

Application News

No. B55

MALDI-TOF Mass Spectrometry

Analysis of Glycopeptides of Monoclonal Antibody Using MALDI-7090 High-Resolution MALDI-TOF MS

Antibodies that are utilized in biopharmaceuticals are often subjected to glycan modification. This glycan is a molecule with high structural heterogeneity consisting of intricately coupled monosaccharides, such as glucose and mannose, and that complex structure is known to play an important role in the functional regulation of proteins. However, information such as the complex structure of the glycan and the site at which the glycan is attached to the protein is not written into the gene beforehand. Rather, it is information that is formed based on the actions of many glycosyltransferases during the process of protein biosynthesis. Therefore, depending on the state of growth of the cells that produce antibodies, it is not uncommon to see such phenomena as the formation of glycans having different structures in spite of the same protein backbone, or the formation of the glycan bond at an unexpected site. Therefore, when an antibody is biosynthesized as a biopharmaceutical, elucidation of both its structure and binding site is important. Mass spectrometers are now regularly used for ascertaining both the binding site as well as the structure of glycans. Here, using the MALDI-7090 high-resolution MALDI-TOF MS with its unique 20 keV high energy fragmentation capability, we introduce an example of analysis to determine the structure and binding site of a glycan bound to an antibody.

■ Preparation of Glycopeptides from a Glycoprotein

First, using a commercially available monoclonal antibody as the sample, reduction and alkylation was conducted in the solution. Then, trypsin was added to this solution, allowing enzymatic digestion to continue for 2 hours. The enzyme-digested solution was then added to a spin column packed with Sepharose CL4B gel previously equilibrated using butanol: ethanol:

water (4:1:1), and the solution was allowed to interact with the gel, permitting adsorption of the glycopeptide to the gel. Then, by washing with the equilibrated solution to remove non-glycosylated peptides, glycopeptides were recovered using aqueous ethanol solution. The recovered glycopeptides were spotted on a target plate using 2,5-dihydroxybenzoic acid (DHB) as the matrix, and analyzed using the MALDI-7090.

■ MS Analysis of the Glycopeptide Fraction

The mass spectrum of the recovered glycopeptide fraction is shown in Fig. 1. Of the detected signals, m/z 2405.72, 2430.75, 2567.72, 2592.73, 2633.74, 2795.74 and 2957.74 were considered to be signals derived from glycopeptides. Because glycans have a non-uniform structure, multiple different glycan structures are present in one peptide backbone. Since a signal in MALDI-TOF MS is associated with a monovalent ion, it is possible to look at mass differences between peaks, and if they match the mass of a glycan moiety, then those peaks are likely glycopeptides.

■ Glycan and Amino Acid Sequence Analysis Using MS/MS

As an example, we selected m/z 2795.74 from among the detected glycopeptide signals, and conducted MS/MS analysis using high-energy CID (Fig. 2). In the MS/MS spectrum, fragment ions derived from the glycan were detected in the region near the precursor ion, and fragment ions derived from the peptide backbone were detected in the lower mass region far from the precursor ion. Based on the fragment ions derived from the glycan, it is likely that the glycan structure on the glycopeptide at m/z 2795.74 is a

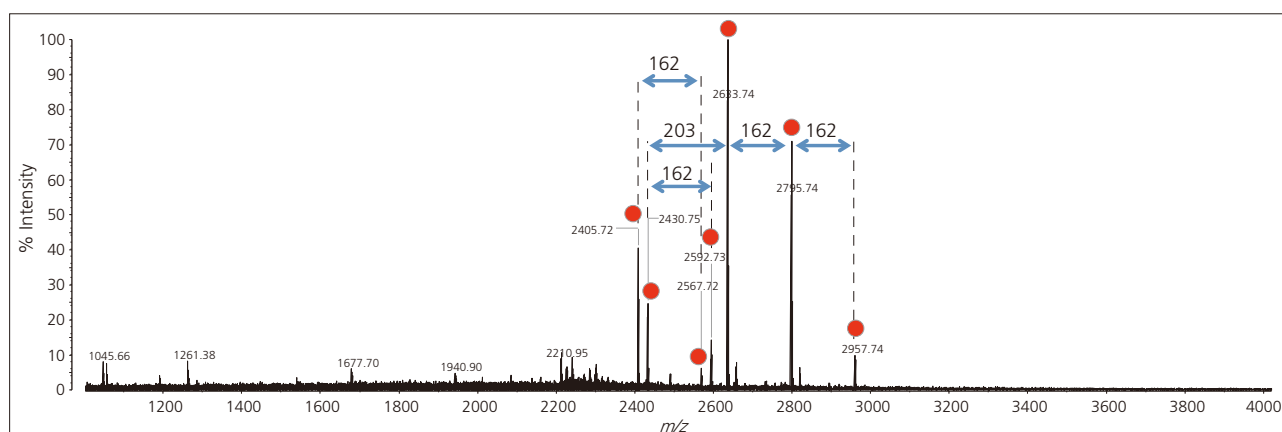


Fig. 1 Mass Spectrum of the Glycopeptide Fraction from Monoclonal IgG
Glycopeptide signals are shown by red circles.

biantennary agalacto/galacto N-linked glycan with core fucose. Furthermore, the amino acid sequence of the peptide backbone attached to this glycan was confirmed as EEQYNSTYR. Because the N-linked glycan binding site motif is known to be -NXS/T-, the binding site of the glycan was confirmed to be N (asparagine) of EEQYNSTYR. Using this strategy, the signals derived from the seven glycopeptides obtained by MS measurement are suggested to have the structures shown in Fig. 3.

