The Effects of Copper and EDTA on the Autoxidation of Phospholipid Emulsions

By Makio MORITA and Masao FUJIMAKI

Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo

Received December 1, 1971

Phosphatidyl ethanolamine and lecithin dispersed in water rapidly absorbed oxygen. The autoxidation was promoted with copper and inhibited with EDTA. Phosphatidyl ethanolamine was more sensitive both to copper and EDTA than lecithin. Their peroxide decomposition under the deaerated condition was promoted with copper and inhibited with EDTA. The copper-catalyzed O_2 uptake of phosphatidyl ethanolamine was lowered, when its peroxide was decreased. Thus, the mechanism of promoting the oxidation with copper may be explained as catalytic supply of initiator radicals from the peroxides by copper.

It has been formerly known that phospholipids readily undergo autoxidation.¹⁾ Lea²⁾ reported that phosphatidyl ethanolamine was as readily oxidized as hundreds times of lecithin. The autoxidation of the phospholipids is the major cause of the oxidized off-odor of dairy products,^{3~5)} and the researchs on the phospholipids autoxidation were mostly concerned with dairy products.^{3~11)} In these reports, the importance of copper and ascorbic acid in the phospholipid oxidation was pointed out^{3~8)} and the main role of phosphatidyl ethanolamine was again emphasized.¹¹⁾

However, the previous investigations were performed from the view of practical purpose and the mechanism of phospholipid autoxidation, especially that of rapid autoxidation in phosphatidyl ethanolamine was not investigated. This paper describes an evidence for the roles of peroxide and transition metal in the rapid autoxidation of phospholipids.

EXPERIMENTALS

Materials. Phosphatidyl ethanolamine and lecithin were separated from crude soya lecithin (Kanto Chem. Co., Ltd.).

The crude lecithin was applied to the column containing silicic acid in chloroform and phosphatidyl ethanolamine was eluted with chloroform-methanol

(95:5).¹²⁾ The sample was pure on a TLC¹³⁾ with detection of iodine vapor and ninhydrin reagent.

Lecithin was separated first by the column chromatography using alumina and ethanol¹⁴⁾ and next by the column chromatography using silicic acid and chloroform-methanol.¹²⁾ For the second column, silicic acid (Kanto Chem. Co., Ltd.) was kept in the atmospheric humidity overnight and reactivated in an oven at 110°C overnight. Lecithin was eluted with chloroform-methanol (2:1 to 1:1) and was pure on the TLC¹³⁾ with detection of iodine vapor and the Dragendorff's reagent.

Methods. Phospholipids were suspended in water, solubilized with the addition of alkali keeping pH below 9, and adjusted to pH 7.5 immediately after solubilized. The emulsion contained some suspended solid.

All experiments were carried out using the 1% emulsion. In the experiments at pH 8.1, 0.3% sodium bicarbonate was added. Copper was added as an aqueous solution of cupric sulfate.

Oxygen uptake was measured by the Warburg's manometry. The samples for the incubation under the deaerated condition were prepared in Pyrex ampoules by degassing in a vacuum line below 10⁻⁴ mmHg and sealing. Peroxide values were measured according to Lea.¹⁵⁾

RESULTS

The O_2 uptakes of phosphatidyl ethanol-

FIG. 1. Oxygen Uptake of Phosphatidyl Ethanolamine Dispersed in Water and the Effects of Copper and EDTA (pH 8.1, 37°C).

▲—▲ control, ×—× 0.1 mg/ml, ○—○ 0.4 mg/ml,
△—△ 2.0 mg/ml.
* EDTA added

FIG. 2. Oxygen Uptake of Lecithin Dispersed in Water and the Effects of Copper and EDTA (pH 8.1, 37°C).

▲—▲ control, ×—× 0.1 mg/ml, ○—○ 0.4 mg/ml,
△—△ 2.0 mg/ml.
* EDTA.

amine and lecithin dispersed in water were affected by the addition of copper or EDTA (ethylenediamine tetraacetic acid) as shown in Figs. 1 and 2. Copper (0.2~5 ppm) much promoted the O_2 uptake of phosphatidyl ethanolamine and EDTA (0.1~2 mg/ml) effectively inhibited it. The effects of copper and EDTA on the O_2 uptake of lecithin were similar to, but smaller than, those of phosphatidyl ethanolamine. The O_2 uptake depended on pH and temperature as shown in Table I. Higher temperature and higher pH value promoted the oxygen uptake. As shown in Fig. 3, the log-log plots of the O_2 uptake rates *vs.* the concentrations of copper added gave the straight lines for phosphatidylethanolamine

