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Synthesis and Antioxidant Properties of an Unnatural Plasmalogen Analogue Bearing a *trans* O-Vinyl Ether Linkage

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Abstract

To assess the antioxidant behavior of *trans*-1, we first synthesized *trans*-allyl ether 4 by opening an (*S*)-glycidol derivative with an (*E*)-alk-2-en-ol, and then produced the unnatural *E*-enol ether 1 by a stereoselective iridium(I)-catalyzed olefin isomerization. Natural *cis*-1 was preferentially degraded by HOCl and was more protective than *trans*-1 against lipid peroxidation induced by a free-radical initiator, demonstrating that the geometry of the 1'-alkenyloxy bond participates in the antioxidant defensive role of 1.

Plasmalogens are a subclass of glycerophospholipids that bear an 1-*O*-alk-1'-(*Z*)-enyl chain at the *sn*-1 position of glycerol (typically a 16-carbon chain), an *sn*-2 fatty acyl chain (typically unsaturated or polyunsaturated), and a polar head group at the *sn*-3 position (predominantly a mixture of phosphoethanolamine and phosphocholine).¹ Plasmalogens comprise about 20% of the total phospholipids in humans; they are especially abundant in human cardiac, skeletal muscle, and nervous tissues, which contain high levels of polyunsaturated fatty acids.² Since plasmalogen-deficient cells were more susceptible to free radical mediated oxidative damage than cells with normal plasmalogen levels,³ and because enol ethers are known to be susceptible to oxidation,⁴ it has been proposed that the *Z*-enol ether functionality, which is unique to plasmalogens, may serve as a trap for reactive oxygen species. The vinyl ether linkage is located in close proximity to the lipid-water interface, and may thus protect the polyunsaturated chains of phospholipids in membranes and lipoproteins from oxidation.⁵ The putative intermediates

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Supporting Information Available: Experimental procedures and NMR spectra for the reported compounds. This material is available free of charge at <http://pubs.acs.org>.

formed by [2+2] and [2+4] cycloaddition of singlet oxygen to this linkage are dioxetane and ene intermediates, respectively, which subsequently undergo degradation to generate a complex mixture of fatty aldehydes (such as pentadecanal), 1-formyl-2-acyl-*sn*-glycerophospholipids, and 1-(*O*-1'-hydroperoxy)-2-acyl-*sn*-glycero-3-phospholipids (Fig. 2).⁶ Decomposition of the allylic hydroperoxides produces plasmalogen epoxides via a radical process, and 1-formyl- and 1-lyso-phospholipids by hydrolytic processes.^{6d,7}

Plasmalogens are more susceptible to oxidation than phosphatidylcholine (PC) and sphingomyelin, the other major membrane phospholipids;⁸ however, sphingomyelin has also been found to inhibit PC peroxidation, albeit less efficiently than plasmalogen, but its long-chain base contains a trans double bond near the membrane-water interface.^{9a} To elucidate the role of the naturally occurring (*Z*)-*O*-vinyl linkage of plasmalogen in protecting polyunsaturated lipids from oxidative degradation, we describe herein the first chemical synthesis of an unnatural analogue of plasmalogen with an (*E*)-*O*-vinyl linkage at the *sn*-1 position (*trans*-**1**, Figure 1)¹⁰ and a comparison of the antioxidant effects of *cis*- and *trans*-**1** in two model systems.

The first model system we investigated was the reaction of *cis*- and *trans*-**1** with hypochlorous acid (HOCl), a highly reactive oxidant and chlorinating agent that is produced endogenously when physiological concentrations of chloride ion are oxidized by the myeloperoxidase-catalyzed decomposition of hydrogen peroxide.¹¹ HOCl reacts with many biological molecules,¹² including the double bonds of lipids, to generate chlorinated products.¹³ Recently, Davies and co-workers showed that the kinetics of vinyl ether oxidation is several orders of magnitude greater than that of aliphatic alkenes.¹⁴ The products of plasmalogen oxidation by HOCl are 2-chloro fatty aldehydes and 1-lysophosphatidylcholine (LPC).¹⁵ Phospholipid chlorohydrins in the *sn*-2 chain of unsaturated LPC molecular species are also formed as secondary reaction products by electrophilic attack of HOCl on alkenyl double bonds.¹⁶ These chlorinated lipid species accumulate in activated neutrophils, monocytes, ischemic myocardium, and human atherosclerotic lesions, and have potential roles in many inflammatory disorders.¹⁷

