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Application News

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MALDI Mass Spectrometer

# Analysis of Glycopeptides Using MALDImini™-1 Compact MALDI Digital Ion Trap Mass Spectrometer

Glycans, which are one post-translational modification of proteins, are molecules with high structural heterogeneity which are formed by complex bonding of glucose, mannose, and other monosaccharides. It is known that their complex structure is related to regulation of protein functions, and various phenomenon can be observed depending on illness and other factors. These include abnormal glycan structures with the protein backbone and the absence of glycan bonding at sites where it is assumed that such bonding should occur. The information concerning complex glycan structures and the binding sites of glycan with the proteins is not coded directly in genes, but is created by the action of a large number of glycosyltransferases, which act in the protein biosynthesis process. Therefore, a direct analysis of the target glycoprotein is necessary in order to understand the structures and binding sites of glycans on glycoproteins. Although mass spectrometers are widely used in this type of analysis, almost all such analyses are performed with large-scale, high performance instruments.

This article reports a glycopeptide analysis using a Shimadzu MALDImini-1 compact MALDI-DIT mass spectrometer equipped with a digital ion trap (DIT), which is an original technology developed by our company.

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## MALDImini-1 Compact MALDI-DIT Mass Spectrometer

The MALDImini-1 (Fig. 1) is a mass spectrometer which combines a MALDI ion source and a digital ion trap. Conventionally, sine wave RF had been used in the ion trap mechanism, but because a large high voltage power source and sine wave RF generator coil for voltage modulation were necessary, devices inevitably tend to be large in scale. In comparison with conventional devices, the size of MALDImini-1 used in this analysis is substantially reduced by installing a "digital ion trap," which is a unique technology of Shimadzu Corporation utilizing rectangular wave RF rather than the conventional sine wave RF. Even with all vacuum pumps built-in, a lightweight and space-saving design was realized.

**Fig. 1 Appearance of MALDImini™-1 Compact MALDI-DIT Mass Spectrometer**

## Preparation of Glycopeptides from Glycoprotein

Using a commercial monoclonal antibody as the sample material, first, reductive alkylation was performed in a solution. Then, trypsin was added to this solution, and enzymatic digestion was performed at 37 °C for 3 h. After enzymatic digestion, the solution was passed through a pipette tip packed with SepharoseCL4B gel, which had been equilibrated in advance with butanol : ethanol : water = 4 : 1 : 1, and glycopeptides were adsorbed on the gel. The peptide component was then removed by washing with the equilibration solution, and the glycopeptides were recovered with an ethanol solution [1]. The recovered glycopeptides were placed on the MALDI target plate, and then overlaid with the matrix solution and dried. DHB (2.5-dihydroxybenzoic acid) was used as the matrix.

## MS Measurement of Glycopeptide Fraction

MS measurements of the recovered glycopeptides were conducted at a scan speed of 4,000 Da/s using the MALDImini-1 MALDI-DIT mass spectrometer. As a result, *m/z* 2269.0, 2431.1, 2593.3, 2634.3, 2796.3, 2958.2, 3119.9, and 3282.3 having mutual glycan-derived mass differences were detected as the main peaks (Fig. 2).

Because glycans are structurally heterogeneous, multiple different glycan structures exist on one peptide backbone. In the MALDI-DIT mass spectrometer, signals readily become univalent ions, and as a result, signals originating from glycopeptides can be identified easily from multiple detected signals by confirming the *m/z* difference between the signals detected by MS measurements, and then searching for signals that coincide with the differences derived from structural components of the glycan.

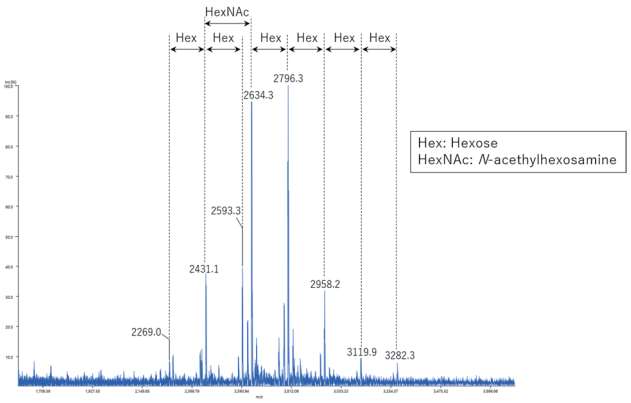


**Fig. 2 Mass Spectrum of Glycopeptide Fraction Extracted from Commercial Antibody**

## Identification of Glycan Structure and Binding Site by $MS^n$

As one example, *m/z* 2796.3 was selected from the glycopeptide-derived signals identified in this manner, and was subjected to MS/MS analysis (Fig. 3). As a result, glycan-derived product ions were detected, and furthermore, a triplet peak having distinctive mass differences (*Δ m/z* 83, 120) of GlcNAc at the root of *N*-glycan was also detected. Based on this fact, it could be understood that the ion of *m/z* value of 1189.7 seen in the MS/MS spectrum is a peptide backbone ion. Next, an $MS^3$ analysis of the peptide backbone ion (*m/z* 1189.7) was performed, and the amino acid sequence of the peptide backbone was confirmed. In addition, $MS^3$ analysis was also performed for a ring cleavage ion (*m/z* 1272.7) of the root GlcNAc which had bonded to the peptide backbone, and it was possible to identify the binding site of the glycan by comparing the analysis result with the $MS^3$ spectrum of the peptide backbone (Fig. 4). From these analysis results, it was suggested that the glycopeptide of the commercial antibody used in this analysis has various structures shown in Fig. 5.

