INTERMEMBRANE TRANSFER AND ANTIOXIDATIVE ACTION OF α-TOCOPHEROL IN LIPOSOMES

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The process of free-radical peroxidation in the lipid phase of membranous structures is regulated mainly or solely by vitamin E, interacting with alkoxy- and peroxy-radicals of lipids and thereby breaking the oxidation chain [12, 13]. Tocopherol is distributed unevenly in the lipid bilayer of membranes, forming clusters and preferentially concentrated in regions with a high content of polyenic phospholipids [1, 11]. The development of oxidative stress is invariably accompanied by a fall in the tocopherol concentration [9, 3, 10], and the resulting antioxidant deficiency in the membranes may be local in character. One way of correcting a local vitamin E deficiency may be through intermembrane transfer of α-tocopherol.

Studies of inhibition of lipid peroxidation (LPO) during intermembrane transfer of tocopherols have proved contradictory. For instance, Niki et al. [8, 7] found that α-tocopherol, incorporated into donor liposomes made from polyunsaturated phospholipids had virtually no effect on the velocity of LPO in acceptor liposomes, whereas Fukuzawa et al. showed that this is valid only when the donor liposomes were obtained from saturated phospholipids [6].

The aim of this investigation was to study the effectiveness of the antioxidative action of α-tocopherol, incorporated into donor monolayer liposomes formed from saturated or unsaturated phospholipids relative to acceptor liposomes composed of brain lipids.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g were used in the experiments. Lipids were isolated from the cerebral cortex by the method in [5]. Monolayer liposomes were obtained by ultrasonic treatment (1 min, 22 kHz, 25°C for dimyristoylphosphatidylcholine and at 0-4°C for unsaturated lipids) of lipid dispersions (0.6 mg/ml) in 0.1M K,Na-phosphate buffer (pH 7.4) until complete clarification of the suspension.

α-Tocopherol was incorporated into liposomes by one of two methods: 1) by addition of an alcoholic solution of α-tocopherol to prepared liposomes or 2) by combined solution of lipids and α-tocopherol in chloroform, evaporating the solvent, and carrying out subsequent dispersion and ultrasonic treatment in buffer as described above.

Incorporation and uniformity of the distribution of α-tocopherol in the liposomes were assessed by a fluorescence method [6]. LPO was induced in the liposomes by a system of Fe^{++} (40 μM) + ascorbate (0.5 mM) after preincubation of the liposomes for the time specified in the text (from 1 to 60 min at 37°C). The content of LPO products was determined by the reaction with 2-thiobarbituric acid, by recording absorption at 535 nm [2]. LPO was induced in mixtures of two types of liposomes: donor (containing α-tocopherol) and acceptor (not containing α-tocopherol).

Reagents. α-Tocopherol and dimyristoylphosphatidylcholine were from "Serva," K_2HPO_4 and NaH_2PO_4 were from "Merck," ascorbate from "Reanal," sodium deoxycholate and 2-thiobarbuturic acid from "Sigma," and chloroform and methanol from "Reakhim."

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TABLE 1. Action of Sodium Deoxycholate on Fluorescence of α-Tocopherol in Liposomes (M ± m)

Lipids of liposomes	Intensity of fluorescence, relative units			
	α-tocopherol in inner and outer monolayers		α-tocopherol in outer monolayer	
	–detergent	+ detergent	–detergent	+ detergent
Brain lipids	4.5 ± 0.3	5.0 ± 0.4	2.8 ± 0.3	4.0 ± 0.4
Egg phosphatidylcholine	4.2 ± 0.3	3.9 ± 0.4	2.1 ± 0.3	4.0 ± 0.3
Dimyristoylphosphatidylcholine	4.4 ± 0.4	4.1 ± 0.3	1.8 ± 0.2	3.9 ± 0.4