Peroxidation of phospholipids containing a polyunsaturated fatty acyl chain such as linolenic acid has been studied frequently by using free-radical initiators, such as the water-soluble thermolabile free-radical generator 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH).¹⁸ As a model system to compare the antioxidant capability of *cis*- and *trans*-**1**, we studied the influence of **1** on the rate and extent of peroxidation of 1-palmitoyl-2-linolenoyl-phosphatidylcholine (16:0-18:2-PC) in liposomes exposed to AAPH. The reaction kinetics were monitored by measuring the formation of conjugated diene lipid hydroperoxides, as monitored spectrophotometrically by the absorbance at 234 nm.⁹ A_{234} is diminished when AAPH-induced free-radical chain initiation in a polyunsaturated acyl chain is suppressed.

Previous reports of the preparation of 1-*O*-alkenyl ether derivatives of glycerol were based on elimination reactions of α -halo cyclic glycerol acetals, which gave low yields and poor stereoselectivity.¹⁹ To construct the glycerol backbone with an *O*-1'-alkenyl chain at the *sn*-1 position, we used (*S*)-glycidol and 1-hexadecanol as the starting materials for the synthesis of *trans*-**1** (see Supporting Information). As shown in Scheme 1, the DPS ether of (*S*)-glycidol (**3**) was employed in a regioselective $\text{BF}_3 \cdot \text{OEt}_2$ -mediated ring-opening reaction²⁰ of (*E*)-octadec-2-en-1-ol (**2**), prepared by DIBALH reduction of ethyl (*E*)-octadecanoate. The expected attack at C-3 provided an 8:1 (separable) mixture of allyl ether **4** and the undesired regioisomer **4a**.

Next, isomerization of the double bond was investigated using transition metal complexes as catalysts.²¹ An initial attempt using $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ ²² as a catalyst resulted in no reaction.

Fortunately, we found that use of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium (I) hexafluorophosphate ($\text{Ir}(\text{COD})[\text{PMePh}_2]_2\text{PF}_6$),²³ after activation with hydrogen for 5 min, resulted in the stereoselective isomerization of allylic ether **4** to enol ether **5** in 2 h at rt, and with the desired E double bond as the only product (Scheme 2). The coupling constant ($J_{\text{H1}'-\text{H2}'} = 12.6$ Hz at δ 6.24 ppm) in the ^1H -NMR spectrum of **5** is indicative of the desired E isomer, which was obtained in 96% yield.²⁴ *sec*-Alcohol **5** was then esterified with oleic acid, using DCC in the presence of DMAP, affording ester **6**. The final steps in the synthesis of *trans*-**1** require conditions that do not cleave the acid-labile and oxidizable vinyl ether moiety or the alkaline-labile acyl ester bond. After the silyl ether in **6** was removed using TBAF (6 equiv) and imidazole (2.5 equiv) in THF at -23°C , the phosphocholine head group was installed at the *sn*-3 position in 54% overall yield without accompanying acyl migration by opening of a cyclic phospholane intermediate in a pressure tube with anhydrous trimethylamine (200 equiv, condensed at -10°C) in MeCN/ C_6H_6 (3:1) in the presence of pyridine.²⁵

We analyzed the HOCl oxidation reaction of *cis*- and *trans*-**1** using increasing concentrations of HOCl. At equimolar concentrations of HOCl and **1**, the extent of plasmalogen oxidation was minimal with both species (data not shown). Figure 3 shows the loss of both *cis*- and *trans*-**1** as the HOCl concentration was increased. At both a 2- and 5-fold molar ratio of $[\text{HOCl}]/[\text{1}]$, the *cis*-vinyl ether linkage was oxidized faster than the *trans* linkage. This selectivity was not observed at a higher HOCl concentration (1.0 mM).

