Structural Elucidation of the Ceramide Moiety of Starfish Gangliosides by Collision-Induced Dissociation of the Sodium Ion Complex

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Collision-induced dissociation (CID) spectra of sodium ion complexes ($[M+Na]^+$ ions), produced by FAB-MS of methyl ester derivatives of ganglioside, indicate the length of the fatty acyl chain of the ceramide moieties without chemical degradation. In the case of a genuine ganglioside, only the fission of the glycosyl linkage of sialic acid was prominently observed.

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

We have been investigating the glycosphingolipids of echinodermata, namely, starfish and sea cucumber, in order to develop novel medicinal resources, and have reported cerebrosides,[1] ceramide-lactosides,[2] sulfatides,[3] and gangliosides,[4] some of which possess biological activities.

On the other hand, to obtain useful information on the structure of minute amounts of natural glycosphingolipids, we have been developing a new mass spectrometrical technique.[5—7] Recently, we have reported the usefulness of the collision-induced dissociation (CID)-MS/MS of $[M+Na]^+$ ions obtained in the (+)-FAB-MS to provide structural elucidation of ceramide, cerebroside, and ceramide-lactoside.[8] Namely, the characteristic fragment ion, originated from the fission of the amide bond of the ceramide part, was observed in the CID spectra of $[M+Na]^+$ ions and indicate the length of the fatty acyl chain of ceramide moieties without chemical degradation. Based on our previous studies, we attempted to apply this method to starfish gangliosides, which have already been isolated and characterized in our laboratory,[9,10] and are known to possess α-hydroxy fatty acid in their ceramide part.

At first, monosialo-ganglioside was examined. When (+)-FAB-MS of acanthaganglioside C (**1**)[9] (Fig. 1) was measured, a $[M+Na]^+$ ion (*m/z* 1617) was observed. However, in the CID spectrum of $[M+Na]^+$ ion, the expected characteristic fragment ion which would normally originate from the fission of the amide bond, was not observed; only the fragment ion at *m/z* 1002, corresponding to the fission of the glycosyl linkage of sialic acid, was prominently observed (Fig. 2). As we have reported,[8] the coordination of a Na^+ ion to the amide nitrogen is essential for the cleavage of the amide linkage of the ceramide part (Fig. 3). Therefore, in the case of **1**, it was presumed that the coordination of a Na^+ ion was disturbed by the effect of a carboxyl group of sialic acid. In order to reduce the negative charge of the carboxyl group, **1** was modified by dimethyl sulfoxide (DMSO)–MeI[11] to give a methyl ester derivative, acanthaganglioside C-ME (**2**). In the IR spectrum, characteristic absorption (1735 cm^{-1}) originating from the ester bond appeared, and the $[M+Na]^+$ ion shifted from *m/z* 1617 to *m/z* 1631 in the (+)-FAB-MS spectrum, which verified that **2** was a methyl ester derivative of **1**. In the CID spectrum of $[M+Na]^+$ ion, obtained in the (+)-FAB-MS of **2**, a highly intense and characteristic fragment ion, which arose from the fission of the amide bond of the ceramide part, was clearly observed at *m/z* 1265 (Fig. 4).

It is thought that the negative charge of the carboxyl group of sialic acid was reduced by methylation, and the Na^+ ion became able to coordinate to the ceramide amide nitrogen. Then, a stable five-membered chelate ring occured (Fig. 3), and the N(amide)–C(amide) bond of the ceramide part cleaved easily.[8]

Meanwhile, glycosphingolipids usually show variations in their fatty acyl and long-chain base moieties to form the molecular species. Therefore, we examined the possibility of application of this method to the molecular species of gangliosides. Ganglioside molecular species AG-2, the parent molecular species of **1**, was modified in the same manner as acanthaganglioside C-ME to obtain the methyl ester derivative AG-2-ME (Fig. 1). In the CID spectrum of each $[M+Na]^+$ ion, obtained in the (+)-FAB-MS of AG-2-ME, highly intense and characteristic fragment ions, arising from the fission of the amide bond of the ceramide part, were clearly observed.

