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Detection of phosphatidylcholine oxidation products in rat heart using quadrupole time-of-flight mass spectrometry

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Abstract

An improved technique for the analysis of phosphatidylcholine (PC) and lyso-phosphatidylcholine (lyso-PC) oxidation products was developed using quadrupole time of flight (Q-TOF) mass spectrometry with electrospray ionization. We separated these products using an HPLC C_8 column with a gradient of methanol and 10 mM aqueous ammonium acetate. Monohydroxides, oxo derivatives, and trihydroxides of palmitoyl-linoleoyl (C16:0/C18:2) PC, stearoyl-linoleoyl (C18:0/C18:2) PC, and oleoyl-linoleoyl (C18:1/C18:2) PC were detected mainly as MH^+ and $[M+Na]^+$ ions in the heart of the intact rat. Using standard synthetic PC-OH (C16:0/C18:2-OH), the lipid extract component was identified as (C16:0/C18:2-OH) PC based on the product ions of ESI–MS–MS and, the PC-OH concentration was quantitated. Four oxidatively modified 1-lyso-phosphatidylcholines (lyso-PCs) were also detected. This is the first report showing the presence of monohydroxides, oxo derivatives, and trihydroxides of (C16:0/C18:2)PC, (C18:0/C18:2)PC, and (C18:1/C18:2) PC in the rat heart.
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Keywords: Phosphatidylcholine oxidation products; Q-TOF; Rat

1. Introduction

The oxidation of linoleic acid hydroperoxide in vivo products as follows [1]. 13-Hydroperoxy-9-*cis*,11-*trans*-octadecadienoic acid formed by the peroxidation of linoleic acid in vivo reacts with hematin to produce *erythro*-11-hydroxy-12, 13-epoxy-9-octadecenoic acid, *threo*-11-hydroxy-12,13-epoxy-9-octadecenoic acid, 9,12,13-trihydroxy-10-octadecenoic acid, 13-oxo-9,11-octadecadienoic acid and 13-hydroxy-9,11-octadecadienoic acid [1]. Similar reactions are believed to occur with phosphatidylcholine (PC) hydroperoxide. Previously, we identified a monohydroperoxide of palmitoyl-lynoleoyl (C16:0/C18:2)PC using quadrupole time of flight (Q-TOF) mass spectrometry [2]. We also detected monohydroperoxides of stearoly-linoleoyl (C18:0/C18:2)PC and oleoyl-linoleoyl (C18:1/C18:2)PC and epoxyhydroxy derivatives of (C16:0/C18:2)PC, (C18:0/C18:2)PC, and (C18:1/C18:2)PC in the rat heart [2]. The aim of this study was to elucidate the molecular structure of oxidation products other than monohydroperoxides and epoxyhydroxy-derivatives of PC and lyso-phosphatidylcholine (lyso-PC) in control rat heart by Q-TOF mass spectrometry with electrospray ionization.

The proposed molecular species of oxidatively modified PC and protonated molecular ions are shown in Table 1. The proposed molecular species of lyso-PC and oxidatively modified lyso-PC are also shown in Table 2.

2. Experimental

2.1. Materials

3,5-Di-*tert*-butyl-4-hydroperoxytoluene (BHT), luminol and cytochrome C (from horse heart, type IV) were purchased

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Table 1
Molecular species of oxidatively modified phosphatidylcholine (PC) and protonated molecular ions

Peak	Molecular species of PC		
	R_1	R_2	MH^+
Standard PC-OH			
1	16:0	Hydroxy-18:2	774.6
Hydroxy-PC			
2	16:0	Hydroxy-18:2	774.6
3a	18:1	Hydroxy-18:2	800.6
4	18:0	Hydroxy-18:2	802.6
Oxo-PC			
5	16:0	Oxo-18:2	772.6
6	18:1	Oxo-18:2	798.6
7	18:0	Oxo-18:2	800.6
Trihydroxy-PC			
8	16:0	Trihydroxy-18:2	808.6
9	18:1	Trihydroxy-18:2	834.6
10	18:0	Trihydroxy-18:2	836.6

from Wako Pure Chemical Co. (Osaka, Japan). 1-Palmitoyl-2-linoleoyl-phosphatidylcholine hydroxide (C16:0/C18:2-OH, PLPC-OH) was prepared by the aerobic oxidation of PLPC with lipoxygenase [3]. PLPC (6 μmol) was dissolved in 20 ml of 0.1 M sodium borate buffer (pH 9) containing 3 mM sodium deoxycholate and mixed with 2 mg of soybean lipoxygenase (Sigma, Type 1B). The solution was stirred for 45 min at room temperature and was passed through a solid phase extraction tube (Supelco LC-18, 1g, Sigma–Aldrich, Tokyo, Japan) to remove bile acid and other components. The eluant was concentrated and PLPC-OOH was purified by HPLC (Superiorex ODS column; 20 mm × 250 mm, 5 μm, Shiseido, Tokyo, Japan) using 0.02% triethylamine in methanol as the mobile phase with a flow rate of 8 ml/min. The corresponding alcohol (PLPC-OH) was purified by solid phase extraction and HPLC separation as described above after the reduction with sodium borohydride. The amount of phospholipid was quantitatively analyzed and found to be 2.20 mg/ml. MS: 774.6 (MH^+, monooxide) and 796.6 ($[M+Na]^+$, monooxide).

