Mode of Loss of Phosphatidylethanolamine in Autoxidation

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It is well known that phosphatidylethanolamine (PE) undergoes rapid autoxidation.^{1,2)} Unsaturated fatty acid residues in PE are more sensitive to oxidation than those in other lipids, *e.g.*, triglycerides, phosphatidylcholine, or free fatty acids. In an emulsion system containing a copper salt, an explanation for this sensitivity has been given by us.³⁾ However, a general explanation has not yet been found. It is also well known that the amino groups of PE are labile in the oxidation. In this study, attempts have been made to evaluate how the loss of the fatty acid residues or the amino groups causes the loss of PE during the oxidation process.

Phosphatidylethanolamine was obtained from commercial crude soybean lecithin by means of preparative thin layer chromatography (TLC). The lecithine (200 mg) was applied in a line to a Kieselgel G layer (20×20 cm and 2 mm thick), the layer was developed twice with chloroform-methanol-acetic acid-water (20:3:1:0.5 v/v), the zone containing the PE was detected with a ninhydrin reagent by the transcription method described previously,⁴⁾ and the purified PE was extracted from the zone with methanol; the whole procedure was performed, as far as possible, under oxygen-free conditions.

Among the fatty acids of the soybean PE used, the main and easily oxidizable fatty acid was linoleic acid. The oxidative decrease in fatty acids may therefore be represented by the decrease in linoleic acid concentrations.

Figure 1 shows the oxidation of PE as a thin film at 60°C under atmospheric conditions in the light place. The film (1 mg/cm²) was made by drying a chloroform solution of PE under reduced pressure. The part of the oxidized sample which behavior on TLC is same as intact PE will be called the unchanged PE and was separated by means of the same TLC as used above. The total oxidized PE consisted of the unchanged PE and other changed parts of PE. After conversion to methyl esters, fatty acids of the unchanged PE and the total oxidized PE were analyzed by means of gas-

FIG. 1. Time Course of Decrease in Fatty Acids, Amino Groups in Total Oxidized PE, and Unchanged PE Concentration during the Oxidation of PE Only. Fatty acids, ○—○; amino groups ●—●; unchanged PE, △···△.

liquid chromatography (GLC) under the following conditions: column, 15% diethyleneglycol adipate on Chromosorb WAW, 3 mm×2 m; oven temperature, 190°C; carrier gas, N₂, 1.2 kg/cm²; detector, hydrogen flame ionization type. The amount of the unchanged PE was calculated from the data obtained by quantitative injection of an aliquot of its methyl ester derivative. The amounts of the fatty acids of the total oxidized PE were also determined by the quantitative injection. For the determination of the residual amino groups, the colorimetric method of Yemm and Cocking⁵⁾ using ninhydrin was modified in order to keep samples soluble as follows: the PE sample was dissolved in methyl cellosolve, and 80% aqueous ethanol was used for dilution before the colorimetric determination. As shown in Fig. 1, the decrease in the amino groups was to some extent smaller than that in the linoleic acid residues. The relationship of the decreasing amounts of the amino groups and of the fatty acid residues in the total oxidized PE, to the decreasing amount of the unchanged PE suggested that, at least in the first 2 hr of oxidation, each loss of an amino group or of a fatty acid residue makes a PE molecule or more not behave as PE, *i.e.*, disappear from the PE zone of the TLC.

A sample of PE (5 mg) was also oxidized with 2.5 ml methyl linoleate (ML) at 60°C in a Warburg flask with shaking under atmospheric conditions in the light place. Before analysis, the oxidized mixture was applied to a silicic acid column, the ML and most of its oxidation products were removed by elution with hexane-ethyl ether (8:2 v/v), and the oxidized PE was then eluted with methanol. The palmitic acid residues in the total

oxidized PE decreased when PE was treated in ML even though under oxygen-free or peroxide-free conditions, *e.g.*, the ratio $C_{16:0}/(C_{18:0}+C_{18:1})$ decreased from 2.0 for the control PE to 0.7 for a PE sample treated with ML under unaerobic conditions for 4 hr. We could not give explanation for the decrease. In order to avoid being influenced by this decrease, the relative content of linoleic acid to those of stearic and oleic acids was calculated and expressed in Fig. 2. Figure 2

FIG. 2. $C_{18:2}$ Content of Unchanged and Total Oxidized PE during Oxidation.

Oxidation of PE only at 60°C in a film, total oxidized PE, ○---○; unchanged PE, ●...●. Oxidation of a mixture of PE and ML at 60°C, total oxidized PE, △—△; unchanged PE, ▲—▲.

shows that, in the oxidation of both PE only and PE in ML, the linoleic acid residues in the unchanged PE decreased always more rapidly than those in the total oxidized PE. This tendency prominently appeared in the case of PE in ML. The small decrease in the linoleyl residues of the total oxidized PE suggested that the main route of PE degradation, of PE in ML, was

the reaction of the amino groups with the oxidation products of ML. In fact, spots positive to ninhydrin test on TLC disappeared rapidly within 2~4 hr oxidation but quantitative analysis for amino groups failed owing to the interference coloring of the oxidation products of ML. The fact that the content of linoleic acid residues decreased more rapidly in the unchanged PE than in the total oxidized PE suggested that the unsaturated fatty acids in the intact PE species are more labile to oxidation than those in PE derivatives, such as are formed by the amino-carbonyl reaction. It is interesting from the point of view of food chemistry that, after PE has been modified in this way, the high susceptibility of its fatty acid residues to oxidation is lost, and the change may cause a decrease in the potential activity of PE in off-flavor formation.

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