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Continuous Flow Liquid Secondary Ion Mass Spectrometric Characterization of Phospholipid Molecular Species

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The positive and negative ion continuous flow liquid secondary ion mass spectrometric (CF-LSIMS) analysis of four major phospholipid classes is reported. Coupling capillary high-performance liquid chromatography with CF-LSIMS proved to be very useful for the structural identification of minor phospholipid species present in complex mixtures. Phospholipid molecular species within a particular class were separated or partially separated on the capillary column. Abundant molecular ions and characteristic fragment ions were produced. Information regarding the molecular mass, the polar head group and the fatty acyl substituents can be easily obtained, with a limit of detection below 1 ng per component. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

Phospholipids play an important role in biological systems; they are major constituents of the lipid bilayer of all cell membranes. The physical and chemical properties of cell membranes are dependent on the phospholipid composition. Alterations in phospholipid molecular species in membranes can result in modification of cell functions. In addition, phospholipids are important sources of arachidonic acid, which can be metabolized via cyclooxygenase or lipoxygenase pathways to produce biologically active prostaglandins or leukotrienes.[1,2] Therefore, finding effective analytical techniques for phospholipids in complex mixtures at the molecular species level is important in biological research.

Glycerophospholipid structures consist of three parts: a glycerol backbone, a polar head group and two fatty chains esterified at the *sn*-1 and *sn*-2 positions. Mass spectrometric characterization of phospholipid molecular species has been a challenging task, owing to the number and diversity of phospholipid species. The conventional gas chromatographic/mass spectrometric (GC/MS) technique is time consuming and often involves multi-step sample preparation procedures (hydrolysis, derivatization).[3,4] With the development of many soft ionization techniques, the analysis of intact phospholipids has become feasible. Among these techniques, fast atom bombardment (FAB) ionization is the most widely used for phospholipid analysis.[5–25] Several groups have demonstrated that useful structural information regarding the polar head group and fatty acyl substituents can be obtained using FAB tandem mass spectrometry (MS/MS). In particular, Murphy's group has successfully used negative-ion FAB-MS/MS to characterize arachidonyl-containing phospholipid species in various cell types.[5] However, owing to the high background ions in FAB mass spectra, the identification of minor phospholipid molecular species in complex mixtures has been very difficult. Thermospray liquid chromatography (LC)/MS has been shown useful for phospholipid analysis,[26,27] providing on-line separation of molecular species, but suffers from an overall lack of sensitivity. Recently, LC/electrospray MS has been utilized for phospholipid analysis;[28–32] structural information could be obtained using MS/MS for subpicomole amounts of phospholipid. These studies, however, used either LC conditions without molecular species separation[29] or direct infusion of phospholipid samples into the electrospray ion source. The primary positive and negative ion spectra were often not informative, with complete structural analysis of phospholipids requiring MS/MS of precursor ions. Identification of minor species present in complex mixtures would still be difficult, especially for isobaric components and species differing by 2 mass units. Also, salts present in biological samples often complicated the analysis, especially for glycerophosphoinositol and glycerophosphoserine species.[30]

This paper describes the first application of capillary high-performance LC (HPLC)/continuous flow liquid secondary ion mass spectrometry (CF-LSIMS) to phospholipid analysis. This technique is similar to that described by Ito *et al.*[33] as frit-FAB and by Caprioli *et al.*[34] as continuous flow FAB. The use of CF-FAB or CF-LSIMS leads to the production of a steady ion beam over a long time, such that the background noise associated with matrix adduct ions is significantly

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reduced and the ion suppression effect is diminished.[35] Coupling capillary HPLC with a liquid secondary ion mass spectrometer provided a convenient and efficient method for detecting phospholipids, especially for minor molecular species present in complex mixtures. On-line chromatographic analysis combined with the remarkable sensitivity of this technique should be very valuable in lipid research.

EXPERIMENTAL

Materials

Standard phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL, USA) or Sigma Chemical (St Louis, MO, USA). L-δ-Glycerophospholcholine (GPC) (lecithin) from egg yolk, glycerophosphoethanolamine (GPC) from bovine liver, glycerophosphoinositol (GPI) from bovine liver and bovine glycerophosphoserine (GPS) were all obtained from Avanti Polar Lipids.

Capillary HPLC

A Waters 600MS HPLC pump was used to supply the main flow. Gradient mobile phase programming was used with a flow rate of 1.0 ml min^{-1}. Eluent A was methanol–propan-2-ol (80:20, v/v) and eluent B and 20 mM ammonium acetate adjusted to pH 5.0 with acetic acid. Glycerol (1.5%) was added to both eluents as the matrix for CF-LSIMS. A linear gradient was run from 93 to 100% A over 20 min for GPC and GPE and from 90 to 100% A over 30 min for GPI and GPS. The capillary HPLC flow was supplied by splitting the man flow to 3 μl min^{-1} using an open split at a Valco tee (Valco Instruments, Houston, TX, USA). The flow was directed through a Valco injector (with an 10 μl loop), and then through a KAPPA Hypersil BDS C_{18} capillary column (100 × 0.3 mm i.d.) (Keystone Scientific, Bellefonte, PA, USA) to the frit probe of the JEOL mass spectrometer. The capillary column was flushed with methanol whenever it was not being used for analysis.

