Structural Determination of Fatty Acyl Groups of Phospholipids by Fast Atom Bombardment Tandem Mass Spectrometry of Sodium Adduct Molecular Ions

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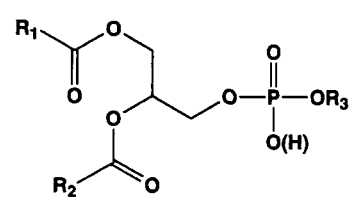
Various classes of phospholipids were investigated for the structural determination of fatty acyl groups by fast atom bombardment tandem mass spectrometry (FAB-MS/MS). Phospholipids were desorbed by FAB as molecules chelated with sodium ion (or ions). Collision-induced dissociation (CID) of intact sodium adduct molecular ions ($[M+Na]^+$, $[M-H+2Na]^+$ or $[M+Na-2H]^-$) produced a series of homologous fragment ions via the charge-remote fragmentation along the fatty acid chains. These ions were found useful to locate the double bond positions even for the polyunsaturated fatty acid chains. The regiospecificity of the acyl chain linkages in phosphatidylcholine (PC) could also be determined based on the ratio of relative abundance of the product ions (*i.e.*, $[M+Na-85-R_2COOH]^+$ *vs* $[M+Na-85-R_1COOH]^+$) in CID-MS/MS of $[M+Na]^+$. These are generated by the loss of fatty acyl groups at *sn*-1 and *sn*-2, respectively, together with the choline group. In all the phospholipid compounds investigated, loss of the fatty acid at the *sn*-2 position was dominant. The present method was applied to the structural determination of molecular species of phosphatidylglycerols (PG) isolated from cyanobacterium *Synechocystis* sp. PCC 6803.

Introduction

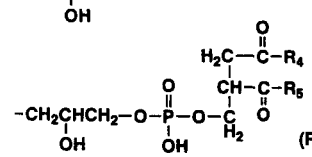
Phospholipids are significant and major components of cell membranes in living organisms due to their amphipathic property.[1] In addition to their structural role in biological membranes, some phospholipids such as phosphatidylinositol and its phosphorylated derivatives, participate in cellular signal transduction processes as second messenger molecules.[2,3] Arachidonic acid released from membrane phospholipids serves as a precursor to potent hormonelike active eicosanoids such as prostaglandins, thromboxanes, and leukotrienes.[4,5] The chemical structures of phospholipids are characterized by two hydrophobic fatty acyl chains esterified at *sn*-1 and *sn*-2 of glycerol, and a polar head group joined at *sn*-3 of glycerol through phosphodiester bond (See Figure 1). The fatty acid composition of lipid mixture in cell membrane has an effect on the degree of fluidity in membrane.[6] Because saturated fatty acids pack well into paracrystalline array in membrane while the kinks in unsaturated fatty acids interfere with this packing, the fluidity of cell membrane increases with the degree of unsaturation in the fatty acids. Thus, the fatty acid compositions of the molecular species of lipid mixture are of great interest.

Fast atom bombardment (FAB) ionization has proven to be a useful technique for mass spectral analysis of phospholipids.[7-9] Also, the usefulness of FAB in combination with tandem mass spectrometry (MS/MS) has been demonstrated for the structural analysis of the molecular species of phospholipids.[10-15] FAB-MS/MS investigation of phospholipids has been focused on the analyses of the negative ions because FAB-MS/MS of positive ions does not provide sufficient information for the structural analysis.[11] In particular, the negative-ion FAB-MS/MS of deprotonated molecules ($[M-H]^-$) and FAB-desorbed fragment ion corresponding to the phosphatidic acid analog was found useful for obtaining information on the fatty acid composition and regiospecificity of fatty acyl chain linkages, respectively.[16] In addition, the CID spectra of FAB-desorbed fatty acid carboxylate anions,[17,18] and GC/MS analysis of the fatty acid methyl ester derivatives prepared by methanolysis[19] were useful in elucidating the structures (especially, positions of unsaturated bonds) of fatty acyl groups. However, phospholipids purified from biological sources are often mixtures of many molecular species which differ in the chain length of fatty acid, and the position and degree of unsaturation of fatty acyl groups.[20,21] For example, if two isomeric acid carboxylates (*e.g.*, oleic and vaccenic) are liberated



R_1, R_2 : Fatty acid chain

R_3	:	$-H$	Phosphatidic acid (PA)
		$-CH_2CH_2NH_2$	Phosphatidylethanolamine (PE)
		$-CH_2CH_2\overset{+}{N}(CH_3)_3$	Phosphatidylcholine (PC)
		$-CH_2\underset{OH}{CH}CH_2OH$	Phosphatidylglycerol (PG)



Cardiolipin (R_4, R_5 : Fatty acid chain)

Figure 1. Structures of phospholipids investigated.

