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Application News

No. B99

MALDI Mass Spectrometry

Analysis of Modification Site of Chemically Modified Antibody Using MALDImini™-1 Compact MALDI Digital Ion Trap Mass Spectrometer

Antibody drug conjugates (ADC), which appeared in the 2000s, are a new class of anti-cancer drugs in which an antibody is bound to a cytotoxic drug. Because they combine the high substrate specificity of the antibody and the effect of a low-molecular drug, ADC are expected to be more effective anti-cancer drugs than the conventional low-molecular drugs. When a different compound is bound artificially to a protein, as in the case of ADC, the binding degree of that compound and its binding site become one of the critical quality properties.

Therefore, as reported in the example in this article, a pseudo ADC was created by artificially binding a low-molecular compound to a standard research antibody, and was then analyzed using a MALDImini-1 compact MALDI digital ion trap (DIT) mass spectrometer.

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Tryptic Digestion and MS Measurement of Antibody

Tryptic digestion of a standard antibody modified with Me-fluorescein-ABNO on the tryptophan residue[1] (Fig. 1, NISTmab, humanized IgG $_K$ monoclonal antibody, RM8671) and an untreated standard antibody (1 μL each) was performed in respective solutions, and the samples were then desalted with a Ziptip® μC18 tip and deposited on the MALDI target plate. The matrix solution (0.5 μL) was overlaid on the sample and dried, and an MS^n analysis was conducted using the MALDImini-1 compact MALDI-DIT mass spectrometer. DHB (2.5-dihydroxybenzoic acid) was used as the matrix.

The modified and unmodified antibodies were measured using the MALDImini-1 (Fig. 2), and their mass spectra were compared. Although almost all ions were common to the two samples, some ions (*m/z* 2416.9, 2430.7, 2452.7, 2560.9) that were detected only in the modified antibody were discovered (Fig. 3).

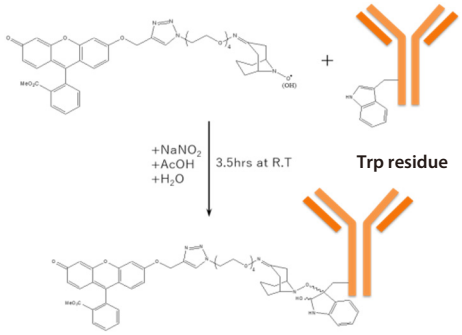

Fig. 1 Me-fluorescein-ABNO Modification of Antibody

Fig. 2 Appearance of MALDImini™-1 Compact MALDI-DIT MS

From the difference in their masses, three of these ions, *m/z* 2416.9, 2430.7, and 2452.7, were thought to be related to a monomolecular CH_2 elimination product or H/Na substitution product. Therefore, a further analysis of *m/z* 2416.9 and *m/z* 2560.9 was carried out, as these were thought to have originated separately from different molecules.

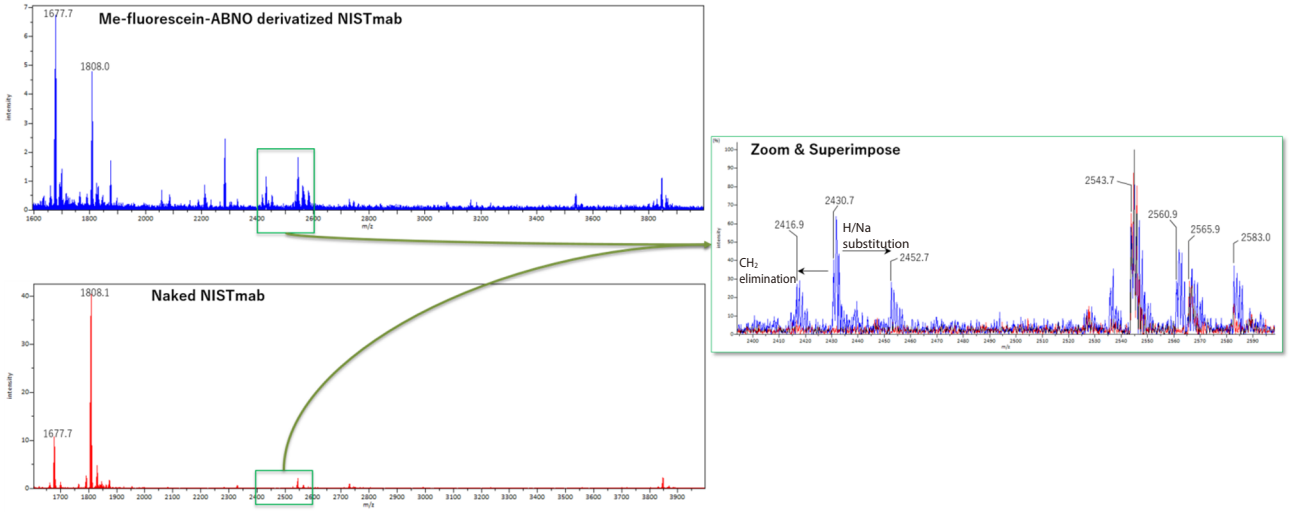

Fig. 3 Comparison of Mass Spectra of Standard Antibodies (Red: Unmodified Antibody, Blue: Modified Antibody)

Analysis of Modification Site by MS^n

First, an MS/MS analysis of *m/z* 2416.9 was performed, and a fragment ion (*m/z* 1677.7), which is supposed to be the peptide backbone from which the modifying group dissociated, was detected (Fig. 4). Because this ion showed the same *m/z* value as the ion detected by MS measurement of the unmodified antibody, MS^3 measurement of the above-mentioned ion obtained by MS/MS analysis of the modified antibody, and MS/MS measurement of ion having the same *m/z* value obtained from the MS spectrum of the unmodified antibody were carried out. When the obtained spectra were compared, the two showed the same fragment patterns. Furthermore, the result of a Mascot MS/MS ion search of these data confirmed that this is a peptide sequence (^{278}FNWYVDGVEVHNAK^{291}) originating from a heavy chain of the antibody.

