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# Platelet-activating Factor (PAF)-like Phospholipids Formed during Peroxidation of Phosphatidylcholines from Different Foodstuffs

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**Previously, we reported that induction of peroxidation of synthetic phosphatidylcholines (PCs) containing a polyunsaturated fatty acid by $Fe^{2+}$-EDTA in the presence of ascorbate resulted in the formation of four types of PCs with an *sn*-2-oxidatively fragmented acyl group, which had platelet-aggregating activity due to interaction with platelet-activating factor (PAF) receptors. These PCs were compounds with a short-chain monocarboxylate, ω-hydroxymonocarboxylate, dicarboxylate, and dicarboxylate semialdehyde residue, respectively. In this study, we investigated the PAF-like lipids formed during peroxidation of PCs from hen egg yolk, salmon roe, sea urchin eggs, and krill in an $FeSO_4$/EDTA/ascorbate system. The platelet-aggregating activities of these oxidized PCs were all inhibited by FR-900452, an antagonist of PAF. The activity of oxidized krill PC, which was equivalent of 89.8±8.8 pmol 16:0-PAF/μmol of starting PC, was about 5 times those of oxidized PCs from salmon roe and sea urchin eggs, and about 50 times that of oxidized hen egg yolk PC. The PAF-like phospholipids that had different combinations of long-chain alkyl or acyl groups with one of the above four types of short-chain acyl groups were identified by gas chromatography-mass spectrometry. The results indicated that foodstuffs that are rich in 1-*O*-alkyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine are potential sources of compounds with high PAF-like activity formed by deleterious lipid peroxidation.**

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Peroxidation of phospholipids containing polyunsaturated fatty acid (PUFA) is known to result in formation of secondary degradation products such as short-chain fatty aldehydes. These compounds have been shown to be produced by chain scission of the PUFA moiety *via* phospholipid hydroperoxides formed as primary products.[1] However, until recently, less attention has been paid to the chemical nature of other classes of cleavage products retaining a phospholipid backbone, although oxidized phospholipids have been reported to have significant biological effects such as enhancement of atherogenesis[2] and augmentation of macrophage growth-stimulating activity.[3] Itabe *et al.*[4] reported that PCs with an azelaoyl group were generated as cytotoxic compounds by the oxidation of PCs containing linoleate with oxyhaemoglobin. Furthermore, Smiley *et al.*[5] demonstrated that some oxidized PCs expressed platelet-activating factor (PAF)-like activity (neutrophil adhesion to vascular endothelial cells), and identified PCs with an oxovaleroyl group as active components. Using gas chromatography-mass spectrometry (GC-MS) and fast atom bombardment-mass spectrometry, we identified four types of PCs with an *sn*-2-oxidatively fragmented acyl group formed during peroxidation of different synthetic PCs with a PUFA by $Fe^{2+}$–EDTA in the presence of ascorbate.[6,7] These were PCs with an *sn*-2-short-chain monocarboxylate (MC), dicarboxylate (DC), dicarboxylate semialdehyde (DCsa), or ω-hydroxymonocarboxylate (HC) group. Furthermore, we showed that oxidized PCs had platelet-aggregating activity *via* PAF receptors and that this activity was mainly due to PCs with a short-chain monocarboxylate group.[7] In this previous study, oxidized 1-*O*-hexadecyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (GPC) was shown to have a much higher platelet-aggregating activity than oxidized 1-*O*-hexadecyl-2-arachidonoyl-GPC or oxidized 1-palmitoyl-2-docosahexaenoyl-GPC. This was found to be because the PAF-like phospholipids derived from 1-*O*-alkyl-2-docosahexaenoyl-GPC had suitable structural requirements for induction of PAF-like activity: an *sn*-1-alkyl ether linkage and an *sn*-2-acyl group of shorter chain length (Fig. 1).