The results of this analysis show that the MALDImini-1 compact MALDI-DIT mass spectrometer has a high $MS^n$ analysis capacity in spite of its small size, and possesses the highest possible performance for obtaining full information for components like glycopeptides which have large molecular sizes and complex structures.

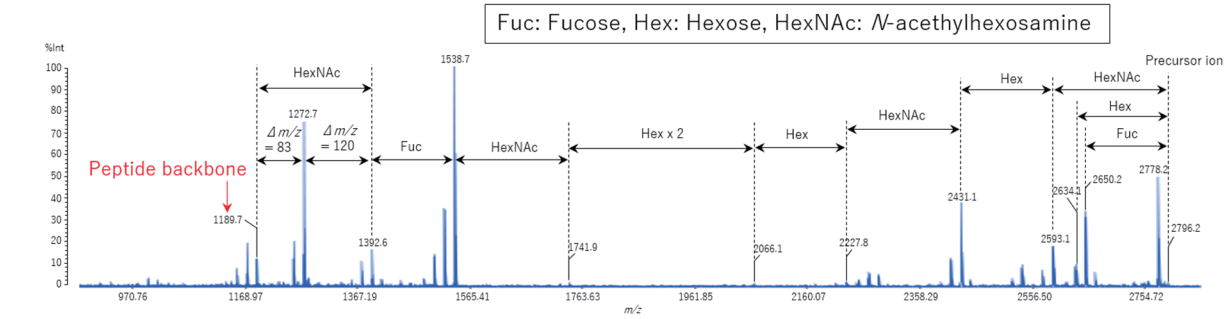


**Fig. 3 MS/MS Spectrum of Glycopeptide (Precursor ion *m/z* 2796.2)**

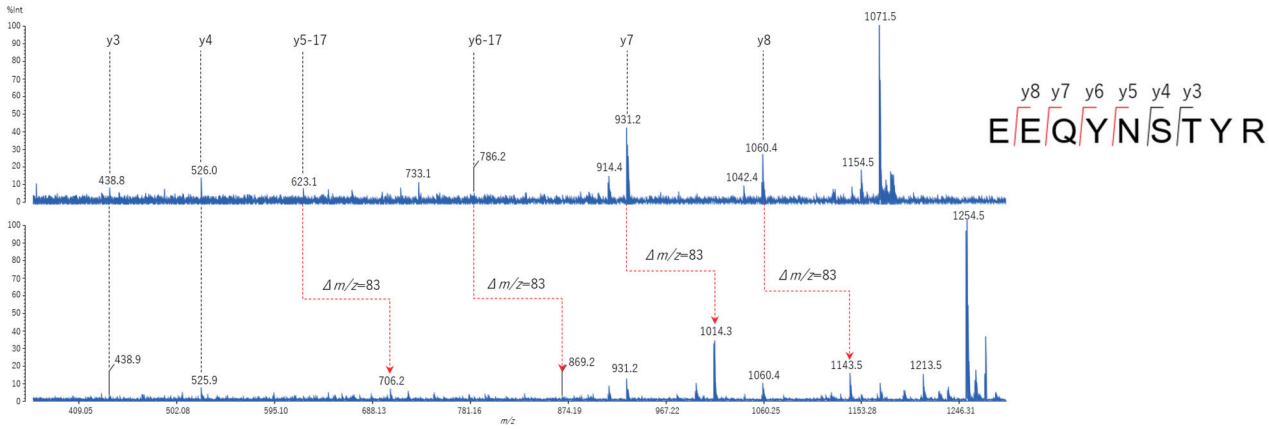


**Fig. 4 $MS^3$ Spectrum of Glycopeptide**
**Top: Peptide Backbone (Precursor ion *m/z* 1189.7),**
**Bottom: Peptide Backbone Containing a Partial Structure of the Root GlcNAc (Precursor ion *m/z* 1272.7)**

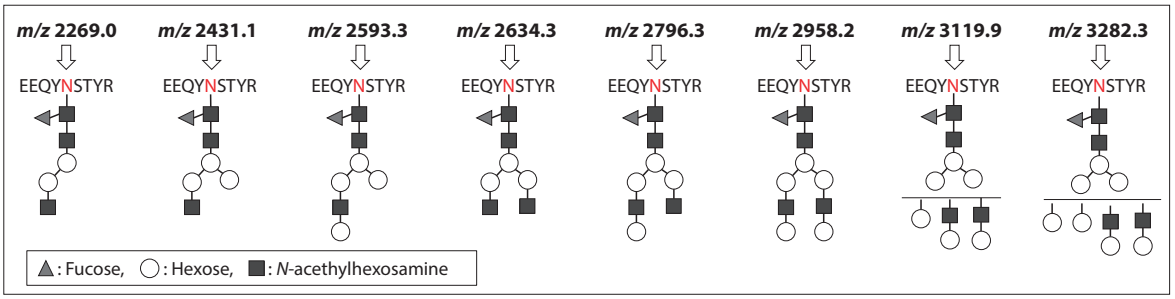


**Fig. 5 Estimated Structures of Commercial Antibody-Derived Glycopeptide (Red: Amino Acid at Glycan Binding Site)**

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References

1) Yoshinao Wada et al., Anal. Chem. 2004, 76, 6560-6565.

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