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INFLUENCE OF IONOL ON THE PERMEABILITY OF LARGE UNILAMELLAR VESICLES IN THE PRESENCE OF EMBRYONIC CALF SERUM

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The influence of various concentrations of an antioxidant – Ionol – (incorporated in the bilayer of large unilamellar liposomes) on the stability of lipoid vesicles in the presence of 10% of embryonic calf serum is considered. The kinetics and temperature dependence of the egress of an intraliposomal marker (6-carboxyfluorescein) from the liposomes has been studied.

The interaction of lipid vesicles (liposomes) with a cell surface or components of the incubation medium leads to a disturbance of the structure of the liposomes. The nature and the mechanism of the destabilization of liposomes depends on the possibility of regulating the processes of peroxide oxidation of lipids. Peroxide oxidation exerts considerable influence on the properties of liposomal membranes: it increases the "rigidity" of the bilayer and its permeability [1], accelerates phospholipid flip-flop [2], and increases the size of the liposomes [3].

Diminution or inhibition of the peroxide oxidation of liposomes can be achieved by introducing antioxidants into the composition of the lipid vesicles. At the present time, among synthetic antioxidants, organic compounds of sulfur – β-mercaptoethanolamine – and phenols – butyl oxidant, butoxyanisole, Ionol, etc. – have found the widest use in medicine and the food industry [4].

In the present paper, we give the results of a study of the influence of various concentrations of the food antioxidant Ionol (2,6-di-*tert*-butyl-4-methylphenol) on the stability of liposomes in the presence of 10% of embryonic calf serum.

An investigation of the properties of liposomes charged with 6-carboxyfluorescein showed that the introduction of Ionol into the composition of the lipid vesicles had a positive influence on the stability of the liposomes in the presence of the serum (Fig. 1). Vesicles of egg lecithin rapidly lost their contents (about half in the first 30 min) in the presence of serum. The incubation of liposomes containing 0.027% of Ionol under the same conditions substantially stabilized the lipid vesicles. An increase in the Ionol concentration to 0.2% led to even greater stabilization of the liposomes, comparable with the stabilization achieved on the addition of cholesterol: in 3 h, about 20% of the carboxyfluorescein included in the liposomes "escaped." Increasing the amount of Ionol incorporated in the liposomes to 2% did not give the expected results: no further increase in the stability of the liposomes in the presence of the serum was observed. A determination of the time of half-egress of the chromophore from the liposomes confirmed these results. It follows from the figure in Table 1 that Ionol stabilizes a membrane in the presence of serum. It is possible that the effect of Ionol, like that of cholesterol, is connected with its condensing action on the lipid bilayer of the vesicles.

With the aim of checking this possibility, we investigated the influence of various concentrations of Ionol introduced into the bilayer of lipid vesicles on the egress of carboxyfluorescein from liposomes as a function of the temperature (Fig. 2). As can be seen from Fig. 2, the addition of 0.02% of Ionol to egg lecithin liposomes had practically no effect on the temperature dependence of the egress of the chromophore from lipid vesicles. However, at Ionol concentrations of from 0.2 to 2.0%, the phase transition temperature rose from +40 to 70°C. Thus, the incorporation of Ionol leads to a shift in the phase transition boundary towards higher temperatures.

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TABLE 1. Influence of Ionol on the Time of Half-Degress $T_{1/2}$ of Carboxyfluorescein from Liposomes in the Presence of Serum

Composition of the liposomes	$T_{1/2}$, h	
	buffer	serum
Egg lecithin	12.86	0.72
Egg lecithin + cholesterol (7:3)	18.01	3.70
Egg lecithin + Ionol (0.02)*	12.20	2.49
Egg lecithin + Ionol (0.2)	23.90	4.09
Egg lecithin + Ionol (2)	25.92	3.57

*In parentheses, the ionol content of the liposomes in percentages by weight.