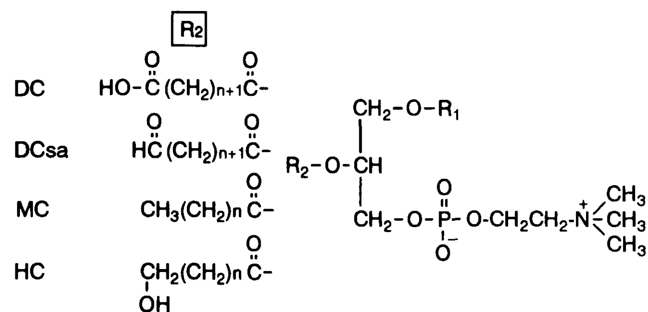


**Fig. 1.** Structures of PAF-like Phospholipids Formed during Peroxidation of PCs from Different Food Stuffs.

DC, $DC_{sa}$, MC, and HC indicate short-chain dicarboxylyl, dicarboxylate semialdehyde, monocarboxylyl, and ω-hydrocarboxylyl group, respectively.

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*Abbreviations*: AA, arachidonic acid; BHT, butylated hydroxytoluene; DC, dicarboxylate; DCsa, dicarboxylate semialdehyde; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FR-900452, 1-methyl-3-(1-[5-methylthiomethyl-6-oxo-3-(2-oxo-3-cyclopenten-1-ylidene)-2-piperazinyl]-ethyl)-2-indolinone; GC-MS, gas chromatography-mass spectrometry; GPC, *sn*-glycero-3-phosphocholine; HC, ω-hydroxymonocarboxylate; MC, monocarboxylate; PAF, platelet-activating factor; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; *t*BDMS, *tert*-butyldimethylsilyl;

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It is known that many marine animals eaten by humans are rich in 1-*O*-alkyl-2-acyl-GPC[8] and that some aquatic animals contain abundant docosahexaenoate (DHA).[9] So, there is a possibility that deleterious lipid peroxidation of such compounds from marine animals might result in the formation of oxidatively fragmented PC with potent PAF-like activity. In this study, we examined this possibility using krill, salmon roe, and sea urchin eggs.

## Materials and Methods

*Materials.* The reagents used and their sources were as follows: butylated hydroxytoluene (BHT), imidazole, and 6-(*p*-toluidino)-2-naphthalenesulfonic acid from Wako Pure Chemical Co. (Tokyo); perdeuterated acetic anhydride and silica gel 60 TLC plates (0.25 mm thickness) from Merck (Darmstadt, Germany); *Bacillus cereus* phospholipase C from Sigma Chemical Co. (St. Louis, MO), and fatty acid methyl ester standards for GLC from Nu-Chek Prep. (Elysian, MN). FR-900452 was kindly donated by Fujisawa Pharmaceutical Co., (Ibaraki, Japan). 1-*O*-Hexadecyl-2-perdeuterated ($d_3$) acetyl-GPC was prepared by treatment of 1-*O*-hexadecyl-2-lyso-GPC with perdeuterated acetic anhydride as described previously.[10] A lipid extract of krill (*Euphausia superba*) was a generous gift from Itano Refrigerated Food Co. (Tokushima, Japan). Eggs of a sea urchin (*Hemicentrotus pulcherrimus*), ripe roes of salmon (*Oncorhynchus keta*), and hen eggs were obtained commercially.

*Preparations of PCs.* Samples of 2 g of salmon roe, sea urchin eggs, and hen egg yolk were homogenized in 12 ml of water at 4°C. The homogenate was mixed with 22.5 ml of chloroform–methanol (1 : 2, by vol.) containing 0.01% BHT, and 7.5 ml of chloroform was added to the mixture for phase separation. After centrifugation at 2000 rpm for 5 min, the chloroform-rich layer was withdrawn, and the remaining aqueous layer was again mixed with 15 ml of fresh chloroform and centrifuged. The chloroform extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml of 80% ethanol and mixed with 10 ml of *n*-hexane for phase separation. After centrifugation at 2000 rpm for 5 min, the upper phase containing triglycerides, cholesterol, and BHT was removed, and the lower layer was evaporated to dryness. The phospholipids in the lower phase were fractionated by Sephadex LH-20 column chromatography with a solvent system of chloroform–methanol (1 : 1, by vol.). The PC-rich eluate was collected and dried, and the residue was dissolved in a small volume of ethanol containing 0.01% BHT and stored at −20°C. Just before use, PC was separated from BHT in this stock solution by TLC with a developing solvent system of chloroform–methanol–28% ammonium hydroxide (65 : 35 : 5, by vol.).

Krill PC was purified from the crude lipid extract of krill in the same way by Sephadex LH-20 column chromatography and TLC.

