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MALDI-TOF Mass Spectrometer MALDI-8020/MALDI-8030

Application News

Amino Acid Sequencing of Synthetic Peptides Using a MALDI-TOF Mass Spectrometer and Protein Sequencer

Kumiko Yamaguchi, Miho Akagi, and Tomoko Kuriki

User Benefits

- Amino acid sequences of peptides can be determined with the MALDI-ISD analysis.
- By combining protein sequencer results with MALDI molecular weight measurements or MALDI-ISD results in a complementary way, all amino acid sequences can be determined regardless of the peptide structure.

■ Introduction

Compared to large molecular weight antibody therapeutics, peptide therapeutics have been attracting attention in recent years because they are more readily absorbed in oral form, and offer higher cell membrane permeability, lower production costs based on chemical synthesis, and other benefits. In order for peptide therapeutics to offer a particular function, they must have a specific amino acid sequence. However, during the synthesis process, by-products can be formed if the peptide bond elongation reaction is accidentally stopped or missed, etc. It is therefore necessary to confirm the amino acid sequence of the final synthesized product.

Technical advances in mass spectrometers now enable high-throughput analysis of amino acid sequences of peptides and proteins by using mass spectrometers and corresponding databases. However, that can result in problems, such as inaccurate or uncertain database search results due to amino acid side chain modifications or other factors causing the mass spectrometry results to differ from the theoretical masses. Also, if the genome database of a biological species is incomplete, amino acid sequencing with a mass spectrometer can be complicated and unreliable.

In contrast, when sequencing amino acids by Edman degradation, respective amino acids are identified one at a time starting from N-terminals, which eliminates problems with mass and database dependence. However, sequencing by Edman degradation has limitations, such as decreased efficiency when analyzing long sequences or difficulty analyzing modified amino acids.

This Application News describes the combined use of sequence information obtained with the MALDI-8030 benchtop MALDI-TOF mass spectrometer and a PPSQ™-50A series protein sequencer with conventional Edman degradation to determine the amino acid sequences in two types of synthetic peptides.

■ Pretreatment and Analytical Conditions

Sample 1: Parathormone

Straight chain peptide with 34 residues

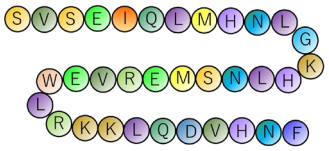

Fig. 1 Amino Acid Sequence of Parathormone

Sample 2: Somatostatin

Cyclic peptide with 14 residues

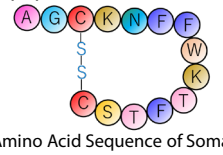

Fig. 2 Amino Acid Sequence of Somatostatin

MALDI-TOF MS Analytical Conditions

Samples were analyzed using the MALDI-8030 (Fig. 3) in positive-ion mode. Molecular weights were measured using an a-cyano-4-hydroxycinnamic acid (CHCA) matrix. MALDI-ISD (in-source decay) was measured using the 1,5-diaminonaphthalene (1,5-DAN) matrix. Samples and matrix solutions were applied to a MALDI plate and dried before measuring.

Fig. 3 Left: Applying Samples and Matrix Solutions to MALDI Plate
Right: MALDI-8030 Benchtop MALDI-TOF Mass Spectrometer

Analysis Using a Protein Sequencer

Standard mixture samples of PTH-amino acids (phenylthiohydantoin derivatives of amino acids) were analyzed using PPSQ-50A series isocratic and gradient protein sequencer systems (Fig. 4). Analytical conditions are provided in Application News No. 01-00521-EN and Application News No. 01-00549-EN. The chromatograms obtained using respective analytical conditions are shown in Figs. 5 and 6.

Fig. 4 PPSQ™-50A Gradient Protein Sequencer System

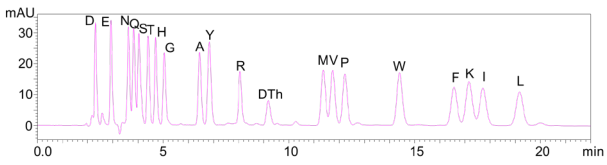

Fig. 5 Chromatogram of Standard PTH-Amino Acid Mixture (25 pmol)
(Isocratic System)

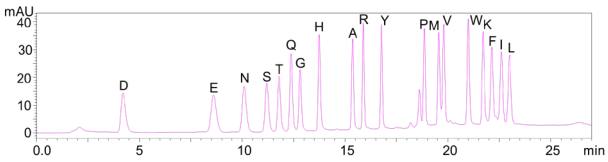

Fig. 6 Chromatogram of Standard PTH-Amino Acid Mixture (10 pmol)
(Gradient System)

Parathormone Analysis Results

The chromatograms obtained from analyzing parathormone using the protein sequencer are shown in Fig. 7. With the protein sequencer, amino acids up to the 33rd residue could be determined. However, it was not possible to identify the amino acid for the 34th residue at the C-terminal because the specifically increased PTH-amino acids could not be detected due to washout from the sample support during Edman degradation.