Mass spectrometry

A JEOL HX110A double-focusing mass spectrometer[36] (EB configuration; JEOL, Boston, MA, USA), equipped with a 10 kV LSIMS source and a cesium ion gun was used. The mass spectrometer was operated in the capillary HPLC/CF-LSIMS mode. Ions were produced by bombardment with a beam of Cs^+ ions (10 keV for the positive ion mode and 15 keV for the negative ion mode), with the ion source accelerating voltage at 10 kV. The resolution was set at 1000. Data acquisition was in either the negative or positive ion mode and the mass spectrometer was scanned at a rate of 4 s from m/z 0 to 1000. The background produced by the glycerol matrix was stable in our system. The background-subtracted mass spectra were therefore obtained by averaging a few scans associated with the maximum of a specific peak in the reconstructed ion chromatogram and subtracting an average matrix ion spectrum from the adjacent chromatographic background.

RESULTS AND DISCUSSION

The separation of intact phospholipid molecular species within a phospholipid class can be accomplished on a C_{18} reversed-phase column, as described by Patton *et al.*[37] Traditionally, complete separation of each intact phospholipid molecular species on a C_{18} column with UV detection was lengthy, and required addition of choline chloride to prevent peak tailing. A modified HPLC gradient using methanol–propan-2-ol (80:20, v/v) and 20 mM ammonium acetate was set up in this study for the separation of intact phospholipid molecular species on the capillary column. Because the major factor governing reversed-phase chromatography of phospholipids is the solubility of the acyl chains in the stationary phase, the retention time of any particular molecular species of either GPE, GPS or GPC is approximately the same, while GPI has a slightly shorter retention time owing to the large number of polar sites on the head group. The well end-capped Hypersil BDS capillary column provided reasonably narrow and symmetrical chromatographic peaks. With MS detection, complete baseline separation of phospholipid molecular species was not crucial, that is, co-eluting species can be identified. This method is simple, rapid and general for all phospholipid classes. For a biological lipid extract, molecular species distribution in each phospholipid class can be rapidly determined using the present technique, after phospholipid class separation using thin-layer chromatography or normal-phase HPLC.[37]

Glycerophosphocholine species (GPC)

Both positive and negative ion FAB analyses of GPC species have been reported by several groups using standard direct-probe FAB.[13–18] The full-scan mass spectra were not informative owing to the interference of the glycerol background in the low-mass region. The structural identification of individual phospholipid molecular species required collision-induced dissociation of each molecular ion, and often required micrograms of sample.[20] The CF-LSIMS technique used in this study offered good sensitivity in detecting GPC species, with full-scan mass spectra being obtained using sub-nanogram amounts of material. Examples were illustrated using a mixture of five synthetic GPC standards. Figure 1(a) shows the reconstructed ion chromatogram (from full scan) of the class specific protonated phosphocholine (m/z 184) for 1 ng of each standard. The five standard GPC species were separated on the capillary column. A representative positive ion full-scan mass spectrum is shown in Fig. 1(b) for 1 ng of 14:0a/20:4 GPC. Abundant MH^+ ion at m/z 754 and fragment phosphocholine ion at m/z 184 were detected and molecular mass and head group information was, there-

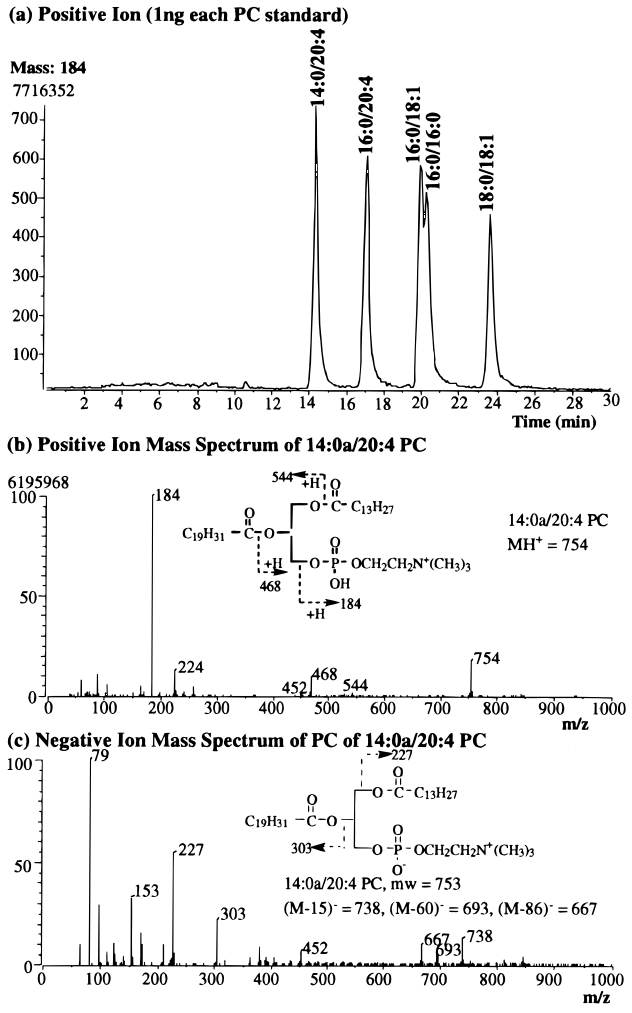

Figure 1. (a) Reconstructed ion chromatogram of phosphocholine ion (*m/z* 184) from positive ion full-scan CF-LSIMS of five synthetic glycerophosphocholine species (1 ng each standard). For HPLC conditions, see Experimental. (b) Positive ion full-scan CF-LSI mass spectrum for 1 ng of 14:0a/20:4 GPC. (c) Negative ion full-scan CF-LSI mass spectrum for 10 ng of 14:0a/20:4 GPC.

fore, readily obtained. The deacylated ions corresponding to the loss of either acyl chain (*m/z* 452, 528) and its ketene analog (*m/z* 468, 544) were also present in lower abundance, thereby giving information about the fatty substituents.

