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Brief Communication

INHIBITORY EFFECT OF ETHANOLAMINE PLASMALOGEN ON IRON- AND COPPER-DEPENDENT LIPID PEROXIDATION

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Abstract—The effect of ethanolamine plasmalogen (EtnPm) on lipid peroxidation was investigated in liposomal suspension of egg yolk phosphatidylcholine. EtnPm inhibited iron- and copper-dependent peroxidation in the presence of preformed hydroperoxides, although it was not effective for radical initiator mediated lipid peroxidation. EtnPm resulted in complete binding of iron to liposomal lipids, suggesting that EtnPm exerts its inhibitory effect on lipid peroxidation through inhibiting preformed peroxide decomposition by trapping transition metal ions.

Keywords—Plasmalogen, Transition metals, Lipid peroxidation, Free radicals

INTRODUCTION

Plasmalogens describe the glycerophospholipids that contain a vinyl ether moiety at the *sn*-1 position and are found in a wide range of tissues in the animal kingdom. The proportion of plasmalogens is relatively high in heart, striated muscle, and nervous tissues, immune cells, neutrophils, and macrophages.[1] Plasmalogens have been suggested as a storage terminal for polyunsaturated fatty acids because eicosapentaenoic acid and docosahexaenoic acid content in the muscles of fish oil-supplemented ruminants is higher in plasmalogens than that of the diacyl subclasses.[2] It is conceivable that animals preferentially conserve highly polyunsaturated fatty acids in plasmalogens to prevent their peroxidation because recently suggested function of ethanolamine plasmalogen (EtnPm) is its role in protecting animal cells against photosensitized killing by acting as a singlet oxygen quencher.[3] The aim of this study is to investigate the effect of EtnPm on the oxidative modification of liposomal suspension of phosphatidylcholine (PtdCho) induced by transition metals in the presence of cumene hydroperoxides.

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EXPERIMENTAL PROCEDURES

Multilamellar liposomes were prepared according to the method of Yoshida et al.[4] Briefly, egg yolk Ptdcho (PC-98HC, Q P. Corp., Tokyo) in a chloroform solution was evaporated to dryness, and the dried lipid film was dispersed in 10 mM Tris-HCl buffer (pH 7.4) by vigorous shaking on a vortex mixer followed by ultrasonication under argon gas in ice bath for 2 min. Final concentration in the liposomal solution was 4 mM PtdCho and 0.4 mM additive phospholipids; PtdCho, dipalmitoylphosphatidylethanolamine (DPPE, Sigma Chemical Co., St. Louis, MO) and ox brain EtnPm composed of 98% alkenyl form (Funakoshi Co., Ltd., Tokyo). Transition metal ion-dependent peroxidation was induced either by the addition of 0.1 mM $FeSO_4$—1 mM ascorbic acid—0.04 mM cumene hydroperoxides (Nacalai Tesque Inc., Kyoto), or by 10 μM $CuSO_4$—0.04 mM cumene hydroperoxides. Free radical mediated peroxidation was induced by the addition of 20 mM 2,2′-azobis (2-aminopropane) dihydrochloride (AAPH)[4] (Wako Pure Chemicals CO., Osaka). Incubation was carried out in water bath at 37°C with constant shaking. The samples were removed at appropriate intervals for lipid peroxide assays. The transition metal ion-dependent oxidation was terminated by adding final concentration of 20 μM butylated hydroxytoluene (BHT) (Nacalai Tesque Inc.)

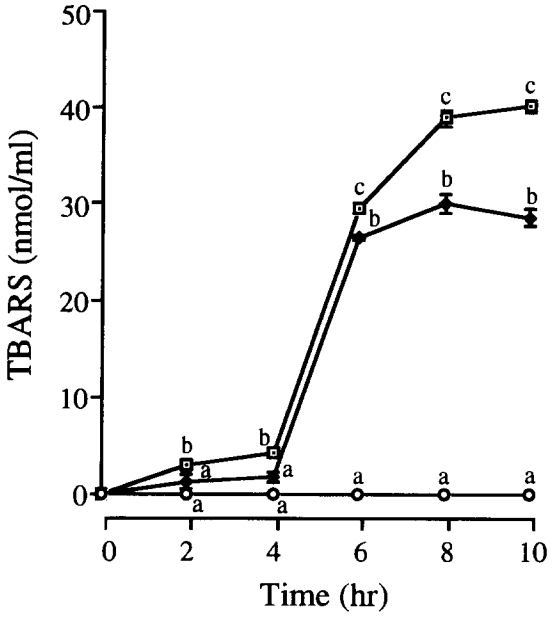

Fig. 1. Effect of EtnPm, DPPE, and PtdCho on iron-dependent peroxidation of PtdCho liposomes. Each point and bar shows mean ± SE for three determinations. Different superscript letters show significant difference at $p < 0.05$. ⊡-PtdCho, ♦-DPPE, ○-EthPm.

