Plasmalogens are a phospholipid molecular subclass that are enriched in the plasma membrane of many mammalian cells. The present study demonstrates that reactive brominating species produced by myeloperoxidase, as well as activated neutrophils, attack the vinyl ether bond of plasmalogens. Reactive brominating species produced by myeloperoxidase target the vinyl ether bond of plasmalogens, resulting in the production of a neutral lipid and lysophosphatidylcholine. Gas chromatography-mass spectrometry and proton NMR analyses of this neutral lipid demonstrated that it was 2-bromohexadecanal (2-BrHDA). In comparison to myeloperoxidase-generated reactive chlorinating species, reactive brominating species attacked the plasmalogen vinyl ether bond at neutral pH. In the presence of a 20-fold molar excess of NaCl compared with NaBr, myeloperoxidase-derived reactive halogenating species favored the production of 2-BrHDA over that of 2-chlorohexadecanal. Additionally, 2-BrHDA was preferentially produced from plasmalogen treated with hypochlorous acid in the presence of NaBr. The potential physiological significance of this pathway was suggested by the demonstration that both 2-BrHDA and 2-bromooctadecanal were produced by PMA-stimulated neutrophils. Taken together, the present studies demonstrate the targeting of the vinyl ether bond of plasmalogens by the reactive brominating species produced by myeloperoxidase and by activated neutrophils, resulting in the production of novel brominated fatty aldehydes.

Activated phagocytes generate a variety of reactive halogenating species that damage adjacent cells as a part of the normal physiological defense function of phagocytes (1, 2). Activation of neutrophil NADPH oxidase during the respiratory burst results in the production of superoxide anions that disproportionate to produce hydrogen peroxide. Myeloperoxidase amplifies the oxidizing potential of hydrogen peroxide by using it as a substrate in the presence of physiological concentrations of halides to produce hypohalous acids and their conjugate bases, which are the predominant oxygen-derived free radicals produced by activated neutrophils (3). Two of the hypohalous acids produced by the myeloperoxidase system in the presence of chlorine and bromine are hypochlorous and hypobromous acids, respectively (4).

Lipids are major targets of the reactive halogenating species produced by activated phagocytes. Reactive chlorinating species released by activated neutrophils attack unsaturated C=CC=− bonds within the aliphatic chains of phospholipids and form chlorohydrins, which ultimately disrupt normal membrane fluid molecular dynamics (5–7). This mechanism may represent a major cytotoxic effect of activated neutrophils. Additionally, reactive chlorinating species produced by myeloperoxidase chlorinate lipoprotein-associated cholesterol, leading to the production of a family of chlorinated cholesterol species including chlorohydrins and dichlorinated products (8). Similarly, reactive brominating species have been shown to target unsaturated fatty acids, resulting in the production of bromohydrins that may serve as markers not only of phagocyte-mediated inflammation but also of specific inflammatory processes mediated by reactive brominating species (9).

Plasmalogens are a phospholipid molecular subclass found in the plasma membrane phospholipid pools of many mammalian tissues (10–14). 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 the solvation of both transmembrane ion channels and transport proteins (15, 16) and the storage of arachidonic acid (12–14, 17). The plasmalogen vinyl ether bond has been shown to be hydrolyzed by plasmalogenase activity (18, 19) and is also susceptible to chemical degradation in vivo. For example, the vinyl ether linkage of plasmalogens terminates the propagation of lipid-free radicals initiated by reactive oxygen species attack on aliphatic alkenes of phospholipids (20, 21). We have recently demonstrated that plasmalogens may represent accessible molecular targets of the membrane-permeable, reactive chlorinating species generated by activated phagocytes, resulting in the production of α-chloro fatty aldehydes (22).

