ROLE OF THE ISOPRENOID CHAIN OF LATERAL MOBILITY OF α-TOCOPHEROL IN THE LIPID BILAYER

N. V. Gorbunov, V. E. Kagan, S. M. Alekseev, and A. N. Erin

UDC 615.356:577.161.3].015.4.07

KEY WORDS: α-tocopherol; biomembranes; lipid peroxidation.

The polyfunctional character of the stabilizing action of α-tocopherol on biomembranes, which includes inhibition of lipid peroxidation (LPO), protection against singlet oxygen, structural stabilization, and protection against the damaging action of phosphorylase A2, has recently been proved experimentally [9-12]. Among the mechanisms listed above, inhibition of LPO, a universal factor in injury to biomembranes in various pathological states, is particularly important. Accordingly, research aimed at studying the molecular mechanism of action of α-tocopherol, which is linked with inhibition of LPO, deserves particular attention. It has been shown, for instance, that the effectiveness of this action in biomembranes in vitro is increased with shortening of the isoprenoid chain of α-tocopherol [4, 5]; enhancement of the effect of inhibition of LPO with shortening of the isoprenoid chain of the chromanols, moreover, takes place only in heterogeneous systems [1], and, in the opinion of the authors cited, it can be explained by an increase in mobility of the chromanol in the lipid bilayer. An increase in rotary and transbilayer mobility with shortening of the isoprenoid chain has been demonstrated experimentally [4, 5]. Data on dependence of lateral mobility of chromanols on the length of their isoprenoid chain could not be found in the literature.

The aim of this investigation was to determine the effect of the length of the isoprenoid chain of α-tocopherol on its lateral mobility in the lipid bilayer of phospholipid membranes, using model compounds differing in the length of their isoprenoid chain.

EXPERIMENTAL METHOD

To solve this problem we used the effect of quenching of the natural fluorescence of the chromanols in the bilayer of lipid membranes (λ_{ex} = 295 nm, λ_{fl} = 325 nm) by spin-labeled lipotropic probes [3, 7]. Stable radicals were used: stearic acid derivatives with an incorporated doxyl fragment attached to the 5th and 12th carbon atom, counting from the carboxyl group (5DC and 12DC), in which the level of insertion of the nitroxyl radical into the lipid bilayer was 6-8 and 18-20 Å respectively [6]. As the control we used a water-soluble ester of 1-oxyl-2,2,5,5,-tetramethylpyrroline-3-carboxylic acid. Spin probes were introduced into a suspension of liposomes in alcoholic solution. The final alcohol concentration did not exceed 1%. EPR spectra were recorded on the small EPR spectrometer manufactured by the Svetlana Leningrad Optical Electronic Factory. Considering that α-tocopherol possesses a condensing property, i.e., reduces the molecular mobility of lipids in membranes [2], in a series of preliminary investigations we estimated the microviscosity of the lipid bilayer in the presence of chromanols (1 molecule of chromanol to 100 molecules of lecithin). As the criterion of membrane microviscosity we used the parameter of excimerization of a pyrene probe [3] and polarization of fluorescence of 1,6-diphenyl-1,3,5-hexatriene (DPHT) [8]. The concentration of the fluorescent probe in the test membranes did not exceed 1 mole %.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. E. Vartanyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 7, pp. 39-41, July, 1991. Original article submitted December 5, 1990.