*Fatty acid analysis.* PCs (1.6 μmol) were transmethylated, and the resultant fatty acid methyl esters were analyzed in a Hitachi 263-70 gas chromatograph with a fused silica capillary column (J & W, DB-225, 30 m × 0.242 mm i.d., 0.25 μm-thickness).[6] The column temperature was kept at 120°C for 1 min, then raised to 220°C at a rate of 10°C/min, and kept at this temperature for 25 min. The carrier gas was helium, and the temperatures of the injection port and flame ionization detector were both set at 250°C. Fatty acid methyl esters were identified by comparing their retention times with those of authentic standards.

*Analysis of subclasses of PCs.* The percentages of PC subclasses were measured by successive degradations of PC with mild alkali and acid.[11] Briefly, native PC (3.2–16.1 μmol) was dissolved in 15 ml of 0.1 M methanolic KOH and the mixture was stirred for 4 h at room temperature. After neutralization of the solution with 1 N HCl, 12.5 ml of water and 15 ml of chloroform were added for phase separation and the mixture was centrifuged. The lipid-phosphate content of the lower phase containing both 1-*O*-alkyl-2-lyso-GPC and 1-*O*-alkenyl-2-lyso-GPC was measured by the method of Chalvardjian and Rudnicki.[12] Next, the lower layer was dried and the residue was dissolved in 28.5 ml of a mixture of chloroform–methanol–water (7.5 : 15 : 6, by vol.) acidified with 5 N HCl and stirred at room temperature for 20 min to decompose 1-*O*-alkenyl-2-lyso-GPC. To the reaction mixture, 7.5 ml each of chloroform and water were added for phase separation follwed by centriguation. The lower phase containing [illegible] between the lipid-phophate contents before and after alkaline hydrolysis. The amount of 1-*O*-alkenyl-2-acyl-GPC was calculated from the difference in the lipid-phosphate-contents in the lower phase before and after acid hydrolysis.

*Fatty acid analysis of 1-O-alkyl-2-acyl-GPC.* Krill PC (16 μmol) was hydrolyzed with phospholipase C from *Bacillus cereus* and the resultant glycerides were acetylated as described previously.[13] The diradyl acetates were separated by TLC,[13] and the fatty acid composition of 1-*O*-alkyl-2-acyl-3-acetyl-glycerol was measured by GLC as described above.

*Peroxidation of PCs.* PCs were peroxidized with $FeSO_4$/EDTA/ascorbate, and the oxidatively degraded phospholipids were analyzed by TLC as reported previously.[6] Briefly, PCs from various samples (2 μmol) were suspended in 8 ml of water acidified to pH 4.0, and the suspensions were sonicated for 15 min in a probe type sonicator (200 W, 9 kHz). The resulting liposomes were incubated with $FeSO_4$ (25 μM), ascorbate (50 μM) and EDTA (25 μM) at room temperature for 2 h. Lipids were extracted from the reaction mixture by the method of Bligh and Dyer[14] after acidification of the aqueous phase with 1 N HCl, and were separated by TLC in a solvent system of chloroform–methanol–28% ammonium hydroxide (65 : 35 : 8, by vol.). With the aid of authentic PC (1,2-dipalmitoyl) and lysoPC (1-palmitoyl), the plate was divided into a zone corresponding to standard PC and a zone from the origin to the area below the standard PC, which was deduced to contain the PAF-like phospholipids. The lipids were recovered from the PC zone of silica gel (PC fraction) by the method of Bligh and Dyer[14] and from the active phospholipid zone of the silica gel (active phospholipid fraction) by the method of Bligh and Dyer[14] after acidification of the aqueous phase.

*Measurement of platelet-aggregating activity.* The active phospholipid fractions by the oxidative degradation of PCs were dispersed in saline with 0.1% bovine serum albumin. Samples of 50 μl of the dispersion were added to 250 μl of a suspension of washed rabbit platelets, and platelet aggregation was monitored as described previously.[7] In some experiments, the platelets were treated with different concentrations of FR-900452 for 1 min before addition of the dispersion. The platelet-aggregating activities of the active phospholipid fractions were calculated as pmol equivalents of PAF ($C_{16:0}$) per 1 μmol of starting PC using a calibration curve for synthetic PAF ($C_{16:0}$).

