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Structural Studies of Gangliosides by Fast Atom Bombardment Ionization, Low-Energy Collision-Activated Dissociation, and Tandem Mass Spectrometry†

Takeshi Kasama and Shizuo Handa*

Laboratory for Biomedical Analysis and Department of Biochemistry, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo, Japan

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ABSTRACT: Negative ion fast atom bombardment, low-energy collision-activated dissociation, and tandem mass spectrometry techniques were applied for the structural elucidation of gangliosides. The mass spectra were simplified by selecting a single molecular ion or fragment ion in the analysis of mixtures, and interference by background signals from the liquid matrix could be avoided. Introduction of collision-activated dissociation produced abundant fragment ions convenient for structural analysis. In the daughter scan mode, ions were produced by cleavage of the glycosidic bonds, and not by cleavage at the sugar ring. These ions all contain ceramide moieties, except the sialic acid fragment ion. In the parent scan mode, product ions resulting from cleavage at the sugar ring were detected beside the ions resulting from cleavage at the glycosidic bonds, and ions of oligosaccharide fragments were also detected. In parent scan mode spectra of gangliosides based on the sialic acid ion, all ions contained a sialic acid residue, and the observed ions were similar to those obtained in the high-energy collision-activated dissociation daughter scan mode. These results indicate the usefulness of low-energy collision-activated dissociation tandem mass spectrometry in the daughter and parent scan modes for the analysis of ganglioside structure, in combination with fast atom bombardment mass spectrometry and high-energy collision-activated dissociation mass spectrometry.

Glycolipids are components of the cell membrane and exhibit characteristic distribution patterns depending on the species, organ, cell type, and developmental stage (Hakomori, 1981). They have vital functions on the cell surface, as antigens (Clausen & Hakomori, 1989), binding sites for microbes (Karlsson, 1989), cell–cell recognition sites (Kojima & Hakomori, 1989), and signals for cell growth (Bremer et al., 1986) and differentiation (Nojiri et al., 1986). For studies in all these areas, characterization of the chemical structure of the glycolipids is a fundamental requirement. In many cases, the content of the glycolipids is low, and the purification is difficult and time-consuming. The development of new techniques which require only a minute amount of the sample and give detailed information is therefore essential. Nuclear magnetic resonance spectroscopy is a powerful method especially for stereochemical and linkage analysis (Sweeley & Nunez, 1985), but it requires relatively large amounts of samples (at least 100 nmol for two-dimensional nuclear magnetic resonance spectrometry) and is almost impossible to apply for the analysis of mixtures. The use of tandem mass spectrometry (MS/MS)[1] has been reported to overcome these difficulties, and it can give valuable information for structural studies. Domon and Costello reported the application of fast atom bombardment high-performance tandem mass spectrometry (MS/MS) for the analysis of glycolipids using high-energy (kiloelectron volts) collision to obtain collision-activated dissociation (CAD) (Domon & Costello, 1988a).

In this report, we show that another type of structural information can be obtained by using MS/MS with low-energy (tens of electron volts) CAD in the daughter scan mode and in the parent scan mode, and we compare the results with those

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* To whom correspondence should be addressed at the Department of Biochemistry Tokyo Medical and Dental University.

[1] Abbreviations: MS, mass spectrometry; MS/MS, tandem mass spectrometry; CAD, collision-activated dissociation; FAB, fast atom bombardment; sialyl2-3nLc$_4$Cer, NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-ceramide; Cer, ceramide.

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obtained by using high-energy CAD.

Materials and Methods

All glycolipids were prepared in this laboratory. Gangliosides GM1 and GD1a were obtained from bovine brain. Ganglioside GM2 was prepared from the brain of a patient with Sandhoff's disease (Handa & Kushi, 1981). Sialyl2-3nLc$_4$Cer was obtained from human placenta (Taki et al., 1988).

FAB mass spectra were obtained on a TSQ 70 triple-stage quadrupole mass spectrometer (Finnegan MAT Inc., San Jose, CA) with a FAB neutral source powered and controlled at 8 kV by a Model B-50 high-voltage power supply unit (ION TECH Ltd., Teddington, U.K.) and an electron multiplier with a 20-kV conversion dinode. Xenon was used as the FAB gas. Five micrograms of a ganglioside dissolved in 1 μL of a chloroform–methanol mixture (1/1, v/v) was loaded on the FAB target with 1 μL of triethanolamine as the matrix. Collision-activated dissociation (CAD) MS/MS studies were performed on the Finnegan TSQ 70 with a 10-eV collision energy and 1.5×10^{-3} torr of argon as the collision gas. Linked scan spectra were obtained on an HX-110 double-focusing mass spectrometer (JEOL, Tokyo, Japan) equipped with a 6-kV FAB gun at 10-kV accelerating voltage and a 20-kV postacceleration detector at constant B/E ratio. Helium was used as the collision gas at a pressure sufficient to reduce the precursor ion signal by one-third. All spectra were recorded at 250 masses/s scan speed on the TSQ-70 and at 20-s slope on the HX-110.

