Xanthine Oxidase Initiated Oxidation of Model Membranes. Effect of Position of Abstractable Hydrogen Atoms in the Bilayer on the Distribution of Products

Ned A. Porter* and Laura S. Lehman

P. M. Gross Chemical Laboratories
Duke University, Durham, North Carolina 27706

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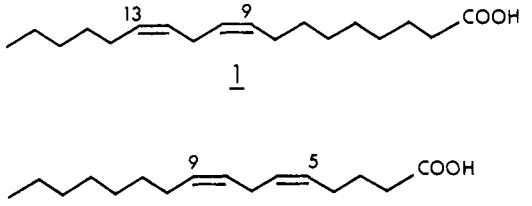
Polyunsaturated phospholipids are abundant in biological membranes, and the random oxidation of these membrane lipids has been suggested to be involved in a variety of pathological events.[1] While considerable effort has been made to establish the mechanisms of initiation of membrane oxidation,[2] most studies have utilized complex or undefined mixtures of phospholipids as model membranes. For this reason, little information about the nature of the oxidation products or the effect of membrane composition on products is available. We report here the xanthine oxidase initiated oxidation of well-characterized phospholipid bilayers. Our experiments focus on the products of oxidation and examine the effect of membrane composition on the distribution of these products.

Five phosphatidylcholines were prepared from linoleic, **1**, lorionic,[3] **2**, and palmitic acids, **3**, by conventional methods.[4]



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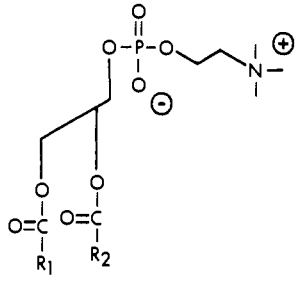
Oxidation of multilamellar vesicles of these phospholipids was initiated (0.3 mM phosphatidylcholine, 37 °C) by 11.6 nM xanthine oxidase,[5] 20 mM acetaldehyde, 50 mM phosphate buffer



DiLinPC : $R_1 = R_2 = 1$
DiLorPC : $R_1 = R_2 = 2$
LinLorPC : $R_1 = 1$; $R_2 = 2$
PLinPC : $R_1 = 3$; $R_2 = 1$
PLorPC : $R_1 = 3$; $R_2 = 2$

(pH 7.0), and 0.1 mM EDTA.[6] Extent of oxidation was monitored by reverse-phase HPLC, and reactions were generally carried out to less than 10% conversion.[7]

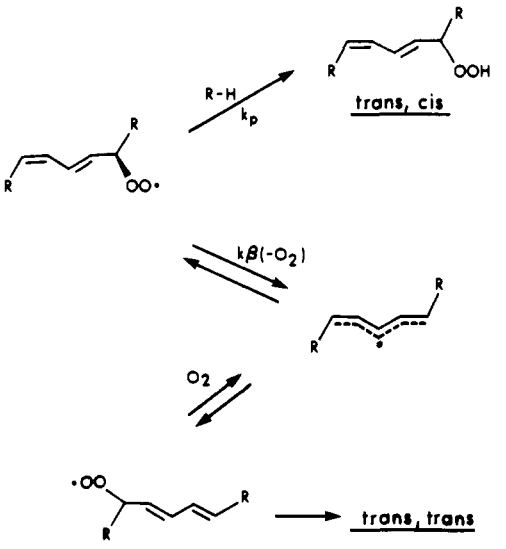
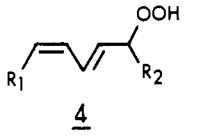


Figure 1.

Peroxide products derived from phospholipid oxidations were reduced (Ph_3P) and converted to fatty acid methyl esters by treatment with CH_3OH/KOH. The eight hydroxymethyl esters formed as oxidation products were analyzed by normal-phase HPLC.[8] The four oxidation products from linoleate have been previously identified as the 9- and 13-substituted *trans,cis*-**4** and



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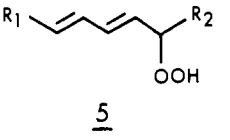
LINOLEATE PRODUCTS

$R_1 = C_5H_{11}$, $R_2 = -(CH_2)_7COOCH_3$
$R_1 = -(CH_2)_7COOCH_3$, $R_2 = C_5H_{11}$



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LORIONATE PRODUCTS

$R_1 = C_7H_{15}$, $R_2 = -(CH_2)_3COOCH_3$
$R_1 = -(CH_2)_3COOCH_3$, $R_2 = C_7H_{15}$

trans,trans-**5** fatty acid methyl esters,[9] and the four comparable products from lorionic acid (9- and 5-substituted) were identified by spectroscopic techniques. The product distributions presented in Table I are corrected for the absorbance of each component.

