

Structural Elucidation of Glycosphingolipids by Collision-Induced Dissociation of Sodium Ion Complex

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Collision-induced dissociation (CID) spectra of sodium ion complexes ($[M+Na]^+$ ions) produced by FAB-MS of glycosphingolipids indicate the length of the fatty acyl chain of ceramide moieties without chemical degradation.

Key words glycosphingolipid; FAB-MS; sodium complex; collision-induced dissociation

To obtain useful information on the structure of minute amounts of natural sphingolipids, a mass spectrometric technique has been developed in our laboratory.^{1–3} The effectiveness of a matrix system comprised of an alkali metal ion on the FAB-MS for the molecular weight

determination of the labile oligoglycosides,⁴ as well as the usefulness of FAB combined with the CID method for the analysis of the metal complexes that can not be isolated from solution,^{5,6} has also been established in our previous studies. In this paper, we report the usefulness

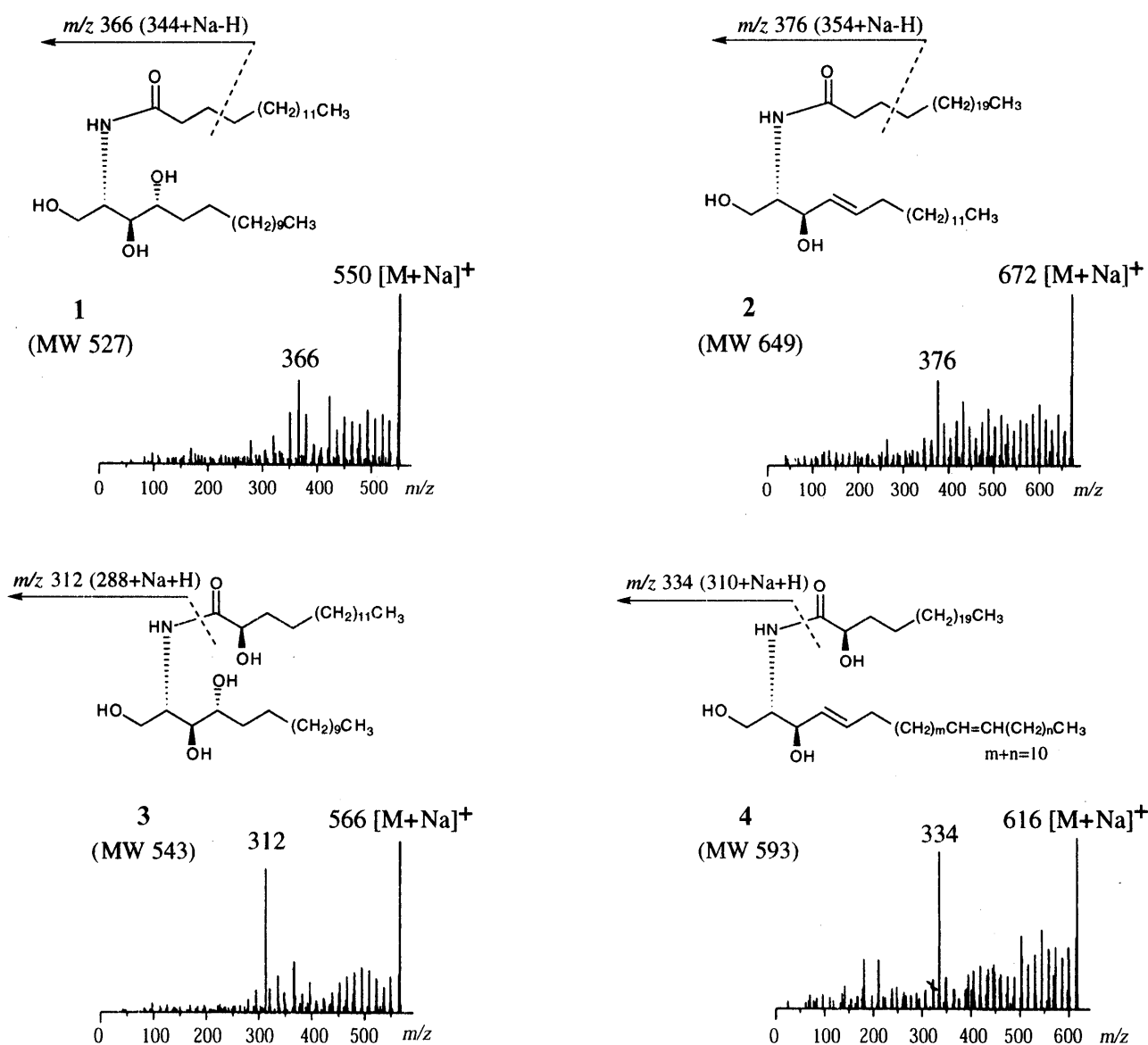


Fig. 1. CID Spectra of $[M+Na]^+$ Ions Obtained in the Positive Ion FAB-MS of Ceramides 1, 2, 3, and 4

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of the CID of the $[M+Na]^+$ ions derived from positive ion FAB-MS for the structural study of glycosphingolipids.

Results and Discussion

The CID spectra of $[M+Na]^+$ ions obtained in the positive ion FAB-MS of the ceramides⁷⁾ **1**, **2**, **3** and **4** were

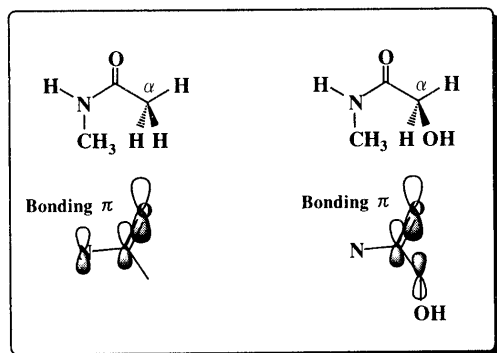


Fig. 2

investigated as a preliminary study (Fig. 1). In the spectra of **1** and **2** possessing non-hydroxylated fatty acids, ions due to the charge remote fragmentations of the fatty acyl chain, e.g., the ions at m/z 366 in **1** and at m/z 376 in **2**, were observed. On the other hand, intense and characteristic fragment ions due to fission of the amide bond were detected in the spectra of **3** and **4** having α -hydroxy fatty acids, namely m/z 312 in **3** and at m/z 334 in **4**. The fragment ions were analogous to those proposed by Adams.⁸⁻¹¹⁾ These phenomena observed in **3** and **4** can