Table 2
Molecular species of lyso-phosphatidylcholine (lyso-PC) and oxidatively modified lyso-PC

Peak	Molecular species of lyso-PC		
	R_1	R_2	MH^+
Oxidized lyso-PC			
11	H	Trihydroxy-18:2	570.3
12	H	Hydroperoxy-18:2	552.3
13	H	Oxo-18:2	534.3
14	H	Hydroxy-18:2	536.3
Lyso-PC			
15	H	18:2	520.3
16	16:0	H	496.3
17	H	18:1	522.3
18	17:0	H	510.3
19	18:0	H	524.3

2.2. Tissue extraction

Six control rats were killed under deep anesthesia induced by pentobarbital sodium (Abbott Lab, Abbott Park, IL, USA). The hearts were dissected on ice and the total lipids were extracted by adding 4 ml of ice-cold chloroform:methanol (3:1, v/v) containing 0.005% BHT to approximately 0.1 g of heart, followed by homogenization under ice-cold conditions. The homogenate was mixed with another 4 ml of chloroform: methanol (3:1, v/v) and 1 ml of distilled water, spun vigorously for 1 min, and then centrifuged at 800 × *g* for 20 min. The chloroform layer was removed by aspiration, concentrated in a rotary evaporator and then dried under a nitrogen stream. The phospholipid fraction was then isolated from the total lipid sample by solid phase extraction. A silica column (Sep-Pak, Waters Co. Milford, MA) of 3 ml capacity packed with aminopropyl-derivatized silica ($-NH_2$) was used. The total lipid sample was dissolved in a small amount of chloroform and layered on the column, which then was flushed with a mixture of 2 ml of chloroform and 1 ml of *iso*-propanol. The column was next flushed with methanol containing 0.005% BHT, giving an eluate consisting mainly of phospholipid. This was concentrated using a rotary evaporator, dried under a nitrogen stream and then dissolved in 150 μl of methanol. A 1 μl portion was injected into the LC–MS.

2.3. LC–MS conditions

An HPLC model 1100 system (Agilent Technologies, Waldbronn, Germany) with a Luna C_8 column (1.0 mm × 150 mm, 5.0 μm; Phenomenex, Torrance, CA) was used. Injections of rat samples (1 μl (MS) and 2 μl (MS/MS)) were made. The column was maintained at 40 °C and eluted at 100 μl/min. The mobile phase consisted of 5% methanol with 10 mM aqueous ammonium acetate (solvent A) and 95% methanol with 10 mM aqueous ammonium acetate (solvent B). Separation was carried out with a linear gradient starting with 90% solvent B followed by ramping up to 100% solvent B at 20 min and then maintaining for 17 min. The total run time was 37 min.

Mass spectrometric analysis was performed on a quadrupole orthogonal acceleration time of flight, Micromass Q-TOF Micro (Waters Corporation, Milford, MA) equipped with an electrospray interface. The instrument was operated in positive ion mode with a capillary voltage of 3200 V and a cone voltage of 40 V. The desolvation gas was set at 600 L/h with a desolvation temperature of 150 °C and source temperature of 80 °C. Full-scan spectra were recorded in profile mode. The range between *m*/*z* 100 and 1000 was recorded at a resolution of 5000 (FWHM) and the accumulation time was 1 s/spectrum. Accurate masses were measured by comparison to a reference compound, Leucine Enkephalin ($[M+H]^+ = 556.2771$ Da) infused into the Lock Spray reference channel. Quantitative analysis was carried out in the selected ion monitoring (SIM) mode. The ion monitored for quantitation was *m*/*z* 774.6.

Table 3
Accurate mass measurement results for compounds found in the rat heart

Peak	R.T. (min)	Measured m/z	Calculated m/z	Composition	Differences[a] (ppm)
1[b]	9.8	774.5657	774.5649	C42H81NO9P	1.0
2	9.5	774.5645	774.5649	C42H81NO9P	−0.5
3a	10.5	800.5818	800.5805	C44H83NO9P	1.6
4	13.9	802.5959	802.5962	C44H85NO9P	−0.4
5	9.8	772.5502	772.5492	C42H79NO9P	1.2
6	10.9	798.5624	798.5649	C44H81NO9P	−3.1
7	13.8	800.5817	800.5805	C44H83NO9P	1.4
8	3.9	808.5715	808.5704	C42H83NO11P	1.4
9	4.3	834.5881	834.5860	C44H85NO11P	2.5
10	5.6	836.6050	836.6017	C44H87NO11P	3.9
11	1.2	570.3427	570.3407	C26H53NO10P	3.5
12	1.3	552.3317	552.3301	C26H51NO9P	2.8
13	1.9	534.3203	534.3196	C26H49NO8P	1.3
14	1.6	536.3356	536.3352	C26H51NO8P	0.7
15	2.3	520.3407	520.3403	C26H51NO7P	0.7
16	3.0	496.3402	496.3403	C24H51NO7P	−0.2
17	3.2	522.3561	522.3560	C26H53NO7P	0.3
18	3.2	510.3532	510.3560	C25H53NO7P	−5.4
19	4.1	524.3710	524.3716	C26H55NO7P	−1.2

[a] Differences in values between measured and calculated.
[b] Standard PC C16:0/C18:2-OH.

