Structural characterization of oxidized phospholipid products derived from arachidonate-containing plasmenyl glycerophosphocholine

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Abstract **Plasmenyl phospholipids are a structurally unique class of lipids that contain a vinyl ether substituent at the *sn*-1 position of the glycerol backbone, imparting unique susceptibility to oxidative reactions that may take place at the cell membrane lipid bilayer. Several studies have supported the hypothesis that plasmalogens may be antioxidant molecules that protect cells from oxidative stress. Because the molecular mechanism for the antioxidant properties of plasmenyl phospholipids is not fully understood, the oxidation of arachidonate-containing plasmalogen-glycerophosphocholine (GPC) was studied using electrospray tandem mass spectrometry after exposure to the free radical initiator 2,2′-azobis(2-amidinopropane)hydrochloride (AAPH). Various oxidized GPC products involving the *sn*-1 position alone (1-formyl-2-arachidonyl lipids and lysophospholipid), oxidation products involving the *sn*-2 position alone (chain-shortened ω-aldehyde radyl substituents at *sn*-2) as well as products oxidized both at the *sn*-1 and *sn*-2 positions were observed and structurally identified. The results of these experiments suggest that oxidation of plasmenyl phospholipids esterified with polyunsaturated fatty acid groups at *sn*-2 likely undergo unique and specific free radical oxidation at the 1′-alkenyl position as well as oxidation of the double bond closest to the ester moiety at *sn*-2.**—Khaselev, N., and R. C. Murphy. **Structural characterization of oxidized phospholipid products derived from arachidonate-containing plasmenyl glycerophosphocholine. *J. Lipid Res.* 2000.** 41: **564–572.**

Supplementary key words plasmenyl phospholipids • arachidonate oxidation • lipid peroxidation • electrospray mass spectrometry • AAPH oxidation • arachidonate plasmalogens • LC/MS • antioxidant plasmalogens

The plasma membrane of mammalian cells is composed of a complex mixture of glycerophospholipids including a unique group of plasmenyl phospholipids also termed plasmalogens (1). This phospholipid subclass has a vinyl ether substituent at the *sn*-1 position of the glycerol backbone imparting several interesting properties including instability to acid (2) as well as susceptibility to oxidative reactions (3–5). The precise role played by plasmalogens in cellular membranes is not entirely understood; however, the concept that these molecules have an antioxidant role in protecting cells from reactive oxygen species has emerged following studies of oxidative stress in intact cells in which plasmalogen biosynthesis had been interrupted (6, 7) as well as from studies of cell-derived phospholipid oxidation (8). Plasmalogens are synthesized within the peroxisome subcellular organelle through the action of dihydroxyacetonephosphate acyl transferase and alkyl dihydroxyacetonephosphate synthase both of which are located within the peroxisomal membrane (9).

The chemical mechanisms responsible for protecting cells from reactive oxygen species are not entirely understood, but recent studies have revealed rather unique products of arachidonoyl-plasmalogen oxidation result after exposure of unilamelar vesicles to hydroxyl radical (N. Khaselev and R. C. Murphy, unpublished results). These studies have revealed that not only is the vinyl ether substituent important in the oxidative degradation of plasmalogen species, but esterified arachidonate present at the *sn*-2 position may also participate in specific oxidative reactions. While previous studies have focused on the importance of the vinyl ether substituent in plasmalogens as a central component in protecting cells from lipid peroxidative events, the fact that naturally occurring plasmalogens contain a relatively high abundance of esterified arachidonate (10, 11) has not been included in the discus-

Abbreviations: for individual GPC molecular species used in this paper: n:jk/s:t GPC (e.g., 16:0p/20:4 GPC), where n is the number of carbons in the *sn*-1 substituent and j is the number of double bonds in the *sn*-1 hydrocarbon chain; k represents the type of *sn*-1 linkage (where p = 1-O-alk-1′-enyl (plasmalogen); and a = acyl; s is the number of carbons and t is the number of double bonds. For oxidized GPC molecular species, additional abbreviations include: p-Ep = 1′-epoxy-3′-enyl; p-OOH = 1′-hydroperoxy-2′-enyl; p-OH = 1′-hydroxy-2′-enyl, OH = *sn*-1 lyso; and eicosanoid nomenclature (21) at the *sn*-2 fatty acyl substituent. The chain shortened *sn*-2 products identified as ω-aldehyde species are indicated as containing a carbonyl group (c) along with the total carbon chain and double bonds indicated.

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sion of mechanisms responsible for the protective effect in terms of cellular toxicity induced by reactive oxygen and free radical species.

In order to gain further insight into the oxidative decomposition of arachidonate-containing plasmalogen-GPC species, the oxidized phospholipid components resulting from the exposure of this one unique plasmalogen-GPC (1-O-hexadec-1′-enyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine) to the free radical initiator AAPH were undertaken. Tandem mass spectrometry and electrospray ionization were used to structurally characterize various oxidized GPC products.

MATERIALS AND METHODS

Materials

1-O-Hexadec-1′-enyl-2-[5,6,8,9,11,12,14,15]-arachidonyl-*sn*-glycero-3-phosphocholine as well as 2-arachidonyl-*sn*-glycero-3-phosphocholine (*sn*-1 lyso-GPC) were synthesized as described previously (N. Khaselev and R. C. Murphy, unpublished results). 2,2′-Azobis-(2-amidinopropane)hydrochloride (AAPH) was a gift from Dr. Joseph McCord (University of Colorado Health Sciences Center, Denver, CO). Arachidonic acid was purchased from Nu-Chek Prep (Elysian, MN). HPLC solvents were purchased from Fisher Scientific (Fair Lawn, NJ) and used for HPLC, extraction, hydrolysis, and oxidation.

