α-TOCOPHEROL: MODIFIER OF THE PHASE STATE OF THE LIPID BILAYER

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A decrease in the content of vitamin E (tocopherols) in biological membranes leads to their injury [3]. Tocopherols, as minor lipid components of biomembranes, are considered to perform mainly the function of antioxidants, capable of interacting with free radicals of lipids [15] and with singlet molecular oxygen [8]. At the same time, tocopherols are known to have the property of stabilizing phospholipid monolayer and bilayer membranes with unsaturated fatty acid residues [11, 12], most probably through Van der Waals interaction between methyl groups of α-tocopherol and cis-unsaturated double bonds of the fatty acids [6]. This interaction must lead to a change in the phase state of the lipid bilayer, which largely determines such important properties of biological membranes as the viscosity of the hydrophobic zone, the passive permeability of the membranes for water, cations, and low-molecular-weight compounds, activity of membrane-bound enzymes, mobility of receptors, etc. [5]. It has been shown on models of phospholipid membranes that introduction of α-tocopherol into the bilayer modifies permeability of the membranes for water [4], chromate, and glucose [7]. It has also been found that α-tocopherol modifies thermotropic phase transitions in the membrane in liposomes made from dipalmitoylphosphatidylcholine [13]. However, all these effects were obtained by the use of high α-tocopherol/lipid ratios. Yet we know that the content of tocopherols in biological membranes does not exceed a few per cent.

The aim of the present investigation was to determine the action of low (near-physiological) concentrations of α-tocopherol on the character of the gel—liquid crystal transition in a lipid bilayer containing free fatty acids.

EXPERIMENTAL METHOD

The reagents used were dipalmitoyl-α-α-phosphatidylcholine (DPPC), from "Fluka," (Switzerland), linoliec acid (LA), from "Koch-Light" (England), D, α-α-tocopherol, from "Serva" (West Germany), and heavy water (D_2O), from "Stohler" (USA). 50 mM of K^+-phosphate buffer was made up in D_2O and kept for several hours to enable substitution of ^1H by ^2D, after which the buffer was dried and redissolved in D_2O (pH 7.1). To obtain liposomes, solutions of the original components in chloroform (DPPC, LA, α-tocopherol) in the corresponding molar proportions were mixed, evaporated on a rotary evaporator, then suspended in D_2O—K^+-phosphate buffer and treated with ultrasound on a "Soniprep" disintegrator (from "MSE," USA) with titanium probe (diameter 9 mm) three times, for 30 sec each time and with 30-sec intervals, at 0°C in an atompshere of argon with an amplitude of 10 μ. The volume of the specimen was 1 ml. Proton magnetic resonance spectra were recorded on a WH-270 spectrometer (from "Bruker," West Germany), using a thermostatically controlled attachment, in ampuls 5 mm in diameter. The specimens were kept at each temperature for 5 min. In all cases measurements were made from lower to higher temperatures. The DPPC concentration was 50 mM in all cases.

EXPERIMENTAL RESULTS

High-resolution ^{1}HNMR spectra of liposomes of different composition at temperatures from 30 to 50°C are shown in Fig. 1. The signals at 0.8, 1.2, and 3.2 ppm belong to protons of terminal CH_3 and $-CH_2$ groups and choline $-N(CH_3)_3$ groups of DPPC respectively. Since signals from protons of DPPC molecules in the gel state increase in width until they fuse completely with the base line, only molecules in the liquid-crystalline state make a contribution to the observed signal. Thus the intensities of the $-CH_3$, $-CH_2$, and $-N(CH_3)_3$ groups in

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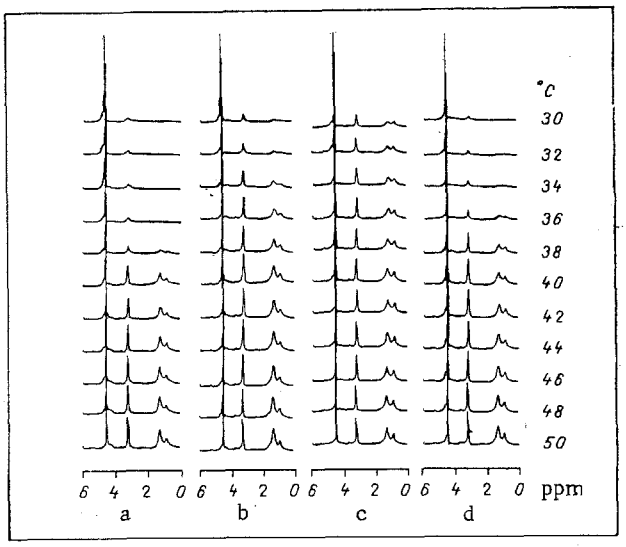

Fig. 1

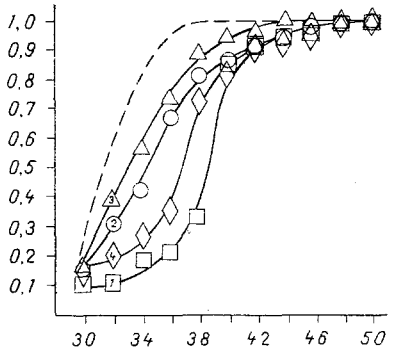

Fig. 2

Fig. 1. ^{1}H NMR spectra of liposomes of different composition at temperatures from 30 to 50°C. a) Liposomes of pure DPPC; liposomes of DPPC + 5 moles % α-tocopherol (molar ratio DPPC:LA:α-tocopherol 100:0:5); c) liposomes of DPPC + 20 moles % LA (100:20:0); d) liposomes of DPPC + 20 moles % LA + 5 moles % α-tocopherol (100:20:5). Scale of chemical shift, in ppm, shown below.

