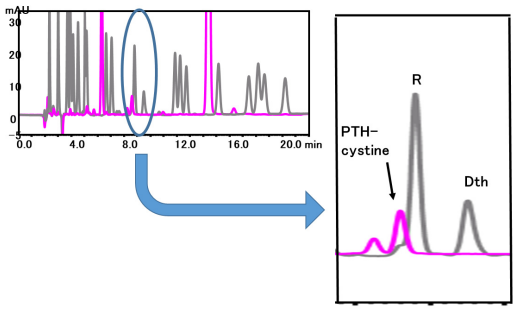

Fig. 2 Chromatograms for Edman Degradation Gray : PTH-Amino Acid Mixture Standard (25 pmol) and Pink : 100 pmol of PTH-Cystine, and Enlargement of Area Indicated in Blue in Fig. 2 (Isocratic System)

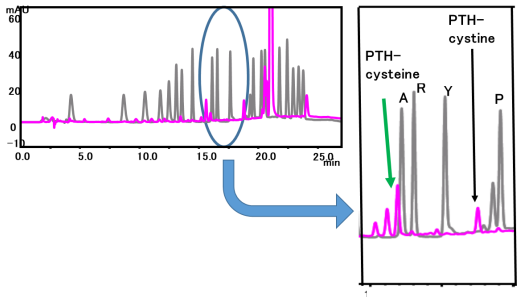

Fig. 3 Chromatograms for Edman Degradation Gray : PTH-Amino Acid Mixture Standard (10 pmol) and Pink : 50 pmol of PTH-Cysteine, and Enlargement of Area Indicated in Blue in Fig. 3 (Gradient System)

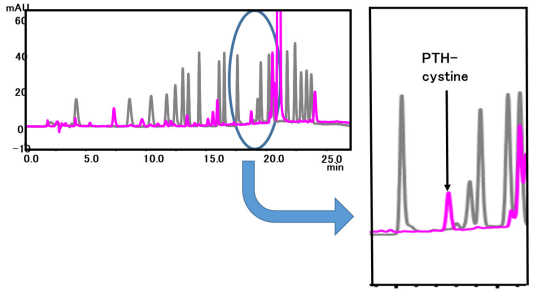

Fig. 4 Chromatograms for Edman Degradation Gray : PTH-Amino Acid Mixture Standard (10 pmol) and Pink : 50 pmol of PTH-Cystine, and Enlargement of Area Indicated in Blue in Fig. 4 (Gradient System)

The elution positions of PTH-cysteine and PTH-cystine in the isocratic system and gradient system could be confirmed from Figs. 1 through 4. Although PTH-cystine was also detected in the sequence analysis of cysteine in Figs. 1 and 3, it is thought that this is attributed to the formation of disulfide bonds by cysteine during Edman degradation. Moreover, because the elution position of PTH-cystine was near that of PTH-arginine in the isocratic system (Fig. 2), automatic estimation by the software is difficult. However, identification is possible by visual inspection.

N-Terminal Amino Acid Sequence Analysis

Here, a sample consisting of 100 pmol of synthetic oxytocin (Peptide Institute, Inc., 4084-v) having one disulfide bond in a molecule was analyzed with a Shimadzu PPSQ-50A protein sequencer system using a polybrene-treated glass fiber disk. Fig. 5 shows the amino acid sequence, together with the raw chromatograms for the 1st cycle and the subtracted chromatograms for the 6th cycle. Because the cysteine in the 1st cycle has bonded with the C-terminal side cysteine in the 6th cycle by disulfide bonding, the PTH-amino acid increased specifically in that cycle was not detected. In the 6th cycle, this was detected as PTH-cystine. However, the PTH-cystine from the 6th cycle could be identified easily from the subtracted chromatogram, as the PPSQ-50A system provides good repeatability of the elution times of each PTH-amino acid.

Conclusion

The PPSQ-50A protein sequencer system enables easy and accurate identification of N-terminal amino acid sequences and can also identify cysteine and cystine. Because cysteine and cystine are degraded more markedly than other amino acids during Edman degradation, a certain sample amount is necessary in order to determine the elution position with a protein sequencer. In the future, this Shimadzu protein sequencer system is expected to be an effective tool for structural analysis in proteomic analysis and evaluation of these substances as synthetic intermediates before they form disulfide bonds during peptide synthesis.

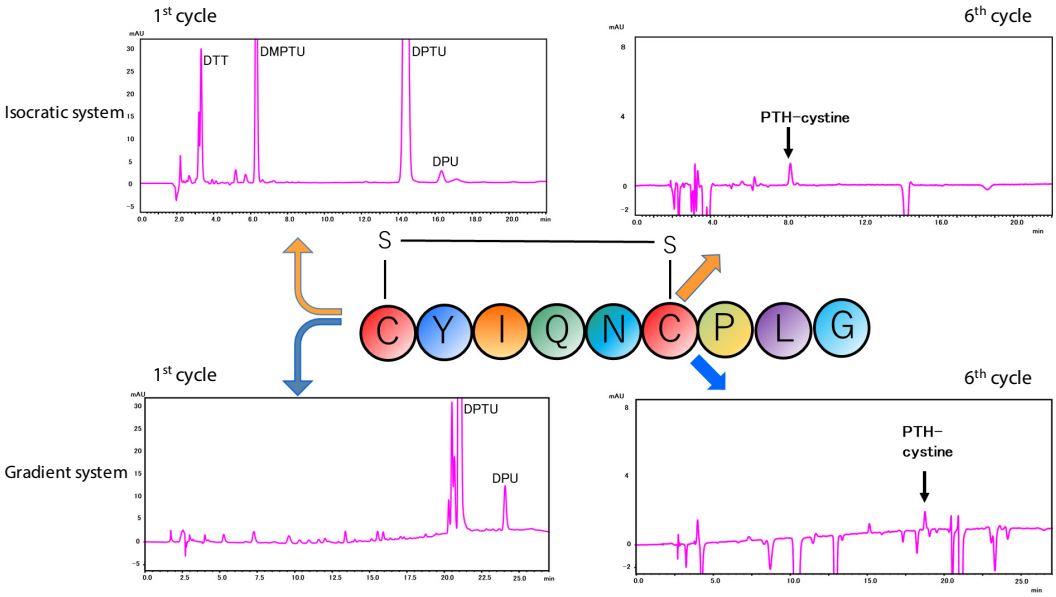

Fig. 5 Amino Acid Sequence of Synthetic Peptide Oxytocine and Chromatograms

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