Abstract The myeloperoxidase-derived metabolite hypochlorous acid (HOCl) promotes the selective cleavage of plasmalogens into chloro fatty aldehydes and 1-lysophosphatidylcholine (LPC). The subsequent conversion of the initially generated LPC was investigated in plasmalogen samples in dependence on the fatty acid residue in the sn-2 position by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry and $^{31}$P NMR spectroscopy. Plasmalogens containing an oleic acid residue in the sn-2 position are converted by moderate amounts of HOCl primarily to 1-lyso-2-oleyl-sn-glycero-3-phosphocholine and at increased HOCl concentrations to the corresponding chlorohydrin species. In contrast, plasmalogens containing highly unsaturated docosahexaenoic acid yield upon HOCl treatment 1-lyso-2-docosahexaenoyl-glycerophosphocholine and glycerophosphocholine.$^{31}$ The formation of the latter product denotes a novel pathway for the action of HOCl on plasmalogens.—Leßig, J., J. Schiller, J. Arnhold, and B. Fuchs. Hypochlorous acid-mediated generation of glycerophosphocholine from unsaturated plasmalogen glycerophosphocholine lipids. J. Lipid Res. 2007. 48: 1316–1324.

Supplementary key words $^{31}$P nuclear magnetic resonance • matrix-assisted laser desorption and ionization time-of-flight mass spectrometry • lysophosphatidylcholine

Olefinic residues of fatty acyl residues of phospholipids are an important target for oxidative damage induced by reactive oxygen species (ROS) known to occur under various pathological conditions, including chronic inflammation, atherosclerosis, aging, and cancer. Some mammalian tissues and cells, such as erythrocytes, kidney, lung, testes, skeletal muscle, brain, heart, and particularly selected animal spermatozoa (e.g., from bull), contain considerable levels of plasmalogen glycerophosphocholine lipids (1). Plasmalogens also represent an important target for ROS (2) and are often considered antioxidants. Although the 1-alkenyl residues in plasmalogens (mainly plasmalogen glycerophosphocholine and glycerophosphoethanolamine) of mammalian cells are usually derived from saturated long-chain fatty aldehydes, the acyl residues are highly unsaturated (2). For instance, in spermatozoa from boar or bull, docosahexaenoic and docosapentaenoic acid residues do nearly occur exclusively (3). Accordingly, the oxidative attack of ROS may affect the vinyl-ether function as well as the olefinic residue in the fatty acid moiety (4). The presence of a vinyl-ether bond makes plasmalogens more susceptible to oxidative damages compared with their 1-acyl analogs (2). This has prompted the hypothesis that plasmalogens may act as ROS scavengers, protecting other phospholipids and lipoprotein particles from oxidative damage (2). Thus, plasmalogens seem to have an antioxidative effect toward many ROS (5).

Hypochlorous acid (HOCl) is generated by polymorphonuclear leukocytes under the catalysis of the enzyme myeloperoxidase (MPO) (6). HOCl reacts with a variety of molecules, including amino acids, proteins, carbohydrates, nucleic acids, and lipids (7–12). In the latter case, chlorohydrins are generated as the primary products when HOCl reacts with the double bonds in unsaturated phosphatidylcholines (12–14). HOCl and the MPO-H$_2$O$_2$-Cl$^-$ system also induce the formation of 2-lysophosphatidylcholines (LPCs) in polyunsaturated phosphatidylcholines, as shown by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) and $^{31}$P NMR spectroscopy (15, 16).

The vinyl-ether bond of plasmalogens is also assumed to represent the preferred target for reactive chlorinating

Abbreviations: 2-D-1-LPC, 1-lyso-2-docosahexaenoyl-sn-glycero-3-phosphocholine; GPC, glycerol-1-phosphocholine; LPC, lysophosphatidylcholine; HOCI, hypochlorous acid; LPc, lysophosphatidylcholine; 1-M-2-LPC, 1-myristoyl-2-lyso-sn-glycero-3-phosphocholine; 2-O-1-LPC, 1-lyso-2-oleoyl-sn-glycero-3-phosphocholine; OAPC$_{plasm}$, 1-O-1’-(Z)octadecenyl-2-archidonyl-sn-glycero-3-phosphocholine; ODPC$_{plasm}$, 1-O-1’-(Z)octadecenyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine; 2-OMPC$_{plasm}$, 1-O-1’-(Z)octadecenyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine; MALDI-TOF MS, matrix-assisted laser desorption and ionization time-of-flight mass spectrometry; MPO, myeloperoxidase; PNA, p-nitroaniline; ROS, reactive oxygen species.