The present study was designed to test whether the vinyl ether bond of the sn-1 aliphatic chain of plasmalogens is susceptible to bromination by reactive brominating species produced by myeloperoxidase and to compare this putative halogenation to that mediated by reactive chlorinating species. The results demonstrate for the first time that myeloperoxidase utilizes micromolar concentrations of bromine at neutral pH, resulting in the production of reactive brominating species that attack the vinyl ether bond of plasmalogens, leading to the production of lysophospholipids and α-bromo fatty aldehydes. Furthermore, these α-bromo fatty aldehydes are produced by...
Products were sequentially extracted into chloroform and subjected to TLC with either Solvent System 1 (petroleum ether/diethyl ether/acetic acid, 90:10:1, v/v/v) or Solvent System 2 (chloroform/acetone/methanol/acetic acid/water, 6:8:2:2:1, v/v/v/v) as mobile phase. Reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring.

FIG. 1. TLC analysis of myeloperoxidase-treated lysoplasmenylcholine. 300 nmol of lysoplasmenylcholine was incubated in the presence or absence of each of the myeloperoxidase (MPO)/HBr generating reagents, including MPO (1 unit), H2O2 (1 mM), and NaBr (100 mM), in 500 µl of 20 mM phosphate buffer, pH 4.0 or pH 7.0 as indicated, at 37 °C for 5 min. Incubations were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and subjected to TLC with either Solvent System 1 (petroleum ether/diethyl ether/acetic acid, 90:10:1, v/v/v) or Solvent System 2 (chloroform/acetone/methanol/acetic acid/water, 6:8:2:2:1, v/v/v/v) as mobile phase. Reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring.

FIG. 2. TLC analysis of myeloperoxidase-treated plasmenylcholine. 200 nmol of plasmenylcholine was incubated in the presence or absence of each of the MPO/HBr generating reagents including MPO (2.5 units), H2O2 (1 mM), and NaBr (100 mM) in 500 µl of 20 mM phosphate buffer, pH 4.0 or pH 7.0 as indicated, at 37 °C for 5 min. Incubations were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and subjected to TLC with either Solvent System 1 (petroleum ether/diethyl ether/acetic acid, 90:10:1, v/v/v) or Solvent System 2 (chloroform/acetone/methanol/acetic acid/water, 6:8:2:2:1, v/v/v/v) as mobile phase. Reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bromide-free hypobromous acid was prepared as previously described (23). The concentration of hypobromous acid was determined spectrophotometrically (ε331 = 315 M⁻¹ cm⁻¹) (24). Hypobromous acid was immediately used for experiments. Chloride-free sodium hypochlorite was prepared in chlorine demand-free and chloride-free glassware by a modification of a previously described method (8, 25). The concentration of sodium hypochlorite was determined spectrophotometrically at 350 nm with a molar absorptivity of 340 M⁻¹ cm⁻¹ (26). Myeloperoxidase was purchased from Calbiochem. Pentafluorobenzyl hydroxylamine was purified by HPLC utilizing a Dynamax Si column (21.4 x 250 mm, 8 µm) and isocratic elution with hexane at a flow rate of 6 ml/min. Utilizing this system, 2-Br-[d₄]-HDA eluted with an Rₜ = 25 min, and the purity was confirmed by TLC, as well as by GC-MS of its PFB oxime derivative (see below).

**Preparation of Lysoplasmenylcholine and Plasmenylcholine**—The lysoplasmenylcholine molecular species, 1-O-hexadec-1′-enyl-GPC, was prepared from bovine heart lecithin and purified as previously described (29). Plasmenylcholine was synthesized by an anhydrous reaction utilizing 1-O-hexadec-1′-enyl-GPC and hexadecanoyl chloride as precursors with dimethylaminopyridine as a catalyst and was purified as previously described (29). Synthetic plasmenylcholine was confirmed by TLC and capillary gas chromatography of the aliphatic constituents. Lysoplasmenylcholine and 16:0-16:0 plasmenylcholine were quantified by capillary gas chromatography.

