Specific Detection of Lysophosphatidic Acids in Serum Extracts by Tandem Mass Spectrometry

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The various molecular species of lysophosphatidic acid (lysoPA) exhibit different abilities to activate the various lysoPA receptors. The composition of lysoPAs is affected by factors such as external stimulants and diseases and their profile can change on an hourly basis. For this reason, studying lysoPAs or using lysoPAs as markers for the survey of disease requires analysis of individual lysoPA species. We examined methods for sensitive and selective detection of lysoPAs in a mixture of serum lipids using precursor ion scanning and neutral loss scanning. Precursor ion scanning of m/z 153 (glycerophosphate—H—H2O) was effective for first screening of all acyl lysoPAs in lipid mixture due to its high sensitivity. This method has also some advantages such as high selectivity, and rapid and easy operation without conventional method by extraction from thin layer chromatography or separation using high performance liquid chromatography. We successfully detected acyl 22:6 (docosahexanoyl) and 22:5 (docosapentaenoyl) lysoPAs in serum using this method.

1. Introduction

Lysophosphatidic acids (lysoPAs) are found in the serum and exhibit numerous activities including stimulation of cell growth, stimulation of platelet aggregation, and induction of cancer cell invasion. In addition, lysoPAs may be useful markers for the early stages of ovary cancer, which is otherwise difficult to detect. The activities of lysoPAs are mediated by G-protein-coupled receptors (GPCRs) including lysophosphatidic acid-1 (LPA1)/endothelial cell differentiation gene-2 (Edg2), LPA2/Edg4, and LPA3/Edg7. These receptors are based on the presence of head groups other than sphingosine head groups at m/z 264, and these product ions are used to detect CERs in lipid mixture using precursor ion scanning. Therefore, characteristic product ions or neutral loss can be used to detect molecules using precursor ion scanning or neutral loss scanning, respectively.

Lipid mixtures from either the serum or plasma were found to contain various lysophospholipids (will be reported elsewhere). Lysophospholipids have one fatty acyl, alkyl, or alkenyl chain in their structure, i.e., lysophosphatidycholines (lysoPCs) and lysophosphatidylethanolamines (lysoPEs), as well as phospholipids having two fatty acyl chains. The structural differences between these lysophospholipids and lysoPAs are based on the presence of head groups other than phosphate. Precursor ion scanning of phosphatidylcholine (PC) revealed product ions for phosphoryl-choline, but precursor ion scanning using one product ion (m/z 184) showed peaks for PCs, indicating that precursor ion scanning of the mass value corresponding to the head group is useful for selective detection of relative phospholipid classes. In contrast, lysoPAs have no characteristic structural fragments such as these head groups. It was reported that precursor ion scanning of m/z 79 ([PO3]−) could be utilized for analysis of lysoPAs. However, it is possible that phosphoric acid will be generated by most classes of phospholipids. Therefore, precursor ion scanning of phosphoric acid would reveal peaks not only for lysoPAs but also for many other phospholipids.
In this paper, we report methods for selective detection of lysoPAs in extracted lipid mixture using precursor ion scanning.

2. EXPERIMENTAL

2.1 Materials

Deionized water was obtained from a Milli-Q water system (Millipore, Milford, MA). All other chemicals were of analytical reagent grade unless otherwise stated. Standard phospholipids, such as diacyl 16:0–16:0 PC and diacyl 16:0–16:0 phosphatidylethanolamine (PE), as well as calf serum were purchased from Sigma Chem. Co. (St. Louis, MO). Other phospholipids, such as PCs of various molecular species, were obtained from Avanti Co. (Alabaster, AL). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Acyl 16:0 lysophosphatidylglycerol (lysoPG) was obtained following addition of excess lipase (Rhizopus delemar) (with phospholipase A1 activity) to diacyl 16:0–16:0 PG by first extraction with ether and water (1:1), and second extraction with chloroform and 30% acetic acid.