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species. It has been shown that the MPO-H$_2$O$_2$-Cl$^-$ system promotes selective oxidative cleavage of the vinyl-ether bond of plasmalogens, liberating α-chloro fatty aldehydes and unsaturated 1-LPC (17, 18). α-Chloro fatty aldehydes at physiologically relevant concentrations induce neutrophil chemotaxis in vitro, suggesting that these compounds possess a role in neutrophil recruitment and signaling function (19). On the other hand, enhanced levels of LPC are known to be cytotoxic. Enhanced LPC formation induces membrane rupture, nuclear expansion, cell lysis, a sustained increase in intracellular Ca$^{2+}$ levels, and the production of ROS with the consequence of cytotoxic injuries and necrosis (20). Because plasmenylcholine is preferentially oxidized in contrast to unsaturated phosphatidylycerol (21), the supposed protective action of plasmalogens as ROS scavengers could be in competition with the damaging effects of the oxidation products. An accumulation of α-chloro fatty aldehydes, including 2-chlorohexadecanal, has been found in activated neutrophils and monocytes as well as in infarcted myocardium and human atheromas (22). The selective oxidative cleavage of plasmalogens in human atherosclerotic lesions, leading to a novel population of unsaturated 1-LPCs, independent of the action of phospholipase A$_2$ (23), possesses proatherogenic properties (18, 24).

Here, we investigated the formation of HOCl-induced degradation products from unsaturated plasmalogen glycerophosphocholine lipids by MALDI-TOF MS and high-resolution $^{31}$P NMR spectroscopy. Effects on mono-unsaturated plasmalogen glycerophosphocholine and plasmalogen glycerophosphocholine esterified with docosahexaenoic acid at the sn-2 position of the glycerol backbone were compared. A novel effect of HOCl, the formation of glycerol-1-phosphocholine (GPC), was established.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals for NMR spectroscopy (sodium cholate, EDTA, and deuterated water with an isotopic purity of 99.6%), buffer preparation (NaH$_2$PO$_4$·H$_2$O, Na$_2$HPO$_4$·2H$_2$O, and Tris), and matrix preparation [p-nitroaniline (PNA)] as well as all solvents (chloroform and methanol) and taurine were obtained in the highest commercially available purity from Fluka Feinchemikalien GmbH (Taufkirchen, Germany). 1-O-1¶-Octadecenyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (ODPC$_{plasm}$) and 1-O-1¶-Octadeccenyl-2-oleoyl-sn-glycero-3-phosphocholine (OOPC$_{plasm}$) as well as the internal standards 1-myristoyl-2-lyso-sn-glycero-3-phosphocholine (1-M-2-LPC), POPC, and 1,2-dipalmitoyl-sn-glycero-3-phosphate were purchased from Avanti Polar Lipids (Alabaster, AL) as CHCl$_3$ solutions and used without further purification.

**Fig. 1.** Positive ion matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) mass spectra of the organic extracts of plasmalogen glycerophosphocholines after incubation with buffer (A) and 5 mM hypochlorous acid (HOCl) (B) for 1 h at room temperature. The HOCl incubation of 1-O-1¶-(Z)octadecenyl-2-oleoyl-sn-glycero-3-phosphocholine (OOPC$_{plasm}$) is shown at left and that of 1-O-1¶-(Z)octadecenyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (ODPC$_{plasm}$) is shown at right. The plasmalogen concentration was 2 mM. The slight intensity differences between the H$^+$ and Na$^+$ adducts in the starting material and product spectra stem from the additional salt in the incubation buffer. Spectra were scaled according to the intensity of 1-myristoyl-2-lyso-sn-glycero-3-phosphocholine (1-M-2-LPC) standard, and the m/z values as well as peak assignments are indicated: 2-D-1-LPC, 1-lyso-2-docosahexaenoyl-sn-glycero-3-phosphocholine; 2-O-1-LPC, 1-lyso-2-oleoyl-sn-glycero-3-phosphocholine; 2-D-1-LPC, 1-lyso-2-oleoyl-sn-glycero-3-phosphocholine.
HOCl incubation and lipid extraction