*GC-MS.* PAF-like phospholipids derived from several PCs were hydrolyzed with phospholipase C and the resultant glycerides were converted to *tert*-butyldimethylsilyl (*t*BDMS) derivatives as described previously.[6,10] The *t*BDMS derivatives were fractionated by TLC with a solvent system of *n*-hexane–ethyl ether (90 : 10, by vol.) after treatment with ethereal diazomethane.[6] The regions of silica gel in the range of $R_f$ 0.04–0.80, in which the *t*BDMS derivatives of glycerides from PAF-like compounds migrated, were scraped off and tested by GC-MS as described previously.[6]

The PCs containing a short-chain monocarboxylate group in active phospholipid fractions were measured by the peak areas on the total ion chromatograms (TIC) using 1-*O*-hexadecyl-2-perdeuterated ($d_3$) acetyl-GPC as an internal standard.

## Results and Discussion

### *Analysis of subclasses and fatty acids of various PCs*

In this study, we used krill, salmon roe, and sea urchin egg PCs as potential marine sources of PAF-like lipids, and hen egg yolk PC for comparison. The subclasses and fatty acid compositions of PCs prepared from these foodstuffs are shown in Tables I and II, respectively. Krill PC was rich in both alkylacyl-GPC subclass and DHA as a PUFA, suggesting that it contains abundant 1-*O*-alkyl-2-docosahexaenoyl-GPC. GLC analysis found that alkylacyl-GPC from krill had the following fatty acid composition: 14 : 0 (2.1%), 16 : 0 (10.6%), 16 : 1 (5.2%), 18 : 0 (4.5%), 18 : 1, n-9 (10.0%), 18 : 2, n-6 (1.3%), 18 : 3, n-6 (0.4%), 18 : 3, n-3 (0.4%), 20 : 3, n-6 (3.7%), 20 : 3, n-3 (2.9%), 20 : 4, n-6 (0.3%), 20 : 5, n-3 (30.8%), 22 : 6, n-3 (21.8%), unknown (6.0%). Sea urchin egg PC was rich in alkylacyl-GPC, but

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hen egg yolk PC contained a significant amount of alkyl-acyl-PC, but the DHA content of PC in salmon roe was much higher than that in hen egg yolk.

*Platelet-aggregating activities of oxidized PCs*

We peroxidized PCs prepared from hen egg yolk, salmon roe, sea urchin eggs and krill in an $FeSO_4$/EDTA/ascorbate system and measured the platelet-aggregating activities of the active phospholipid fractions containing oxidatively fragmented PCs with an *sn*-2-short-chain acyl moiety. All these phospholipid fractions induced aggregation of washed rabbit platelets. FR-900452, an antagonist of PAF, inhibited the platelet-aggregating effects of these fractions to similar extents in a concentration-dependent manner (data not shown), suggesting that the observed platelet aggregations were mediated *via* PAF receptors on the platelets. The activity of oxidized krill PC was about 5 times those of oxidized PCs from salmon roe and sea urchin eggs, and 50 times that of oxidized hen egg yolk PC (Table III).

*GC-MS of PAF-like phospholipids formed during peroxidation of PCs*

To identify the PAF-like phospholipids formed during peroxidation of PCs from various foodstuffs, we took the active phospholipid fractions separated by TLC of the oxidized PCs and separated them by GC-MS as described in Marerials and Methods. Figures 2-A, B, C, and D show total ion chromatograms (TIC) of the *t*BDMS derivatives of glycerides obtained by the phospholipase C hydrolysis of the active phospholipid fractions of hen egg yolk, salmon roe, sea urchin egg, and krill PCs, respectively. Based on previous findings on mass spectrometric analysis of PAF-like phospholipids derived from synthetic PC[6,7] and in a bovine brain lipid extract,[15-17] the peaks on TICs were identified as shown in Table IV. Several major peaks on the TIC shown in Fig. 2-A were assigned to the *t*BDMS derivatives of diacylglycerols containing $MC_{8:0}$, $DC_{9:0}$, or $HC_{8:0}$. Since the chain lengths of the *sn*-2-acyl moieties in major PAF-like phospholipids were found to depend on the double bond vicinal to the ester linkage of PUFA in the parent PC,[6,7] the PAF-like phospholipids containing $MC_{8:0}$, $DC_{9:0}$, and $HC_{8:0}$ are thought to be formed during peroxidation of PC with a linoleate moiety, which is a major PUFA in hen egg yolk PC (Table IV).

