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Application News

No. B103

Protein Sequencer

Amino Acid Sequence Analysis of Peptides and Proteins with Modified Amino Acid Using PPSQ™-50A Isocratic System

Introduction

Protein identification with a mass spectrometer (MS) and search engine utilizing genomic databases has now become the main stream in analysis of proteins. Although the proteins in the genomic databases are registered as precursor proteins, the expressed proteins in living cells are modified after translation and have various functions. However, since there are differences in the theoretical mass number of the precursor protein and the mature protein modified after translation, the score and reliability of search results obtained by the MS analysis and search engine is sometimes low. Moreover, identification of amino acid sequences by MS without using databases is both quite complex and difficult. On the other hand, a protein sequencer using the conventional Edman degradation method obtains highly reliable sequencing results, and amino acid sequences can be identified easily even in case the database is inadequate. This article introduces an example of amino acid sequence analysis of a sample containing a modified amino acid by using the PPSQ-50A isocratic system.

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Identification of Side-Chain Modified Lys

Many of the proteins expressed in living cells are acetylated by post-translational modifications of an α amino group at the N-terminus after translation. N terminal acetylation of proteins occurs in more than about 80% of human proteins. This acetylation is one of post-translational modifications that occurs not only in the amino group at the N-terminus, but also in Lys residue with side-chain amino group. Histone, which is a component of chromosomes, is an example of a protein that contains acetylated Lys. The Lys residue of histone is either acetylated or methylated. It is known that methylation of the Lys residue of histone is involved in control of transcription. It is considered that determining how a Lys residue having a certain number in the histone sequence is modified is one of the methods for elucidating the expressions and functions of various cells.

First, we investigated whether the analysis of PTH-acetylated Lys and PTH-trimethylated Lys can be performed with the PPSQ-50A isocratic system. Table 1 shows the analytical conditions. Fig. 1 shows the chromatograms of a PTH-amino acid standard mixture and PTH-acetylated Lys, and Fig. 2 shows an enlarged view of the part indicated by the blue circle in Fig. 1.

Table 1 Analytical Conditions

Column	:	Wakopak™ Wakosil® PTH-II (250 mm L, 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

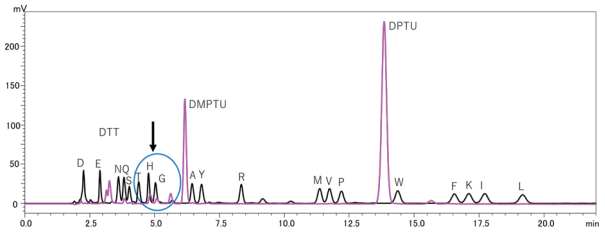

Fig. 1 Chromatograms of PTH-Amino Acid Standard Mixture (25 pmol) (Black) and PTH-Acetylated Lys (Red)

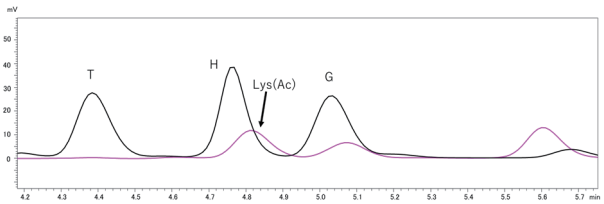

Fig. 2 Chromatograms of PTH-Amino Acid Standard Mixture (25 pmol) (Black) and PTH-Acetylated Lys (Red) (Showing Enlarged View of Part in Blue Circle in Fig. 1)

From these results, the PPSQ-50A isocratic system can easily identify the PTH-acetylated Lys (PTH-Lys(Ac)) because the peak top of PTH-Lys(Ac) has been separated from PTH-His.

Next, Fig. 3 shows the chromatograms of the PTH-amino acid standard mixture and PTH-trimethylated Lys, and Fig. 4 shows an enlarged view of the part indicated by the blue circle in Fig. 3.

