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Application News

MALDI-QIT-TOF Mass Spectrometry No.B11

MALDI-MS^n Analysis Method to Assign Regioisomeric Structures of Multi-Modified Cyclodextrins

Cyclodextrins (hereafter, CyD) are cyclic oligosaccharides made up of D-glucose units linked α 1-4, and depending on the number of glucose units, are named α (6 glucose units), β (7 glucose units), or γ (8 glucose units)-CyD. CyD is shaped like a doughnut with a hollowed out center, endowing this molecule with the characteristic that allows hydrophobic substances (guest molecules) that do not usually dissolve in aqueous solution to be "hosted" in this hollow, and thereby dissolved. Owing to this characteristic, the commercial use of cyclodextrins has exploded; with applications in food, pharmaceutical, agricultural and chemical industries.

However, molecules that can become "guests" and the solvents that can be dissolved are limited to only the naturally occurring CyDs. Accordingly, various types of modifications have been applied to CyDs, and on-going research is expanding their application range.
A modern challenge is determining where among the multiple glucoses in the structure the modification should be targeted, and how to verify the modification site of an artificially synthesized modified CyD.
For instance, in a β-CyD consisting of 7 glucoses, if a modification is to be made at any of the OH groups in position 6 of glucose, 1 - 7 different modified CyDs could exist based on the number of modification positions. Furthermore, if 2 - 5 modifications are introduced simultaneously, positional isomerism will occur, resulting in the existence of 19 types of modified CyD.

The synthesis and purification of this type of modified CyD are difficult, but determining the positional isomer of the successfully synthesized molecule as well as its identification are also difficult tasks.
Up to now, NMR has often been used to conduct structural analysis to determine the structures of these molecules, but this requires a high degree of refinement and a large quantity of synthesized molecules, making it unsuitable for small scale screening of synthesized molecules.
In this article, we introduce a simple, fast technique for identifying positional isomers of modified CyDs even for small amounts of synthesized molecules.