Oxidation procedure

The arachidonate plasmenyl phosphocholine 16:0p/20:4-GPC (0.5 μmol) was suspended in 50 mm PBS (pH 7.4) to a final concentration of 2 mm by vortexing and sonication. AAPH dissolved in phosphate-buffered saline (50 mm, pH 7.4) was added to this phospholipid suspension for a final concentration of 10 mm. The reaction was carried out at 37°C at various times indicated in the text. The incubation was stopped by immersion in an ice bath and the addition of chloroform–methanol according to Bligh and Dyer (12). The chloroform layer was taken to dryness under nitrogen, and aliquots were taken for direct analysis by electrospray tandem mass spectrometry.

Electrospray ionization mass spectrometry and tandem mass spectrometry (MS/MS)

On line RP-LC/MS/MS analysis of the chloroform layer from oxidized plasmalogen was performed by using a 5 μ Columbus C-18 100A (1.0 × 150 mm) column (Phenomenex, Torrance, CA) connected to a UV monitor (photodiode array) on line just prior to the electrospray interface. The HPLC was operated at a flow rate of 50 μL/min with the mobile phase consisted of methanol–water–acetonitrile 60:20:20 (v/v/v) containing 1 mm ammonium acetate as solvent A, and 1 mm methanolic ammonium acetate solution as solvent B eluting 0% B to 100% in 40 min followed by isocratic elution of 100% B for 20 min. A postcolumn split was used to yield a 25 μL/min flow into the mass spectrometer and 25 μL/min to a fraction collector.

The Sciex API III$^+$ triple quadruple mass spectrometer (PE-Sciex, Toronto, Canada) was operated in the positive ion mode with the orifice at +70 V, using collision-induced dissociation and tandem mass spectrometry to obtain a total ion current profile of those precursor ions which generated *m/z* 184 corresponding to the phosphocholine ion characteristic to all GPC species (13). LC/MS/MS analysis in negative mode (with the orifice at −100 V) was used to identify the structure of oxidation products by collision-induced decomposition of [M–15]$^-$ ions obtained as a result of decomposition of [M+OAc]$^-$ ions with high orifice potential (14). Collision-induced dissociation (CID) was performed by using 30 eV and a collision gas thickness (argon) of 230×10^{13} molecules/cm^2. A curtain gas flow of 1.6 L/min and spray temperature of 400°C were used.

Saponification and multiple reaction monitoring (MRM) analysis

An aliquot of the oxidized plasmalogen mixture was dried under a dry nitrogen stream and then hydrolyzed by the addition of 0.5 mL of 0.5 m methanolic sodium hydroxide at room temperature for 2 h. The reaction mixture was acidified to pH 5 with 10% acetic acid and fatty acids were extracted twice with ethyl acetate (1 mL). Combined ethyl acetate fractions were taken to dryness, resuspended in 30% methanol solution, and analyzed by using on-line RP-LC/MS/MS.

MRM analysis were performed with monitoring the transitions *m/z* 317 → 203 for 5-oxo-ETE; *m/z* 319 → 115 for 5-HETE; *m/z* 319 → 151 for 9-HETE; *m/z* 319 → 155 for 8-HETE and 8,9-ETE; *m/z* 319 → 167 for 11-HETE; *m/z* 319→ 191 for 5,6-ETE; *m/z* 319 → 208 for 12-HETE and 11,12-EET; *m/z* 335 → 115 for 5,6-diHETE; *m/z* 335 → 127 for 8,15-diHETE; *m/z* 335 → 195 for 5,12-diHETE; *m/z* 335 → 201 for 5,15-diHETE based on the collision-induced decomposition mass spectra of each eicosanoid (15). A dwell time of 450 ms was used for each ion transition for a total scan time of 3.0 s for a selection of eight mass transitions in each MRM protocol. The spray voltage was −3,000 V; the orifice potential was set at −60 V, with an offset of 20 eV.

RESULTS

Reverse phase LC/MS/MS analysis of the 1-O-hexadecyl-1′-enyl-2-arachidonyl-*sn*-glycero-3-phosphocholine (16:0p/20:4-GPC) oxidized for 3 h in the presence of AAPH was performed in the positive ion mode acquiring all precursors of the ion *m/z* 184 which corresponds to the phosphocholine ion characterized for all GPC phospholipids (13). Several major as well as minor glycerophosphocholine species were observed where each individual component of the mixture yielded a prominent *m/z* 184 (**Fig. 1**). The major component in the mixture was unreacted 16:0p/20:4-GPC also indicated by the elution retention time and

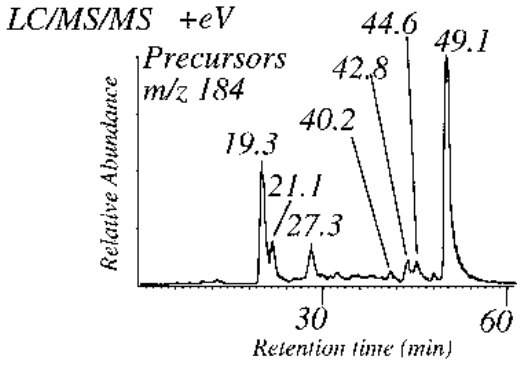

Fig. 1. Reverse phase HPLC and tandem mass spectrometric analysis (LC/MS/MS) of glycerophosphocholine lipids obtained after oxidation of 1-O-hexadec-1′-enyl-2-arachidonoyl-GPC (16:0p/20:4-GPC) with 10 mm AAPH. Precursor ion scans for the GPC specific cation, *m/z* 184, indicate the elution of specific oxidized products from the reverse phase HPLC column.

mass spectral characteristics (see below) of this molecule by monitoring UV absorbance at 206 nm at 49 min. Other products of radical oxidation had less lipophilicity compared to the starting material. The most abundant of these products which yielded *m/z* 184 eluted from the column from 19–30 min and also had some ultraviolet absorption at 206 nm. The UV absorption profile at 270 and 235 nm (data not shown) revealed elution of several components having UV chromophores corresponding to conjugated triene and conjugated diene containing phospholipids, respectively, eluting between 40–48 min which were also detected as glycerophosphocholine lipids generating *m/z* 184 (Fig. 1).