Results

As the FAB sensitivity for underivatized ganglioside is more favorable in the negative ion mode than the positive ion mode, the present work was performed in the negative ion mode. The structure of the gangliosides, and the assignment of the ion peaks are shown in the insets of the figures.

The FAB mass spectrum of ganglioside GM2 showed two deprotonated molecular species at m/z 1410 and 1382 due to the presence of both sphingenine (d18:1) and icosasphingenine (d20:1) as long-chain bases. The intensities of the molecular ion peaks reflected the contents of these long-chain bases. As already reported in the case of brain gangliosides of a patient with Sandhoff's disease, the content of sphingenine is higher than that of icosasphingenine (Handa & Kushi, 1981). Some fragment ions containing ceramide moieties could be observed (m/z 1091 and 564), but other fragment ions were of low intensity. Fragments representing the sugar moiety alone were difficult to find except for m/z 671 and 290.

The low-energy CAD daughter scan mode spectrum of GM2 is shown in Figure 1A. The precursor ion was the deprotonated molecule $(M - H)^-$ at m/z 1382. The abundant product ions at 1091, 888, 726, 564, and 290 result from the cleavage of the glycosidic bonds between the anomeric carbon and oxygen, and contain ceramide moieties. Mass differences of these ions from the molecular species represent sialic acid, hexosamine, hexose, and hexose, and are useful for the sugar sequence analysis.

The low-energy CAD parent scan mode spectrum of GM2 based on the sialic acid ion at m/z 290 is shown in Figure 1B. All the ions retain the sialic acid residue with (m/z 1410, 1382, 1207, and 1179) or without ceramide moieties (m/z 833, 817, 729, 671, and 655). These ions result from cleavage at the glycosidic bond except m/z 729, which is produced by cleavage of the galactose ring.

In the FAB spectrum of ganglioside GM1 (Figure 2A), the molecular species (m/z 1572 and 1544) and a series of fragment ions that result from the sequential cleavage of the glycosidic bonds are obtained in pairs with the mass difference of 28 due to the presence of almost equivalent amounts of sphingenine and icosasphingenine in the bovine brain gangliosides (m/z 1410, 1382; 1281, 1253; 1207, 1179; 1119, 1091; 916, 888; 754, 726; and 592, 564). Fragment ions representing the sugar moiety without the ceramide residue are also apparent (m/z 290, 833, and 995).

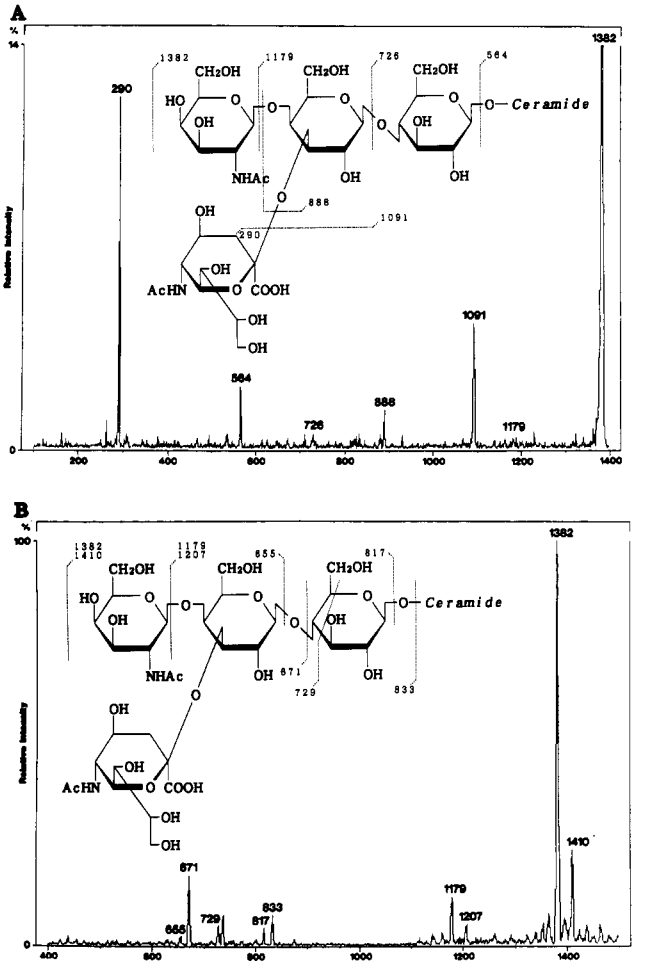

FIGURE 1: Mass spectra of ganglioside GM2. (A) Low-energy CAD daughter scan mode spectrum. The precursor ion is $(M - H)^-$ at m/z 1382. (B) Low-energy CAD parent scan mode spectrum based on the ion at m/z 290. Experimental details are presented under Materials and Methods.