In the negative ion mode, the overall sensitivity for detecting GPC species was lower than that in the positive ion mode. The negative ion full-scan mass spectrum of 14:0a/20:4 PC (10 ng injected on-column) is shown in Fig. 1(c). The deprotonated molecule $[M - H]^-$ was absent from the spectrum and, instead, ions at *m/z* 695, 721 and 766 corresponding to $[M - 15]^-$, $[M - 60]^-$ and $[M - 86]^-$ were observed, similar to those reported previously.[14,15,18] These ions are believed to originate from matrix–ion adduct to the target molecule.[5,18] In the low-mass region, the diagnostic phosphate ion PO_3^- at *m/z* 79 and the dehydrated glycerophosphate ($C_3H_6PO_5$) ion at *m/z* 153 were present. In the fatty acid residue region, abundant carboxylate anions at *m/z* 255 and 303 facilitated the identification of the substituents at the *sn*-1 and *sn*-2 positions. Using the CF-LSIMS technique, information regarding the phospholipid molecular mass, head group and fatty substituents can be readily obtained from the positive and negative ion full-scan mass spectra of subnanogram amounts of phospholipids without the need for MS/MS. The *sn*-1 and *sn*-2 positional isomers cannot be distinguished directly based on their CF-LSIMS primary spectra alone; they co-elute and have similar mass spectra, and the relative abundances of the two carboxylate anions did not indicate any preferential release of one carboxylate anion over the other. Identification of positional isomers will still require MS/MS of precursor ions, e.g. $[M - 15]^-$, which has been reported to yield more abundant R^2COO^- than R^1COO^-.[14]

The potential of CF-LSIMS for the structural determination of individual phospholipid molecular species in small amounts of biological samples was demonstrated using commercially available egg lecithin. Figure 2(a) shows the reconstructed ion chromatogram of the class specific protonated phosphocholine (*m/z* 184) for egg lecithin (10 ng total injected on column). The positive and negative ion mass spectra of a minor peak (labeled A) are shown in Fig. 2(b) and (c). This minor

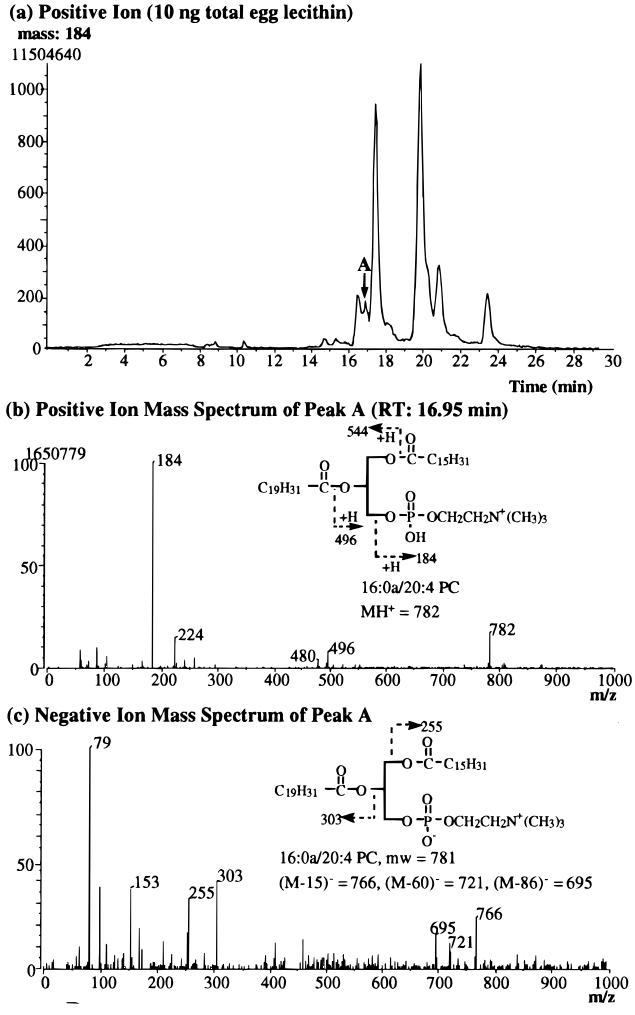

Figure 2. (a) Reconstructed ion chromatogram of phosphocholine ion (*m/z* 184) from positive ion full scan CF-LSIMS of egg lecithin (10 ng total injected on-column). (b) Positive ion full-scan mass spectrum of a minor peak (labeled A), 16:0a/20:4 GPC. (c) Negative ion full-scan mass spectrum of a minor peak (labeled A), 16:0a/20:4 GPC.