An aliquot of the corresponding plasmalogen glycerophosphocholine lipid dissolved in chloroform was evaporated to dryness. Multilamellar liposomes were prepared by dissolving the phospholipid film in 10 mM phosphate buffer (pH 7.4) and vortexing vigorously for 30 s. A stock solution of NaOCl was kept in the dark at 4°C. Its concentration was determined at pH 12 using $e_{290} = 350 \text{ M}^{-1} \text{ cm}^{-1}$ for $\text{HOCl}$ (25). NaOCl was diluted with 10 mM phosphate buffer (pH 7.4) immediately before use. Liposomes (2 mM phospholipid) were incubated with varying concentrations of sodium hypochlorite for 1 h at pH 7.4. Adding an excess of taurine stopped the incubation. To extract the lipids, the same volume of a chloroform-methanol mixture (1:1, v/v) was added (26). Both the organic and the aqueous layers were used for further analysis.

MALDI-TOF MS measurements

Positive ion MALDI-TOF mass spectra were acquired on a Bruker Daltonics Autoflex workstation (Bremen, Germany). The system uses a pulsed nitrogen laser emitting at 337 nm. The extraction voltage was 20 kV, and 200 single laser shots were averaged for each mass spectrum. To enhance the spectral resolution, spectra were recorded in the reflector mode under “delayed extraction” conditions (27, 28). Internal standards were added for quantitative analysis (27–29). POPC and 1-M-2-LPC were added to the chloroform phase before MALDI-TOF MS. Furthermore, GPC amounts were estimated by comparison with the intensities of selected matrix peaks (28, 30). Organic or aqueous phases of the reaction extracts were applied to the MALDI target using PNA as the matrix (31). For the investigation of the organic and aqueous phases, the “low-mass gate” (matrix suppression) was set to m/z 315 and 150, respectively. All spectra were processed using the software Flex analysis version 2.2 provided by Bruker.

$^{31}$P NMR spectroscopic measurements

The dried organic and aqueous layers were redissolved as described previously (32, 33) in 50 mM Tris (pH 7.65) containing 200 mM sodium cholate and 5 mM EDTA. As a concentration standard, 1,2-dipalmitoyl-sn-glycerol-3-phosphate was added. After intense vortexing of the 0.5 ml samples, $^{31}$P NMR spectra were recorded in 5 mm NMR tubes on a Bruker DRX-600 spectrometer operating at 242.88 MHz for $^{31}$P. All measurements were performed using a direct “broad-band” NMR probe at 37°C and Waltz-16 composite pulse decoupling to eliminate $^{31}$P-$^1$H coupling. Pulse intervals of the order of $T_1$ permit the quantitative analysis of phosphorus resonance integral intensities (34).

Other NMR parameters were as follows: experiment time, 2–8 h; data size, 8 k; $60^\circ$ pulse (7 ms), pulse delay of 2 s; and line broadening of 1 Hz. Chemical shifts were referenced to the most intense resonance of OOPCplasm at $\delta = -0.60$ ppm and $m/z$ 229.0.

Fig. 2. Positive ion MALDI-TOF mass spectra of the aqueous extracts of plasmalogen glycerophosphocholines incubated with 10 mM HOCl at 1 h room temperature. A: Laser desorption spectrum of the pure p-nitroaniline (PNA) matrix. B, C: Spectra of OOPCplasm (B) and ODPCplasm (C). Spectra were scaled according to the most intense matrix peak at m/z 229, and the m/z values as well as peak assignments are indicated. The same experimental parameters were used in all cases. GPC, glycerol-1-phosphocholine.

Fig. 3. Changes in the concentrations of ODPCplasm (open circles), OOPCplasm (closed circles), 2D-1-LPC (open triangles), and 2-O-1-LPC (closed triangles) (A) as well as GPC derived from ODPCplasm (open squares) and OOPCplasm (closed squares) (B) as a function of HOCl concentration. Plasmalogens and 1-lyso-2-fatty acyl glycerophosphocholine lipids were determined from the peak intensities in the MALDI-TOF mass spectra with reference to the intensities of the peaks of POPC and 1-M-2-LPC used as standards (A). The sum of all individual adducts (H$^+$ and Na$^+$) was used as the intensity measure. In the case of GPC, the intensity ratio of the GPC peak at m/z 280.1 and the PNA matrix peak at 229.0 is indicated (B). Error bars represent SD of three independent measurements. The curved lines were not derived from a mathematical model but only represent spline curves to guide the eye.
ODPc_plasm at δ = −0.62 ppm. All peak assignments were confirmed by comparison with the shift of commercially available reference compounds. Spectra were processed using the software 1D WIN-NMR version 6.2® (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) including the deconvolution (II) routine for peak area determination.

