

Application News

MALDI-TOF Mass Spectrometry

No.B24

Analysis of O-linked Glycopeptide Using AXIMA Resonance™, Multiple Stage Mass Spectrometry - Sensitivity / Analysis -

Along with the growing use and advances in proteomics research, it has become clear that many proteins, when subjected to a type of modification, become biologically active molecules.

This protein modification is generally referred to as posttranslational modification, and many types of modification have been identified, including phosphorylation, methylation, etc. Among these, sugar chain modifications are receiving attention as new biomarkers for a variety of diseases such as cancer. However, when these proteins which have undergone

Here we introduce an evaluation of ion detection sensitivity in analysis of an actual sample consisting of an O-linked glycopeptides, in addition to analysis of the glycosylation site.

For the sample, we used the O-linked glycopeptide (MW: 1517.55) which binds the Core 3 structure (GlcNAc β 1-3GalNAc α 1-) with threonine, the fifth amino acid in the partial sequence "AHGVTSAPDTR" of the MUC1 mucin protein (Fig. 1). Stepwise dilutions of this sample were deposited on a MALDI target plate, and after matrix solution (DHB: 2,5-dihydroxybenzoic acid) was spotted on each sample dilution, the spots were dried and MS analysis was conducted (Fig. 2) according to the conditions shown in Table 1. In addition to the typically used stainless steel MALDI plate, we also investigated ion detection using the μ Focus MALDI plate*, equipped with 600 μ m diameter measurement wells (Fig. 3). Due to the surface treatment applied to μ Focus MALDI plates*, sample solution can be confined to a small area within the measurement wells, allowing ultra-small quantities of sample to be measured efficiently in this very useful plate. The results of these measurements confirm that detection of glycopeptide to the 1 fmol level is possible using the AXIMA Resonance™ with the selection of an appropriate MALDI plate.

In addition, by not limiting analysis to simple MS and MS/MS, but extending analysis to MS $_n$ ($n \geq 3$), it is possible to identify the site where the glycan binds to the peptide.

Here, the glycopeptide molecular ion obtained in MS analysis was used as the precursor ion in conducting MS/MS analysis, making it possible to learn the composition of the glycan linked to the peptide (Fig. 4). Further, the ion of the peptide part obtained in MS/MS analysis was used in MS $_3$ analysis (MS $^{3-}$), and the ion with one sugar linked to the peptide was used as the precursor ion in MS $_3$ analysis (MS $^{3-}$). By conducting comparative analysis of these, it was

sugar chain modification are analyzed using mass spectrometry, their detection sensitivity is extremely low compared to that of glycopeptides, with the result that they are currently difficult to analyze.

The AXIMA Resonance™ has a detection sensitivity of 500 amol when conducting MS using peptides, and furthermore, since it is equipped with a mechanism for conducting multiple-stage mass spectrometry, it can be expected to play a powerful role in analysis of complex posttranslational modifications involving sugar chains.

possible to identify the site where the glycan binds to the target peptide (Fig. 5).

As described above, by conducting MS/MS spectral analysis of the sample selected here, and then comparing the spectra obtained in MS $_3$ analysis, we were able to confirm that the Core 3 structure suggesting a disaccharide structure is linked to threonine, the fifth amino acid in the peptide sequence.

The AXIMA Resonance™, with its high sensitivity, high resolution as well as its ability to conduct highly accurate multiple-stage mass spectrometric analysis, is extremely useful for this type of analysis.

