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Application News

No. B86

MALDI-TOF Mass Spectrometry

Simplified Mass Measurement of Chemically-Modified Antibodies: Determination of the Presence of the Number of Modifications Using a Linear Benchtop MALDI-TOF MS

Antibody drug conjugates (ADC), a type of pharmaceutical composed of an antibody bound to a drug, appeared in the 2000s with the expectation they would serve as more effective anti-cancer drugs than previous small-molecule pharmaceuticals through the combination of the antibody's high selectivity and the availability of a small-molecule drug. With a small number of products already available in the marketplace, the degree to which and where binding occurs are important characteristics to determine quality when compounds are artificially bound to proteins, as in the case of ADCs.

This article introduces an example of analyzing the pseudo ADC, which was created by artificially binding low-molecular compounds to a standard research antibody, using a benchtop MALDI-TOF MS.

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Mass Spectrometry of a Low-Molecular Compound-Modified Antibody in Non-Reduced Form

Using the method of Seki et al. [1], a standard antibody modified with Me-fluorescein-ABNO on the tryptophan residue (Fig. 1, NISTmab, humanized IgG κ monoclonal antibody, RM8671) and an untreated standard antibody (0.5 µL each) were respectively mixed with an equal volume of matrix solution (10 mg/mL sinapinic acid in 50 % acetonitrile, and 0.1 % trifluoroacetic acid), and then deposited on the MALDI target plate to undergo mass spectrometric analysis. The "MALDI-8020" benchtop MALDI-TOF MS (Fig. 2) was used for analysis.

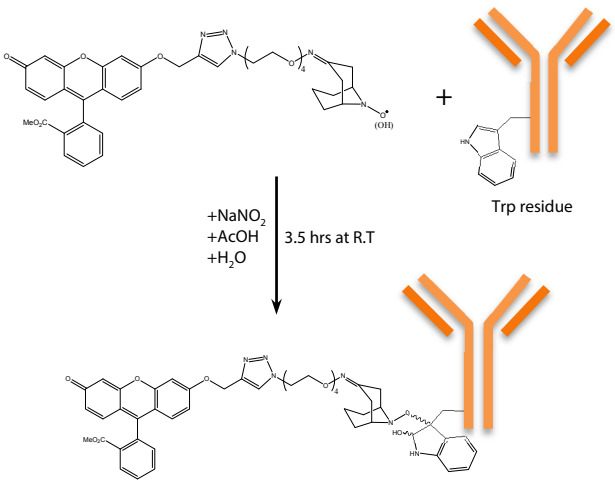

Fig. 1 Me-fluorescein-ABNO Modification of Antibody

Fig. 2 Appearance of "MALDI-8020" Benchtop MALDI-TOF MS

Fig. 3 shows a comparison of the MS spectra of the modified antibody and untreated antibody in non-reduced form. A signal indicating mass differences of about three modifier groups was detected for the modified antibody compared to the untreated antibody.

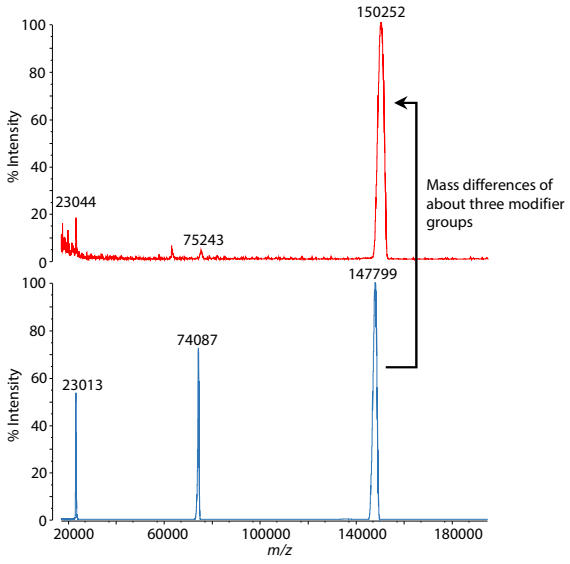

Fig. 3 Mass Spectra of Modified Antibody (top) and Untreated Antibody (bottum) Antibody

Mass Spectrometry of Antibodies After Reduction Treatment

50 mM DTT aqueous solution (1 µL) was added to both the modified antibody and untreated antibody (4 µL each) and then reduction treatment was performed for one hour at 57 °C. Each reaction solution (0.5 µL) was then deposited on the MALDI target plate and overlaid with 0.5 µL of matrix solution (10 mg/mL sinapinic acid in 50 % acetonitrile, and 0.1 % trifluoroacetic acid), to undergo mass spectrometric analysis.