2.2 Extraction of lipids from the serum

Extract cartridges (Oasis HLB, Hydrophilic–Lipophilic Balance, 30 mg, Waters) were first conditioned by rinsing with methanol (1 mL) and water (1 mL). As a standard, acyl 14:0 lysoPA (200 pmol/μL) in methanol (20 μL) was dried under a gentle stream of nitrogen. Incubated calf serum (100 μL) was added to the standard. The sample was loaded onto the cartridge, and the cartridge was centrifuged at 1000 rpm for 5 min (about 20 μL/min) and washed with water (1 mL) and chloroform (300 μL). The sample was then eluted with methanol (200 μL).

2.3 ESI-MS/MS analysis of phospholipids

Product ion scanning, precursor ion scanning, and neutral loss scanning were performed on a programmable pump model 305 (Gilson; Middleton, WI) equipped with a 20 μL-sample loop and a hybrid quadruple and linear ion trap mass spectrometer, Q TRAP™ TC/MS/MS system (Applied Biosystems, Foster City, CA). A total of 25 μL of sample was subjected to each analysis. The solvent system was used at a flow rate of 100 μL/min. Collision energy was 5–130 eV. The mobile phase consisted of methanol–28% aqueous ammonium–water (1000:1:1, pH 11).

2.4 Calibration curves for analysis of quantitative alteration

In order to obtain calibration curves for concentration, efficiency of ionization proportional to fatty acyl chain length, and efficiency of ion suppression, various concentrations of a mixture of equimolar amounts of acyl 12:0, 14:0, 16:0, 18:0 lysoPAs were added to a mixture of serum lipids prepared with HLB. As standard, acyl 14:0 lysoPE was added to the samples. Samples were dried under a gentle stream of nitrogen, dissolved in methanol, and were then measured using precursor ion scanning of m/z 153 at 30 eV in negative ion mode. The concentrations of each lysoPA used were 0, 0.25, 0.5, 2.5, 5, and 10 pmol/μL. Twenty microliters of injected sample contained the lipid mixture from 20 μL of serum. The relative intensities of each lysoPA calculated using average intensities of them and lysoPE standard. Calibration curves were obtained from the concentrations and the calculated intensity of each lysoPA.

2.5 Identification of individual molecular species of phospholipids by MS data

Using theoretical mass data, we identified the individual molecular species of each phospholipid class, such as lysoPCs and lysoPEs. Differences between the observed experimental mass values of individual monoisotopic peaks and their theoretical values were typically less than 0.4. Italic numerical values (e.g., m/z 153.0) indicate the theoretical mass values, while non-italicized numerical values (e.g., m/z 153.1) indicate experimental mass values.

Lyso phospholipids were designated as follows: acyl 16:1 lysoPA (16:1 in Fig. 5), where 16 indicates the number of carbon atoms of the acyl group at the sn-1 or sn-2 positions, and 1 indicates the number of double bonds at the sn-1 or sn-2 positions, and alk 16:1 lysoPA, where alk 16:1 indicates alkyl (alkyl ether) 16:1 and/or alkenyl (vinyl ether) 16:0.

3. Results and Discussion

3.1 Product ion scanning of lysoPA

LysoPA has glycerophosphate (GP), while 2-lysoPA (Fig. 1) and 1-lysoPA have a fatty acid (FA) ester at the sn-1 and sn-2 positions, respectively. LysoPAs were detected as [M−H]− in negative ion mode (Fig. 2a). In Fig. 2b, the peaks corresponding to [GP−H−H2O]− (m/z 153.0) and [PO3]− (m/z 79.0) were detected by product ion scanning of [M−H]− (m/z 409.3, m/z 409.2) of lysoPA at 30 and 80 eV. LysoPAs have no characteristic structural fragments, such as a head group. We performed product ion scanning of various phospholipids at various collision energies in order to explore the differences between lysoPAs and other phospholipids. Figure 2c shows that the optimal collision energy for generation of [GP−H−H2O]− in negative ion mode was 20–30 eV. The intensity of [PO3]− was almost constant at 50–130 eV. The rate of generation for [PO3]− was 30% that for [GP−H−H2O]−. The FA ion is one of the main product ions from most glycerol-phospholipids but not from lysoPAs. Product ions from lysoPAs included a weak peak intensity for FA, and a strong peak intensity for [GP−H−H2O]−.

