Characterization of the Molecular Species of Glycerophospholipids from Rabbit Kidney: An Alternative Approach to the Determination of the Fatty Acyl Chain Position by Negative Ion Fast Atom Bombardment Combined with Mass-analysed Ion Kinetic Energy Analysis

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An alternative approach to identifying fatty acid chain position in the molecular species of glycerophospholipids has been studied and developed. The fatty acyl groups esterified to the glycerol backbone in isomeric glycerophosphatidyl-choline, -serine and -ethanolamine as well as glycerophosphatidic acid can be detected by the presence of a pair of anions derived from phosphatidic acid parent ions (M minus the polar head group in glycerophospholipids), designed to be \([M - \text{polar head} - R_1\text{COOH}]^-\) and \([M - \text{polar head} - R_2\text{CO} - H]^-\), produced by negative ion fast atom bombardment combined with mass-analysed ion kinetic energy analysis. Because of the significant abundance of \([M - \text{polar head} - R_1\text{COOH}]^-\) anion, fatty acid chains differing by 2 Da can be distinguished by accurate measurements of the electrostatic voltage related to this ion. Three-volt differences can be evidenced. Using this approach, the molecular species of glycerophosphatidyl-choline, -serine, -ethanolamine and -inositol from rabbit kidney were characterized after the separation of both class and species by normal and reversed-phase high-performance liquid chromatography, respectively. We identified 11 arachidonoyl-containing molecular species of glycerophospholipids and the other 17 lipid molecules in this biological material. A couple of 1-alkenyl-2-arachidonoyl-sn-glycerol-3-phosphoethanolamine species, identified as plasmalogen GPE 16:0-20:4 and plasmalogen GPE 18:0-20:4, were found for the first time in rabbit kidney.

**INTRODUCTION**

Lipids are important components of biological systems. The most abundant lipids in mammalian cell membranes are glycerophospholipids, and their structures consist of three parts: a glycerol backbone, a phosphorylated alcohol making the phosphate polar heads, and two fatty chains with a number of carbon atoms (between 14 and 22), esterified to the sn-1 and sn-2 positions of the glycerol backbone.

Prostaglandins, thromboxanes, leukotrienes and hydroxyeicosatetraenoic acids are the oxygen-containing metabolites of arachidonic acid, known to participate in a number of pathological processes. Some studies have reported that kidney injury is indicated by the remarkable increase of the metabolites in kidney tissues in which a high content of arachidonic acid-containing molecular species of glycerophospholipids are present. Synthesis of these metabolites is partly initiated by the release of free arachidonic acid from the glycerophospholipid species containing this fatty acid chain at the sn-2 position by phospholipase A2 enzymolysis.

In order to better understand the metabolic relationship between potential precursors and these metabolites, it is necessary to characterize such molecular species in kidney cells, especially the exact location of the arachidonic acid esterified to the hydroxyl groups in the glycerol backbone. Although the principle that the polyunsaturated fatty acid groups in natural glycerophospholipid species are preferentially located at the sn-2 position has been widely accepted, the evidence for the first determination of the lipid molecules from a new animal species is requisite.

The usual approach to identifying the location of the fatty acyl chains involves: (i) isolation of glycerophospholipid classes by thin-layer chromatography or normal-phase high-performance liquid chromatography (NP-HPLC) on the basis of their polar head groups; (ii) separation of molecular species by reversed-phase HPLC (RP-HPLC) based on level of unsaturation as well as carbon chain length; and (iii) to

1052-9306/92/120655-12 $11.00 © 1992 by John Wiley & Sons, Ltd.
identification of fatty acid position by gas chromatographic analysis of the fatty acid methyl esters after enzymatic and chemical treatments of the purified glycerophospholipid species.

In recent years, fast atom bombardment (FAB) mass spectrometry has proved to be highly powerful in the characterization of glycerophospholipids and the location of the fatty acid chains in glycerophosphatidylcholine (GPC) species could be determined utilizing the intensity differences of carboxylated fragment ions produced by negative ion FAB combined with high- and low-energy collisionally activated dissociation (CAD) of the parent ions in the high-mass region. Meanwhile, researchers also elucidated the decylated anions of GPC in the spectra produced by negative ion FAB CAD of phosphatidic acid ion (M – choline), for the identification and suggested that this approach is insensitive to the nature of the fatty acyl chains.