ESI-MS analyses of the reaction mixture with 1 mM HOCl demonstrated that the other product of HOCl oxidation is LPC containing an oleic acid moiety (m/z 544.24) (Figure 4). The chlorohydrin of this material, formed by reaction with HOCl,¹⁵ is a by-product with m/z 596.18. In these spectra, m/z 794.35 is the unreacted *cis*- or *trans*-**1**. These spectra were acquired in the presence of dilute NaOH, which facilitates the production of the observed sodiated adduct ions.

Plasmalogens are known to protect membrane phospholipids against radical-induced damage.^{1a} Therefore, we next evaluated the ability of *cis*- and *trans*-**1** to protect 16:0-18:2-PC from radical-mediated peroxidation in liposomes. The liposomes, which were prepared with 80 mol % 16:0-18:2-PC and 20 mol% *cis*- or *trans*-**1**, were subjected to oxidation by 3.3 mM AAPH. The oxidation of 16:0-18:2-PC²⁶ was monitored by the increase in absorbance at 234 nm, a measure of conjugated diene hydroperoxide formation in the *sn*-2 fatty acyl group of PC.²⁷ Figure 5 shows that *cis*-**1** inhibited the rate of AAPH-mediated oxidation of 16:0-18:2-PC by 33% whereas *trans*-**1** inhibited the rate by only 12%, based on the slopes of the reaction curves in the linear range (0–100 min). Similar data were obtained when 1 mM AAPH was used to induce oxidation (data not shown).

We also determined the effect of *cis*- and *trans*-**1** on 16:0-18:2-PC oxidation in liposomes in the presence of 50 μM Cu^{2+} as the oxidizing agent, which facilitates free-radical chain propagation.²⁸ We found that *trans*-**1** was completely ineffective in protecting 16:0-18:2-PC oxidation whereas *cis*-**1** inhibited PC oxidation by 45% (results not shown).

In summary, the first chemical synthesis of *trans*-**1**, an unnatural analog of plasmalogen bearing a *trans* *O*-vinyl linkage at the *sn*-1 position, has been achieved. A key step in the synthesis involves *E* stereoselective enol ether formation of **5** via an iridium(I)-mediated olefin isomerization of *O*-allyl ether **4**. The kinetic data (Figure 3 and Figure 5) indicate that the geometry of the alkenyl ether linkage of plasmalogen plays a significant role in plasmalogen's antioxidant properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structures of naturally occurring *cis*-1 and the unnatural *trans*-1 analogue.

Figure 2. Structures of 1-formyl- and 1-(*O*-1'-hydroperoxy)plasmalogen derivatives formed on reaction of *cis*-1 with reactive oxygen species.

Figure 3.

Disparate *cis*- and *trans*-1 oxidation by HOCl.

The bars show the amounts of enol ether remaining (left panel) and the amount of 2-chloro-aldehyde produced (right panel) (both in nmoles) after treatment with various HOCl concentrations (in mM) for 5 min at 37 °C. Data are the mean $\pm$ SD of 3 experiments. See S.I. for experimental details.

Figure 4.

ESI-MS of the HOCl oxidation products.

cis- or *trans*-1 (50 nmol) was incubated in 500 μ L of 20 mM phosphate-buffered saline containing 0.1 mM diethylenetriaminepentaacetic acid (pH 7.0) in the presence or absence of 1 mM chloride-free NaOCl for 5 min at 37 $^{\circ}$ C. After the reactions were terminated by addition of MeOH, products were extracted into CHCl_3 . Reaction products and precursor *cis*-or *trans*-1 were resuspended in 500 μ L of MeOH/ CHCl_3 (4:1) containing 1 μ M NaOH and analyzed by ESI-MS in the direct infusion mode at a flow rate of 3 μ L/min. Samples were analyzed in the positive ion mode with detection of their sodiated adducts.

Figure 5.
Effect of *cis*- and *trans*-1 on the oxidation of 16:0-18:2 PC induced by AAPH.

Scheme 1.
Preparation of Allyl Ether 4