The low-energy CAD daughter scan mode spectrum of ganglioside GM1 (Figure 2B) is simpler than the FAB spectrum (Figure 2A) due to the single molecular species of ceramide moiety obtained by selecting the deprotonated molecule at m/z 1544 as the precursor ion. The fragmentation pattern is similar to that of ganglioside GM2, representing the sequential cleavage of the glycosidic bonds.

The linked scan mass spectrum obtained by high-energy CAD of the ion at m/z 1544 at constant B/E ratio (Figure 2C) shows more complicated fragment ions and is very similar to the reported high-energy CAD daughter scan mode spectrum (Domon & Costello, 1988a). In contrast to the low-energy CAD daughter scan MS/MS spectrum, fragment ions with (m/z 1382, 1253, and 1179) and without the ceramide moiety (m/z 995, 979, 891, 833, 308, and 290) all retain the sialic acid residue, except the ion m/z 1253, which is the asialo-GM1 fragment ion. A fragment ion resulting from sugar ring cleavage was also detected (m/z 891) as well as ions due to cleavage at the glycosidic bonds.

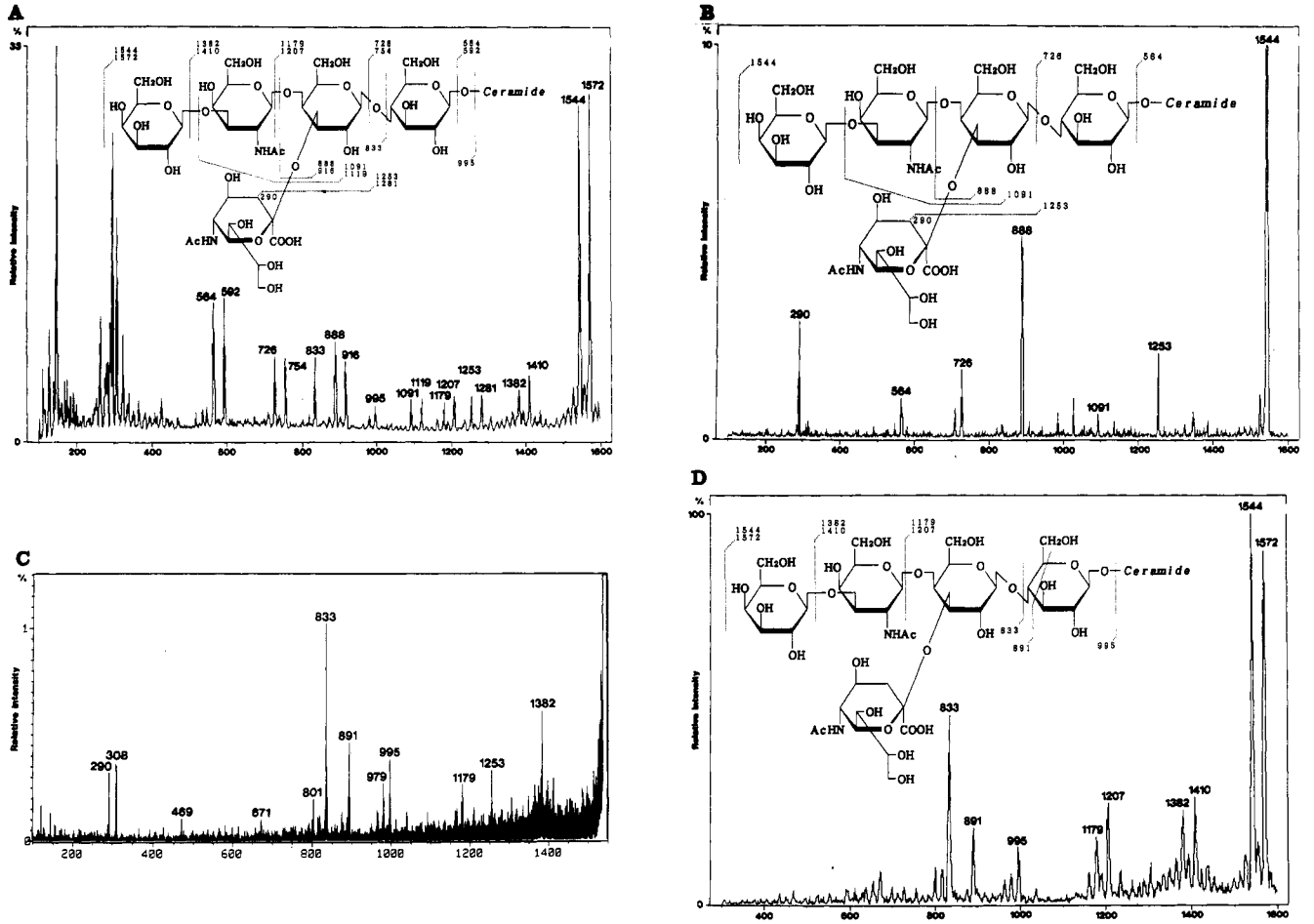

FIGURE 2: Mass spectra of ganglioside GM1. (A) Negative ion FAB MS spectrum. (B) Low-energy CAD daughter scan mode spectrum. The precursor ion is $(M - H)^-$ at m/z 1544. (C) High-energy CAD linked scan spectrum of the ion $(M - H)^-$ at m/z 1544 at constant B/E ratio. This spectrum was taken with HX110. (D) Low-energy CAD parent scan mode spectrum based on the ion at m/z 290. Experimental details are presented under Materials and Methods.

The low-energy CAD parent scan mode spectrum of GM1 based on the sialic acid ion, m/z (Figure 2D), shows a quite different ion pattern from that of the daughter scan mode spectrum (Figure 2B), but is rather similar to the reported high-energy CAD daughter scan mode spectrum (Domon & Costello, 1988a) and the high-energy CAD linked scan spectrum (Figure 2C). All the ions retain the sialic acid residue, and the fragment ion resulting from cleavage of the sugar ring was also detected (m/z 891). When the parent ions contained the ceramide moieties, they appeared as a pair of ions with a 28 mass difference due to the long-chain base (m/z 1572, 1544; 1410, 1382; and 1207, 1179).

In the FAB spectrum of ganglioside GD1a, the molecular species were deprotonated and sodium adduct ions $(M - 2H + Na)^-$ at m/z 1857 and 1885.