Next, the application of this method to the disialo-ganglioside molecular species LLG-3 (**3**) (Fig. 1), which was obtained from the starfish *Linckia laevigata* ,[10] was attempted. **3** was converted to the dimethyl ester derivative LLG-3-DME (**4**) in the same manner as AG-2-ME. Its structure was confirmed by an IR spectrum (1735 cm^{-1}, ester) and by two methoxy signals (δ_H 3.56) in the ^{1}H-NMR spectrum of **4**. In the CID of **4**, each $[M+Na]^+$ ion, obtained in the (+)-FAB-MS, provided highly intense characteristic fragment ions, too. In Fig. 5, an example of the CID spectrum is described.

By using the above mentioned technique, the structure of the ceramide part of mono- and disialo-gangliosides possessing an α-hydroxy fatty acyl moiety in their ceramide part can be determined without chemical degradation. This method is useful for the structural elucidation of minute amounts of starfish gangliosides, and is worthy to note.

Experimental

NMR spectra. we refer to previous paper.[9a]; Positive ion FAB-MS and CID spectra. All mass spectra were acquired with a JMS-SX/SX102A four sector type tandem mass spectrometer (JEOL Ltd., Tokyo) of BE/BE geometry, which was controlled by a JEOL DA-7000 data system. Positive ion FAB-MS were obtained using only the first spectrometer (MS1). The spectra were measured under the following conditions: xenon atom beam, 5 kV; ion source accelerating potential, 10 kV; matrix, *m*-nitrobenzyl alcohol+NaCl. The $[M+Na]^+$ ions were selected as precursor ions and then achieved high energy (10 kV) collision with argon molecules in the third field-free region.

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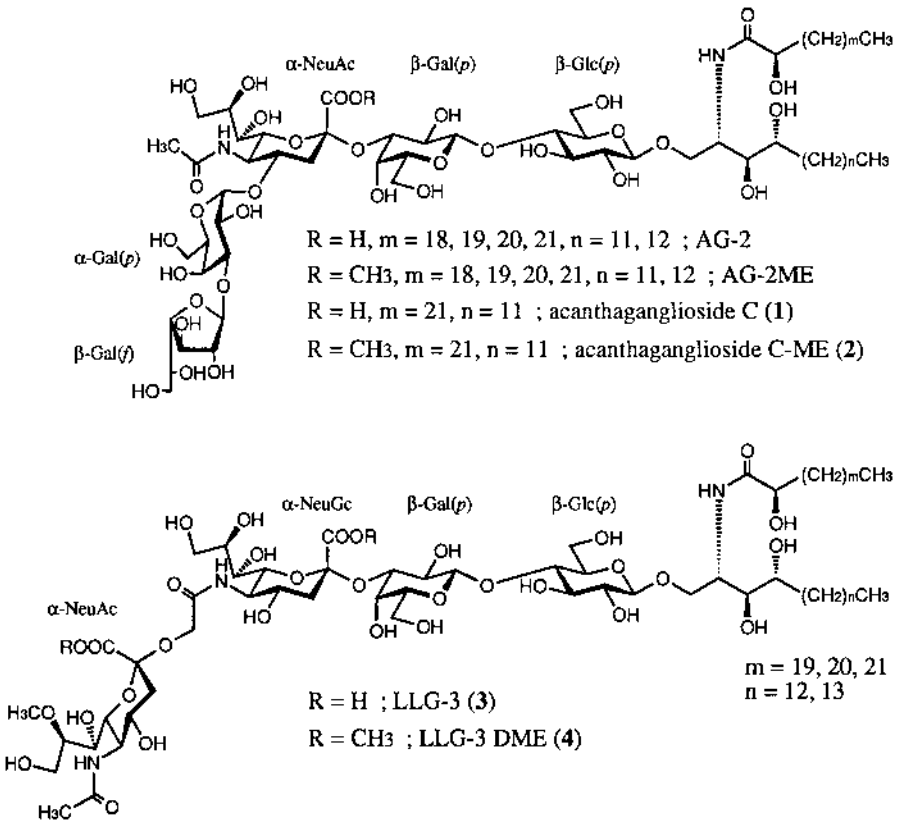

