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Structural Characterization of Sulfoquinovosyl, Monogalactosyl and Digalactosyl Diacylglycerols by FAB-CID-MS/MS

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The glycolipids such as sulfoquinovosyl diacylglycerol (SQDG), isolated from wild-type cyanobacterium *Synechocystis* sp. PCC 6803, and monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), obtained from whole wheat flour, have been investigated by using fast atom bombardment tandem mass spectrometry (FAB-MS/MS). Collision-induced dissociation (CID) of sodium-adduct molecular ions $[M - H + 2Na]^+$ from SQDG and $[M + Na]^+$ from MGDG and DGDG generates ions which are characteristic of the structure of the polar head group as well as the fatty acid composition. In particular, the charge-remote fragmentation along the fatty acid chains observed in the high-mass range was useful to locate the double-bond positions. The major component ion ($[M - H + 2Na]^+$ at *m/z* 865) of SQDG contains oleic and palmitic acids at the *sn*-1 and *sn*-2 positions, respectively. The predominant molecular ion ($[M + Na]^+$ at *m/z* 801) of MGDG contains two linoleic acids. One of the two major species of DGDG contains palmitic and linoleic acids ($[M + Na]^+$ at *m/z* 939) at the *sn*-1 and *sn*-2 positions, while the other contains two linoleic acids ($[M + Na]^+$ at *m/z* 963). The utility of FAB-MS/MS in the structural determination of glycolipids in mixtures of biological origin has been demonstrated. © **1997 by John Wiley & Sons, Ltd.**

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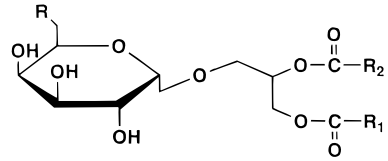
KEYWORDS: sulfolipid; galactolipid; fast atom bombardment; tandem mass spectrometry; charge-remote fragmentation

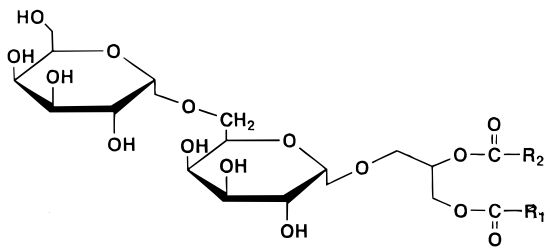
INTRODUCTION

Sulfolipids and galactolipids, which are glycolipids containing sulfonic acid and galactose respectively, have been regarded as the predominant lipid components of the photosynthetic membrane in plants, algae and various bacteria.[1,2] The three most common glycolipids are sulfoquinovosyl diacylglycerol [1,2-diacyl-3-*O*-(α-6′, 6-deoxyaldohexopyranosyl-6′-sulfonic acid)-*sn*-glycerol; SQDG], monogalactosyl diacylglycerol [1,2-diacyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol; MGDG] and digalactosyl diacylglycerol [1,2-diacyl-3-*O*-(α-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl)-*sn*-glycerol; DGDG]. Their chemical structures are characterized by two non-polar fatty acyl chains bonded to the glycerol backbone with a polar head group of sulfoquinovose, monogalactose and digalactose, respectively (Fig. 1). In higher plants these are particularly abundant in the thylakoid membrane of chloroplasts, in which MGDG represents up to 50 mol.%, DGDG ~30 mol.% and SQDG ~4 mol.% of polar lipids.[3] Based on the exclusive distribution of the glycolipids in the chloroplasts, they have been thought to play a critical role in photosynthesis. Also, sulfolipids have attracted considerable interest in the past few years owing to their activity against the human immunodeficiency virus.[4]

Since glycolipids differ mainly in the chain length and the position and degree of unsaturation of fatty acyl groups, it is difficult to separate and purify a naturally



SQDG : R = SO_3H
MGDG : R = OH



DGDG

Figure 1. Structures of SQDG, MGDG and DGDG. The acyl groups designated R_1 and R_2 are attached at the *sn*-1 and *sn*-2 positions of the glycerol backbone respectively.

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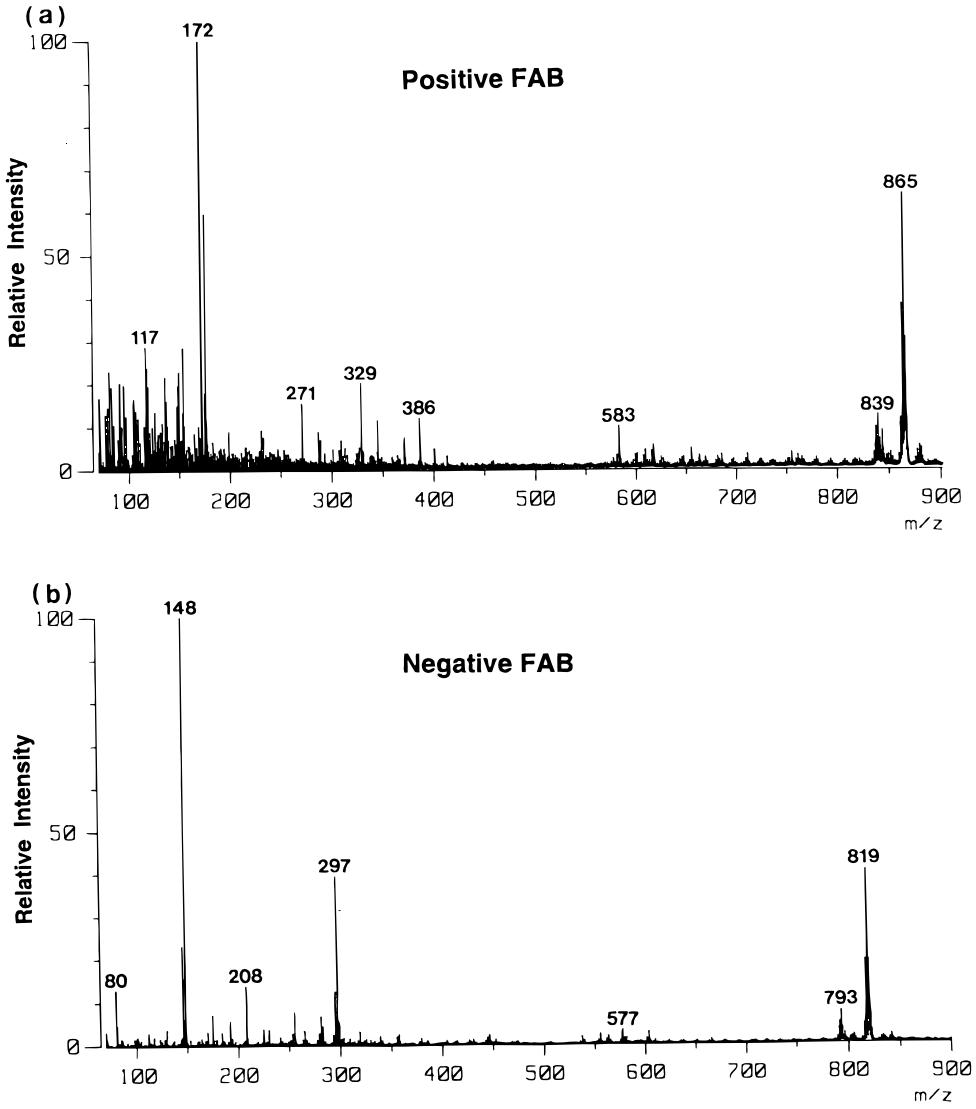