3. Results

Accurate masses of 19 components of PC and lyso-PC oxidation products in rats were measured (a single run) using the Q-TOF system. The results of the measurements are shown in Table 3.

The following possible products of phosphatidylcholine oxidation are shown in Fig. 1. (A) 13-Hydroxy-PC and 9-hydroxy-PC; (B) 13-oxo-PC and 9-oxo-PC; and (C) 9,12, 13-trihydroxy-PC.

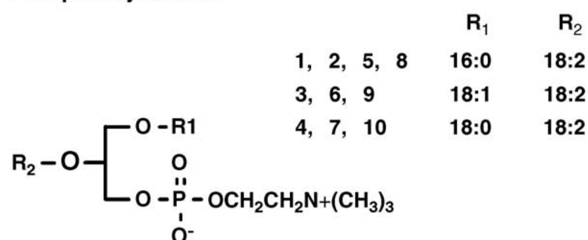
Fig. 2 shows mass chromatograms obtained from 5 to 16 min by LC–MS. Mass chromatograms of standard PC-OH (column 1) and rat extracts (columns 2, 3, and 4) are shown in Fig. 2. Coinciding peaks are seen originating from the MH^+ of standard PC-OH (C16:0/C18:2-OH) at m/z 774.6 at a retention time of 9.8 min, from the MH^+ of the hydroxy derivative of PC C16:0/C18:2 at m/z 774.6 with a retention time of 9.5 min (column 2), from the MH^+ of the hydroxy derivative of PC C18:1/C18:2 at m/z 800.6 (column 3), and from the MH^+ of PC C18:0/C18:2 at m/z 802.6 (column 4), respectively. The ESI mass spectra of standard PC-OH and peak 2 showed MH^+ (m/z 774.6), $[M-H_2O+H]^+$ (m/z 756.6), and $[M+Na]^+$ (m/z 796.5) ions, as shown in Fig. 3. The ESI mass spectrum of peak 3a appearing at 10.5 min show MH^+ (m/z 800.6), $[M-H_2O+H]^+$ (m/z 782.6), and $[M+Na]^+$ (m/z 822.6) ions, and that of peak 4 MH^+ (m/z 802.6), $[M-H_2O+H]^+$ (m/z 784.6), and $[M+Na]^+$ (m/z 824.6) ions (Fig. 3). The peak appearing at 13.8 min (Fig. 2, column 3) without an ion at m/z 782.6 was not estimated as hydroxyl-PC (C18:1/C18:2).

Mass chromatograms of oxo-PC from rat extracts (columns 5, 6 and 7) are shown in Fig. 2. Coinciding peaks are seen originating from the MH^+ of oxo derivatives of PC C16:0/C18:2 at m/z 772.6 with retention time of 9.5 min (column 5), of PC C18:1/C18:2 at m/z 798.6 of 10.9 min (column 6), and PC C18:0/C18:2 at m/z 800.6 of 13.8 min (column 7). The ESI mass spectra of peaks 5, 6, and 7 showed MH^+ and $[M+Na]^+$ ions as follows: 5, 772.6 and 794.5; 6, 798.6 and 820.6; 7, 800.6 and 822.6 (Fig. 3).

Fig. 4 shows the product ion profiles for standard PC-OH and peaks 2, 5, and 7. For standard PC-OH, the base peak was m/z 756.6. The breakdown product lost 18 mass units, corresponding to dehydration; a small peak was seen at m/z 184.1, corresponding to choline phosphate; this supported the assignment as phosphatidylcholine hydroxide. For peak 2, the base peak was m/z 756.6; a small peak attributed to fragment ion appeared at m/z 184.1. Thus, peak 2 was identified as PC C16:0/C18:2-OH. For peaks 5 and 7, the base peak was m/z 772.6 and 800.6, respectively with small peaks associated with fragment ions appearing at m/z 756.6 and 784.6, respectively. Each breakdown product lost 16 mass units, corresponding to deoxygenation. Moreover the peaks 5 and 7 had fragment ions at m/z 184.1.

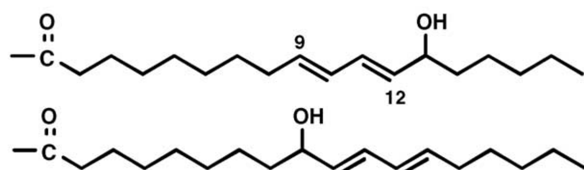
Thus, peaks 5, 6, and 7 were assumed to be the oxo derivatives of PC C16:0/C18:2, PC C18:1/18:2, and PC C18:0/C18:2, respectively. Fig. 5 shows mass chromatograms of rat extracts from 0 to 8 min (A) and from 0 to 5 min (B). Coinciding peaks are seen originating from MH^+ of trihydroxy derivatives of PC C16:0/18:2, PC C18:1/C18:2, and PC C18:0/C18:2 at m/z 808.6, 834.6, and 836.6, respectively. The ESI mass spectra of peaks 8, 9, 10 showed MH^+ and $[M+Na]^+$ ions (Fig. 6 upper column). In Fig. 6, the lower column shows the product ion profiles for peaks 8 and 10. The base peak showed m/z 808.6 and 836.6, respectively, and small peaks associated with fragment ions appeared at m/z 790.6, 772.6, and 754.6 for peak 8 and at 818.6, 800.6, and 782.6 for peak 10. These breakdown products lost 18 mass units. Moreover, peaks attributed to fragment ions appeared