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from two different components of mixture, it is impossible to determine which of the two components of mixture contain the carboxylate ions.[12] In this case, use of MS/MS/MS technique with a four-sector instrument has been helpful.[22] However, structural information decreases with the degree of unsaturation. It is widely accepted that the technique is useful for fatty acids containing three double bonds at the most.[14,23]

Recently, we reported that the positions of the double bonds in the fatty acid chains of glyceroglycolipids could be determined from a series of charge-remote fragmentation occurring along the fatty acid chains which generate peaks in the high-mass regions of the CID spectra of sodium adduct molecular ions ($[M+Na]^+$ or $[M-H+2Na]^+$).[24] As an extention of the previous work, we report in this paper the structural analysis for the fatty acyl groups in various classes of phospholipids using FAB tandem mass spectrometry. Also, the utility of the FAB-MS/MS for the structural determination of phospholipids in mixtures of biological origin is demonstrated.

Experimental

Materials. All phospholipids used for this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Samples of phosphatidylglycerol (PG) were purified from total lipid extract of cyanobacterium *Synechocystis* sp. PCC 6803. All solvents and reagents were the highest grade commercially available.

Isolation of Phosphatidylglycerols from Cyanobacteria. Cyanobacterium *Synechocystis* sp. PCC 6803 was grown in BG11 media containing 10 mM glucose at 28 °C under continuous fluorescence light. Cyanobacterial cells harvested from the liquid suspension were blended in 300 mL of chloroform/methanol/water (1 : 1 : 1, v/v/v). The total lipids were extracted by the method of Bligh and Dyer.[25] The final extracts in chloroform phase were fully evaporated and the residue was reconstituted in 2 mL of chloroform/methanol (1 : 1, v/v). According to the method of Douce and Joyard,[26] the extract was purified by two-dimensional TLC on silica plate (Whatman, Hillsboro, OR, USA) using chloroform/methanol/water (65 : 25 : 4, v/v/v) as solvent for dimension I and chloroform/acetone/methanol/acetic acid/water (10 : 4 : 2 : 2 : 1, v/v/v/v/v) for dimension II. The PG band was scraped from the plate after identification under ultraviolet light and eluted with chloroform/methanol (1 : 1, v/v).

Fast atom bombardment mass spectrometry. FAB mass spectra were taken with the first (MS-1) of the two mass spectrometers of a JMS-HX110/110A tandem mass spectrometer (JEOL, Tokyo, Japan) using a JMS-DA 9000 data system. The ion source was operated at 10 and − 10 kV accelerating voltage in the positive- and negative-ion modes, respectively, with the mass resolution of 1000 (10% valley). Ions were produced by fast atom bombardment using the cesium ion gun operated at 22 kV. Approximately 10 μg of each sample dissolved in chloroform/methanol (1 : 1, v/v) was mixed with 1 μL of 3-nitrobenzyl alcohol (NBA, 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. Sodium adduct molecular ions were generated for the samples in NBA matrix saturated with NaI (Sigma). Calibration was performed with CsI (Sigma) in the positive-ion mode and Ultramark 1621 (PCR, Florida, USA) in the negative-ion mode as standards.

For FAB exact mass measurements, high voltage scanning method at a resolution 10000 (10% valley) was employed. Each sample (1 μL) was mixed with 1 μL of 1% solution of polyethylene glycol (PEG 300, Sigma) in glycerol containing 1% TFA as an internal standard.[27]

Tandem mass spectrometry. MS/MS was carried out by using the 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 two scans was carried out. MS-1 was operated at the resolution adjusted so that only the ^{12}C-species of the precursor ions 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 the positive-ion mode, and with a solution of CsI in water/glycerol (2 : 1, v/v) in the negative-ion mode.[28]

Results and Discussion

The typical positive-ion FAB mass spectrum of phos-

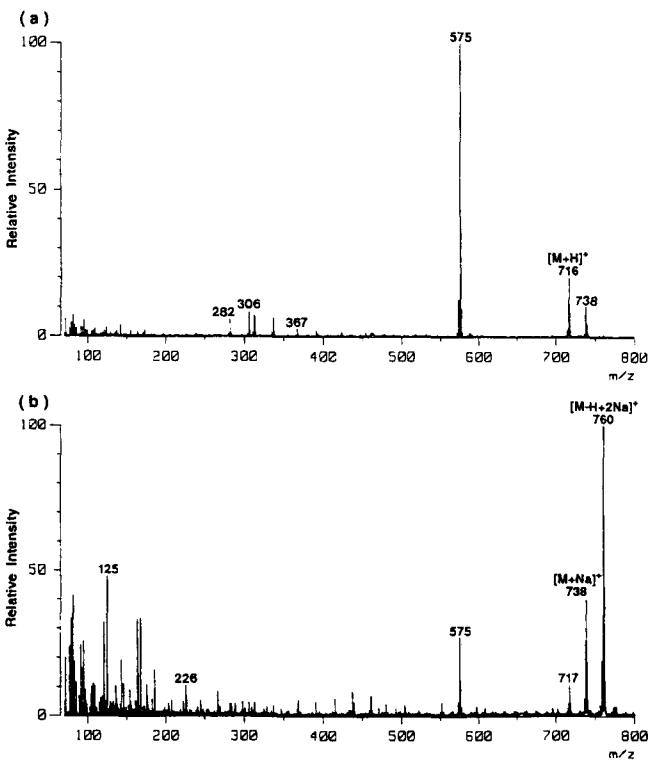

Figure 2. Positive-ion mass spectra obtained by fast atom bombardment of 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylethanolamine in (a) pure NBA and (b) NBA matrix saturated with NaI, respectively.

