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Protein Sequencer PPSQ™-50A Series
MALDI-TOF Mass Spectrometer MALDI-8020/MALDI-8030

Application News

Amino Acid Sequence Analysis of Peptides Containing Modified Amino Acids Using The PPSQ™-50 Gradient System

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User Benefits

- Amino acid sequences from the N-terminus can be reliably identified even in minute sample volumes.
- Combined with MALDI-ISD analysis, information on the C-terminus and modified amino acids can be obtained.
- Amino acid sequences of proteins that are not registered in genome databases can be easily determined.

■ Introduction

Proteins expressed in vivo are post-translationally modified to have various functions. In recent years, attention has been focused on peptide therapeutics, which are medium-molecular drugs, and peptides containing non-natural amino acids that have been modified on the side chains of natural amino acids have been synthesized as the constituent amino acids. The inclusion of non-natural amino acids causes changes in various physical properties and steric structures, resulting in peptides with unique functions that can be used as pharmaceuticals. Analytical methods for peptide drug characterization include amino acid sequence analysis methods on protein sequencers using Edman degradation and MS analysis and search engines, each of which can be difficult to use with a single method to analyze modified amino acids.

In this report, we present an example of accurate amino acid sequence analysis of a peptide containing modified amino acids by combining mass and sequence information obtained from in-source decay (ISD) measurements by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and N-terminal amino acid sequence information from the PPSQ-50A gradient system. The following is an example of accurate amino acid sequence analysis of a peptide containing modified amino acids by combining mass and sequence information obtained from in-source decay (ISD) measurements by TOF MS and N-terminal amino acid sequence information obtained from the PPSQ-50A gradient system.

■ Identification of Lys with modified side chains

Peptide drugs, one of the middle-molecular drugs, are expected to become novel drugs as research and development is accelerated by the development of new drug discovery technologies. Peptide drugs, like macromolecular drugs, have few side effects and, like small-molecular drugs, can be produced relatively inexpensively by chemical synthesis, combining the advantages of both. However, there are also issues such as the fact that these peptide drugs are easily degraded when administered into the body due to their structure and their low permeability through cell membranes. These issues have been solved by changing the physical properties and steric structure of the peptides by using non-natural amino acids as the constituent amino acids or by cyclizing the peptides.

In this report, we describe the amino acid sequence analysis of a synthetic peptide containing Lys with a modified side chain. The sample was a synthetic fragment peptide derivative of histone H4 [Lys(Ac)12/16,Lys(Me3)20]-Histone H4 (1-25) with the side chain of Lys at residues 12 and 16 modified with an acetyl group (Ac) and the side chain of Lys at residue 20 with three methyl groups (Me) GSGSK (Biotin) (AnaSpec, Inc., CA) (Fig. 1). First, a PTH-amino acid mixture standard was analyzed using the PPSQ-50A gradient system. The analytical conditions are shown in Table 1 and the chromatograms in Fig. 2. The elution positions of the modified Lys residues were then confirmed (Fig. 3). PTH-Lys(Ac) and PTH-Lys(Me3) could be detected without overlap with the elution positions of the other PTH-derivatized natural amino acid peaks.

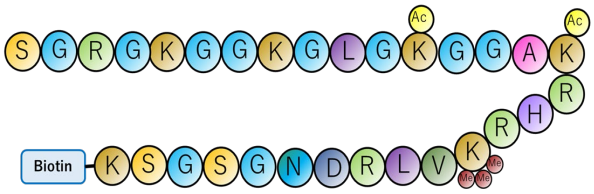

Fig. 1 Amino acid sequence of a synthetic fragment peptide derivative of histone H4