In the present study, first we confirm the latter approach by examining two pairs of GPC isomers (samples 13–16 in Table 1) using negative ion FAB combined with mass-analysed ion kinetic energy (MIKE) analysis, and then develop it as a method to distinguish the fatty acid position in the molecular species of other glycerophospholipids (Table 1), including glycerophosphatidic acid (GPA; samples 1–4), glycerophosphatidylserine (GPS; samples 9–12), glycerophosphatidylethanolamine (GPE; samples 5–8) and glycerophosphatidylinositol (GPI; sample 17), by the determination of the fatty acyl chain esterified to the position based on the appearance of the doublet significant fragments, \([M – \text{polar head} – \text{R}_2\text{COOH}]^-\) and \([M – \text{polar head} – \text{R}_1\text{CO} – \text{H}]^-\) anions, derived from the phosphatidic acid parent ions (M minus the polar head groups). This complementary means has been used to characterize the molecular species of glycerophospholipids from rabbit kidney.

### MATERIALS AND METHODS

#### Chemicals

1. Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (GPC 16:0–18:1), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (GPC 18:1–16:0), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (GPC 18:0–18:1), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (GPC 18:1–18:0) and the bovine liver GPI were obtained from Sigma Company (St. Louis, Missouri, USA). HPLC-grade methanol and ethanol (Carlo Erba, Milan, Italy) as well as trifluoroacetic ammonium salt (Janssen, Geel, Belgium) and diethanolamine (Fluka, Buchs, Switzerland) were used in this study.

#### Preparation of the molecular species isomers

1-Palmitoyl-2-oleoyl-sn-glycerol-3-phosphoserine (GPS 18:0–18:1), 1-oleoyl-2-palmitoyl-sn-glycerol-3-phosphoserine (GPS 18:1–16:0), 1-stearoyl-2-oleoyl-sn-glycerol-3-phosphoserine (GPS 18:0–18:1) and 1-oleoyl-2-stearoyl-sn-glycerol-3-phosphoserine (GPS 18:1–18:0) were prepared by the described procedure. After extraction of the lipids from the reacted solution by the method of Folch, the GPS and GPA were purified on a silica-gel thin-layer chromatographic plate (E. Merck, Frankfurt, Germany) using a mixture of ethyl acetate–chloroform–propanol–methanol–water (25:25:25:10:9, by volume) as the developing system.

### Table 1. Structures of the molecular species of glycerophospholipids used in this study

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<thead>
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<th>Sample</th>
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<th>(\text{R}_2)</th>
<th>(X)</th>
<th>Abbreviation</th>
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<td>H</td>
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</tr>
<tr>
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<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>H</td>
<td>GPE 18:1–16:0</td>
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<tr>
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<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>H</td>
<td>GPE 18:0–18:1</td>
</tr>
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<td>4</td>
<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>H</td>
<td>GPE 18:1–18:0</td>
</tr>
<tr>
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<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>Ethanolamine</td>
<td>GPE 16:0–18:1</td>
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<tr>
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<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>Ethanolamine</td>
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<tr>
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<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>Ethanolamine</td>
<td>GPE 18:0–18:1</td>
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<tr>
<td>8</td>
<td>(C_{16}H_{33})</td>
<td>(C_{18}H_{37})</td>
<td>Ethanolamine</td>
<td>GPE 18:1–18:0</td>
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<tr>
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<td>Serine</td>
<td>GPE 16:0–18:1</td>
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<td>Choline</td>
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<tr>
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<td>(C_{16}H_{33})</td>
<td>Choline</td>
<td>GPE 18:1–18:0</td>
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<td>17</td>
<td>(C_{16}H_{33})</td>
<td>Inositol</td>
<td>GPE 18:0–20:4</td>
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</table>