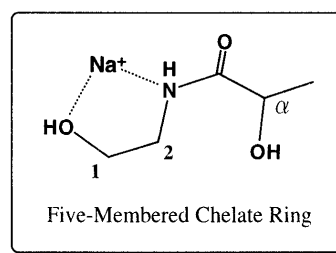


Fig. 3

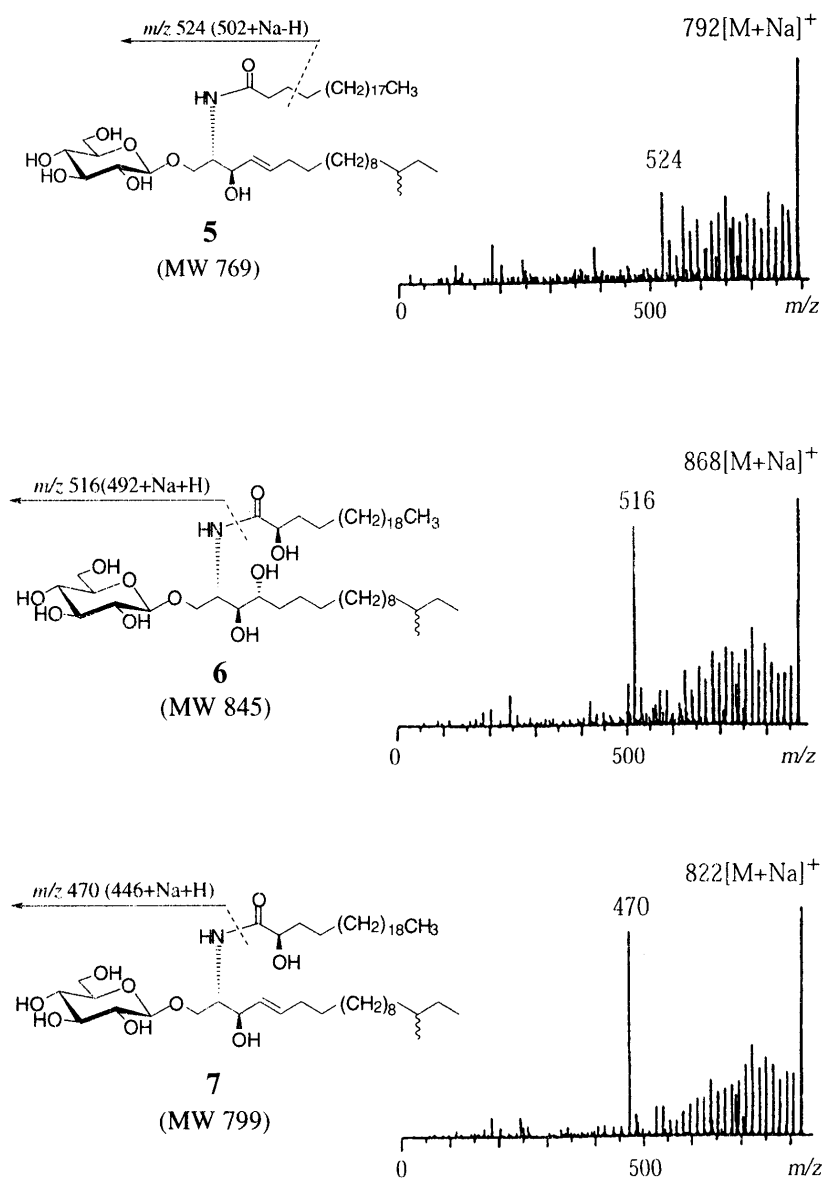


Fig. 4. CID Spectra of $[M+Na]^+$ Ions Obtained in the Positive Ion FAB-MS of Cerebrosides **5**, **6**, and **7**