RESULTS

Two selected plasmalogen glycerophosphocholines (ODPc_plasm and OOPc_plasm) were treated separately with increasing concentrations of HOCl. Plasmalogen species with oleyl and docosahexaenoyl residues in the sn-2 position were chosen because significant differences in the yield of 2-LPC are known to occur when differently saturated diacyl-glycerophosphocholine lipids react with HOCl (15, 16). Selected examples of positive ion MALDI-TOF mass spectra of the organic phase of chloroform-methanol extracts of the reaction mixture are shown in Fig. 1. The top spectra (Fig. 1A) represent the starting material (in the absence of HOCl), and the bottom spectra (Fig. 1B) correspond to the organic extracts of plasmalogen after reaction with 5 mM HOCl. The plasmalogen concentration was 2 mM. Untreated OOPc_plasm (left traces) gives two major peaks at m/z 772.6 and 794.6, corresponding to the neutral molecule cationized by H⁺ and Na⁺, respectively. These peaks disappear nearly completely upon treatment with a 2.5 molar excess of HOCl. New peaks arise in the low mass range at m/z 522.3 and 544.3, corresponding to the H⁺ and Na⁺ adducts of 1-lyso-2-oleoyl-sn-glycero-3-phosphocholine (2-O-1-LPC). The mass spectra of ODPC_plasm (right traces) change in a similar way by treatment with HOCl. The peaks at m/z 818.6 and 840.6, corresponding to the H⁺ and Na⁺ adducts of the starting material, disappear completely upon treatment with HOCl, and at m/z 568.3 and 590.3, peaks of 1-lyso-2-docosahexanoyl-sn-glycero-3-phosphocholine (2-D-1-LPC) appear concomitantly. The spectra in Fig. 1 also contain additional peaks at m/z 468.3 and 490.3, corresponding to the proton and sodium adducts of 1-M-2-LPC, and at m/z 760.6 and 782.6, corresponding to POPC. Both substances were added to the organic plasmalogen extracts as internal standards to allow a reliable quantitative evaluation of the reaction products.

Fig. 4. Positive ion MALDI-TOF mass spectra of the chloroform phases of the organic extracts of plasmalogen glycerophosphocholines incubated with 2.5 mM HOCl (A), 5 mM HOCl (B), or 10 mM HOCl (C) for 1 h at room temperature. The plasmalogen concentration was 2 mM in all cases. Spectra at left correspond to OOPc_plasm, and spectra at right correspond to ODPC_plasm. Spectra were scaled according to the intensity of the 1-M-2-LPC standard, and the m/z values and peak assignments are indicated.
The aqueous extracts of both plasmalogens incubated with HOCl were also analyzed by MALDI-TOF MS (Fig. 2). New peaks appeared at $m/z$ 258.1 and 280.1 in the HOCl-treated (10 mM) OOPCplasm (Fig. 2B) and ODPCplasm (Fig. 2C) samples, in contrast to the untreated controls (data not shown). These peaks correspond to the proton and sodium adducts of GPC. It is evident that the yield of GPC upon HOCl incubation of ODPCplasm (Fig. 2C) exceeds that of OOPCplasm (Fig. 2B). All other peaks in Fig. 2 are caused by the PNA matrix, as indicated by the spectrum of the pure PNA matrix (Fig. 2A). Of course, these data are not absolutely reliable, because matrix peak intensities are influenced by many other parameters. However, these data were confirmed by another independent method ($^{31}$P NMR; see below).

To evaluate the relative contributions of 1-lyso-2-fatty acyl glycerophosphocholine lipids and GPC formed as a result of the incubation of HOCl with plasmalogens in more detail, products were analyzed in relation to the concentrations of HOCl in the incubation mixture (Fig. 3). Peaks of interest were referenced to the intensities of the internal standards [i.e., the sum of the intensities of the proton and sodium adducts of 1-myristoyl-2-lyso-sn-glycero-phosphocholine ($m/z$ 468.3 and 490.3) for 1-lyso-2-fatty acyl glycerophosphocholines and the sum of the intensities of the proton and sodium adducts of 1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine ($m/z$ 760.6 and 782.6) for the plasmalogen glycerophosphocholines]. The most pronounced PNA matrix peak at $m/z$ 229 served as a reference for the formation of GPC (Fig. 3B). Although not yet comprehensively established for PNA, this approach offers different advantages, particularly the fact that there is no need to add a reference compound.