The identification of the individual molecular species within this oxidized plasmalogen mixture was carried out in the negative ion mode using a relatively high orifice potential to obtain the choline dimethylated $[M-15]^-$ species (14). As glycerophosphocholine lipids have both a quaternary ammonium cation moiety and a very acidic phosphodiester anion, the appearance of negative ions required either removal of the quaternary nitrogen or the addition of another anionic site in the phospholipid for a total of two negative charges. Oxidized GPC lipids readily formed acetate adduct ions in the presence of acetate buffers and the formation of $[M-15]^-$ anions can be generated from these acetate adduct ions by collisional activation in the high-pressure region of the electrospray interface before the quadruple mass analyzer (14). The molecular weight of each product obtained as a result of the oxidation of the starting plasmenyl-GPC was further confirmed from the observation of the corresponding $[M-15]^-$ (**Table 1**). During the second HPLC separation and negative ion electrospray analysis, fractions were collected and each oxidation product was subsequently analyzed by collision-induced decomposition of the corresponding $[M-15]^-$ in order to determine the fatty acyl substituent present in each oxidized product (15).

The most abundant oxidation product generated a $[M-15]^-$ anion observed at *m/z* 528.4 which was consistent with a loss of the entire *sn*-1 alkenyl chain during the oxidation process (**Fig. 2**). The CID spectrum of *m/z* 528.4 yielded an abundant ion at *m/z* 303 corresponding to the intact carboxylate anion of arachidonic acid and ion at *m/z* 242 corresponding to the loss of the *sn*-2 arachidonoyl moiety as a neutral ketene (16). These data were consistent with the major product (eluting at 19.3 min) being the *sn*-1 lyso-GPC (2-arachidonoyl-*sn*-glycero-3-phosphocholine).

The CID spectrum of the ion at *m/z* 556.4 $[M-15]^-$ from the fraction which eluted at 21 min (**Fig. 3**) yielded an abundant ion at *m/z* 303 corresponding to the arachidonate carboxylate anion as well as an ion at *m/z* 270 corresponding to the loss of the *sn*-2 arachidonate as a neutral ketene species. Because this oxidation product generated a $[M + H]^+$ ion at *m/z* 572.5 which decomposed by CID to *m/z* 184, there was no modification of the phosphocholine portion of the molecule by the free radical oxidation. The mass of this oxidation product was 28 daltons higher than that of the major product identified as the *sn*-1 lyso-GPC consistent with 1-formyl-2-arachidonyl-

TABLE 1 Oxidized glycerophosphocholine products formed by AAPH-oxidation of 16:0p/20:4-GPC identified by tandem mass spectrometry

Retention Time[a]	UV[b]	$[M + H]^+$	$[M-15]^-$	Product Ions after CID of $[M-15]^-$ (Relative Abundance)					Structural Assignment[h]
				A[c]	B[d]	C[e]	D[f]	E[g]	
min	*nm*	*m/z*	*m/z*						
19.3	206	544.4	528.5	303(100)	242(20)	224(18)	—	—	OH/20:4-GPC
21.1	206	572.4	556.4	303(100)	270(13)	—	—	—	1:0a/20:4-GPC
27.3	—	578.5	562.4	115(100)	464(25)	446(5)	—	—	16:0p/5:0c-GPC
27.3		578.5	562.5	115(100)	464(25)	446(5)	—	—	16:0p/5:0c-GPC
26.4		592.5	576.5	115(100)	478(20)	—	253(45)	340(11)	16:1p-OOH/5:0c-GPC
26.9		592.5	576.5	129(100)	464(26)	—	—	—	16:0p/6:0c-GPC
30.8		644.5	628.4	181(100)	464(32)	137(36)	—	—	16:0p/10:2c-GPC
31.3		630.5	614.4	167(100)	464(43)	123(50)	—	—	16:0p/10:2-GPC
40.2	235	798.6	782.6	317(100)	464(28)	764(22)	—	—	16:0p/5HPETE-GPC
42.8	235,206	798.6	782.6	303(94)	496(5)	—	271(100)	253(27)	16:0p-OOH/20:4-GPC
42.8	270	782.5	766.6	319(75)	464(21)	191(26)	—	—	16:0p/5-ETE-GPC
43.6	270	814.6	798.6	335(100)	480(24)	780(25)	253(23)	—	16:0p-OH/5,12-diHETE-GPC
44.2	270	814.6	798.6	335(100)	480(26)	780(21)	255(24)	—	16:0p-Ep/5,12-diHETE-GPC
44.6	206,235	782.6	766.6	303(100)	480(6)	—	253(46)	528(10)	16:0p-OH/20:4-GPC
45.4	206	782.6	766.6	303(100)	480(6)	—	255(47)	528(9)	16:0p-Ep/20:4-GPC
45.7	206	782.6	766.6	303(100)	—	259(5), 596(4)	—	—	16:0p/20:4-N-oxide-GPC
49.1	206	766.6	750.6	303(100)	464(18)	—	—	—	16:0p/20:4-GPC

[a] RP-HPLC retention time using column and mobile phase as in Fig. 1.
[b] UV absorption maximum.
[c] *sn*-2 carboxylate anion.
[d] Loss of ketene from *sn*-2.
[e] Other abundant ions.
[f] *sn*-1 alkoxide anion.
[g] Loss of ketene from *sn*-1.
[h] Abbreviations for individual oxidized GPC molecular species see footnote on title page.

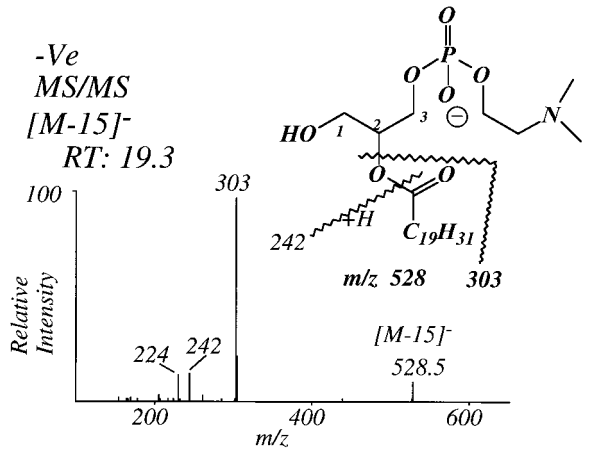

Fig. 2. Collision-induced decomposition of the [M−15]$^-$ ion derived from the major product eluting from the HPLC (19.3 min) and identified as lyso-2-arachidonoyl-GPC (OH/20:4-GPC). Suggested origins of the major product ions are indicated in the structure of this metabolite.

