Preventive Action of Phospholipids on Decomposition of Methyl 13-Hydroperoxyoctadecadienoate

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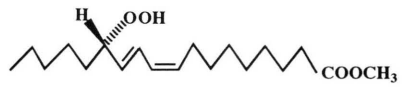
The effects of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine on the thermal decomposition of methyl 13-hydroperoxyoctadecadienoate were investigated in a chloroform/methanol mixture. Among the phospholipids tested, phosphatidylserine inhibited the thermal decomposition of methyl 13-hydroperoxyoctadecadienoate to about 30% after exposure to 60 °C for 48h wheareas only 10% remained in the absence of the phospholipid for the same heating period. Addition of eugenol with phosphatidylserine showed high synergistic activity which prevented the decomposition of the hydroperoxide to a higher extent under the same conditions. Phosphatidylserine was also found to inhibit iron – accelerated decomposition of methyl 13-hydroperoxyoctadecadienoate. Moreover, the decomposition of the hydroperoxide accelerated by ascorbic acid was effectively prevented by a combination of eugenol and phosphatidylserine.

Introduction

A number of studies have been reported on the toxicity and nutritive problems associated with lipid peroxides produced during the autoxidation of food oils (Kaneko *et al.,* 1987; Imagawa *et al.,* 1983). Lipid hydroperoxides, the primary oxidation products formed by radical-initiating autoxidation or by catalysis with various lipoxygenases are found ubiquitously in the biological kingdom, and numerous secondary oxidation products such as ketones, epoxides and aldehydes formed by decomposition of lipid hydroperoxides are thought to be the substances most responsible for the toxicity (Schauenstein 1967, Summerfield and Tappel 1983). Interaction of lipid hydroperoxides and their oxidation products with proteins and other components have an important impact on flavor stability and texture during processing, cooking and storage. Recent studies suggest that hydroperoxy unsaturated fatty acids themselves are not so much toxic and can be metabolized in the digestive tract (Ohfuji and Kaneda 1973, Terao *et al.,* 1993). Therefore, it is important to prevent the decomposition of lipid hydroperoxides once formed. However, very little information on that problem is available despite of the large number of investigations done on the toxicity of degradation products of lipid peroxides.

We recently reported in this Journal that phenolic antioxidants can effectively prevent the thermal decomposition of methyl 13-hydroperoxyoctadecadienoate (ML-OOH) as a model example of lipid hydroperoxide in methanolic system (Alam *et al.,* 1996). In the present study effects of phospholipids are investigated on the activity of eugenol, a typical phenolic antioxidant, in the prevention of ML-OOH decomposition.



Methyl 13-hydroperoxyoctadecadienoate (ML-OOH)

Materials and Methods

Linoleic acid was purchased from Tokyo Kasei Chemical Co (Japan), soybean lipoxygenase, distearoyl phosphatidylcholine (PC), distearoyl phosphatidylethanolamine (PE) and dipalmitoyl phosphatidylserine (PS) were purchased from Sigma Chemical Co. $Fe(NO_3)_3 \cdot 9H_2O$, eugenol and ascorbic acid were obtained from Nacalai Tesque Inc. (Tokyo, Japan). ML-OOH was prepared in a pure state by enzymatic oxidation of linoleic acid using soybean lipoxygenase and the carboxyl group was methylated with diazomethane followed by silica gel column purification (Baba *et al.,* 1990). The

Abbreviation: PC, Distearoyl phosphatidylcholine; PE, distearoyl phosphatidylethanolamine; PS, dipalmitoyl phosphatidylserine; ML-OOH, methyl 13-hydroperoxyoctadecadienoate; AA, ascorbic acid.

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decomposition experiment was conducted in the dark at 60 °C in a mixture of $CHCl_3$ / MeOH (2:1) containing ML-OOH (20 mg, 60 mM) and phospholipid (4.5 mM) with a final volume of 1 ml, and an aliquot (0.5µl) of the solution was analyzed at different time intervals. The remaining undecomposed hydroperoxide (%) was determined by analyzing the reaction mixture with silica gel TLC (R_f = 0.29, hexane/ethyl acetate, 9:1 v/v; 3 times). The spot intensity was determined at 234 nm by a densitometer as reported previously (Alam *et al.*, 1996). The amount of the remaining hydroperoxides (%) in each experiment is shown in Figs. 1, 2 and 3.