Fig. 1

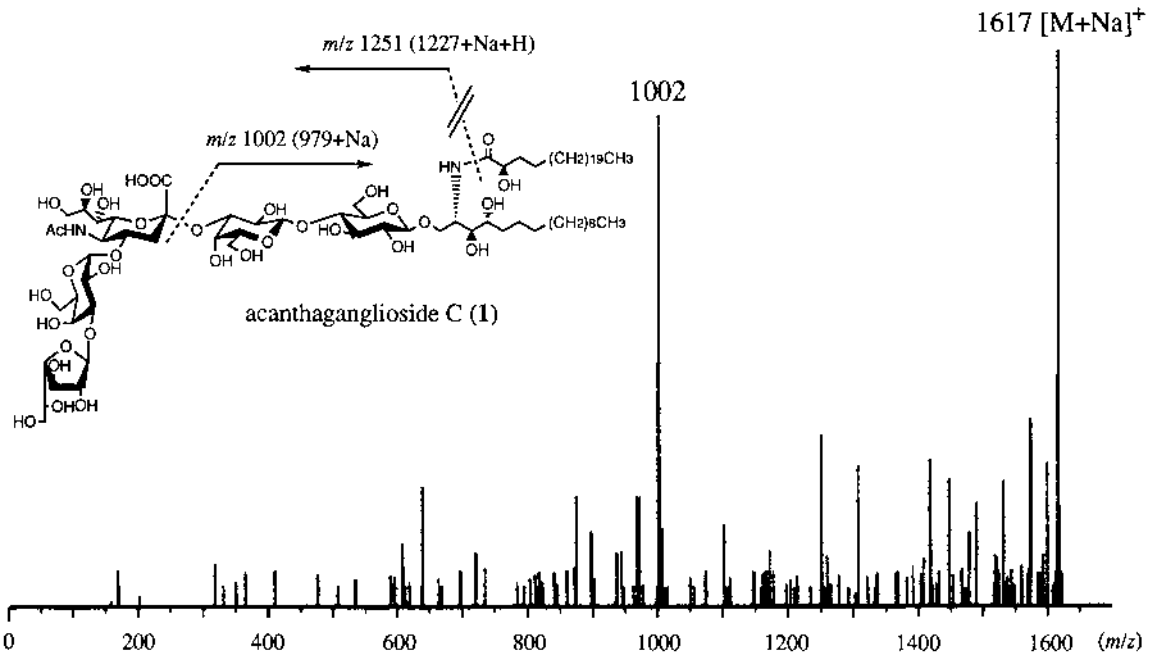

Fig. 2. CID Spectrum of $[M+Na]^+$ Ion Obtained in the Positive Ion FAB-MS of **1**

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 the CID spectra.; IR, JASCO IR-410 infrared spectrophotometer. Spectra were taken as KBr pellets; column chromatography was carried out with Cosmosil 140C_{18}PREP (Nacalai Tesque) or Silica gel 60 (0.063—0.200 μm, Merck).

Methyl Esterification of the Sialic Acid of Gangliosides A few mg of gangliosides were dissolved in 1 ml of DMSO, and 0.2 ml of MeI was added to this solution. After stirring for 30 min at room temperature, the reaction mixture was diluted with 10 ml of 50% MeOH and applied to RP-CC (Cosmosil 140C_{18}PREP) (1 i.d.×3 cm) prewashed with 50% MeOH. The column was washed with 20 ml of 50% MeOH to remove CH_3I and DMSO, and the esters were eluted with 30 ml of $CHCl_3$–MeOH (1 : 1). The eluate was concentrated and purified by Si-CC ($CHCl_3$: MeOH : H_2O=6 : 4 : 0.5), if necessary.