TABLE I. O_2 UPTAKE (μ l/30 mg·h) OF PHOSPHATIDYL ETHANOLAMINE AND LECITHIN AND THE EFFECTS OF pH, TEMPERATURE, COPPER, AND EDTA

	pH 8.1 37°C	pH 7.5	
		37°C	50°C
Phosphatidyl ethanolamine			
control	27.4	6.1	61.0
with 1 ppm copper	237.2	141.5	399.6
with 0.4 mg/ml EDTA	4.1	3.0	5.4
Lecithin			
control	12.5	12.5	50.8
with 1 ppm copper	75.8	53.5	140.2
with 0.4 mg/ml EDTA	12.5	6.0	15.8

FIG. 3. Log-Log Plot of O_2 Uptake *vs.* Copper Concentration Added (pH 7.5, 37°C).

and lecithin, the values of the slopes being 0.42 and 0.23, respectively.

Both the O_2 uptakes of phosphatidyl ethanolamine and lecithin in the presence of 2 ppm copper were not influenced by the exchange

TABLE II. PEROXIDE VALUES (mmoles/1000 g) OF THE PHOSPHOLIPIDS INCUBATED AT 50°C FOR 20 HR (pH 7.5)

	Without additive	With 1 ppm copper	With 2 mg/ml EDTA
Phosphatidyl ethanolamine			
control (without incubation)	213	—	—
incubated without O_2	124	0	189
incubated with O_2	803	229	228
Lecithin			
control (without incubation)	876	—	—
incubated without O_2	667	469	738
incubated with O_2	769	369	986

of the head space gas from air to oxygen.

The peroxide values of phosphatidyl ethanolamine and lecithin were listed in Table II. When the phospholipids were incubated under the deaerated condition, their peroxide values decreased in both the cases of phosphatidyl ethanolamine and lecithin. The decreases were promoted by the addition of copper but were inhibited by the addition of EDTA. Figure 4 gives the copper-catalyzed O_2 uptakes in the samples of phosphatidyl ethanolamine which were preincubated under the deaerated condition without and with 1 ppm copper. The control (1) which was not preincubated consumed oxygen linearly against time. The samples (2) (3) which had been preincubated at 50°C under the deaerated condition without and with 1 ppm copper initially absorbed oxygen in the low rate corresponding to their lowered peroxide concentrations. These uptake rates were gradually elevated as the oxidation was advanced.

DISCUSSION

In the measurement of the autoxidation of lipids, the O_2 uptake is suitable for the purpose of studying the mechanism. Although the peroxide value gives alternative informations, its increase does not correspond to the degree of the oxidation, especially in the case of phospholipids.²¹ As TBA value (thio-barbituric acid value) represents the final products of the autoxidation, it is not suitable for the investigation of the mechanism.

The aqueous emulsions of phosphatidyl ethanolamine and lecithin rapidly absorbed oxygen. The oxidations were assumed to be catalyzed with transition metal because they were promoted by the addition of copper and inhibited by that of EDTA as in shown in Figs. 1, 2 and Table I.

It has been known that phosphatidyl ethanolamine undergoes more easily autoxidation than lecithin does²¹ but the oxidation of phosphatidyl ethanolamine must be, rigorously, charac-

FIG. 4. Copper-Catalyzed O_2 Uptake of Phosphatidyl Ethanolamine after Decomposition of its Peroxide (pH 7.5, 37°C, with 1 ppm copper).

Preincubation for decomposition of the peroxide was carried out under the deaerated condition at 50°C for 20 hr.

terized by its extremely high sensitivity to both copper and EDTA. The effects of copper and EDTA on the oxidation of phosphatidyl ethanolamine are surprisingly large and consequently it must be emphasized in foods that the presence of transition metals and chelating substances which affect the activity of the transition metals is very important for the phospholipid autoxidation.

Attempts were made to clarify why copper promotes the autoxidation. In the presence of 2 ppm copper, the rate of O_2 uptake was not affected by the exchange of the head space gas from air to oxygen in both the cases of phosphatidyl ethanolamine and lecithin. This exchange makes $[O_2]$ five times. This result indicates that the rate-regulating step is not affected by the O_2 concentration.