In the low-energy CAD daughter scan mode spectrum of ganglioside GD1a with the ion $(M - 2H + Na)^-$ at m/z 1857 as the precursor ion, monosialo fragment ions (m/z 1566, 1544, 1404, and 1201) were abundant, and the intensities of the other fragment ions were not sufficient to provide structural information. When the spectrum was taken with the monosialo fragment ion at m/z 1544 as the precursor ion, a further ion series resulting from the asialo fragment ions was detected (m/z 1253, 1091, 888, and 726).

In Figure 3A, the FAB spectrum of sialyl2-3nLc$_4$Cer is shown. Ceramide moieties of this glycolipid are composed of sphingenine and long-chain fatty acids (mainly lignoceric acid, behenic acid, and stearic acid), and the molecular and fragment ions contain the set of ions reflecting this heterogeneity of the ceramide moieties. The abundant fragment ions result from single glycosidic bond cleavage on either side of glycosidic oxygen atoms, and the ions resulting from cleavage at the anomeric carbon side contain the ceramide moieties while the other ions do not.

When the ion at m/z 1628 was selected as the precursor ion, the low-energy CAD daughter scan mode spectrum was simplified. The ions resulting from sequential cleavage of the glycosidic bonds at the anomeric carbon side from the nonreducing end were detected, and retain the ceramide moiety (Figure 3B).

Discussion

FAB mass spectrometry is a powerful technique for the structural analysis of gangliosides. However, the isolation and purification of gangliosides from tissues are often difficult, and gangliosides are usually isolated on the basis of differences of the sugar moieties, so a preparation for mass analysis is often a mixture of different gangliosides or at least a mixture of several molecular species of a special ganglioside with mixed ceramide moieties. To overcome these problems, methods for direct coupling of chromatographic separation and mass spectrometry, such as high-performance liquid chromatography/MS (Kushi et al., 1989) and thin-layer chromatography/MS (Kushi & Handa, 1985; Kushi et al., 1988, 1990; Handa & Kushi, 1988), have been developed. Although molecular weight information can almost always be obtained by FAB, structurally significant fragment ions may be absent or of such low abundance that they are obscured by the