#### Preparation of biological samples

Total lipids were extracted from the cortex (4 g) of the kidney from a New Zealand rabbit (3 months old, weight 2.7 kg) by the method of Folch. Pure glycerophospholipid classes were prepared using the reported procedure. Briefly, the lipids in chloroform–methanol
(6:4, by volume) were isolated into two fractions on a short column containing Q-Sepharose anion-exchange resin (Pharmacia, Uppsala, Sweden) using (i) chloroform–methanol (7:3, by volume) for the elution of non-acidic classes and (ii) acetic acid–chloroform (5:1, by volume) for the elution of acidic classes. The rabbit GPE, GPC, GPS and GPI were further purified on an aninopropyl-bonded HPLC column (Chrompack, LiChrosorb NH2, 10 µm, 46 × 250 mm, Middelburg, The Netherlands) using a mixture of ethanol–0.9 M phosphoric acid (85:15 for GPS and GPI, and 90:10 for GPE and GPC, respectively) as the mobile phase.14 The molecular species within each class were separated on a C18 HPLC column (Ultraphere-ODS, 5 µm, 4.6 × 25 cm, Altex Scientific, Berkeley, California, USA) with a mixture of methanol–ethanol (6:4):water (91:9) containing 7 mM trifluoroacetic acid ammonium salt as the mobile phase, at a flow rate of 1.0 ml min⁻¹ (for the GPE and GPC) and of 0.6 ml min⁻¹ (for the GPS and GPI), respectively.19,20 The column was kept at 35°C. A Perkin-Elmer HPLC system (Connecticut, USA), consisting of a series of 3B pump and LC-75 detector, was used in the study. The wavelength of detection was 205 nm. Twenty microliters of the sample dissolved in chloroform was loaded on the column. The acid in the NP-HPLC fractions were removed by the method of Foleh. The molecular species containing the RP-HPLC fractions were extracted from the residue by chloroform after drying of the solvent by nitrogen.

Percentage of the glycerophospholipid classes

Percentage analysis of the rabbit kidney GPC, GPE, GPS and GPI was carried out by the phosphoric assay after thin-layer chromatographic separation of the classes.11

Mass spectrometry

All mass spectra were obtained on a VG ZAB-2F (VG Analytical, Manchester, UK), equipped with FAB gun, static FAB ion source, standard FAB probe and data processing and recording systems. The bombarding gas was xenon, with kinetic energy of 7-8 keV. Parameters used for MIKE analysis were: (i) electrostatic scan regions were from 400 to 950 V for 18:0–18:1 and 18:1–18:0 isomers, or from 100 to 950 V for the others, respectively, (ii) response time was 100 ms; and resolution was 500. Most samples were dissolved in chloroform–methanol–water (48:4:0.2), and the FAB probe was introduced into the mass spectrometer after precipitation of the solution on diethanolamine matrix.19 The GPE samples for MIKE analysis were mixed with diethanolamine in order to increase the intensities of [M − 43]⁻ ions of the GPE.

RESULTS AND DISCUSSION

Verification of the analytical approach by examining GPC isomers

Fenwick and co-workers31 first reported the positive ion FAB analysis of GPC molecular species containing the two identical fatty acyl chains esterified to the sn-1 and sn-2 positions and elucidated their protonated molecules in the high-mass region (m/z 600 to 900), deacyl and deacyloxy cations in the intermediate-mass region (m/z 350 to 550) and the choline phosphate ion at m/z 184 in the mass spectra for the characterization of GPC species. Using this method, Ohashi31 studied GPC molecular species containing the two different fatty acyl groups by secondary ion mass spectrometry and observed the intensity differentiation of the deacyl and deacyloxy doublet cations for the distinction of GPC species isomers. Because of the limitation of positive ion FAB in analysing various glycerophospholipids (for example, the cations are absent in the positive ion FAB mass spectra of GPI33 and GPE34), Munster and Budzikiewicz34 described the analysis of GPC molecular species by negative ion FAB mass spectrometry. Triplet ions, [M − 15]⁻, [M − 60]⁻ and [M − 86]⁻, corresponding to the molecular weight of GPC species (Scheme 1), the deacylated anions and the carboxylated fragments in the spectra were provided for the structural identification.