Fig. 1. Structure of acyl 16:0 2-lysophosphatidic acid (lysoPA) and schema of its product ions from deprotonated molecule of lysoPA. Deprotonated molecule of lysophosphatidic acids (lysoPAs) is detected in negative ion mode. Italic numerical values (e.g., m/z 153.0) indicate theoretical mass values. GP, glycerophosphate; FA, fatty acid.
3.2 Precursor ion scanning and neutral loss scanning for standard lysoPAs

We explored a specific and sensitive method that detects all molecular species of lysoPAs. In negative ion mode, the main product ion of lysoPAs from [M−H]− was [GP−H−H2O]−, and the secondary product ion was [PO3]−, as shown in Figs. 2b and c. Standard acyl 12:0 lysoPA was analyzed using precursor ion scanning of m/z 199 (m/z 199.0) at 40 eV, m/z 79 (m/z 79.0) at 80 eV, and m/z 153 (m/z 153.0) at 30 eV (Fig. 3a). Small amounts of deprotonated molecule of acyl 12:0 lysoPA (m/z 353.2) were detected in precursor ion scanning of m/z 199. The precursor ion scanning of [M−H]− of FA is one of the major methods for detecting most glycerolphospholipids, but not for detecting lysoPAs. Precursor ion scanning of m/z 153 was more sensitive than that of m/z 79 or 199, thus showing that the sensitivity of precursor ion scanning depends on the sensitivity of the product ion (Fig. 2b).

The [GP−H−H2O]− was generated from [M−H]− of lysoPAs with neutral loss of FA on product ion scanning. Acyl lysoPAs have an ester bond, while alk (alkyl and alkenyl) lysoPAs have an ether bond. Acyl lysoPAs generate neutral loss of FA, and the [GP−H−H2O]− is produced, while this ion does not appear in alk lysoPAs. This suggests that precursor ion scanning of m/z 153 ([GP−H−H2O]−) does not detect alk lysoPAs. Precursor ion scanning of m/z 79 is able to detect alk lysoPAs, but amounts of alkyl-acyl (alkyl) and alkenyl-acyl (alkenyl) phospholipids (lyso phospholipids) are substantially less than those of diacyl (acyl) phospholipids (lyso phospholipids) in mammalian sources, and thus it is difficult to detect alk lysoPAs by precursor ion scanning of m/z 79 due to its low sensitivity in detecting lysoPAs.

Standard acyl 12:0 lysoPA was analyzed using neutral loss scanning of 200 u at 30 eV (Fig. 3a). Generation of a product ion at m/z 153 ([GP−H−H2O]−) from acyl 12:0 lysoPA (m/z 353.2) is equal to the generation of a neutral loss of 200 u (200.2−353.2−153.0, acyl 12:0 FA) from acyl 12:0 lysoPA. The sensitivity of neutral loss scanning of 200 u was 70% that of precursor ion scanning of m/z 153, thus show-
ing that the sensitivity of precursor ion scanning is better than that of neutral loss scanning.

Production ion scanning of lysoPAs resulted in [GP – H – H2O]−, regardless of fatty acyl chain length (data not shown). Deprotonated molecules of acyl 12:0 (m/z 353.4, m/z 353.2) and 14:0 (m/z 381.3, m/z 381.2) lysoPAs were detected when the mixture of acyl 12:0 and 14:0 lysoPAs was analyzed using precursor ion scanning of m/z 153 (Fig. 3b, left). Acyl 12:0 lysoPA was detected by neutral loss scanning of 200 u, while acyl 14:0 lysoPA was not detected (Fig. 3b, middle). Acyl 14:0 lysoPA was detected by neutral loss scanning of 228 u (228.2–381.2–153.0, acyl 14:0 FA) (Fig. 3b, right). These results showed that precursor ion

![Graph](image)