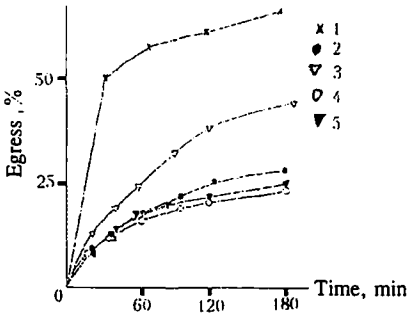

Fig. 1

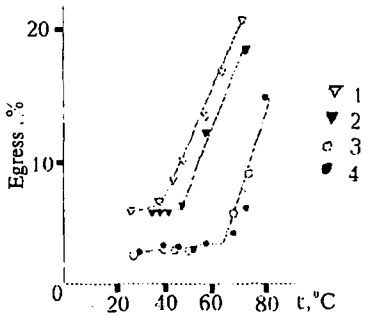

Fig. 2

Fig. 1. Egress of carboxyfluorescein from liposomes with various concentrations of Ionol in a medium containing 10% of embryonic calf serum. Composition of the liposomes: *1*) egg lecithin; *2*) egg lecithin + cholesterol (molar ratio 7:3); *3*) egg lecithin + Ionol, 0.02%; *4*) the same, 0.2%; *5*) the same, 2%.

Fig. 2. Temperature dependences of the release of carboxyfluorescein from egg lecithin liposomes containing various amounts of Ionol. Composition of the liposomes: *1*) egg lecithin; *2*) egg lecithin + 0.02% of Ionol; *3*) the same, 0.2%; *3*) the same, 2%.

Thus, the use of the antioxidant Ionol as a stabilizing agent in phosphatidylcholine vesicles enables the rate of egress of the contents of these liposomes to be regulated.

EXPERIMENTAL

Egg lecithin was obtained by L. I. Barsukov's method [5]. Large unilamellar vesicles were obtained by the reversed-phase method [6].

As an intraliposomal marker we used 6-carboxyfluorescein previously purified as in [7]. The 6-carboxyfluorescein was boiled with activated carbon in ethanol, followed by filtration through filter paper and precipitation with cold ethanol–water (1:2). After washing with water and drying, an orange powder was obtained, which was dissolved in water (250 mM, pH 7.4) and titrated with 1 N NaOH. The resulting solution of carboxyfluorescein was passed through a column (5 × 40 cm) of Sephadex LH-20 at room temperature and the carboxyfluorescein was eluted with distilled water in the form of a dark red fraction after a brown nonfluorescing fraction.

To eliminate nonincorporated carboxyfluorescein, the liposomes were passed through a minicolumn of Sephadex G-25.

Microcolumns were prepared from cylinders of 5-ml plastic syringes, and they were filled with the gel, placed in centrifuge tubes, and centrifuged in the following regime: 2-5 min gradually from 0 to 3000 *g*, 3 min at 3000 *g*, 2-3 min

gradually slowing to a stop. On a column prepared in this way was deposited 0.2 ml of a liposomal preparation and it was centrifuged under the same regime. As a result, the liposomes passed through the column and accumulated in the test tube with practically no change of concentration, and the substances not bound to them were retained in the gel.

The egress of dye from the liposomes was determined as in [8]. For this, 1 ml of phosphate buffer, or of a mixture of buffer with serum, was added to a quartz cell and heated to the required temperature. Then 10 μl of liposomes containing carboxyfluorescein was added and the mixture was rapidly stirred. The intensity of fluorescence was measured after predetermined intervals. At the end of the experiment, 0.1 ml of a 20% solution of sodium deoxycholate was added to destroy the liposomes and complete the release of the dye remaining in them. The percentage release of the chromophore R_t at time t was calculated from the formula

$$R_t=100(F_t-F_0)/[(F_{max}\times 1.1)-F_0],$$

where F_0 is the intensity of fluorescence of 1 ml of buffer plus 10 μl of chromphore-containing liposomes at time 0, F_t is the intensity of fluorescence of 1 ml of buffer plus 10 μl of chromophore-containing liposomes at time t, and F_{max} is the intensity of fluorescence of the solution after the addition of 0.1 ml of sodium deoxycholate.

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