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EFFECT OF FUNCTIONAL GROUPS OF TOCOPHEROL MOLECULES ON MICROVISCOSITY OF MITOCHONDRIAL LIPIDS

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Despite the great interest of many investigators in the role of natural antioxidants (AO) in the regulation of lipid peroxidation, their effect on the structural lability of biological membranes has received little study. This is an urgent problem also because there is no general agreement on the mechanism of the stabilizing action of AO on membrane structures.

The aim of this investigation was to study changes in viscosity of mitochondrial membrane lipids depending on the concentration of α-tocopherol (TP) and of its synthetic analog without the phythyl side chain, 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMHC), which has antiradical activity virtually equal to that of TP [2], in the mitochondria (MCh).

EXPERIMENTAL METHOD

MCh, isolated by differential centrifugation from mouse liver [4] were suspended in incubation medium containing TP or PMHC within the concentration range $2.3 \cdot 10^{-6}$-$2.3 \cdot 10^{-3}$ M, 0.25M sucrose, and 2 mM $MgCl_2$, pH 7.2-7.4. AO were added in the form of ethanol solutions (the ethanol concentration did not exceed 3%), after which the incubation medium was sonicated in the cold for 3-5 min by means of the UZDN-2T ultrasonic disintegrator at 44 kHz and 0.5 A. The MCh were kept in the incubation medium for 1 h at 4°C, with periodic shaking. They were then sedimented by centrifugation, and washed twice with 0.25M sucrose (pH 7.2-7.4), contain-

TABLE 1. Structural Transformation Temperature and Effective Activation Energy Values in Mitochondrial Lipids after Incubation with TP and PMHC

Concentration of TP and PMHC in incubation medium, M	T_s, °C		E_a, kcal/mole		
			temperature range		
	transformation I	transformation II	10—25 °C	25—40 °C	40—60 °C
control	23	42	3,1	0,41	1,93
TP:					
$2,3 \cdot 10^{-6}$	26	43	4,0	0,72	1,93
$2,3 \cdot 10^{-5}$	31	47	3,3	0,74	1,47
$2,3 \cdot 10^{-4}$	32	45	2,35	1,07	1,50
$2,3 \cdot 10^{-3}$	34	47	2,57	0,95	1,77
PMHC:					
$4,5 \cdot 10^{-6}$	42		3,53	—	1,5

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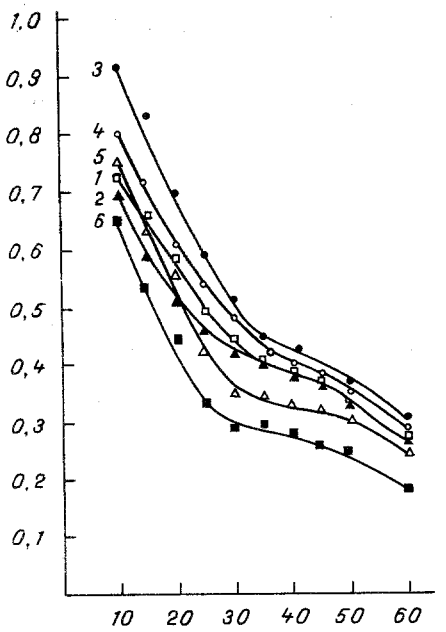

Fig. 1

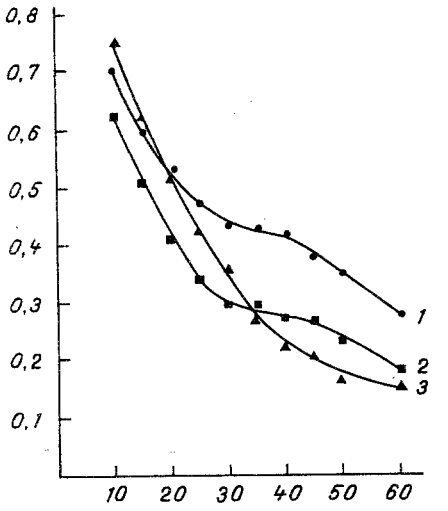

Fig. 2

Fig. 1. Viscosity of mitochondrial lipids as a function of TP concentration. 1) Control; 2) native MCh; 3, 4, 5, 6) TP in concentrations of $2.3\times$ (10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) M, respectively. Here and in Fig. 2: abscissa, temperature (in °C); ordinate, spin correlation time of probe $\times 10^{-10}$ (in sec).

Fig. 2. Changes in viscosity of lipids of MCh incubated with TP and PMHC, depending on temperature. 1) Control; 2) incubation with TP ($2.3\cdot10^{-6}$ M); 3) incubation with PMHC ($4.5\cdot10^{-6}$ M).

ing 2 mM $MgCl_2$. MCh kept under analogous conditions,but in medium without AO, served as the control.

