Plasmalogens contain a vinyl ether bond linking the sn-1 aliphatic chain to the glycerol backbone of this predominant phospholipid molecular subclass, which is found in many mammalian tissues. The present study demonstrates that the vinyl ether bond of plasmalogens is a molecular target of the reactive chlorinating species produced by myeloperoxidase. Analysis by thin layer chromatography revealed that reactive chlorinating species produced by myeloperoxidase target the vinyl ether bond of the plasmalogen, lysoplasmenylcholine (1-O-hexadecyl-1'-enyl-sn-glycero-3-phosphorylcholine), resulting in the production of a neutral lipid. Capillary gas chromatographic analyses demonstrated that the neutral lipid generated from lysoplasmenylcholine was neither hexadecanal nor did it contain masked hexadecanal (i.e. the vinyl ether) because the dimethyl acetel of hexadecanal produced by acid methanolation derivatization was no longer present. Electrospray ionization mass spectrometry of the myeloperoxidase-generated neutral lipid product was consistent with the production of a 16-carbon fatty aldehyde containing one chlorine atom. Furthermore, proton NMR analysis indicated that this neutral lipid product was a 2-chloro-fatty aldehyde. Additional structural analysis of this neutral lipid by gas chromatography-mass spectrometry of the underivatized product as well as its pentafluorobenzyl oxime-derivative product was consistent with the neutral lipid being 2-chlorohexadecanal. The reactive chlorinating species, hypochlorous acid and chlorine gas, both attacked the vinyl ether bond of lysoplasmenylcholine resulting in the production of 2-chlorohexadecanal. The production of 2-chlorohexadecanal was dependent on the presence of the plasmalogen masked aldehyde (i.e. the vinyl ether) in the substrate because the free fatty aldehyde, hexadecanal, was not converted to 2-chlorohexadecanal by the reactive chlorinating species generated by myeloperoxidase. Taken together, the present studies demonstrate for the first time the targeting of the vinyl ether bond of plasmalogens by the reactive chlorinating species produced by myeloperoxidase resulting in the production of novel chlorinated fatty aldehydes.

Activated phagocytes generate a variety of reactive oxidizing and chlorinating species that damage target cells (1, 2). The activation of neutrophil NADPH oxidase during the respiratory burst results in the production of superoxide anions that dismutate to produce hydrogen peroxide. Myeloperoxidase, which is released by activated neutrophils, amplifies the oxidizing potential of hydrogen peroxide by using it as a substrate in the presence of physiological concentrations of chloride to produce hypohalous acid (HOCl) and its conjugate base (ClO\(^{-}\)), which are the predominant oxidants produced by activated neutrophils (3). The cytotoxic reactions mediated by hypohalous acid include the chlorination of amines and unsaturated lipids as well as oxidative bleaching of heme groups and iron sulfur centers (4–8). Additionally, hypohalous acid can react with primary amines to produce another group of chlorinating intermediates, the chloramines (R-NHCl) (9). Chlorine gas has also been shown to be released from activated neutrophils and to contribute to many of the chlorination reactions previously attributed to hypohalous acid (10). Thus, phagocytes release an arsenal of reactive chlorinating and oxidizing species, which include HOCl, ClO\(^{-}\), Cl\(_2\), and chloramines.

Lipids are major targets of the reactive chlorinating species produced by activated phagocytes. For example, the chlorinating agents released by activated neutrophils attack the unsaturated C=C bonds within the aliphatic chains of phospholipids (6). Formation of chlorohydrins within the aliphatic groups of phospholipids are disruptive to normal membrane fluid molecular dynamics and could represent a major cytotoxic effect of activated neutrophils (11, 12). Additionally, reactive chlorinating species produced by myeloperoxidase chlorinate lipoprotein-associated cholesterol to lead to the production of a family of chlorinated cholesterol species including chlorohydrins and dichlorinated products (13).