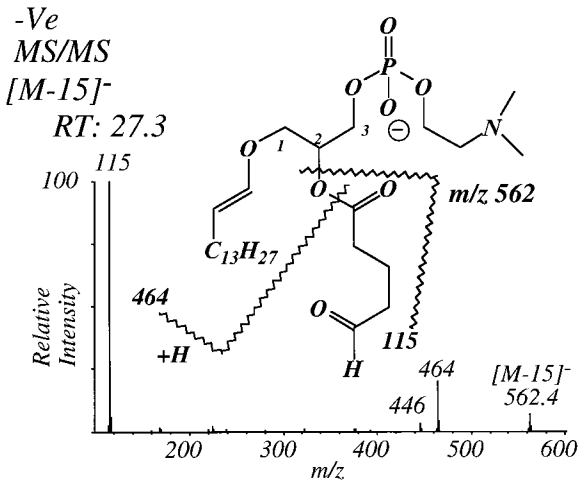

Fig. 4. Collision-induced decomposition of the [M−15]$^-$ ions derived from the oxidation product eluting from the RP-HPLC at 27.3 min and identified as 1-O-hexadec-1′-ene-2-[5-oxo]pentanoyl-GPC (16:0p/5:0c-GPC). Suggested origins of the major product ions are indicated in the structure of this metabolite.

sn-glycero-3-phosphocholine as a result of the oxidation of the oxidation of the *sn*-1 carbon chain and decomposition of the hydroperoxy acetyl and oxidation of the 1′-carbon atom to a formyl group.

The products that eluted from the HPLC between 26 and 27 min generated abundant [M−15]$^-$ ions at *m/z* 562.4 and two isobaric species with *m/z* 576.5, revealing several metabolites in these fractions. These [M−15]$^-$ ions, as well as ions that eluted between 30–31 min, were identified as products of oxidation of the arachidonate substituent followed by subsequent chain shortening of the *sn*-2 substituent into a series of aldehydes esterified to the *sn*-2 position (Table 1). The CID mass spectrum of the most abundant [M−15]$^-$ ion at *m/z* 562.4 yielded a very abundant ion at *m/z* 115 corresponding to 5-oxo-pentanoate carboxylate anion and an ion at *m/z* 464 corresponding to the loss of the same *sn*-2 substituent as a neutral ketene species (**Fig. 4**).

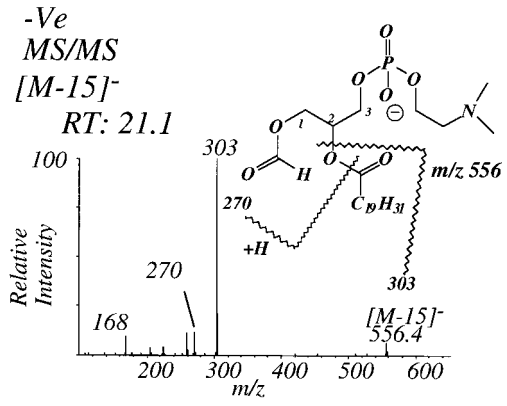

Fig. 3. Collision-induced decomposition of the [M−15]$^-$ ions derived from the oxidation product eluting from the RP-HPLC at 21.1 min and identified as 1-formyl-2-arachidonoyl-GPC (1:0a/20:4-GPC). Suggested origins of the major product ions are indicated in the structure of this metabolite.

Mass spectrometric analysis of the HPLC fraction between 40 and 45 min revealed elution of numerous products of oxidation of both radyl substituents at *sn*-1 or *sn*-2 positions. The fractions eluting at 40.2 and 42.8 min contained components that had abundant and identical [M−15]$^-$ ions at *m/z* 782.6 corresponding to the addition of two oxygen atoms to the starting plasmenyl-GPC. Tandem mass spectrometry permitted discrimination between those two isobaric ions. Collision-induced dissociation of the ion at *m/z* 782.6 which eluted at 40.2 min (**Fig. 5A**) yielded an abundant ion at *m/z* 317 corresponding to the carboxylate anion from the *sn*-2 position, containing one oxygen atom. The ion at *m/z* 464 corresponded to the loss of the arachidonate and two oxygen atoms as a neutral ketene, strongly supporting the presence of a non-oxidized plasmenyl chain at the *sn*-1 position. As two oxygen atoms were added to the intact plasmenyl-GPC, this carboxylate anion was most likely a dehydration product ion of the arachidonoyl hydroperoxide radyl group. As previously observed, hydroperoxy arachidonate metabolites readily lose H_2O to yield the corresponding ketene species at *m/z* 317 (17).

Tandem mass spectrometry of the fraction that eluted at 42.8 min yielded very abundant ions at *m/z* 303 and 271 (Fig. 5B). As the ion at *m/z* 303 was consistent with the presence of non-oxidized arachidonate esterified at the *sn*-2 position, the addition of two atoms of oxygen were most likely present at the *sn*-1 position. The ion at *m/z* 271 confirmed that the *sn*-1 plasmenyl moiety underwent oxidation with formation of a 1′-hydroperoxide which might stabilize the alkoxide anion as a leaving group from the *sn*-1 position during collisional activation. Further elimination of a neutral water molecule from the alkoxide anion derived from *sn*-2 would yield the ion at *m/z* 253.