Phosphatidylcholine

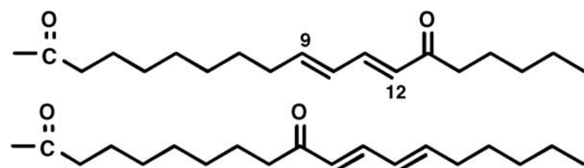


R2:

(A) Hydroxy



(B) Oxo



(C) Trihydroxy

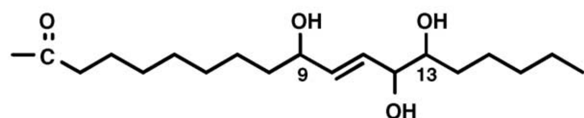


Fig. 1. Possible products of phosphatidylcholine oxidation.

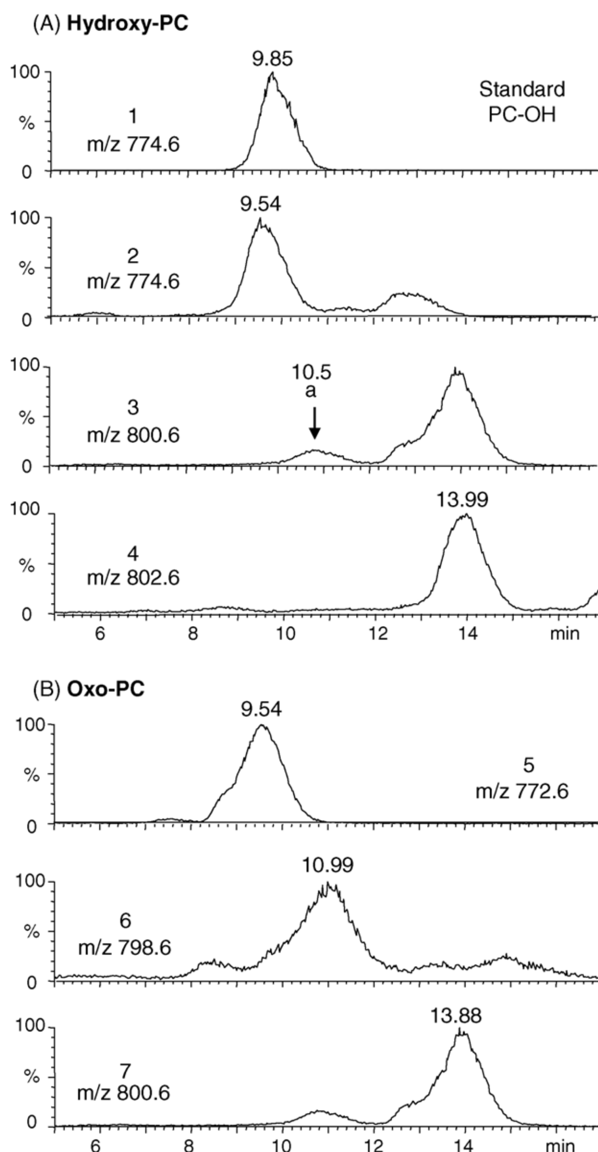


Fig. 2. Mass chromatograms of standard PC-OH and lipid extracts from 5 to 16 min using LC–MS.

at *m/z* 184.1. Thus, peaks 8, 9, and 10 were assumed to be the trihydroxy derivatives of PC C16:0/C18:2, PC C18:1/18:2, and PC C18:0/18:2, respectively.

In Fig. 5(B), coinciding peaks are seen originating from MH^+ of the trihydroxy derivative of 1-lyso-PC C18:2 at *m/z* 570.3 with retention time of 1.1 min (column 11), from MH^+ of the hydroperoxy derivative of 1-lyso-PC C18:2 at *m/z* 552.3 with retention time of 1.3 min (column 12), from MH^+ of the oxo derivative of 1-lyso-PC C18:2 at *m/z* 534.3 with retention time of 1.4 min (column 13), and from MH^+ of hydroxy derivative of 1-lyso-PC C18:2 at *m/z* 536.3 with retention time of 1.6 min. Fig. 7 shows ESI mass spectra of peaks 11–14. For peak 11 the mass spectrum showed MH^+ (*m/z* 570.3), $[M+Na]^+$ (*m/z* 592.3), and $[M+K]^+$ (*m/z* 608.3). For peak 12 MH^+ (*m/z* 552.3), $[M+Na]^+$ (*m/z* 574.3), $[M+K]^+$ (*m/z* 590.3), for peak 13 MH^+ (*m/z* 534.3), $[M+Na]^+$ (*m/z* 556.2), for peak 14 MH^+ (*m/z* 536.3), $[M+Na]^+$ (*m/z* 558.2), and $[M\text{-}H_2O+H]^+$ (*m/z* 518.3). Thus, peaks 11, 12, 13, and 14 were assumed to be the trihydroxy derivative of 1-lyso-PC C18:2, 1-lyso-PC C18:2-OOH, 1-lyso-PC C18:2-oxo, and 1-lyso-PC C18:2-OH, respectively.