Many mammalian tissues are enriched with plasmalogen phospholipid molecular species in their plasma membrane phospholipid pools (14–18). Plasmalogens possess a masked aldehyde, vinyl ether linkage between the sn-1 aliphatic chain and the glycerol backbone and may have an important role in both the solvation of transmembrane ion channels and transport proteins (19–21) as well as the storage of arachidonic acid (16–18, 22) that is released by calcium-independent phospholipase A\(_2\) (23, 24) and subsequently used for eicosanoid synthesis (25). Although plasmalogenase activities have been reported that are responsible for the degradation of the plasmalogen vinyl ether bond, this bond is relatively stable to enzymatic degradation (26, 27). Alternatively, the vinyl ether bond of plasmalogens may be susceptible to chemical degradation in vivo. For example, the vinyl ether linkage of plasmalogens terminates the propagation of the production of lipid free radicals initiated by reactive oxygen species attacking aliphatic alkenes of phospholipids (28, 29).
Plasmalogens, which are localized in the plasma membranes of mammalian cells (17), likely would be highly accessible molecular targets of the membrane-permeable, reactive chlorinating species generated by activated phagocytes. Accordingly, the present study tested the hypothesis that the vinyl ether of the sn-1 aliphatic chain of plasmalogens would be susceptible to chlorination by reactive chlorinating species produced by myeloperoxidase. The results herein demonstrate for the first time that reactive chlorinating species produced by myeloperoxidase are reactive with the masked aldehyde, vinyl ether linkage of plasmalogens resulting in the production of lysophospholipids and α-chloro-fatty aldehydes (see Scheme I). Furthermore, the production of α-chloro-fatty aldehydes by reactive chlorinating species generated by myeloperoxidase is specific for the masked aldehyde found in plasmalogens and is not produced from free fatty aldehydes or carboxy ester-linked aliphatic groups found at the sn-2 carbon of the plasmalogen glycerol backbone.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chloride-free sodium hypochlorite was prepared in chlorine-demand free and chloride-free glassware by a modification of a method described previously (13, 30). The concentration of sodium hypochlorite was determined spectrophotometrically ($\epsilon_{292} = 350 \text{ M}^{-1} \text{ cm}^{-1}$) (31). Sodium hypochlorite was immediately used for the experiments. Myeloperoxidase was purchased from Calbiochem. Pentafluorobenzyl hydroxylamine was purchased from Aldrich. Hexadecanoyl chloride was purchased from Nu-Chek Prep. All other reagents and chemicals were purchased from either Aldrich, Sigma, or Fisher.

**Preparation of Lysoosphosphatidylcholine and Phosphatidylcholine**—The lysoosphosphatidylcholine molecular species, 1-O-hexadec-1'-eny-PG, was prepared from bovine heart lecithin and purified as described previously (32). Plasmenylcholine was synthesized by an anhydrous reaction from plasmalogen.
Targeting of Plasmalogens by Reactive Chlorinating Species

Plasmalogen Treatment with Myeloperoxidase-derived Reactive Chlorinating Species: Analysis of Reaction Products by Thin Layer Chromatography and Capillary Gas Chromatography—Incubations were performed in chlorine-demand-free and chloride-free reaction vessels. In a typical assay 200 or 300 nmol of either lysoplasmenylcholine or plasmenylcholine was incubated in 300 μl of phosphate-buffered saline comprised of 100 mM NaCl, 20 mM NaPO₄, 0.1 mM diethylenetriaminepentaacetic acid (pH 4.0–7.0) in the presence of the indicated amounts of myeloperoxidase, H₂O₂, chlorine gas, or chloride-free sodium hypochlorite for the indicated intervals at 37 °C. The reaction products were subjected to TLC with chloroform/acetone/methanol/acetic acid/water (6:8:2:1 v/v/v/v) as mobile phase. The reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring (inset A). In parallel studies silica from the developed TLC plate was zonally scraped, as indicated (inset A), and TLC resolved reaction products were analyzed for inorganic phosphate and aliphatic groups (inset B) as described under “Experimental Procedures.” For capillary GC analysis, the indicated peak (FAME of palmitic acid) had an identical retention time with that of authentic FAME of palmitic acid.