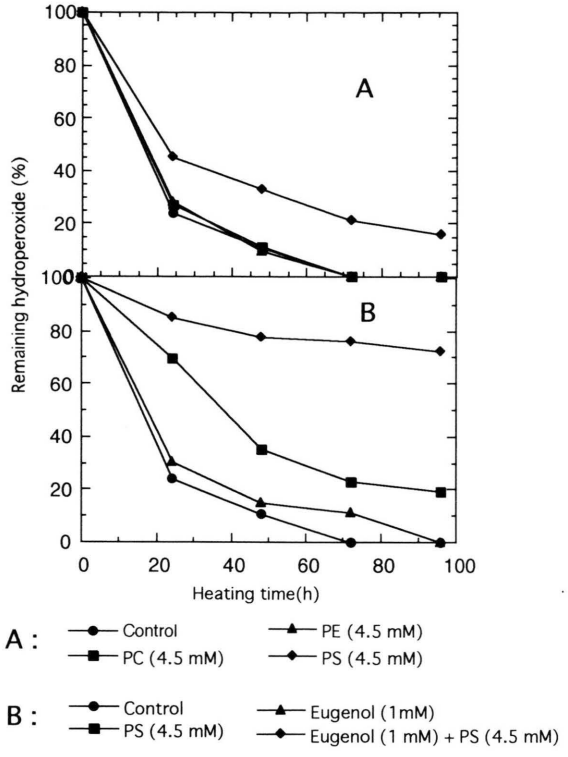

Fig. 1. (A) Preventive action of phospholipids, and (B) preventive action of phosphatidylserine with eugenol on decomposition of methyl 13- hydroperoxyoctadecadienoate (ML-OOH).

Results and Discussion

Fig. 1A shows the preventive activity of the three kinds of phospholipids on the thermal decomposition of ML-OOH (60 °C) . As can be seen from the figure PC and PE among the three phospholipids described above, could not prevent the decomposition of ML-OOH in comparison to PS by which more than 20% of ML-OOH remained after 72 h. Usually, phenolic antioxidants prevent autoxidation of unsaturated lipids and the same trend is also followed in prevention of decomposition of ML-OOH. Accordingly, the compounds that can not prevent autoxidation also can not prevent the decomposition of ML-OOH (Alam *et al.*, 1996). Therefore, it is expected that phospholipids that can not prevent autoxidation of oil may not be able to prevent decomposition of ML-OOH. In their experiments on the autoxidation of perilla oil Kashima *et al.* (1991) observed that none of the phospholipids (PC, PE and PS) show an antioxidant effect. In the present study, however, some preventive activity of PS was observed in the decomposition of ML-OOH. This difference may partly be due to the fact that the phospholipids used by Kashima *et al.*, were isolated from bovine brain which contains phospholipids having both saturated and unsaturated acyl groups, and it has been reported that phospholipids composed of unsaturated fatty acyl groups may rather exert prooxidant effect (Hussain *et al.*, 1986). The phospholipids used in this study contained only saturated fatty acyl group. It can be observed from Fig. 1-B that although PS (4.5 mM) could inhibit the decomposition of ML-OOH to a smaller extent eugenol (1 mM) was not effective when added alone with ML-OOH, whereas they prevent ML-OOH decomposition very efficiently when both are

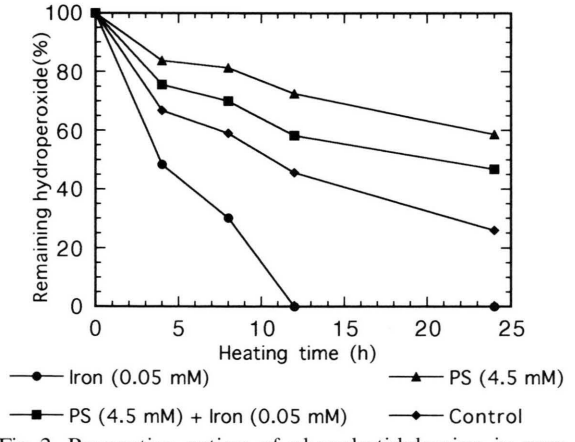

Fig. 2. Preventive action of phosphatidylserine in presence of iron on decomposition of methyl 13- hydroperoxyoctadecadienoate (ML-OOH).