peak (A) showed MH^+ at m/z 782 in the positive ion mode and $[M-15]^-$, $[M-60]^-$ and $[M-86]^-$ at m/z 766, 721 and 695, respectively, in the negative ion mode. Two carboxylate anions at m/z 255 and 303 corresponding to the *sn*-1 and *sn*-2 substituents were also detected. This peak was therefore identified as 16:0a/20:4 PC. The identification of diacyl, alkylacyl and plasmalogen species was based on the molecular ion and *sn*-1 and *sn*-2 carboxylate anions observed, and also on assumption that the *sn*-1 and *sn*-2 groups have an even number of carbon atoms. For diacyl species, both carboxylate anions corresponding to *sn*-1 and *sn*-2 substituents were present in the negative ion mode. For alkylacyl and plasmalogen species, only one carboxylate anion corresponding to *sn*-2 acyl substituents was observed, because the *sn*-1 group did not cleave to form an anion. The alkylacyl and plasmalogen species were not distinguishable based on their mass spectra alone. They could be distinguished, however, by acid hydrolysis. Plasmalogen species are believed to be acid labile and converted into lyso-phospholipids upon acid treatment, whereas the diacyl and alkylacyl species are acid stable.[18] Using the CF-LSIMS technique, 14 GPC species present in egg lecithin were readily identified from small amounts of material, as listed in Table 1. Some minor species had very weak mass spectra, making the identification of the acyl groups difficult. In those cases, only the total number of carbon atoms and total number of unsaturations were determined, based on the molecular ion observed. Many molecular species were resolved on the capillary column. Isobaric components such as 18:2a/18:2 GPC and 16:0a/20:4 GPC, which cannot be easily distinguished using standard direct-probe FAB, can be easily identified by HPLC/CF-LSIMS, because of their facile separation on the capillary column.

Glycerophosphoethanolamine species (GPE)

The CF-LSIMS characterization of GPE species was first illustrated for synthetic GPE standards. The standard GPE species were separated or partially separated on the capillary column, as shown in Fig. 3(a), the reconstructed ion chromatogram of the class characteristic protonated phosphoethanolamine (m/z 142) for 1 ng of each standard. The representative positive ion full-scan mass spectrum for 1 ng of 18:2a/18:2 GPE is shown in Fig. 3(b). Abundant MH^+ at m/z 740 and $[M+H-141]^+$ at m/z 599 corresponding to the loss of neutral phosphoethanolamine were observed. Fragment ions (m/z 142 and 44) resulting from the polar head group were also detected. Molecular mass and head group information is, therefore, easily obtained from the positive ion full-scan mass spectrum.

In the negative ion mode, as shown in Fig. 3(c) for 18:2a/18:2 PE (10 ng injected on-column), abundant $[M-H]^-$ at m/z 738 and carboxylate anion at m/z 279 corresponding to the substituents at the *sn*-1 and *sn*-2 positions were detected, similar to those reported previously.[21,22] Information regarding the *sn*-1 and *sn*-2 substituents is, therefore, readily available from the negative ion full-scan mass spectrum, although *sn*-1 and *sn*-2 positional assignments could not be made.

The application of capillary HPLC/CF-LSIMS for the identification of phospholipid molecular species in complex mixtures was also demonstrated using GPE derived from bovine liver. The CF-LSIMS negative ion detection of this mixture (100 ng total injected on-column) revealed many GPE species, as shown in Fig. 4(a), the reconstructed ion chromatogram of phosphoethanolamine anion (m/z 140). A representative negative ion full-scan mass spectrum of a minor peak

Table 1. Identification of phosphatidylcholine (PC) molecular species in egg lecithin by CF-LSIMS

Retention time (min)	Negative ion $[M-15]^-$, $[M-60]^-$, $[M-86]^-$	Positive ion $[M+H]^+$	Diacyl[a]	Alkylacyl[b]	Plasmalogen[b]
15.00	740, 695, 669	756	16:1a/18:2		
15.51	766, 721, 695	782	18:2a/18:2		
16.59	716, 671, 645	732	16:0a/16:1		
16.59	742, 697, 671	758	16:1a/18:1		
16.59	790, 745, 719	806	16:0a/22:6		
16.95	766, 721, 695	782	16:0a/20:4		
17.46	742, 697, 671	758	16:0a/18:2		
18.18	730, 685, 659	746		34:1	34:0
18.18	792, 747, 721	808	16:0a/22:5		
19.69	744, 699, 673	760	16:0a/18:1		
20.12	794, 749, 723	810	18:0a/20:4		
20.63	770, 725, 699	786	18:0a/18:2		
21.49	758, 713, 687	774		36:1	36:0
23.30	772, 727, 701	788	18:0a/18:1		

[a] *n*1:*d*1/*n*2:*d*2 represents the arbitrary assignment of the *sn*-1 and *sn*-2 carbon chain (*n*1, *n*2) with the total number of unsaturations in both radyl groups (*d*1, *d*2). The lower-case letter following *sn*-1 radyl group indicates the type of linkage at *sn*-1, either a, e or p, representing acyl, ether or vinyl ether, respectively.

[b] *n*:*d* represents the total number of carbon atoms in both radyl groups (*n*) and total number of unsaturations in both groups (*d*). The vinyl ether degree of unsaturation in plasmalogens is not added to the value of *d*. Both alkylacyl and plasmalogen species having the same *sn*-2 fatty acyl group have similar mass spectra and diffentiation between alkylacyl and plasmalogen was not possible based on mass spectra alone.