The mean concentration of PC C16:0/C18:2-OH in the rat heart determined by SIM was 35.1 ± 10.3 nmol/g wet weight ($n = 6$).

We investigated the validation of this assay method. The calibration curve was linear in the range of 10–100 nmol/ml. The intra-series precision of the present method was 7.0% coefficient of variation (C.V.) ($n = 5$). The extraction recovery was determined by adding PC-OH at 80 nmol to tissue. The recovery was 65%.

4. Discussion

In the present study, we applied LC–MS and LC–MS–MS systems to analyze the oxidation products of PC and lyso-

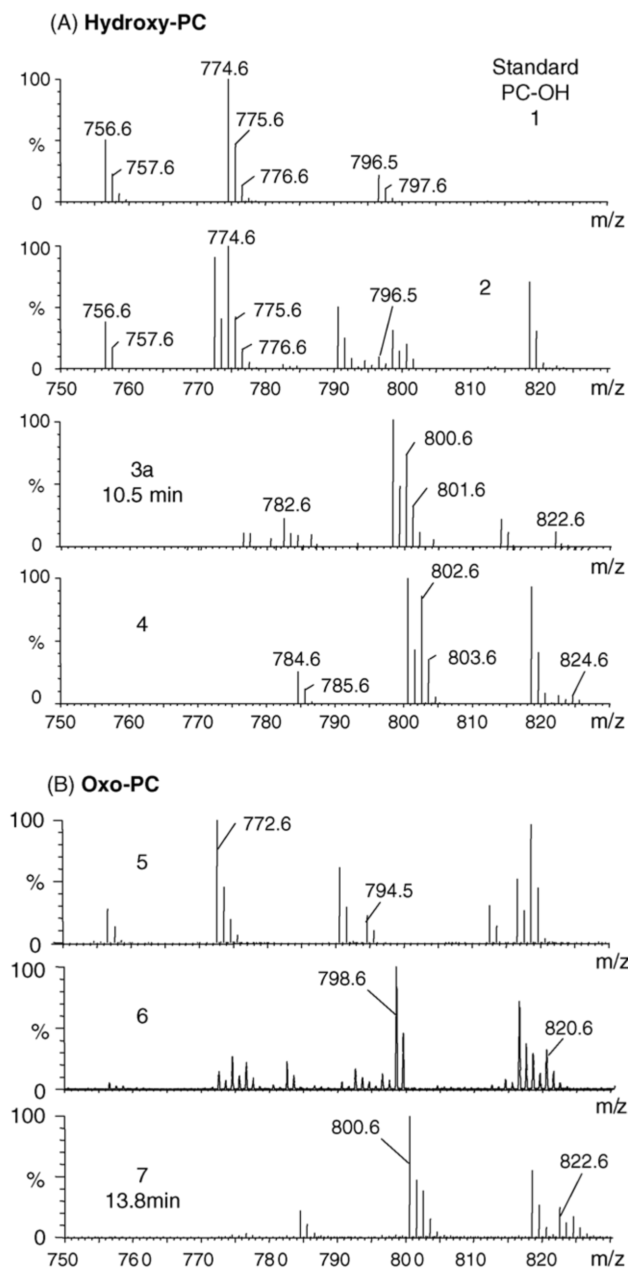


Fig. 3. ESI-mass spectra of standard PC-OH and peaks 2–7.

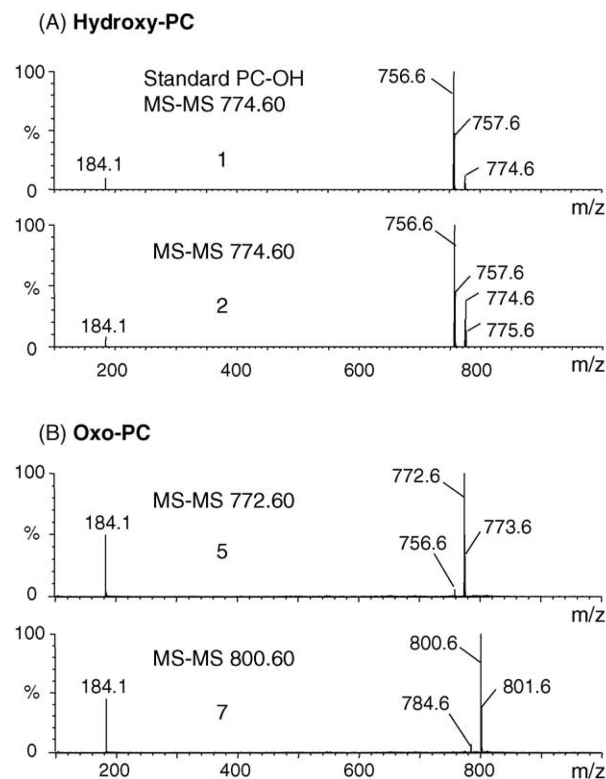


Fig. 4. Product ion spectra on LC–MS–MS of the $[M+H]^+$ ion of standard PC-OH and peaks 1, 2, 5 and 7.