Table 1 Analytical 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	

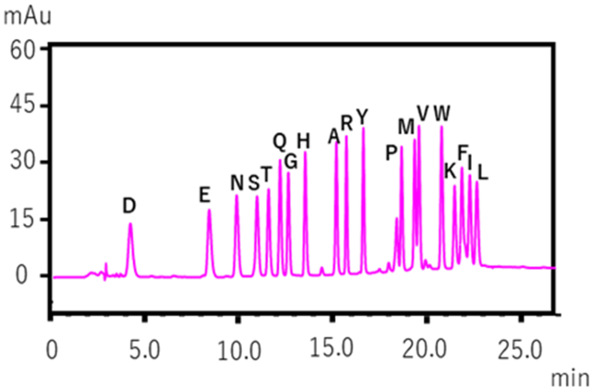

Fig. 2 Analytical results of PTH-amino acid standard mixture (10 pmol)

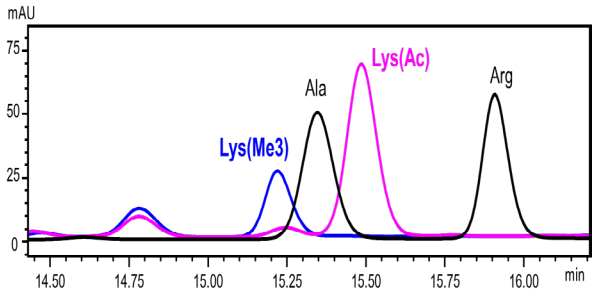

Fig. 3 Chromatogram of Edman degradation of modified amino acids
Black: PTH-Ala, Peach: Acetyl lysine (Lys (Ac)), Blue: Trimethyl lysine (Lys (Me3))

Next, 50 pmol of the sample was placed on a polybrene-treated glass fiber disk, dried, and subjected to amino acid sequence analysis (Fig. 4). The 12/16th Lys (Ac) and 20th Lys (Me3) could be identified in each cycle. It was difficult to identify the C-terminal Lys because it is modified with biotin and also because the sample is washed out with each successive Edman degradation. In protein sequencers, when the elution position of the modified amino acid residue is near the elution position of the natural amino acid, as shown in Fig. 3, identification of PTH-amino acids may be difficult due to the influence of sample adulteration. In such cases, integrating MALDI-ISD measurements can provide more reliable amino acid sequence results. Fig. 5 shows the results of ISD analysis using the MALDI-8030 benchtop MALDI-TOF MS system. This analysis method using MALDI-ISD measurement is relatively easy to perform due to its short analysis time and minimal pretreatment requirements. Furthermore, the inherent difficulty in identifying C-terminal amino acids during Edman degradation—which results from sample washout—can be overcome by employing MALDI-ISD, facilitating the identification of the entire amino acid sequence.

On the other hand, there are some issues such as the inability to distinguish between leucine and isoleucine, which have the same mass, and the complexity of analyzing amino acid sequences that are not registered in the database, making it useful to use in combination with a protein sequencer.

■ Conclusions

Using the PPSQ-50A series protein sequencer, it is now possible to identify the amino acid sequence of synthetic peptides with not only natural-type amino acids but also amino acids with modified side chains, and to combine this with MALDI-ISD measurements to obtain more reliable results. In the future, peptide drugs produced by chemical synthesis are expected to include modified amino acids and cyclic peptides in their composition in order to increase their activity, specificity, and in vivo stability. These results suggest that amino acid sequencing methods using protein sequencers, in combination with MALDI-ISD measurements, may be useful in the development and confirmation of synthesis of peptide drugs.