**Plasmenylcholine Treatment with Myeloperoxidase-derived Reactive Halogenating Species: Analysis of Reaction Products by Thin Layer Chromatography and Capillary Gas Chromatography**—In a typical assay 200 or 300 nmol of either lysoplasmenylcholine or plasmenylcholine was incubated in 300 µl of phosphate buffer supplemented with 100 mM NaBr or selected concentrations of NaBr and/or NaCl, 20 mM NaPO₄, 0.1 mM diethylthiurammoniateacetic acid, pH 4–7, in the presence or absence of indicated amounts of myeloperoxidase, H2O2, bromine, or hypobromous acid for indicated intervals at 37 °C. Reactions were...
mination by the addition of methanol, and reaction products were extracted into chloroform by the method of Bligh and Dyer (30). Reaction products were separated by TLC utilizing silica gel 60 Å plates (Whatman) and a mobile phase comprised of petroleum ether/ethyl ether/acetic acid (90:10:1, v/v/v) for the separation of neutral lipids. Alternatively, polar lipid reaction products were separated by TLC utilizing silica gel 60 Å plates (Whatman) and a mobile phase comprised of chloroform/methanol/acetic acid/water (6:2:2:1, v/v/v/v). Reaction products on TLC plates containing an aldehyde or a masked aldehyde were visualized by charring concentrated sulfuric acid-treated plates. In some cases, reaction products or TLC-purified reaction products were extracted from silica by a modified Bligh and Dyer technique (30). TLC-purified reaction products were then either subjected to inorganic phosphate analysis (31) or capillary gas chromatography following their derivatization with pentafluorobenzyl hydroxylamine (see below) or methanolic HCl (70 °C for 30 min). In some cases, acid methanalysis-derivatized products were subjected to capillary gas chromatography utilizing a Supelco SP-2330 column and detected by FID under conditions previously described (32). Alternatively, acid methanalysis-derivatized products were subjected to GC-MS with electron impact ionization.

Gas Chromatography-Mass Spectrometric Analyses of Pentafluorobenzoxime 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 300 μl of ethanol followed by the addition of 300 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. Reaction products were diluted with 1.2 ml of water, extracted into cyclohexane/diethyl ether (4:1, v/v), and resuspended in 30–100 μl of petroleum ether prior to GC-MS analysis. Samples were analyzed on a DB-1 column (12.5 m, 0.2-mm inner diameter, 0.33-μm methyl silicone film coating, from 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 3.5 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 5988A 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.

Proton NMR Analyses—Proton NMR spectrometry and analyses were performed at ambient temperature in CDCl₃ using a Varian Unity 300 spectrometer (7.05T). 1H shifts were referenced to tetramethylsilane.

FIG. 3. Analysis of TLC-purified lysophosphatidylcholine produced by myeloperoxidase treatment of plasmalogen. 200 nmol of plasmalogen was incubated in the presence of the MPO/HOBr generating reagents, including MPO (2.5 units), H₂O₂ (1 mM), and NaBr (100 mg/ml) in 500 μl of 20 mM phosphate buffer, pH 4.0, at 37 °C for 5 min. Reaction products were analyzed by TLC utilizing this TLC system and was then subjected to sequential acid methanalysis and capillary gas chromatographic analysis, as described under “Experimental Procedures.” Peaks 1–3 are the solvent, the di-methyl acetal of hexadecanal, and the unknown derivatized reaction product, respectively.

FIG. 4. Capillary gas chromatographic analysis of myeloperoxidase-treated lysoplasmenylcholine. 200 nmol of lysoplasmenylcholine was incubated with (panels B and C) or without (panel A) 0.6 unit of myeloperoxidase in the HOBr generating buffer system at pH 4.0 and 37 °C for 5 min. Lipid reaction products were extracted and subjected to acid methanalysis, and derivatives were analyzed by capillary gas chromatography with FID detection. For panel C, the reaction product with an Rₜ = 0.58 on TLC in Solvent System 1 was first purified utilizing this TLC system and was then subjected to sequential acid methanalysis and capillary gas chromatographic analysis, as described under “Experimental Procedures.” Peaks 1–3 are the solvent, the di-methyl acetal of hexadecanal, and the unknown derivatized reaction product, respectively.
Neutrophil Activation—Whole blood (50 ml) was taken from healthy volunteers and anticoagulated with EDTA (final concentration 5.4 mM) prior to the isolation of neutrophils using a Ficoll-Hypaque gradient, as previously described (33). Pelleted neutrophils were resuspended in Hanks’ balanced salt solution (HBSS), pH 7.3, supplemented with either 100 μM, 500 μM, or 5 mM NaBr and immediately subjected to experimental protocols. Neutrophils (0.5 × 10⁶ cells/ml) were treated with or without (control) 200 nM PMA for 30 min at 37 °C. Reactions were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and subjected to TLC, as described under “Experimental Procedures.” The TLC-purified material having an Rf = 0.58 was dried and resuspended in petroleum ether prior to GC-MS with electron impact ionization detection, as described under “Experimental Procedures.” Ions and ion fragments (the mass spectrum) are shown of the major peak eluting at 6.55 min (see inset A for total ion current chromatogram).