Fig. 2. Graphs of temperature dependence of relative intensity of signal of protons of choline group in liposomes of different composition. Abcissa, temperature (in °C); ordinate, relative signal intensity (I/I_{50}). Curves 1-4 correspond to spectra a, b, c, and d in Fig. 1. Broken line shows expected change in relative signal intensity of choline group in liposomes with 100:20:5 composition in the case of additive chaotropic action of LA and α-tocopherol.

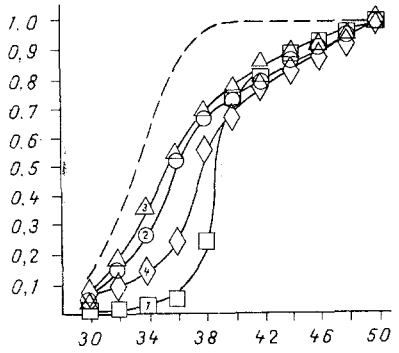

Fig. 3

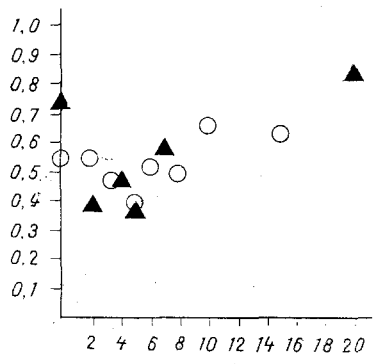

Fig. 4

Fig. 3. Graphs of temperature dependence of relative signal intensity of protons of methylene groups in liposomes of different composition. Legend as to Fig. 2.

Fig. 4. Changes in relative intensity of choline signal at 36°C depending on quantity of α-tocopherol in membrane, for bilayer containing 15 moles % (empty circles) and 20 moles % (filled triangles) of LA. Abscissa, content of α-tocopherol (in %); ordinate, relative signal intensity (I/I_{50}).

the spectrum are proportional to the fraction of these groups in the liquid-crystalline state, and temperature dependences of these intensities give some idea of the character of the gel—liquid crystal transition in the membrane. Introduction of 5 moles % of α-tocopherol into liposomes from DPPC modifies the character of the gel—liquid crystal transition considerably, shifting the transition temperature and increasing its width (Fig. 1b). Introduction of 20 moles % of the chaotropic agent LA into the bilayer had a similar action. Meanwhile

combined introduction of LA and α-tocopherol in the same concentrations into the bilayer resulted in a picture of melting of the bilayer which was much closer to that observed for pure DPPC (Fig. 1d). The same but rather weaker effect was observed after introduction of 15 moles % of LA and 5 moles % of α-tocopherol into the bilayer.

The relative intensity (i.e., the ratio of signal intensity at the given temperature to its intensity at 50°C) of signals of choline and methylene groups of DPPC in liposones of different composition as a function of temperature is shown in Figs. 2 and 3, and the broken line demonstrates how the signals ought to change during a rise of temperature if LA and α-tocopherol, on introduction into the bilayer, had an additive chaotropic action on the membrane. The experimental curve obtained for the transition indicates that if α-tocopherol and LA were introduced simultaneously into the lipid bilayer the chaotropic effect of each compound was abolished, due to the formation of complexes of α-tocopherol with LA [1].

It must, however, be noted that introduction of larger quantities of α-tocopherol caused a considerable chaotropic effect, which could not be compensated by interaction between α-tocopherol and LA. Dependence of relative signal intensity of choline protons at 36°C on the quantity of α-tocopherol introduced into a bilayer containing LA is illustrated in Fig. 4. As Fig. 4 shows, an increase in the α-tocopherol concentration in the bilayer to 4-6 moles % reduced the chaotropic effect due to the presence of both 15 moles % and 20 moles % of LA in the bilayer. However, a further increase in the α-tocopherol concentration in the bilayer led to a considerable increase in mobility of DPPC, expressed as an increase in the intensity of the DPPC signals in the NMR-spectrum of the liposomes. This result is in harmony with the fact that under normal conditions the fraction of α-tocopherol in biological membranes does not as a rule exceed 1 mole %.

It can be concluded from the experimental data that the stabilizing action of α-tocopherol on the phase state of the lipid bilayer of membranes is one of the mechanisms by which the recently demonstrated ability of α-tocopherol to protect biological membranes against the injurious action of free fatty acids, through the formation of complexes of α-tocopherol with fatty acids [1, 2], may be effected. This mechanism probably has an essential role in the stabilizing action of vitamin E in pathological states such as ischemia, hypoxia, and stress injury, in which the level of free fatty acids in biomembranes is sharply increased [9, 10, 14].

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