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THE EFFECT OF ANTIOXIDANTS ON THE
PEROXIDATION OF BUMOLECULAR PHOSPHOLIPID
MEMBRANES

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Bimolecular Phospholipid Membranes

AUTHOR: Yu. A. Zilber, G. Ya. Dubur, K. K. Kumsar and
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Methods using artificial model systems, especially liquid phospholipid crystals, micelles, are applied in the field of investigation of structure and function of biological membranes. Most often, phospholipid membranes are prepared from lecithin, the content of which reaches 25% in individual types of biological membranes [1, 2]. The physical-chemical properties of phospholipid molecules lie at the base of the process of micelle formation in water solutions. A bimolecular phospholipid layer has a structure and function which are similar to those of a biological membrane [3, 4]. The composition of a phospholipid molecule includes unsaturated and saturated fatty acids. Normal function and structure of the membrane depends on the ratio of saturated and unsaturated fatty acids in the phospholipid molecule [2]. As is known, unsaturated fatty acids are subject to oxidative and, eventually, destructive processes. Membranes in which phospholipid molecules undergoing peroxidation are present lose their characteristic properties. Their permeability and electrical conductivity increase [5].

A possible protection of membranes, by use of synthetically created peroxidation inhibitors, from the adverse action of oxidation processes could disclose great promise for providing for preservation of the membrane structure and function, as well as of regulation of their permeability and enzyme reactions.

Materials and Method

Lecithin (Olaynskiy chemical reagent works) was used as material for preparation of phospholipid micelles. It was purified by silica gel column chromatography before use [3]. The purity of the lecithin fraction obtained was tested by thin-layer chromatography, and the concentration, by dry weight [3]. The pure material was stored in benzene solution at -20°C . Micelle preparation was carried out in 0.15 M KCl solution, with a calculated 15 μmole of lecithin per ml [6]. After introduction of the lecithin into the 0.15 M KCl, the flask was mechanically agitated for a period of 30 minutes. The prepared

micelles remained at a temperature of 4°C overnight. All procedures, beginning with lecithin purification, were carried out in a nitrogen atmosphere.

Change in concentration of dissolved oxygen in the reaction system was determined polarographically in a cuvette with a rotating platinum electrode, in the presence of an oxidation reaction initiator. Recording was carried out on a EPP-09 MZ automatic recording potentiometer. Hemoglobin (Hb), at a concentration of $2 \cdot 10^{-5}$ M, served as catalyst for the phospholipid micelle oxidation processes. The cuvette was thermostatted for measurement of O_2 content, with the temperature maintained at up to 45°C. 2,6-Di-tert-butyl-4-methylphenol (BOT) and 2,2,4-trimethyl-6-ethoxy-1,2-dihydroquinoline (ethoxyquin) were used as antioxidants. The BOT and ethoxyquin were first purified by vacuum distillation, and the BOT was again recrystallized before the experiment.

Results and Their Discussion

A high oxygen consumption by phospholipid micelles, in the presence of catalyst (Hb), can be foreseen from the data obtained (Table). Similar data on O_2 consumption by emulsions of various fatty acids are encountered in the literature [7, 8]. These literature data are evidence that, in our reaction system, the question is precisely of fatty acid peroxidation, since unsaturated and saturated fatty acids are included in the composition of the lecithin molecule. Consumption of 35% of the total oxygen in a reaction system with lecithin micelles takes place in a period of 20 seconds on the average. This time fluctuates from test to test within the limits of 9.0-37.5 seconds. The irregular oxygen consumption rate in various tests, in which identical operating conditions were observed, can be explained by the degree of purity of the lecithin fraction obtained, contamination of the lecithin by oxidation process catalysts and inhibitors and by previous autooxidation of fatty acids in the presence of oxygen during storage.

The data which we obtained demonstrates a low capability for oxygen use in work with micelles from unpurified lecithin. The oxidation reaction rate in use of unpurified lecithin is much lower than in micelles of pure material.

As is known, peroxidation processes are very easily subjected to catalytic and inhibiting actions of certain compounds. The results of the effect of the BOT and ethoxyquin antioxidants on the O_2 consumption rates by lecithin micelles are summarized in the Table. BOT at a concentration of $1 \cdot 10^{-5}$ M decreases the rate of oxidation processes by half. The second antioxidant, ethoxyquin, is a stronger inhibitor of the peroxidation process. In this case, the oxygen consumption rate at a $1 \cdot 10^{-5}$ M antioxidant concentration is decreased on the average by 2/3-3/4 of the control rate.

Moore and others [5] used BOT at a $4.5 \cdot 10^{-5}$ M concentration as an antioxidant for protection of phospholipid membranes. The antioxidant concentration mentioned is sufficient to noticeably decrease sodium ion

Inhibiting action of antioxidants on oxygen consumption by bimolecular phospholipid micelles

No.	Time in which 35% of O ₂ is consumed, sec			
	Control	Ethoxyquin 1·10 ⁻⁵ M	BOT 1·10 ⁻⁵ M	Inhibition of oxidation processes by antioxidants
			21.5	2.1 %
			22.5	2.2 %
			23.5	2.7 %
			25.5	3.5 %

Reaction mixture compositions: 1) 1.5 ml lecithin micelles, lecithin concentration 15 μ mole/ml;
 2) hemoglobin, 0.05 ml, $6 \cdot 10^{-4}$ M;
 3) antioxidants at a final concentration of $1 \cdot 10^{-5}$ M.

[Translator's note: Commas in tabulated figures are equivalent to decimal points.]

diffusion from liquid lecithin crystals. The authors demonstrated, by carrying iodometric tests for the presence of peroxides, that the formation of peroxides is the principal change in diffusion of ions.

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