Further investigations20–24 indicated that the abundant carboxylated ions arising from low and high-energy CAD of the [M − 15]⁻ ion (see Scheme 1) showed an intensity ratio of 1:3 for the fatty acyl chains esterified to the sn-1 and sn-2 positions,24 and the location of the fatty acid chains in the GPC species can be determined. Furthermore, Jensen,20 Hayashi,22 Huang25 and their co-workers elucidated two pairs of the anions derived from CAD of [M − 86]⁻ parent, assigned to be [M − 86 − R₁CO − H]⁻ and [M − 86 − R₂COOH]⁻ as well as [M − 86 − R₁CO − H]⁻ and [M − 86 − R₂COOH]⁻. The fatty acid location in GPC species can also be characterized on the basis of

\[\text{CH}_2\text{-O-CO-R}_1\]
\[\overset{\text{O}}{\text{CH}_2\text{-O-CO-R}_2}\]
\[\overset{\text{O}}{\text{CH}_2\text{-O-P-O-CH}_2\text{-CH}_2\text{-N}(\text{CH}_3)_2}\]
\[\overset{\text{O}}{\text{O}}\]

\[\text{(M-15)⁻}\]

\[\text{CH}_2\text{-O-CO-R}_1\]
\[\overset{\text{O}}{\text{CH}_2\text{-O-CO-R}_2}\]
\[\overset{\text{O}}{\text{CH}_2\text{-O-P-O-CH}_2\text{-CH}_2\text{-N}(\text{CH}_3)_3}\]
\[\overset{\text{O}}{\text{O}}\]

\[\text{(GPC)}\]

\[\overset{\text{O}}{\text{CH}_2\text{-O-P-O-CH}_2\text{-CH}_2}\]
\[\overset{\text{O}}{\text{O}}\]

\[\text{(M-60)⁻}\]

\[\text{CH}_2\text{-O-CO-R}_1\]
\[\overset{\text{O}}{\text{CH}_2\text{-O-CO-R}_2}\]
\[\overset{\text{O}}{\text{CH}_2\text{-O-P-O-CH}_2\text{-OH}}\]
\[\overset{\text{O}}{\text{O}}\]

\[\text{(M-86)⁻}\]

\[\text{Scheme 1}\]
the different abundances of the anions. We fully confirm the latter approach by negative ion FAB combined with MIKE analysis of GPC species isomers (samples 13–16 in Table 1).

Figure 1 shows the MIKE spectra of \([M-86]^-\) ion generated by negative FAB ionization of (a) 16:0–18:1 and (b) 18:1–16:0 species. The two significant anions at \(m/z\) 391 and 409 as well as \(m/z\) 417 and 435, related to \([M-86-R_2COO^-]^-\) and \([M-86-R_2COOH^-]^-\) ions, are easily recognized. Compared with \([M-86-R_2COO^-]^-\) and \([M-86-R_2COOH^-]^-\) anions, the formation of the former pair of anions, due to the loss of the sn-2 fatty acyl chain from the species, is more preferable. The MIKE spectra of \([M-86-R_2COO^-]^-\) ions at \(m/z\) 419 and 417, corresponding to 18:0 and 18:1 acyl chains esterified to the sn-1 position. Three-unit differences in the voltage are easily differentiated.

In contrast to the reported data\(^{20-24}\) and our results (not shown) obtained by negative ion FAB with high- and low-energy CAD or FAB MIKE analysis of \([M-15]^-\) and \([M-60]^-\) ions of GPC species, the intensities of the carboxylated peaks derived from \([M-86]^-\) ion are relatively weak and show opposite ratio. But \([M-86-R_2COO^-]^-\) and \([M-86-R_2COOH^-]^-\) ions produced by FAB CAD or FAB MIKE measurements of the \([M-86]^-\) ion of GPC are present and show high abundance.

The distinction of 18:0–18:1 and 18:1–18:0 isomers by negative ion FAB MIKE analysis without CAD is considered to be easier to achieve, because the increase in relative intensity of the \([M-86-R_2COO^-]^-\) peak resulting from CAD, accompanying the peak enlargement due to the higher kinetic energy release under such conditions, may extend overlapping on the two ions with 3 V differences and make accurate measurement difficult. The results obtained indicated that \([M-86]^-\) ionic species, corresponding to phosphatidic acid, are also the useful parent ions for the determination of the fatty acid position in GPC species by using negative ion FAB with tandem mass spectrometry.