The PAF-like phospholipids formed during peroxidation of slamon roe PC were separated into three groups (Fig. 2-B, Table IV). One included 1-long-chain acyl-PCs with $MC_{3:0}$, $DC_{4:0}$, and $HC_{3:0}$, and was regarded as the oxidation products of PCs having a PUFA with a double bond vicinal to the ester linkage at positions $C_4$ and $C_5$ such as DHA. The second consisted of PCs with $MC_{4:0}$, $DC_{5:0}$, and $HC_{4:0}$, which were deduced to be generated from PC having a PUFA with a double bond vicinal to the ester linkage at positions $C_5$ and $C_6$, mainly eicosapentaenoic acid (EPA). We detected several species of PCs with short-chain monounsaturated hydroxymonocarboxylate groups in oxidized salmon roe PC.

Both 1-*O*-alkyl and 1-acyl type PCs with a $DC_{5:0}$, $MC_{4:0}$, or $HC_{4:0}$ were detected as major PAF-like phospholipids by GC-MS of oxidized sea urchin egg PC (Fig. 2-C, Table IV). These were probably derived from both PCs containing arachidonate (AA) or EPA with a double bond at positions $C_5$ and $C_6$. The major PAF-like phospholipids formed by oxidation of krill PC were classified into two groups: 1-*O*-alkyl and 1-acyl PCs with $MC_{3:0}$, $DC_{4:0}$, and $DCsa_{4:0}$ derived from PCs containing DHA, and 1-*O*-alkyl and 1-acyl PCs with $MC_{4:0}$, $DC_{5:0}$, $DCsa_{5:0}$, and $HC_{4:0}$ derived from PCs containing EPA (Fg. 2-D, Table IV).

We failed to assess PAF-like activity of individual molecular species of PCs in these foodstuffs, because the separation was unsatisfactory. A previous study on synthetic PCs containing a PUFA demonstrated that oxidized PCs with an ether linkage at the *sn*-1 position showed much higher platelet-aggregating activities than the

**Table I.** Subclass Composition of PCs from Food Stuffs

| PC | Diacyl | Alkylacyl | Alkenylacyl |
|---|---|---|---|
| | | % | |
| Hen egg yolk | 99.2±0.2 | 0.8±0.1 | <0.1 |
| Salmon roe | 98.8±0.2 | 1.2±0.2 | <0.1 |
| Sea urchin egg | 57.5±1.1 | 41.5±0.3 | 1.0±0.8 |
| Krill | 77.0±1.2 | 23.0±1.2 | <0.1 |

Values are means±SE for four experiments.

**Table II.** Fatty Acid Composition of PCs from Food Stuffs

| Fatty Acid | Hen egg yolk | Salmon roe | Krill | Sea urchin egg |
|---|---|---|---|---|
| | | % | | |
| 14:0 | —[a] | 1.3±0.4 | 1.3±0.1 | 1.9±0.2 |
| 16:0 | 36.0±0.4 | 20.7±1.7 | 22.8±1.6 | 7.7±1.1 |
| 16:1 | 1.4±0.1 | 1.6±0.4 | 1.1±0.1 | 2.1±0.2 |
| 18:0 | 11.1±0.3 | 10.9±0.3 | 1.0±0.3 | 2.2±0.1 |
| 18:1 (n-9) | 28.5±0.1 | 16.3±1.6 | 5.2±0.5 | 0.6±0.1 |
| 18:1 (n-7) | 0.3±0.03 | 1.9±0.2 | 4.7±0.2 | 4.8±0.9 |
| 18:2 (n-6) | 16.2±0.9 | — | 1.9±0.03 | 1.2±0.3 |
| 18:3 (n-3) | — | — | 0.9±0.04 | — |
| 18:3 (n-6) | — | — | 0.8±0.1 | — |
| 20:1 (n-6) | — | — | — | 8.0±1.5 |
| 20:1 (n-3) | — | — | — | 3.5±0.5 |
| 20:2 (n-6) | — | — | — | 19.1±1.8 |
| 20:2 (n-3) | — | — | — | 7.4±0.5 |
| 20:3 (n-6) | — | — | 0.5±0.3 | 1.0±0.3 |
| 20:3 (n-3) | — | — | 4.5±1.0 | — |
| 20:4 (n-6) | 2.3±0.4 | — | 0.4±0.2 | 15.1±0.8 |
| 20:5 (n-3) | — | 15.3±1.2 | 32.9±1.0 | 17.0±1.0 |
| 22:5 (n-3) | — | 4.4±0.6 | 0.8±0.4 | — |
| 22:6 (n-3) | 3.7±0.4 | 22.2±1.2 | 19.4±1.4 | 1.0±0.3 |
| Unknown | 0.5±0.5 | 5.5±0.5 | 1.6±0.4 | 7.3±2.7 |