Products derived from the *sn*-1 hydroperoxide such as epoxy- and hydroxy-compounds were also identified as mi-

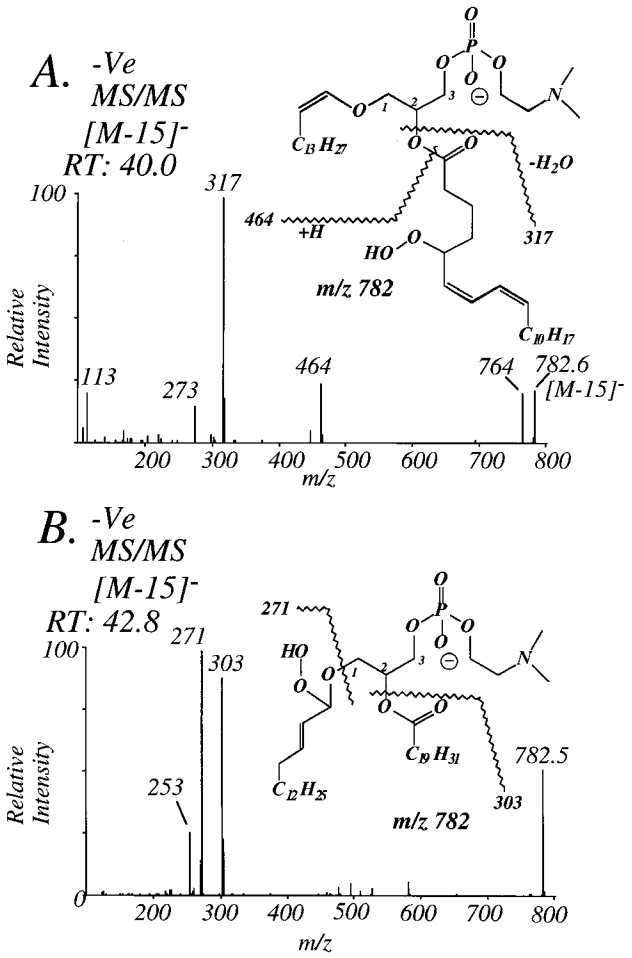

Fig. 5. Collision-induced decomposition of the [M−15]⁻ ions derived from the oxidation products eluting from the RP-HPLC at (A) 40.0 min and identified as 1-O-hexadec-1′-ene-2-[5-hydroperoxy]eicosatetraenoyl-GPC (16:0p/5-HpETE-GPC) and the isobaric (B) 1-O-hexadec-1′-hydroperoxy-2′-ene-2-arachidonoyl-GPC (16:0p-OOH/20:4-GPC) which eluted at 42.8 min.

nor oxidation products of plasmalogen-GPC. For example, the CID mass spectrum of *m/z* 766.6 derived from the component that eluted at 44.6 min resulted in abundant ion at *m/z* 303 corresponding to the arachidonate anion. Loss of arachidonate as a neutral ketene led to the formation of the ion observed at *m/z* 480. This hemiacetal-type molecule underwent an unexpected cleavage with formation of a,β-unsaturated carboxylate anion at *m/z* 253. This latter reaction probably occurred by a Kornblum-DeLaMare type elimination mechanism (18) with concomitant loss of arachidonic acid and 1-propenyl-phosphatidyl choline as neutral molecules. The ions at *m/z* 528 would correspond to the *sn*-1 alkoxide anion as a result of the loss of a neutral a,β-unsaturated aldehydes from the hemiacetal (**Fig. 6**). A similar fragmentation pattern was observed for analogous hemiacetal compounds with [M−15]⁻ at *m/z* 576.5, 798.6, and 798.6 eluting at 26.4, 42.8, and 43.6 min, respectively (Table 1).

Tandem mass spectrometric analysis of HPLC fractions which eluted between 44 and 46 min revealed several mono- and dioxygenated eicosatetraenoic acids esterified

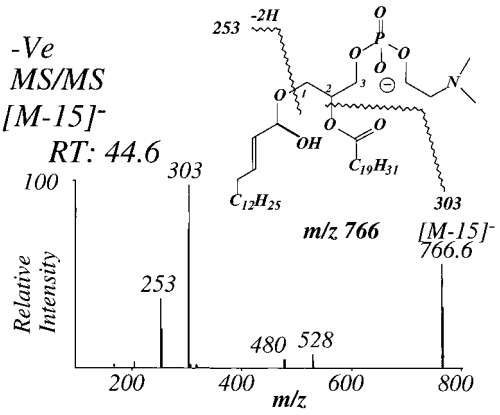

Fig. 6. Collision-induced decomposition of the [M−15]⁻ ions derived from the oxidation products eluting from the RP-HPLC at 44.6 min and identified as 1-O-hexadec-1′-hydroxyl-2′-ene-2-arachidonoyl-GPC (16:0p-OH/20:4-GPC). Suggested origins of the major product ions are indicated in the structure of this metabolite.

at the *sn*-2 position of phosphatidylcholine with an additional oxygen atom at the *sn*-1 position. For example, the CID mass spectrum of the ion at *m/z* 798 which eluted at 43.6 min (**Fig. 7**) revealed abundant ion products at *m/z* 335 consistent with the carboxylate anion for a dihydroxy-eicosatetraenoic acid (*m/z* 335). The ion at *m/z* 480 corresponded to the loss of the *sn*-2 substituent as a neutral ketene, confirming that two oxygen atoms were covalently bound to the arachidonyl moiety esterified at *sn*-2 position. The ion at *m/z* 253 corresponded to the loss of *sn*-1 moiety as a result of oxidation of vinyl double bond and subsequent transformation into an a,β-unsaturated hemiacetal.