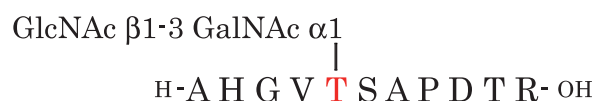


Fig. 1 O-linked Glycopeptide Sample

Table 1 Measurement Conditions

Conditions 1) for Standard Stainless MALDI Plate	
Matrix	: 10 mg/ml DHB (2,5-dihydroxybenzoic acid) in 50 % CH ₃ CN, 0.05 % TFA (0.5 μ l)
Laser Power	: 77 - 90
Laser Shots	: 2 shots/profile
Accumulation Profile	: 100 profiles
Conditions 2) for μ Focus MALDI Plate*	
Matrix:	: 2.5 mg/ml DHB (2,5-dihydroxybenzoic acid) in 50 % CH ₃ CN, 0.05 % TFA (0.5 μ l)
Laser Power	: 85 - 104
Laser Shots	: 2 shots/profile
Accumulation Profile	: 100 profiles

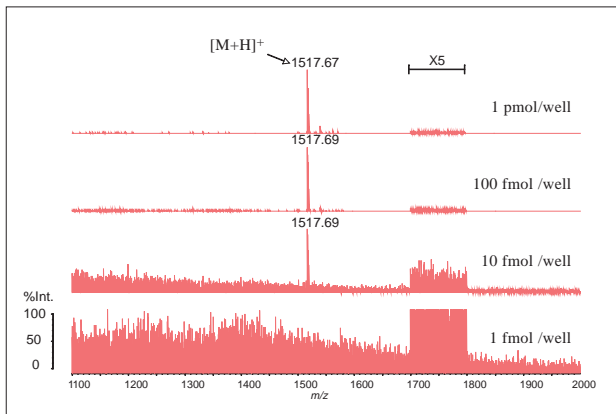


Fig. 2 Ion Detection of O-linked Glycopeptide on Standard Stainless MALDI Plate

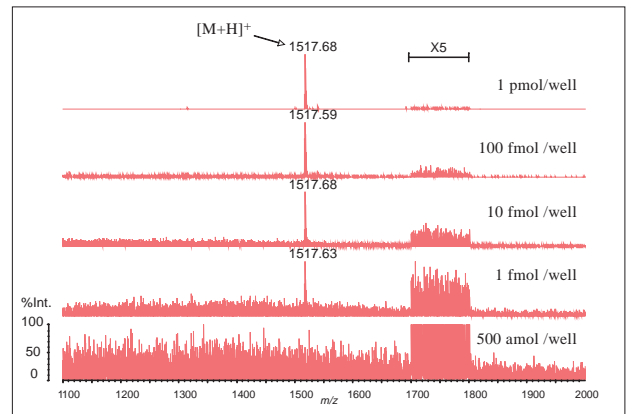
