Positive Ion Fast Atom Bombardment Mass Spectrometric Analysis of the Molecular Species of Glycerophosphatidylserine

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Received March 27, 1990

The structure of 1,2-dipalmitoyl-sn-glycero-3-phosphoserine was analyzed by positive ion fast atom bombardment mass spectrometry and collisional activation mass-analyzed ion kinetic energy spectroscopy. The molecular weight, the polar-head group, and the fatty acid composition of this species were identified by the appearance of protonated and solvated protonated species ions, diglyceride and monoglyceride fragment ions. After purification of glycerophosphatidylserine from bovine brain and rat kidney by normal phase high-performance liquid chromatography, molecular species were identified by either positive or negative ion fast atom bombardment mass spectrometry. The study suggests that negative ion fast atom bombardment ionization is a more powerful tool for the identification of the molecular species of glycerophosphatidylserine from biological samples. Positive ion fast atom bombardment represents a useful alternative for analysis of major molecular species in natural glycerophosphatidylserine.

Glycerophosphatidylserine (GPS) is a phospholipid present in mammalian cell membranes, known to participate in a number of biochemical processes (1–3). Since the function of GPS appears to be determined by the nature of each of its lipid components (4–7), knowledge of the structure of its molecular species is essential to an understanding of its role in cellular events. Structural information of the molecular species of GPS and other glycerophospholipids includes (i) molecular weight; (ii) the structure of the polar-head group; (iii) the composition of the fatty acyl chains and their position esterified to the glycerol backbone; and (iv) the position of double bond(s) in the unsaturated fatty acyl groups.

Mass spectrometry has proved to be particularly useful for the identification of the molecular species of glycerophospholipids (8), and soft ionization techniques are suitable for analysis of intact molecules of GPS species. Mass spectrometric analysis of GPS species using positive ion desorption chemical ionization (9) and chemical ionization (10) have been discussed. Since its inception, fast atom bombardment (FAB) mass spectrometry has been used to analyze GPS, and Fenwick et al. (11) first reported data on positive ion FAB analysis of GPS species. These results, however, were insufficient because of the lack of significant information that readily identifies GPS species, such as quasi-molecular ions. Recent reports on positive ion californium-252 plasma (12) and thermospray desorption analysis (13) of intact GPS species have been shown to yield the intense quasi-molecular ions of the sodium adduct type and fragments that are also indicative of the structure of GPS molecular species.

Negative ion FAB ionization is especially effective in the analysis of GPS (14). Due to an improvement in the sample preparation technique of FAB mass spectrometry, a good negative ion mass spectrum with abundant, clear-cut quasi-molecular ions of GPS species and fragments for structural elucidation could be obtained (15).

In the present investigation, we report an analysis of the molecular species of GPS by positive ion FAB and
collisional activation mass-analyzed ion kinetic energy (CA MIKE) spectroscopy. Application of positive ion FAB ionization, together with negative ion FAB mass spectrometry to identify the molecular species of GPS from rat kidney is described.

MATERIALS AND METHODS

Chemicals

Phospholipid classes including glycerophosphatidylethanolamine (GPE), lysophosphatidylethanolamine (Lyso-PE), glycerophosphatidylserine (GPS), lysophosphatidylserine (Lyso-FS), lysophosphatidylcholine (Lyso-PC), and glycerophosphatidylinositol (GPI) were prepared according to described procedures (15). Glycerophosphatidic acid (GPA) was prepared from glycerophosphatidylcholine (GPC) by treatment with phospholipase D. 1,2-Dipalmitoyl-sn-glycero-3-phosphoserine (GPS di 16:0) was obtained from Nova-BioChem (Laufelfingen, Switzerland); HPLC grade ethanol, chloroform, methanol, tetrahydrofuran, and analytical grade phosphoric acid were purchased from Carlo Erba (Milan, Italy). Diethanolamine (DEA) was obtained from Fluka AG (Buchs, Switzerland). Sep-Pak C18 cartridges were from Waters Associates (Milford, MA). Bovine brain phospholipids were obtained from our laboratories.