phatidylethanolamine (PE) shown in Figure 2(a) displays abundant protonated molecule ($[M+H]^+$, m/z 716), monosodiated ion ($[M+Na]^+$, m/z 738), and fragment ions. The most intense peak at m/z 575 corresponds to the diglyceride ion generated by the loss of phosphoethanolamine head group.[20] As noted in Figure 2(b), fast atom bombardment of PE in a matrix of NBA saturated with NaI produces an intense peak of disodiated molecular ion ($[M-H+2Na]^+$, m/z 760). The absolute abundance of disodiated ion in Figure 2(b) is comparable to that of $[M+H]^+$ ion in Figure 2(a). The CID spectrum of $[M-H+2Na]^+$ ion of 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoethanolamine (16 : 0/18 : 2-PE) is shown in Figure 3. The compositions of each fatty acyl groups will be denoted by the number of carbon atoms: number of double bonds. For example, 16 : 0/18 : 2 means the fatty acyl groups at the *sn*-1 and *sn*-2 positions are palmitoyl and linoleoyl groups, respectively. In contrast to the low-energy CID spectrum of $[M+H]^+$ reported previously in which the diglyceride ion was the sole product,[29] the high-energy CID spectrum of $[M-H+2Na]^+$ ion shows a variety of product ions. The product ions appearing below m/z 200 are formed by the fragmentation of the phosphoethanolamine head group. There are $PO_3Na_2^+$ (m/z 125), $H_2PO_4Na_2^+$ (m/z 143), $[HOP(O)(ONa_2)OCH_2]^+$ (m/z 156), $[CH_3OP(O)(ONa_2)OCH{=}CH_2]^+$ (m/z 183), and $[HOP(O)(ONa_2)OCH_2CH_2NH_2]^+$ (m/z 186). In addition, an intense peak is observed at m/z 168 which corresponds to disodiated five-membered amidophosphane.[30] Presence of these ions indicates that the positive charge is localized at the phosphate group due to the attachment of two sodium ions. The remainder of peaks in the spectrum are produced by the cleavages of fatty acyl chains and display the spectral pattern very similar to that observed in the CID spectra of sodium adduct molecular ions of glyceroglycolipids reported previously.[24] The fragmentation pathways are illustrated in Figure 3 also and nomenclatures are taken from Ref. 24. The series of homologous product ions in high-mass region arise from the charge-remote decompositions along the fatty acid chains *via* 1,4-elimination as observed in the CID spectra of $[M-H]^-$ ions of free fatty acids previously.[31] These ions give information about the positions of double bonds as well as the composition of each fatty acid group. Each pair of abundant G and I ions provides information on the compositions of two fatty acyl groups. The homologous product ions can be divided into two groups; m/z 674, 660, 646, 632, 618, 604, 590 and 576 ($^3I_1$) due to saturated palmitic acyl chain (16 : 0), and 676, 662, 648, 636, 622, 608, 594, 580, 566 and 552 ($^3I_2$) due to unsaturated linoleic acyl chain (18 : 2). The ions at m/z 744, 730, 716, 702 and 688 are common to the cleavage of both fatty acyl groups. Each group begins with the product ion (m/z 744) corresponding to the loss of CH_4 from the alkyl-terminus side and ends with an abundant ^3I ion corresponding to stable α,β-unsaturated ester. A series of the product ions in each group show the same tendency of relative peak intensities as well as the mass difference between neighboring ions with those observed in the negative-ion CID spectra[24] of carboxylate of standard palmitic and linoleic acids, respectively.