Viscosity of the membrane lipids and heat-induced structural changes in them were determined by the spin probe method, by measuring the spin correlation time of the probe τ_s [3]. The EPR spectra were recorded on an EPR-E-4 spectrometer (Varian, USA). The stable iminoxyl radical 2,2,6,6-tetramethyl-4-capryloyloxypiperidine-1-oxyl was used as the probe.

EXPERIMENTAL RESULTS

The viscosity of the lipids of the control MCh was found to be worse than viscosity of native MCh (freshly isolated) at temperatures up to 30°C, but with a further rise of temperature the difference disappeared (Fig. 1). Incubation of MCh in medium containing $2.3\cdot10^{-3}$ and $2.3\cdot10^{-4}$ M TP led to an increase in viscosity of the lipids, which was greater when TP was present in a concentration of $2.3\cdot10^{-3}$ M (Fig. 1). With a decrease in the TP concentration to $2.3\cdot10^{-5}$ M, the viscosity of the lipids of the experimental MCh was reduced compared with that in the control and the native MCh. The greatest decrease in viscosity in the lipids was found with TP in a concentration of $2.3\cdot10^{-6}$ M.

With an increase in the TP concentration the effective activation energy (E_a) increased within the temperature range from 25 to 40°C and the structural transformation temperature (T_s) rose (Table 1).

Since the TP concentration in the incubation medium ($2.3\cdot10^{-6}$ M) was about equal to that in lipids of native MCh [7, 10], incubation of MCh was subsequently carried out in a medium containing this concentration of TP.

After incubation of MCh with TP and PMHC, in equal concentrations, it was found that more TP than PMCH is introduced into the lipids. The quantity of intercalated AO was determined by the chemiluminescence method, which gives a rough idea of the AO concentration in lipids [1]. A concentration twice as high as that for TP, namely $4.5\cdot10^{-6}$ M, was therefore chosen for the analog.

A comparative study of the effect of AO on the viscosity of mitochondrial lipids showed that TP and PMHC lower the viscosity of the lipids below the control level; the effect of the analog of lipids was stronger than that of TP, i.e., it increased the flowability of the mitochondrial lipids more strongly (Fig. 2).

Simultaneously with the change in viscosity of lipids, AO also changed T_s. Two structural transformations were found in lipids of the control MCh (Table 1). After incubation with TP, two transformations also were observed, but after incubation with PMHC only one transformation took place. Values of T_s after incubation with AO shifted into the region of higher values. The value of E_a for MCh was higher in the experiment than in the control (Table 1).

On incubation of MCh with AO (in physiological concentrations) the viscosity of the lipids decreased, temperatures of structural transformation shifted into the region of higher values, and E_a rose.

Another interesting fact was the increased effectiveness of the action of AO on viscosity of lipids with a decrease in its dose, and a change in the character of its action with a change in concentration, are other interesting facts. AO, when introduced in small quantities, "liquify" lipids, whereas in large quantities, they increase their viscosity. Differences in the action of AO may be due to the two ways in which they affect membrane structure: by direct interaction with fatty acid residues of phospholipid molecules, and indirectly — through interaction with the protein component of the membranes. In this connection it is worth noting that the same concentrations of TP has been shown [9] to increase the viscosity of liposomes made from lipids isolated from platelets, but they liquify lipids composing platelet membranes.

In our view, changes in viscosity of lipids under the influence of AO in low (physiological) concentrations take place with the aid of receptors. The presence of TP-binding proteins in membranes was demonstrated previously [5, 6, 8].

The increase in viscosity may be due to the fact that when the receptors of the "residues" are saturated with AO, the unbound AO increases viscosity because of incorporation into the lipid bilayer, and in that case the presence of the phytyl side chain plays a definite role.

The experiments showed that an important role in the change in viscosity of mitochondrial lipids under the influence of tocopherols with different structure is played by the chromane ring, which is found in the molecules of these substances. The phytyl side chain of TP is responsible for the change in temperatures of structural transformations taking place in the lipid bilayer.

The experiments thus showed that TP, in small doses, liquifies the lipid bilayer of mitochondrial membranes, whereas in large doses, it structurizes them. It is suggested that mitochondrial membranes contain receptors for AO, by means of which the latter, in physiological concentrations, modify viscosity of the membrane lipids.

On the basis of the experimental results described above it can be postulated that TP exerts its influence on viscosity of membrane lipids in two ways: through binding with a receptor and thorugh its direct distribution in the lipid bilayer. In one way an increase in the AO concentration leads to an increase in the viscosity of the lipids, whereas in the other way it leads to liquifaction of the lipid bilayer.

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