Plasmalogen Treatment with Myeloperoxidase-treated lysoplasmenylcholine. 200 nmol of lysoplasmenylcholine was incubated in the presence of the MPO/HOCl generating reagents including MPO (2.5 units), H₂O₂ (1 mM), and NaCl (100 mM) in 500 μl of 20 mM phosphate buffer (pH 4.0) at 37 °C for 5 min. The incubations were terminated by the addition of methanol, and the reaction products were sequentially extracted into chloroform and subjected to TLC with chloroform/acetone/methanol/acetic acid/water (6:8:2:1 v/v/v/v) as mobile phase. The reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring (inset A). In parallel studies silica from the developed TLC plate was zonally scraped, as indicated (inset A), and TLC resolved reaction products were analyzed for inorganic phosphate and aliphatic groups (inset B) as described under “Experimental Procedures.” For capillary GC analysis, the indicated peak (FAME of palmitic acid) had an identical retention time with that of authentic FAME of palmitic acid.

Electrospray Ionization Mass Spectrometric and Proton NMR Analyses—The reaction products from the treatment of lysoplasmenylcholine with reactive chlorinating species generated by the myeloperoxidase system were subjected to HPLC, capillary gas chromatographic analysis as described under “Experimental Procedures.” Peaks 1, 2, and 3 are the solvent, the dimethyl acetal of hexadecanal, and the unknown derivatized reaction product, respectively.

**Fig. 3.** Analysis of TLC-purified lysophosphatidylcholine produced by myeloperoxidase treatment of plasmenylcholine. 200 nmol of plasmenylcholine was incubated in the presence of the MPO/HOCl generating reagents including MPO (2.5 units), H₂O₂ (1 mM), and NaCl (100 mM) in 500 μl of 20 mM phosphate buffer (pH 4.0) at 37 °C for 5 min. The incubations were terminated by the addition of methanol, and the reaction products were sequentially extracted into chloroform and subjected to TLC with chloroform/acetone/methanol/acetic acid/water (6:8:2:1 v/v/v/v) as mobile phase. The reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring (inset A). In parallel studies silica from the developed TLC plate was zonally scraped, as indicated (inset A), and TLC resolved reaction products were analyzed for inorganic phosphate and aliphatic groups (inset B) as described under “Experimental Procedures.” For capillary GC analysis, the indicated peak (FAME of palmitic acid) had an identical retention time with that of authentic FAME of palmitic acid.

**Fig. 4.** Capillary gas chromatographic analysis of myeloperoxidase-treated lysoplasmenylcholine. 200 nmol of lysoplasmenylcholine was incubated with (B and C) or without (A) 0.6 unit of myeloperoxidase in the HOCl generating buffer system at pH 4.0 at 37 °C for 5 min. The lipid reaction products were extracted and subjected to acid methanalysis, and derivatives were analyzed by capillary gas chromatography with FID detection. For C, the reaction product with an Rᵣ of 0.46 on TLC in solvent system 1 was first purified utilizing this TLC system and then was subjected to sequential acid methanalysis and capillary gas chromatographic analysis as described under “Experimental Procedures.” Peaks 1, 2, and 3 are the solvent, the dimethyl acetal of hexadecanal, and the unknown derivatized reaction product, respectively.