The initial peroxy radicals derived from diene fatty acids such as linoleic or lorionic acid may either[10] (1) abstract hydrogen atom from substrate to give trans,cis products or (2) undergo β scission to eventually form the thermodynamically more stable trans,-trans-hydroperoxide products (Figure 1). The distribution of trans,cis and trans,trans products clearly reflects this competition. Thus, (trans,cis)/(trans,trans) product ratios are found to be greater than 1.0 for diunsaturated lecithins (entries 1–3) while those lecithins having one saturated chain gave (trans,cis)/(trans,trans) ratios less than 1.0 (entries 4–6). Lecithins containing only one unsaturated chain have a lower bilayer concentration of readily abstractable hydrogens, and the pathway leading to trans,cis products (Figure 1) is less competitive with β scission

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(3) *cis*-5,8-Hexadecadienoic acid has been given the trivial name lorionic acid. This previously unreported fatty acid was prepared by the coupling of the dimagnesium bromide salt of 5-hexynoic acid and 1-bromodec-2-yne in the presence of Cu(I). Hydrogenation of the resulting diyne in the presence of Lindlar's catalyst gave the desired cis diene.

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(5) Xanthine oxidase was a gift from Dr. I. Fridovich.

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(8) At low extent of oxidation the only products detected were the phospholipid hydroperoxides. Oxidations were monitored between 1% and 15% oxidation.

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Table I. Product Distribution of Polyunsaturated Lipid Autoxidation[a]

			linoleates				lorionates				(t,c)/(t,t)	
expt	lipid	n^c	13-t,c	13-t,t	9-t,c	9-t,t	9-t,c	9-t,t	5-t,c	5-t,t	linoleate	lorionate
1	DiLorPC	4					1.00	0.54	0.56	0.34		1.77 ± 0.04
2	DiLinPC	6	1.00	0.77	0.99	0.79					1.28 ± 0.05	
3	LinLorPC	2	1.00	0.89	1.01	1.03	1.18	0.92	0.69	0.71	1.05 ± 0.01	1.15 ± 0.02
4	PLorPC	8					1.00	1.16	0.73	1.06		0.78 ± 0.04
5	PLinPC	6	1.00	1.55	0.99	1.63					0.63 ± 0.04	
6	PLin:PLorPC (1:1)	8	1.00	1.70	1.03	2.05	1.11	1.59	1.00	1.54	0.54 ± 0.03	0.67 ± 0.03
7	Lin acid.Lor acid[b]		1.00	0.43	0.84	0.38	0.52	0.27	0.45	0.23	2.3	1.9

[a] Product mixtures containing both linoleate and lorionate are referred to 13-*t*,*c*-linoleate = 1.0. In oxidations of lorionate alone, products are referred to 9-*t*,*c*-lorionate = 1.00. Conditions of oxidation as noted in text. [b] Autoxidation of a 1:1 mixture of linoleic acid and lorionic acid (0.12 M each) in 1.5 M cyclohexadiene in benzene. [c] Number of analyses for each experiment.

for these substrates compared to phospholipids containing two unsaturated acyl groups.[9b]

The following results from xanthine oxidase studies of bilayer oxidation are also noteworthy:

(1) The products of xanthine oxidase initiated bilayer oxidation are identical with those found in the spontaneous autoxidation of phospholipid bilayers. Product distribution for enzymatic oxidation at pH 7 was virtually identical with that obtained by spontaneous oxidation. *The distribution of products is independent of mode of initiation and depends only on bilayer constituents.* The xanthine oxidase reaction was, of course, significantly faster than spontaneous oxidation.

