Application of Fast Atom Bombardment Collision-induced Dissociation Tandem Mass Spectrometry for Structural identification of Glycerolipids Isolated From Marine Sponge

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Abstract: Two types of glycerolipids [monoacylglycerols (MAG) and cyclitols] were isolated by reversed phase high-performance liquid chromatography from the methanol extracts of a marine sponge, and analyzed by fast atom bombardment mass spectrometry (FAB-MS) in positive-ion mode. FAB mass spectra of these compounds yielded protonated molecules $[M + H]^+$ and abundant sodiated molecules $[M + Na]^+$ from a mixture of 3-nitrobenzyl alcohol and NaI. The structures of these compounds were elucidated by FAB-collisional-induced dissociation (CID)-tandem mass spectrometry. We carried out collision-indused dissociation (CID) of these lipids in B/E-linked scan mode. The CID B/E-linked scan of $[M + H]^+$ and $[M + Na]^+$ precursor ions resulted in the formation of numerous characteristic product ions through a series of dissociative processes. The product ions formed by charge-remote fragmentation (CRF) provided important information for the identification of the acyl chain structure substituted at the glycerol backbone. Some of the product the ions were diagnostic for the presence of a glycerol backbone or acyl chain structure.

Key words: Glycerolipids, Marine sponge, Collisional-induced dissociation (CID), Tandem mass spectrometry

Introduction

Glycerolipids generally contain saturated fatty acids with even carbon numbers, whereas those extracted from animals are found to possess fatty acids with odd numbered and branched chains.[1] Monoacylglycerols (MAGs) are hydrolysis products of triacylglycerols (TAGs) or diacylglycerols (DAGs) and minor components of most plants and animal tissues.[2] Cyclitols[3] occur in plants and animals and are widely known to inositols. Cyclitols can form complexes with phospholipids and phosphoric esters containing glycerol moiety. These compounds are involved in specific functions, including signal transduction, cell recognition, regulation of cell growth, differentiation and programmed cell death.[4] It is important to elucidate the structures of glycerolipids and, in particular, of the fatty acids on the glycerol backbone in order to understand lipid metabolism.

To study the trace amounts of glycerolipids extracted from biological samples and natural products, several analytical methods have been pursued.[5] Mass spectrometry (MS) using various ionization methods such as electron ionization (EI),[6] chemical ionization (CI),[7] electrospray ionization (ESI),[8] atmospheric pressure chemical ionization (APCI),[9] and fast atom bombardment (FAB)[10,11] has been popularly used for the structural determination of glycerolipids extracted from natural products.

Recently, LC-ESI-MS/MS has been applied to the separation and identification of mixtures of glycerolipids and also used to obtain structural and quantitative information using low-energy collision-induced dissociation (CID).[12] Especially, FAB-MS has been used to characterize various glycerolipids over the past decades, this method offers many advantages for structure elucidation due to its high-energy CID capability. FAB-MS yields abundant $[M + H]^+$ and/or $[M + \textit{alkali metal}]^+$ ions and gives a stable spectrum for a considerable time. In general, the alkali metal cationization method provides significantly higher sensitivity than protonation and the $[M + Na]^+$ ions can assist the characterization of a molecule in MS/MS analysis. FAB-MS/MS has provided important structural information for compounds that have a similar repetitive structural moiety. Both charge-driven fragmentation (CDF) and charge-remote fragmentation (CRF) processes are major dissociation routes. The CDF process, which involves exchange of hydrogen atom and cleavage of a weak bond, provides information on particular functional groups. The CRF process provides information regarding chain length, alkyl and hydroxyl branching positions, and degree of unsaturation for fatty acids and glycerolipids.[13]

We have isolated several glycerolipids from marine sponge and determined their chemical structures by FAB-MS. The

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structural determination of glycerolipids was carried out by FAB-CID B/E-linked scan of their sodiated molecules. In addition, the dissociation patterns of $[M+Na]^+$ ions were proposed on the basis of interpretation of their CID spectra. The diagnostic ions proposed in this study will be helpful for the identification of compounds extracted from natural products.

