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Chromatographic Methods for the Analyses of 2-Halofatty Aldehydes and Chlorohydrin Species of Lysophosphatidylcholine

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Abstract

Plasmalogens are targeted by hypohalous acids resulting in the production of 2-chlorofatty aldehydes, 2-bromofatty aldehydes and chlorohydrin species of lysophosphatidylcholine. These novel lipids have required the development of techniques for their purification and quantification. Thin layer chromatography, high performance liquid chromatography and gas chromatography of these lipids and their derivatives have provided a battery of tools for their analyses. These lipids have been quantified using flame ionization detection, electron impact ionization mass spectrometry, chemical ionization mass spectrometry, and electrospray ionization mass spectrometry including tandem mass spectrometry.

Keywords

Plasmalogens; Fatty aldehydes; chlorohydrins; myeloperoxidase; eosinophil peroxidase; reactive chlorinating species

1. Introduction

Plasmalogens are an abundant phospholipid subclass found in mammalian tissues including those of the cardiovascular system [1–5]. Plasmalogens possess a vinyl ether bond that is susceptible to attack by reactive oxygen species [6–9]. Reactive oxygen species are produced by the innate immune system [10]. Neutrophils, monocytes, and macrophages use the enzyme, myeloperoxidase, to amplify the oxidizing potential of the reactive oxygen species, hydrogen peroxide, by converting it to the strong oxidant, hypochlorous acid. Similarly, hypobromous acid is produced by a parallel pathway by eosinophils through the activity of eosinophil peroxidase. Plasmalogens are targeted by neutrophil, monocyte and macrophage myeloperoxidase-derived hypochlorous acid leading to the production of 2-chlorofatty aldehyde and unsaturated species of lysophosphatidylcholine, which can be further targeted by reactive chlorinating species leading to the production of chlorohydrins of lysophosphatidylcholines [11–14]. Additionally, plasmalogens are targeted by eosinophil peroxidase-derived hypobromous acid leading to the production of 2-bromofatty aldehyde [15,16]. Several molecular species of these chlorinated and brominated lipid species have been identified in chemical reactions, isolated cell studies, coculture experiments, in-vivo models of ischemia/reperfusion injury and in atherosclerotic human tissue [11–19].

At present the primary halogenated lipids that have been identified in biological tissues include 2-chlorofatty aldehydes, 2-bromofatty aldehydes and chlorohydrins of

lysophosphatidylcholine. These are unique oxidized lipids species that are produced as a result of peroxidase activities at the site of tissue injury or infection. The accumulation of these lipids during tissue injury implies that they may have a role in injury. One possible mechanism that chlorinated lipids could accelerate tissue injury is through the neutrophil chemoattractant properties of 2-chloropalmitaldehyde as well as the ability of chlorohydrins of lysophosphatidylcholine to elicit surface expression of P-selectin [14,19]. Collectively, these actions may contribute to neutrophil infiltration in injured tissues. Alternatively, these unique lipids, or their metabolites, may serve as biomarkers of disease. Accordingly, it has been critical to develop methods to rapidly identify and quantify these lipid species.

Scheme I shows: 1) the pathways for the production of chlorinated lipids derived from the attack of plasmalogens by reactive chlorinating species (RCS), and 2) chromatographic strategies used to identify and quantify these novel chlorinated lipids. Similar chromatographic strategies can be applied to brominated lipids produced by the attack of plasmalogens by hypobromous acid. This review will provide details of the chromatographic methods used for the purification and quantification of these lipids. Chlorinated and brominated fatty aldehydes are readily resolved by conventional thin layer chromatography (TLC) techniques and have been quantified at the low fmol levels using gas chromatography (GC) with negative ion chemical ionization of their pentafluorobenzyl (PFB) oxime derivatives. Both 2-chlorofatty aldehyde and 2-bromofatty aldehyde can also be converted their dimethyl acetal derivatives and detected by either flame ionization detector (FID), electron impact ionization mass spectrometry, or positive ion chemical ionization mass spectrometry. Chlorohydrin molecular species of lysophosphatidylcholine (LPC-ClOH) are readily quantified using both liquid chromatography/mass spectrometry (LC/MS) and direct infusion electrospray ionization-mass spectrometry (ESI-MS) techniques employing specific neutral loss scanning for the loss of chlorine and trimethylamine.