Figure 2. (a) Positive-ion and (b) negative-ion FAB mass spectra of SQDG isolated from wild-type cyanobacterium *Synechocystis* sp. PCC 6803. The peaks at *m/z* 148 and 297 are due to the matrix.

occurring mixture of glycolipids. Hence, few direct methods for the structural analysis of these substances have been reported. An early mass spectrometric study of Budzikiewicz *et al.*[5] employed electron impact ionization to analyze the peracetylated derivatives of SQDG, MGDG and DGDG. Gage *et al.*[6] reported the use of fast atom bombardment mass spectrometry (FAB-MS) and fast atom bombardment collison-induced dissociation tandem mass spectrometry (FAB-CID-MS/MS) in the negative-ion mode, which employed the two-sector B/E linked scan method to analyze mixtures of SQDG in lipids from two different sources. However, owing to the poor resolution of the precursor ions, it was not possible to obtain information about the position of the double bond in the fatty acyl group from the CID spectra.

In this paper we present results from our investigation on the fragmentation of the sodium-adduct precursor ions ($[M - H + 2Na]^+$ or $[M + Na]^+$) of SQDG, MGDG and DGDG using four-sector tandem mass spectrometry. It will be demonstrated that a complete structural analysis, including the identification of the head groups, the composition of the two fatty acyl groups and the double-bond positions, is possible with FAB-CID-MS/MS.

EXPERIMENTAL

Materials

Monogalactosyl diacylglycerol, digalactosyl diacylglycerol and standard fatty acids such as linoleic and oleic acids were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Information on the fatty acid compositions and the double-bond positions for MGDG and DGDG were not available from the commercial source. Sulfoquinovosyl diacylglycerol was extracted from culture media of wild-type cyanobacterium *Synechocystis* sp. PCC 6803 with a mixture of chloroform/methanol/water (1:2:1 by volume). The extract was purified by two-dimensional TLC on silica plates (Whatman, Hillsboro, OR, USA) using chloroform/

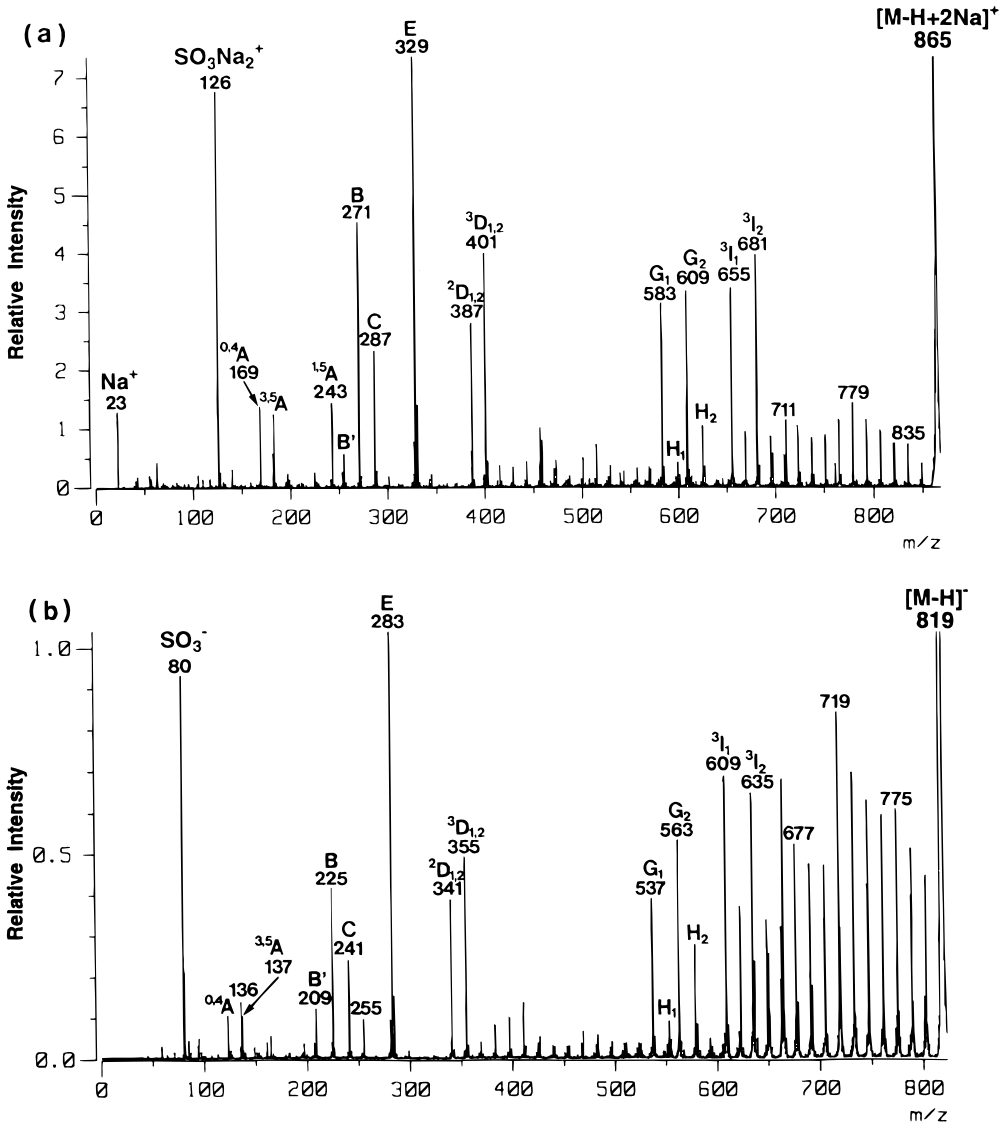

Figure 3. (a) Positive-ion FAB tandem mass spectrum of $[M - H + 2Na]^+$ (at *m/z* 865) and (b) negative-ion FAB tandem mass spectrum of $[M - H]^-$ (at *m/z* 819) for major molecular species of SQDG.