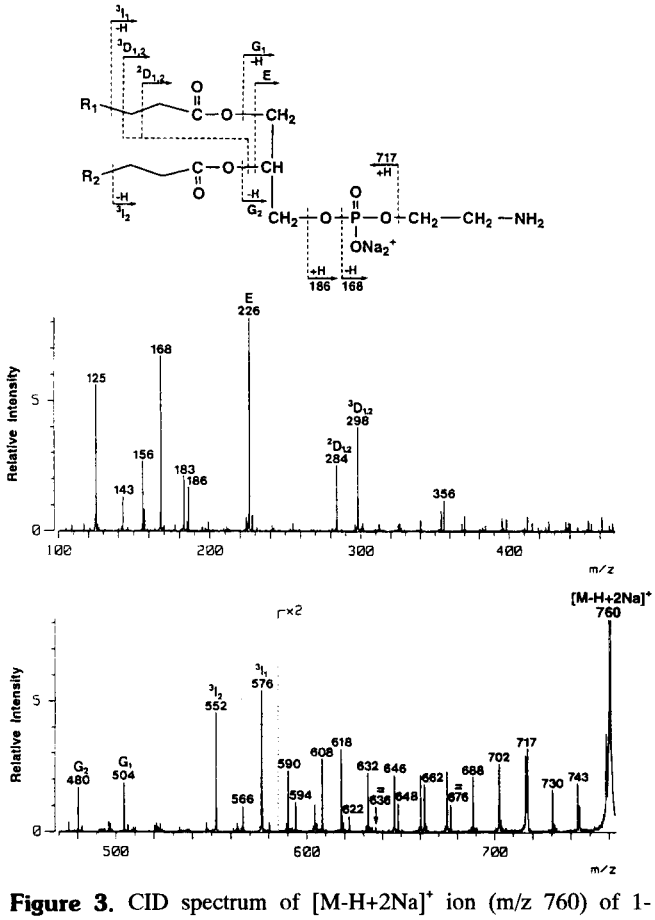

Figure 3. CID spectrum of $[M-H+2Na]^+$ ion (m/z 760) of 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylethanolamine and proposed fragmentation pathways for product ions. Nomenclatures are taken from Ref. 24. All the product ions are attached with two sodium ions. The sign (=) above some peaks indicates the double bond position.

Even for the phospholipids with polyunsaturated fatty acyl group, the product ions due to the charge-remote fragmentation along the highly unsaturated acid chain are observed. High-mass region of the CID spectrum of $[M-H+2Na]^+$ parent ion (m/z 769) of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphatic acid (18 : 0/20 : 4-PA) is shown in Figure 4(a). For the ease of explanation, the CID spectra of $[M-H+2Na]^+$ (m/z 349) and $[M-H]^-$ (m/z 303) of arachidonic acid are shown in Figures 4(b) and (c), respectively. The decomposition patterns of $[M-H+2Na]^+$ and $[M-H]^-$ ions of highly unsaturated fatty acid are significantly different. Locating double bonds by CID of $[M-H]^-$ ions of polyunsaturated acids becomes impossible for fatty acids containing four or more double bonds [Figure 4(c)].[14,23] In contrast, the fragmentations of $[M-H+2Na]^+$ ion of arachidonic acid are distinctly indicative of double bond positions [Figure 4(b)]. A series of homologous ions due to charge-remote cleavages of arachidonic acid group (20 : 4) in high-mass region of CID spectrum of $[M-H+2Na]^+$ parent ion for 18 : 0/20 : 4-PA appear at m/z 537, 551, 565 (not found), 577, 591, 605, 617, 631, 645, 657, 671, 685, 697, 711, 725, 739 and 753. Their spectral feature is very similar to that of product ions at m/z 117, 131, 145 (not found), 157, 171, 185, 197, 211, 225, 237, 251, 265, 277, 291, 305, 319 and 333 observed in the CID spectrum of $[M-H+2Na]^+$ ion of free arachidonic acid. Hence, these results show that the CID spectral analysis of sodium adduct molecular ion pro-

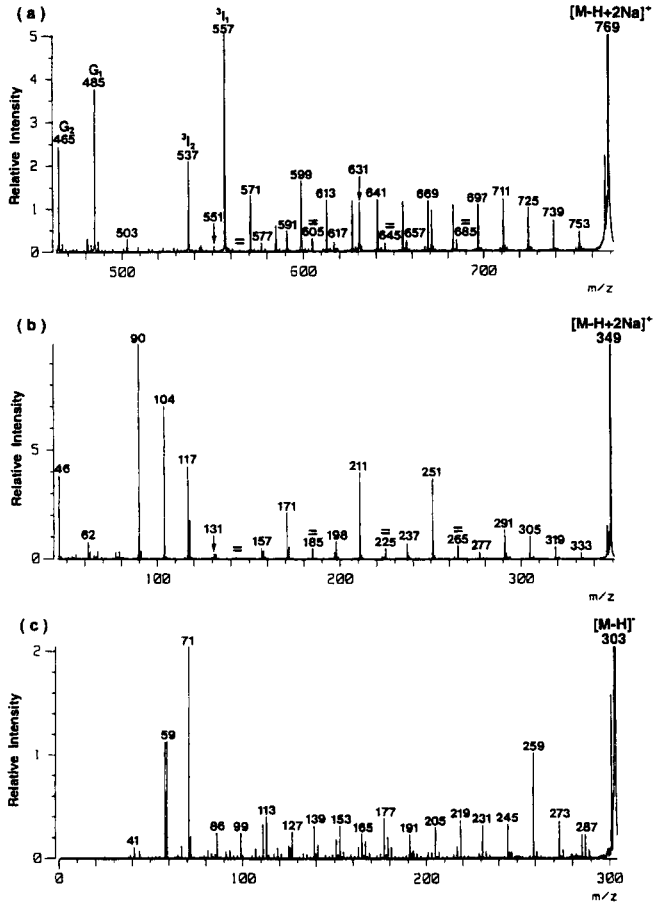

Figure 4. (a) High-mass region of the CID spectrum of $[M-H+2Na]^+$ ion (m/z 769) of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphatic acid. CID spectra of (b) $[M-H+2Na]^+$ (m/z 349) and (c) $[M-H]^-$ (m/z 303) of free arachidonic acid, respectively.

vides information on the positions of double bonds even when four double bonds are on the fatty acyl group of intact phospholipid precursor.