(2) In mixed bilayers of linoleate or lorionate phospholipids, extent of oxidation of these two fatty acyl groups is equivalent. Thus, oxidation of a 1:1 mixture of PLinPC and PLorPC gives linoleate and lorionate oxidation products in a nearly 1:1 ratio (Table I, entry 6). This result is in marked contrast to results obtained in bulk-phase oxidation where linoleate appears to be oxidized nearly twice as readily as does lorionate (Table I, entry 7). In previous bulk-phase fatty acid oxidation studies, we have reported that hydrogen atoms near the tail of a fatty acid chain are more abstractable than those near the head. For example,[10] autoxidation of a mixture of arachidonic and linoleic acids gave relative H atom donating abilities as follows: arachidonic C-7 = 0.86, C-10 = 0.70, C-13 = 1.33; linoleate C-11 = 1.0. Singlet oxygen oxidation of arachidonic acid in bulk phase also gives rise to analogous hydroperoxide products with a similar preference for products formed at the tail of the unsaturation.[11] We suggest that the skewed product distribution in bulk phase must result from some conformational bias that exposes the tail of the fatty acid toward oxidation. In bulk phase then, linoleate is more readily oxidized than is lorionate. In the bilayer, on the other hand, where fatty acyl chains are known to have extended conformations, hydrogen atoms near the head group (lorionate) are as abstractable as those near the tail (linoleate).

(3) The relative orientation of fatty acyl hydrogen atom donor and peroxy free radical in the bilayer influences the course of oxidation. This can be seen by comparing the (trans,cis)/(trans,trans) ratios observed in entries 1 and 2 (Table I) with the ratio shown in entry 3. Recall that this product ratio serves as a measure of available abstractable hydrogen atoms (Figure 1). Lorionate is a beter donor to peroxy radicals derived from lorionic phospholipid ((trans,cis)/(trans,trans)$_{Lor}$ = 1.77, entry 1) than is linoleate ((trans,cis)/(trans,trans)$_{Lor}$ = 1.15, entry 3). Similarly, linoleate is a better donor to peroxy radicals derived from linoleate phospholipid than is lorionate. It thus appears that *peroxy radicals derived from lorionate prefer reaction with lorionate while peroxy radicals derived from linoleate prefer reaction with linoleate.*

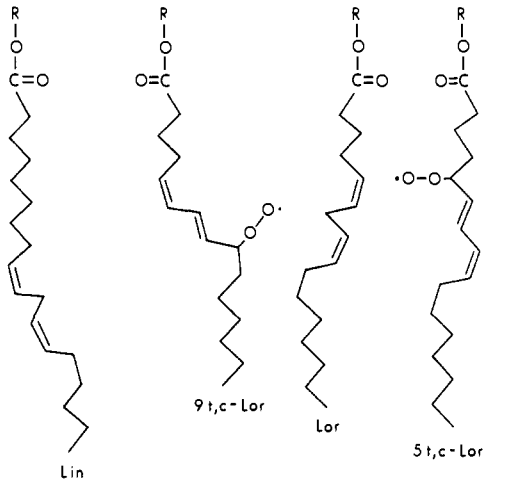

Figure 2.

Similar effects are observed in mixtures of PLinPC and PLorPC (entries 4–6).

A reasonable explanation for this observation rests on the orientation of linoleate or lorionate peroxy radicals in the bilayer relative to oxidizable substrate.[12] The reaction hydrogen atoms of the lorionic and linoleic lecithins occupy different positions in the hydrophobic region of the bilayer (Figure 2). Exchange of one lorionate acyl substituent (DiLorPC) by a linoleate (LinLorPC) introduces the saturated portion of linoleate into the region of the bilayer occupied by the lorionate peroxy radicals. Linoleate thus acts as an inert diluent to the region of the bilayer (although not as inert as is palmitate) occupied by lorionate peroxy radicals.

We note that while the foregoing arguments constitute a framework for understanding product distribution in membrane-phase oxidation, the effect of other variables (membrane fluidity, dissolved oxygen profile) on the oxidation products remains to be clarified. In this regard, explanations for the following observations would appear to require more experimentation: (1) (trans,cis)/(trans,trans) ratios for lorionate products are consistently higher than those for linoleate products; (2) products formed at the 9-position of lorionate exceed those formed at the 5-position (9:5 = 1.1–1.80) while 13:9 ratios are close to 1.0 for linoleate products.

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(12) For examples of orientational controlled reactivity in organized bilayers, see: (a) Czarnicki, M.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *101*, 3675. (b) Gupta, C.; Radhakrishnan, R.; Gerber, G.; Olsen, W.; Quay, S.; Khorana, H. G. *P. Nat. Acad. Sic. U.S.A.* **1979**, *76*, 2595.