An analysis of the *m/z* 2560.9 ion was also carried out in the same manner, and it was found that the modifying group also existed in a peptide sequence (^{305}WSVLTVLHQDWLNGK^{320}) that originated from a heavy chain of the antibody (Fig. 5).

Based on these facts, it was suggested that this chemical modification existed in the tryptophan residues in the above-mentioned two peptide sequences.

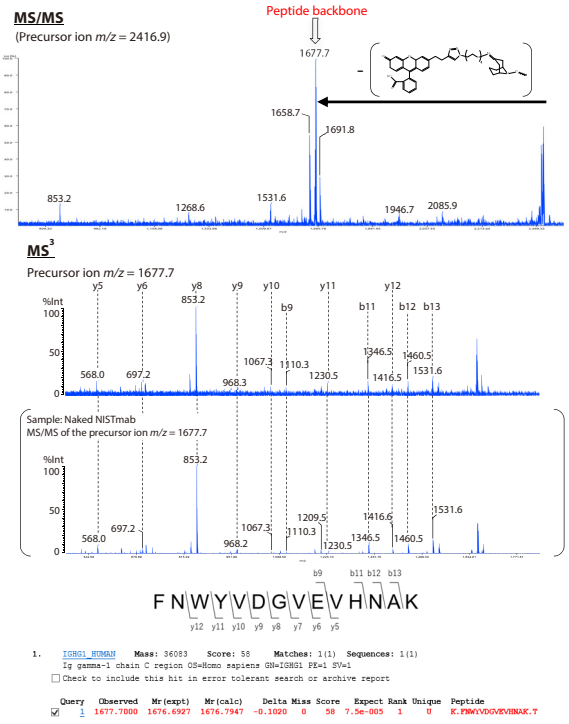

Fig. 4 MS^n (n=2, 3) Spectra and Results of Mascot MS/MS Ion Search

The fact that a single antibody molecule was a complex molecule comprising two heavy chain molecules and two light chain molecules suggested that a maximum of four chemical modifications has occurred this time. This is also consistent with the result (existence of three chemical modifications in one antibody molecule) obtained in another experiment (Application News No. B86).

The results of this analysis demonstrated that the MALDImini-1 compact MALDI-DIT mass spectrometer has high MS^n analysis capability in spite of its small size, and has the highest possible performance for obtaining information from modified peptides.

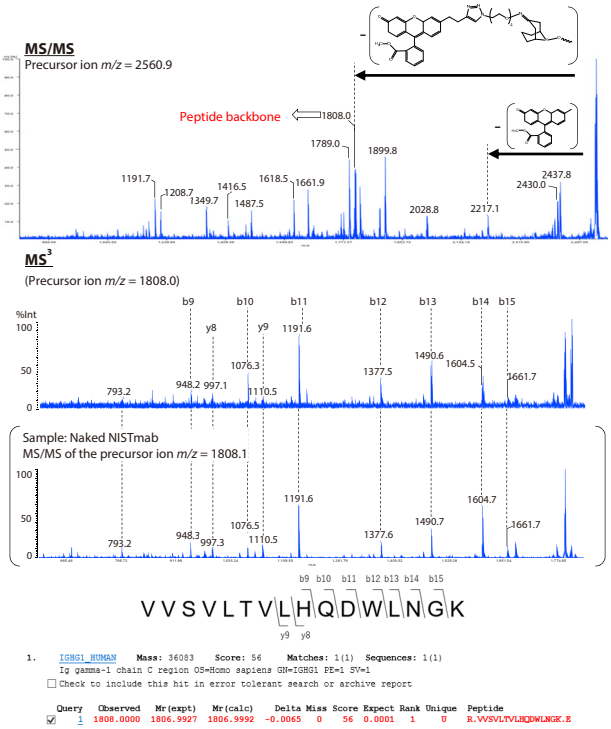

Fig. 5 MS^n (n=2, 3) Spectra and Results of Mascot MS/MS Ion Search

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYN

PSLKDRLTISKDTSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV

VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD

TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD

WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD

IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT

QKSLSLSPGK

ME-fluorescein-ABNO

ME-fluorescein-ABNO

Fig. 6 Amino Acid Sequence of Heavy Chain and Modification Sites (Bold: Amino Acid Sequence of Peptide Confirmed by Mascot MS/MS Ion Search, Red: Modification Site of ME-fluorescent-ABNO)

Reference

(1) Yohei Seki, Takashi Ishiyama, Daisuke Sasaki, Junpei Abe, Youhei Sohma, Kounosuke Oisaki, and Motomu Kanai, Transition Metal-Free Tryptophan-Selective Bioconjugation of Proteins. *J. Am. Chem. Soc.* 2016, 138 (34), 10798-801.

Acknowledgements

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