2. 2-Halofatty aldehydes

2.1. Preparation of halofatty aldehydes

2.1.1 2-Chloropalmitaldehyde preparation—2-Chloropalmitaldehyde can be readily prepared by treating lysoplasmeylcholine with hypochlorous acid. Chloride-free sodium hypochlorite is prepared in chlorine-demand free and chloride-free glassware by a modification of a previously described method [20,21]. The concentration of sodium hypochlorite determined spectrophotometrically ($\epsilon_{292} = 350 \text{ M}^{-1} \text{ cm}^{-1}$) [22]. Sodium hypochlorite is immediately used for 2-chloropalmitaldehyde production. The lysoplasmeylcholine molecular species, 1-O-hexadec-1'-enyl-*sn*-glycero-3-phosphorylcholine (GPC), is prepared from bovine heart lecithin and purified as previously described [23]. Typical 200 nmol of lysoplasmeylcholine is incubated in 300 μl of 20 mM phosphate buffered saline containing 0.1 mM diethylenetriaminepentaacetic acid (pH=4.0) in the presence of 1 mM chloride-free sodium hypochlorite for 30 min at 37° C. Reactions are terminated by the addition of methanol and reaction products are extracted into chloroform by the method of Bligh and Dyer [24]. Additionally, 2-chloro-[7,7,8,8-*d*₄]-palmitaldehyde and 2-chloropalmitaldehyde can be synthesized as previously described [14].

2.1.2. 2-Chlorofatty aldehyde production by activated human neutrophils—Also, 2-chlorofatty aldehydes can be produced by activated human neutrophils. Whole blood (50 ml) drawn from healthy volunteers and anticoagulated with EDTA (final concentration 5.4 mM) can be used to isolate neutrophils on a Ficoll-Hypaque gradient as previously described [27]. Pelleted neutrophils resuspended in Hank's balanced salt solution (0.5×10^6 cells/ml) can be stimulated with phorbol myristate acetate (PMA) or fMLP resulting in 2-chlorofatty aldehyde production [14].

2.1.3. 2-Bromopalmitaldehyde preparation—2-Bromopalmitaldehyde can be prepared by treating lysoplasmenylcholine with hypobromous acid. Bromide-free hypobromous acid can be prepared as previously described [25]. The concentration of hypobromous acid is determined spectrophotometrically ($\epsilon_{331} = 315 \text{ M}^{-1} \text{ cm}^{-1}$) [26]. Hypobromous acid is immediately used for the preparation of 2-bromopalmitaldehyde. 200 nmol of lysoplasmenylcholine is incubated in 300 μl of 20 mM phosphate buffer supplemented with 100 mM NaBr, and 0.1 mM diethylenetriaminepentaacetic acid (pH 4.0) in the presence of 1mM hypobromous acid for 30 min at 37° C. Reactions are terminated by the addition of methanol and reaction products are extracted into chloroform by the method of Bligh and Dyer [24].

2.2. Lipidomics of 2-halofatty aldehydes

2.2.1. Thin layer chromatography and high performance liquid chromatography of halogenated fatty aldehydes—Halogenated fatty aldehydes can be separated by TLC utilizing silica gel 60Å plates (Whatman) and a mobile phase comprised of petroleum ether/ethyl ether/ acetic acid (90/10/1, v/v/v). Figure 1 shows a thin layer chromatography plate that has been sprayed with concentrated sulfuric acid and then charred following separation of 2-bromopalmitaldehyde (lanes 1 & 2), 2-iodopalmitaldehyde (lanes 3 & 4), and 2-chloropalmitaldehyde (lane 5). The R_f for 2-chloropalmitaldehyde in this system is 0.45. In comparison, the R_f for the non-halogenated fatty aldehyde, palmitaldehyde, is 0.35 (data not shown). Also shown in Figure 1 is the separation of 2-bromopalmitaldehyde ($R_f = 0.54$) as well as 2-iodopalmitaldehyde ($R_f = 0.63$). This thin layer chromatography method is useful for rapidly confirming the production of these halogenated fatty aldehydes as well as for the purification of synthetic halogenated fatty aldehydes. This method can be modified to use small silica gel TLC plates for rapidly screening synthetic reactions and can be modified to purify > 1 g quantities using preparative silica TLC plates (e.g., 1 mm thickness). 2-Chloropalmitaldehyde can also be purified by HPLC utilizing a Dynamax Si column (21.4 $\times$ 250 mm; 8 μm) and gradient elution with hexane as the initial mobile phase and hexane/chloroform (90/10, v/v) as the final mobile phase at a flow rate of 6 mL/min. Utilizing this system, 2-chloropalmitaldehyde elutes in 3% chloroform and the purity can be confirmed by TLC, as well as by GC/MS (see Section 2.2.2.).

