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MSn Analyses for Tryptophan-Conjugated ADC Mimic by Miniature MALDI Digital Ion Trap Mass Spectrometer (MALDI-DIT-MS)

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Overview

Analysis of modification sites of chemically modified antibody Using MALDImini-1 Compact MALDI Digital Ion Trap Mass Spectrometer

Introduction

Antibody drug conjugates (ADCs), in which drugs are conjugated to antibodies, combine the high selectivity of antibodies with the effects of small molecule drugs to make it more effective than conventional low molecular weight drugs. It appeared in the 2000s as a drug expected to have cancer effects.
When artificially binding another compound to a protein, such as ADC, the binding degree of that compound and its binding site become one of the critical quality properties..
In this experiment, we created a pseudo-ADC in which a low molecular weight compound was artificially bound to a research standard antibody, and modified group binding site analysis was attempted using the compact MALDI-DIT mass spectrometry "MALDImini™ -1." (Fig.1)

Figure 1　MALDImini-1 MALDI DIT MS

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Methods

Standard antibody (NISTmAb, Humanized IgGκ monoclonal antibody, RM8671) in which Me-fluorescein-ABNO has been modified to tryptophan residue according to Seki et al.[1] (Fig.2), and untreated standard antibody (10 µg each) respectively internally tryptic digests and desalted with a Ziptip ® µC18 chip were loaded onto a MALDI target plate. Furthermore, after accumulating and drying the matrix solution (0.5 µL), MSn analysis was performed using the compact MALDI-DIT mass spectrometry "MALDImini-1". The matrix used was DHB (2.5-fihydroxybenzoic acid).

[1] Seki, Y. *et al.*: J. Am. Chem. Soc. 2016, **138**, pp 10798-10801.

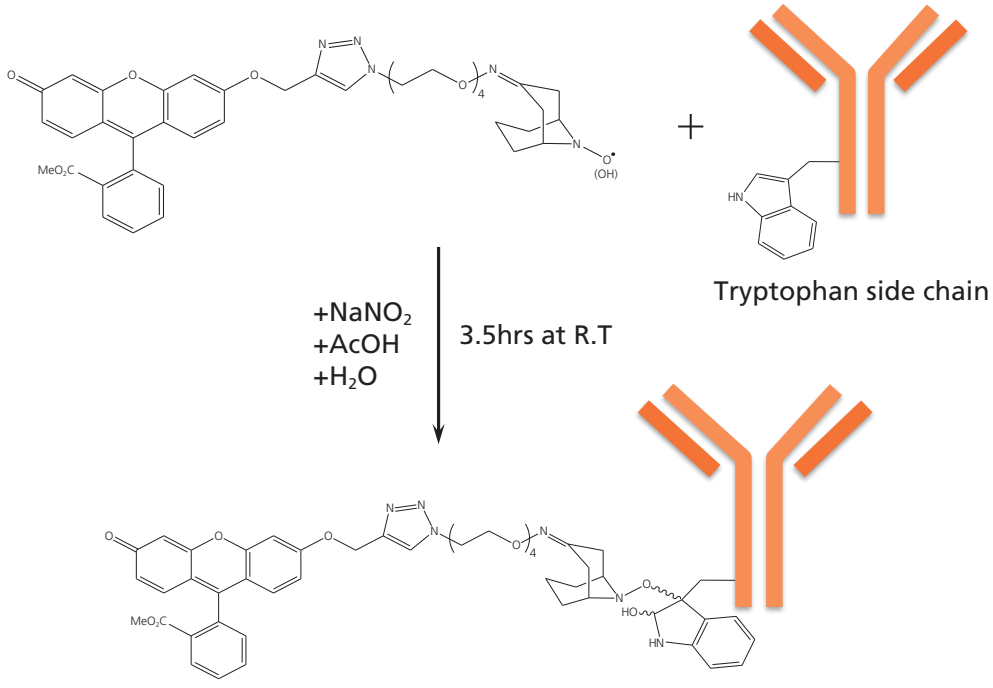

Figure 2 methyl fluorescein-ABNO conjugation to an antibody

Results

The modified and unmodified antibodies were measured using MALDImini-1, and their mass spectra were compared. Although almost all ions were common to both, some of the ions were detected only with the modified antibody (*m/z* 2416.9, 2430.7, 2452.7, 2560.9) (Fig. 3). Therefore, we decided to further analyze *m/z* 2416.9 and *m/z* 2560.9 that were considered to be from different molecules among the ions obtained only with the modified antibody.