methanol/water (65:25:4 by volume) as solvent for dimension I and chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1 by volume) for dimension II. Further details of the extraction and purification procedure are described elsewhere.[7]

Fast atom bombardment mass spectrometry

FAB mass spectra were taken with the first (MS-1) of the two mass spectrometers of a JMS-HX110A/110A tandem mass spectrometer (JEOL, Tokyo, Japan) using a JMS-DA9000 data system. The ion source was operated at 10 and −10 kV accelerating voltage in the positive- and negative-ion modes, respectively, with a mass resolution of 1000 (10% valley). Ions were produced by fast atom bombardment using a cesium ion gun operated at 22 kV. Approximately 10 μg of sample dissolved in chloroform/methanol (1:1 by volume) was mixed with 1 μl of *m*-nitrobenzyl alcohol (Sigma) in the positive-ion mode and with 1 μl of triethanolamine (BDH, Poole, Dorset, UK) in the negative-ion mode on the FAB probe tip. Calibration was performed with CsI (Sigma) in the positive-ion mode and Ultramark 1621 (PCR, Florida, USA) in the negative-ion mode as standards.

Tandem mass spectrometry

MS/MS was carried out using a four-sector instrument with the $E_1B_1E_2B_2$ configuration. Collision-induced dissociation of the precursor ions selected with MS-1 (E_1B_1) occurred in the collision cell located between B_1 and E_2 and floated at 3.0 kV (or −3.0 kV). Both MS-1 and MS-2 were operated as double-focusing instruments. The collision gas, helium, was introduced into the collision chamber at a pressure sufficient to reduce the precursor ion signal by 70%. Signal averaging with several scans was carried out. MS-1 was operated at a resolution adjusted so that only the ^{12}C species of the precursor ions to be analyzed was transmitted. MS-2 was operated at a resolution of 1000 and was calibrated with a mixture of CsI, NaI, KI, RbI and LiCl (Sigma) in

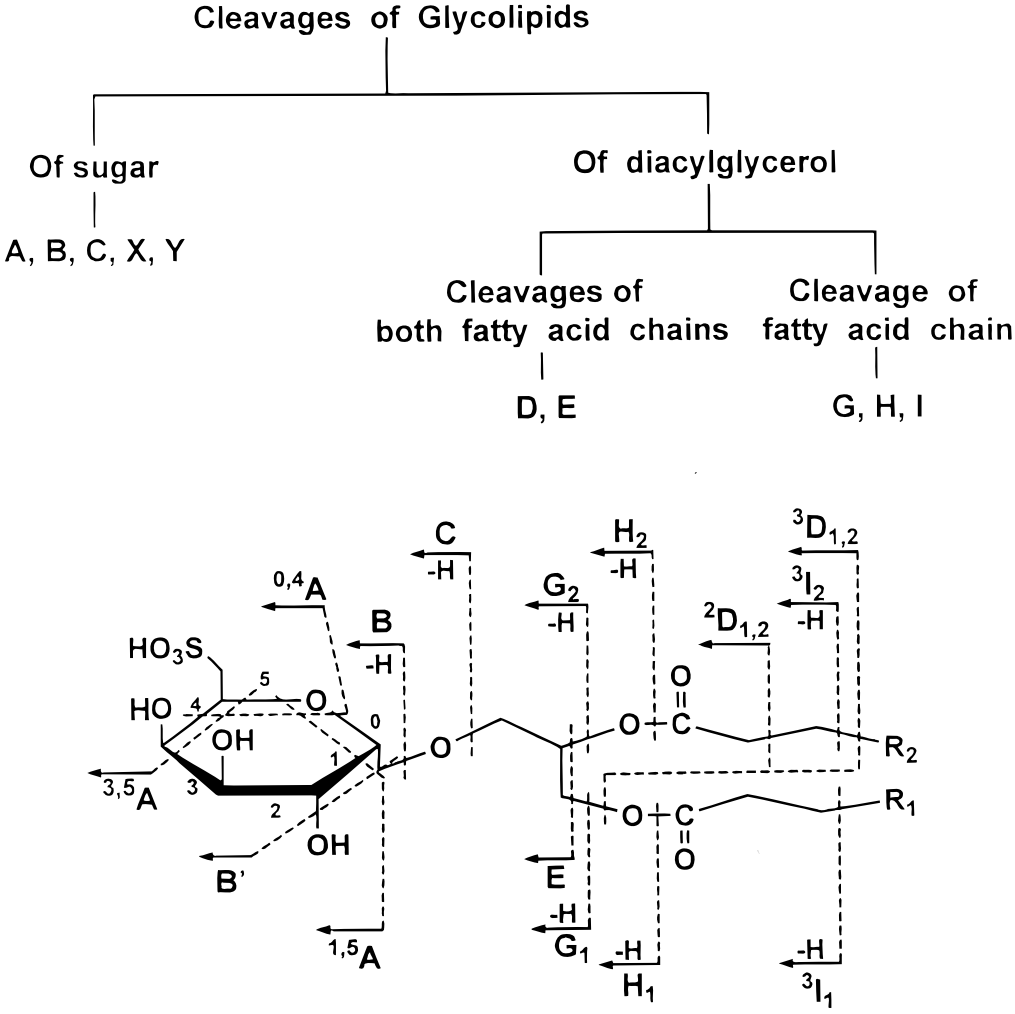

Figure 4. Nomenclature for cleavages of glycolipids and fragmentation scheme of SQDG. The nomenclature proposed by Costello and co-workers for glycosphingolipids has been adopted with minor changes. For the cleavages of fatty acyl chains the subscript in the symbol represents the relative position (*sn*-1 or *sn*-2) of the cleavage in the fatty acyl chain and the superscript the cleaved bond position relative to the carbonyl carbon of the fatty acyl group. In the proposed positive ions there are two sodium ions attached at the terminal sulfonic group.

the positive-ion mode and with a solution of CsI in water/glycerol (2:1 by volume) in the negative-ion mode.