**Fig. 3.** Comparison of intensities of precursor ion scanning and neutral loss scanning for lysoPAs. a) Deprotonated molecule of acyl 12:0 lysoPA (m/z 353.1) was detected using precursor ion scanning of m/z 199 ([acyl 12:0 FA]−) at 40 eV ("Pre199"), at m/z 79 ([PO3]−) at 80 eV ("Pre79"), and at m/z 153 ([GP – H – H2O]−) at 30 eV ("Pre153") in negative ion mode, when 10 pmol/μL of acyl 12:0 lysoPA was used. In neutral loss scanning of 200 u (200.2–353.2–153.0, [acyl 12:0 FA]) and at 30 eV ("NL200"), [M – H]− (m/z 353.1) for acyl 12:0 lysoPA was also detected. Pre; precursor ion scanning, NL; neutral loss scanning. b) Dependence of fatty acyl chain length on detection of lysoPAs. A mixture of acyl 12:0 and 14:0 lysoPAs (10 pmol/μL) was analyzed using precursor ion scanning of m/z 153 ([GP – H – H2O]−, "Pre153") and neutral loss scanning of 200 u (200.2–353.2–153.0, [acyl 12:0 FA], "NL 200") and 228 u (228.2–381.2–153.0, [acyl 14:0 FA], "NL228") in negative ion mode (30 eV). The ion of m/z 353 is [M – H]− of acyl 12:0 lysoPA and m/z 381 is [M – H]− of acyl 14:0 lysoPA.

<table>
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Each value from “PC” to “cPA” shows the range of collision energy (CE) to detect each indicated lipid using precursor ion scanning of m/z 79 ([PO3]−) and 153 ([GP – H – H2O]−) in negative ion mode (−). Each value indicated for “lysoPA” shows the optimal CE to detect lysoPAs by precursor ion scanning of each mass value. PC; phosphatidylcholine, PAF; platelet-activated-factor, PE; phosphatidylethanolamine, PI; phosphatidylinositol, PS; phosphatidylserine, PG; phosphatidylglycerol, PA; phosphatidic acid, cPA; cyclic PA.

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Table 1. Optimal Collision Energies to Detect Phospholipids and Lysophospholipids by Precursor Ion Scanning of m/z 79 and 153 in Negative Ion Mode

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scanning of m/z 153 can be applied for detecting in all lysoPAs with different fatty acyl chain length, but neutral loss scanning of individual FA is only applied for lysoPA with each individual fatty acyl chain length.

3.3 Precursor ion scanning of m/z 79 and 153 for lysoPAs in lipid mixture

Precursor ion scanning of m/z 79 ([PO₃]⁻/c8140) detects all molecular species of lysoPAs. However, Table 1 shows that precursor ion scanning of m/z 79 at 80 eV detects all phospholipids indicated in the table. Several molecular species of cyclic PAs, lysoPGs, lysoPEs, and lysophosphatidylserines have the same m/z values as lysoPAs. This indicates that precursor ion scanning of m/z 79 is not specific for lysoPA detection.

Precursor ion scanning of m/z 153 (GP−H−H₂O⁻) at 30 eV detects acyl lysophosphatidylinositol, phosphatidylserines, acyl lysophosphatidylserines, acyl lysoPGs, PAs, acyl cyclic PAs, and acyl lysoPAs (Table 1). Acyl 16:0 lysoPG (m/z 483.2) and acyl 22:5 lysoPA (m/z 483.3), and acyl 16:1 lysoPG (m/z 481.3) and acyl 22:6 lysoPA (m/z 481.2) are detected at the same mass value. Therefore, when acyl 22:5 and 22:6 lysoPAs do not exist at higher concentrations than acyl 16:0 and 16:1 lysoPGs, respectively, it is difficult to determine acyl 22:5 and 22:6 lysoPAs by precursor ion scanning of m/z 153.

Precursor ion scanning of m/z 79 ([PO₃]⁻) detects numerous phospholipids in addition to lysoPAs. Precursor ion scanning of m/z 153 ([GP−H−H₂O⁻]) is the available method for detection of lysoPAs if amounts of acyl 16:0 and 16:1 lysoPGs in the sample are less than the limit of detection.

3.4 Analysis of quantitative alteration of lysoPAs

Quantitative “determination” of all molecular species of lysoPAs is troublesome because preparation of standards for all species of lysoPAs and a calibration curve for each sample is necessary (due to ion suppression effects by the matrix). We therefore tested the quantitative “alteration” (not “determination”) of relative amounts, which does not require standards for all lysoPA species.