Experimental

Extraction and isolation

The glycerolipids were extracted with MeOH from the frozen marine sponges. The MeOH-soluble fraction was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to yield aqueous MeOH- and *n*-hexane-soluble fractions. A portion of the *n*-hexane fraction was subjected to Silica gel 60 (15-40 μm) column chromatography and eluted with a gradient solvent system of 100% CH_2Cl_2 to 100% MeOH to afford 20 fractions. Each fraction was further purified by RP-HPLC (YMC packed J'sphere ODS-H80 column, 250 × 10 mm, 4 μm, 80 Å).

Fast atom bombardment mass spectrometry

FAB mass spectra were recorded with a JMS-700 Mstation mass spectrometer (JEOL, Tokyo, Japan) using a MS-MP9020D data system. The ion source was operated at 10 kV accelerating voltage with a mass resolution of 2000 (10% valley). The primary beam was produced by using a xenon atom gun operated at 10 kV. Samples were dissolved in MeOH or CH_2Cl_2 and mixed with 1 μL of 3-nitrobenzyl alcohol (NBA, Sigma, St. Louis, MO, USA) or glycerol (JUNSEI, Japan) on a FAB probe tip. Sodiated molecules $[M+Na]^+$ were generated from the samples in the NBA matrix saturated with NaI (Sigma). Calibration was performed with Ultramark 1621 (PCR, Gainesville, FL, USA) in positive-ion modes.

B/E-linked scan mass spectrometry

B/E-linked scan experiments were carried out using a JMS-700 (JEOL, Tokyo, Japan) double-focusing instrument with B/E configuration. Precursor ions were accelerated at 10 kV. Product ions were obtained by collisional activation in the first field-free region. Helium was introduced into the collision chamber at a pressure sufficient to reduce the precursor ion signal by 70%. B/E-linked scans were acquired at a resolution of 2000 and were calibrated with Ultramark 1621 in positive-ion mode.

Results and Discussion

FAB spectra of glycerolipids

The glycerolipids isolated from the marine sponge are classified by specific types of acyl substituents with differences in hydrocarbon chain length and in the presence and position of unsaturation. Though not shown here, the FAB-mass spectra of these glycerolipids can be characterized based on the protonated molecules $[M+H]^+$ and sodium-adducted molecules $[M+Na]^+$ or $[M+2Na-H]^+$ ions. For all the glycerolipids, $[M+Na]^+$ ions are more abundant than $[M+H]^+$ ions due to the high affinity of the sodium ion for the nonbonding electrons of the hydroxyl groups. Thus, the sodium cationization method can facilitate the characterization of the molecular structure in tandem mass spectrometric analysis.

FAB-CID spectra of $[M+Na]^+$ ions for monoacylglycerols

The FAB-CID-MS/MS spectra of $[M+Na]^+$ of monoacylglycerols I, II and III are shown in Figure 1. The product ions appearing below *m/z* 120 are formed as common ions by the fragmentation of the sodiated glycerol backbone. The characteristic ions at *m/z* 113 $[C_3H_6O_3Na]^+$, and *m/z* 97 $[C_3H_6O_2Na]^+$ are formed by hydrogen migration cleavage between the glycerol backbone and the fatty acyl chain substituted at the glycerol moiety. Another characteristic ion at *m/z* 84 $[C_2H_5O_2Na]^+$ is produced by the direct cleavage between *sn*-2 and *sn*-3 in sodiated glycerol backbone. Especially, the appearance of the ions at *m/z* 84 and 97 implies that the fatty acyl group does not link at *sn*-2 position of glycerol backbone.

Otherwise, the series of product ions appeared above *m/z* 160 are mainly formed by charge remote fragmentation (CRF) of acyl chains substituted at the glycerol backbone. CRF of acyl chain result in parallel losses of C_nH_{2n+2} units along hydrocarbon chain via 1,4-elimination[14] or a charge-driven process[15,16] beginning at the acyl terminus. The spectral pattern of these ions immediately gives information on the presence and location of the double bond and branch position as well as the composition of acyl groups.[17] In our previous studies,[17,18] structural information on glycerolipids that have similar repetitive structural moiety are readily obtained by charge remote fragmentations resulting in FAB-MS/MS analysis.