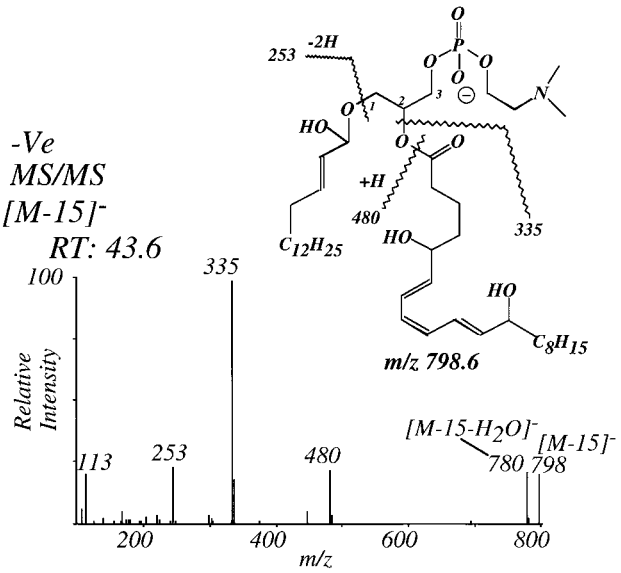

Fig. 7. Collision-induced decomposition of the [M−15]⁻ ions derived from the oxidation products eluting from the RP-HPLC at 43.6 min and identified as 1-O-hexadec-1′-hydroxy-2′-ene-2[5,12-dihydroxy]eicosatetraenoyl-GPC (16:0p-OH/5,12-diHETE-GPC). Suggested origins of the major product ions are indicated in the structure of this metabolite.

In order to obtain detailed information of mono- and dihydroxyeicosatetraenoic acyl substituents formed by AAPH oxidation, oxidized plasmenyl-GPCs in the fractions eluting from the HPLC between 40 and 46 min were saponified and free oxidized fatty acids were analyzed by on-line LC/MS/MS using a negative mode multiple reaction monitoring protocol. Product ions derived from *m/z* 319 and 335, corresponding to the addition of one and two oxygen atoms to the arachidonate moiety, respectively, were monitored using specific transitions for each eicosanoid (see Methods). As shown in **Fig. 8**, MRM analysis revealed the elution of a mixture of isomeric HETEs, EETs, and diHETEs. The major products appeared to be 5-HETE and 11-HETE products. However, 5-oxo-ETE, 9-HETE, 8-HETE, 8,9-ETE, 5,6-ETE, and 5,12-diHETE were present in significant amounts. Other minor components including 12-HETE, 11,12-ETE, 5,15-diHETE, 5,6-diHETE, and 8,15-diHETE were definitely observed, but in very low amounts.

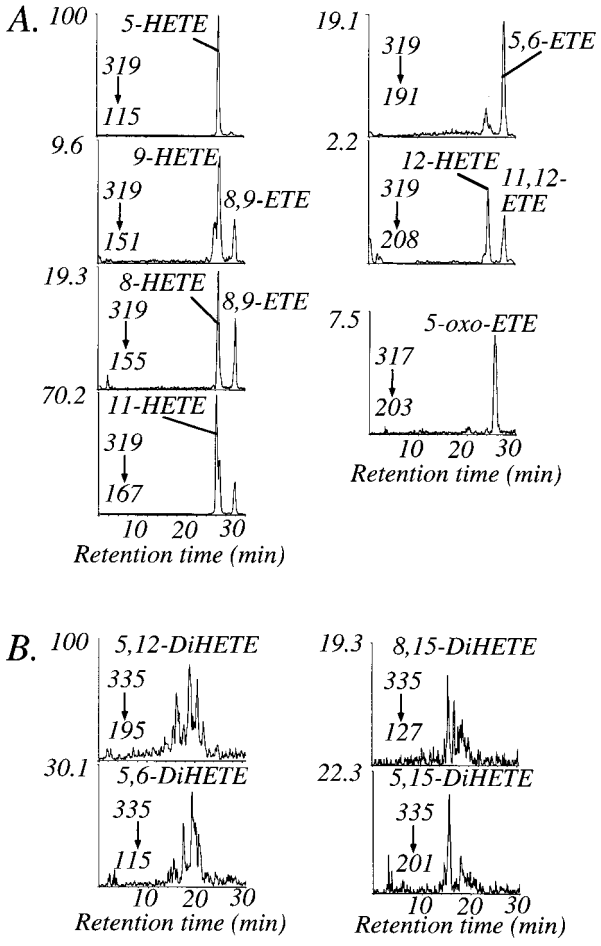

Fig. 8. Multiple reaction monitoring after collision-induced dissociation of specific carboxylate anions obtained after saponification of the oxidized phospholipids eluting between 40–43 min in the RP-HPLC separation of the arachidonoyl plasmalogen (Fig. 1). (A) The elution of monooxygenated derivatives of arachidonic acid as the corresponding carboxylate anions are indicated at the corresponding retention times and ion transitions. (B) Elution of dioxygenated derivatives of arachidonic acid indicated at the appropriate retention times and ion transitions.

DISCUSSION

The plasmenyl glycerophospholipids are unusually susceptible to free radical lipid peroxidation. While early work had related this unique reactivity of plasmalogen phospholipids solely to the vinyl ether double bond at the *sn*-1 position (5–7), an alternative picture has merged where there is participation of both radyl substituents in oxidation reactions when a polyunsaturated fatty acyl group is esterified at the *sn*-2 position as well (9). A high proportion of plasmalogen molecular species contains polyunsaturated fatty acids esterified at this position, and in particular arachidonic acid (10, 11). While there is still only an incomplete understanding of the precise role played by these unique phospholipids in the cellular membrane, it is clear that these compounds do protect cells from damaging free radical reactions. As we previously observed, the arachidonoyl portion of plasmenyl-GPC was oxidized at carbon-5 almost exclusively after exposure of unilamellar to hydroxyl radical generated by the Fenton-type reaction (9). In turning attention to products derived from the reaction of plasmenyl-GPC with peroxyl radicals (AAPH) under aerobic conditions, somewhat of a different and rather diverse number of reaction products were observed including a 1,2-diacyl lipid, a lysophospholipid, oxidation products involving the *sn*-1 position alone, oxidation products involving the *sn*-2 position alone, chain-shortened ω-aldehyde radyl substituents as well as products which were oxidized both at the *sn*-1 and *sn*-2 positions. Ester hydrolysis products were not found as was the case for hydroxyl radical initiation of lipid peroxidation (N. Khaselev and R. C. Murphy, unpublished results).