First, MS/MS analysis was performed on *m/z* 2416.9. As a result, a fragment ion (*m/z* 1677.7) was detected (Fig.4), which appeared to be a peptide backbone after the modification group was eliminated. And this ion showed the same *m/z* value as the ion detected in the unmodified antibody. The MS^3 spectrum of the modified antibody and the MS / MS spectrum of the unmodified antibody showed the same fragment pattern. Furthermore, when these data were searched by Mascot MS/MS, it was confirmed that it is a peptide sequence (^{278}FNWYVDGVEVHNAK^{291}) derived from the heavy chain of the antibody.

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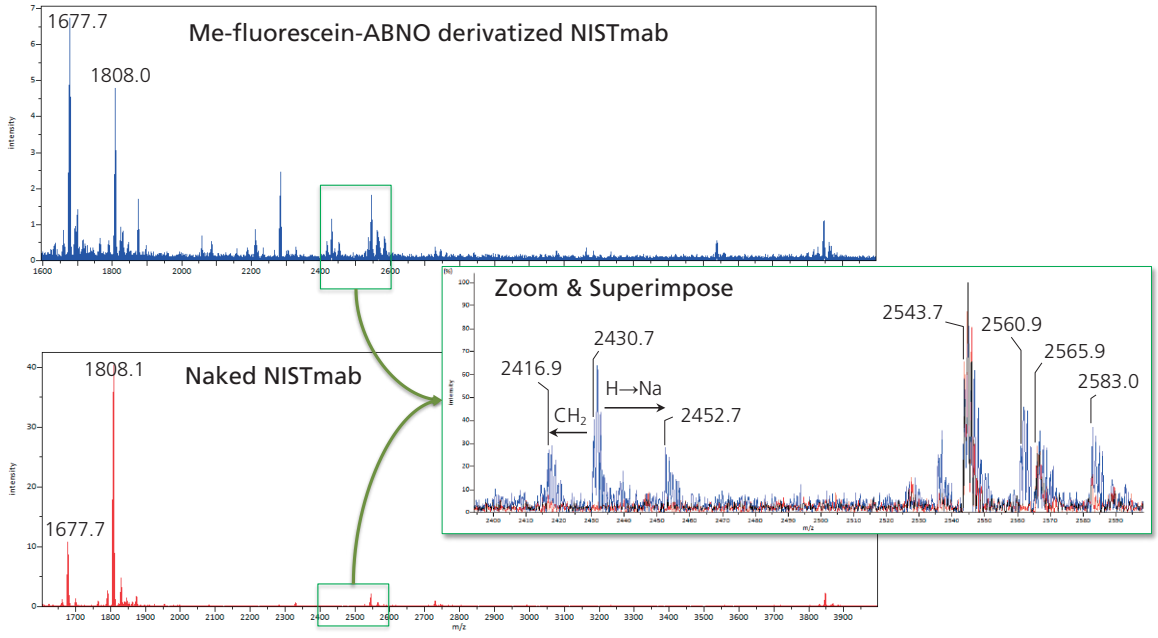

Figure 3 MS spectra of trypsinized antibody (blue : modified, red : unmodied)

Similarly, MS/MS measurement was performed for the *m/z* 2560.9 ion. As compared the MS^3 measurement result for the peptide backbone (*m/z* 1808.0) by MS/MS measurement of the modified antibody, with the MS/MS measurement results of the ions having the same *m/z* value by MS measurement of the unmodified antibody, both showed similar fragment patterns, and further, it was found that the peptide sequence (^{305}VVSVLTVLHQDWLNGK^{320}) derived from the heavy chain of the antibody was obtained by MS/MS ions search (Fig.5). This suggests that the chemical modification used here is present in the tryptophan side chain in the above two peptide sequences.