Extraction and Isolation of Glycerophosphatidylserine in Rat Kidney

Approximately 50 mg of fresh kidney cortex from a Sprague-Dawley rat (10 weeks old) were homogenized in 3 ml of chloroform:methanol (1:2, v/v) and allowed to stand for 1 h at 4°C. The suspension was then centrifuged at 3000 rpm for 15 min. The pellet was extracted and centrifuged again as before. To 6 ml of the crude extract was added 8 ml of methanol:water (1:1, v/v), and the solution was mixed to homogeneity. After centrifugation, the solution was poured onto a Sep-Pak C18 cartridge, which was washed with 3 ml of methanol before use. Column elution was carried out under normal pressure with an elution speed of 0.6–0.8 ml/min. Another 8 ml of methanol:water (1:1, v/v) was added to the eluate, mixed, and passed through the same column. After that, the column was washed with 1 ml water. Polar contaminants and gangliosides in the crude extract were removed by the procedures cited (16). GPS, GPI, and small amounts of other phospholipids were eluted with two portions of 1.5 ml methanol:water (12:1, v/v), and this eluate was used for purification of GPS by normal-phase high-performance liquid chromatography (HPLC).

High-Performance Liquid Chromatography

A Perkin–Elmer liquid chromatography system (series 3B pump and LC-75 detector) was employed for purification of GPS from bovine brain and rat kidney, using an aminopropyl HPLC column (Alltech, 5 μm, 4.6 mm × 15 cm) with ethanol:phosphoric acid 1 M (89:11, v/v) as the mobile phase, at a flow rate of 1 ml/min. The wavelength of detection was at 205 nm. All samples were dissolved in tetrahydrofuran:water (70:30, v/v). GPS-containing HPLC fractions were collected, and the acid containing the fractions was removed by the method of Bligh and Dyer (17). After continuous use for several days, it was found necessary to wash the HPLC column with ethanol:phosphoric acid 1 M (80:20) for 10 h at a flow rate of 0.2 ml/min.

Fast Atom Bombardment Mass Spectrometry and Collision Spectroscopy

All spectra were obtained on a VG ZAB-2F mass spectrometer (VG Analytical, Manchester, UK) with its data processing system (VG PDP11/250) operating in both positive and negative ion modes. Xenon atoms at 8 keV were used for FAB analysis. Resolution of the instruments was 2000. CA MIKE spectra were obtained on the same instrument by collision of preselected ionic species with helium in the cell placed in the second field-free region. The pressure of the helium collision gas was adjusted to reduce the parent ion beam intensity by 50%.

Sample Preparation for FAB Analysis

DEA was used as the liquid matrix. GPS samples were dissolved in chloroform:methanol:water (58:4:0.4). The solutions were made to 2.5 μg/μl for GPS di 16:0 and 1.2 μg/μl for purified GPS. A 2 μl sample of each was transferred to the surface of the liquid matrix (15) with a microsyringe, and the solvent was evaporated before the sample probe was introduced into the ion source.

RESULTS AND DISCUSSION

Positive Ion FAB and CA MIKE Analysis of GPS di 16:0

A positive ion FAB mass spectrum of GPS species has not been previously reported. In order to better understand the decomposition modes under positive ion FAB ionization, we first studied an individual molecular species. Figure 1A shows the positive ion FAB mass spectrum of GPS di 16:0. Abundant peaks were observed at m/z 736 ([M + H]+) and at m/z 841 ([M + H + DEA]+), corresponding to protonated and solvated protonated GPS di 16:0 ions. The formula of the latter was verified by appearance of the peak (M + H + TEA)+ when triethanolamine (TEA) was used as the matrix. The ion at m/z 551 is formed by loss of the serine phosphate.
from this species. Clear fragments related to the fatty acyl groups esterified at sn-1 and sn-2 positions of the glycerol backbone, which were observed in positive ion spectra of thermospray, californium-252 plasma, chemical and chemical desorption ionization, could be observed at m/z 313 (monoglyceride ion) in the spectrum. CA MIKE analysis of the protonated molecule (Fig. 1B) confirms these identifications. Another ionic species at m/z 631 ([M - 105]+), corresponding to the loss of serine, appeared in the CA MIKE spectrum.

Positive Ion FAB Analysis of the Molecular Species of Bovine Brain GPS

In contrast to negative ion FAB (not shown) and other positive ion mass spectra of the GPS species, the background elicited by the liquid matrix in positive ion FAB mass spectrum is obvious. The limit of detection of positive ion FAB analysis of GPS species was 1.2 µg based on a signal-to-noise (S/N) greater than 5. A constant ion current of the species can be maintained for 5 min when 2 µl of the solution (5-µg sample) was loaded onto the liquid matrix. The key to achieving positive ion FAB analysis of GPS species is presumably the fact that GPS can exist in ion form, and that the ionized molecules easily leave the surface of the matrix under FAB desorption. The desorption mechanism of the sample on the liquid matrix has been discussed (15,18). Valuable structural information concerning the GPS species provided by positive ion FAB and CA MIKE spectrometry methods includes, therefore: (i) molecular weight; (ii) characterization of the polar-head group; and (iii) fatty acid composition.