FIG. 5. Gas chromatography-mass spectrometry of the lysoplasmenylcholine/myeloperoxidase reaction product. Lysoplasmenylcholine (200 nmol) was incubated in the presence of the MPO/HOBr generating system, which includes MPO (0.6 unit), H₂O₂ (1 mM), and NaBr (100 mM) in 300 μl of 20 mM phosphate buffer, pH 4.0, at 37 °C for 5 min. Incubations were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and subjected to TLC, as described under “Experimental Procedures.” The TLC-purified material having an Rf = 0.58 was dried and resuspended in petroleum ether prior to GC-MS with electron impact ionization detection, as described under “Experimental Procedures.” Ions and ion fragments (the mass spectrum) are shown of the major peak eluting at 6.55 min (see inset A for total ion current chromatogram).

FIG. 6. Gas chromatography-mass spectrometry of the pentafluorobenzyl oxime derivative of the lysoplasmenylcholine/myeloperoxidase reaction product. Lysoplasmenylcholine (200 nmol) was incubated in the presence of the MPO/HOBr generating system, which includes MPO (0.6 unit), H₂O₂ (1 mM), and NaBr (100 mM) in 300 μl of phosphate buffer, pH 4.0, at 37 °C for 5 min. Incubations were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and converted to their pentafluorobenzyl oxime derivatives, as described under “Experimental Procedures.” Derivatization products were subjected to capillary gas chromatography utilizing a DB-1 column and negative chemical ionization mass spectrometry for detection. The mass spectrum of peak 2 of the total ion current chromatogram (inset A) is shown. Inset A shows the total ion current chromatogram of the PFB product of the myeloperoxidase reaction. Peaks 1 and 2 represent the syn and anti isomers of the pentafluorobenzyl oxime derivative of the lysoplasmenylcholine/myeloperoxidase reaction product. Inset B illustrates the structure and putative fragmentation patterns of the pentafluorobenzyl oxime of 2-bromohexadecanal, the product of the lysoplasmenylcholine/myeloperoxidase reaction.
The vinyl ether bond of plasmalogens has recently been shown to be a target of the reactive chlorinating species produced by myeloperoxidase (22). The present studies were directed at determining whether the vinyl ether bond of plasmalogens is preferentially targeted by reactive brominating species, compared with reactive chlorinating species. As a first step, lysoplasmenylcholine (1-O-hexadec-1-ENyl-GPC) was incubated with myeloperoxidase in phosphate buffer containing hydrogen peroxide and sodium bromide. TLC analysis of the reaction products revealed the loss of lysoplasmenylcholine in the presence of myeloperoxidase, hydrogen peroxide, and sodium bromide at both pH 4 and pH 7 (Fig. 1, Solvent System 2). Concomitant with lysoplasmenylcholine loss, a neutral lipid was produced that migrated with an Rf of 0.20 (Fig. 1, Solvent System 2). This neutral lipid did not co-migrate with either 2-chlorohexadecanal (Rf 0.06) or multiple molecular species of fatty acid (Rf range for palmitic, palmitoleic, and arachidonic acid = 0.18–0.20) in this TLC system. The loss of lysoplasmenylcholine was dependent on the presence of a complete reaction mixture comprised of active myeloperoxidase (thermal treatment of myeloperoxidase-ablated lysoplasmenylcholine), hydrogen peroxide, and sodium bromide (Fig. 1).