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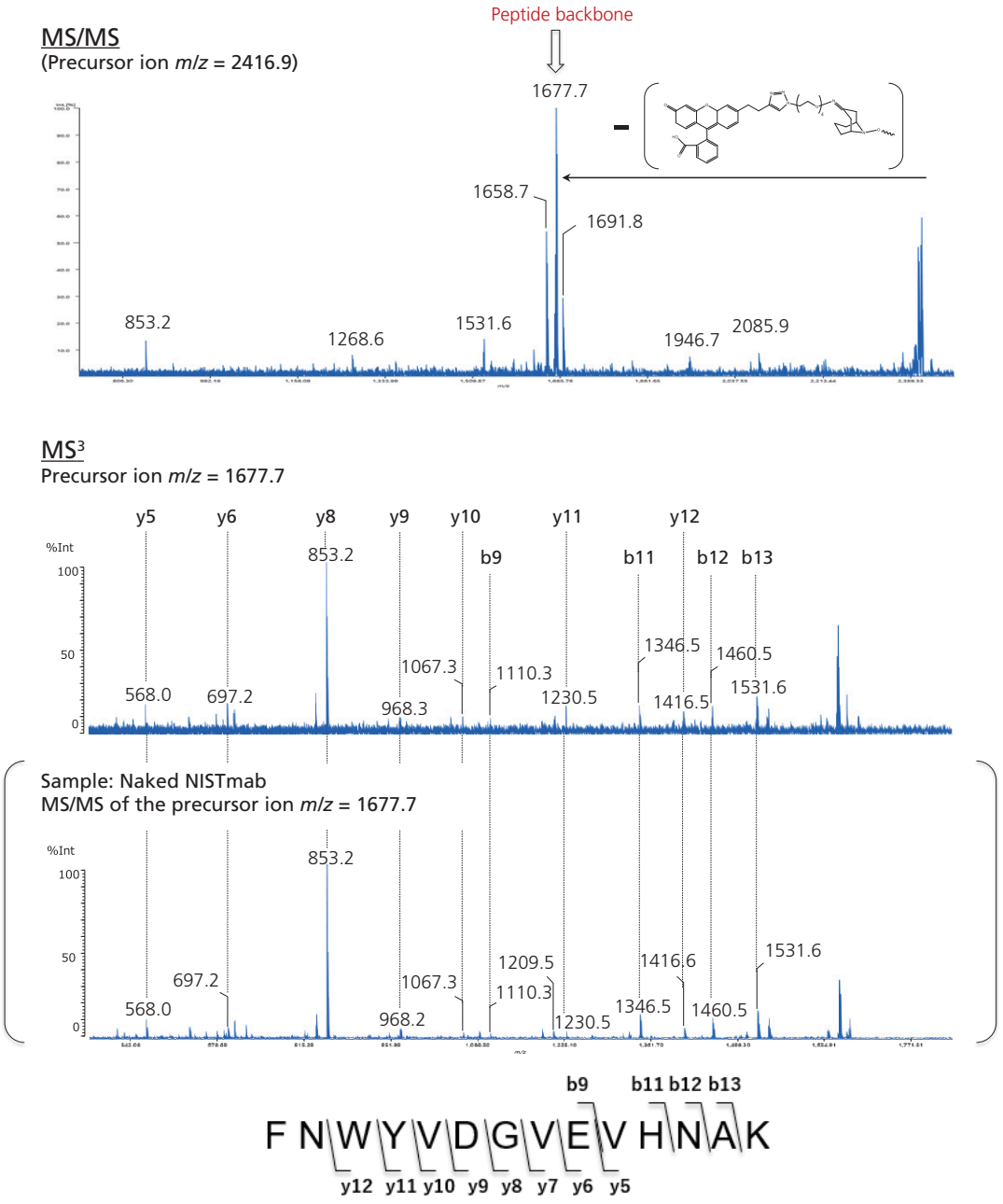

1. IGHG1_HUMAN Mass: 36083 Score: 58 Matches: 1(1) Sequences: 1(1)
Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1
☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
☑ 1	1677.7000	1676.6927	1676.7947	-0.1020	0	58	7.5e-005	1	U	K.FNWYVDGVEVHNAK.T