RESULTS AND DISCUSSION

The positive- and negative-ion FAB mass spectra of SQDG isolated from wild-type cyanobacterium *Synechocystic* sp. PCC 6803 display abundant peaks for $[M - H + 2Na]^+$ and $[M - H]^-$ of the individual molecular species present, respectively (Fig. 2). Several molecular ion peaks are attributed to various sulfolipids with different fatty acid composition. Referring to the previous negative-ion FAB investigations of spinach and bacterial sulfolipids,[6] two peaks at *m/z* 839 and 865 in the positive-ion FAB mass spectrum [Fig. 2(a)] are interpreted as sodium-adduct molecular ions of sulfoquinovosyl diacylglycerols containing two acyl groups corresponding to a total fatty acid composition (carbon atoms:double bonds) of 32:0 and 34:1, respectively. The CID tandem mass spectra of the major ions $[M - H + 2Na]^+$ (at *m/z* 865) appearing in the positive-ion FAB spectrum and $[M - H]^-$ (at *m/z* 819) in the negative-ion FAB spectrum are shown in Fig. 3(a) and 3(b), respectively. The presence of $[M - H + 2Na]^+$ instead of $[M + Na]^+$ in the positive-ion FAB spectrum is due to the acidity of the sulfonic acid group at position 6 of the sugar unit. Namely, the positive charge is located at the terminal sulfonic group. This is further supported by the presence of a prominent peak at *m/z* 126 in the positive-ion CID spectrum of $[M - H + 2Na]^+$ shown in Fig. 3(a), which can be assigned to the $SO_3Na_2^+$ ion. Almost all the product ions appearing in the CID spectrum are found to be produced by fragmentations remote from the positive charge localized at the sulfonic group. The fragmentation pattern of the $[M - H]^-$ ion in the negative-ion CID spectrum shown in Fig. 3(b) is very similar to that obtained in the positive-ion mode. The main differences are that all the fragment ions in the former are shifted to lower mass by 46 u (corresponding to Na_2) and the product ions in the high-mass region are more abundant. The fragmentation pathways leading to some major product ions in the negative-ion CID spectrum have been proposed by Gage *et al.*[6] It has been attempted here to establish a more comprehensive frag-

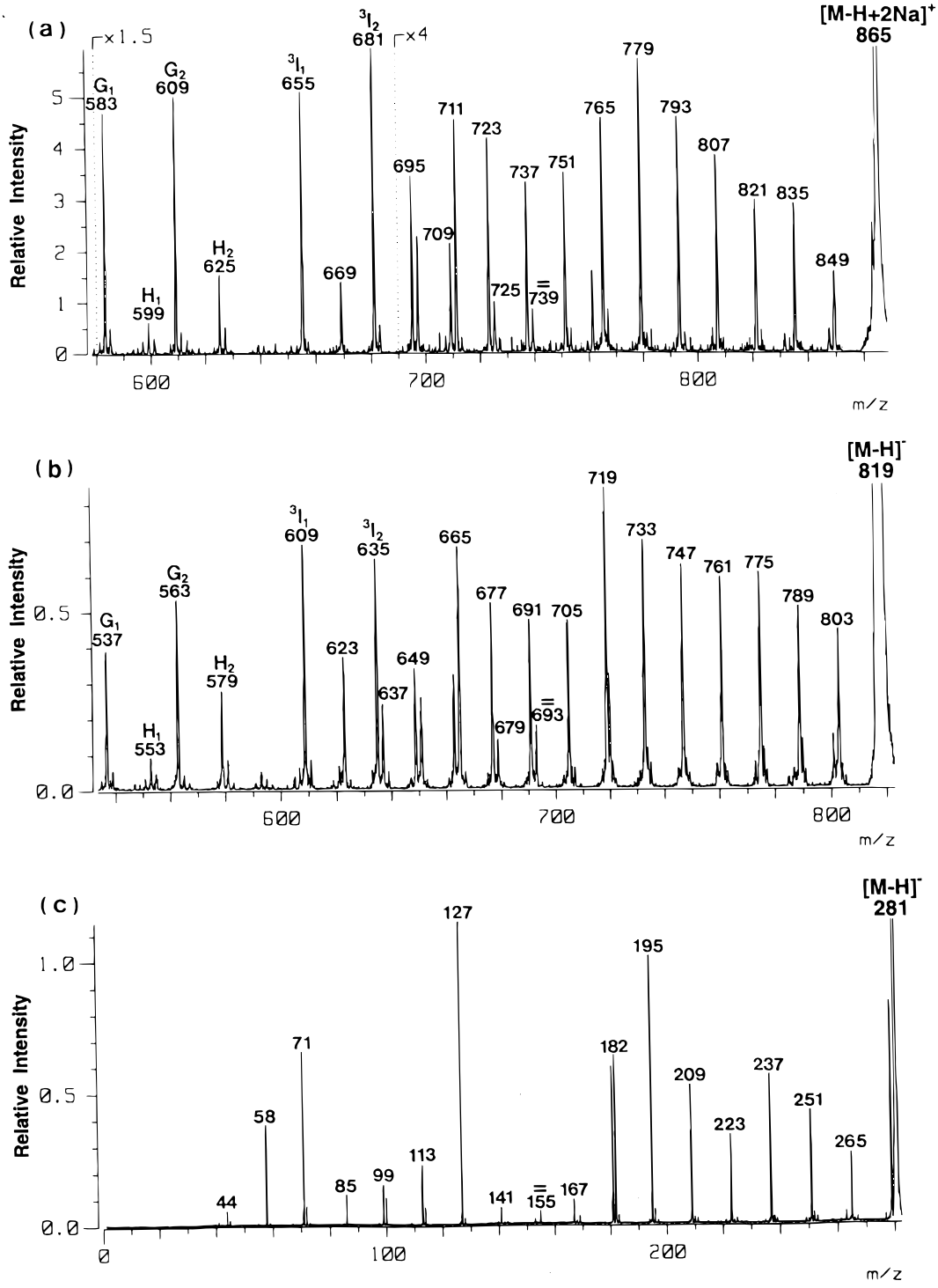

Figure 5. High-mass region of (a) positive-ion FAB CID mass spectrum of $[M-H+2Na]^+$ (at *m/z* 865) and (b) negative-ion FAB CID mass spectrum of $[M-H]^-$ (at *m/z* 819) for SQDG and (c) negative-ion FAB CID mass spectrum of $[M-H]^-$ (at *m/z* 281) of oleic acid. An equal sign above a peak indicates the double-bond position.

mentation mechanism which can explain the appearance of most of the major product ions in both the positive- and negative-ion CID spectra. The results are summarized in Fig. 4. The nomenclature proposed by Costello and co-workers[8,9] is adopted. Based on this analysis, we can divide the product ions into two types, one generated by the cleavage of the head group (i.e. the sugar moiety) and the other by the cleavage of the fatty acyl chains, as illustrated in Fig. 4. All the product ions observed in the CID spectrum of $[M-H+2Na]^+$ correspond to sodium-attached ions. In the low-mass region of the positive-ion CID spectrum shown in Fig. 3(a), product ions characteristic of the head group are found, as in the CID spectra of $[M+Li]^+$ of neutral glycosphingolipids.[10] The product ions generated by the cleavages of the sugar unit are observed at *m/z* 169 (^{0,4}A), 183 (^{3,5}A) and 243 (^{1,5}A). The other product ions at *m/z* 271 (B), 287 (C) and 329 (E) in this region