Closer examination of the products derived from the AAPH-dependent oxidation of arachidonate-containing plasmenyl-GPC suggested that unique hydroperoxide chemistry took place during oxidation at the 1′-alkenyl position at *sn*-1 as well as the 5′ position at the *sn*-2 arachidonoyl group. As suggested in **Fig. 9**, the formation of the *sn*-1 hydroperoxy hemiacetal would likely be unstable and decompose by several different reaction pathways including homolytic oxygen–oxygen bond cleavage of the hydroperoxide group leading to alkoxy radical species. These intermediate oxygen-centered radicals could have additional reaction fates including abstraction of a hydrogen atom forming a hemiacetal that could be readily hydrolyzed to yield the major oxidation product, namely the *sn*-1 lyso-arachidonoyl-GPC observed at 19.3 min. As outlined in Fig. 9, further abstraction of the hydroxyl hydrogen atom under CID conditions would result in the formation of *sn*-1 α,β-unsaturated hemiacetal (44.6 min) that yielded an unexpected ion at *m/z* 253 by the elimination of arachidonic acid and 1-propenyl-phosphatidyl choline as neutral molecules in a Kornblum-DeLaMare elimination process (18). A more complicated reaction involving allylic alkoxyl radical cyclization would lead to an epoxide radical species that could subsequently abstract a hydrogen atom leading to the epoxy ether observed to elute from the HPLC at 45.4 min. Epoxy ether products of plasmalogen oxidation have been previously described (19).

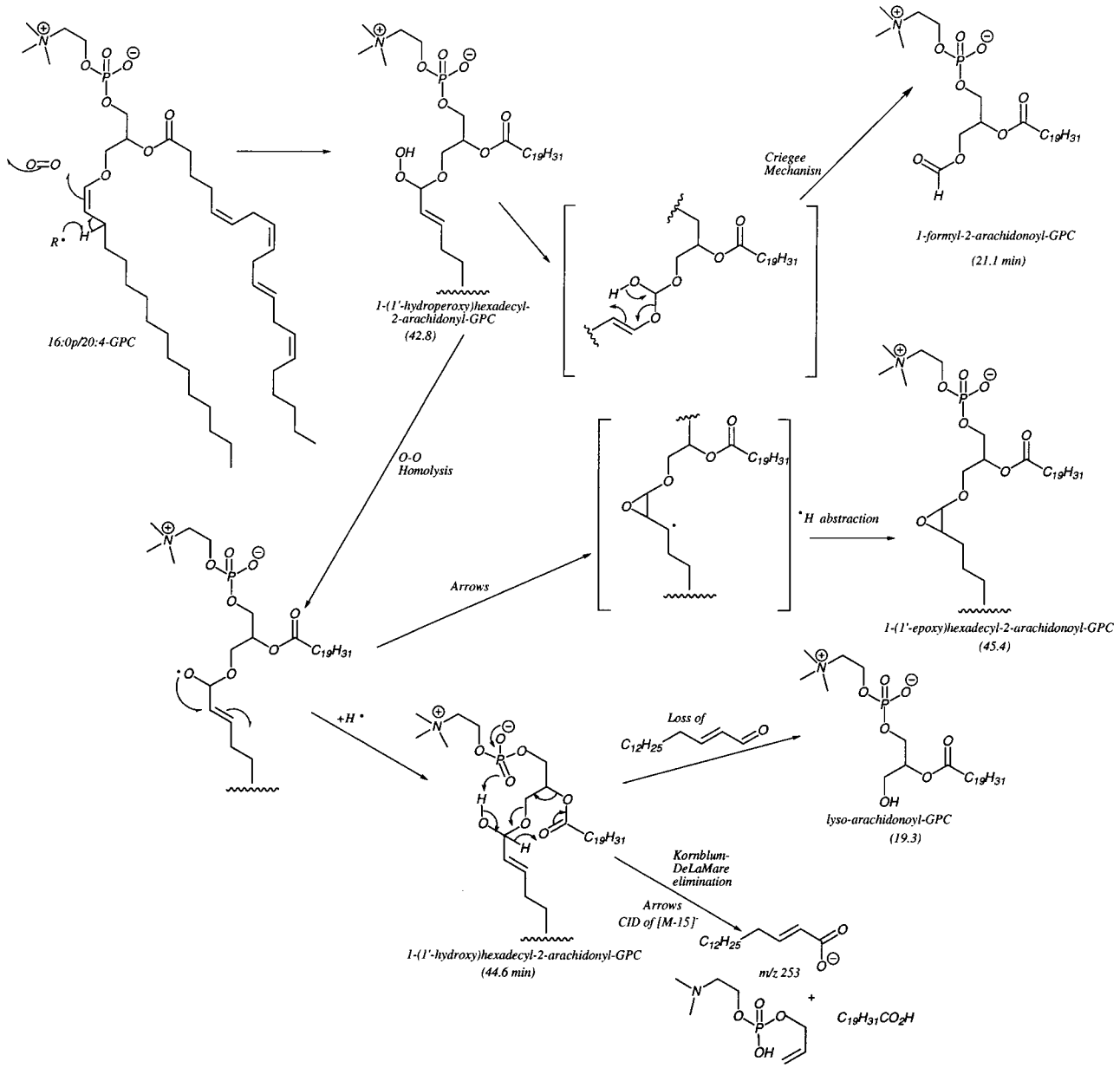

Fig. 9. Proposed mechanism for the formation of specific oxidized products of 1-O-hexadec-1′-ene-2-arachidonoyl-GPC (16:0p/20:4-GPC) after initiation of oxidation at the vinyl ether substituent.

One of the most interesting products observed was the abundant formation of 1-formyl-2-arachidonoyl-GPC eluting from HPLC at 21.1 min. A possible mechanism explaining the formation of this product would involve the migration of the vinyl group which is known to be a facile reaction of allylic hydroperoxides and termed the Hoch rearrangement (20). The resulting allylic ortho ester could then rearrange through a Criegee mechanism to directly form the 1-formyl-2-arachidonoyl-GPC and pentadecanal (Fig. 9).