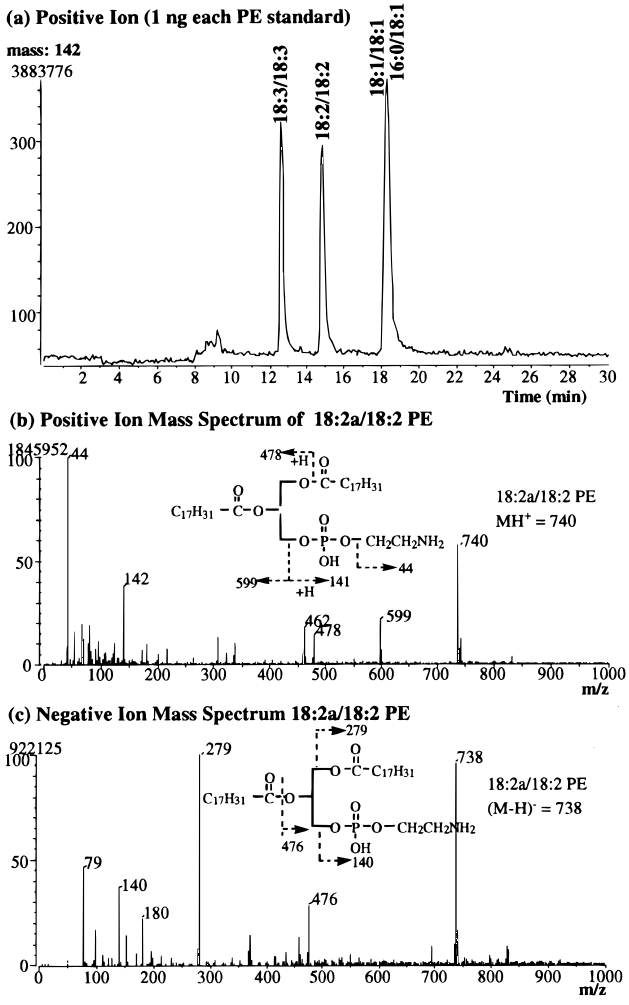

Figure 3. (a) Reconstructed ion chromatogram of protonated phosphoethanolamine (*m/z* 142) from positive ion full-scan CF-LSIMS of four synthetic GPE species (1 ng each standard). (b) Positive ion full-scan mass spectrum for 1 ng of 18:2a/18:2 GPE. (c) Negative ion full-scan mass spectrum for 10 ng 18:2a/18:2 GPE.

(labeled A) in this mixture is shown in Fig. 4(b). In the low-mass region, the diagnostic phosphate ion PO_3^- at *m/z* 79, the phosphoethanolamine ion at *m/z* 140 and an ion at *m/z* 180 resulting from the loss of both *sn*-1 and *sn*-2 substituents were observed. In the molecular ion region, abundant $[M - H]^-$ at *m/z* 670 and a minor peak at *m/z* 627 corresponding to the loss of $CH{=}CHNH_2$ were detected. In the fatty acid residue region, only one ion at *m/z* 279 was observed. The mass of the other fatty substituent can be calculated from the mass difference between the $[M - H]^-$ (670) and the GPE backbone (180) plus the carboxylate anion observed (279), and was found to be 211, indicating an ether linkage at the *sn*-1 or *sn*-2 position. This minor species was therefore identified as either 14:1e/18:2 PE or 14:0p/18:2 PE. Using the CF-LSIMS technique, more than 30 PE species were readily identified from bovine liver PE, and are listed in Table 2.

Glycerophosphoinositol species (GPI)

CF-LSIMS characterization of glycerophosphoinositol species is illustrated using a GPI mixture derived from

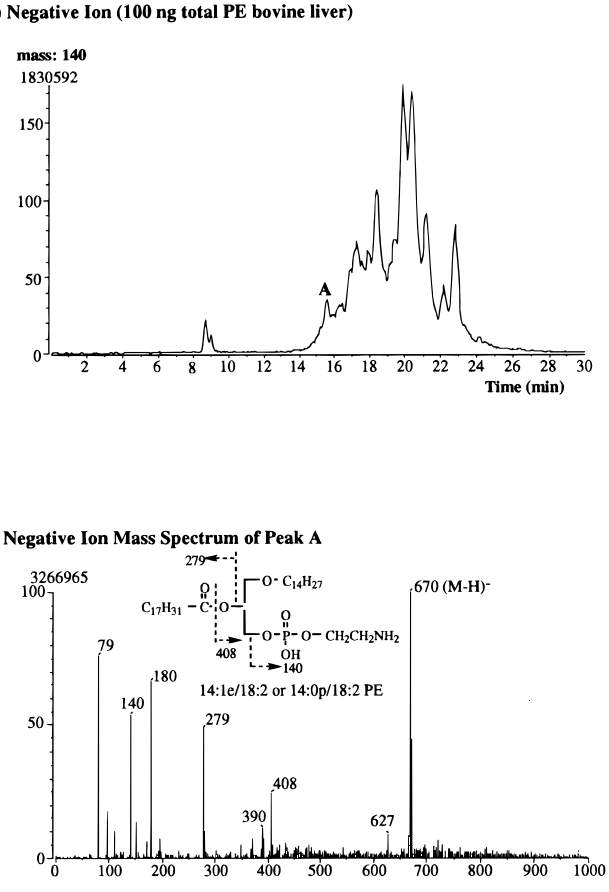

Figure 4. (a) Reconstructed ion chromatogram of phosphoethanolamine anion (*m/z* 140) from negative ion full-scan CF-LSIMS of bovine liver glycerophosphoethanolamine (100 ng total injected on-column). (b) Negative ion full scan-mass spectrum of a minor peak (labeled A), 14:1p/18:2 or 14:0p/18:2 GPE.