 0007-4888/91/0007-0946$12.50

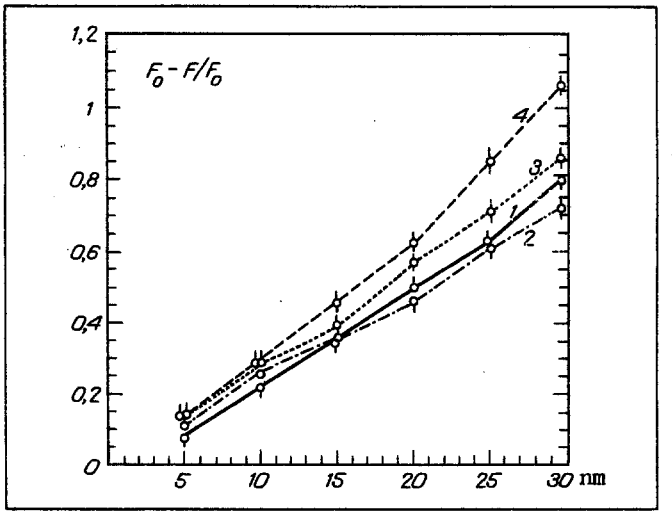

Fig. 1. Quenching of fluorescence of α-tocopherol (1) and of chromanols C11 (2), C6 (3), C1 (4) by 5DC as a function of concentration, between Stern—Volner coordinates.

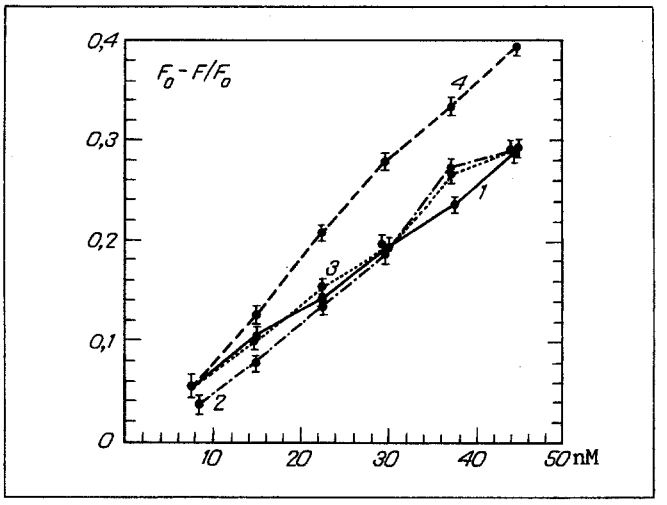

Fig. 2. Quenching of fluorescence of α-tocopherol and of chromanols C11, C6, and C1 by 12DC as a function of concentration, between Stern—Volmer coordinates. Legend as to Fig 1.

Monolayer liposomes were prepared by injecting a 10% solution of egg lecithin with the addition of chromanols (with the addition of fluorescent probes in the control) into a buffer solution of 5 mM Tris-HCl (pH 7.4), containing 100 mM NaCl (at 37°C), using a Hamilton microliter syringe. The final concentration of liposomes did not exceed 300 μg in 1 ml of buffer solution. Fluorescence measurements were carried out in a rectangular (1 × 1 cm) quartz cuvette on a Hitachi-850 spectrofluorometer (Japan) using slits with a spectral width of 5 nm. The spectra were corrected with rhodamine B. The temperature of the incubation medium was 37°C and the accuracy of thermostating was ±0.5°.

The following reagents were used: D,L-α-tocopherol (C16) from "ICN Pharmaceuticals" (USA), 5 DC, 12DC, DPHT, and Tris-HCl buffer from "Sigma" (USA), pyrene from "Serva" (West Germany), egg lecithin from the Khar'kov Experimental Factory; α-tocopherol analogs, differing in the length of their carbon chain (C11, C6, and C1 — pentamethylhydroxychromane) were synthesized at the Moscow Institute of Fine Chemical Technology. The remaining reagents were of Soviet origin and of the chemically pure grade.

The experimental results, given in Figs. 1 and 2, indicate twice the standard deviation. Coefficients of correlation were not below 0.99. The results were analyzed by computer using the Statgraph program package (version 3.0).

EXPERIMENTAL RESULTS

The chromanol nucleus, responsible for fluorescence of α-tocopherol, is located in the lipid bilayer close to the phase separation boundary [7]. It has been suggested that the effect of 5DC is connected primarily with lateral displacement of the doxyl fragment and chromanol, whereas the 12DC effect is also due to transbilayer mobility of the interacting components.