Peaks of both plasmalogens decreased continuously with increasing HOCl concentrations. No plasmalogens were detected at HOCl concentrations $> 5 \times 10^{-3}$ mol/l. There were no significant reactivity differences between ODPCplasm and OOPCplasm. 1-Lyso-2-fatty acyl glycerophospholipid lipids (2-D-1-LPC from ODPCplasm and 2-O-1-LPC from OOPCplasm) were clearly detected at $2 \times 10^{-3}$ mol/l and greater HOCl concentrations. The highest yields of 2-D-1-LPC and 2-O-1-LPC were detected at $2.5 \times 10^{-3}$ mol/l and $5 \times 10^{-3}$ mol/l HOCl, respectively. Using still higher HOCl concentrations, the yields of both LPCs decreased continuously.

The formation of GPC was observed only at $5 \times 10^{-3}$ mol/l and greater HOCl concentrations. A pronounced formation of GPC occurred only in the case of

**Fig. 5.** $^{31}$P NMR spectra (242.88 MHz) of the combined organic and aqueous extracts of plasmalogen glycerophosphocholine after incubation with buffer (A) or 1 mM (B), 5 mM (C), 7.5 mM (D), or 10 mM (E) HOCl for 1 h at room temperature. The plasmalogen concentration was 2 mM in all cases. OOPCplasm spectra are shown at left, and ODPCplasm spectra are shown at right. Spectra were scaled according to the 1,2-dipalmitoyl-sn-glycero-3-phosphate standard resonance (peak outside the shown range at 3.15 ppm), and the peak assignment and chemical shifts are indicated. The signal-to-noise ratio differs slightly between the individual spectra because not exactly the same number of scans was accumulated in all spectra.
ODPC\textsubscript{plasm} but not in the OOPC\textsubscript{plasm} samples. This is not surprising, because it was already shown that phosphatidylcholine lipids with only one double bond under the influence of HOCl give much smaller yields of the 2-lysoacyl glycerophosphocholine lipids compared with higher unsaturated species (15, 16). One additional product was detected in the plasmalogen mass spectra at HOCl concentrations $> 5 \times 10^{-3}$ mol/l. This product corresponds to the monochlorohydrin derived from 2-O-1-LPC with peaks at m/z 574.3 and 596.3 for the proton and sodium adducts, respectively (Fig. 4, left traces). Using ODPC\textsubscript{plasm} only traces of the corresponding chlorohydrin species were detected at m/z 620.3 for the proton adduct (Fig. 4, right traces).

Besides the plasmalogens discussed to this point, a third plasmalogen [1-O-1-(Z)octadecenyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OAPC\textsubscript{plasm})] is still commercially available and was also used. The same incubation and quantification procedures were used to evaluate the relative contributions of 1-LPC and GPC formed from OAPC\textsubscript{plasm} (data not shown). OAPC\textsubscript{plasm} incubations with HOCl gave complementary results. No plasmalogens were detected at HOCl concentrations $> 5 \times 10^{-3}$ mol/l. LPC lipids were also clearly detected at $10^{-3}$ mol/l and greater HOCl concentrations. The formation of GPC from OAPC\textsubscript{plasm} started at $5 \times 10^{-3}$ mol/l HOCl. At $10^{-2}$ mol/l HOCl, the relative GPC moiety derived from OAPC\textsubscript{plasm} was 1.01, corresponding to an average value of OOPC\textsubscript{plasm} and ODPC\textsubscript{plasm} (Fig. 3B).