The CID spectrum of $[M+Na-2H]^-$ ion (m/z 1470) of bovine heart cardiolipin is shown in Figure 5. Its structure is characterized by the combination of phosphatidylglycerol (PG) and phosphatidic acid (PA) through the phosphoester bond between glycerol head group and phosphoric acid group as shown in Figure 1. Only one series of charge-remote product ions along fatty acid chains such as m/z 1454, 1440, 1426, 1412, 1398, 1386, 1372, 1358, 1346, 1332, 1318, ..., etc. appears in the high-mass region, which is similar to the one observed in the CID spectrum of $[M-H]^-$ ion of linoleic acid.[24] Thus, this result and the presence of 18 : 2 carboxylate ion at m/z 279 indicate that all four fatty acyl groups are linoleoyl chains. The same conclusion was made in the previous studies.[12,30] In addition to the above series, the radical cations generated by the homolytic cleavages along the fatty acid chains are observed at m/z 1455, 1441, 1427, 1413 and 1373. Among these ions, the peaks at m/z 1413 and 1373 display abundances higher than those of neighboring peaks due to the conjugation of the resulting radical sites with the double bond. Each of the sodiated product ions observed at m/z 853, 871, 911, 969 and 983 can be correlated to the fragments at m/z 168, 186, 226, 284

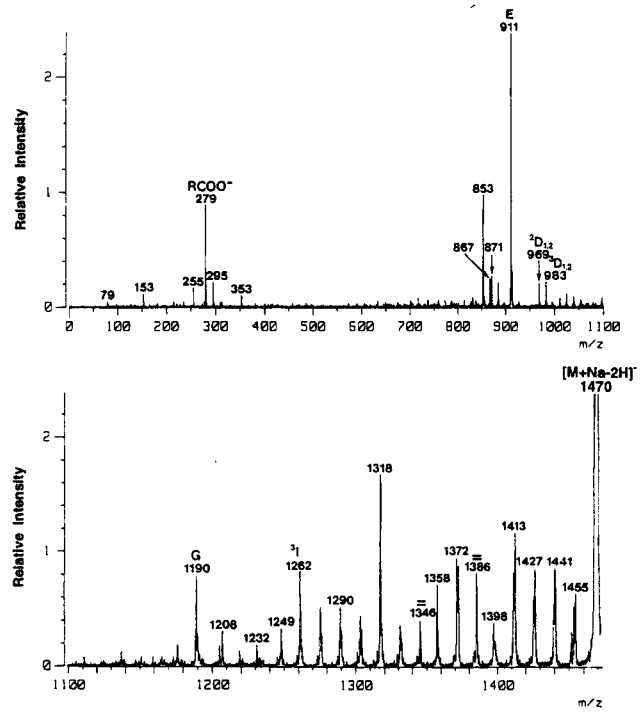

Figure 5. Negative-ion FAB tandem mass spectrum of $[M+Na-2H]^-$ parent ion (m/z 1470) of bovine heart cardiolipin. All the product ions are attached with a sodium ion.

and 298 in the CID spectrum of PE shown in Figure 3, respectively.

FAB of phosphatidylcholine (PC) in a matrix of NBA saturated with NaI results in abundant $[M+Na]^+$ ion. Due to the zwitterionic property that the positive and negative charges are on the quaternary nitrogen atom of choline and oxygen atom of phosphate group, respectively, $[M-H+2Na]^+$ can not be generated. The CID spectrum of $[M+Na]^+$ ion (m/z 782) of 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (18 : 1/16 : 0-PC) is shown in Figure 6. The choline and sodiated five-membered cyclophosphane ions characteristic of phosphocholine head group appear at m/z 86 and 147, respectively. The fragmentation pathways for the production of other major ions in the spectrum can be easily assigned by comparing with those for the product ions observed in the CID spectra of $[M-H+2Na]^+$ ions of phospholipids mentioned above. However, the product ions at m/z 415 and 441 are not observed in their CID spectra and will be discussed later. There are also a series of high-mass fragment ions formed by charge-remote cleavages of fatty acyl chains.