Development of the analytical approach

Analysis of glycerophosphoserine. Jensen\(^{20}\) and co-workers first reported the negative ion FAB CAD analysis of the \([M-H]^-\) ion from a single GPC species and observed the fragments of \([M-87]^-\), related to phosphatidic acid species (M–serine), \([M-87-R_2COOH^-]^-\) and carboxylated groups. We found, in the negative ion FAB MIKE spectra, that: (i) the carboxylated ions derived from \([M-H]^-\) precursors are present (Fig. 2(a)); (ii) the intensity of the \([M-87-R_2COO^-]^-\) and \([M-87-R_2COOH^-]^-\) ions derived from \([M-87]^-\) ions of 16:0–18:1 and 18:1–16:0 species (Fig. 2(b) and (c)) are more abundant than those derived from their \([M-H]^-\) ions (Fig. 2(a)). 18:0–18:1 and 18:1–18:0 isomers can be distinguished by the appearance of \([M-87-R_2COO^-]^-\) anions (Fig. 2(d) and (e)). Although \([M-87]^-\) ions of GPE species show relatively lower intensities than their \([M-H]^-\) peaks (three to five times lower), \([M-87-R_2COOH^-]^-\) anion derived from the former is yielded easily.

Analysis of the other isomers. Isomeric GPA species (samples 1–4) were used to verify the results obtained above. An identical pattern of ions, as GPC and GPS, can be observed in their negative ion FAB MIKE spectra (not shown). This approach is also effective in the identification of the GPE isomers (samples 5–8) and GPI species (sample 17).
The molecular species analysis of rabbit kidney glycerophospholipids

Arachidonic acid metabolites have played a role in the regulation of renal blood flow, glomerular filtration rate and urinary concentration. The studies reported that the synthesis of these metabolites occurred in the stimulated kidney cells. Our previous work described the profile of the arachidonic acid-containing molecular species of glycerophospholipids from rat kidney. In the following part of the study, we present structural information on the composition of the molecular species within the major glycerophospholipids, GPC, GPE, GPS and GPI, from rabbit kidney and the location of arachidonic acid and other fatty chains in the species molecules.

Glycerophosphatidylcholine GPC is one of the major classes (33%) of rabbit kidney glycerophospholipids. Figure 3 shows the RP-HPLC separation of rabbit kidney GPC species. Each HPLC peak was analysed by FAB mass spectrometry and negative ion FAB MIKE spectrometry. We identified ten molecular species (Table 2), including two molecules containing arachidonic acid.
Table 2. The molecular species of rabbit kidney GPC by negative ion FAB and FAB MIKE analyses

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<td>409 (20)</td>
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<td>673</td>
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<td>699</td>
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* Peak numbers correspond to those shown in Fig. 3.

Numbers in parentheses are the absolute intensities of the ions (arbitrary units).

sn-1/sn-2.

M.n code is due to total carbons: unsaturation.

chains esterified to the sn-2 position. Figure 4 shows the mass spectra of the RP-HPLC fractions 3 + 4. Ions at m/z 782 and m/z 758 (Fig. 4(a)) relate to the protonated molecules of 36:4 and 34:2 species. Triplet ions of the two lipids, ions at m/z 766 (781 – 15), 721 (781 – 60) and 695 (781 – 86), as well as m/z 742 (757 – 15), 697 (757 – 60) and 671 (757 – 86), can also be observed in the negative ion FAB mass spectrum (Fig. 4(b)). The fragments generated by negative ion FAB MIKE analysis of the peaks at m/z 695 (781 – 86) from 36:4 species and m/z 697 (757 – 60) from 34:2 species (Fig. 4(c)) are derived from the two parent ions. Anions at m/z 391 ([M – 86 – R₂COOH]⁻) and 409 ([M – 86 – R₂CO – H]⁻), derived from the ion at m/z 695

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Figure 4. Positive (a) and negative (b) ion FAB spectra of the HPLC peaks 3 + 4 of the GPC (see Fig. 3) and the negative ion MIKE mass spectrum (c) of the selected ions from FAB of GPC 16:0-20:4 and GPC 16:0-18:2.
Carboxylated fragments derived from the \([M - 60]^-\) ion of 34:2 species (m/z 697 in Fig. 4(c)), at m/z 255 (palmitic acid; 16:0) and m/z 279 (linoleic acid; 18:2), and \([M - 86 - R_1COOH]^-\) anion derived from its \([M - 86]\) precursor at m/z 671, provide information on the location of the two fatty acyl chains in this molecule. It is 1-palmitinoyl-2-linoleoyl-sn-glycerol-3-phosphocholine (GPC 16:0-18:2). Table 2 lists the rabbit kidney GPC molecular species identified by this approach. Location of the fatty acyl chains in a few minor species could not be determined because of lower intensities of their parent ions. The difference of rabbit kidney GPC from rat kidney GPC is in the percentage distribution of the class. The profiles of the molecular species in both materials are quite similar.10