The major products involving only oxidation of the arachidonoyl substituent at the *sn*-2 position involved predominant oxidation at carbon-5 as found previously (N. Khaselev and R. C. Murphy, unpublished results). The 5-hydroperoxy eicosatetranoyl ester which was observed as one of the major dioxygenated arachidonate species eluting at 40.0 min most likely was a precursor of a product that underwent oxygen–oxygen bond homolysis leading to the 5-hydroxyeicosatetraenoate ester observed eluting from the HPLC at 42.7 min (**Fig. 10**). Perhaps the most curious product was the identification of the C_5-ω-aldehyde ester as one of the major products eluting at 27.2 min. Once again, this product was likely a result of the migration of the vinyl substituent of this allylic hydroperoxide (Hoch rearrangement) followed by Criegee rearrangement with intermediate formation of a hemiacetal that would decompose to the aldehyde attached to the glycerophospholipid backbone and a conjugated dienal eliminated as a neutral product (Fig. 10).

The result of these detailed structural studies of the free

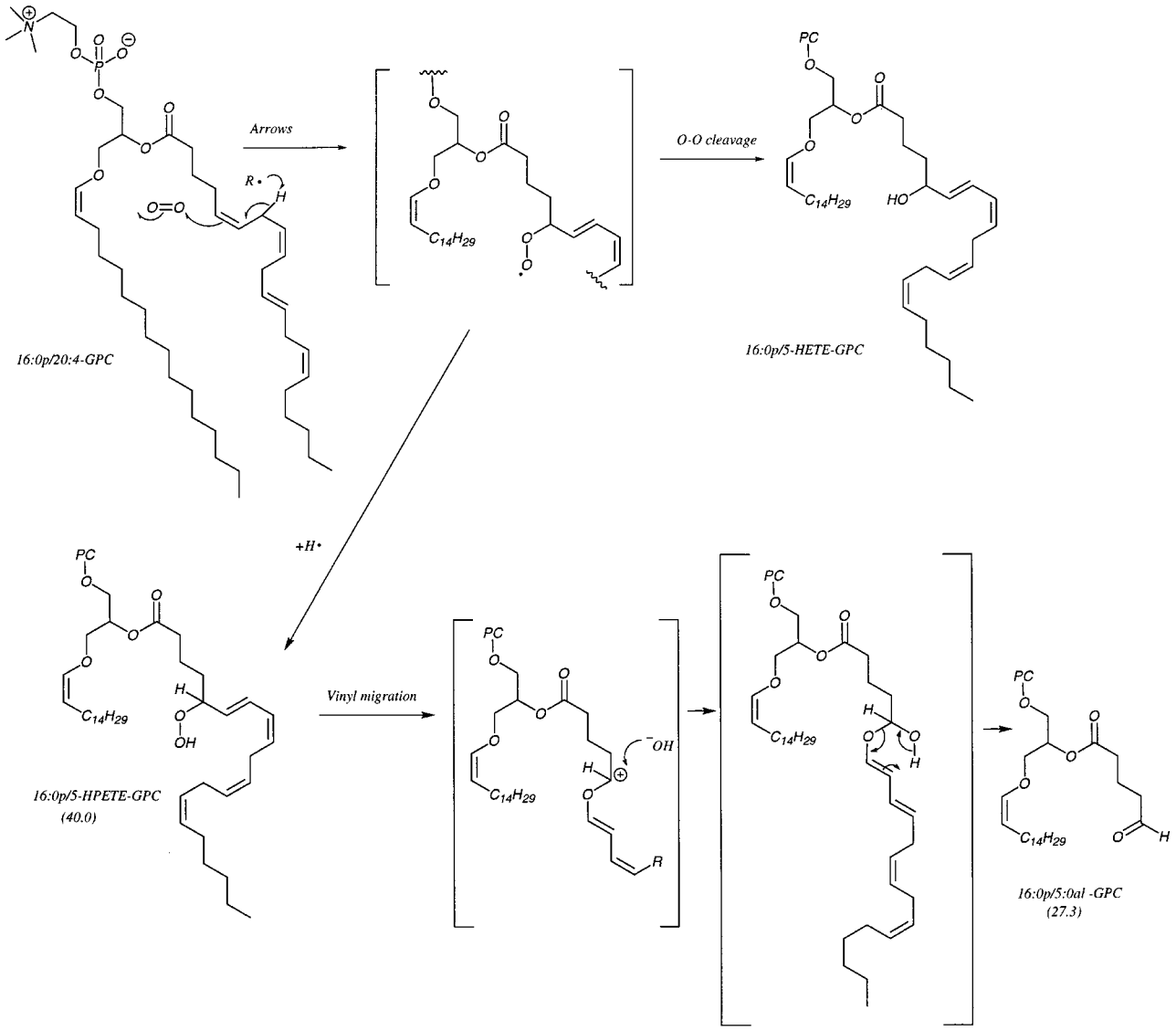

Fig. 10. Proposed mechanism for the formation of specific oxidized products of 1-O-hexadec-1′-ene-2-arachidonoyl-GPC (16:0p/20:4-GPC) after initiation of oxidation of the arachidonoyl radyl group at *sn*-2. The vinyl group migration indicated has been described as the Hock (20) rearrangement.

radical-induced peroxidation of arachidonoyl plasmenyl-GPC has revealed several new families of radical-derived oxidized phospholipids. The formation of these different products of lipid peroxidation suggest that several chemical mechanisms are involved in their formation. The major products of oxidation appeared to be related to the chemical reactivity of the vinyl ether double bond of the plasmenyl *sn*-1 carbon atom leading to a 1′-hydroperoxy unsaturated hemiacetal. However, oxidation of the arachidonoyl group also occurred to a large extent, suggesting that the chemical reactivities of the plasmalogen vinyl ether and the bisallylic methylene groups of arachidonic acid are similar in terms of susceptibility to free radical reactions. The number of possible radical reactions taking place within the arachidonoyl plasmenyl-GPC may, in part, be responsible for the ultimate slowing of radical propagation through the formation of more stable radical products (less energetic) that finally terminate the radical reaction process when encountering antioxidant molecules dissolved in the plasma membrane.

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