Chiral Recognition in the Inclusion Complex of Cyclodextrins with N-Dansyl-Phenylalanine Observed by Linked Scan FAB-MS Spectrometry

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The cyclodextrin(CD) inclusion complex showed a chiral recognition observed by linked scan FAB-MS. The ratio, R, of the daughter ionic peak strength to the precursor ionic peak strength using the linked scan FAB-MS method was suggested to express a parameter for the stability of CD inclusion complex. The ratios between R_D and R_L were determined in inclusion complexes consisted of D-and L-*N*-dansyl-phenylalanines with various CDs. As for this parameter R derived from the linked scan FAB-MS ionic peaks, the wide application is possible to the inclusion complexes and the evaluation of the chiral recognition in various inclusion complexes.

Introduction

In recent years the characterization of molecular complexes that form with weak interactions using mass spectrometry has been paid much attention, such complexes include the protein-sugar,[1] crown ether-amino acid,[2] and cyclodextrin-drug.[3] Ohashi *et al.*[4] successfully observed the molecular ions of the complex between β-CD and nileprost, a derivative of prostacyclin, in both the positive and negative ion modes of FAB. The complex formed between β-CD and β-blocker can also be seen in the positive-ion FAB mode. They discovered that the complexation suppressed the reaction process of reduction or hydrolysis that occurred under FAB conditions. Sorokine *et al.*[5] reported the characterization of polymethylated CD (PMCD) inclusion complexes such as hydrocortisone-PMCD and phenylalanine-PMCD in the presence of ammonium ion by ESI-MS. Sawada *et al.*[6] reported the chiral recognition of enantiomeric alkyl ammonium ions for the first time by detection of the molecular ion peak of the host-guest complex in FAB-MS. Recently Selva's group reported CD complexes[7]. These findings may establish mass spectrometry as a tool for investigating host-guest interactions in biomolecular systems. However, while there are various and delicate experimental conditions in preparing the samples, the detection of such complexes in satisfied reproducibility by mass spectrometry does occur. We report here that new use of linked scan FAB-MS method for CD complexes can characterize the CD complex clearly and the evaluation method of chiral recognition.

Experimental

The host cyclodextrins used in this study included α-, β-, and γ-cyclodextrins and also mono

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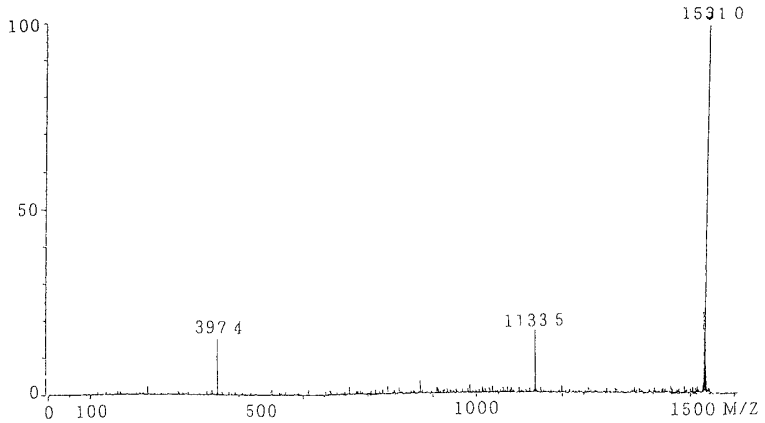


Fig. 1 Detection of the inclusion complexed ion by FAB-MS spectorometry
FAB-MS conditions; negative mode, molar ratio of β-CD: Dns-D-Phenylalanine=1 : 4, Matrix; diethanolamine: DMSO=2 : 5(v/v), Accelaration voltage; 8 kV, Scan mass range; 0-1700, Scan speed; 30.0 sec, Gun high volt; 2 kV, Emission current; 5 mA, FAB gun gas; Xenon, MS system; JEOL-JMS-SX-102 A

-6-deoxy-6-aminocyclodextrin (ACD).[8] The evaluation of chiral recognition was detected using enatiomeric D- and L-*N*-dansyl-phenylalanines (Dns-Phe). The usual negative ion FAB mass spectrum of the complex, measured using a double focused mass spectrometer (JEOL SX-102A) with diethanolamine as the matrix. Linked scan FAB-MS analyses were submitted under the following conditions; A stock solution consisted of 20 μl of 2.64 (10^{-2} M β-CD in DMSO, 20 μl of 1.06×10^{-1} M Dns-Phe in DMSO in a molar ratio of 1 : 4 and 8 μl of diethanolamine were prepared. For the FAB-MS measurement, 3 μl of the stock solution was put on the FAB target using a micro-injector. A FAB gun gas using xenon with an acceleration voltage of 8 kV with negative mode and Helium was used as the collision gas in a JEOL SX-102A instrument.

