Determination of phospholipid base structure by CA MIKES mass spectrometry

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Summary The fast atom bombardment spectra of three phospholipids containing a secondary, tertiary, and quaternary nitrogenous base attached to a 1,2-dipalmitoyl-sn-glycero-3-phosphate skeleton were shown to each contain an ion derived from the intact nitrogenous base. The collisional activation mass analyzed ion kinetic energy spectroscopy (CA MIKES) spectrum produced from each of these ions was shown to be unique for the particular base. The technique thus provides a non-destructive rapid identification of the phosphorylated base of phospholipids by collisional activation mass analyzed ion kinetic energy spectroscopy (CA MIKES) (16, 17). This technique produces a mass spectrum that is characteristic for the particular phosphorylated base of an individual phospholipid. It permits the unambiguous identification of the hydrophilic moiety and as a test it was used to confirm the structure of the major phospholipid stored in the liver of a patient with Niemann-Pick disease (sphingomyelin abnormality) (18).

Supplementary key words phosphocholine • phosphomethylethanolamine • phospho-N,N-dimethylethanolamine • sphingomyelin • Niemann-Pick disease

Phospholipids are major membrane components of all eukaryotic and prokaryotic cells. Individual cells contain a great number of molecular species that vary according to the nature of the bound fatty acids and the hydrophilic group. The most common hydrophilic groups are primary and quaternary nitrogenous bases, generally ethanolamine and choline, respectively, although smaller amounts of the corresponding secondary and tertiary bases (N,N-dimethylethanolamine and N,N-dimethylethanolamine, respectively) (1, 2), as well as neutral hydrophilic groups, such as inositol and glycerol, and amphiphatic groups such as serine are also present (3).

The confirmation of the structure of an isolated phospholipid is based primarily on chromatographic identification of the hydrophilic group released from the parent lipid by either acid or alkaline hydrolysis (1, 2, 4). More recently, however, mass spectrometric analysis of the degraded lipids has been employed with varying success. Electron impact ionization can provide useful information on phospholipid structure (5, 6), but the complexity of the spectra produced from the large numbers of molecular species present, even in a single phospholipid class derived from natural sources, makes the interpretation of data quite difficult.

The softer techniques of chemical ionization (7, 8), field desorption (9-12), and more recently positive (13, 14) and negative ion (15) fast atom bombardment produce less complex spectra. However, it should be emphasized that the assignment of base structure by these techniques is largely dependent on the identification of fragments produced from individual bases. In complex mixtures there may be many fragments in the region of interest in the mass spectrum and therefore accurate structural assignment is not always possible.

In this study we report on the results of our investigations on the determination of phospholipid structure using collisional activation mass analyzed ion kinetic energy spectroscopy (CA MIKES) (16, 17). This technique produces a mass spectrum that is characteristic for the particular phosphorylated base of an individual phospholipid. It permits the unambiguous identification of the hydrophilic moiety and as a test it was used to confirm the structure of the major phospholipid stored in the liver of a patient with Niemann-Pick disease (sphingomyelin abnormality) (18).

MATERIALS

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phospho-N,N-dimethylethanolamine (DPDMPE), 1,2-dipalmitoyl-sn-glycero-3-phospho-N,N-dimethylethanolamine (DPMMPE), and bovine brain sphingomyelin were obtained from Sigma Chemical Co., St. Louis, MO. Sphingomyelin (SM) was also isolated from the liver of a patient with Niemann-Pick Type A disease (19).

METHODS

A chloroform solution (2 mg/ml) of each of the phospholipids (DPPC, DPDMPE, DPMMPE, and SM) was evaporated on the fast-atom bombardment probe tip of the mass spectrometer and then covered with a drop of glycerol. Mass spectra were measured on a Vacuum Generators ZAB 2F mass spectrometer operating in the positive ion fast-atom bombardment mode. Argon gas was used in the source with a primary beam energy of 8 keV.

Abbreviations: CA MIKES, collisional activation mass analyzed ion kinetic energy spectroscopy; DPPC, phosphatidylcholine; 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPDMPE, 1,2-dipalmitoyl-sn-glycero-3-phospho-N,N-dimethylethanolamine; DPMMPE, 1,2-dipalmitoyl-sn-glycero-3-phospho-N,N-dimethylethanolamine; SM, sphingomyelin.
RESULTS AND DISCUSSION

Although each phospholipid gave a complex spectrum, the spectra of DPPC, DPDMPE, and DPMMPE showed ions at m/z 184, 170, and 156, respectively, attributable to the corresponding nitrogenous bases. These ions were analyzed by the CA MIKES technique. In this technique the spectrometer magnet allows only the transmission of the ion to be investigated into a collision cell containing neutral helium at a pressure of $2 \times 10^{-7}$ torr. Under these conditions the ion undergoes a single collision with helium. Product ions formed by fragmentation of the collisionally activated ions are analyzed.

The CA MIKES spectrum of the ion at m/z 184 in the mass spectrum of DPPC is shown in Fig. 1A. It contains a peak at m/z 184 ($M^+$) and the following peaks which can be rationalized as representing charged fragments that result from the loss, from DPPC, of the groups in parentheses, at m/z 169 ($-\text{CH}_3$), 166 ($-\text{H}_2\text{O}$), 125 ($-(\text{CH}_3)_3\text{N}$), 86 ($-\text{H}_2\text{PO}_4$), and 60 ($-\text{C}_2\text{H}_4\text{PO}_4$).

The CA MIKES spectrum of the ion with m/z 170 in the mass spectrum of DPDMPE (Fig. 2) is consistent with the structure of phospho-$\text{N},\text{N}$-dimethylethanolamine, with peaks at m/z 155 ($-\text{CH}_3$), 138 ($-\text{H}_2\text{O}$), and 58 ($-\text{H}_2\text{PO}_4$). With DPMMPE the peaks at 141 ($-\text{CH}_3$), 138 ($-\text{H}_2\text{O}$), and 58 ($-\text{H}_2\text{PO}_4$) in the CA MIKES spectrum of the ion with m/z 156 (Fig. 3) are again consistent with an ion generated from phospho-$\text{N}$-methyl-ethanolamine.
Positive ion fast-atom bombardment mass spectra of bovine brain sphingomyelin and of sphingomyelin isolated from a patient with Niemann-Pick Type A disease showed an ion with m/z 184 in each case. The respective CA MIKES spectra, Fig. 1B and 1C, confirm the identity of the nitrogenous base as phosphocholine. Indeed, the similarities between Fig. 1A–C show the extent to which this technique produces mass spectra characteristic of the phosphorylated base of the phospholipid and the strength of the technique in the unambiguous identification of the hydrophilic moiety.

In the practical application of this methodology, amounts of material on the order of several hundred micrograms are sufficient, the majority of which can be recovered after separation from the glycerol matrix. It is expected that prior purification by either thin-layer or high pressure liquid chromatography of the phospholipid would be performed; although CA MIKES mass spectrometry exhibits excellent discrimination, the presence of other components in the glycerol matrix can affect ionization and hence detectability of the phospholipid. The technique is thus unsuitable for the quantitation of components of mixtures of phospholipids. The extension of the technique to the identification of other glycerophospholipids with different hydrophilic groups would only be possible if a protonated species of the hydrophilic group could be generated in the source of the mass spectrometer.

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REFERENCES