Acanthaganglioside C-ME (2) Amorphous powder. IR (KBr) cm^{-1}:

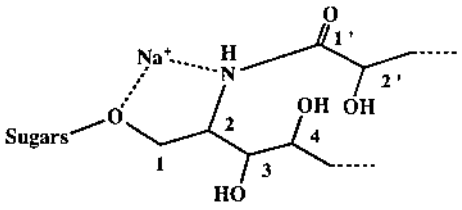

Fig. 3. Five Membered Chelate Ring

3390 (OH), 1735 (ester), 1650, 1550 (amide). Positive ion FAB-MS *m/z*: 1631 $[M+Na]^+$, 656[ceramide]. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 8.73 (1H, d, J=8.9 Hz, –N<u>H</u>COCH$_3$), 8.50 (1H, d, J=9.2 Hz, NH), 3.39 (3H, s, –COOC<u>H</u>$_3$), 2.17 (3H, s, –NHCOC<u>H</u>$_3$), 0.86 (6H, t, J=6.8 Hz, –CH$_3$).

AG-2-ME Amorphous powder. IR (KBr) cm^{-1}: 3390 (OH), 1735 (ester), 1650, 1550 (amide). Positive ion FAB-MS *m/z*: 1575, 1589, 1603, 1617, 1631, 1645 $[M+Na]^+$ series. ^{1}H-NMR (270 MHz, C_5D_5N) δ: 8.81 (1H, d, J=7.6 Hz, –N<u>H</u>COCH$_3$), 8.53 (1H, d, J=8.9 Hz, NH), 3.36 (3H, s, –COOC<u>H</u>$_3$), 2.17 (3H, s, –NHCOC<u>H</u>$_3$), 0.84 (6H, t, J=6.4 Hz, –CH$_3$).

LLG-3-ME (4) Amorphous powder. IR (KBr) cm^{-1}: 3390(OH), 1735 (ester), 1650, 1550 (amide). Positive ion FAB-MS *m/z*: 1615, 1629, 1643, 1657 $[M+Na]^+$ series. ^{1}H-NMR (270 MHz, C_5D_5N) δ: 9.05 (1H, d, J=8.9 Hz, –N<u>H</u>COCH$_2$–), 8.81 (1H, d, J=8.6 Hz, –NHCOCH$_3$), 8.54 (1H, d, J= 9.2 Hz, NH), 3.56 (6H, s, –COOC<u>H</u>$_3$), 3.34 (3H, s, –OC<u>H</u>$_3$), 1.84 (3H, s,