Normally, as glycans fragment more easily than peptides when using low-energy CID, fragment ions

originating from the peptide can barely be detected by MS/MS using low-energy CID. Therefore, there is a need for a cleavage mechanism other than CID, such as ECD (electron capture dissociation).

However, the MALDI-7090 enables simultaneous analysis of not only the glycan structure but also the peptide sequence of glycopeptides by enabling high energy-CID at 20 keV while still retaining high resolution of the fragment ions. The MALDI-7090 provides both high-energy and high-resolution MS/MS data, making it an ideal instrument for qualitative analysis of glycopeptides.

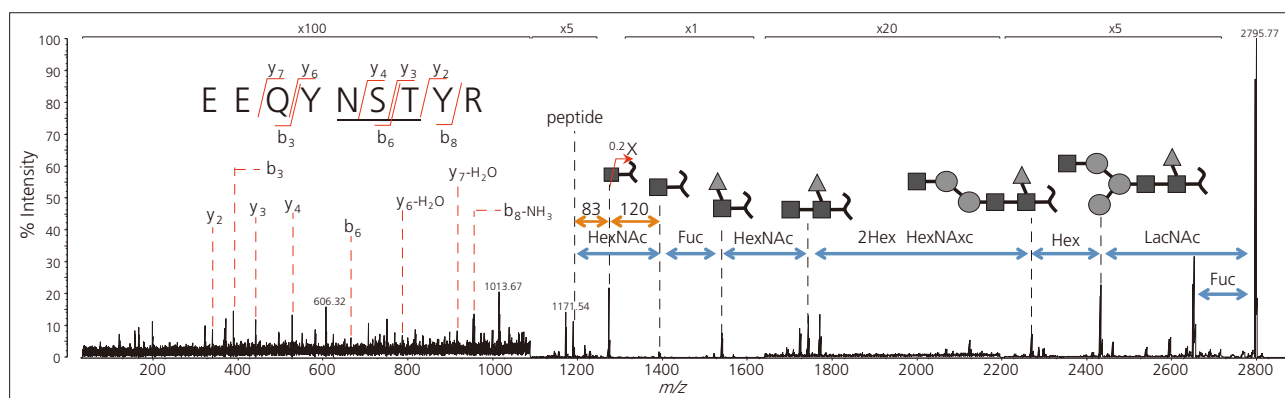


Fig. 2 High Resolution MS/MS Analysis of the Glycopeptide (m/z 2795.74) from Monoclonal IgG
The peptide sequence EEQYNSTYR was confirmed by MS/MS analysis for precursor m/z 2795.74. The consensus sequence of the N-glycan binding site is underlined. HexNAc, Fuc, Hex, and LacNAc are the abbreviations for N-acetylhexosamine, fucose, hexose, and N-acetylglucosamine, respectively.

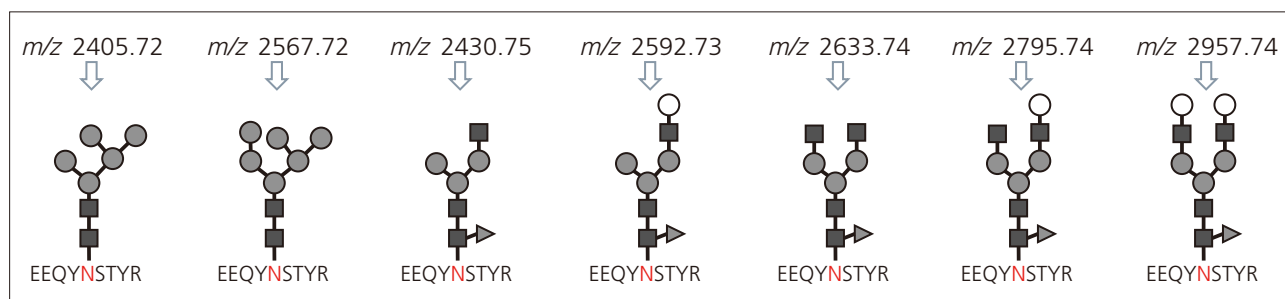


Fig. 3 Identified Glycopeptide Structures from Monoclonal IgG
The structures of all seven N-linked glycans attached to EEQYNSTYR are represented. The amino acid residue of the N-glycan binding site is shown in Red.
Symbols: ■ = N-acetylhexosamine, ○/● = Hexose, ▲ = Fucose

[Acknowledgment]

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