PC. We can estimate chemical structures of PC oxidation products that are the metabolites of PC and that were expected previously from a chemical reaction with hematin and linoleic acid [1]. We have decided composition formula in these limited compounds by using mass spectrometry. When we observe dehydrations of three molecules occurred from MS/MS spectrum, for example, we decide that the compound is trihydroxy-PC. Thus, we can estimate chemical structures from composition formula by the measured and calculated mass of precursor and product ions.

The mass accuracy obtained using the Q-TOF system was good, with differences within 3.9 ppm (with one exception of 5.4 ppm) between the calculated values of known molecular masses of sample compounds and their measured values. These results were nearly the same as those obtained using multiple sprayer nano-ESI–MS combined with nano-HPLC [4].

First, we detected hydroxides of palmitoyl-linoleoyl-PC and stearoyl-linoleoyl-PC (hydroxyl derivatives of PC) in the heart of a control rat. Using a standard synthetic compound we confirmed the identification of this product as PC C16:0/C18:2-OH based on the product ions of MS–MS. When palmitoyl-linoleoyl-PC is oxidized with soybean lipoxygenase, 1-palmitoyl-2-(13-hydroperoxy-9-*cis*,11-*trans*-octadecadienoyl)-phosphatidylcholine is selectively prepared [5]. Thus, standard compound is 13-hydroxy-PC, while PC-OH in biological samples may be 13-hydroxy-PC or 9-hydroxy-PC.

Moreover, we detected the hydroxide of oleoyl-linoleoyl PC in the rat, since C36:3 is an abundant unsaturated lipid present in the heart. Thus, this is the first report showing the presence of monohydroxides of (C16:0/C18:2)PC, (C18:0/C18:2)PC, and (C18:1/C18:2)PC in the heart of the intact rat.

1-Palmitoyl-2-linoleoyl-PC and 1-palmitoyl-2-arachidonyl-PC are oxidized by the myeloperoxidase (MPO)–H_2O_2–NO_2^-system to form monohydroxides of PC, for example, 15-hydroxyeicosatetraenoate (HETE) PC, and 13-hydroxyoctadecadienoate (HODE) PC. They serve as high-affinity ligands for macrophage scavenger receptor CD 36 [6]. Some studies have used LC–ESI–MS–MS

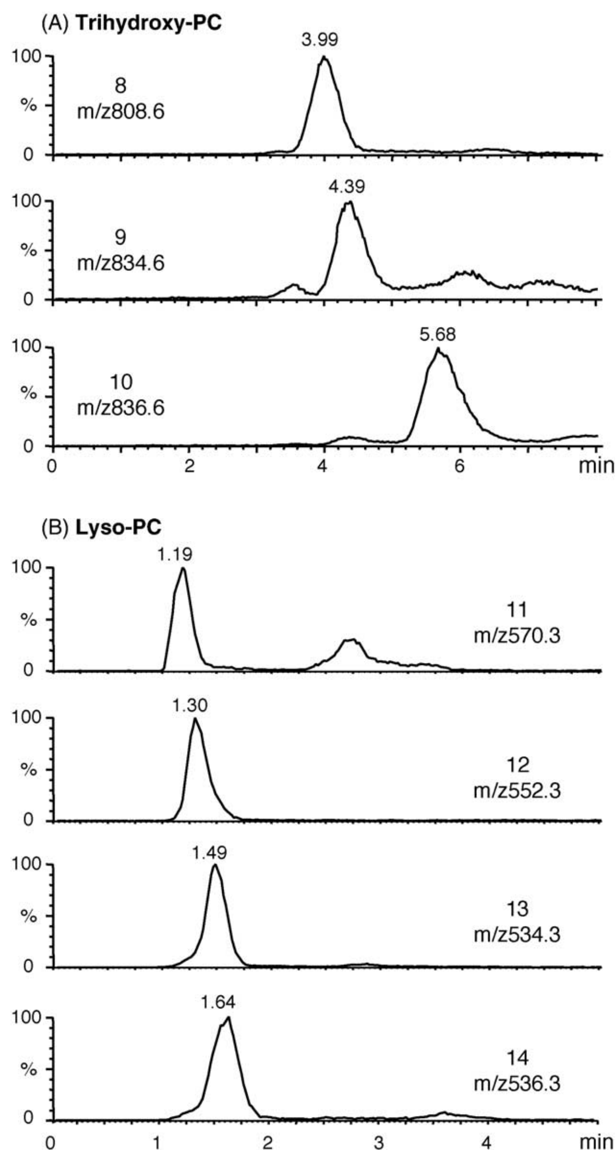


Fig. 5. Mass chromatograms of lipid extract from 0 to 8 min using LC–MS.