2.2.2. Gas chromatography of halogenated fatty aldehydes and their derivatives—Halofatty aldehydes and their derivatives can be readily separated by capillary gas chromatography and detection by either flame ionization detector (FID) or mass spectrometer. Figure 2A shows the total ion current chromatogram using (electron impact ionization) of underivatized 2-chloropalmitaldehyde which has a $R_t = 5.9$ min. The mass spectrum of 2-chloropalmitaldehyde from electron impact ionization is shown in Figure 2B. This GC/MS can be used in conjunction with purification techniques including preparative HPLC (as described in Section 2.2.1.) to confirm purification of 2-chloropalmitaldehyde.

2-Halofatty aldehydes can be derivatized to their respective dimethyl acetals prior to gas chromatography. Figure 3 and Figure 4 show the gas chromatograms of the dimethyl acetals of 2-chloropalmitaldehyde and 2-bromopalmitaldehyde. Panel A of each of these figures shows the chromatograms employing FID of acid methanolysis derivatized products that are subjected to capillary gas chromatography utilizing a Supelco SP-2330 column under conditions previously described [29]. Under the isothermal conditions employed (190° C), the dimethyl acetal of 2-chloropalmitaldehyde and 2-bromopalmitaldehyde elutes at 9.1 min and 11.2 min, respectively. Since GC/FID is common instrumentation available to lipid biochemists, it should be appreciated that this technique can be readily employed for the characterization of halogenated fatty aldehyde production in biological tissues. Panels B and C of Figure 3 and Figure 4 show the chromatograms employing positive ion chemical ionization mass

spectrometry and electron ionization mass spectrometry, respectively, for these dimethyl acetals. The mass spectra for these dimethyl acetals using positive ion-chemical ionization mass spectrometry (insets of Panel B of Figure 3 and Figure 4) show a base peak that is the dimethyl acetal derivative – methoxy ion. The mass spectra for these dimethyl acetals using electron impact ionization mass spectrometry (insets of Panel C of Figure 3 and Figure 4) show a base peak $m/z = 75$, which is the McLafferty rearrangement ion. For these analyses using the mass spectrometer as the detector, acid methanolysis products were separated on a DB-1 column (see Fig. 2 for details regarding this column) using either positive ion-chemical ionization with methane as the reagent gas or electron impact ionization. For the separations of dimethyl acetals on the DB-1 column, the GC oven was maintained at 120° C for 2 min and increased at a rate of 25° C/min to 270° C. The oven temperature was held at 270° C for an additional 2 min. It is important to note that derivatization to dimethyl acetals of bromopalmitaldehyde using acid catalyzed methanolysis must be performed at lower temperatures (60° C) for a shorter reaction interval (1 hr) to minimize Cl exchange with the aldehyde Br.

The most sensitive technique to quantify 2-chloropalmitaldehyde or 2-bromopalmitaldehyde includes capillary gas chromatography with negative ion-chemical ionization mass spectrometry of their pentafluorobenzyl oxime derivatives. Employing a deuterated internal standard and selected ion monitoring scanning the sensitivity of this method is in the low femtomol range (e.g., injected on column) [17]. Figure 5 shows the structure and the mass spectrum of the pentafluorobenzyl oxime of 2-chlorostearaldehyde (an 18 carbon 2-chlorofatty aldehyde). m/z 316 is the most predominant structurally-informative fragment ion of this derivative since this fragment contains the 2-chlorostearaldehyde residue of the derivative. Figure 6 shows the selected ion monitoring (SIM) chromatograms for m/z 288 (pentafluorobenzyl oxime of 2-chloropalmitaldehyde), m/z 292 (pentafluorobenzyl oxime of 2-chloro-*d*₄-palmitaldehyde, internal standard) and m/z 316 (pentafluorobenzyl oxime of 2-chlorostearaldehyde) from lipid extracts from unstimulated neutrophils (Panel A) and PMA-stimulated neutrophils (Panel B). The area under the peak of the natural 2-chloropalmitaldehyde can be compared to that from the 2-chloro-*d*₄-palmitaldehyde, internal standard. It should be appreciated that these chromatograms show two peaks for each SIM which represent the *anti* and *syn* isomers of the PFB oxime of each aldehyde. We have previously used this method to determine that 2-chloropalmitaldehyde and 2-chlorostearaldehyde accumulate in activated neutrophils and monocytes as well as in infarcted myocardium and human atherosclerotic lesions [13,14,17,18].