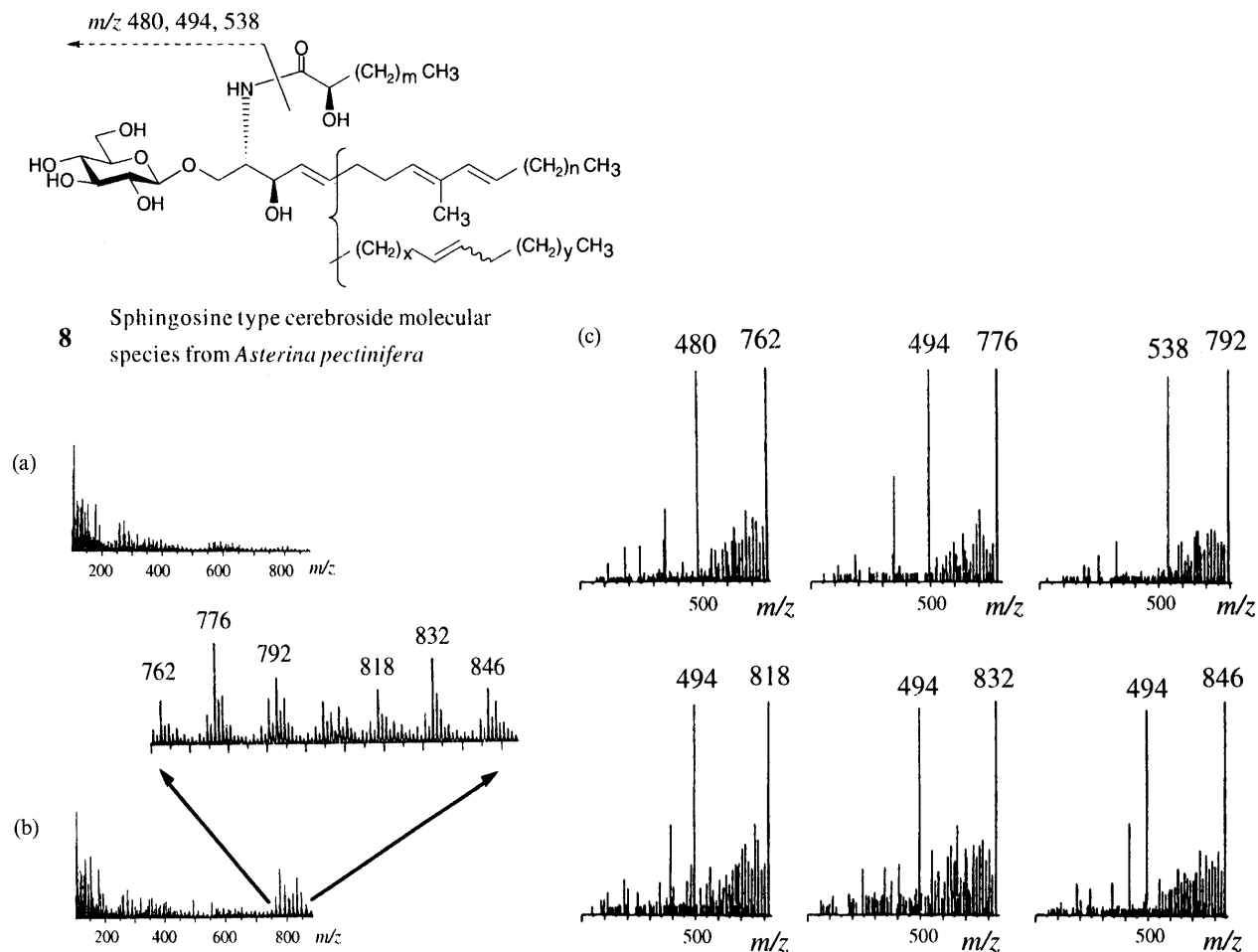


Fig. 5. Positive Ion FAB Mass Spectra of **8** with (a) *m*-NBA and (b) NaCl/*m*-NBA as a Matrix, (c) CID Spectra of $[M+Na]^+$ Ions Obtained in the Positive Ion FAB-MS of