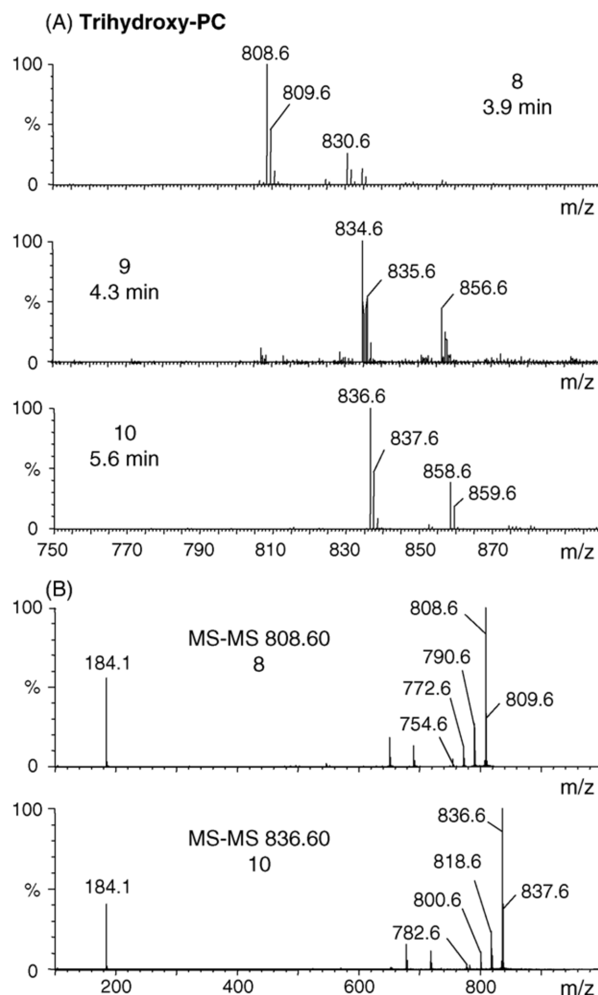


Fig. 6. ESI-mass spectra of peaks 8–10 and product ion spectra on LC–MS–MS of the $[M+H]^+$ ion of peaks 8 and 10.

techniques to examine the structure of oxidized products of arachidonic acid in glycerophospholipids from red blood cell ghosts treated with *t*-butylhydroperoxide [7,8] and oxidized products of 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphoryl-choline in human aortic endothelial cells [9]. These products were 5-, 8-, 9-, 12-, and 15-HETE [7,8] and 5-, 12- and 15-hydroperoxyeicosatetraenoate (HPETE) [7] and 15-hydroperoxy (15-HPETE) derivatives of plasmalogen [8]. Furthermore, studies on the peroxidation of 1-palmitoyl-2-linoleoyl PC revealed that the oxidation products were 9- and 13-hydroperoxyoctadecadienoate (HPODE), 9- and 13-hydroxyoctadecadienoate (HODE) [7], 13-hydroxy [HODE] and 13-hydroperoxy [HPODE] PC [8]. Accordingly, PC hydroxides (hydroxyl derivatives of PC) have been studied following treatment with oxidizing agents.

Phosphatidylcholine hydroperoxide (PCOOH) is reduced to PC-OH by glutathione peroxidase and apolipoprotein A-I, A-II, and B-100 [10,11]. In vitro, apolipoprotein B-100 in human plasma reduces PCOOH to PC-OH, but glutathione peroxidase dose not [12]. On the other hand, PCOOH is oxidized to form oxo-PC. Next we detected oxo derivatives of PC in the rat. LC–MS and LC–MS–MS studies revealed that they were the oxo derivatives of (C16:0/C18:2)PC, (C18:1/C18:2)PC, and (C18:0/C18:2)PC. To our knowledge they have not been detected yet, since they are unstable. Instead, 1-palmitoyl-2-(9′-oxo-nonanoyl)-phosphatidylcholine was identified as an oxidized phospholipid derivative in calf lung surfactant exposed to ozone by electrospray tandem mass spectrometry, giving rise to an abundant negative ion species $[M-15]^-$ being observed at *m/z* 634 [13].

After the incubation of submitochondrial particles, phospholipids were hydrolyzed by phospholipase A_2. In the fatty acid constituents analyzed by GC–MS and LC–MS, derivatives of the hydroperoxide of linoleic acid such as keto, hydroxy, trihydroxy, and hydroxyepoxy compounds were observed [14].

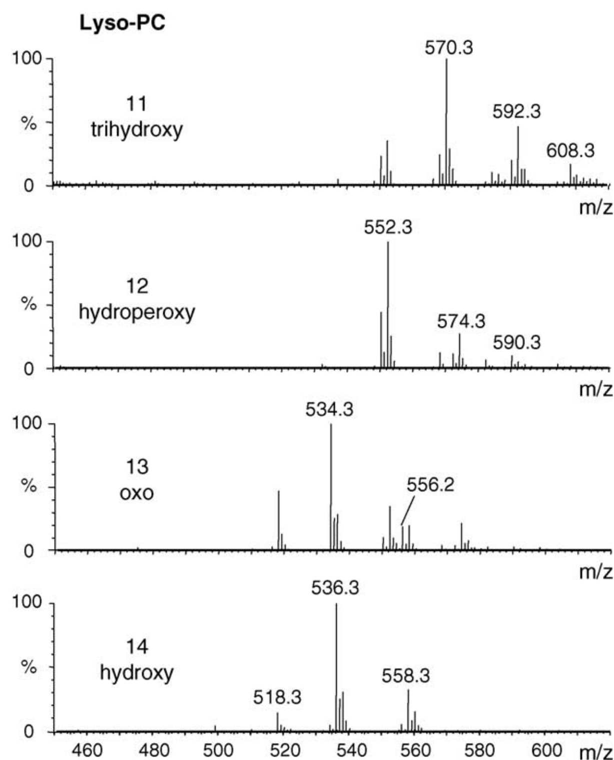


Fig. 7. ESI-mass spectra of peaks 11–14.