**Table III.** Platelet-aggregating Activities of Active Phospholipid Fractions Obtained by Oxidation of PCs from Various Food Stuffs

| PC | Platelet-aggregating activity |
|---|---|
| | Equivalents of $C_{16:0}$ PAF as pmol/$\mu$mol PC |
| Hen egg yolk | 1.7±0.6 |
| Salmon roe | 17.8±1.9 |
| Sea urchin egg | 17.2±4.0 |
| Krill | 89.8±8.8 |

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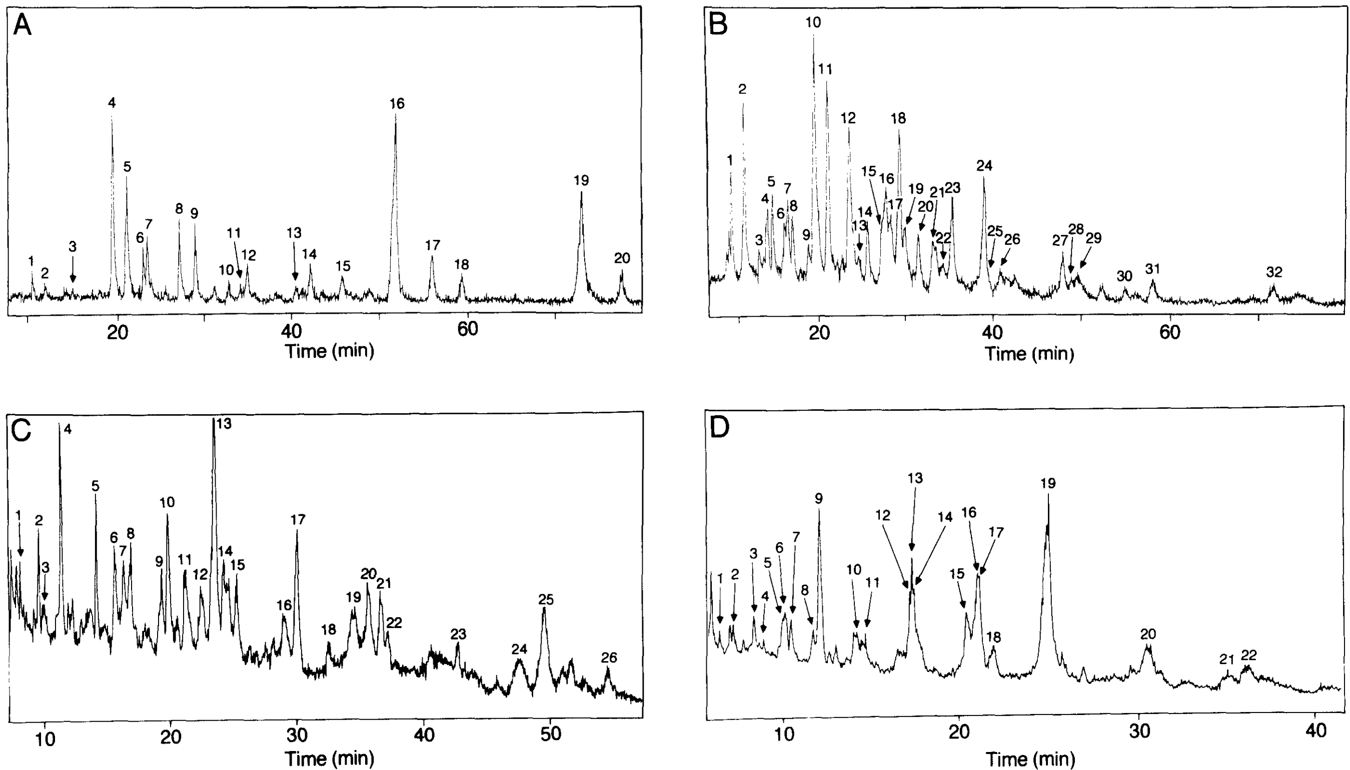


**Fig. 2.** GC-MS Fractions of Phospholipidic Secondary Products Generated during Peroxidation of Egg Yolk (A), Salmon Roe (B), Sea Urchin Egg (C), and Krill (D) PCs with $Fe^{2+}$-EDTA in the Presence of Ascorbate.