Similar studies were performed utilizing the plasmaldehyde molecular species, 1-O-hexadec-1-ENyl-2-hexadecanoyl-GPC, as the target phospholipid of the reactive brominating species generated by myeloperoxidase. TLC analysis of the reaction products of this reaction demonstrated plasmalogen loss in the presence of the complete myeloperoxidase reactive brominating species system at both pH 4 and pH 7 (Fig. 2, Solvent System 2), concomitant with the production of a neutral lipid, which again migrated with an Rf of 0.58 (Fig. 2, Solvent System 1). Because the sulfuric acid-charring detection method employed in these TLC analyses detects aldehydes and masked aldehydes but does not readily detect lysophosphatidylethanolamine, further analyses were performed to determine that lysophosphatidylethanolamine was also produced by the action of reactive brominating species attacking plasmalogens containing an ester-linked sn-2 aliphatic group. Silica corresponding to the region to which lysophosphatidylethanolamine migrates on the TLC plates that were developed in Solvent System 2 (as well as the remaining silica on the plate) was zonally scraped and extracted (Fig. 3, inset A). The presence of lysophosphatidylethanolamine in the...
extract from this silica was confirmed by the presence of inorganic phosphate (Fig. 3) and a palmitic acid residue, as determined by the detection of palmitic acid methyl ester by capillary GC analysis following acid methanolysis (Fig. 3, inset B).

The neutral lipid reaction product from the myeloperoxidase-mediated degradation of plasmalogens was further characterized by capillary gas chromatography. Capillary gas chromatography of the acid methanolysis derivatives of the reaction products from lysylplasmenylcholine treatment with reactive brominating species generated by myeloperoxidase resulted in the production of a unique acid methanolysis derivative product with an Rf = 12.3 min (Fig. 4, panel B, peak 3). In contrast, the dimethyl acetal of hexadecanoyl (Rf = 5.6 min; Fig. 4, panel A, peak 2) was the only acid methanolysis derivative of untreated lysylplasmenylcholine (i.e., not treated with the myeloperoxidase-generated reactive brominating species). Furthermore, acid methanolysis and subsequent capillary gas chromatography of the TLC-purified neutral lipid (e.g., Rf = 0.58 in Fig. 1, Solvent System 1) demonstrated that this lipid gave rise to the acid methanolysis product with an Rf = 12.3 min (Fig. 4, panel C, peak 3). Further studies demonstrated that this product of the myeloperoxidase reaction was only produced from vinyl ether-containing lipids (i.e., plasmalogens) and was not produced from lysophosphatidylcholine or hexadecanoyl choline (data not shown). Both the neutral lipid produced from lysylplasmenylcholine treatment with the myeloperoxidase reactive brominating species generating system and the acid methanolysis product of the neutral lipid were subjected to GC-MS utilizing electron impact ionization. The ionization spectrum of the derivatized compound included the putative parent ions that would be expected from a-hexadecanoyl (2-BrHDA) (Fig. 5). These ions at m/z 318 and 320 are present at a 1:1 ratio characteristic of monobrominated molecules (due to the ratio of the natural isotopic abundance of 79Br and 81Br at 1:1). The ionization spectrum of the acid methanolysis product of the neutral lipid showed a predominant base ion at m/z 75 that is a signature ion of dimethylacetalts, which are acid methanolysis products of masked aldehydes (i.e., the vinyl ether bond of plasmalogens) and free aldehydes (data not shown).