Positive Ion FAB Analysis of the Molecular Species of Bovine Brain GPS

A new HPLC procedure is described for separation of phospholipid classes. Seven classes could be separated within 20 min on an aminopropyl column by using ethanol:phosphoric acid 1 M (89:11, v/v) as the mobile phase (Fig. 2). With this method, bovine brain GPS was purified (Fig. 2B) from bovine brain phospholipids.

We analyzed the molecular species of bovine brain GPS using negative ion FAB mass spectrometry (15) and the major species of the GPS used in this study is 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoserine (GPS 18:0-18:1). Figure 3 shows a positive ion FAB mass spectrum of this class. Ions at m/z 790 ([M + H]+) and at m/z 895 ([M + H + DEA]+) correspond to GPS 18:0-18:1. The principal fragment is the diglyceride ion at m/z 605, which is formed by loss of the serine phosphate from the ion at m/z 790. Monoglyceride fragments corresponding to fatty acid chains appeared at m/z 341
FIG. 2. Normal-phase HPLC separation of a mixture of individual classes (A) and of bovine brain phospholipids (B), using an aminopropyl column with ethanol:phosphoric acid 1 M (89:11) as the mobile phase; uv detection at 205 nm. The numbers at the top of the peaks correspond to retention time in minutes. (S.F., solvent front.)

(stearic acid) and m/z 339 (oleic acid). Other minor molecular species of the GPS could not be identified from the positive ion spectrum due to the high chemical background.

The results suggest that negative ion FAB mass spectrometry is a more powerful method by which to identify the molecular species of GPS from biological samples: less background is derived from the liquid substrate,
Identification of the Molecular Species of GPS from Rat Kidney

GPS comprises a minor class (4%) of phospholipids in rat kidney (19). Profile of its molecular species has not been described before. After a simple isolation of acidic phospholipids from other major classes (GPC 40%, GPE 27%, and SPH 17%), purification of the GPS class was achieved on an aminopropyl HPLC column as described under Materials and Methods (Fig. 4A). From negative ion FAB analysis (Fig. 4B), the major molecular species of rat kidney GPS was identified as GPS 38:4 (ion at m/z 810). Loss of the serine from the species gives an ion at m/z 723. The fragment ions at m/z 283 (stearic acid) and at m/z 303 (arachidonic acid) furnish information regarding the composition of the fatty acids in this species. The position of the two fatty acyl groups esterified to the glycerol backbone could not be determined from the spectrum alone. However, it is most likely GPS 18:0–20:4 since the unsaturated fatty acyl groups in natural glycerophospholipids are preferentially located at the sn-2 position. The positions of the double bonds in the 20:4 fatty acid remain undetermined. Complementary data from positive ion FAB analysis (Fig. 4C), ions at m/z 812 ([M + H]⁺), m/z 917 ([M + H + DEA]⁺), m/z 627 ([M – 185]⁺), and m/z 341 (stearic acid) corresponding to the protonated GPS 18:0–20:4 ion, its solvated protonated peak, the diglyceride ion resulting from loss of the serine phosphate, and the monoglyceride fragment related to fatty acid, support this determination. Negative ion FAB spectrum also provides information on minor molecular species of GPS from the rat kidney. They are probably GPS 18:0–18:1 (m/z 788) and GPS 18:0–18:2 and GPS di 18:1 (m/z 786), accompanying ions at m/z 281 (oleic acid) and m/z 279 (linoleic acid). But the complementary data of the minor species were not obtained from positive FAB anal-
MASS SPECTROMETRIC ANALYSIS OF GLYCEROPHOSPHATIDYLSERINE

ysis. This differs mainly in the detection sensitivity of two methods. Negative FAB appears to be at least 10 times more sensitive than positive FAB in identification of GPS molecular species in biological samples. An excess of GPS molecules deposited on the liquid matrix, while accompanying the increase in chemical background, may lead to reduced sensitivity (18,15).

In conclusion, structural analysis of GPS species by FAB mass spectrometry (i) requires no modification of standard equipment and needs no special accessories, (ii) uses low amounts of sample, and (iii) generates a stable ion current. Negative ion FAB ionization is a more powerful tool for the identification of GPS species in biological samples, while positive ion FAB mass spectrometry represents a complementary means for analysis of major species in natural GPS.

ACKNOWLEDGMENTS

The authors thank R. Mariot for technical assistance and B. Corey for editorial assistance.

REFERENCES