### Gas Chromatography-Mass Spectrometric Analyses of Pentfluoro-

benzylxime-derivative Products—GC-MS was performed on reaction products or TLC-purified reaction products either directly or following derivatization with acidic methanol or pentafluorobenzyl hydroxylamine. In brief, derivatization with pentafluorobenzyl hydroxylamine was performed by resuspending the reaction products in 100 μl of ethanol followed by the addition of 100 μl of 6 mg/ml pentafluorobenzyl hydroxylamine in water. The ethanolic-water mixture was vortexed for 5 min at room temperature and allowed to further incubate at room temperature for an additional 25 min. The reaction products were extracted into cyclohexane/diethyl ether (4:1 v/v) and resuspended in 30–100 μl of petroleum ether prior to GC-MS analysis. The samples were analyzed on a DB-1 column (125 m, 0.2-mm inner diameter, 0.33-μm methyl silicone film coating; P. J. Cobert, St. Louis, MO) utilizing a Hewlett Packard 5890 gas chromatograph. The injector temperature and the transfer lines were maintained at 250 °C. The GC oven was maintained at 150 °C for 1 min and increased at a rate of 30 °C/min to 270 °C. The oven temperature was held at 270 °C for an additional 2 min. All spectra were acquired on a Hewlett Packard 5998A mass spectrometer (Palo Alto, CA) and operated in the negative chemical ionization mode with methane as the reagent gas. The source temperature was set at 150 °C. The electron energy was 240 eV, and the emission current was 300 μA.
RESULTS

It was predicted that the vinyl ether bond of plasmalogens represents a molecular target of the reactive chlorine species generated by myeloperoxidase that are produced during phagocyte activation. This hypothesis was first tested by incubating lysoplasmenylcholine with myeloperoxidase in phosphate-buffered saline containing hydrogen peroxide. TLC analysis of the reaction products revealed that lysoplasmenylcholine (1-O-hexadec-1'-enyl-GPC) was degraded in the presence of myeloperoxidase, hydrogen peroxide, and phosphate-buffered saline (pH 4.0) (Fig. 1, Solvent System 2). Concomitant with lysoplasmenylcholine degradation, a neutral lipid was produced that migrated with an \( R_f \) of 0.46 in a solvent system that resolves neutral lipids (Fig. 1, Solvent System 1). This neutral lipid did not co-migrate with either hexadecanal (\( R_f = 0.57 \)) or fatty acid (\( R_f = 0.18, 0.19, \) and 0.20 for palmitic, palmitoleic, and arachidonic acid, respectively) in this TLC system. The degradation of plasmalogens was dependent on the presence of a complete reaction mixture comprised of active myeloperoxidase (thermal treatment ablated plasmalogen degradation), hydrogen peroxide, and sodium chloride (Fig. 1). Additionally, the degradation of plasmalogens by the complete myeloperoxidase reaction mixture occurred at pH 4.0 with little degradation at pH 7.0.

Similar studies were performed utilizing the plasmenylcholine molecular species, 1-O-hexadec-1'-enyl-2-hexadecanoyl-GPC, as the target phospholipid of the reactive chlorinating species generated by myeloperoxidase. TLC analysis of the reaction products of this reaction demonstrated that plasmenylcholine was degraded (Fig. 2, Solvent System 2) concomitant

FIG. 5. Chemical and enzymic treatments of plasmenylcholine, lysoplasmenylcholine, lysophosphatidylcholine, and palmitaldehyde. The indicated lipids (200 nmol) were incubated at 37 °C for 5 min in 20 mM phosphate buffer with either no further additions (A), 1.5 units of myeloperoxidase (with 1 mM H\(_2\)O\(_2\) and 100 mM NaCl) (B), 4.16 mM NaOCl (C), or 4.16 mM Cl\(_2\) (D). The reactions were terminated with the addition of methanol. The lipids were extracted and subjected to acid methanalysis, and the acid methanalysis-derivatives were subjected to capillary gas chromatography as described under “Experimental Procedures.” Peaks 1, 2, 3, and 4 are the solvent, the dimethyl acetal of hexadecanal, the fatty acid methyl ester of palmitic acid, and the unknown derivatized reaction product, respectively.
with the production of a neutral lipid that again migrated with an \( R_t \) of 0.46 (Fig. 2, Solvent System 1). Similar to lysoplasmenylcholine degradation, the degradation of plasmenylcholine was dependent on the presence of the complete myeloperoxidase system at pH 4.0. Further analyses revealed that the reaction products that were separated by TLC utilizing solvent system 2 also included lyso phosphatidylcholine. For these analyses, silica corresponding to the region that lyso phosphatidylcholine migrates on the TLC plates was scraped and extracted. The presence of lyso phosphatidylcholine in the extract from this silica was confirmed by the presence of phosphate as determined by a Bartlett phosphate assay (Fig. 3) and a palmitic acid residue as determined by the detection of the methyl ester of palmitic acid by capillary GC analysis following acid methanalysis (Fig. 3, inset B). It should be appreciated that the sulfuric acid-charring detection method employed in these TLC analyses detects aldehydes and masked aldehydes but does not readily detect lyso phosphatidylcholine. Taken together, these results demonstrate the specificity of the attack of the vinyl ether-linked aliphatic group of plasmenylcholines by myeloperoxidase-mediated reactive chlorinating species.