Thirdly, we detected trihydroxy derivatives of (C16:0/C18:2)PC, (C18:1/C18:2)PC, and (C18:0/C18:2)PC in the heart of a control rat. We confirmed these structures based on the product ions of MS–MS, since dehydration of the three molecules occurs.

Finally, we detected oxidation products of lyso-PC, namely, trihydroxy, hydroperoxy, oxo, and hydroxy derivatives of lyso-PC in the heart of a control rat. To our knowledge, trihydroxy and hydroperoxy derivatives have not hitherto been detected. Lyso-PC and platelet-activating factor-like oxidized phospholipids induce apoptosis of rat vascular smooth muscle cells via temporary membrane distortion [15]. Using negative ion full-scan ESI–MS analysis, PC and lyso-PC were detected in human blood as the formate adduct $[M+45]^-$ [16]. In addition, 5-hydroxy-8-oxo-6-octenoic acid ester of 2-lyso-PC (HOOA-PC) and 5-keto-8-oxo-6-octenoic acid ester of 2-lyso-PC (KOOA-PC) were identified using high performance liquid chromatography with on-line electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS) [17].

Phosphatidylchoine hydroperoxide (PCOOH) in plasma was detected and reported previously [18]. Other oxidation products of phosphatidylchoine may be detected in plasma.

5. Conclusion

We were able to measure the accurate masses of PC and lyso-PC oxidation products in rat extract using Q-TOF–ESI–mass spectrometry with a lock spray interface. Furthermore, the presence of hydroxides, oxo, and trihydroxy derivatives of PC was confirmed.

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References

[1] T.A. Dix, L.J. Marnett, J. Biol. Chem. 260 (1985) 5351.
[2] J. Adachi, N. Yoshioka, R. Funae, H. Nushida, M. Asano, Y. Ueno, J Chromatogr. B 806 (2004) 41.
[3] Y. Nagata, Y. Yamamoto, E. Niki, Arch. Biochem. Biophys. 329 (1996) 24.
[4] Y. Takahashi, T. Morita, Y. Ueda, J. Chromatogr. B 776 (2002) 31.
[5] H. Arai, A. Nagao, J. Terao, T. Suzuki, K. Takama, Lipids 30 (1995) 135.
[6] E.A. Podrez, E. Poliakov, Z. Shen, R. Zhang, Y. Deng, M. Sun, P.J. Finton, L. Shan, B. Gugiu, P.L. Fox, H.F. Hoff, R.G. Salomon, S.L. Hazen, J. Biol. Chem. 277 (2002) 38503.
[7] L.M. Hall, R.C. Murphy, Anal. Biochem. 258 (1998) 184.
[8] R.C. Murphy, N. Khaselev, T. Nakamura, L.M. Hall, J. Chromatogr. B. 731 (1999) 59.
[9] N. Leitinger, A.D. Watson, K.F. Faul, A.M. Fogelman, J.A. Berliner, Adv. Exp. Med. Biol. 433 (1997) 379.
[10] Y. Yamamoto, Free Radic. Res. 33 (2000) 795.
[11] R. Mashima, Y. Yamamoto, S. Yoshimura, J. Lipid Res. 39 (1998) 1133.
[12] K. Hayashida, K. Iwasaki, T. Makino, R. Mashima, Y. Yamamoto, S. Yoshimura, Tokai J. Exp. Clin. Med. 26 (2001) 131.
[13] C. Uhlson, K. Harrison, C.B. Allen, S. Ahmed, C.W. White, R.C. Murphy, Chem. Res. Toxicol. 15 (2002) 896.
[14] H. Iwase, T. Takatori, M. Nagao, H. Niijima, K. Iwadate, Y. Matsuda, M. Kobayashi, Free Radic. Biol. Med. 24 (1998) 1492.
[15] K. Kogure, S. Nakagawa, A. Tsuchie, A. Tokumura, K. Fukunaga, Chem. Phys. Lipids 126 (2003) 29.
[16] S. Uran, A. Larsen, P.B. Jacobsen, T. Skotland, J. Chromatogr. B 758 (2001) 265.
[17] E.A. Podrez, E. Poliakov, Z. Shen, R. Zang, Y. Deng, M. Sun, P.J. Finton, L. Shan, M. Feffraio, D.P. Hajjar, R.L. Silverstein, H.F. Hoff, R.G. Salomon, S.L. Hazen, J. Biol. Chem. 277 (2002) 38517.
[18] J. Adachi, N. Yoshioka, R. Funae, Y. Nagasaki, T. Naito, Y. Ueno, Lipids 39 (2004) 891.