The MALDI mass spectrum for measuring the molecular weight of parathormone is shown in Fig. 8. Based on the difference between the molecular weight of parathormone determined from Fig. 8 and the molecular weights for the first 33 residues determined with the protein sequencer (147.2), the amino acid in the 34th residue at the C-terminal could be estimated to be Phe.

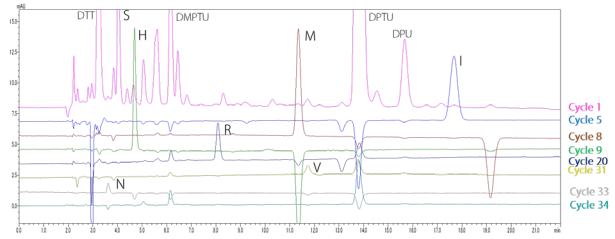

Fig. 7 Chromatogram of Parathormone

Fig. 8 MALDI Mass Spectrum of Parathormone
(Molecular Weight Measurement)

Edman degradation amino acid sequencing results (protein sequencer)

Next, a MALDI-ISD measurement of parathormone and a Mascot (Matrix Science) database MS/MS ion search were performed.

MALDI-ISD (in-source decay) refers to the fragmentation that occurs simultaneously or immediately after laser irradiation inside the ion source during ionization. Fragment ions are generated when the peptide backbone is cleaved by the interaction between the ISD matrix and the peptide sample. Consecutive fragment ions are generated with relatively little dependence on the amino acid sequence, making it suitable for sequence analysis. MALDI-ISD can also be useful for obtaining sequencing information from samples that do not generate enough fragments by enzyme digestion and for which peptide mass fingerprinting (PMF) is minimally effective.

The MALDI-ISD mass spectrum of parathormone is shown in Fig. 9. Parathormone was identified by searching the database for fragment ions derived from the observed MALDI-ISD fragments.

Based on MALDI-ISD, amino acids up to the 8th residue from the N-terminal could not be identified, but the entire amino acid sequence of parathormone could be identified by using the protein sequencer results in Fig. 7 as a complement.

If sequences cannot be identified by the database search using MALDI-ISD fragments, de novo sequencing must be used. This directly reads mass differences of fragment ions, but it cannot distinguish between Ile and Leu isomers. Since protein sequencers can easily distinguish between the isomers, it is useful to use both MALDI-ISD and protein sequencer results in combination.

Parathormone Amino Acid Sequencing Using Protein Sequencer Results in Combination with MALDI-ISD Results

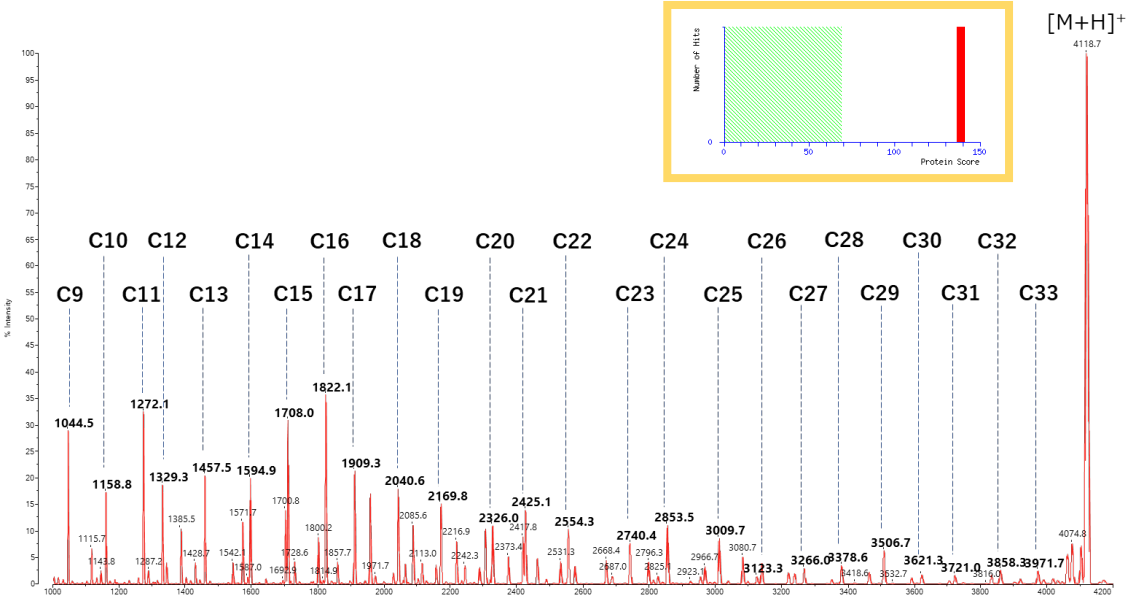

Fig. 9 MALDI-ISD Mass Spectrum of Parathormone (Inset: Mascot Database Search Results)

Somatostatin Analysis Results

Fig. 10 shows the chromatograms obtained from using a protein sequencer to analyze somatostatin, a cyclic peptide with 14 residues. The protein sequencer enabled amino acid sequences to be determined up to the 12th residue.