Table 1. Structures of both fatty acyl groups of glycolipids interpreted from FAB-CID-MS/MS

Compound	MW	$[M+Na]^+$ or $[M-H+2Na]^+$	$[M-H]^-$	Structure[a]
SQDG	820.5	865.5	819.6	18:1(oleic)/16:0
	818.5	863.5	817.5	18:2(linoleic)/16:0
	794.5	839.5	793.5	16:0/16:0
	792.5	837.5	791.5	16:1(palmitoleic)/16:0[b]
DGDG	940.6	963.5	939.5	18:2(linoleic)/18:2(linoleic)
	916.6	939.5	915.5	16:0/18:2(linoleic)
MGDG	778.6	801.5	777.4	18:2(linoleic)/18:2(linoleic)

[a] The structure of each fatty acyl group at *sn*-1/*sn*-2 positions, respectively.
[b] Regiospecificity of fatty acyl groups not determined.

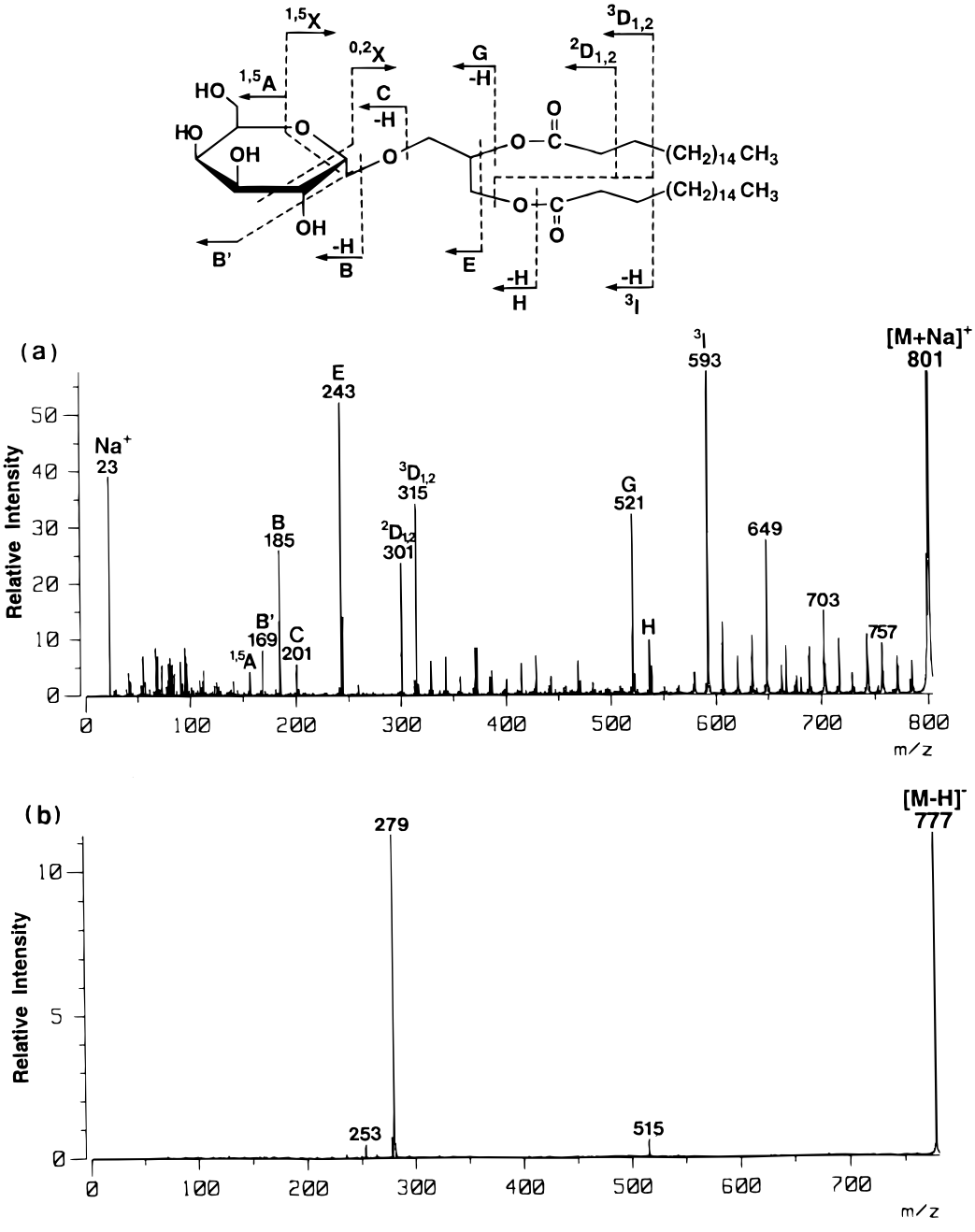

Figure 6. (a) Positive-ion FAB tandem mass spectrum of $[M+Na]^+$ (at *m/z* 801) and (b) negative-ion FAB tandem mass spectrum of $[M-H]^-$ (at *m/z* 777) for MGDG.