The MS/MS spectra of $[M+Na]^+$ ions for monoacylglycerols I, II and III are shown in Figure 1. From observing MS/MS spectra, the common abundant ion at *m/z* 156 is formed by homolytic cleavage between C-2 and C-3 of the fatty acyl group. This specific ion with even mass number is radical cation formed elimination of hydrogen radical for charge-mediated fragmentation suggested by Wysocki and Ross.[15] However, the formation of other fragment ions of the fatty acyl group may be explained by 1,4-H_2 elimination of CRF process. The major peaks at *m/z* 169 and 183 appeared as common ions are formed by successive cleavage between C-3, C-4 and C-5, respectively, of the fatty acyl group.

In Figure 1(A), monoacylglycerol I is identified to have a saturated fatty acyl chain without branched group based on appearance of the peaks differing in 14 mass unit corresponding to CH_2. In Figure 1(B), CRF is observed for methyl branched compound with enhanced fragmentation at the branched position and suppressed fragmentation at the branched methyl position. The presence of a branched methyl group at monoacylglycerol II is clear from 28 Th gap between the relatively large peaks *m/z* 295 and 323. From this fragmentation pattern, the position of methyl branch of monoacylglycerols

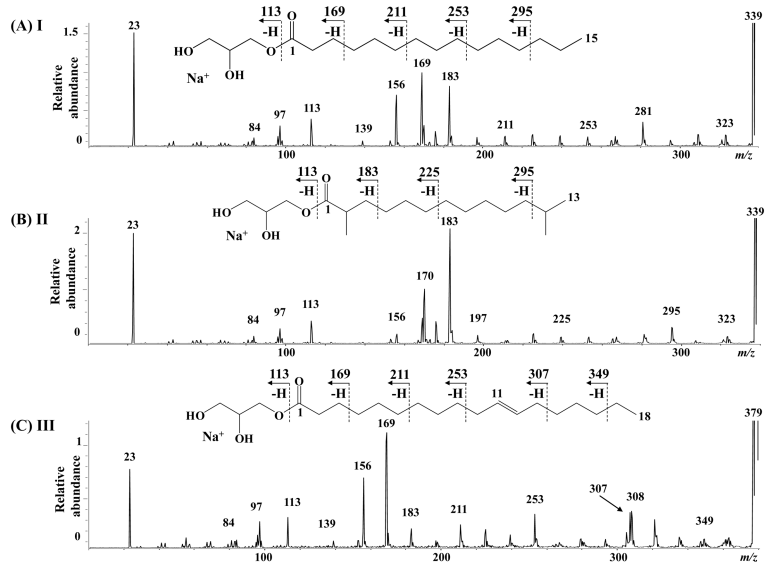


Figure 1. FAB-CID spectra of $[M+Na]^+$ ions for the monoacylglycerols (A) I, (B) II, and (C) III in positive-ion mode.

II can be assigned as the C-12. The sodiated molecule $[M+Na]^+$ ion of monoacyglycerol III is observed at *m/z* 379, indicating heptadecenoyl group with one double bond attached at glycerol backbone. As shown in Figure 1(C), allylic cleavage is observed as enhance peaks at *m/z* 307 and 308, indicating the location of the double bond at C-11. The peak intensities of fragment ions between at *m/z* 308 and 253 in CID-MS/MS spectrum are greatly suppressed due to the presence of double bond at C-11. The characteristic CRF patterns of monoacylglycerols with double bond exhibit relatively weak fragment ions with 12 Th mass differences, augmenting the evidence for the location of the double bond.

FAB-CID spectra of $[M+Na]^+$ ions for cyclitols

Typical FAB-CID-MS/MS spectra of $[M+Na]^+$ of three cyclitols are shown in Figure 2. The product ions appearing below *m/z* 250 are formed as common ions by fragmentation of the glycerol backbone attached to the cyclitol ring. The product ions at *m/z* 229 and 245 are formed by cleavage of the ether bond between the glycerol backbone and the alkyl chain substituted at the glycerol moiety. The product ions at *m/z* 229 and 245 are confirmed to be the alkyl chain attached to *sn*-3 position of the glycerol backbone. When the alkyl chain is substituted at *sn*-2 position of the glycerol backbone, the ions at *m/z* 245 and 213 could not be formed. The product ions at *m/z* 155 and 171 could be formed by cleavage of the ether linkage of the cyclitol attached to the glycerol backbone. Another common ion at *m/z* 113, corresponding to $[C_3H_6O_3Na]^+$, could be produced by direct cleavage of the cyclitol ring. The dissociation patterns of the $[M+Na]^+$ ions of the cyclitols are summarized in Scheme 1. These characteristic ions can be used as diagnostic ions for the presence of cyclitol ring substituted at glycerol backbone.