Presumably, the 13th residue, Ser, and the 14th residue, Cys, could not be identified due to the decreased yield of PTH-amino acids caused by the side chain loss of Ser and especially Cys residues during Edman degradation. However, it also might be due to washout from the sample support during Edman degradation.

To identify all the amino acid sequences in somatostatin, a MALDI-ISD measurement followed by the Mascot database MS/MS ion search was carried out. The MALDI-ISD mass spectrum of somatostatin is shown in Fig. 11.

Although the database search found some candidate sequences, the scores were low and lacked reliability (data not shown). This was probably because the relatively low molecular weight of somatostatin provided minimal fragment information. In such cases, amino acid sequences are predicted by reading the *m/z* intervals between detected ISD fragments.

MALDI-ISD generated sequential fragment ions indicating 9th to 14th residues from the N-terminal. All the amino acid sequences of somatostatin were therefore identified by using the protein sequencer results in Fig. 10 in combination with the MALDI-ISD results.

It should be noted that the MALDI-ISD can easily identify Cys amino acid residue, which can be particularly difficult to identify based on Edman degradation.

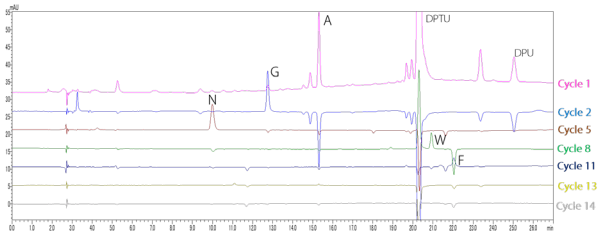

Fig. 10 Chromatograms of Somatostatin

■ Conclusion

By combining protein sequencer results with MALDI molecular weight measurements or MALDI-ISD results, all the amino acid sequences were determined in parathormone and somatostatin, which have linear and cyclic structures, respectively.

By combining data obtained from multiple instruments, all the amino acid sequences of the target peptides can be obtained regardless of the peptide structure.

The complementary use of MALDI-ISD and protein sequencer results will be useful for sequencing amino acids in synthetic peptides.

Related Application News Articles

1. Analysis of Long-Chain Amino Acid Sequences Using a Protein Sequencer—Isocratic System —
   Application News No. 01-00521-EN
2. Analysis of Long-Chain Amino Acid Sequences Using a Protein Sequencer—Gradient System —
   Application News No. 01-00549-EN
3. Protein Analysis Platform
   Combining the powerful capabilities of MALDI-TOF MS (MALDI-8020) and Edman Sequencing (PPSQ™-50A Gradient System) for accurate N-terminal sequence of peptides—
   Application News No.B105

Somatostatin Amino Acid Sequencing Using Protein Sequencer and MALDI-ISD Results in Combination

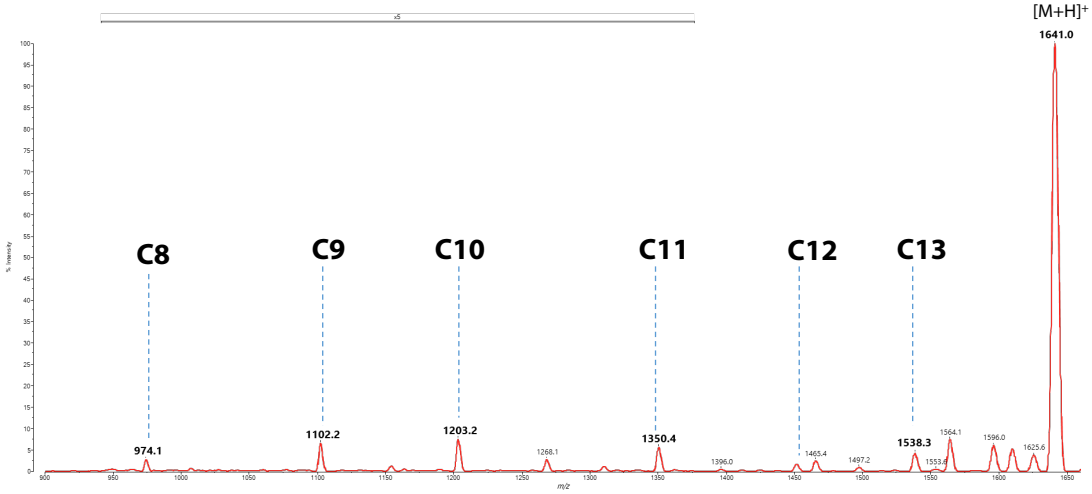

Fig. 11 MALDI-ISD Mass Spectrum of Somatostatin

01-00776-EN First Edition: Jan. 2025

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