Further structural information of the putative monobrominated hexadecanoyl species produced from plasmalogens by reactive brominating species was obtained by further GC-MS analyses as well as proton NMR analyses. Because the likely brominated reaction product of the myeloperoxidase reaction with plasmalogens contains an aldehyde carbonyl, the carbonyl-reactive derivatization agent, pentafluoro benzyl hydroxylamine, was used to produce the pentafluorobenzyl oxime of the aldehyde for GC-MS analyses utilizing negative chemical ionization. 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 lysylplasmalogens (1-O-hexadec-1’-enyl-sn-glycero-3-phosphocholine) with myeloperoxidase, H2O2, and NaBr resulted in the production of two peaks consistent with the production of the syn and anti isomers of the PFB oxime derivative of 2-BrHDA (Fig. 6, inset A). The fragmentation pattern of the second peak is shown in Fig. 6. The molecular ion (M+) of the PFB oxime of 2-BrHDA is observed at m/z 513 (Fig. 6). Again, monobromination of this compound was confirmed by identifying the isotopic cluster (M+–M–2 of 1/1 at m/z 513 and 515). One of the major fragments at m/z 332 is also monobrominated, because an ion of equal intensity was also observed at m/z 334. It should be recognized that the remaining fragmentation pattern is completely consistent with the structure of the PFB oxime of 2-BrHDA shown in Fig. 6, inset B. Additionally, NMR data further supported the most likely structure of the TLC-purified reaction product as 2-BrHDA. In particular, the aldehyde proton is both split by its sole neighboring proton on the α-carbon and shifted downfield because of the neighboring bromine (9.43 parts/million). Also, there is a downfield shift of the brominated methylene of the α-carbon that is split by three neighboring protons. Taken together, this NMR data in conjunction with the GC-MS analysis of both the nonderivatized and derivatized neutral lipid product support the neutral lipid product as 2-BrHDA.

The specificity of the degradation of plasmalogens by myeloperoxidase-generated reactive brominating species was further assessed in reactions that included the myeloperoxidase...
inhibitors sodium azide, catalase, and 3-aminotriazole. Sodium azide, catalase, and 3-aminotriazole inhibited myeloperoxidase-mediated degradation of the vinyl ether bond (Fig. 7). Additionally, the specificity of the reaction of the myeloperoxidase-generated reactive bromine 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, 2-BrHDA (Fig. 7).

Hypobromous acid is the initial product of the myeloperoxidase reaction in the presence of bromide, and molecular bromine can be produced from hypobromous acid (34). Accordingly, further experiments were performed that demonstrate that molar equivalents of either hypobromous acid or molecular bromine mimic the degradation of lysoplasmenylcholine by the reactive-brominating species generated by myeloperoxidase. Data shown in Fig. 8 demonstrate that at neutral pH hypobromous acid and molecular bromine both degrade plasmalogens, resulting in the production of 2-BrHDA.

Because data shown in Figs. 1 and 2 demonstrated that 2-BrHDA was produced under both acidic and neutral conditions, the pH dependence of this reaction was further characterized utilizing lysoplasmenylcholine as the vinyl ether substrate. The targeting of the vinyl ether linkage by the complete myeloperoxidase brominating system was optimal at pH 7 and was nearly half-maximal even at pH 8.4 (Fig. 9). This pH optimum at neutral pH is in sharp contrast to the myeloperoxidase chlorination system attack on the vinyl ether bond, which has a pH optimum at acidic pH of less than 5 (22). Additionally, substantial 2-BrHDA was produced by the myeloperoxidase/reactive brominating species generating system at neutral pH utilizing submillimolar concentrations of NaBr (Fig. 10). Further comparisons were made between chlorination and bromination of the plasmalogen vinyl ether bond by reactive halogenating species produced by myeloperoxidase. At pH 4.0, 2-BrHDA production by reactive brominating species produced by myeloperoxidase in the presence of 100 mM NaBr was not decreased by increasing amounts of NaCl (Fig. 11A). In fact, 2-chlorohexadecanal was only detected if 100 mM NaCl was present (Fig. 11A). In contrast, at pH 4.0, 2-chlorohexadecanal production by reactive chlorinating species produced by myeloperoxidase in the presence of 100 mM NaCl was attenuated by increasing amounts of NaBr (Fig. 11B). In the presence of 0.75 mM NaBr and 100 mM NaCl, more 2-BrHDA than 2-ClHDA was produced by myeloperoxidase-derived reactive
halogenating species (Fig. 11B). In the presence of 5 mM NaBr and 100 mM NaCl, 10-fold more 2-BrHDA was produced compared with 2-ClHDA (Fig. 11B). Further studies show that chlorination of the vinyl ether bond mediated by HOCl at pH 7.0 is modified in the presence of NaBr, resulting in the production of significantly more 2-BrHDA compared with 2-ClHDA (Fig. 12).