The TLC-purified neutral lipid reaction product from the myeloperoxidase-mediated degradation of plasmenylcholines was further characterized by capillary gas chromatography. Capillary gas chromatography of the acid methanalysis-derivatives of lysoplasmenylcholine treated with reactive chlorinating species generated by myeloperoxidase resulted in the production of a unique acid methanalysis-derivative product with an \( R_t \) value of 9.3 min (Fig. 4B, peak 3). In contrast, the dimethyl acetal of hexadecanal \((R_t = 5.6 \text{ min); Fig. 4A, peak 2}\) was the only acid methanalysis-derivative of untreated lysoplasmenylcholine.
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(i.e. not treated with the myeloperoxidase-generated reactive chlorinating species). Furthermore, acid methanolysis and subsequent capillary gas chromatography of the TLC-purified neutral lipid (e.g. $R_f = 0.46$ in Fig. 1, Solvent System 1) demonstrated that this lipid gave rise to the acid methanolysis product with an $R_f$ of 9.3 min (Fig. 4C, peak 3). Further studies demonstrated that this product of the myeloperoxidase reaction was only produced from vinyl ether containing lipids (i.e. plasmalysenylcholine and lysoplasmenylcholine) and was not produced from lysophosphatidylcholine or hexadecanal (Fig. 5). This later finding that hexadecanal was not chlorinated by myeloperoxidase-generated reactive chlorinating species, in contrast to plasmalogens, underscores the specificity of the targeting of the masked aldehyde, vinyl ether bond of plasmalogens by reactive chlorinating species generated by myeloperoxidase. The data shown in Fig. 5 also demonstrate the specificity for the attack of the vinyl ether bond of plasmalogens by chemical treatments with the initial product of myeloperoxidase, hypochlorous acid, as well as chlorine gas (Fig. 5, C and D). Thus, it is likely that the chemical mediators of plasmalogen degradation generated by the myeloperoxidase reaction are hypochlorous acid and/or chlorine gas.

The specificity of the degradation of plasmalogens by myeloperoxidase-generated reactive chlorinating species was further assessed in reactions that included the inhibitors sodium azide, catalase, and 3-amino triazole. Sodium azide, catalase, and 3-amino triazole inhibited myeloperoxidase-mediated degradation of the vinyl ether bond (Fig. 6). Additionally, the specificty of the reaction of the myeloperoxidase-generated reactive chlorine species toward the vinyl ether bond of plasmalogens was demonstrated because destruction of the vinyl ether bond of plasmalogens by treatment with hydrochloric acid fumes for 10 min ablated the production of the myeloperoxidase-mediated neutral lipid product (Fig. 6).

Because the data shown in Figs. 1 and 2 demonstrated differential degradation of plasmalogens at pH 4.0 and 7.0, the pH dependence of this reaction was further characterized utilizing lysoplasmenylcholine as the vinyl ether substrate. The breakdown of the vinyl ether linkage by the complete myeloperoxidase chlorinating system was optimal at acidic pH but was significantly active at pH 6.0 with half-maximal activity at pH 5.5 (Fig. 7). Because hypochlorous acid is the initial product of myeloperoxidase and because chlorine gas can be produced from hypochlorous acid at acidic pH (10), further experiments were performed to determine whether molar equivalents of NaOCl or chlorine gas could mimic the degradation of lysoplasmalogenylcholine by the reactive-chlorinating species generated by myeloperoxidase. The data shown in Fig. 8 demonstrate that, at neutral pH, NaOCl and chlorine gas degrade plasmalogens resulting in the production of the same neutral lipid produced by the myeloperoxidase reaction toward plasmalogen.