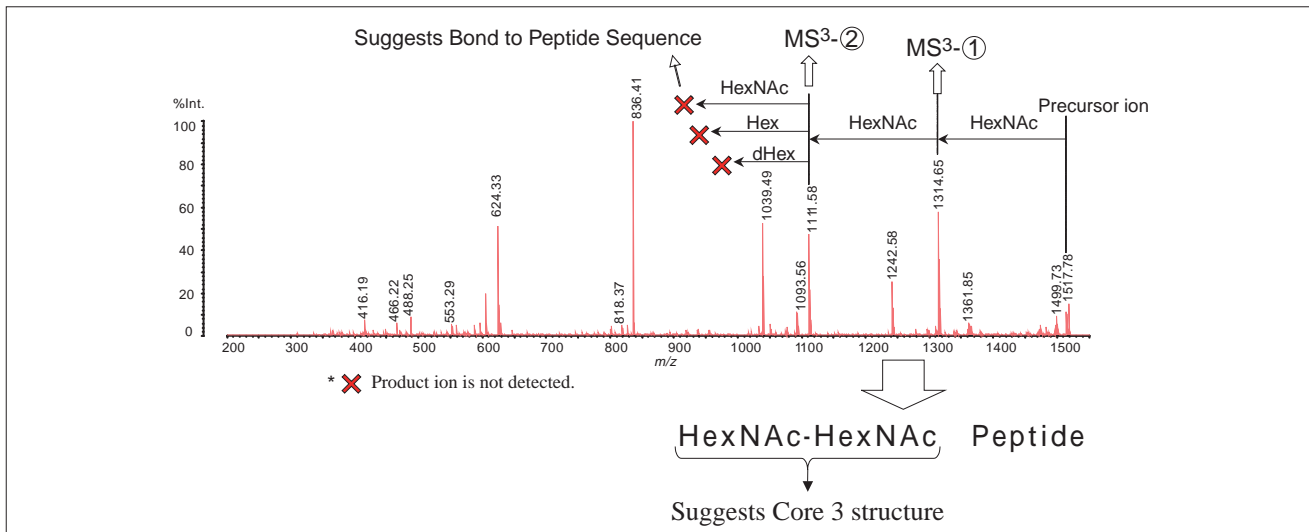
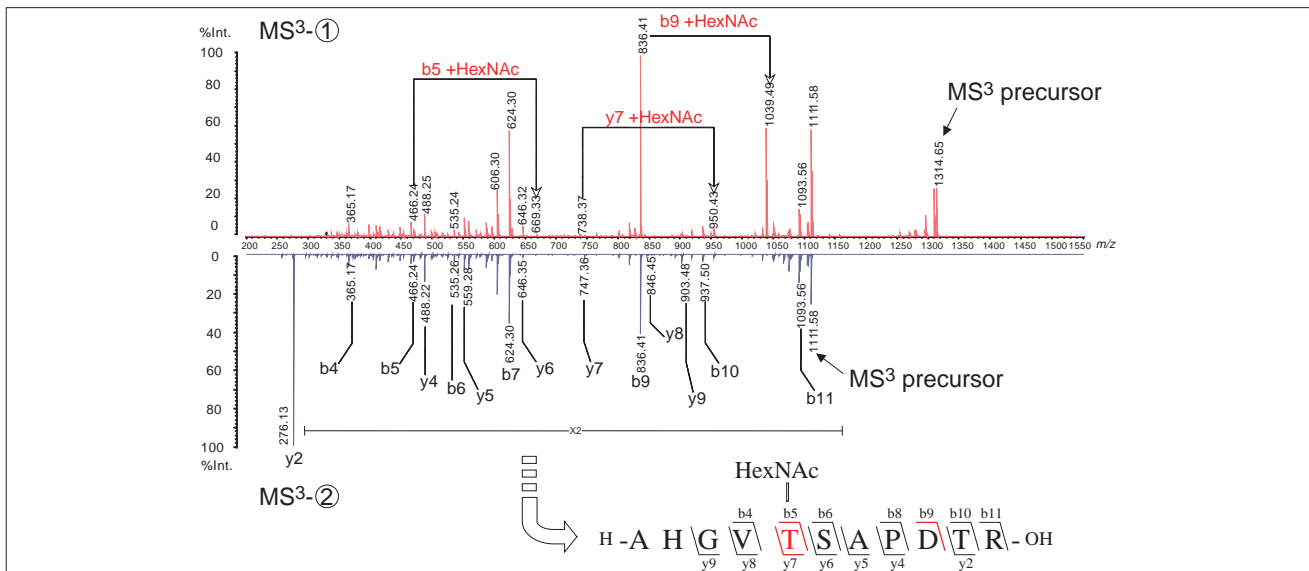
Fig. 3 Ion Detection of O-linked Glycopeptide on μ Focus MALDI Plate*

Fig. 4 Confirmation of The Glycan Sequence by Analysis of MS/MS Spectrum

Fig. 5 Confirmation of Peptide Sequence and Determination of Glycosylation Site by Comparative Analysis of MS³ Spectra

[Acknowledgment]

We wish to offer our appreciation to Dr. Ito of the Advanced Industrial Science and Technology (AIST) Research Center for Medical Glycoscience for kindly providing the sample used in this analysis.

* μ Focus MALDI plates are a product of Hudson Surface Technology, Inc.



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