2-Bromopalmitaldehyde also can be quantified using deuterated 2-bromopalmitaldehyde as an internal standard and derivatization to their pentafluorobenzyl oximes that are detected by negative ion chemical ionization mass spectrometry following capillary gas chromatography. This technique has previously been employed to quantify 2-bromopalmitaldehyde in activated neutrophils and eosinophils [15,16].

3. Chlorohydrins of lysophosphatidylcholine

3.1. Synthesis of chlorohydrins of lysophosphatidylcholine

The plasmalogen choline glycerophospholipid, 1-O-hexadec-1'-enyl-2-heptadec-10'-enoyl (16:0–17:1)-GPC, can be synthesized by an anhydrous reaction utilizing 1-O-hexadec-1'-enyl-GPC and heptadec-10'-enoyl chloride as precursors with dimethylaminopyridine as a catalyst [23]. 16:0–17:1 plasmalogen molecular species is then treated with 10-fold molar excess HOCl to simultaneously cleave the vinyl ether bond and form 17:0 LPC-CIOH, which can be purified by HPLC and quantified by Bartlett [28]. Also chlorinated products of 1-O-octadec-9', 12'-enoyl-GPC (18:2 LPC) are prepared by treating 0.4 mM of this lysophospholipid with either 0.4 or 2 mM HOCl for 5 min. These reactions are stopped by the addition of chloroform/

methanol (2:1 v/v) and lipids are extracted into the organic layer by the method of Bligh and Dyer [24]. Extracted reaction products are dried under N₂ and are stored in chloroform under N₂ prior to lipidomic analyses of the chlorohydrins of lysophosphatidylcholine.

3.2. Lipidomics of chlorohydrins of lysophosphatidylcholine

3.2.1. Reversed phase-liquid chromatography/mass spectrometry—Chlorohydrin molecular species of lysophosphatidylcholine have been identified and quantified by LC/MS and direct infusion using ESI-MS [12,19]. Figure 7A shows an LC/MS chromatogram of the products of 0.4 mM 1-octadec-9', 12'-enoyl-glycero-3-phosphocholine treatment with 0.4 mM hypochlorous acid. The three peaks represent the 1-octadec-9', 12'-enoyl-GPC (18:2 LPC) (peak 1), the monochlorohydrin of 1-octadecenoyl-GPC (peak 2) and the dichlorohydrin of 1-octadecenoyl-GPC (peak 3). The insert of Figure 7, Panel A shows that only 18:2 LPC is present in samples that are not treated with HOCl prior to LC/MS analyses (Control). In contrast, 18:2 LPC treatment with 2 mM HOCl results in a shift with an increase in dichlorohydrin/monochlorohydrin species compared to 18:2 LPC treatments with 0.4 mM HOCl (the chromatogram shown in Panel A). The mass spectra of each peak are shown in Panels B-D, respectively. Each of these mass spectra demonstrated the presence of the protonated and sodiated adducts (separated by 22 mass units) of the LPC molecular species. The mass spectrum of the monochlorohydrin LPC species (Panel C) shows m and $m + 2$ ions present at a 3 to 1 ratio which is indicative of monochlorinated molecules due to the natural distribution of ³⁵Cl and ³⁷Cl. Furthermore the mass spectrum of the dichlorohydrin LPC species (Panel D) shows m , $m + 2$, and $m + 4$ ions at a 9 to 6 to 1 ratio that indicates a dichlorinated molecule.