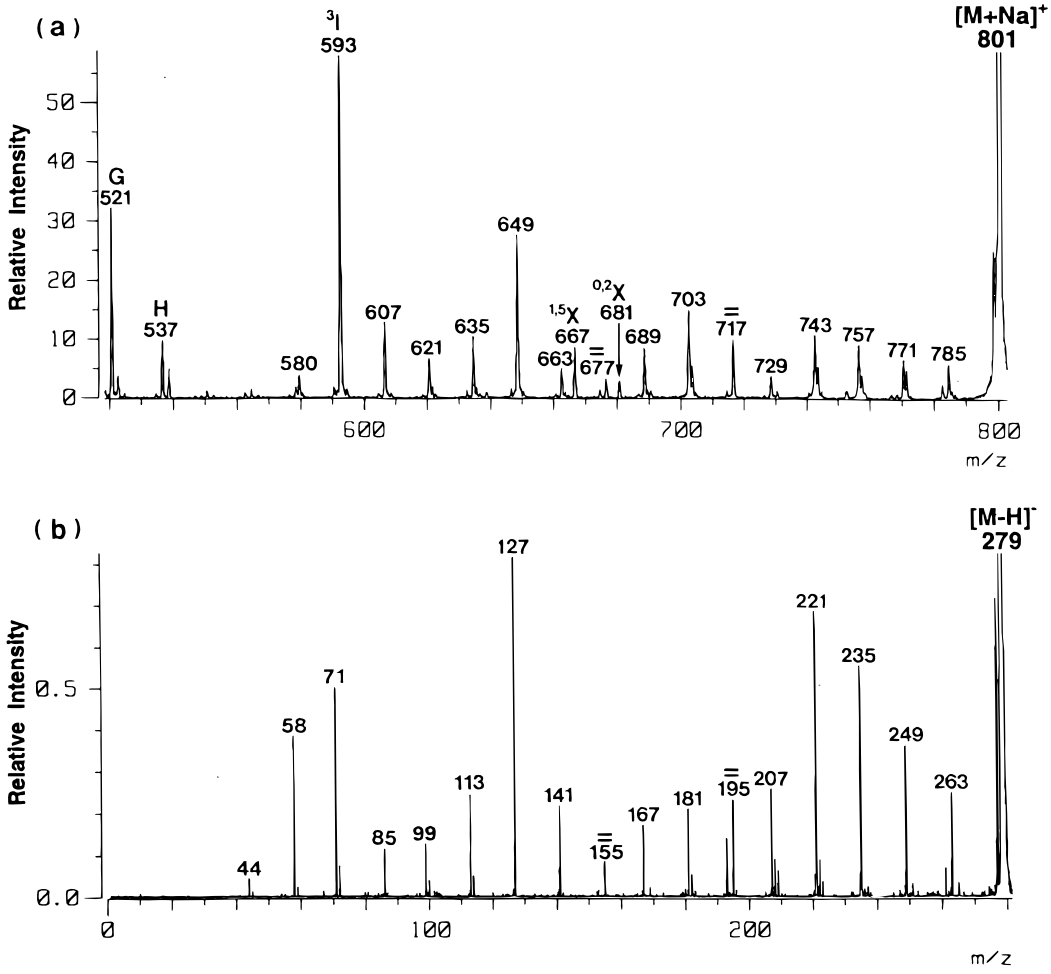

Figure 7. (a) High-mass region of CID mass spectrum of $[M + Na]^+$ at *m/z* 801 generated by fast atom bombardment of MGDG and (b) negative-ion FAB CID mass spectrum of $[M - H]^-$ (at *m/z* 279) of linoleic acid.

can be easily identified by referring to the previous assignments[6] for the corresponding ions at *m/z* 225 (B), 241 (C) and 283 (E) in the negative-ion CID spectrum. Also, the concurrent cleavages of the C(2)—OH bond of the sugar unit and the interglycosidic bond result in the product ion at *m/z* 255 (B′), as shown in Fig. 4. Finally, two prominent ions resulting from the concurrent cleavage of the bond β or γ to the carbonyl group of one fatty acyl chain and the cleavage of the ester bond of the other fatty acyl chain are observed at *m/z* 387 ($^2D_{1,2}$) and 401 ($^3D_{1,2}$), respectively. The product ions observed in the high-mass region are mainly produced by the fragmentation of fatty acyl groups. Among these ions, two prominent product ions are observed at *m/z* 583 (G_1) and 609 (G_2) which result from the loss of each fatty acyl group as the corresponding free fatty acid (—RCOOH). Also, the other two ions at *m/z* 599 (H_1) and 625 (H_2) result from the loss of each fatty acyl group as the corresponding aldehyde (—RCOH). Thus, these ions provide information about the fatty acid composition. Based on the fact that position 2 of the glycerol backbone is occupied almost exclusively by 16 carbon fatty acids for prokaryotic organisms such as cyanobacteria,[3,11] the major molecular species of SQDG contains 18:1 and 16:0 (palmitic) acids at the *sn*-1 and *sn*-2 positions, respectively. An important spectral feature in the high-mass region of the positive-ion CID spectrum is the presence of a homologous series appearing at *m/z* 655, 669, 681, 683, 695, 697, ... These correspond to the elimination of alkene and H_2 by charge-remote fragmentation of the fatty acyl chains. Similar fragmentations along hydrocarbon chains remote from the charge site have previously been used for the determination of the double-bond position.[12]

Figure 5 shows the high-mass region of the CID spectra of SQDG obtained from $[M - H + 2Na]^+$ at *m/z* 865 and $[M - H]^-$ at *m/z* 819. The negative-ion CID spectrum of deprotonated oleic acid (9-octadecenoic acid) with *m/z* 281 is also shown for ease of explanation. The fragmentation along the alkyl chain of a fatty acyl group results in a series of product ions generated by the loss of C_nH_{2n+2} via 1,4-elimination, with the neighboring peaks in the series separated by 14 u. The presence of a double bond in the chain reduces the neighboring peak separation to 12 u. A similar series is observed for $[M - H]^-$ of a free fatty acid also [Fig. 5(c)].[13] These homologous ions in Fig. 5(a) generated by the charge-remote fragmentation of each fatty acid chain can be divided into two groups, *m/z* 737, 723, 709, 695 and 681 ($^3I_2$) due to a saturated fatty acyl group (16:0) and *m/z* 739, 725, 711, 697, 683, 669 and 655 ($^3I_1$) due to an unsaturated fatty acyl group (18:1). The ions at *m/z* 849, 835, 821, 807, 793, 779, 765 and 751 are common to the cleavage of both fatty acyl groups.