**Glycerophosphatidylethanolamine.** GPE is the other abundant class (27%) in rabbit kidney lipids. Its molecular species usually exist in diacyl, alkenylacyl and alkacylacyl forms. Figure 5 shows the RP-HPLC separation of the GPE species. Components-containing the HPLC fractions were characterized by negative ion FAB and FAB MIKE analyses. Thirteen molecular species, including five major molecules containing arachidonic acid chain at sn-2, were identified. Figure 6(a) shows the negative ion FAB mass spectrum of the RP-HPLC fraction 7. Two abundant ions can be observed at m/z 740 and 766. The latter corresponds to the deprotonated molecule of the 38:4 species. Peaks at m/z 283 (stearic acid; 18:0) and m/z 303 (arachidonic acid; 20:4) give the fatty acid composition of this species. Based on both the presence of \([M - 43 - R_2COOH]^-\) anion derived from its \([M - 43]^-\) peak (Fig. 6(c)) and the intensity differences of the carboxylated fragments (Fig. 6(b)) derived from its \([M - H]^-\) ion, produced by negative ion FAB MIKE analysis, this species is determined as 1-stearoyl-2-arachidonoyl-sn-glycerol-3-phosphoethanolamine (GPE 18:0-20:4). The ion at m/z 740 is due to 1-oleoyl-2-linoleoyl-sn-glycerol-3-phosphoethanolamine (GPE 18:1-18:2), identified by the peaks at m/z 281 (oleic acid; 18:1) and m/z 279 (linoleic acid; 18:2) in Fig. 6(a), \([M - 43 - R_2COOH]^-\) anion at m/z 418 derived from its \([M - 43]^-\) parent (not shown) and gas chromatographic analysis of the fatty acid methyl esters of this RP-HPLC fraction. Figure 7(a) shows the mass spectrum of the HPLC peak 9. The ion at m/z 750 is most likely the deprotonated molecule of plasmalogen GPE 38:4 (1-alkenyl-2-acyl-sn-glycerol-3-phosphoethanolamine). The appearance of the peak (Fig. 7(b)) at m/z 303 (arachidonic acid; 20:4) and the anions (Fig. 7(c)) at m/z 404 (\([M - 43 - R_2COOH]^-\)) and 422 (\([M - 43 - R_1CO - H]^-\)), derived from negative ion FAB MIKE analyses of its \([M - H]^-\) and \([M - 43]^-\) parent ions, confirm the fact that only one acyl chain (arachidonic acid) is present in the molecule, and it is esterified to the sn-2 position, because non-carboxylated fragment is absent in the negative ion FAB spectrum.12 This species is identified as plasmalogen GPE 18:0-20:4. The other two abundant species containing arachidonic acid chain at sn-2 are 1-palmitoyl-2-arachidonoyl-sn-3-phosphoethanolamine (GPE 16:0-20:4; RP-HPLC peak 3) and its plasmalogen analogue (plasmalogen GPE 16:0-20:4; RP-HPLC peak 5) as well as a minor species, 1-linoleoyl-2-arachidonoyl-sn-3-phosphoethanolamine (GPE 18:2-20:4; RP-HPLC peak 1). Table 3 lists the composition of rabbit kidney GPE species. Compared with rat kidney GPE,10 the main difference is that 1-alkenyl-2-arachidonoyl-GPE species are present as the major components in rabbit kidney GPE.