The materials in peaks were identified as shown in Table IV.

**Table IV.** Molecular Species of PAF-like Phospholipids Formed by Oxidation of PCs from Food Stuffs

| Peak No. | Hen egg yolk | Salmon roe | Sea urchin egg | Krill |
|---|---|---|---|---|
| 1 | 16:0/3:0 (MC) | 16:0/3:0 (MC) | 14:0/4:0 (MC) | O-14:0/3:0 (MC) |
| 2 | 16:0/4:0 (MC) | 16:0/4:0 (MC) | O-16:0/4:0 (MC) | O-14:0/4:0 (MC) |
| 3 | 18:0/3:0 (MC) | 14:0/4:0 (DC) | 16:0/3:0 (MC) | 14:0/4:0 (MC) |
| 4 | 16:0/4:0 (DC) | 18:1/3:0 (MC) | 16:0/4:0 (MC) | O-16:0/3:0 (MC) |
| 5 | Unknown | 18:0/3:0 (MC) | O-18:0/4:0 (MC) | O-16:0/4:0 (MC) |
| 6 | 16:0/8:0 (MC) | 18:1/4:0 (MC) | 14:0/5:0 (DC) | Unknown |
| 7 | 16:0/5:0 (DC) | 16:0/4:0 (DCsa) | 18:1/4:0 (MC) | 16:0/3:0 (MC) |
| 8 | 16:0/3:0 (HC) | 18:0/4:0 (MC) | 18:0/4:0 (MC) | O-14:0/4:0 (DC) |
| 9 | 18:0/4:0 (DC) | 16:1/4:0 (DC) | 16:0/4:0 (DC) | 16:0/4:0 (MC) |
| 10 | 16:0/4:0 (HC) | 16:0/4:0 (DC) | O-16:0/5:0 (DC) | O-14:0/5:0 (DC) |
| 11 | 18:0/8:0 (MC) | Unknown | Unknown | O-16:0/4:0 (DCsa) |
| 12 | 18:0/5:0 (DC) | 16:0/5:0 (DC) | 20:2/4:0 (MC) | 16:0/4:0 (DCsa) |
| 13 | 18:0/3:0 (HC) | 18:0/4:0 (DCsa) | 16:0/5:0 (DC) | O-16:0/4:0 (DC) |
| 14 | 16:0/8:0 (DC) | Unknown | 20:1/4:0 (MC) | O-16:0/5:0 (DCsa) |
| 15 | 16:0/9:0 (DCsa) | 16:0/3:0 (HC) | Unknown | 16:0/4:0 (DC) |
| 16 | 16:0/9:0 (DC) | 18:1/4:0 (DC) | Unknown | O-16:0/5:0 (DC) |
| 17 | Unknown | 18:1/4:0 (DC) | O-18:0/5:0 (DC) | 16:0/5:0 (DCsa) |
| 18 | 16:0/7:0 (HC) | 18:0/4:0 (DC) | Unknown | Unknown |
| 19 | 16:0/8:0 (HC) | Unknown | 18:1/5:0 (DC) | 16:0/5:0 (DC) |
| 20 | 18:0/9:0 (DC) | Unknown | 18:0/5:0 (DC) | Unknown |
| 21 | | 16:0/4:0 (HC) | Unknown | 16:0/4:0 (HC) |
| 22 | | 18:1/5:0 (DC) | Unknown | 18:1/5:0 (DC) |
| 23 | | 18:0/5:0 (DC) | O-18:0/4:0 (HC) | |
| 24 | | 16:0/6:1 (HC) | 20:2/5:0 (DC) | |
| 25 | | 18:1/3:0 (HC) | 20:1/5:0 (DC) | |
| 26 | | 18:0/3:0 (HC) | 20:0/5:0 (DC) | |
| 27 | | 16:0/7:1 (HC) | | |
| 28 | | 18:1/4:0 (HC) | | |
| 29 | | 18:0/4:0 (HC) | | |
| 30 | | 18:1/6:1 (HC) | | |
| 31 | | 18:0/6:1 (HC) | | |
| 32 | | 18:0/7:1 (HC) | | |

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corresponding oxidized 1-acyl PCs, and that the activities of oxidized PCs were mainly due to MC-PCs.[7] From these findings, active components in the oxidized PCs were suggested to be MC-PCs formed from alkylacyl-GPC.