bovine liver. The CF-LSIMS positive ion detection of this mixture (20 ng total injected on-column) revealed several GPI species, as shown in Fig. 5(a), the reconstructed ion chromatogram of protonated phosphoinositol (*m/z* 261). The positive ion full-scan mass spectrum of a major GPI species (labeled A, 18:0a/20:4 GPI) in this mixture is shown in Fig. 5(b). Molecular ions corresponding to MH^+ at *m/z* 887 and $[M + NH_4]^+$ at *m/z* 904 were detected. The diglyceride ion at *m/z* 627, corresponding to the loss of phosphoinositol from MH^+, was detected as a major fragment ion. In addition, the phosphoinositol ion at *m/z* 261 was observed, in addition to several low-mass fragment ions resulting from the inositol group. Molecular mass and head group information on GPI species was, therefore, readily obtained from the positive ion full-scan CF-LSI mass spectrum. Positive ion FAB analysis of GPI species has not been discussed in detail in the literature, the protonated molecular ion having been either undetectable or present in low abundance.[22]

The CF-LSIMS negative ion detection of the same GPI mixture revealed more structural information. The negative ion mass spectra of GPI species obtained by CF-LSIMS were similar to those reported previously using standard direct-probe FAB-MS/MS.[22–24] As shown in Fig. 5(c) for the major GPI species 18:0a/20:4, an abundant $[M - H]^-$ ion at *m/z* 885 and a less abundant ion at *m/z* 723 corresponding to the loss of

Table 2. Identification of phosphatidylethanolamine (PE) molecular species in bovine liver PE by CF-LSIMS

Retention time (min)	Negative ion $[M-H]^-$	Positive ion $[M+H]^+$	Diacyl	Alkylacyl	Plasmalogen
14.72	682	684	32:4		
14.72	718	720		14:1e/22:6	14:0p/22:6
14.93	670	672		32:3	32:2
15.08	694	696		14:1e/20:4	14:0p/20:4
15.08	738	740	18:2a/18:2		
15.58	670	672		14:1e/18:2	14:0p/18:2
15.58	720	722		36:6	36:5
15.58	708	710	34:5		
15.58	712	714	34:3		
16.02	696	698		16:2e/18:2	16:1p/18:2
16.31	684	686	32:3		
16.31	708	710	34:5		
16.31	762	764	16:0a/22:6		
16.88	738	740	16:0a/20:4		
17.17	764	766	16:0a/22:5		
17.17	714	716	16:0a/18:2		
17.53	740	742	18:1a/18:2		
17.53	672	674		32:2	32:1
17.89	722	724		16:1e/20:4	16:0p/20:4
17.89	764	766	38:5		
18.40	698	700		16:1e/18:2	16:0p/18:2
18.40	748	750		16:1e/22:5	16:0p/22:5
18.83	686	688	14:0a/18:2		
18.83	766	768	16:0a/22:4		
18.83	728	730		36:2	36:1
19.41	716	718	16:0a/18:1		
19.41	742	744	18:1a/18:1		
19.41	790	792	20:3a/20:3		
19.84	766	768	18:0a/20:4		
20.34	742	744	18:0a/18:2		
20.34	750	752		16:1e/22:4	16:0p/22:4
20.34	792	794	18:0a/22:5		
21.14	726	728		36:3	36:2
21.14	768	770	18:0a/20:3		
21.14	700	702		34:2	34:1
21.14	730	732		36:1	36:0
21.14	750	752		18:1e/20:4	18:0p/20:4
22.15	794	796	18:0a/22:4		
22.80	744	746	18:0a/18:1		
23.45	778	780		40:5	40:4
24.17	728	730		18:1e/18:1	18:0p/18:1

[inositol $-H_2O$] was observed. Carboxylate anions at m/z 283, 303 were detected. Ions at m/z 601, 619 corresponding to loss of arachidonic acid and its ketene analog and ions at m/z 581 and 599 arising from loss of stearic acid and its ketene analog were also present. These ions allowed the arbitrary assignment of fatty acyl substituents at the *sn*-1 and *sn*-2 positions. In addition, several specific GPI related negative ions at m/z 259 (inositol phosphate), 241 [inositol phosphate $-H_2O]^-$ and 299 $[M-H-R^1COO-R^2COO]^-$ were produced. The GPI species present in PI bovine liver identified by positive and negative ion CF-LSIMS are listed in Table 3.