LysoPAs stuck fast to peek, deactivated silica, teflon, and stainless steel (relative rate; 1.0, 0.9, 0.7, and 1.7, respectively). The capillary tubing in the Q-Trap is stainless steel. Quantitative alteration of lysoPAs thought to be difficult at low flow rates. Sano et al. performed quantitative determination of lysoPAs

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Fig. 4. Calibration curve for analysis of quantitative alteration. a) A mixture of the indicated equimolar amounts of acyl 12:0, 14:0, 16:0, and 18:0 lysoPAs added to lipid mixture from the calf serum was analyzed. The concentrations and peak intensities of acyl 12:0 lysoPA are shown. b) The calibration curves for acyl 12:0, 14:0, 16:0, and 18:0 lysoPAs are shown (solid line). A mixture of the indicated equimolar amounts of acyl 12:0 and 14:0 lysoPAs without other lipids was analyzed using precursor ion scanning of m/z 153 in negative ion mode (dotted line).
using LC/MS at 500 μL/min. Our system gave the high sensitivity and sharp peaks on the ion chromatogram for lysoPAs at a flow rate of 100 μL/min.

The calibration curve using precursor ion scanning for quantitative measurement of CERS was reported to show high linearity, and the calibration curve using LC/MS for quantitative measurement of lysoPAs was reported to show linearity at 2–100 pmol/injection. We examined the linearity of the calibration curve using precursor ion scanning of m/z 153.

In order to perform quantitative alteration of lysoPAs extracted from calf serum, a mixture containing the indicated concentrations of standard lysoPAs was added to a mixture of lipids from calf serum that was not incubated (incubated serum contains large amounts of lysoPAs). When the various concentrations of acyl 12:0 lysoPA were analyzed, the concentrations and peak intensity showed linearity at 0.25–5 pmol/μL (Figs. 4a and b). Acyl 14:0 lysoPA showed linearity at 0.25–5 pmol/μL, while acyl 16:0 and 18:0 lysoPAs showed linearity at 0.25–10 pmol/μL (Fig. 4b, solid line). The limit of detection of acyl 12:0 was less than 250 fmol/μL. The limits of detection of acyl 14:0, 16:0, and 18:0 lysoPAs were less than 0.5–1.5 pmol/μL (data not shown). The obtained calibration curves indicated in Fig. 4 show the relatively narrow dynamic ranges.

In order to examine the ion suppression effect by matrix in the sample, a mixture of the indicated concentrations of lysoPAs without the mixture of serum lipids was analyzed. The peak intensity of lysoPAs added to the lipid mixture was about 80% of that of

Fig. 5. Molecular species of lysoPAs in the incubated calf serum. After incubation for 48 hours, a mixture of lipids was prepared from calf serum using solid phase extraction HLB (a and c). Acyl 14:0 lysoPA was added before extraction as a standard (10 pmol/μL). The mixture was subjected to precursor ion scanning of m/z 153 at 30 eV in negative ion mode for analysis of lysoPAs. A standard solution of acyl 18:1 lysoPG ([M-H]-, m/z 509.3) was analyzed using precursor ion scanning of m/z 153 and m/z 281 (acyl 18:1 FA) in negative ion mode (b). Figure 5c shows the contribution of acyl 16:1 lysoPG to the peak at m/z 481.3 and a comparison with acyl 16:0 lysoPG at m/z 483.3. The left figure in 5c shows a magnified region of the small spectrum in Fig. 5a at m/z 475–490. The middle figure shows a spectrum using precursor ion scanning of m/z 253 at 40 eV ("Pre253") for detection of acyl 16:1 lysoPG (m/z 481.2). The right figure shows a spectrum using precursor ion scanning of m/z 255 at 40 eV ("Pre255") for detection of acyl 16:0 lysoPG (m/z 483.3).
lysoPAs alone due to the ion suppression effects of matrix (Fig. 4b, dotted line).

The linearity in the calibration curve obtained by precursor ion scanning of m/z 153 can be applied to analysis of quantitative “alteration” of lysoPAs.

3.5 Precursor ion scanning for lysoPAs from incubated calf serum

It was reported that lysoPAs were increased through incubation of serum, and their levels remained almost constant over 24–50 hours. LysoPAs content in the purchased serum varies widely based on serum preservation. We analyzed lysoPAs in serum incubated for 24 and 48 hours, and lysoPAs levels appeared to become constant at that time period.