Results and Discussion

The intense peak corresponding to complex $[\beta\text{-CD}+\text{Dns-Phe}-\text{H}]^-$ was observed at m/z 1531.6. The peaks of β-CD at m/z 1133.5, Dns-Phe at m/z 397.4 and Dns-Phe dimer at m/z 796.4 were also observed as shown in Fig. 1.

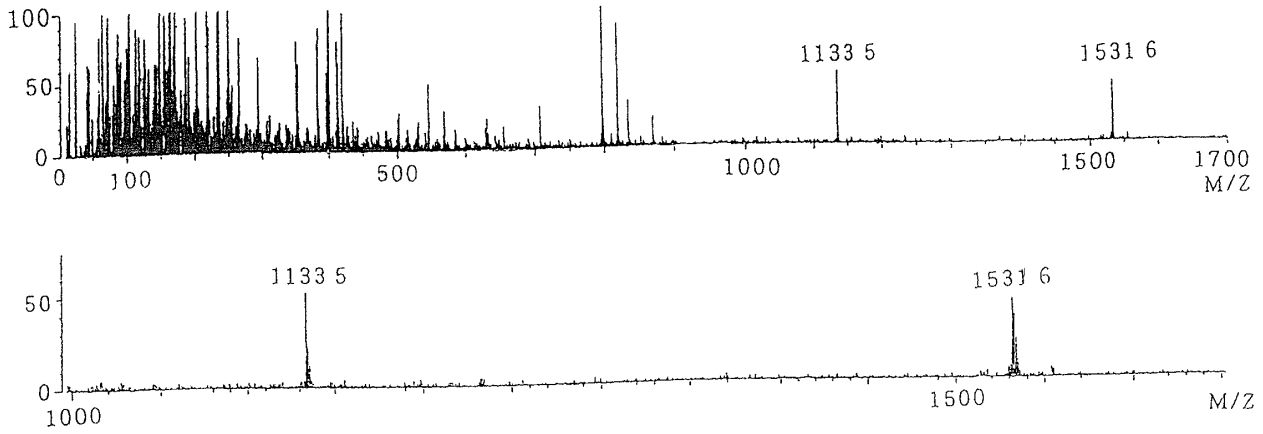
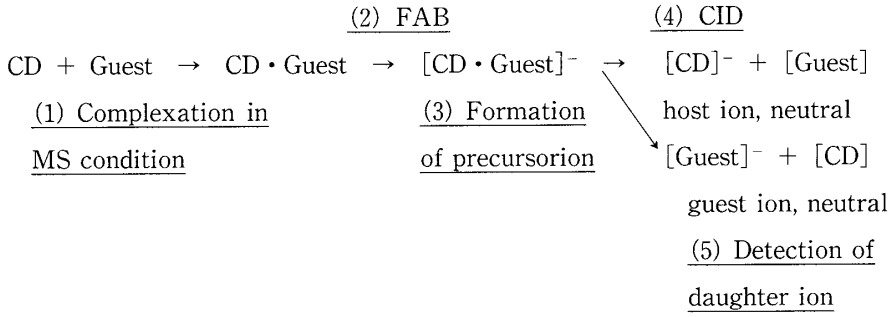


Fig. 2 Detection of the precursor ion peak and daughter ion peak by linked scan-FAB-MS spectrometry
Linked scan conditions; B/E scan, Collision gas of CA chamber side; Helium, all other conditions were the same as shown in Fig. 1.

However, the peak intensity of fragment ions was detected with the strength overlapped both from the starting material and the dissociated component of the complex. The net ion amount generated only from the complex can not be correctly determined. Therefore, by using the linked -scan method in FAB-MS, the ratio of precursor ion peak strength toward the daughter ion peak strength was adopted to express the stability of the complex. The result of linked scan FAB-MS of the complex between β-CD and Dns-Phe is shown in Fig. 2.