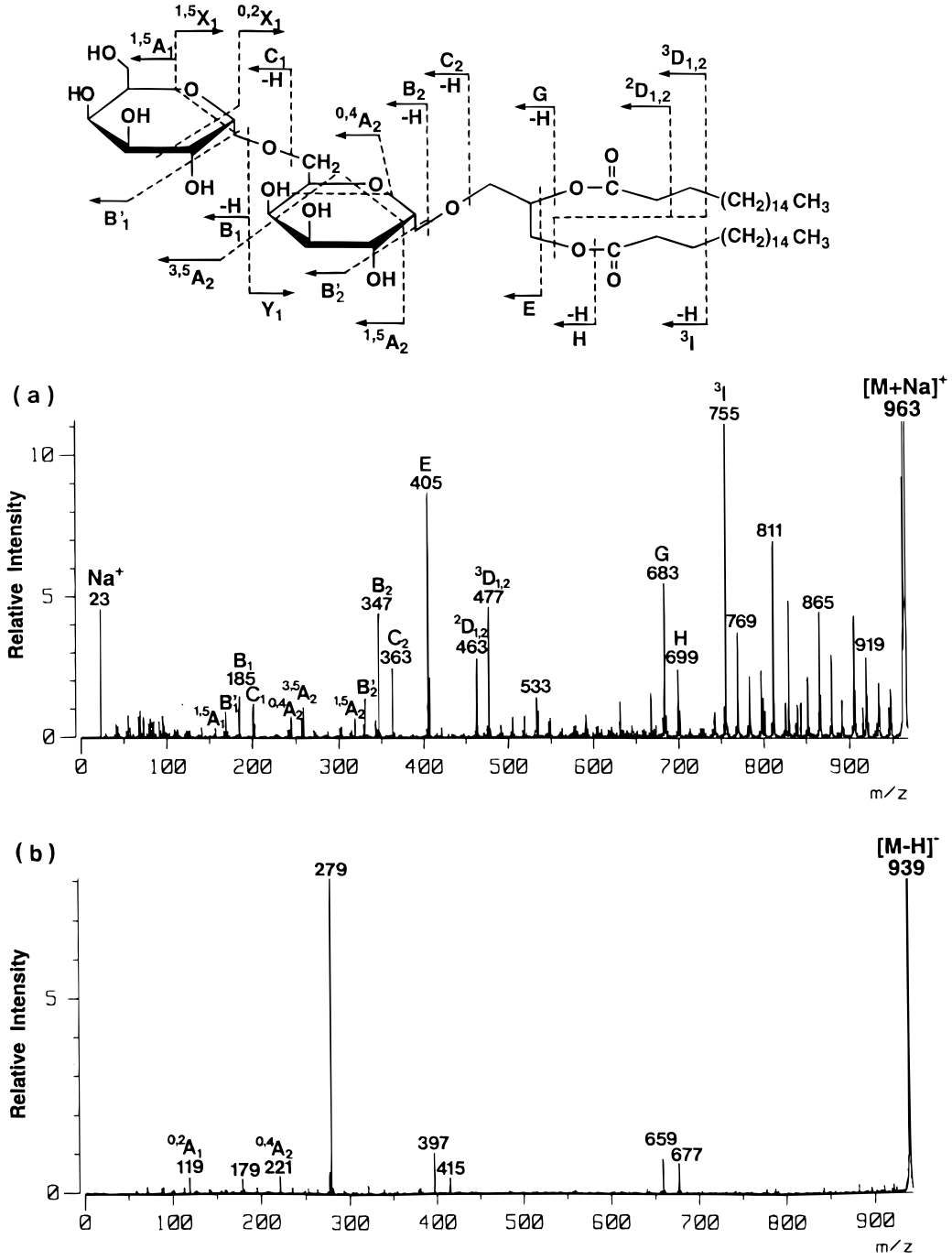

Figure 8. (a) Positive-ion FAB tandem mass spectrum of $[M + Na]^+$ (at *m/z* 963) and (b) negative-ion FAB tandem mass spectrum of $[M - H]^-$ (at *m/z* 939) for DGDG.

Thus, comparing the homologous ions produced by the fragmentation of the 18:1 fatty acyl group with those observed in Fig. 5(c), the position of the double bond in the 18:1 fatty acid chain is the same as that in the oleic acid. The negative-ion CID spectrum of the deprotonated molecular ion shown in Fig. 5(b) is very similar to that obtained in the positive-ion mode. The intensities of the corresponding peaks in the series in Fig. 5(a) and 5(c) are also comparable except for the *m/z* 655 generated by the loss of $C_{15}H_{30}$. This means that the relative peak intensities in addition to the mass difference between neighboring ions are also helpful in the determination of the double-bond position in a fatty acid chain. In addition to the major component of SQDG already analyzed, there are several other species, as can be noticed by the presence of minor peaks in the molecular ion regions of both the positive- and negative-ion FAB spectra. Among these, the peak at *m/z* 861 in the positive-ion FAB spectrum (or at *m/z* 815 in the negative-ion FAB spectrum) arises from a mixture of compounds with the fatty acid composition 18:3/16:0 and 18:2/16:1. Except for this species, the structural analysis carried out for the major species is applicable. The results are summarized in Table 1.

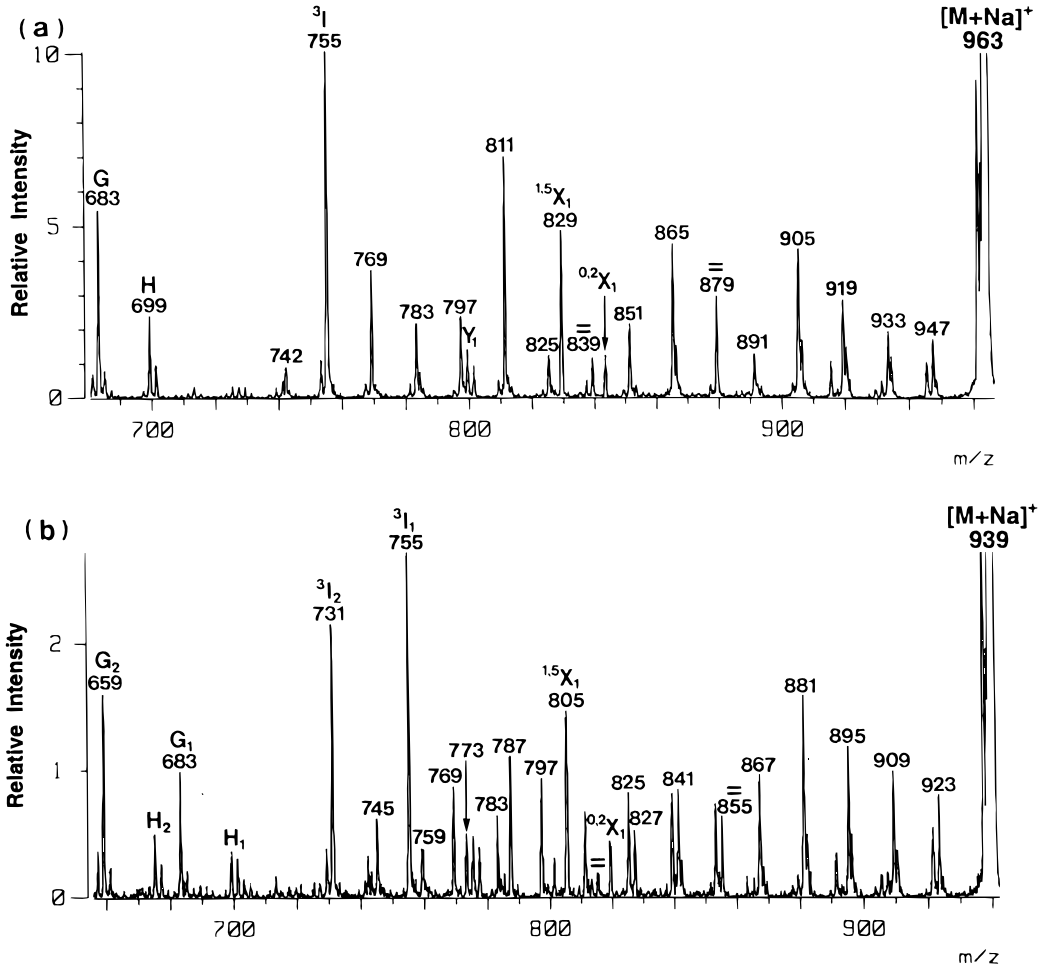

Figure 9. High-mass region of CID tandem mass spectra of $[M + Na]^+$ at *m/z* (a) 963 and (b) 939 generated by fast atom bombardment of DGDG.