Next, 2-BrHDA and 2-bromooctadecanal (2-BrODA) production by intact neutrophils was determined. For these experiments, unstimulated (control) and PMA-stimulated human neutrophils were incubated in HBSS supplemented with 5 mM NaBr, and the production of 2-BrHDA and 2-BrODA was determined by GC-MS of their respective PFB oximes. Fig. 13 shows the chromatogram of the ion extraction of m/z 414, 442, and 418 for the PFB oximes of authentic 2-BrHDA, 2-BrODA, and 2-Br-[d₄]-HDA, respectively. Co-migration of the ion extraction of m/z 79 is also shown for each molecule to provide further evidence of the presence of bromine. Equivalent amounts of m/z 81 in respect to m/z 79 were also observed for each of these compounds (data not shown). Fig. 13, A and B, shows the chromatograms of the extracted ions of m/z 414, 418, and 442 for the PFB oximes of the brominated fatty aldehydes present in control and PMA-stimulated neutrophils. These

![Graph showing 2-BrHDA and 2-ClHDA production](image)

**FIG. 12.** Targeting of plasmalogens by reactive brominating species produced from hypochlorous acid and bromide. 200 nmol of lysoplasmenylcholine was incubated in the presence of either 0.5 or 1 mM hypochlorite ion in the presence of NaBr (1 mM) in 300 μl of phosphate buffer, pH 7, at 37 °C for 5 min. Lipid reaction products were extracted and subjected to acid methanolysis in the presence of 1-hexadecanoyl-GPC (internal standard), and derivatives were analyzed by capillary gas chromatography with FID detection, as described under "Experimental Procedures." Values are the means ± S.E. of at least three independent experiments.

**FIG. 13.** Negative ion chemical ionization GC-MS analysis of PFB oximes of 2-BrHDA and 2-BrODA derived from 2-BrHDA and 2-BrODA produced by PMA-stimulated neutrophils. 0.5 × 10⁶ neutrophils/ml were suspended in HBSS supplemented with 5 mM NaBr and were treated with either 200 nM PMA (B) or only vehicle (A) for 30 min at 37 °C. Reactions were terminated by the addition of methanol containing 200 pmol of 2-Br-[d₄]-HDA, and the reaction products were extracted into chloroform. Subsequently, 2-bromo fatty aldehydes were purified by TLC, as described under "Experimental Procedures." Following conversion to their PFB-oximes, GC-MS analysis using NICI for extracted ion m/z 414 (for 2-BrHDA, peak 2), m/z 418 (for 2-Br-[d₄]-HDA, peak 2d, internal standard), m/z 442 (for 2-BrODA, peak 4), and m/z 79 (for the presence of Br) was performed for the control and PMA-treated neutrophil samples. For these spectra, the relative intensities of m/z 414, 418, and 442 for the control group and the PMA-treated group are scaled against the one ion within each group that has the greatest intensity, which is set at 100%. Panel C shows the extracted ions m/z 414 and 79 for authentic PFB oxime of 2-BrHDA (peaks 1 and 2 for the syn and anti isomers), m/z 418 and 79 for authentic PFB oxime of 2-Br-[d₄]-HDA (peaks 1d and 2d for the syn and anti isomers), and m/z 442 and 79 for authentic PFB oxime of 2-BrODA (peaks 3 and 4 for the syn and anti isomers).
Supplemented with either 100 neutrophils stimulated with PMA and incubated with HBSS, neutrophils supplemented with either 100μM or 500μM NaBr. Additionally, 2-BrODA are produced in PMA-stimulated neutrophils incubated in HBSS supplemented with 5mM NaBr. These ions are observed at the predicted mass of 2-BrHDA, utilizing the neutral lipid with pentafluorobenzyl hydroxylamine followed by GC-MS analysis with negative chemical ionization was consistent with the derivatization of 2-BrHDA to its pentafluorobenzyl oxime. In particular, these analyses also demonstrated the presence of bromine as a fragment ion of the derivative. Last, proton NMR analysis provided additional structural information, including the presence of an aldehyde as well as α-bromination. Taken together, analyses by GC-MS and proton NMR have provided structural information determining that 2-BrHDA is produced from myeloperoxidase-mediated degradation of the plasmalogen, 1-0-hexadec-1'-enyl-GPC.