Reaction products of plasmalogens subsequent to the reaction with HOCl were additionally analyzed by $^{31}$P NMR spectroscopy. For this purpose, the aqueous and CHCl\textsubscript{3} phases were evaporated to dryness, combined, and redissolved in aqueous sodium cholate. This was done to have a suitable reference to the mass spectra for when the aqueous and CHCl\textsubscript{3} layers were investigated separately. The $^{31}$P NMR spectra of OOPC\textsubscript{plasm} (left traces) and ODPC\textsubscript{plasm} (right traces) after incubation with different concentrations of HOCl are shown in Fig. 5. In Fig. 5A, the $^{31}$P NMR spectra of both plasmalogen glycerophosphocholine lipids incubated with pure buffer in the absence of HOCl are shown as reference. The only peak appearing in both spectra represents the resonance of the plasmalogens (~0.60 and −0.62 ppm) and proves the absence of even small amounts LPC and GPC.

NMR spectra of OOPC\textsubscript{plasm} (left traces) and ODPC\textsubscript{plasm} (right traces) after treatment with HOCl are shown in Fig. 5B–E). The intensities of the plasmalogen resonances decrease when increasing amounts of HOCl are used. Additional peaks corresponding to LPCs (2-O-1-LPC and 2-D-1-LPC) are detected at ~0.16 and ~0.18 ppm, respectively, in the presence of 1 mM HOCl (Fig. 5B). A further increase of the LPC resonance intensities was observed at 5 mM HOCl (Fig. 5C). Finally, at still higher HOCl concentrations (7.5 and 10 mM), the intensity of the LPCs decreases (Fig. 5D, E). One should note that a partial migration of the fatty acid residue from the sn-2 to the sn-1 position takes place in mixed detergent-phospholipid micelles (35). Thus, a second resonance of the correspond-
sn-glycero-3-phosphate as an internal standard (31P NMR chemical shift = 3.15 ppm; not shown in Fig. 5). With increasing HOCl concentrations, the concentrations of both plasmalogens decrease (Fig. 6A). The concentrations of 2-O-1-LPC and 2-D-1-LPC exhibit maxima at ~5 × 10^{-3} M HOCl, with a slightly higher value in the case of 2-O-1-LPC. A more pronounced formation of GPC from ODPC_plasm compared with OOPC_plasm is obvious at higher HOCl concentrations (Fig. 6B). Thus, NMR data indicate a comparable behavior to that shown by mass spectrometry.

**DISCUSSION**

The formation of α-chloro fatty aldehydes from plasmalogens subsequent to treatment with HOCl is well documented (13, 14). Here, we focused our attention on the fate of the other products of this reaction: on LPCs bearing an acyl chain in the sn-2 position. In Fig. 7, the proposed two-stage pathway of the reaction of HOCl with plasmalogens is shown. Our data clearly indicate that the vinyl-ether bond in both plasmalogen samples is the preferred target for HOCl. We were able to detect the formation of 2-O-1-LPC from OOPC_plasm and 1-lyso-2-arachidonoyl-sn-glycero-3-phosphocholine from OAPC_plasm as well as of 2-D-1-LPC from ODPC_plasm at relatively low (10^{-3} M) HOCl concentrations by MALDI-TOF MS and 31P NMR spectroscopy. Secondary reaction products resulting from both LPCs were detected only at 5 mM HOCl and higher concentrations. In the case of 2-O-1-LPC, the formation of the corresponding chlorohydrin derivative dominates, whereas GPC is the preferred product from 2-D-1-LPC. This is in accordance with our previous observation that HOCl cleaves unsaturated fatty acyl chains from phosphatidylcholines. The yield of LPC increases considerably with the degree of unsaturation of the fatty acyl residues in the PC (15, 16). High amounts of LPCs were only formed from phospholipids containing arachidonoyl or docosahexaenoyl residues, and a possible mechanism to explain these findings has been proposed (15).

According to our results, GPC is formed from unsaturated plasmalogens (especially from higher unsaturated ones, such as OAPC_plasm and ODPC_plasm) by sequential cleavage of the sn-1 and sn-2 residues. Weak chlorohydrin formation could be observed particularly in the OOPC_plasm sample, but the generation of LPC lipids and glycrophosphocholine was considerably higher. Furthermore, we cannot exclude the existence of additional pathways. For instance, Thompson et al. (36) found upon photooxidation of plasmalogens a cleavage of the alkenyl-ether in the sn-1 position upon the formation of the lyso-and formyl-glycerophosphocholine. In contrast, oxidation along the fatty acid residue in the sn-2 position was also shown to occur under the influence of free radicals (37, 38). Such products are not observed in the presence of HOCl or are beyond our detection limits.