3.2.2. Electrospray ionization-mass spectrometry of lysophosphatidylcholine chlorohydrins—Chlorohydrins of lysophosphatidylcholine can also be detected using tandem mass spectrometry without LC. Figure 8 illustrates the utility of direct infusion methods with ESI-MS employing MS/MS techniques. Panel A of Figure 8 shows the survey scan of the chlorohydrin molecular species of 17:0 LPC (m/z 582.14). An additional $M + 2$ ion is observed (m/z 584.14), that is one third the size of the m/z 582.14 ion since it contains ³⁷Cl. Panel B of Figure 8 shows the collisionally-activated dissociation (CAD) of the m/z 582.14 ion. Two predominant fragment ions are observed at m/z 487.21 and 363.18 which are $\{[M + Na]^+ - 95\}$ and $\{[M + Na]^+ - 219\}$ representing the loss of HCl in addition to trimethylamine and phosphocholine, respectively. Another major fragment ion is m/z 523.19 which is from the loss of only the trimethylamine from the choline polar head group. This CAD analyses indicated that choline glycerophospholipid molecular species containing chlorohydrins can be detected and quantified by direct infusion ESI-MS/MS with neutral loss scanning of 95. The loss of the trimethylamine is common for sodiated adducts of choline glycerophospholipids [4,23]. In fact, the addition of NaOH to the solvent in direct infusion analyses leads to a predominance of sodiated adducts rather than a mixture of sodiated and protonated species. Panel C of Figure 8 shows the neutral loss 95 scan for the chlorohydrin molecular species of 17:0 LPC which shows the ³⁵Cl containing ion, m/z 582.18. Neutral loss 97 scanning of this molecule shows the ³⁷Cl containing ion, m/z 584.18 (not shown). This direct infusion technique employing neutral loss scanning has been used to quantitate LPC chlorohydrin species in biological samples and this neutral loss mechanism has been used in principle to perform multiple selected reaction monitoring scans of LC eluents to quantify LPC-ClOH in biological samples [12, 19].

4. Discussion

Plasmalogens are a critical phospholipid molecular subclass found in the membrane compartments of many of the cells of the cardiovascular system [1–5]. Over 60 % of the phospholipid composition of the plasma membrane of canine heart cells is comprised of

plasmalogens [29]. Furthermore plasmalogens are enriched in smooth muscle, endothelial cells, neutrophils, monocytes, macrophages, spermatozoa and neural tissue [30,31]. This phospholipid molecular subclass has been proposed to play an important role in normal membrane function as well as serve as endogenous anti-oxidants [6–9,32,33]. It is now clear that the vinyl ether linkage contained in plasmalogens is readily targeted by reactive halogenating species [11,14–16,34]. Furthermore, the vinyl ether bond is much more reactive with RCS compared to alkene bonds [12,35].

The primary products of reactive halogenating species targeting plasmalogens are 2-halofatty aldehydes and unsaturated molecular species of LPC [11,15]. It is likely that plasmalogen ethanolamine molecular species are attacked by reactive halogenating species which would yield aldehydes in the vicinity of the free amine of ethanolamine potentially leading to Schiff base adducts [36]. Also, the unsaturated lysophosphatidylcholine molecular species can be targeted by reactive halogenating species leading to halohydrin molecular species, such as LPC-CIOH. This review highlights the chromatographic techniques that have been employed to characterize these important, recently discovered, halogenated lipid species. These techniques have been employed to show that 2-chloropalmitaldehyde accumulates in activated neutrophils, activated monocytes, LDL targeted by monocytes, infarcted myocardium, and human atherosclerotic lesions [11–18]. 2-Bromopalmitaldehyde can be produced by neutrophils under conditions employing millimolar concentrations of bromide [15]. Under these conditions myeloperoxidase produces reactive brominating agents or alternatively ClBr. In contrast eosinophil peroxidase selectively uses micromolar (physiological) levels of bromide in physiological solutions containing millimolar concentrations of chloride resulting in reactive brominating species that lead to 2-bromopalmitaldehyde [16]. Additionally, the chlorohydrins of 1-0-octadec-9'-enoyl-GPC and 1-0-hexadec-7'-enoyl-GPC have been shown to accumulate in human atherosclerotic lesions [19]. Although 1-0-octadec-9,12'-enoyl-GPC is shown to form chlorohydrins and dichlorohydrins when treated with hypochlorous acid, chlorohydrins derived from polyunsaturated molecular species of LPC have not been detected in biological tissues and it is likely that polyunsaturated LPC-CIOH are unstable products. Recently it has been shown that polyunsaturated species of lysophosphatidylcholine when targeted by hypochlorous acid results in loss of the aliphatic chain and the production of glycerophosphorylcholine [37].