mixed together with ML-OOH. In this case, about 75% ML-OOH remained undecomposed after 72 h, whereas PS alone prevented for about 20% only. This may be regarded as a synergistic effect of PS on the activity of the phenolic antioxidant, eugenol. Relevant to this finding Koga and Terao (1995) reported that phospholipids increased the radical-scavenging activity of α-tocopherol, also a typical phenolic antioxidant, in a bulk oil model system and it contributed to keep the oil fresh for a longer time.

Fig. 2 shows that the presence of iron greatly accelerated the decomposition of ML-OOH. ML-OOH diminished completely within 12 h although about 45% of the ML-OOH remained undecomposed in the control run. In the presence of PS and iron, however, about 60% ML-OOH remained undecomposed and without iron 70% ML-OOH remained. This clearly indicates that PS could also prevent very effectively the iron-accelerating decomposition even without any antioxidant. Usually, edible oils may contain a trace of iron or other metals. Koga and Terao (1994) found that ferric nitrate accelerated accumulation of ML-OOH by the oxidation of methyl linoleate in the model system of lard which comprised of methyl linoleate and methyl laurate, and vitamin E (α- tocopherol) could suppress the accumulation induced by iron. It is known that metals such as iron are mostly responsible for the initiation of autoxidation of edible oils (Pokorny, 1987). The reaction of iron with lipid hydroperoxides yields chain initiating lipid alkoxyl radicals (LO·) or lipid peroxyl radicals (LOO·) (Schaich, 1992). Possibly the polar head group of PS can bind to the ionic iron suppressing iron-induced decomposition of fatty acid hydroperoxides. Yoshida *et al.* (1991) showed that iron is trapped most efficiently by dipalmitoylphosphatidylserine among PS, PE and PC liposomes. PS has a net negative charge at the polar group that can easily trap iron, which leads to inhibition of hydroperoxide decomposition.

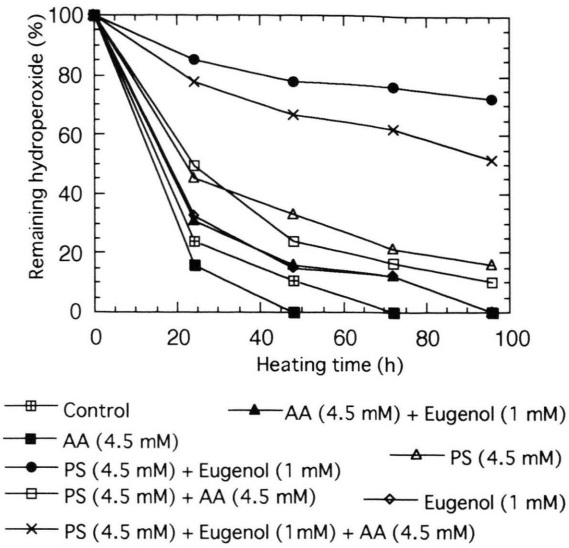

Fig. 3. Preventive action of phosphatidylserine and eugenol on ascorbic acid accelerated decomposition of methyl 13-hydroperoxyoctadecadienoate (ML-OOH).

Fig. 3 shows that ascorbic acid (AA) accelerated the decomposition of ML-OOH and this effect can not be prevented by PS or eugenol alone. But a combination of PS and eugenol was found to prevent effectively the accelerated decomposition of ML-OOH. Although AA is known to be a good radical scavenger but, due to its acidic nature, it rather accelerates decomposition of ML-OOH and phenolic antioxidants including butylated hydroxytoluene (BHT) could not prevent the decomposition (Alam *et al.*, 1996).

The present study clarifies the synergistic effect of PS with eugenol, in the thermal decomposition of unsaturated fatty acid hydroperoxide methyl ester and the preventive effect of PS itself against the iron – accelerated decomposition of the same hydroperoxide. These findings may give some information on the role of phospholipids in food quality control.

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