the Main Constituents of **8**

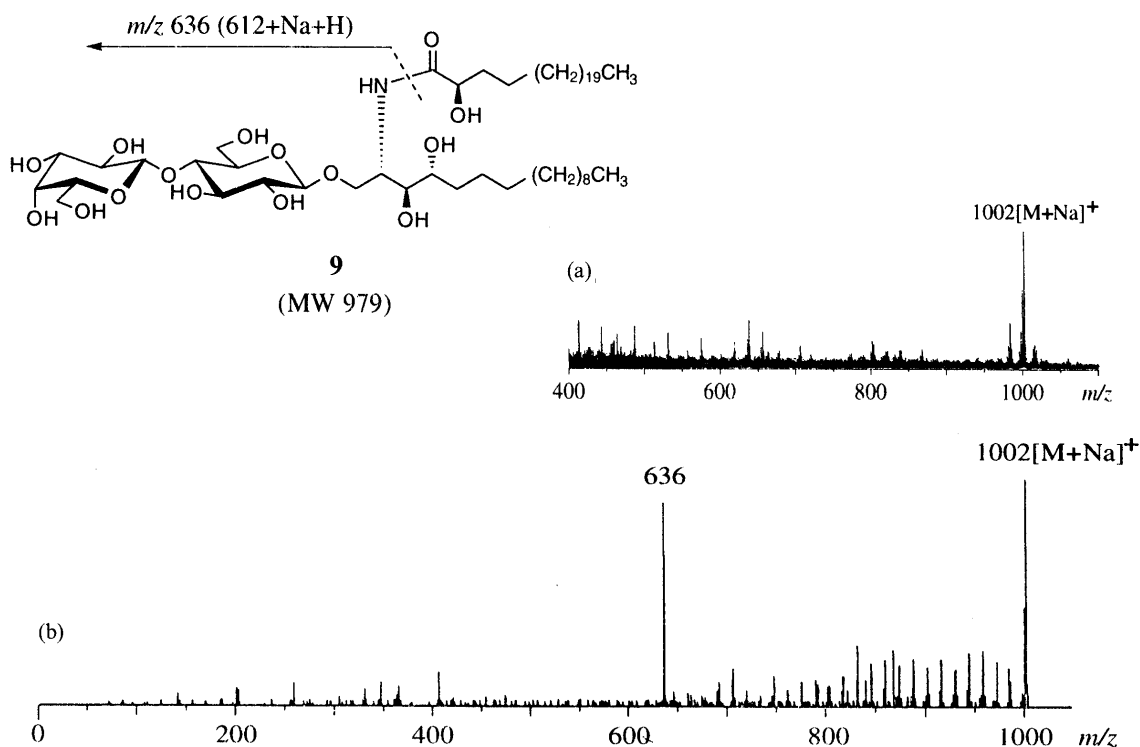


Fig. 6. (a) Positive Ion FAB Mass Spectrum of **9** and (b) CID Mass Spectrum of $[M+Na]^+$ Ion Obtained in the Positive FAB-MS of **9**