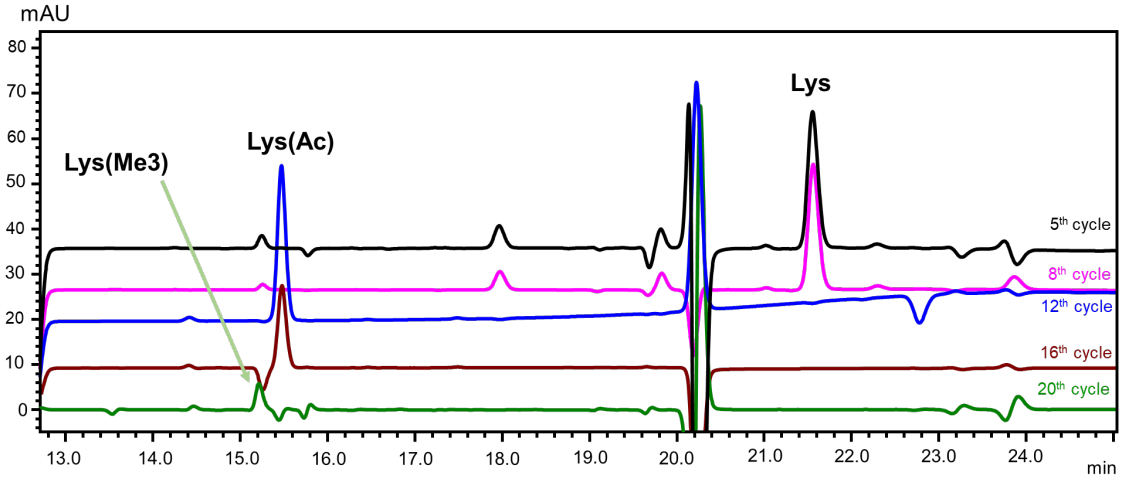

Fig. 4 Results of amino acid sequence analysis by protein sequencer (subtracted chromatogram)

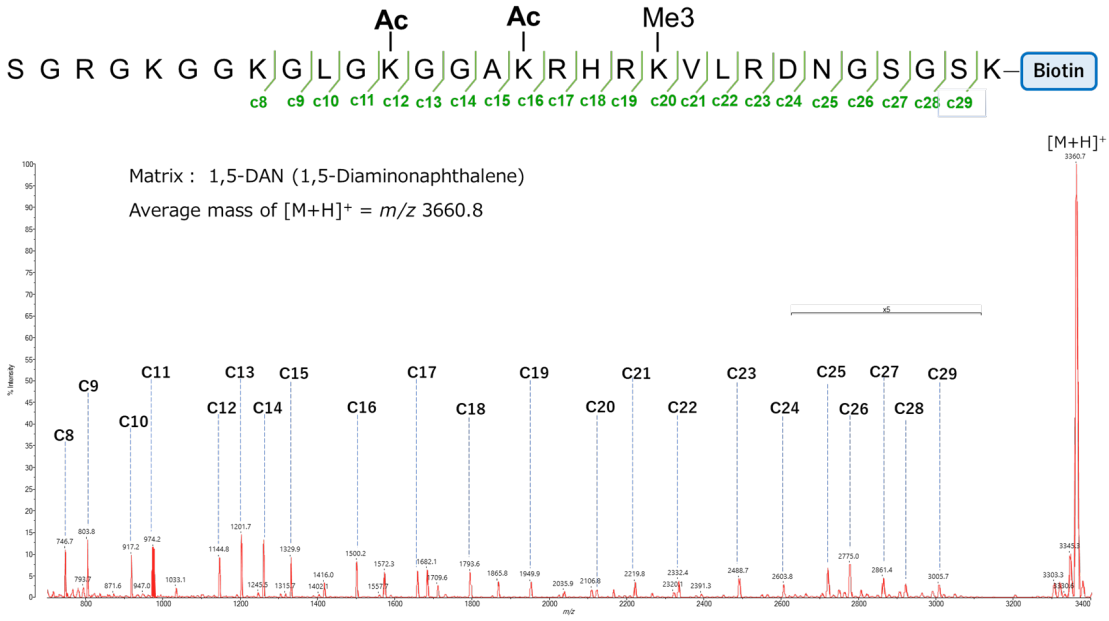

Fig. 5 MALDI-ISD mass spectrum of synthetic peptide Histone H4 fragment using MALDI-8030

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