The positive-ion FAB mass spectrum (not shown) of MGDG follows a similar trend to that of SQDG. The molecular ion region of this spectrum is dominated by the peak at *m/z* 801, which corresponds to $[M + Na]^+$ containing the fatty acyl chains with a total of 36 carbons and four unsaturations. The positive- and negative-ion CID tandem mass spectra of $[M + Na]^+$ (at *m/z* 801) and $[M - H]^-$ (at *m/z* 777) are shown in Fig. 6(a) and 6(b), respectively. The fragmentation pathway for $[M + Na]^+$ of MGDG is similar to that for $[M - H + 2Na]^+$ of SQDG, most of the peaks in Fig. 6(a) being generated by the charge-remote fragmentations of the head group and the fatty acyl groups. The proposed pathway is shown in the same figure. Information on the double-bond positions in the fatty acyl chain is also available from the spectrum, which will be explained later. The CID spectrum of $[M - H]^-$ shown in Fig. 6(b) is much simpler than that of $[M + Na]^+$. However, useful structural information concerning the fatty acid compositions is available from the spectrum, as in a previous study of phospholipids.[14,15] The peak at *m/z* 279 can be assigned to a carboxylate ion with the composition 18:2. The appearance of only one carboxylate ion peak means that the compositions of the two fatty acyl chains are the same. Then the loss of one fatty acyl group in the form of its ketene analog (R=C=O) would result in a peak at *m/z* 515, in complete agreement with the spectrum. Further loss of the other fatty acyl moiety of the *m/z* 515 ion as the ketene analog yields an ion at *m/z* 253.

Figure 7(a) shows the high-mass region of the CID spectrum of $[M + Na]^+$ of MGDG. The CID spectrum of $[M - H]^-$ of linoleic acid (9,12-octadecadienoic acid) with *m/z* 279 is also shown in Fig. 7(b). The neutral loss pattern in the homologous ion series appearing at *m/z* 785, 771, 757, 743, 729, 717, 703, 689, 677, 663, ... in Fig. 7(a) is identical with that at *m/z* 263, 249, 235, 221, 207, 195, 181, 167, 155, 141, ... in Fig. 7(b). This suggests that the double-bond positions in the two fatty acyl chains of MGDG are the same as those in the linoleic acid.

The above spectral interpretation can be extended to DGDG which carries a disaccharide moiety. The positive-ion FAB mass spectrum (not shown) of DGDG shows two abundant peaks at *m/z* 939 and 963 which are $[M + Na]^+$ ions of the molecular species present. Two $[M - H]^-$ ions are also observed at *m/z* 915 and 939 in the negative-ion FAB spectrum (not shown). The *m/z* 963 and 939 peaks in the positive- and negative-ion spectra, respectively, are due to a molecular species with a total fatty acid composition of 36:4. The remaining pair is due to a molecular species with the 34:2 composition. The CID spectra of $[M + Na]^+$ (at *m/z* 963) and $[M - H]^-$ (at *m/z* 939) are shown in Fig. 8(a) and

8(b), respectively. Their spectral patterns are more complicated than those of MGDG because of the fragmentation of the disaccharide sugar unit. Almost all the product ions observed in Fig. 8(a) can be assigned by referring to the pathways presented for SQDG and MGDG. The proposed pathway for the fragmentation of $[M + Na]^+$ of DGDG is also shown in Fig. 8. In the negative-ion CID spectrum of $[M - H]^-$ at *m/z* 939, a prominent carboxylate ion peak appears at *m/z* 279 just as in the spectrum of MGDG [Fig. 6(b)]. Hence, both the fatty acyl chains of $[M - H]^-$ (at *m/z* 939) of DGDG have 18:2 compositions as in MGDG.

Figures 9(a) and 9(b) show the high-mass region of the CID spectra of $[M + Na]^+$ of DGDG at *m/z* 963 and 939, respectively. The fragmentation pattern in the high-mass region of the CID spectrum of the *m/z* 963 ion is the same as that of MGDG. Thus, this $[M + Na]^+$ ion contains two linoleic acids as the fatty acid chains. Homologous ions appearing in the CID spectrum of the *m/z* 939 ion shown in Fig. 9(b) can be divided into two groups, *m/z* 853, 839, 825, 811, 797, 783, 769 and 755 ions due to a saturated fatty acyl chain (16:0) and *m/z* 855, 841, 827, 815, 801, 787, 773, 759, 745 and 731 ions due to a doubly unsaturated fatty acyl chain (18:2). The ions at *m/z* 923, 909, 895, 881 and 867 are common to the cleavage of both fatty acyl groups. The neutral losses in the second homologous series are identical with those for linoleic acid. Thus the $[M + Na]^+$ ion at *m/z* 939 contains palmitic (16:0) and linoleic (18:2) acids which are likely to be attached at the *sn*-1 and *sn*-2 positions, respectively, according to previous studies.[3,11] Since all the ions corresponding to the cleavage of the sugar moiety have Na^+ attached, the positive charge is probably localized on the sugar moiety, especially on the terminal sugar.

CONCLUSION

The localization of the sodium ion (or ions) on the sugar moiety in sodium-adduct molecular ions of glycolipids induces charge-remote fragmentations which are useful to determine the structure of the fatty acyl group as well as the head group and fatty acid composition. In particular, the fragmentation along the hydrocarbon chains remote from the charge site allows the determination of the double-bond position in the fatty acyl groups. Thus, a complete structural analysis of the fatty acyl groups as well as the head group of the glycolipids was possible. Also, investigation on SQDG has demonstrated the potential of the present method for the structure elucidation of individual components present in a mixture of compounds with different fatty acyl groups in samples of biological origin. We are expanding our studies to the determination of the structures of phospholipids[7] and diverse lipids isolated from cyanobacteria.

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