A mixture of lipids from calf serum incubated for 24 hours was analyzed using precursor ion scanning of m/z 153 and 79. Acyl 22:6 lysPA was detected at m/z 481.3 (m/z 481.2) on precursor ion scanning of m/z 153 (data not shown). Acyl 18:0 lysoPE was detected at m/z 480.4 (m/z 480.3) in samples concentrated at 1 or 15 times by evaporation on precursor ion scanning of m/z 79. This indicates that precursor ion scanning of m/z 79 is unable to specifically determine acyl 22:6 lysPA, because acyl 18:0 lysoPE with one 13C was also strongly detected at m/z 481.4.

3.6 Determination of acyl 22:6 lysoPA in calf serum

Using precursor ion scanning of m/z 153, a peak corresponding to acyl 22:6 lysoPA was detected at m/z 481.4 (m/z 481.5) in the lipid mixture extracted from incubated calf serum (48 hours) using HLB (Fig. 5a). Peaks corresponding to acyl 22:6 and 22:5 lysoPAs were also detected at m/z 481.1 (m/z 481.5) and 483.5 (m/z 483.5), respectively, when scan time was 1 s and the scanning range was changed from m/z 375–542 (167 u) to m/z 450–510 (60 u) (small spectrum in Fig. 5a).

For estimation of the presence of acyl 16:0 and 16:1 lysoPGs, several precursor ion scanning runs were performed. Acyl 18:1 lysoPG (m/z 509.4, m/z 509.3) was detected by precursor ion scanning of FA (m/z 281, m/z 282.1) at 40 eV, and this method was 35 times more sensitive than precursor ion scanning of [GP−H−H2O]− (m/z 153) in negative ion mode (Fig. 5b). We applied this method to confirm the proportion of acyl 16:1 and 16:0 lysoPGs in the peak intensity at m/z 481 and 483, respectively.

Peaks corresponding to acyl 16:1 and 16:0 lysoPGs were weakly detected at m/z 481.1 (m/z 481.2) and 483.5 (m/z 483.3) when the same sample was analyzed using precursor ion scanning of m/z 253 and 255, respectively (Fig. 5c). This indicates that acyl 22:6 and 22:5 lysoPAs corresponded to about 97 and 76% of the total peak intensity at m/z 481.1 and 483.5, respectively, detected using precursor ion scanning of m/z 153.

4. Conclusion

In incubated calf serum, acyl 16:0, 18:2, 18:1, 18:0, and 20:4 lysoPAs were detected using precursor ion scanning of m/z 153, and we were particularly successful in detecting acyl 22:6 and 22:5 lysoPAs using this method.

Precursor ion scanning of m/z 153 was detected not only acyl lysoPAs but also acyl lysophosphatidylinositol, phosphatidylserines, acyl lysophosphatidylserines, acyl lysoPAs, PAs, cyclic PAs. In these lipids, [M−H]− of acyl 16:0 and 16:1 lysoPAs have the same mass value with [M−H]− of acyl 22:5 (m/z 483) and 22:6 lysoPAs (m/z 481), respectively. Therefore, when acyl 22:5 and 22:6 lysoPAs do not exist at higher concentrations than acyl 16:0 and 16:1 lysoPAs, respectively, it required not only precursor ion scanning of m/z 153 but also precursor ion scanning of m/z 255 and 253 for the determination of acyl 22:5 and 22:6 lysoPAs.

Precursor ion scanning of m/z 153 appears to be effective as a first screening technique for acyl lysoPAs in lipid mixtures due to its high sensitivity, selectivity, and rapid and easy operation without HPLC or TLC, and precursor ion scanning of m/z 79 is effective as a second screening technique for alkyl and alkenyl lysoPAs.

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References

8) G. Liebisch, W. Drobnik, M. Reil, B. Trumbach, R. Arnecke, B. Olgemoller, A. Roscher, and G. Schmitz, J.

Keywords: ESI-MS, Precursor ion scanning, Serum, Lyso-phosphatidic acids, Docosahexaenoyl