In contrast, the series of product ions appearing above *m/z* 250 arise mainly from charge-remote fragmentation (CRF) of the alkyl chain substituted at the glycerol moiety. As shown in Figure 2(B), CRF of compound II, with a double bond in the alkyl chain, gives characteristic ions at *m/z* 424 and 372 produced by allylic α-cleavage and the position of the double bond can be assigned at C-11 from these fragmentation patterns. Allylic cleavage with even mass is observed as enhanced peaks at *m/z* 424 and 372, indicating the location of the double bond. The characteristic CRF patterns of the cyclitol derivative with a double bond exhibit relatively weak fragment ions with 12u mass differences, augmenting the evidence for the location of the double bond. As can be seen in Figure 2(C), the presence of a branch point is clear from the 28u gap between the relative large peaks at *m/z* 371 and 399. From this fragmentation pattern, the position of methyl branch can be assigned as the C-10 position. These fragmentation patterns are closely resemble those found for alkyl chain of lysophosphatidylcholines, as reported in our previous study[17] and can be useful for the identification of unknown cyclitol derivatives extracted from natural products.

Conclusions

Glycerolipids with unique structures were isolated from a marine sponge and investigated by FAB-MS and CID B/E-linked scan. The collision-induced dissociation of $[M+Na]^+$ ions gives various product ions that allow characterization of the sugar ring, glycerol backbone, and fatty acyl chain moiety. In particular, CRFs of the $[M+Na]^+$ ion provide information on the length of the hydrocarbon chain and the locations of the methyl branch, double bond, and the position of the hydroxyl substituent. Such FAB-CID B/E-linked scan of $[M+Na]^+$ ions provides a good avenue for determining the molecular structure of glycerolipids extracted from marine sponge. Compared with

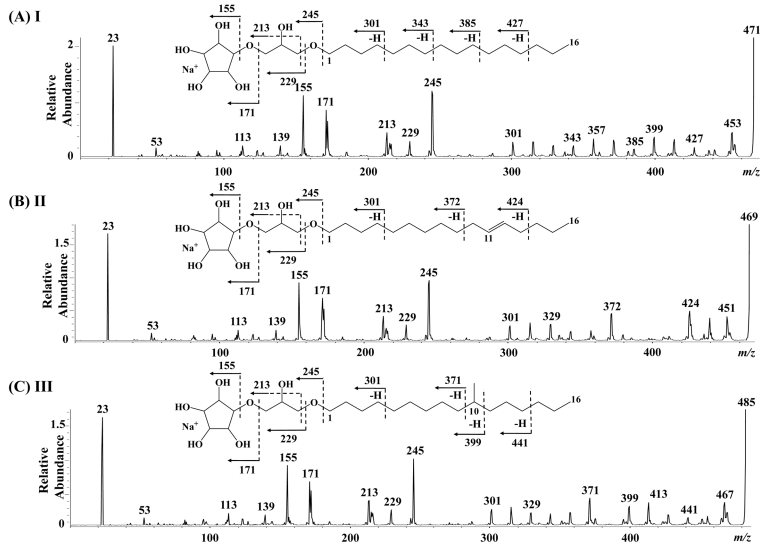
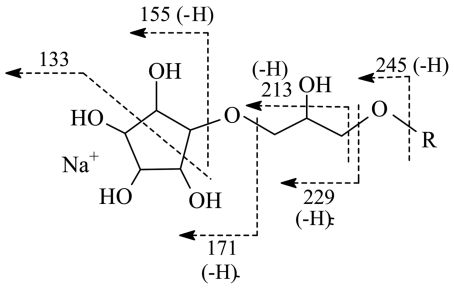


Figure 2. FAB-CID spectra of $[M+Na]^+$ ions for the cyclitols (A) I, (B) II, and (C) III in positive-ion mode.



Scheme 1. FAB-CID-MS/MS fragmentation patterns of the sodiated molecule $[M+Na]^+$ ions of cyclitol derivatives.

ESI-CID-MS/MS, the high-energy FAB-CID B/E-linked scan can be helpful for the structural determination of any unknown glycerolipids isolated from natural products. This study will also be useful for the clarification of biosynthesis and metabolism of glycerolipids in marine natural products.

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