and 2 mM disodium ethylenediaminetetraacetic acid (Na_2-EDTA). Free radical-mediated peroxidation was terminated by immersing the samples in liquid nitrogen.[5] All samples were kept at −70°C until to be assayed within several hours. Lipid peroxidation initiated by transition metal ions was evaluated by either thiobarbituric acid (TBA) assay[6] or ferrous oxidation xylenol orange (FOX) assay.[7] Free radical-mediated peroxidation was evaluated by FOX assay.

Liposomes composed of 4 mM PtdCho and 0.4 mM additive phospholipids were subjected to iron-dependent peroxidation for 2 h as described earlier, but without addition of Na_2-EDTA. The reaction mixture (0.9 ml) was partitioned with 2 ml of chloroform/methanol (1:1, vol/vol), and the organic layer was replaced with xylene. Iron concentration in the organic phase was determined with high performance inductively coupled plasma (ICP) mass spectrometer (MS) (PMS 2000, Yokogawa Electric Co., Tokyo) equipped with electrothermal vaporizer according to the manufacturer.

Statistical analyses were carried out by Duncans new multiple range test.[8]

RESULTS AND DISCUSSION

Iron-ascorbate-dependent peroxidation was measured according to the TBA assay method. As shown in Figure 1, TBA reactive substances (TBARS) in the PtdCho liposome increased markedly after 4 h incubation. This relatively long time lag may be due to the fact that phosphatidylcholine used for the liposome preparation might contain a substance prolonging an initiation of peroxidation reaction. EtnPm completely suppressed the elevation of TBA reactive substances throughout the incubation period. DPPE was not as effective as EtnPm to suppress the elevation of TBARS although the formation of TBARS at 6, 8, and 10 h was significantly lower in the DPPE liposome as compared to the PtdCho liposomes. These data suggest that vinyl ether moiety is requisite for suppressing the elevation of TBARS. Subsequently, copper ion-dependent peroxidation was measured according to FOX assay procedure, which determines oxidized ferric ions.[7] As shown in Figure 2, copper ion-dependent lipid peroxidation of PtdCho liposome proceeded by 6 h, but markedly increased subsequently. EtnPm resulted in a gradual elevation of the FOX reactive substances (FOXRS), and the extent was markedly lower than that of PtdCho liposomes at 10 and 12 h incubation. The elevation of FOXRS of DPPE liposomes was lower than that of PtdCho liposomes, but the extent was greater than that of EtnPm liposomes. When egg yolk PtdCho hydroperoxides[4] were used as preformed hydroperoxides instead of cumene hydroperoxides, EtnPm was also effective to suppress the elevation of lipid hydroperoxide formation, (unpublished observation).

The effect of EtnPm on free radical-mediated perox-

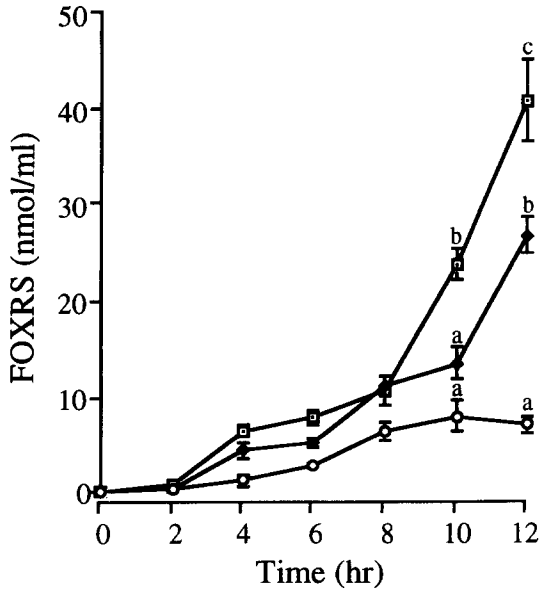

Fig. 2. Effect of EtnPm, DPPE, and PtdCho on copper-dependent peroxidation of PtdCho liposomes. Each point and bar shows mean ± SE for three determinations. Different superscript letters show significant difference at $p < 0.05$. ⊡-PtdCho, ♦-DPPE, ○-EthPm.