Since MC-PCs with an *sn*-1-*O*-hexadecyl group were the predominant species, we measured the yields of MC-PCs with an *sn*-1-*O*-hexadecyl residue and *sn*-2-short-chain monocarboxylate moiety in oxidized krill and sea urchin egg PCs as their *t*BDMS derivatives by GC-MS. 1-*O*-Hexadecyl-2-deuterated acetyl-GPC was used as an internal standard. As shown in Table V, the yields of 1-*O*-hexadecyl-2-propionyl-GPC and 1-*O*-hexadecyl-2-butyryl-GPC were 0.0035% and 0.0049% (or 0.0076%), respectively, of the starting krill PC. The yield of 1-*O*-hexadecyl-2-butyryl-GPC was 0.02% of the starting sea urchin egg PC, but no significant amount of 1-*O*-hexadecyl-2-propionyl-GPC was detected. The platelet-aggregating activities of synthetic 1-*O*-hexadecyl-2-propionyl-GPC and 1-*O*-hexadecyl-2-butyryl-GPC were 1.16 times and 0.02 times that of PAF ($C_{16:0}$).[7] Consequently, the PAF-like activities of these two phospholipids formed by oxidation of krill PC are equivalent to those of 40.6 pmol (PC with an *sn*-2-propionate) and 1.0 pmol (PC with an *sn*-2-butyrate) of 16:0-PAF (Table V). The sum of the estimated values accounted for about half the observed platelet-aggregating activity (74.9 pmol). Thus the fraction of oxidized krill PC may also contain some other PAF-like phospholipids that were not identified in our GC-MS analysis. The PAF-like activity of 1-*O*-hexadecyl-2-butyryl-GPC formed by oxidation of sea urchin egg PC is equivalent to 4.0 pmol of 16:0-PAF (Table V). The remaining activity would be mainly due to 1-*O*-octadecyl-2-butyryl-GPC.

In conclusion, we demonstrated the formation of PAF-like phospholipids during peroxidation of PCs from different foodstuffs. The PCs derived from foodstuffs that have high contents of both alkylacyl-GPC and DHA, such as krill PC, were sources of phospholipids with potent PAF-like activities. However, the occurrence of PAF-like lipids in some stored foods is still speculative and requires further investigation.

**Table V.** Quantitative Analysis of 1-*O*-hexadecyl-PCs Containing a Short-chain Monocarboxylate Group in Active Phospholipidic Fractions Obtained by Oxidation of PCs from Krill and Sea Urchin Egg

| | % of starting PC | |
|---|---|---|
| | 1-*O*-Hexadecyl-2-propionyl-PC | 1-*O*-Hexadecyl-2-butyryl-PC |
| Krill | | |
| Exp. 1 | 0.0035 | 0.0049 |
| ( 74.9 pmol)[a] | (40.6 pmol)[b] | (1.0 pmol)[c] |
| Exp. 2 | 0.0035 | 0.0076 |
| (101.0 pmol)[a] | (40.6 pmol)[b] | (1.5 pmol)[c] |
| Sea urchin egg | —[d] | 0.020 |
| ( 8.9 pmol)[a] | | (4.0 pmol)[c] |

[a] Platelet-aggregating activity of active phospholipid fraction (equivalents of $C_{16:0}$ PAF as pmol/μmol PC) measured by the bioassay.

[b,c] Platelet-aggregating activity of 1-*O*-hexadecyl-2-propionyl-GPC and 1-*O*-hexadecyl-2-butyryl-GPC (equivalents of $C_{16:0}$ PAF as pmol/μmol PC), respectively. Values were estimated from the yields and platelet-aggregating activities of synthetic 1-*O*-hexadecyl-2-propionyl-GPC and 1-*O*-hexadecyl-2-butyryl-GPC.

[d] Not detected.

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