The initial structural information on the neutral lipid produced from lysoplasmenylcholine (1-O-hexadec-1'-enyl-sn-glycero-3-phosphocholine) by the myeloperoxidase-catalyzed reaction was procured by electrospray ionization mass spectrometry. The chloroform extract of the reaction products was subjected to electrospray ionization mass spectrometry. The chloroform extract of the reaction products was subjected to electrospray ionization mass spectrometry in the negative ion mode as described in detail under “Experimental Procedures.” The TLC-purified material having an $R_f$ of 0.5 was dried and resuspended in petroleum ether prior to GC-MS with electron impact ionization as described under “Experimental Procedures.” The ions and ion fragments (the mass spectrum) of the major peak eluting at 7.25 min are shown.

**Fig. 9.** Electrospray ionization mass spectrometry of myeloperoxidase-generated reaction products. Lysoplasmenylcholine (200 nmol) was incubated in the presence of the MPO/HOCl generating system, which includes MPO (0.6 unit), H$_2$O$_2$ (1 mM), and NaCl (100 mM) in 300 μl of 20 mM phosphate buffer (pH 4.0) at 37 °C for 5 min. The incubations were terminated by the addition of methanol, and the reaction products were sequentially extracted into chloroform and subjected to electrospray ionization mass spectrometry in the negative ion mode as described in detail under “Experimental Procedures” (A). The negative ion at m/z 273 was subjected to mass-mass analysis (B).

**Fig. 10.** Gas chromatography-mass spectrometry of the lysoplasmenylcholine/myeloperoxidase reaction product. Lysoplasmenylcholine (200 nmol) was incubated in the presence of the MPO/HOCl generating system, which includes MPO (0.6 unit), H$_2$O$_2$ (1 mM), and NaCl (100 mM) in 300 μl of 20 mM phosphate buffer (pH 4.0) at 37 °C for 5 min. The incubations were terminated by the addition of methanol, and the reaction products were sequentially extracted into chloroform and subjected to TLC as described under “Experimental Procedures.” The TLC-purified material having an $R_f$ of 0.5 was dried and resuspended in petroleum ether prior to GC-MS with electron impact ionization as described under “Experimental Procedures.” The ions and ion fragments (the mass spectrum) of the major peak eluting at 7.25 min are shown.
having an TLC as described under “Experimental Procedures.” The TLC-purified material having an \( R_f = 0.5 \) was suspended in CDCl\(_3\) and subjected to proton NMR spectroscopic analyses as described under “Experimental Procedures.” The peak assignments are depicted in the inset.

likely that the myeloperoxidase-catalyzed degradation of thevinyl ether bond of plasmalogens resulted in the production of a monochlorinated hexadecanial species. The undervatized product of the myeloperoxidase reaction with lysoplasmenylcholine was also subjected to GC-MS with electron impact ionization. A single peak was found at 7.25 min that had an apparent parent ion \( m/z = 274 \) with an associated ion approximately one-third the size at \( m/z = 276 \) (Fig. 10), which was consistent with the electrospray ionization mass spectrometry data.