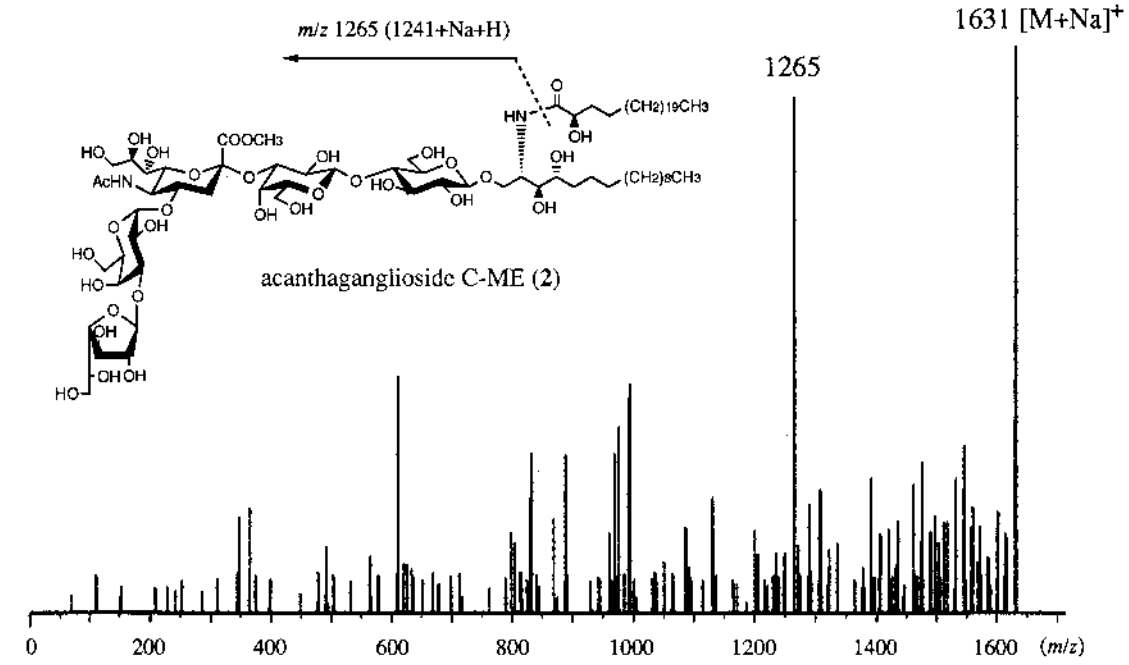

Fig. 4. CID Spectrum of $[M+Na]^+$ Ion Obtained in the Positive Ion FAB-MS of **2**

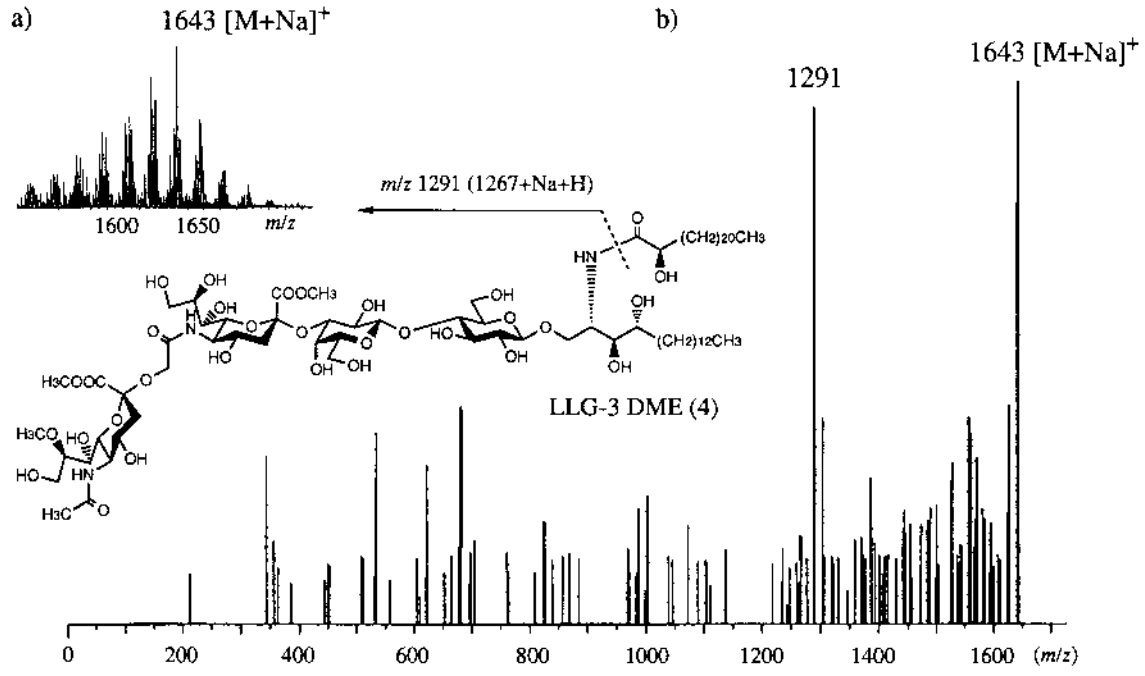

Fig. 5. a) Positive Ion FAB-MS of **4**, b) CID Spectrum of $[M+Na]^+$ Ion Obtained in the Positive Ion FAB-MS of **4**

–NHCOC$\underline{H}_3$), 0.84 (6H, t, J=6.4 Hz, –CH_3).

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