Multiple techniques are described in this review. Conventional TLC and HPLC using silicic acid stationary phase can be used to purify 2-halo fatty aldehydes. These aldehydes can be visualized on TLC plates by either spraying with concentrated sulfuric acid and charring or alternatively by saturating TLC plates with water. This later method is particularly useful for the purification of large quantities of aldehyde and it is not destructive in nature. Conventional GC/FID of dimethyl acetal derivatives is used for quantifying synthetic 2-halofatty aldehydes as well as confirming their purity. Ultimately verification of structure of biologically-produced and synthetic 2-halofatty aldehydes has been made by GC/MS techniques of the underivatized (electron impact ionization), dimethyl acetal derivative (electron impact ionization and positive ion-chemical ionization) and pentafluorobenzyl oxime derivatives (negative ion-chemical ionization). It should be noted that underivatized 2-halofatty aldehydes are not stable under ESI-MS methods. In contrast ESI-MS can be used for the detection and quantification of chlorohydrin molecular species of LPC.

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Figure 1. TLC Analysis of 2-Halofatty aldehydes

Approximately 50 nmol each of 2-bromopalmitaldehyde (lanes 1 and 2), 2-iodopalmitaldehyde (lanes 3 and 4) and 2-chloropalmitaldehyde (lane 5) in 30– 50 μ l of chloroform was subjected to TLC using silica gel 60Å plates as a stationary phase and a mobile phase comprised of petroleum ether/ diethyl ether/acetic acid (90/10/1, v/v/v). Aldehydes on developed TLC plates were visualized by sulfuric acid treatment and charring.

Figure 2. GC/MS of 2-Chloropalmitaldehyde

Approximately 2 pmol of 2-chloropalmitaldehyde in 1 μ l of petroleum ether was injected in pulsed splitless mode onto a DB-1 column (12.5 m, 0.2 mm i.d., 0.33 μ m methyl silicone film coating; P.J. Cobert, St. Louis, MO) utilizing a Hewlett Packard 6890 gas chromatograph and detected by electron impact ionization mass spectrometry using a Hewlett Packard 5988A mass spectrometer (Palo Alto, CA). The injector temperature and the transfer lines were maintained at 250° C. The GC oven was maintained at 150° C for 3.5 min and increased at a rate of 25° C/min to 310° C. The oven temperature was held at 310° C for an additional 5 min. Panel A is the total ion current (TIC, expressed as % intensity of base peak) with 2-

chloropalmitaldehyde eluting at 5.9 min. The mass spectrum of this peak (expressed as % abundance of the base ion) is shown in Panel B.

Figure 3. GC/FID and GC/MS of the Dimethyl Acetal of 2-Chloropalmitaldehyde

Panel A: Approximately 100 pmol of the dimethyl acetal of 2-chloropalmitaldehyde and 200 pmol of the methyl ester of arachidic acid in 1 μ l of petroleum ether was injected with a 50 to 1 split onto the GC column and detection was by flame ionization. Peaks 1–3 are the solvent peak, the dimethyl acetal of 2-chloropalmitaldehyde and the methyl ester of arachidic acid, respectively. Panel B: Approximately 2 pmol of the dimethyl acetal of 2-chloropalmitaldehyde in 1 μ l of petroleum ether was injected in pulsed splitless mode onto the GC column and detected by positive ion-chemical ionization mass spectrometry. Under these conditions, the dimethyl acetal eluted at 7.0 min. The mass spectrum of this peak is shown in the inset. Panel C: Approximately 2 pmol of the dimethyl acetal of 2-chloropalmitaldehyde in 1 μ l of petroleum

ether was injected in pulsed splitless mode onto the GC column and detected by electron impact ionization mass spectrometry. Under these conditions, the dimethyl acetal eluted at 6.7 min. The mass spectrum of this peak is shown in the inset.