Glycerophosphoserine species (GPS)

Analysis of the molecular species of GPS by positive ion FAB was first described by Chen *et al.*,[25] where more than 1 μg of a single standard GPS was needed to obtain a good positive ion mass spectrum. A similar mass spectrum was obtained for sub-nanogram amounts of GPS using the CF-LSIMS technique. This is illustrated using a GPS mixture derived from bovine brain. Figure 6(a) shows the reconstructed ion chromatogram of the class characteristic serine ion (m/z 88) for the GPS mixture (10 ng total injected on-column). The positive ion full-scan mass spectrum of a major species (labeled A, 18:0a/18:1 GPS) in this mixture is shown in Fig. 6(b). The protonated molecule MH^+ at m/z 790 and the diglyceride ion $[M+H-185]^+$ at m/z 605, which was formed by the loss of phosphoserine, were observed in high abundance. The serine ion at m/z 88 and an ion at m/z 226 corresponding to the loss of both *sn*-1 and *sn*-2 substituents were produced and were diagnostic of the phosphoserine species.

Similarly to other phospholipid classes, the negative ion full-scan CF-LSI mass spectrum of GPS species, as

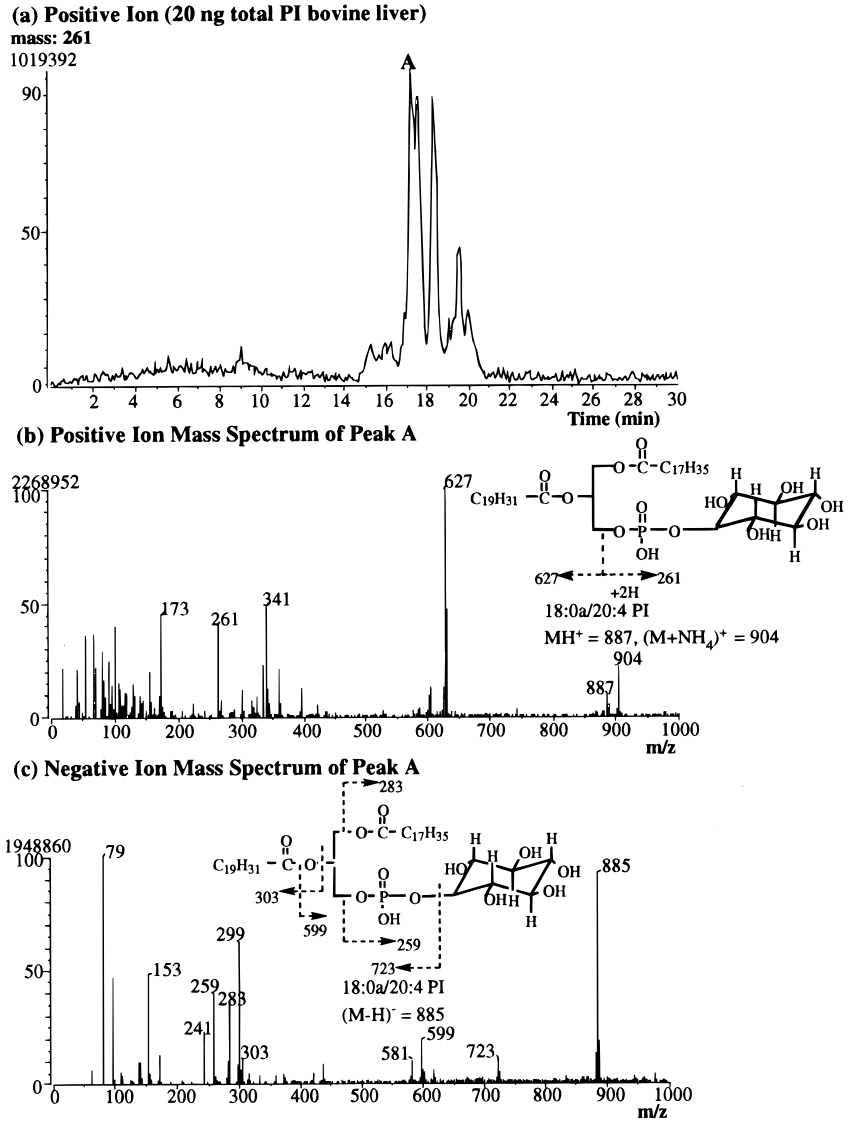

Figure 5. (a) Reconstructed ion chromatogram of protonated phosphoinositol (*m/z* 261) from positive ion full-scan CF-LSIMS of bovine liver glycerophosphoinositol (20 ng total injected on-column). (b) Positive ion full-scan mass spectrum of peak labeled A, 18:0a/20:4 GPI. (c) Negative ion full-scan mass spectrum of peak labeled A, 18:0a/20:4 GPI.

shown in Fig. 6(c) for 18:0a/18:1 GPS (labeled A), was more useful in providing information on the fatty substituents. Carboxylate anions (*m/z* 281 and 283) confirmed the fatty acyl substituents at the *sn*-1 and *sn*-2 positions. Using HPLC/CF-LSIMS, several species present in GPS derived from bovine brain were identified, as shown in Table 4.