The results obtained (Fig. 4) did not indicate any change in the modified antibody or untreated antibody with respect to the light chains (*m/z* ~23,160). However, with respect to the heavy chains (*m/z* ~51,000 & ~52,000) a signal indicating a mass shift of one modifier group and two modifier groups was detected for the modified antibody compared to the signal from the untreated antibody.

The results shown in Fig. 3 & 4 demonstrate that for the modified antibody used in this experiment: (1) addition of three Me-fluorescein-ABNO groups was most prolific, (ii) chemical modification occurred only on the antibody's heavy chains, and furthermore, (iii) the modification occurred only at one site on one heavy chain and two sites on the other (Fig. 5).

While there were a total of 22 tryptophan residues where Me-fluorescein-ABNO could potentially bind on the standard antibody used in this experiment, molecules of the antibody with only thee modified tryptophan residues were the most abundantly detected species. This is probably due to the steric configuration of the antibody restricting access of Me-fluorescein-ABNO.

A key feature of the "MALDI-8020" benchtop MALDI-TOF MS is the rapid sample introduction, allowing users to start their analysis a few minutes after sample introduction, leading to increased productivity. We have demonstrated how, using the "MALDI-8020", it is possible to rapidly and conveniently determine the degree of small-molecule modification using a modified monoclonal antibody as an example.

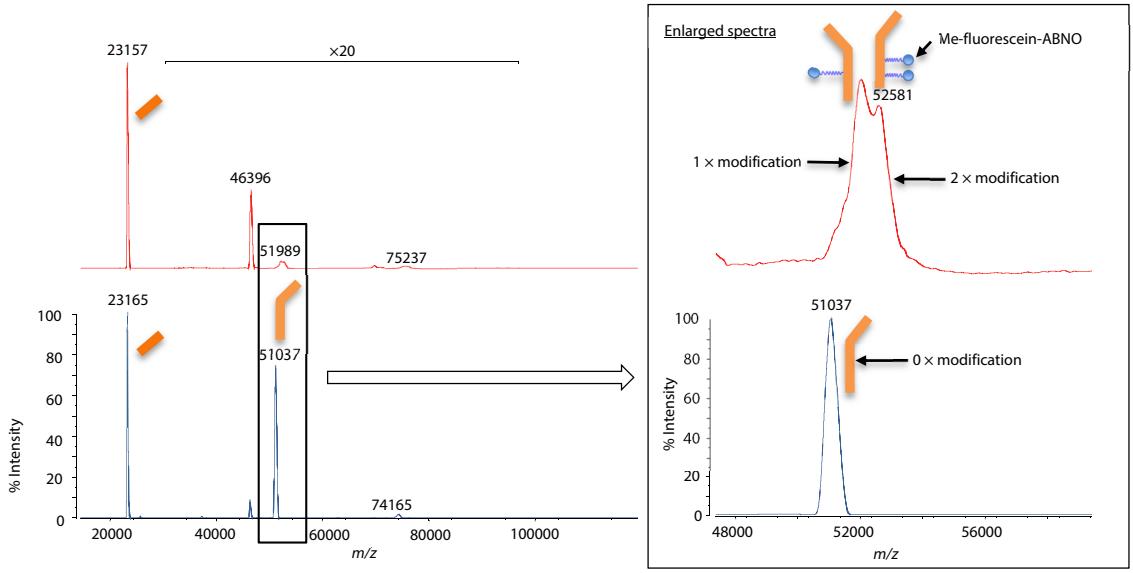

Fig. 4 Mass Spectra of Modified Antibody (top) and Untreated Antibody (bottum) After Reduction Treatment

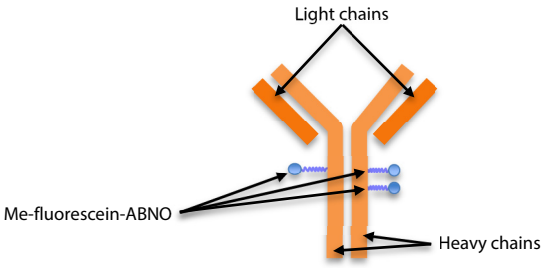

Fig. 5 Diagram of Modified Antibody Presumed to Be the Major Component in This Analysis

References
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

Acknowledgments
We would like to thank the Synthetic Organic Chemistry Laboratory at the Graduate School of Pharmaceutical Sciences, University of Tokyo, for preparing the antibody modified with Me-fluorescein-ABNO.

First Edition: Mar. 2019

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