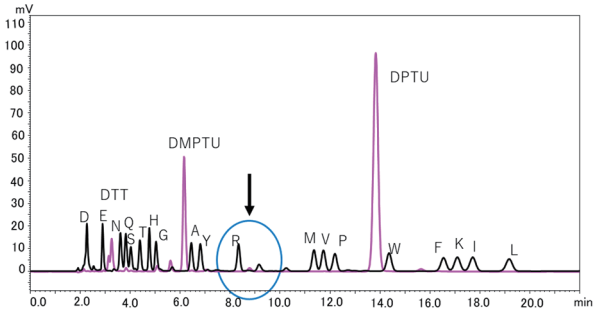

Fig. 3 Chromatograms of PTH-Amino Acid Standard Mixture (25 pmol) (Black) and PTH-Trimethylated Lys (Red)

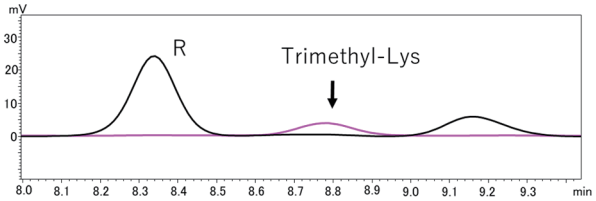

Fig. 4 Chromatograms of PTH-Amino Acid Standard Mixture (25 pmol) (Black) and PTH-Trimethylated Lys (Red) (Showing Enlarged View of Part in Blue Circle in Fig. 3)

Analysis of N-Terminal Amino Acid Sequence

In this experiment, we used a synthetic fragment peptide derivative, [Lys(Ac)12/16, Lys(Me3)20]-Histone H4 (1-25)-GSGSK (Biotin) (AnaSpec. Inc., CA), which is one histone. Here, 20 pmol of the sample was analyzed using a glass fiber disk after polybrene treatment. Fig. 5 shows the amino acid sequence and the subtracted chromatograms of the Lys residues. The PPSQ-50A isocratic system has good reproducibility of the elution time of each PTH-amino acid using the subtracted chromatograms of the 5th, 10th, 15th, and 20th cycle, the unmodified and modified Lys residues were easily identified.

Conclusion

The PPSQ-50A isocratic system enables easy and accurate identification of N-terminal sequences and can also identify modified amino acids. Thus, this can be considered an effective system as a tool for identification of post-translational modifications in protein analyses.

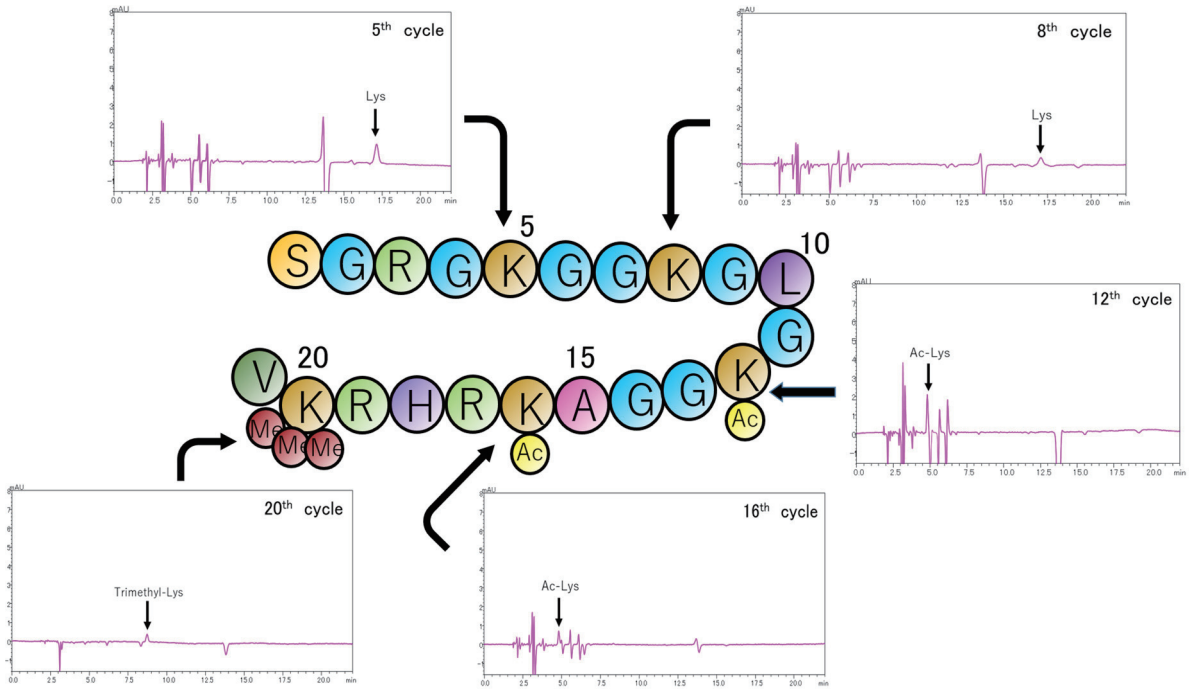

Fig. 5 Partial Amino Acid Sequence of Synthetic Fragment Peptide Derivative of Histone H4 and Its Chromatogram

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