Daughter ions of Dns-Phe and β-CD that were disassembled from the precursor ion of the complex were detected in very excellent sensitivity. This process is shown in Scheme 1.



Scheme 1 The processes (1)~(5) of linked scan FAB-MS to observe the relative ratio of the ionic peak strength, R, as a parameter of complex stability

The value R, a relative ratio of ionic peak strength, is defined as the sum of the peak strength for two kinds of daughter ions as the denominator, and the peak strength of the precursor complex ion as the numerator as shown in the following equation;

$$R=\frac{\Sigma\text{precursor ionic peak strength}}{\Sigma\text{daughter ionic peak strength}}$$

In the present case,

$$R=[CD \cdot Guest]^-/([CD]^-+[Guest]^-)$$

The present adopted parameter R should show the stability type for the complex. The parameter R_D/R_L can be suggested as a chiral recognition when R_D and R_L were separately measured for the D, L-guests. The results are summarized in Table 1.

There were not large changes in the relative ratio of ionic peak strength R when the β-CD (host)-Dns-Phe (guest) molar ratio was changed from 0.30 to 4.0. The constant R_D and R_L values were obtained near 0.70. However, R was influenced by the amount of the matrix, the flow rate of the collision gas and the concentration of the complexed components. Under the same measurement

Table 1 Chiral recognition evaluated by the relative ratio of ionic peak strength R observed by linked scan FAB-MS

Guest	Host				
	α-CD	β-CD	γ-CD	ACD	maltoheptaose
Dns-D-Phe	2.852	1.986	3.720	3.949	1.764
Dns-L-Phe	6.983	2.730	3.501	7.599	1.697
R_D/R_L	0.408	0.727	1.059	0.512	1.039

$R=[complex]^-/([Guest]^-+[Host]^-)$, which is defined also in the experimental section. Linked scan conditions were shown in the caption of Fig. 1 and Fig. 2.

conditions, R_D and R_L, the ratio for the enatiomeric Dns-Phe in the presence of α-, β-, and γ-CD and ACD were determined as summarized in Table 1. The ratio R_D/R_L becomes larger in the order of α-, β- and γ-CD from 0.408 to 1.06. A distinct chiral recognition is not observed in the case of the γ-CD host ($R_D/R_L=1.06$). This means that the different size of each cavity should be related to the difference in the chiral recognition capability. ACD that has a single amino group on the C-6 position showed a slightly higher L-selectivity ($R_D/R_L=0.512$) than β-CD itself ($R_D/R_L=0.727$).

It was examined whether the precursor ion was essentially the product of chemical reaction with covalent bonding or simply the inclusion compound due to hydrogen bonding and van der Waals interaction. Evidences for the inclusion complex of the precursor were observed as follows; (1) The differences in R_D/R_L among α-, β-, γ-CD were found out. Therefore, the size of a cavity is sharply reflected on the inclusion complexation. (2) When used maltoheptose, ring-opened straight oligosaccharide chain, weak and practically equal values of R_D and R_L ($R_D/R_L=1.04$) were observed. (3) CD in vacuum state is estimated in the distorted conformation and at the higher potential energy. Therefore, the stabilization by inclusion is probable. (4) The product ion can be stabilized by a capsule effect in a host. The formation of the inclusion complex in the present case is supported by these reasons. On the other hand, as for the explanation for the product due to covalent bond, the ionization energy of FAB-MS is very large compared to the EI-MS method. The inclusion complex seems to be too unstable to exist in the FAB-MS measurement. However, there is no evidence to support any reaction product in this research.

Conclusions

The ratio, R, of the sum of the daughter ionic peak strength to the sum of the precursor ionic peak strength was suggested to express the stability of the CD inclusion complex using the linked-scan FAB-MS method. The ratios between R_D and R_L were determined using the inclusion complexes between various CDs host and the D-, L-Dns-Phe guests. The CD inclusion complex showed a chiral recognition observed by linked-scan FAB-MS. As for this parameter R derived using the linked-scan FAB-MS ionic peaks, the application is possible to the inclusion complexes widely and the evaluation of the chiral recognition in inclusion complexes.

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