Figure 4 MS^n spectra of *m/z* 2416.9

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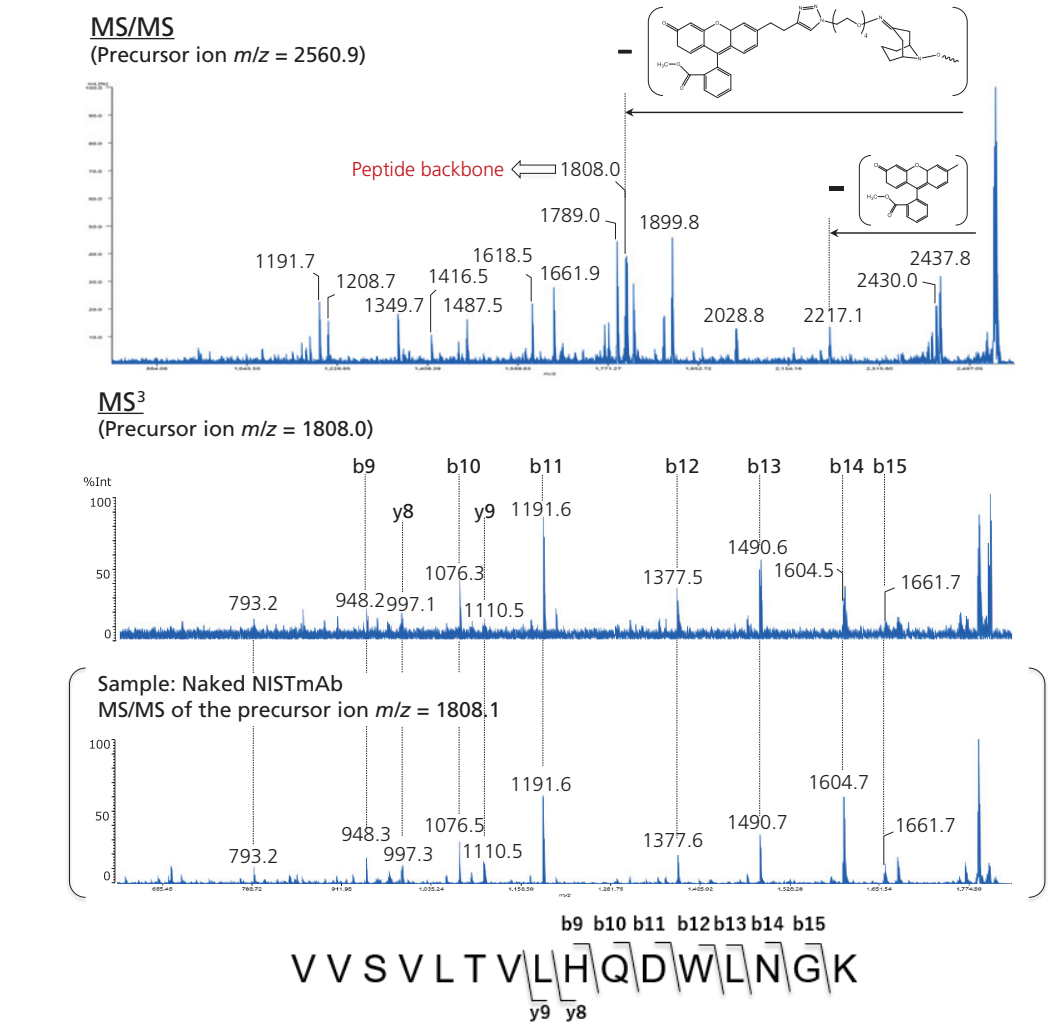

Figure 5 MSⁿ spectra of *m/z* 2560.9

Amino acid sequence of the Heavy chain

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKNQVVLK
VTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

ME-fluorescein-ABNO
ME-fluorescein-ABNO

Figure 6 Amino acid sequence of the heavy chain and position of the conjugation sides

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Conclusions

We analyzed an Tryptophan-Conjugated ADC mimic using the miniature MALDI-DIT-MS focusing on position and number of the conjugation sites. MS^n function of the miniature MALDI-DIT-MS could be a useful tool for quality assessment of next generation ADC with tryptophan conjugated.

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