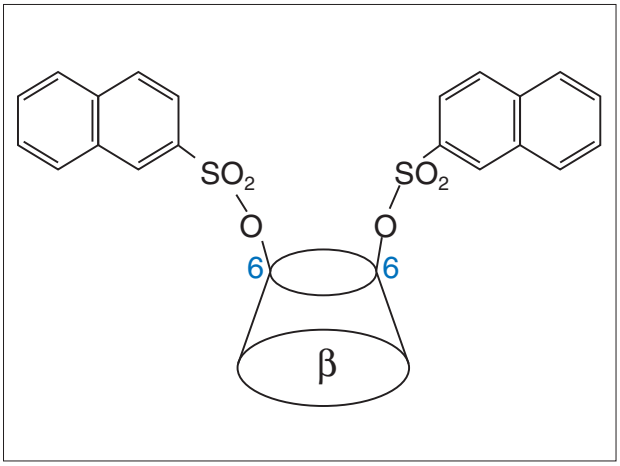


Fig.1 Structural Model of the 6-O-2-naphthalenesulfonyl-β-CyD

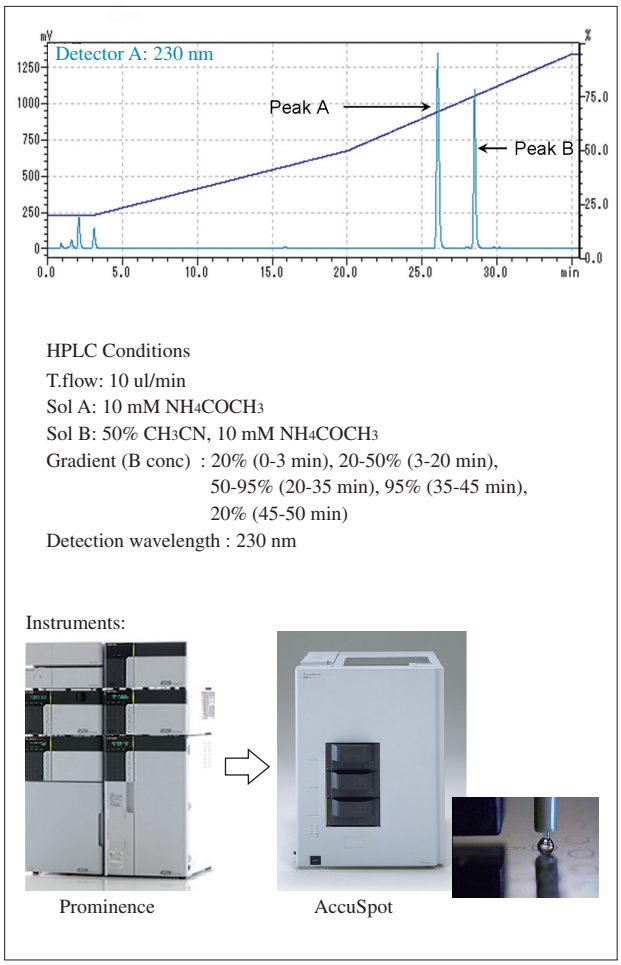


Fig.2 HPLC Chromatogram of 6-O-2-naphthalenesulfonyl-β-CyDs

For the sample, we used a molecule in which two out of the seven OH groups at position 6 in the 7-glucose β-CyD were replaced with sulfonyl groups (6-O-2-naphthalenesulfonyl) (Fig. 1).
Three types of positional isomers exist in this molecule, an AB form, an AC form, and an AD form of isomer.
The two isomers of 6-O-2-naphthalenesulfonyl-β-CyD in the chemically synthesized mixture were separated using a Prominence high-performance liquid chromatograph, and the online-connected AccuSpot MALDI plate spotting system was used to automatically dispense samples and matrix solution (2,5-dihydroxybenzoic acid) on the MALDI plate (Fig. 2).

MS analysis using the AXIMA-QIT MALDI-QIT-TOF MS was conducted on the wells corresponding to the elution positions of the two chromatogram peaks (A, B) separated by the Prominence from among the multiple sample spot wells on the MALDI plate, and the Cationaized molecule ($[M+Li]^+$ *m/z* 1521.57) that originated from the sample was detected (Fig. 3).
It is expected from the value of the molecular ion detected in MS analysis that the original compound has two modification groups in the molecule, and we conducted MS^2 analysis in order to confirm this. Here, desorption of the position-6 sulfonyl group in the ion trap and the formation of an ethyl cross-link between position 6 and position 3 suggested that a fragment ion was detected (Fig. 4).

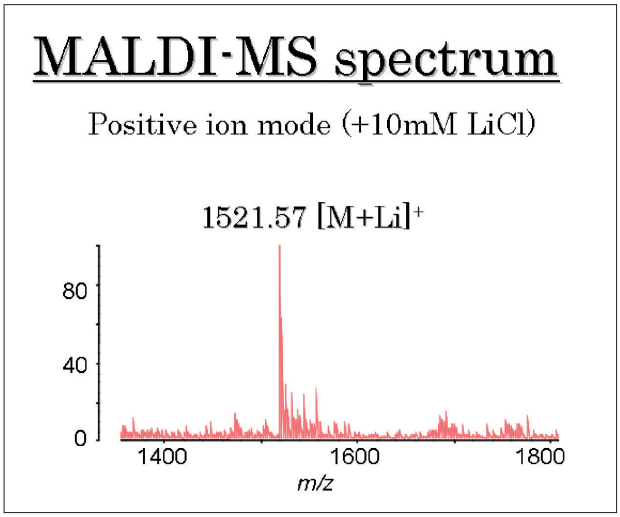


Fig.3 Mass spectrum of Modification 6-O-2-naphthalenesulfonyl-β-CyD (Matrix: 10 mg/mL DHB in HPLC grade water)