Further structural information of the putative monochlorinated hexadecanal species produced from plasmalogens by reactive chlorinating species was obtained by proton NMR analyses as well as GC-MS analyses. The NMR data shown in Fig. 11 demonstrate that the most likely structure of the TLC-purified reaction product is 2-chlorohexadecanal. In particular, the aldehyde proton (Fig. 11, arrowhead e) is split by its sole neighboring proton, the proton on the \( \alpha \)-carbon, and there is a downfield shift of the chlorinated methylene that is split by three neighboring protons (Fig. 11, arrowhead d). There is also a shift in the methane groups neighboring the chlorinated methylene group (Fig. 11, arrowhead c). Because the likely chlorinated reaction product of the myeloperoxidase reaction with plasmalogens contains an aldehyde carbonyl, the carbonyl-reactive derivatization agent, pentafluorobenzyl hydroxylamine, was used to produce the pentafluorobenzyl oxime of the aldehyde for GC-MS analyses. This derivatization is very useful because of the excellent electron capturing properties of the pentafluorobenzyl group and the characteristic fragmentation patterns that are observed by subjecting pentafluorobenzyl (PFB) oxime-derivatives to capillary gas chromatography with negative ion chemical ionization mass spectrometry. GC-MS analyses on a DB-1 column in the negative ion mode of the PFB oxime-derivative of the reaction product from incubations of lysoplasmenylcholine (1-O-hexadec-1'-enyln-sn-glycero-3-phosphocholine) with myeloperoxidase, \( \mathrm{H}_2\mathrm{O}_2 \), and NaCl resulted in the production of two peaks consistent with the production of the \( \text{syn} \)- and \( \text{anti} \)-isomers of the PFB oxime-derivative of 2-chlorohexadecanal (Fig. 12, A and C). The fragmentation pattern of the second peak is shown in Fig. 12B. The molecular ion (M\(^+\)) of the PFB oxime of 2-chlorohexadecanal is observed at \( m/z \) 469 (Fig. 12B). Again, monochlorination of this compound was confirmed by identifying the isotopic cluster (M\(^+\)/M\(^-\) + 2 of 3:1 at \( m/z \) 469 and 471). One of the major fragments at \( m/z \) 288 is also monochlorinated, because an ion of one-third the intensity was also observed at \( m/z \) 290. It should be recognized that the remaining fragmentation pattern is completely consistent with the structure of the 2-chlorohexadecanal PFB-derivative shown in Fig. 12C. Taken together, these data, in conjunction with that from electrospray ionization mass spectroscopic analyses, demonstrate that 2-chlorohexadecanal is the product of myeloperoxidase-mediated degradation of the vinyl ether bond of the plasmalogen, 1-O-hexadec-1'-enyln-sn-glycero-3-phosphocholine.

FIG. 11. Proton NMR of the neutral lipid product generated from myeloperoxidase treatment of lysoplasmenylcholine. Lysoplasmenylcholine (200 nmol) was incubated in the presence of the MPO/HOCl generating system, which includes MPO (0.6 unit), \( \mathrm{H}_2\mathrm{O}_2 \) (1 mM), and NaCl (100 mM) in 300 \( \mu \)l of 20 mM phosphate buffer (pH 4.0) at 37 °C for 5 min. The incubations were terminated by the addition of methanol, and the reaction products were sequentially extracted into chloroform and subjected to TLC as described under “Experimental Procedures.” The TLC-purified material having an \( R_f = 0.5 \) was suspended in CDCl\(_3\) and subjected to proton NMR spectroscopic analyses as described under “Experimental Procedures.” The peak assignments are depicted in the inset.

DISCUSSION

Neutrophil activation results in the production of cytotoxic, bacteriocidal compounds that contribute to protection from invading foreign organisms such as bacteria (1, 2, 36). The combination of hydrogen peroxide production and the release of myeloperoxidase by the activated neutrophils results in the production of hypochlorous acid. Hypochlorous acid is the predominant cytotoxic agent produced by the activated neutrophils and is in equilibrium with the production of chlorine gas, which is favored under acidic conditions (10). Both cholesterol and the unsaturated aliphatic groups of phospholipids are targets of these reactive chlorinating species produced by activated neutrophils through the action of myeloperoxidase (13). In the present study, the plasmalogen vinyl ether bond has been shown to be a target of the reactive chlorinating species produced by myeloperoxidase resulting in the release of an \( \alpha \)-chlooro-fatty aldehyde from the aliphatic chain attached to the sn-1 position of the plasmalogen glycerol backbone (Scheme I).