In the structural analysis of phospholipids, it is important to determine the relative positions of the two different fatty acyl moieties in the compounds. Jensen *et al.*[11,12] pointed out that the intensity of the $[R_2COO]^-$ carboxylate ion formed from the $[M-15]^-$ precursor of PC is always greater than that of the $[R_1COO]^-$. But Huang *et al.*[16] found that this empirical rule was unreliable in cases where the fatty acyl group esterified at *sn*-2 contained more than three double bonds or where short-chain fatty acid was esterified at *sn*-1 or *sn*-2 positions. As an alternative, they proposed to use the abundance ratio (*i.e.*, $[M-86-R_2COOH]^-/[M-86-R_1-COOH]^-$) of the product ions due to the loss of two fatty

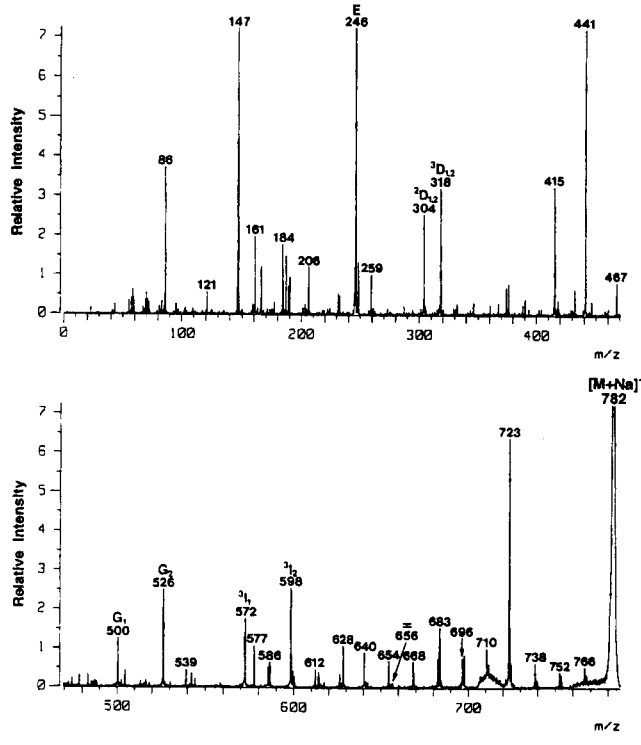

Figure 6. CID spectrum of $[M+Na]^+$ ion (m/z 782) of 1-oleoyl-2-palmitoyl-*sn*-3-glycero-phosphocholine.

acyl groups in the CID spectrum of the $[M-86]^-$ ion (PC minus choline), which is larger than 1. In the present study, abundant product ions that contain information about the regiospecificity of fatty acyl linkages are observed in the CID spectra of $[M+Na]^+$ of PC. These fragments correspond to sodiated 1,2- or 2,3-unsaturated lysophosphatidic acids produced by the loss of either one of the two fatty acyl groups at *sn*-1 and *sn*-2 positions as free acid together with the choline group. In CID spectrum of 18:1/16:0-PC shown in Figure 6, they appear at m/z 415 and 441 due to the loss of free oleic or palmitic acid together with the choline group, respectively. The proposed formulas of the ions of m/z 415 and 441 are confirmed as $C_{19}H_{37}O_6PNa$ (theoretical, 415.2225; observed, 415.2224) and $C_{21}H_{39}O_6PNa$ (theoretical, 441.2382; observed, 441.2379), respectively, by high resolution mass measurements. Intensity of the product ion that corresponds to the loss of *sn*-2 acyl component (m/z 441) is greater than that of the ion derived from *sn*-1 component (m/z 415). This trend is generally observed in the CID spectra of $[M+Na]^+$ of other PCs. Hence, $[M+Na-85-R_2COOH]^+/[M+Na-85-R_1COOH]^+$ ratio can be used to determine the regiospecificity of fatty acyl groups. In addition, two fragments (G_1 and G_2) in the same spectra corresponding to sodiated 1,2- and 2,3-unsaturated lysophosphatidylcholine display similar trend and can be used as the secondary guideline in the determination of the regiospecificity. As was mentioned above, this empirical rule is obeyed for all the commercially available PC compounds including those containing polyunsaturated fatty acyl groups or those containing the two fatty acyl groups with widely different chain lengths (See Table 1). To compare the validity of the empirical rules mentioned above,[11,12,16] the above ratios have been measured by using four-sector tandem mass spectrometry. The results are summarized in Table 1. It should be noted that some exceptions to the empirical rule are observed for the ratios obtained from the negative-ion FAB-MS/MS. In contrast, the ratios obtained from the positive-ion spectra are consistently larger than 1. This trend is due to the stability of the product ion by the conjugation with the phosphate moiety of the double bond formed.[16] Also, studies with all other phospholipid classes have found that the same empirical rule is valid for the corresponding fragment ratios. This indicates that the present method to determine the fatty acyl positions using the CID-MS/MS spectrum of $[M+Na]^+$ is generally valid for all the classes of phospholipids.