CONCLUSION

The analysis of phospholipids is a complex and time-consuming task. CF-LSIMS provided a useful and effective way of detecting intact phospholipid molecular species. The technique has several advantages over conventional methods, one of which is its low detection limit, providing the ability to identify minor species in small amount of biological mixtures. We are currently applying this technique to the identification of arachidonyl-containing phospholipid molecular species in human U937 cells. Using sub-nanogram amounts of phospholipid, information regarding the molecular mass, the polar head group and the fatty acyl substituents can be obtained from the positive and negative ion full-scan CF-LSI mass spectra, allowing the phospholipid species to be readily identified. In the positive ion mode, all phospholipid classes showed abundant MH^+ and low-mass ions regarding the polar head group. In the negative ion mode, abundant $[M - H]^-$ ions were observed for PE, PI and PS molecular species, while $[M - 15]^-$, $[M - 60]^-$ and $[M - 86]^-$ were observed for PC species. Most importantly, abundant carboxylate anions were observed for all classes, allowing the determination of the fatty acids esterified to the *sn*-1 and *sn*-2 positions.

Coupling capillary HPLC and CF-LSIMS is particularly useful for the rapid identification of phospholipid molecular species within a complex mixture. The Hypersil C_{18} capillary column provided good if not

Table 3. Identification of phosphatidylinositol (PI) molecular species in bovine liver PI by CF-LSIMS

Retention time (min)	Negative ion $[M-H]^-$	Positive ion $[M+H]^+$, $[M+NH_4]^+$	Diacyl	Alkylacyl	Plasmalogen
14.86	857	859, 876	16:0a/20:4		
15.15	833	835, 852	16:0a/18:2		
15.15	883	885, 902	18:1a/20:4		
15.51	859	861, 878	18:1a/18:2		
15.87	871	873, 890		38:4	38:3
15.95	883	885, 902	18:0a/20:5		
16.23	847	849, 866		36:2	36:1
16.23	859	861, 878	36:3		
16.23	885	887, 904	38:4		
16.67	835	837, 854	16:0a/18:1		
17.10	885	887, 904	18:0a/20:4		
17.46	861	863, 880	18:0a/18:2		
17.40	911		40:5		
18.25	887	889, 906	18:0a/20:3		
18.97	913	915, 932	18:0a/22:4		
19.41	863	865, 882	18:0a/18:1		
20.27	889	891, 908	18:0a/20:2		

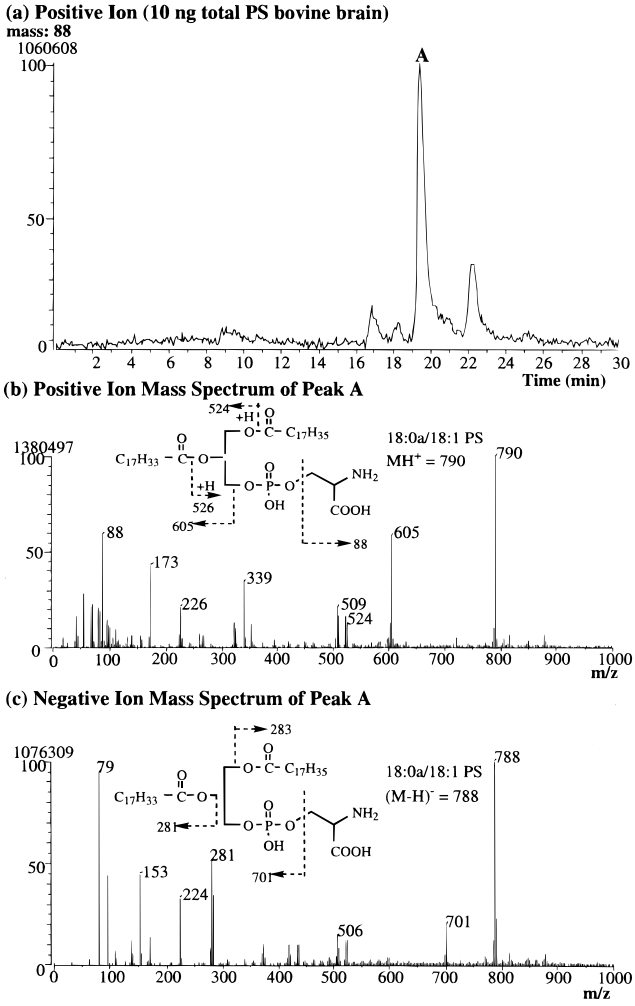

Figure 6. (a) Reconstructed ion chromatogram of serine ion (*m/z* 88) from positive ion full-scan CF-LSIMS of bovine brain glycerophosphoserine (10 ng total injected on column). (b) Positive ion full-scan mass spectrum of peak labeled A, 18:0a/18:1 GPS. (c) Negative ion full-scan mass spectrum of peak labeled A, 18:0a/18:1 GPS.

Table 4. Identification of phosphatidylserine (PS) molecular species in bovine brain PS by CF-LSIMS

Retention time (min)	Negative ion $[M-15]^-$, $[M-60]^-$, $[M-86]^-$	Positive ion $[M+H]^+$	Diacyl	Alkylacyl	Plasmalogen
17.03	760	762	34:1		
17.03	786	788	18:1a/18:1		
18.40	774	776		18:0e/18:1	
19.62	788	790	18:0a/18:1		
20.34	814	816	38:2		
20.92	802	804		38:1	38:0
20.92	840	842	40:3		
22.29	816	818	18:0a/20:1		
22.29	842	844	20:1a/20:1		

complete separation of molecular species within a particular phospholipid subclass. Molecular species ranging over 20-fold relative abundance were identified easily from the positive and negative ion LSI mass spectra of a particular phospholipid class. Isobaric isomers were separated, and can be easily distinguished.

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