If initiator radicals are supplied from the peroxide accumulated by the catalytic action of copper, it is well explained that the O_2 uptake is not affected by the oxygen concentration, and no accumulation of the peroxide

EDTA inhibited both the peroxide decomposition (Table II) and the autoxidation (Figs. 1 and 2). It follows that the rate of the decomposition of the peroxide well corresponds to the rate of the O_2 uptake.

If the peroxide exists in low concentration, the rate of the copper-catalyzed oxidation will be lowered. In Fig. 4, two samples of phosphatidyl ethanolamine had been preincubated without and with copper under the deaerated condition, the peroxide values being lowered to 60% and 0%, respectively, and the initial rates of the O_2 uptake were lowered according to the peroxide values. The rates were recovered gradually by continuing the oxidation which may accumulate the peroxide. On the contrary, the control sample which contained the sufficient peroxide kept, from the beginning to the end of the experiment, the high and constant rate of the O_2 uptake.

From the assumption of steady-state, the rate of the initiation is equal to that of the termination in the chain reaction (1)~(5).

which has been reported²¹ is also well explained.

If copper catalytically decomposes the peroxide and supplies the initiator, the peroxide will be produced and, simultaneously, consumed, and thus a relatively unchanged concentration of the peroxide will be kept. However, in the absence of oxygen, copper promoted the decomposition of the peroxides and EDTA inhibited it as is shown in Table II. These effects were remarkable on phosphatidyl ethanolamine. When copper was added, the peroxide decomposition was promoted as is shown in the cases without O_2 of Table II, and the autoxidation was also promoted as is shown in Figs. 1 and 2. The addition of

It is reasonable that the termination rate (5) is proportional to $[RO_2\cdot]^2$ and the O_2 uptake is proportional to the propagation rate (4) or $[RO_2\cdot]$. If the initiation rate is assumed to be proportional to $[Cu]$, the following relationship should be led;

$$\text{rate of } O_2 \text{ uptake} \propto [Cu]^{1/2} \quad (i).$$

From Fig. 3, phosphatidyl ethanolamine gave the experimental value of the slope, 0.42, which well agrees with 1/2 of (i). Lecithin gave the slope value of 0.23 in Fig. 3, which is far from the relationship of (i).

The reason why a trace of copper added to aqueous phase of the phospholipid emulsions

catalytically decomposes the peroxide may be that copper is attracted by the polar functional group onto the interface and probably, undergoes some activation, and the peroxide group on the fatty acid moiety also is attracted by its polarity onto the interface.

Different effectiveness of copper on phosphatidyl ethanolamine and lecithin may be responsible for the different functional groups, glycerylphosphoryl ethanolamine and glycerylphosphoryl choline. It is the most interesting subjected in future what mechanism makes copper such an effective catalyst in phosphatidyl ethanolamine.

REFERENCES

- 1) E. Chargaff, M. Ziff and D. Rittenberg, *J. Biol. Chem.*, **144**, 343 (1942).
 - 2) C. H. Lea, *J. Sci. Food Agr.*, **8**, 1, (1957).
 - 3) A. M. Swanson and H. H. Sommer, *J. Dairy Sci.*, **23**, 201 (1940).
 - 4) M. Van der Waarden, *Research in Holland*, **1947**, 155.
 - 5) F. E. Van Haeften and J. W. Pette, *Neth. Milk Dairy J.*, **7**, 41 (1955).
 - 6) R. R. Riel and H. H. Sommer, *J. Dairy Sci.*, **38**, 1215 (1955).
 - 7) R. L. King and W. L. Dunkley, *ibid.*, **42**, 897 (1959).
 - 8) T. Richardson and P. L. Guss, *ibid.*, **48**, 523 (1965).
 - 9) A. M. El-Negoumy, *ibid.*, **48**, 1406 (1965).
 - 10) A. M. El-Negoumy and P. S. Ku, *ibid.*, **50**, 961 (1967).
 - 11) J. P. O'Mahony and W. F. Shipe, *ibid.*, **53**, 636 (1970).
 - 12) E. J. Barron and D. J. Hanahan, *J. Biol. Chem.*, **231**, 493 (1958).
 - 13) V. P. Skipski, R. E. Peterson and M. Barclay, *J. Lipid Res.*, **3**, 467 (1962).
 - 14) D. J. Hanahan, M. B. Turner and M. E. Jayko, *J. Biol. Chem.*, **192**, 623 (1961).
 - 15) Official and Tentative Methods of the American Oil Chemist's Society (1945~1956).
-