Table 1. Effect of the regiospecificity of the acyl chain linkages on product ion relative intensities in CID-MS/MS of the $[M-15]^-$, $[M-86]^-$ and $[M+Na]^+$ parent ions generated by FAB of phosphatidylcholine (PC) molecular species

Compound[a]	$[M-15]^-$ parent	$[M-86]^-$ parent		$[M+Na]^+$ parent	
	$[R_2COO]^-/[R_1COO]^-$	$-R_2COOH/-R_1COOH$	$-R_2{=}C{=}O/-R_1{=}C{=}O$	$[M+Na-85-R_2COOH]^+/[M+Na-85-R_1COOH]^+$	$-R_2COOH/-R_1COOH$
14:0/18:1	2.21	3.50	3.64	3.53	2.34
16:0/10:0	1.59	1.58	3.70	3.59	1.84
16:0/12:0	1.67	3.23	3.03	3.04	1.86
16:0/16:1	1.98	3.03	2.49	2.98	1.84
16:0/18:1	1.89	4.00	5.42	2.93	2.26
16:0/18:2	1.75	2.33	2.00	3.70	1.96
16:0/20:4	1.02	4.08	2.32	3.45	2.82
16:0/22:6	0.41	5.00	5.50	3.26	1.06
18:0/18:1	2.19	2.53	2.55	3.21	1.31
18:1/2:0	0.30	0.87	0.95	1.39	N.M.[b]
18:1/16:0	1.79	3.48	5.53	3.41	1.98
18:1/18:0	1.57	4.94	5.10	2.77	2.00
18:0/18:2	1.78	4.10	3.48	3.52	1.91
18:0/20:4	1.20	3.00	3.78	3.43	2.61
18:0/22:6	0.38	4.81	5.29	3.35	3.37
16:0/pyr-6	2.08	1.48	1.17	1.96	1.24
16:0/pyr-10	2.34	1.95	1.74	2.97	1.85

[a] Unsaturated fatty acyl groups were designated by the convention, carbon:double bond number (*i.e.*, 16:1 (palmitoleoyl), 18:1 (oleoyl), 18:2 (linoleoyl), 20:4 (arachidonoyl), 22:6 (all-*cis*-4,7,10,13,16,19-docosahexaenoyl). Pyr-6, [(pyren-1-yl)hexanoyl], pyr-10, [(pyren-1-yl)decanoyl]. [b] Not measured due to spectral overlap.

The positive-ion FAB mass spectrum of phosphatidylglycerol (PG) isolated from cyanobacterium *Synechocystis* sp. PCC 6803 shown in Figure 7(a) displays several peaks of molecular species. The major molecular peak at m/z 791 is interpreted as $[M-H+2Na]^+$ with the total fatty acid composition of 34:2. The CID spectrum of $[M-H+2Na]^+$ parent ion at m/z 791 is shown in Figure 7(b). The intense peak at m/z 199 is identified as the disodiated five- or six-membered hydroxycyclophosphane. The spectral pattern is very similar to that of 16:0/18:2-PE shown in Figure 3. In particular, each homologous ion resulting from the charge-re-

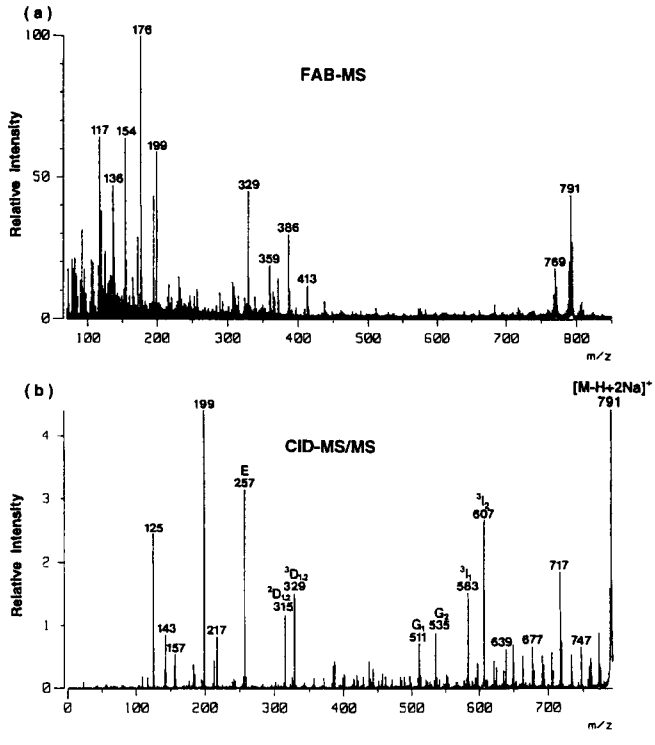

Figure 7. (a) Positive-ion FAB mass spectrum of phosphatidylglycerol isolated from cyanobacterium *Synechocystis* sp. PCC 6803. (b) Positive-ion CID spectrum of disodiated molecular ion ($[M-H+2Na]^+$, m/z 791) in (a).