The attack of plasmalogens by reactive halogenating species produced by myeloperoxidase also results in the production of lysophospholipids such as lysophosphatidylcholine (Scheme I). The present studies demonstrate for the first time the production of lysophospholipids at neutral pH from intact phospholipids through a phospholipase-independent mechanism. Lysophospholipids with an acyl group at the sn-2 position likely would be converted to diacyl phospholipid by esterification at the sn-1 position utilizing acyl CoA. Alternatively, they would be converted to diacyl phospholipid following 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). Thus, the net effect of the acylation of monoacyl lysophospholipid produced from plasmalogen represents a biochemical mechanism for the conversion of plasmalogens to diacyl phospholipids. It is also possible that appreciable amounts of lysophosphatidylcholine could accumulate, which could have profound effects on the host cell through the activation of several protein kinases and the inhibition of membrane transport proteins (38–41).

In contrast to chlorination of the plasmalogen vinyl ether bond, bromination occurs at neutral pH. Additionally, at submillimolar bromide concentrations, the myeloperoxidase reactive brominating species generating system has the capacity to generate reactive species directed toward the vinyl ether bond. It should be appreciated that normal plasma concentrations of bromide have been reported to range from 20–150 μM (42). Furthermore, bromide is a long lasting metabolite of halothane (t1/2 12–25 days), and plasma bromide levels rise to 5 mM during and following halothane anesthesia (43). Thus, the present results demonstrate that the production of α-bromo fatty aldehydes by the myeloperoxidase/reactive brominating species generating system, as well as by PMA-stimulated neutrophils, occurs at physiologically relevant bromide concentrations.
Plasmalogen Attack by Reactive Brominating Species

Similar to the findings of Heinecke and co-workers (44), the present results demonstrate that bromide in the presence of hypochlorous acid leads to the production of brominated products. This selectivity for bromination in the presence of hypochlorous acid was further exemplified by the preferential production of 2-BrHDA over 2-CIHDA in the presence of a 100-fold molar excess of chloride over bromide. This preference for bromide has been reported for nucleoside base bromination and, in fact, in the presence of chloride and bromide, heterodihalogenated products have been observed (44). However, heterohalogenated species were not observed in the present study because this is a monohalogenated molecule.

The present studies suggest 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 and potentially reactive α-bromo fatty aldehydes (Scheme I). It is likely that α-bromo fatty aldehydes form Schiff bases with primary amines and thus potentially modify protein structure and function. The metabolic fate of reactive α-bromo fatty aldehydes remains to be resolved. However, it is possible that they may be oxidized to fatty acids by fatty aldehyde dehydrogenase (45). Although others have demonstrated that plasmalogens can terminate the cytotoxicity of reactive oxygen species (20, 21), it is possible that they may target the cytotoxicity of reactive halogenating species to the plasma membrane, leading to the production of α-bromo fatty aldehydes and 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 hypohalous acids and prevent them from interacting with other targets such as proteins and nucleic acids that could potentially lead to greater long-term damage to the host cell. The role of targeting of the plasmalogens by reactive halogenating species as a protective or deleterious mechanism remains to be resolved as the extent of damage elicited by the products, lysophospholipids and α-halo fatty aldehydes, is examined.

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