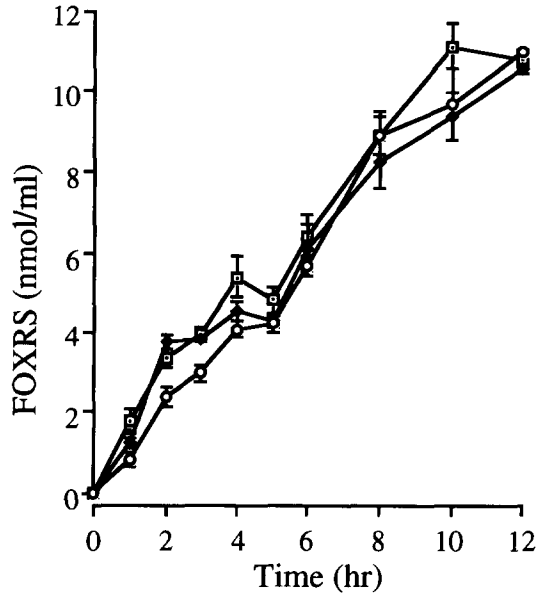

Fig. 3. Effect of EtnPm, DPPE, and PtdCho on AAPH-mediated lipid peroxidation of PtdCho liposomes. Each point and bar shows mean ± SE for three determinations. Different superscript letters show significant difference at $p < 0.05$. ⊡-PtdCho, ♦-DPPE, ○-EthPm.

idation was subsequently measured with AAPH, which yields radicals at known and reproducible rates on thermolysis.[9] As shown in Figure 3, EtnPm did not possess any significant inhibitory action on lipid hydroperoxide formation estimated by FOX reactive substances, and all the liposome preparations exhibited elevation of FOX reactive substances to the same extent.

As shown in Table 1, 90% of iron incubated with EtnPm liposome was recovered in organic phase when the incubation was stopped immediately, and all iron was recovered in the organic phase when incubated for 2 h. On the contrary, the recovery of iron in the organic phase was approximately 10% when incubated in PtdCho and DPPE liposomes. These results thus indicate that EtnPm containing liposomes have a remarkably higher affinity for iron.

It has been proposed that transition metal ions (copper, iron) initiate lipid peroxidation by generation of highly reactive peroxyl (LOO•) and alkoxyl (LO•) radicals from the decomposition of preformed lipid hydroperoxides (LOOH), which subsequently initiate new round of radical chain oxidation according to the following scheme.[10]

$$LOOH + Cu^{2+}/Fe^{3+} \rightarrow LOO^{\bullet} + H^{+} + Cu^{+}/Fe^{2+} \quad (1)$$

$$LOOH + Cu^{+}/Fe^{2+} \rightarrow LO^{\bullet} + OH^{-} + Cu^{2+}/Fe^{3+} \quad (2)$$

Iron-ascorbate and copper-dependent oxidation appears to follow the reactions (2) and (1), respectively. Reaction (2) is usually faster than reaction (1), although the exact rates are affected by the chemical nature of the ligands to the metals. This study showed that EtnPm containing liposomes have a strong capacity to chelate transition metal ions and thereby prevent subsequent formation of peroxyl and alkoxyl radicals as shown in reactions (1) and (2). The fact that vinyl ether linkages of EtnPm are easily cleaved by mercuric ion[1] may explain the affinity of EtnPm for transition metal ions. In addition, vinyl ether linkages of EtnPm may facilitate interaction between liposomes and transition metals since EtnPm has been postulated to stay in the non-bilayer phase in the presence of high amounts of lipids that prefer the bilayer arrangement.[11] Our results therefore suggest that EtnPm possesses an antioxidant activity in transition metal ion-dependent lipid peroxidation in the presence of preformed hydroperoxides, thereby protecting peroxidation of highly polyunsaturated fatty acids in the tissue and immune cells.

Table 1. Iron Binding Capacity of EtnPm, DPPE, and PtdCho Liposomes

	Type of Liposomes		
Incubation Time	EtnPm	DPPE	PtdCho
	(% recovery of added iron in organic phase)		
0 h	91.7 ± 0.7[a]	8.3 ± 0.3[b]	10.3 ± 0.3[c]
2 h	100 ± 0.0[a]	7.5 ± 0.1[b]	12.3 ± 0.2[c]

Values are means ± SE for three separate determinations. Different superscript letters show significant difference at $p < 0.05$.

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