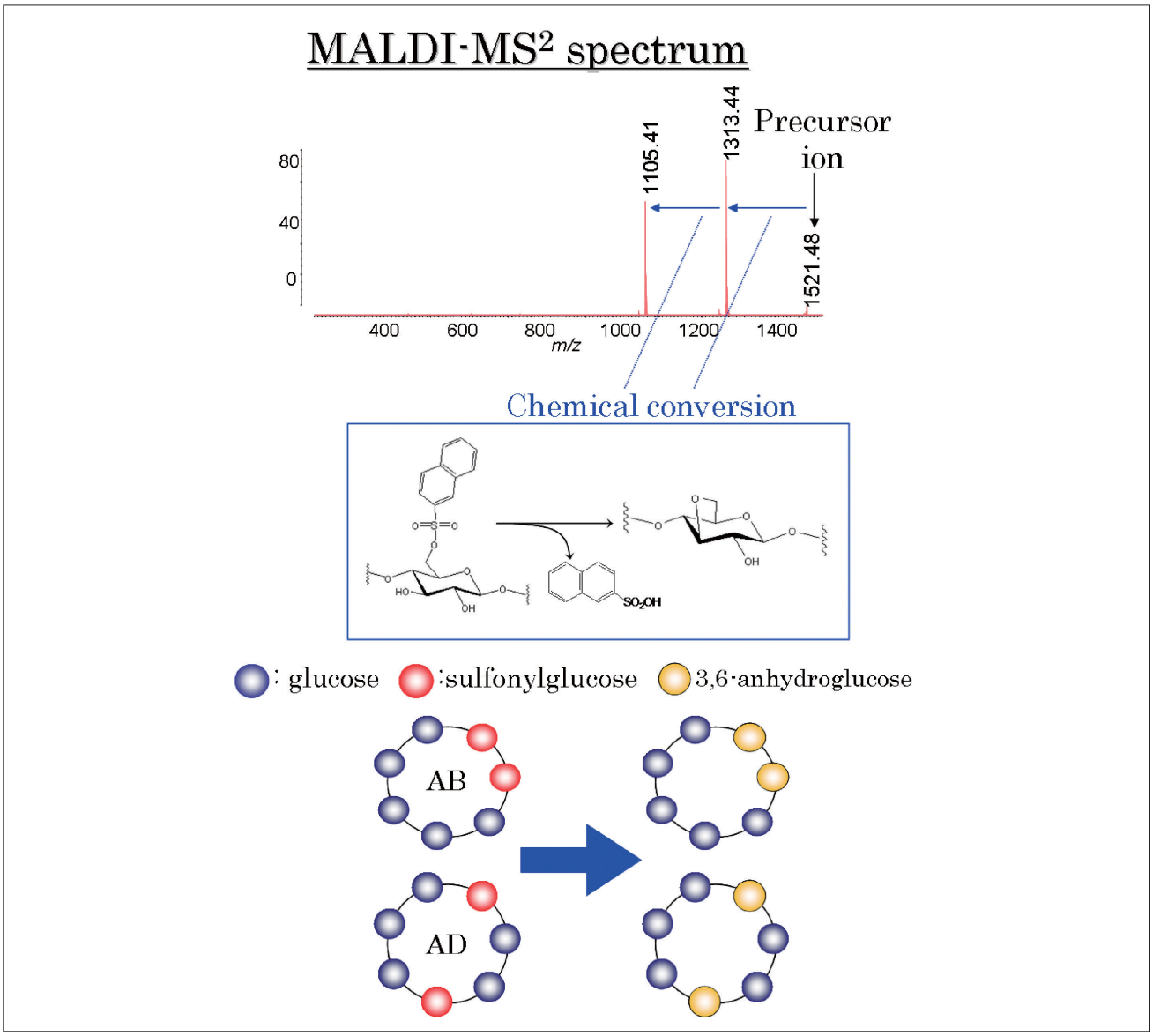


Fig.4 MS^2 Spectrum of Modification 6-O-2-naphthalenesulfonyl-β-CyD

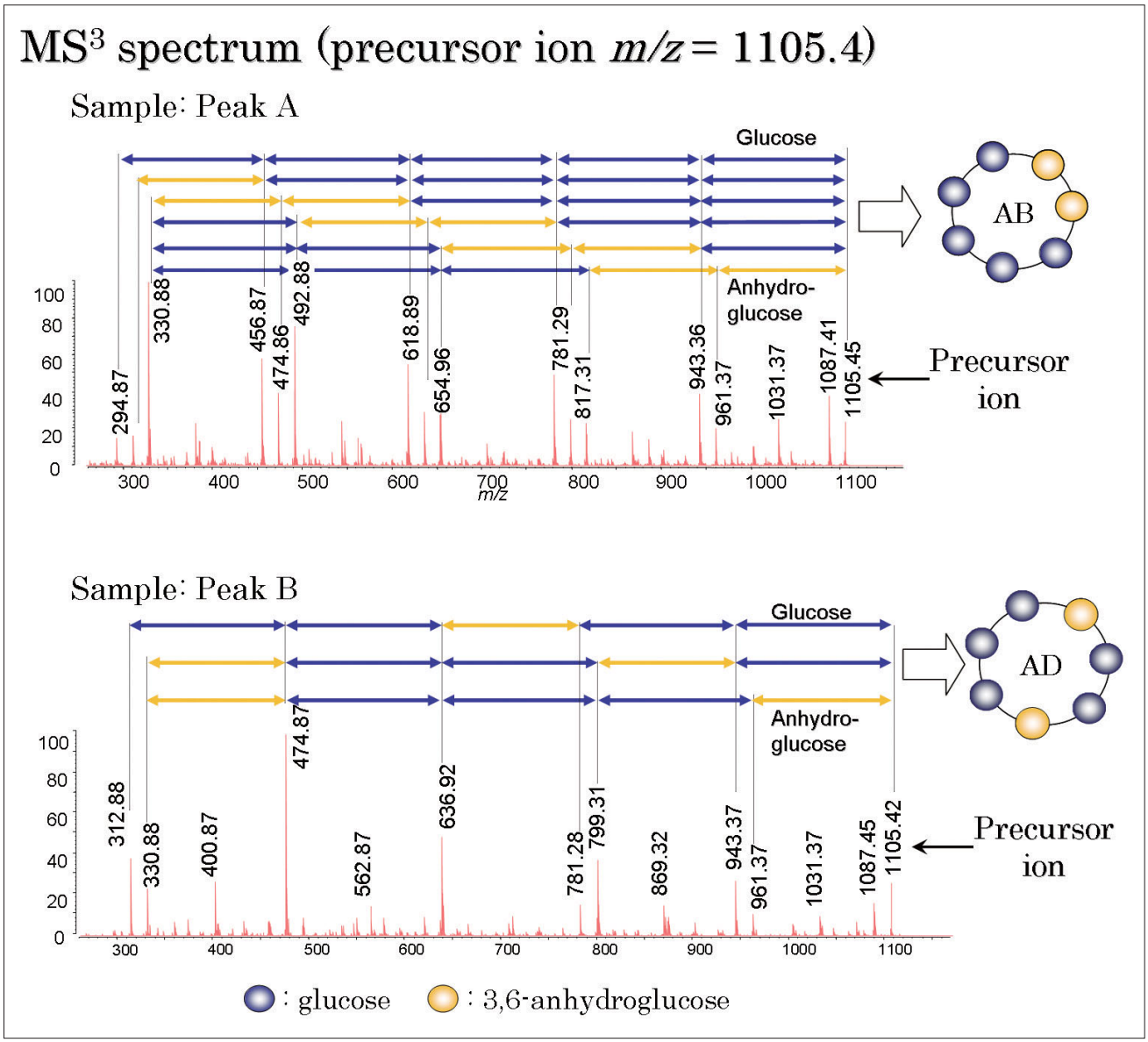


Fig. 5 Identification of the Positional Isomer Using Fragment Ion Assignment in MS³ Spectra of Modification 6-O-2-naphthalenesulfonyl-β-CyD

The results of MS and MS² analysis confirmed that the modified CyD used as the sample had indeed undergone modification with replacement of two position-6 OH groups with sulfonyl groups. Next, among the fragment ions detected in MS² analysis, we conducted MS³ analysis of the fragment ion (*m/z* 1105.4) that had lost all of its modification groups (Fig. 5).