be interpreted as follows: When a hydroxy group was introduced at the α position of the amide carbonyl, the double-bond character of the N(amide)-C(amide) bond is expected to decrease; this is supported by an extended Hückel molecular orbital (EHMO) calculation (Fig. 2).¹²⁾ Thus the amide nitrogen was presumed to possess sp^3 -like character rather than sp^2 as would usually be expected in a normal amide group, and therefore we considered that the sp^3 -nitrogen could be coordinated to the Na^+ ion of the stable five-membered chelate ring as shown in Fig. 3. Consequently the N(amide)-C(amide) bond of **3** and **4** was thought to be cleaved easily. It can generally be accepted that the preferred sites for the metal ion complexation of ceramides are on the oxygen of the amide and/or hydroxy group.¹¹⁾ Also, the amide nitrogen does not usually coordinate to a metal ion as long as an amide proton is attached to the nitrogen. However, in order to interpret the fission of the amide bond, we considered that the coordination between the amide nitrogen and Na^+ ion should have occurred.

Next, the application of this method to the structural elucidation of the ceramide moieties of cerebrosides¹³⁾ was attempted (Fig. 4). Ions due to the charge remote fragmentation of the fatty acyl chain, e.g., the ion at m/z 524, were observed in the CID spectrum of the $[\text{M} + \text{Na}]^+$ ion of compound **5** possessing the non-hydroxylated fatty acid. On the other hand, in the CID spectra of the $[\text{M} + \text{Na}]^+$ ions of compounds **6** and **7** having α -hydroxy fatty acids, we observed prominent fragment ions at m/z 516 in **6** and at m/z 470 in **7** that originated from the cleavage of the amide bond. These results indicated that the CID of the $[\text{M} + \text{Na}]^+$ ions of the cerebrosides can provide useful information about the structures of the ceramide parts without chemical degradation.

The above-mentioned FAB combined with the CID method was applied to the characterization of a cerebroside molecular species **8**¹⁴⁾ composed of α -hydroxy fatty acids and sphingosines. As shown in Fig. 5, the molecular mass and the fatty acid and long-chain base compositions of the main constituents of the molecular species **8** were estimated without acid hydrolysis.

Furthermore, the method was proved to be applicable to the structural elucidation of lactosylceramide, namely, the characteristic fragment ion at m/z 636 due to fission of the amide bond was clearly detected in the CID spectrum of the $[\text{M} + \text{Na}]^+$ ion obtained in the positive ion FAB-MS of compound **9**¹⁵⁾ (Fig. 6). In ongoing studies, we are finding that our method can be applied to a wide range of natural glycosphingolipids.¹⁶⁾

The present results further emphasize the utility of the FAB combined with the CID method for the structure elucidation of sphingolipids.

Experimental

The conditions for the measurements of mass spectra were as follows: All mass spectra were acquired with a JMS-SX/SX102A four sector type tandem mass spectrometer (JEOL Ltd., Tokyo) of BE/BE geometry. The FAB mass spectra were obtained by using only the first spectrometer. A methanol/chloroform (1:1, v/v) solution of the sample was prepared at a concentration of ca. 10 $\mu\text{g}/\mu\text{l}$, and the solution (1 μl) was mixed with 1 μl of *m*-nitrobenzyl alcohol (*m*-NBA) saturated with NaCl on a stainless-steel tip. Ions were produced by bombardment with a neutral xenon atom at 5 kV. The $[\text{M} + \text{Na}]^+$ ions were selected as precursor ions and then were achieved high energy (10 kV) collision with argon molecules in the third field-free region. The argon pressure was sufficient to attenuate the primary ion beam by 50%. The fragment ions were dispersed by the second spectrometer and the spectra were recorded as CID spectra.

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