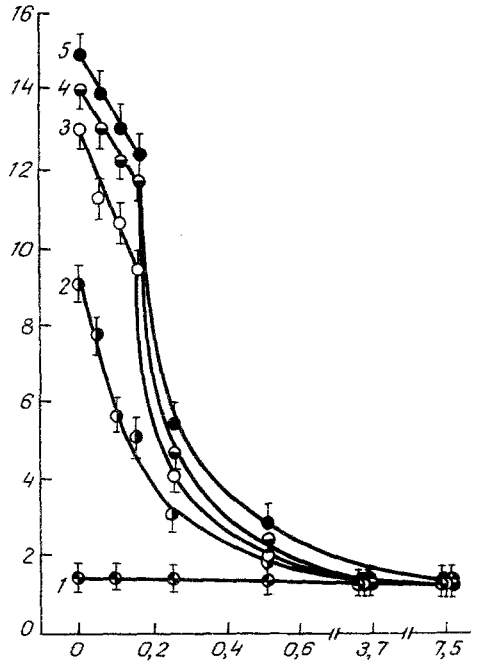

Fig. 1. Accumulation of LPO products in monolamellar liposomes composed of brain lipids (0.6 mg/ml) in the presence of α-tocopherol. 1-5) Time of induction of LPO in liposomes: 0, 5, 15, 30, 60 min respectively. Abscissa, concentration of α-tocopherol (μM); ordinate, LPO products (mmoles MDA/mg lipids).

EXPERIMENTAL RESULTS

In a preliminary series of experiments the uniformity of distribution of α-tocopherol in monolayers of the liposomes was verified. For this purpose the intensity of fluorescence of tocopherol in the liposomes was compared in the presence and in the absence of the detergent, sodium deoxycholate. The results of the measurements of the intensity of fluorescence of tocopherol, incorporated into one or both monolayers of liposomes differing in their lipid composition are given in Table 1. It can be concluded that in the concentration used (7.5 μM) α-tocopherol, incorporated into both monolayers, does not form clusters, i.e., it is in the monomeric form. Subsequent experiments used the procedure of incorporation of α-tocopherol into both monolayers (see "Experimental Method").

Curves showing accumulation of LPO products in liposomes of brain lipids in the presence of α-tocopherol are illustrated in Fig. 1. Within the range from 0.05 to 2 μM concentration-dependent inhibition of LPO is observed, whereas in concentrations exceeding 2 μM,