Several lines of evidence confirmed that the \( \alpha \)-chloro-fatty aldehyde, 2-chlorohexadecanal, was produced from the attack on the vinyl ether bond of the plasmalogen 1-O-hexadec-1'-enyln-GPC by reactive chlorinating species. First, utilizing electrospray ionization mass spectrometry in the negative ion mode two molecular ions were observed at the predicted mass (m – 1) of 2-chlorohexadecanal. These ions at \( m/z \) 273 and \( m/z \) 275 were present at a 3:1 ratio, which is a signature for a monochlorinated compound because of the 3:1 natural abundance of \( ^{35}\text{Cl} \) and \( ^{37}\text{Cl} \), respectively. Additionally, mass-mass analysis of the \( m/z \) 273 ion demonstrated the presence of \( ^{35}\text{Cl} \) at
A similar 3:1 ratio was also observed when the underivatized product of myeloperoxidase treatment of lysoplasmenylcholine was subjected to GC-MS analysis utilizing electron impact ionization (m/z 274 and 276). Second, proton NMR analysis provided additional structural information including the presence of an aldehyde as well as a-chlorination. Finally, derivatization of the neutral lipid with pentafluorobenzyl hydroxylamine followed by GC-MS analysis with negative chemical ionization was consistent with the derivatization of 2-chlorohexadecanal to its pentafluorobenzyl oxime. Taken together, analyses by electrospray ionization mass spectrometry, proton NMR, and GC-MS have provided structural information to determine that 2-chlorohexadecanal is produced from myeloperoxidase-mediated degradation of the plasmalogen, 1-O-hexadec-1-enyl-GPC.

Myeloperoxidase-mediated degradation of plasmalogens also results in the production of lysophospholipids including lysophosphatidylcholine (Scheme I). The myeloperoxidase-mediated degradation of plasmalogens represents a unique mechanism to generate lysophospholipids that is independent of the action of phospholipases. The production of lysophospholipids with an acyl group at the sn-2 position likely would be short-lived either because of esterification at the sn-1 position utilizing acyl CoA or because of nonenzymic acyl migration of the sn-2 position aliphatic group to the sn-1 position followed by esterification at the sn-2 position utilizing acyl CoA or a fatty acid donated from a phospholipid mediated by the activation of a transacylase (37). The net effect of the acylation of monoacyl lysophospholipid produced from plasmalogen represents a biochemical mechanism for the conversion of plasmalogens to diacyl phospholipids. Alternatively, if appreciable amounts of lysophosphatidylcholine accumulate, it likely could have profound effects on the host cell because multiple physiological functions have been attributed to the production and accumulation of lysophosphatidylcholine in cells, including the activation of several protein kinases, the induction of arrhythmias in the heart, and the inhibition of membrane transport proteins (38–41).

Plasmalogens are considered to be antioxidants because the vinyl ether bond is susceptible to attack by oxidants but terminates rather than propagates oxidation (28, 29). In contrast, the present study suggests that plasmalogens might promote the cytotoxic potential of phagocytic cells through a mechanism that involves both the loss of plasma membrane plasmalogens and the production of bioactive lysophospholipids as well as...
potentially reactive α-chloro-fatty aldehydes (Scheme I). Whereas plasmalogens can terminate the cytotoxicity of reactive oxygen species, it is possible that they may target the cytotoxicity of the reactive chlorinating species, hypochlorous acid, to the plasma membrane leading to the production of α-chloro-fatty aldehydes that themselves may be cytotoxic. On the other hand, it is possible that plasmalogens may serve as protective agents to the host cell if they effectively quench hypochlorous acid and prevent it from interacting with other targets such as proteins and nucleic acids, which could potentially lead to greater damage to the host cell. Whether plasmalogen degradation in host cell plasma membranes via attack by reactive chlorinating species is ultimately deleterious or advantageous to the host cell remains to be resolved.

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