mote cleavage along the fatty acid chains corresponds to its counterpart in the PE case, including the product ion (m/z 717) corresponding to the phosphatidic acid analog. Thus, as in the case of Figure 3, this species [m/z 791 in Figure 7(a)] contains the palmitic and linoleic acids as two fatty acyl groups. From the relative abundances of peaks at m/z 415 and 439 observed in the CID spectrum of $[M+Na]^+$ (spectrum not shown), linoleic acid (18 : 2) is attached at *sn*-1 and palmitic acid (16 : 0) at *sn*-2 positions. This fatty acyl linkage is in agreement with the empirical rule that the *sn*-2 position of a glycerol backbone is occupied almost exclusively by 16 carbon fatty acids for prokaryotic organisms such as cyanobacteria.[32] The results of mass spectrometric analysis for the other molecular species are summarized in Table 2. In the structural analysis of the fatty acyl groups of molecular species in a biological mixture, present method seems to be superior to the previous one where the negative-ion CID-MS/MS of FAB-desorbed fatty acid carboxylate was used

Conclusion

The results presented in this study demonstrated the usefulness of FAB-CID-MS/MS of sodium adduct molecular ions for the structural determination of fatty acyl groups of phospholipids. Collision-induced dissociation (CID) of sodium adduct molecular ions ($[M+Na]^+$, $[M-H+2Na]^+$ or $[M+Na-2H]^-$) produced ions which are characteristic of the structure of a polar head group as well as the fatty acid

Table 2. Structures of two fatty acyl groups in phosphatidylglycerol (PG) isolated from cyanobacterium *Synechocystis* sp. PCC 6803

Compound	$[M-H+2Na]^+$	$[M-H]^-$	Structure[a]
PG	793.5	747.4	18:1(oleic)/16:0(palmitic)
	791.5	745.4	18:2(linoleic)/16:0(palmitic)
	789.4	743.3	18:3(linolenic)/16:0(palmitic)

[a] The double bond positions and regiospecificity of fatty acyl groups are determined from the CID spectra of $[M-H+2Na]^+$ and $[M+Na]^+$ ions, respectively.

composition. In particular, charge-remote fragmentations of intact precursor ions along the fatty acyl chains provided information on the double bond positions even for the polyunsaturated fatty acyl groups. In the FAB-CID-MS/MS studies of $[M+Na]^+$ parent ions, we found a empirical rule to determine the regiospecificity of fatty acyl groups, which is valid for all the phospholipids investigated. Hence, the present method which utilizes the CID-MS/MS spectrum of sodium adduct molecular ion seems to be superior to the previous one utilizing negative-ion MS/MS for the structural determination of individual molecular species of phospholipids isolated from biological sources.

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Theoretical Studies on the Acid-Catalyzed Hydrolysis of Sulfinamide

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Ab initio calculations were carried out on the gas phase acid-catalyzed hydrolysis reactions of sulfinamide using the 3-21G* basis sets. Single point calculations were also performed at the MP2/6-31G* level. The first step in the acid-catalyzed hydrolysis of N-methylmethanesulfinamide, I, involves protonation. The most favorable form is the O-protonated one, II, which is then transformed into a sulfurane intermediate, III, by addition of a water molecule. The reaction proceeds further by an intramolecular proton transfer from O to N (TS 2), which is followed by N-S bond cleavage (TS3) leading to the final products. The rate determining step is the N-S bond cleavage (TS3) at the RHF/3-21G* level, whereas it becomes indeterminable at the MP2/6-31G* //3-21G* level of theory. However, the substituent effect studies with N-protonated N-arylmethanesulfinamide, XIII, at the MP2/6-31G*//3-21G* level support the N-S bond breaking step as rate limiting.

Introduction

Nucleophilic substitution reactions are one of the most important class of reactions in mechanistic organic chemistry, and substitutions at carbon centers have been studied extensively both experimentally and theoretically.[1] In a direct displacement (S_N2) reaction at carbon, an attacking nucleophile forms a trigonal bipyramid type transition state (TS), which eventually leads to a product with inversion. Nucleophilic displacement at a second row element, sulfur is known to proceed through more complex processes.[2] Two possible pathways have been proposed for the nucleophilic displacement at sulfur: two-step mechanism involving an addition intermediate (sulfurane), and concerted displacement (S_N2) involving a TS without intermediate formation. Addition intermediate formation has been ruled out in the base-catalyzed hydrolysis of N-mesitylbenzenesulfinamides based on the substituent effect studies,[3] and also the base-catalyzed reactions of cyclic sulfinate esters are reported to occur by a concerted S_N2 mechanism.[4] However, synthesis of a sulfurane-like intermediate[5] has led to predict the possible existence of such intermediate.

A useful means of elucidating the reaction mechanism involving sulfur atom is the test of ^{18}O exchange[6]; ^{18}O exchange is possible only through sulfurane intermediate formation. Recently Okuyama *et al.*,[7] have reported the ^{18}O exchange in the acid-catalyzed reactions of sulfinamide and proposed the existence of such an intermediate. They argued that the reaction proceeds through a sulfurane intermediate and the rate determining step changes depending on the substituent, based on the experimental results of pH-rate profiles for various substituents which showed a break

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