Figure 4. GC/FID and GC/MS of the Dimethyl Acetal of 2-Bromopalmitaldehyde

Panel A: Approximately 30 pmol of the dimethyl acetal of 2-bromopalmitaldehyde and 50 pmol of the fatty acid methyl ester of palmitic acid in 1 μ l of petroleum ether was injected with a 50 to 1 split onto the GC column and detection was by flame ionization. Peaks 1–3 are the solvent peak, the fatty acid methyl ester of palmitic acid and the dimethyl acetal of 2-bromopalmitaldehyde, respectively. Panel B: Approximately 2 pmol of the dimethyl acetal of 2-bromopalmitaldehyde in 1 μ l of petroleum ether was injected in pulsed splitless mode onto the GC column and detected by positive ion-chemical ionization mass spectrometry. Under these conditions, the dimethyl acetal eluted at 7.4 min. The mass spectrum of this peak is shown in the inset. Panel C: Approximately 2 pmol of the dimethyl acetal of 2-bromopalmitaldehyde

in 1 μ l of petroleum ether was injected in pulsed splitless mode onto the GC column and detected by electron impact ionization mass spectrometry. Under these conditions, the dimethyl acetal eluted at 7.0 min. The mass spectrum of this peak is shown in the inset.

Figure 5. GC/MS of the Pentafluorobenzyl Oxime Derivative of the 2- Chlorostearaldehyde
 Panel A: The structure and putative fragmentation patterns of the pentafluorobenzyl oxime of 2-chlorostearaldehyde. Panel B: 2-Chlorostearaldehyde was converted to its pentafluorobenzyl oxime derivatives and subjected to GC/MS with detection employing negative ion-chemical ionization. Shown is the mass spectrum of the pentafluorobenzyl oxime of 2-chlorostearaldehyde.

Figure 6. GC/MS analysis of PFB oximes of 2-Chlorofatty aldehydes produced by PMA-stimulated neutrophils

0.5×10^6 neutrophils were treated with either 200 nM PMA or vehicle only for 30 min at 37°C . Reactions were terminated by the addition of methanol containing 20 pmol 2-chloro- $[d_4]$ -palmitaldehyde and the reaction products were extracted into chloroform. Following conversion to their PFB-oximes, SIM GC/MS analysis using negative ion-chemical ionization for $m/z = 288$, $m/z = 292$ and $m/z = 316$ (as indicated) was performed for the control (A) and PMA-treated (B) neutrophil samples, respectively.

Figure 7. Chlorohydrin formation of 18:2 LPC

Panel A: Chromatographic separation of 18:2 LPC (0.4 mM) treated with equimolar HOCl. Panels B-D - ESI-MS spectra derived from Panel A showing protonated and sodiated ions from 18:2 LPC (RT: 7.8–8.4), 18:1 LPC-ClOH (RT: 4.6–5.1), and 18:0 LPC-diClOH (RT: 2.5–3.4), respectively. Insets show chromatograms of control or 5-fold molar excess HOCl-treated 18:2 LPC. All chromatograms show relative abundance of positive ions over time ranging 0–30 minutes. 18:2 LPC and the products of 18:2 LPC treatment with HOCl were suspended in 100 μ L of methanol and subjected to LC/MS (25 μ L injection volume). Lipid molecular species were separated using a 15 cm $\times$ 2.1 mm, 5 μ m Discovery® HS C18 HPLC column (Supelco) and an initial mobile phase comprised of methanol/water/acetonitrile (60/20/20, v/v/v) containing 1 mM ammonium acetate with a gradient to 1 mM methanolic ammonium acetate solution over 5 min followed by isocratic elution in this mobile phase for an additional 35 min. The flow rate was 0.2 mL/min.

Figure 8. ESI-MS/MS analysis of LPC chlorohydrin

One micromolar of the chlorohydrin of 17:0 LPC in 4/1 methanol/chloroform containing 0.5 μ M NaOH was subjected to ESI-MS in the direct infusion mode at a flow rate of 3 μ l/min using a Thermo Electron TSQ Quantum Ultra instrumentation. Samples were analyzed in the positive ion mode. Panel A shows the survey scan acquired over 3 min. Panel B shows the CAD scan for 582, the chlorohydrin of 17:0 LPC containing ^{35}Cl . The structure and fragmentation scheme are shown in the inset. Panel C shows the neutral loss scan of 95 for this sample, which is from the loss of the choline trimethylamine and HCl from the chlorohydrin. Tandem mass spectrometry was with a collision energy 32 eV and at 1 atmosphere argon as collision gas.

Scheme I.
Chromatographic Strategies for Reactive Chlorinating Species (RCS)-derived Plasmalogen Oxidation Products