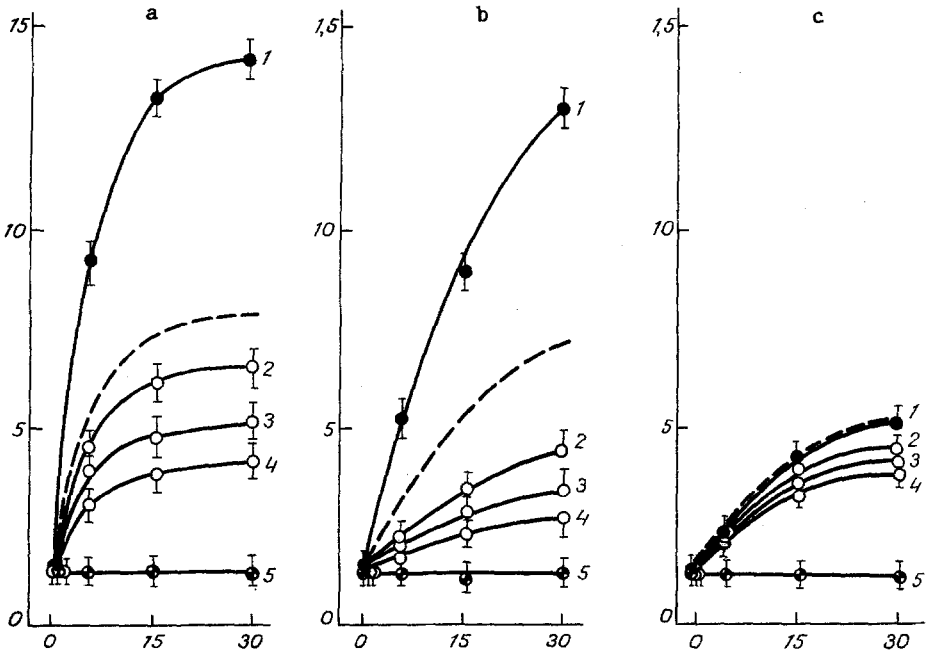

Fig. 2. Accumulation of LPO products in acceptor monolamellar liposomes composed of brain lipids in the absence (1) and in the presence (2-5) of donor monolamellar liposomes (containing 7.5 μM α-tocopherol) differing in chemical composition. a) Liposomes from brain lipids, b) liposomes from egg phosphatidylcholine, c) liposomes from dimyristoylphosphatidylcholine. 2-4) Time of preincubation of donor and acceptor liposomes, 1, 30, and 60 min respectively; 5) α-tocopherol incorporated into both types of liposomes in a concentration of 3.75 μM; broken line indicates theoretical curve of accumulation of LPO products in absence of intermembrane transfer of α-tocopherol from donor to acceptor liposomes. Abscissa, incubation time of LPO (min); ordinate, LPO products (mmoles MDA/mg lipids).

accumulation of LPO products did not take place at all under the conditions used. Since as a result of intermembrane transport of α-tocopherol its concentration in donor liposomes must fall and, consequently, their sensitivity to LPO inducers must change, concentrations of α-tocopherol to be chosen for which a reduction by a half (as a result of migration into acceptor liposomes until the development of equilibrium) caused complete inhibition of LPO as before. This condition is satisfied by concentrations of α-tocopherol above 4 μM (Fig. 1) In the experiments described below, a standard concentration of 7.5 μM was chosen.

To record the antioxidative action of α-tocopherol due to its intermembrane transfer we used mixtures of donor (containing α-tocopherol) and acceptor (not containing α-tocopherol) liposomes. The results of determination of LPO products in liposomes after different times of preincubation (before addition of LPO inducers) are given in Fig. 2. Curve 1 shows the kinetics of LPO in the absence of α-tocopherol and curve 5 the same in the presence of α-tocopherol (in a concentration 7.5 μM) in the mixture of liposomes. When α-tocopherol was incorporated only into donor liposomes (7.5 μM) complete inhibition of LPO was not recorded (curves 2-4). These experimental curves lie beneath the theoretical curve (broken line), corresponding to the case of complete absence of α-tocopherol migration from donor into acceptor liposomes (i.e., the development of LPO only in acceptor liposomes). Since 7.5 μM of α-tocopherol (and also half of this amount) induce 100% inhibition of LPO in donor liposomes, the inhibition of LPO observed took place entirely in acceptor liposomes. This effect of LPO inhibition in acceptor liposomes was evidently intensified with an increase in the duration of preincubation of the donor and acceptor liposomes, and also in the course of incubation of the mixture of liposomes with LPO inducers (5-30 min). However, during preincubation for 60 min (curve 4) accumulation of LPO products differed from curve 5 in Fig. 2a, which reflects the kinetics of LPO in a mixture of liposomes into which 3.75 μM of α-tocopherol is incorporated, i.e., 100% inhibition was not observed. It can be concluded that in the course of 60 min the concentration of α-tocopherol in the acceptor liposomes at least did not reach 2 μM (i.e., the minimal concentration giving 100% inhibition of LPO).

Similar results were obtained when liposomes from egg phosphatidylcholine were used as the donors (Fig. 2b). On the other hand, when saturated donor liposomes from dimyristoylphosphatidylcholine were used, although the antioxidative effect in the system increased with time, it was much weaker (Fig. 2c).

The results show that the efficacy of the antioxidative action of vitamin E depends essentially on its location in the lipid bilayer of saturated or unsaturated phospholipids. The inhibitory action of α-tocopherol can be realized only when transferred into acceptor liposomes. This transfer can take place either through fusion of donor and acceptor liposomes followed by lateral diffusion of α-tocopherol, or by intermembrane migration during contact between the two types of liposomes. The possibility of fusion of donor and acceptor liposomes was tested in a special series of experiments. For this purpose donor liposomes containing α-tocopherol only in their inner monolayer and aceptor liposomes in the internal space of which potassium ferricyanide was incorporated, were obtained. During fusion of both types of liposomes fluorescence of α-tocopherol ought to disappear. However, during fusion of donor and acceptor liposomes for at least 60 min this does not happen unless LPO products accumulate in them. The appearance of the latter can probably stimulate fusion of liposomes [4].

The results thus show that LPO in acceptor liposomes is inhibited by intermembrane transfer of α-tocopherol, and that the efficacy of the two processes is determined by the degree of unsaturation of the liposomal lipids.

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