The sensitivity of the plasmalogen vinyl-ether bonds emphasizes their impact as physiological prime targets for HOCl and subsequent 1-lyso-2-fatty acyl glycerophosphocholine and GPC formation. In the majority of plasmalogens, the sn-1 position consists mainly of hexadecenyl or octadecenyl residues, whereas the sn-2 position is es-

**Fig. 7.** Proposed two-stage process of the cleavage of the sn-1 plasmalogen glycerophosphocholine accompanied by the generation of 1-lyso-2-fatty acyl glycerophosphocholine and α-chloro fatty aldehydes. In the second step, a cleavage of the polyunsaturated fatty acid at the sn-2 position upon the formation of GPC, or alternatively upon the formation of chlorohydrins, takes place. R_1, octadecenyl residue; R_2, unsaturated acyl residue.
terified with polyunsaturated fatty acids and the head
group is usually either ethanolamine or choline (1, 2). Diff-
erent tissues and cell types contain significant amounts of
plasmalogens. Brain possesses the highest content of
plasmalogen glycerophosphoethanolamine, followed by
spermatozoa, whereas heart has a higher content of
plasmalogen glycerophosphocholine (3, 39). As glycer-
ophosphoethanolamine contains an additional amino group
that reacts readily with HOCl, plasmalogen glycerophos-
phocholine was used exclusively in this study to investi-
gate the influence of the alkenyl ether at sn-1 and the fatty
acid composition in the sn-2 position.

The reactive chlorinating species HOCl is known to be
produced from H₂O₂ and Cl⁻ in the presence of MPO,
an enzyme released by activated neutrophils and macro-
phages (6). Released MPO mediates, in part, the destruc-
tion of foreign organisms by producing toxic chlorinating
species (40). In contrast, HOCl also targets host tissues
and participates in cardiovascular pathophysiology (e.g.,
atherosclerosis) (18, 19) and other inflammatory diseases
(e.g., rheumatoid arthritis) (41). Plasmalogens have been
proposed not only as antioxidants but also as mediators of
membrane dynamics and the storage of polyunsaturated
fatty acids and lipid mediators (1, 2). Besides their involve-
ment in plasmalogen-deficiency disorders, plasmalogens are
also implicated in many diseases, including aging,
Alzheimer’s disease, heart diseases, and/or myocardial in-
farction (1, 2). Recently, HOCl has been shown to target
the vinyl-ether bond of plasmalogen glycerophospho-
choline, resulting in α-chloro fatty aldehydes and unsatu-
rated 1-LPCs (17–19). LPC formation is known to be
cytotoxic; thus, the proposed protective role of plasmalo-
gens against oxidative damage potentially could be in
competition with the damaging effects of the released LPC
lipids (20). Therefore, following the fate of the 1-LPCs
bearing an acyl chain at the sn-2 position is essential in
understanding the putative role of plasmalogen glycer-
ophospholipids as scavenger molecules.

The formation of glycerophosphocholine from plasmalo-
gens under the influence of HOCl denotes a novel reac-
tion pathway. Remarkably, GPC is generated in the absence
of any phospholipase activities (i.e., exclusively under the in-
fluence of HOCl). Although GPC is unequivocally an im-
portant metabolite, there is yet no established correlation
between increased GPC levels and different processes such
as inflammation and ROS-mediated pathologies.

This study provides clear evidence that GPC formation
occurs when ODPCplasm is incubated with a slight excess of
HOCl (e.g., 5 mM HOCl). This is close to physiologically
relevant HOCl concentrations, although the in vivo sta-
tionary HOCl concentration is always near zero, because
many potential targets are available. HOCl is produced con-
tinuously at inflammatory sites by MPO, and calculations
revealed that 2–4 × 10⁹ leukocytes produce 100–140 µmol
of HOCl during 1 h (42). Furthermore, the same reac-
tion would take place at reduced HOCl concentrations if
the plasmalogen concentrations were decreased concomi-
tantly. Thus, under inflammatory conditions, HOCl may
be capable of triggering GPC generation throughout. In

the case of polyunsaturated plasmalogen, ODPCplasm
GPC is the main product besides 2-D-1-LPC at increased
HOCl concentrations. These results confirm the previ-
ously underestimated role of plasmalogens as scavenger
molecules in biological cells and tissues. GPC generation
may be of particular relevance in animal spermatozoa
(e.g., bull or boar), because these cells have very high
plasmalogen moieties that consist primarily of docosahexa-
enoic acid residues.

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