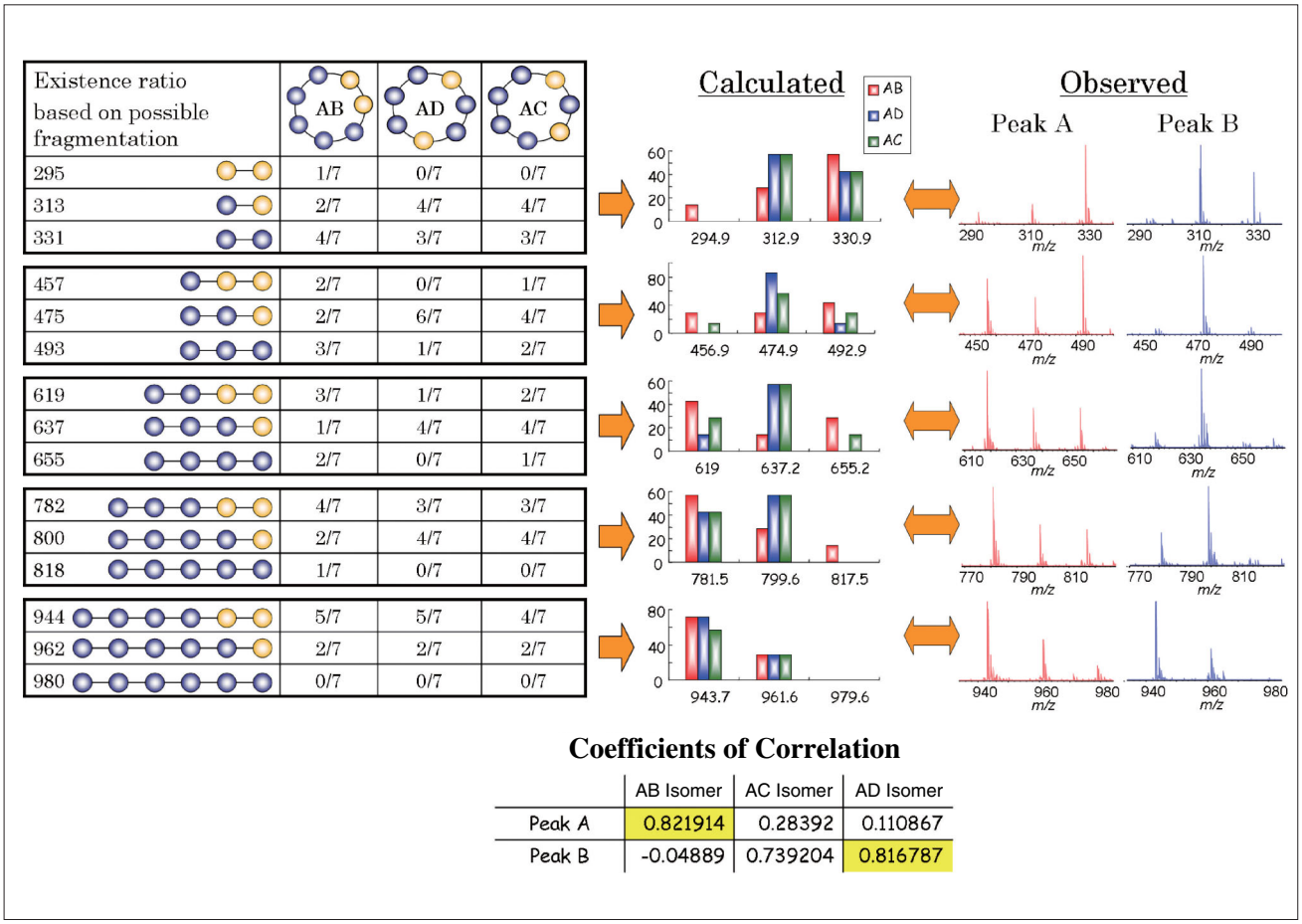
As for the fragment ions that lost all of their modification groups, since an ethyl cross-link is formed between position 6 and position 3 simultaneous with the loss of the modification group, as mentioned above, the mass of the glucose unit in the non-modified glucose of 162 (integer) changes to 144 (integer). By reading this change from the MS³ spectrum, in many cases it becomes possible to identify the position of the modified glucose. In this analysis, by assigning the fragment ion obtained in MS³ analysis, we were able to verify that the chromatogram peaks A and B were the AB and AD isomers, respectively.

Correct assignment of all corresponding fragmentation in MS³ analysis can be a very tedious assignment. Here we present one method of conducting positional isomer identification of modified CyDs without conducting detailed ion assignment (Fig. 6)[1].

The number of glucose units and modification groups in a synthesized modified CyD can be determined from the *m/z* values of molecular ions generated in MS analysis and from the number of fragment ions obtained in MS² analysis (in this analysis, 7 glucose units and 2 sulfonyl groups).

Fig. 6 shows possible combinations of glucose units and the calculated/observed existence ratio of the ions that are obtained in MS³ analysis. (In this analysis, since the ion is detected as $[M+Li]^+$, the mass becomes that of the combined glucose units plus the mass of Li.) In this way, the obtained theoretical existence ratio and mass are compared with the actual mass and ion intensity obtained in MS³ analysis, and by calculating their correlation coefficients, the modification CyD positional isomers can be identified.

By using a combination of HPLC and Multiple-stage mass spectrometry (MS^n) as described here, it becomes possible to identify positional isomers even if only a small amount of synthetic modified CyD is available.



Coefficients of Correlation

	AB Isomer	AC Isomer	AD Isomer
Peak A	0.821914	0.28392	0.110867
Peak B	-0.04889	0.739204	0.816787

Fig.6 Identification of the Positional Isomer Using Fragment Ion Existence Ratio in MS^3 Spectra of 6-O-2-naphthalenesulfonyl-β-CyD

[Reference Material]
1) S. Sforza et.al. *J. Am. Chem. Soc.* 2003, 14, 124-135.

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