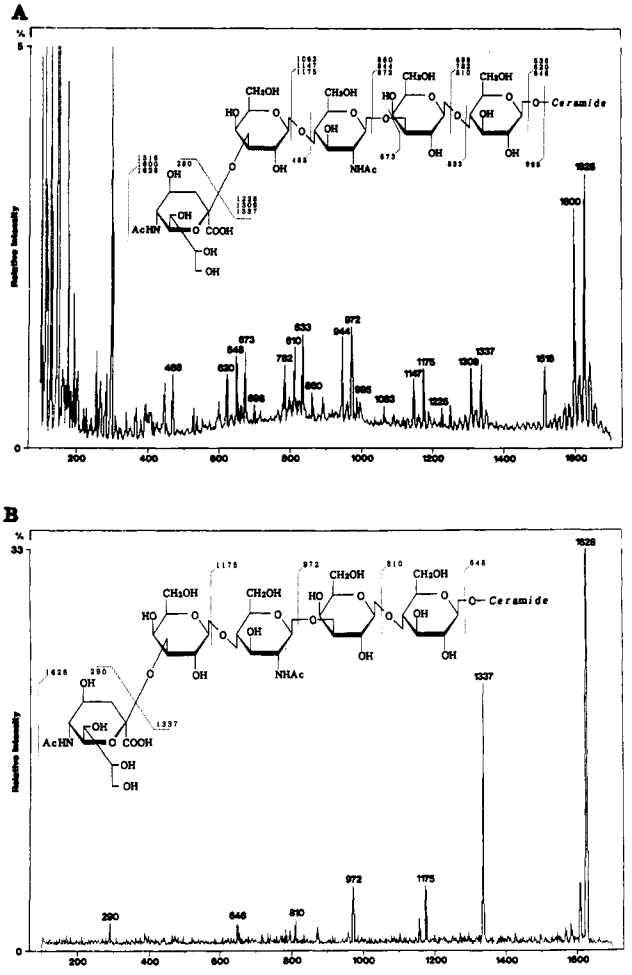

FIGURE 3: Mass spectra of the ganglioside sialyl2-3nLc$_4$Cer. (A) Negative ion FAB MS spectrum. (B) Low-energy CAD daughter scan mode spectrum. The precursor ion is $(M - H)^-$ at m/z 1628. Experimental details are presented under Materials and Methods.

background signals from the liquid matrix, especially in the low-mass region. Tandem mass spectrometry can overcome these difficulties by allowing the choice of a suitable precursor ion, and making it easy to identify the origin of the fragment ions. Introduction of CAD produces abundant fragment ions for structural analysis (Carr et al., 1985), and a systematic nomenclature for carbohydrate fragmentation in FAB MS/MS spectra of glycoconjugates was presented (Domon & Costello, 1988b). It was reported that high-energy CAD spectra obtained in the positive ion mode provide information on the ceramide portion, whereas the negative ion spectra provide more information regarding the sequence of the sugar portion (Domon & Costello, 1988a). Derivatization of the ceramides and neutral glycosphingolipids offers an approach to amplify the structural information content of the positive ion FAB MS/MS spectrum (Domon et al., 1990).

In the present study, we found several differences between high- and low-energy CAD MS/MS spectra. In the daughter scan mode of low-energy CAD, no sugar ring cleavage was observed, in contrast to the reported findings in the case of the high-energy CAD method. Such cleavage of the sugar ring was detected in the parent mode of low-energy CAD. In the low-energy CAD daughter scan mode, fragment ions without sialic acid residues were produced, while in the parent scan mode spectra of gangliosides based on the sialic acid ion all the ions retained sialic acid residues, as in the high-energy CAD daughter scan mode spectra. This paper is the first report on the application of parent scan mode MS/MS for the analysis of gangliosides. The results indicate that the use of low-energy CAD MS/MS analysis in daughter and parent scan modes is informative for the structure analysis of gangliosides, in combination with FAB and high-energy CAD methods.

Since isomers give identical mass spectra, it is difficult to get the information about the anomeric configuration and linkage position of the surgars by mass spectrometry. FAB MS combined with chromium trioxide oxidation is a valuable method for assigning anomeric configuration of pyranose sugars (Khoo & Dell, 1990). Use of the low-energy CAD method allowed stereochemical assignment of sugar subunits (Mueller et al., 1988) and determination of linkage positions in oligosaccharides (Laine et al., 1988). Further development of a new strategy is necessary for this problem.

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Registry No. Ganglioside GM$_1$, 104443-62-1; ganglioside GM$_2$, 104443-57-4; sialyl 2-3nLc$_4$Cer, 71833-57-3.

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