**Glycerophosphatidyl-serine and -inositol.** GPS (4%) and GPl (6%) are the two minor classes in rabbit kidney lipids. Their molecular species were separated by RP-HPLC (Fig. 8). Three species in GPS were identified. Figure 9(a) shows the negative ion FAB mass spectrum of the RP-HPLC fraction 3. The abundant ion appears at m/z 786, corresponding to the deprotonated molecule of GPS 36:2 species. The ion at m/z 700 is due to the loss of serine from this species. Location of the two fatty acyl chains is identified by negative ion FAB MIKE analysis. The presence of the anions at m/z 420 (\([M - 87 - R_1COOH]^-\)) and m/z 424 (\([M - 87 - R_1COOH]^-\)) indicates that the 18:0 group is esterified to the sn-1 position. Gas chromatographic methyl ester analysis of RP-HPLC fraction 3 supports the identification of the fatty acid composition. This species is 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoserine (GPS 18:0-18:2). Two minor species are 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoserine (GPS 16:0-20:4; RP-HPLC peak 1), on the basis of its deprotonated molecule at m/z 782 and the intensity differences of carboxylated fragments generated by negative ion FAB MIKE analysis of its \([M - H]^-\) ion (not shown), and
Figure 6. Negative ion FAB mass spectrum (a) of the HPLC peak 7 of the GPE (see Fig. 5) as well as the negative ion MIKE mass spectra of [M – H]− ion (b) and [M – 43]− fragment (c) from FAB of GPE 18:0–20:4.

1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoserine (GPS 18:0–20:4; RP-HPLC peak 2), based on the ion at m/z 810 ([M – H]−) and 724 ([M – serine]−) in Fig. 9 as well as [M – 87 – R1 – COOH]− anion derived from negative ion FAB MIKE analysis of the ion at m/z 724.

The molecular species of rabbit kidney GPI (the RP-HPLC fraction 1) was characterized by negative ion FAB and FAB MIKE analysis as 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoinositol (GPI 18:0–20:4), based on the ions at m/z 885 (the deprotonated molecule) and m/z 724 ([M – inositol]−) in Fig. 10(a) as well as the anions at m/z 420 ([M – 162 – R2 COOH]−) and 438 ([M – 162 – R2 – CO – H]−) in
Figure 7. Negative ion FAB mass spectrum (a) of the HPLC peak 9 of the GPE (see Fig. 5) as well as the negative ion MIKE mass spectra of \([M - H]^-\) ion (b) and \([M - 43]^+\) fragment (c) from FAB of plasmalogen GPE 18:0-20:4. D peaks are due to diethanolamine matrix.

Figure 8. RP-HPLC separation of the molecular species of glycerophosphatidylerine (a) and inositol (b) from rabbit kidney. The HPLC conditions are described under Materials and methods. Peaks were numbered in the sequence of elution and were identified by FAB mass spectrometry.
Table 3. The molecular species of rabbit kidney GPE by negative ion FAB and FAB MIKE analyses

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* Peak numbers correspond to those shown in Fig. 5.
Numbers in parentheses are the absolute intensities of the ions (arbitrary units).
M : N code is due to total carbons : unsaturation.
Plasmalogen GPE molecular species.

Figure 9. Negative ion FAB mass spectrum (a) of the HPLC peak 3 of the GPS (see Fig. 8(a)) and the negative ion MIKE mass spectrum of [M - 87]- fragment (b) and [M - 43]- from FAB of GPS 18:0-18:2. D peaks are due to diethanolamine matrix.
**CONCLUSION**

Location of the fatty acyl chains in GPC molecular species can be identified by the determination of the fatty acyl group esterified to the sn-1 position on the basis of \([M - \text{choline} - R \text{COOH}]^-\) and \([M - \text{choline} - R \text{CO} - H]^-\) anions derived from negative ion FAB MIKE analysis of phosphatidic acid ionic species (M minus choline group). Because of the significant abundance of the \([M - \text{choline} - R \text{COOH}]^-\) anion, the distinction of the molecular species differing by 2 Da is achieved based on the differences of the electrostatic voltage of this ion.

The study further demonstrated that this approach is also effective in the identification of other glycerophospholipids, including GPS, GPA, GPE and GPI, and in the characterization of the molecular species of glycerophospholipids from biological material. The major disadvantage of this approach in identifying natural glycerophospholipid species is that the analysis cannot be carried out in the case of lack of separation of the molecular species by RP-HPLC, because the parent ions, corresponding to the phosphatidic acid fragment, are not easy to select. However, it also makes identification of the minor molecular species in the RP-HPLC fractions difficult. More than 2 μg of sample are required.

The present data indicated that 1-alkenyl-2- arachidonoyl-sn-glycerophosphoethanolamine (plasmalogen GPE molecular species) is more abundant in rabbit kidney than in rat kidney. This diversity should be taken into account in studies on renal pathology and biochemistry when different animal species are used.
REFERENCES