In fact, as the data given in Figs. 1 and 2 show, dependence of quenching of fluorescence of the chromanols on the concentration of 5DC and 12DC added to the suspension is linear in character between Stern–Volmer coordinates. It must be pointed out that the presence of the ethyl ester of 1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxylic acid in the liposome suspension in concentrations up to values 10 times the maximal concentration of 5DC and 12DC, did not change the intensity of fluorescence of the chromanols.

Quenching of fluorescence of chromanols C6, C11, and C16 (Fig. 2) by the action of 12DC has only weak differences, and is evidently caused by fluctuation changes in the doxyl fragment along the normal to the surface of the lipid bilayer of the membranes, whereas the hydrocarbon chain of these chromanols stabilizes the position of the chromophore group close to the polar groups of phospholipids. This interpretation of the results does not contradict data already published showing that transbilayer diffusion of chromanols is restricted by the presence of the hydrocarbon chain [4, 5]. This conclusion also is confirmed by the sharp increase in quenching of fluorescence under the influence of 12DC during the transition from C16, C11, and C6 to C1. In fact, the absence of a hydrocarbon chain in C1 leads to a significant increase in its transbilayer displacement [4, 5] and correspondingly increases the likelihood of interaction with the doxyl fragment of 12DC.

Quenching of fluorescence of the chromanols by 5DC (Fig. 1) was significantly higher than by 12DC (Fig. 2), possibly due to the close proximity of the nitroxyl group of 5DC and the chromophore groups within the thickness of a single monolayer [7]. It is an essential fact that differences in the effects of quenching are found in the presence of 5DC in the series C16, C11, C6, C1 (Fig. 2). This result can be explained (taking the whole of the previous analysis into account) by differences in the lateral mobility of these chromanols, due to significant shortening of the isoprenoid chain during the transition from C16, C11 to C6, and then to C1.

These differences in the lateral mobility cannot be explained by differences in the effect of chromanols on the microviscosity of the bilayer. According to estimates of microviscosity of the lipid matrix of the liposomal membranes the presence of chromanols did not evoke significant changes – either in the orderliness of the lipid acyl groups or their lateral mobility. In fact, polarization of fluorescence of DPHT in the presence of chromanols in the membranes differed only a little from the control, at 0.240 ± 0.004. Very small changes were recorded when the parameter of excimerization of pyrene was estimated, for this varied within limits of the control value 0.17 ± 0.01.

It thus follows from all the results described above that lengthening the isoprenoid chain reduces lateral mobility of chromanols in the bilayer. This is evidently the reason for the reduction of antioxidative activity in heterogeneous systems in the series C1, C6, C11, C16.

LITERATURE CITED

1. E. B. Burlakova, E. N. Kukhtina, I. K. Sarycheva, et al., Biokhimiya, **47**, No. 6, 987 (1982).
2. N. V. Gorbunov, V. I. Skrypin, V. A. Tyurin, et al., Biol. Nauki, No. 7, 27 (1987).
3. A. D. Dergunov, A. S. Kaprel'yants, and D. N. Ostrovskii, Biokhimiya, **46**, No. 8, 1499 (1981).
4. A. N. Erin, N. G. Davitashvili, V. N. Kagan, et al., Biokhimiya, **53**, No. 4, 591 (1988).
5. V. E. Kagan, F. A. Serbinova, R. A. Bakalova, et al., Dokl. Akad. Nauk SSSR, **295**, No. 3, 728 (1987).
6. O. M. Ponasenko, A. I. Deev, I. B. Deev, et al., Biofizika, **30**, No. 5, 817 (1985).
7. F. J. Aranda, A. Coutinho, M. N. Berberan-Santas, et al., Biochim. Biophys. Acta, **985**, No. 1, 23 (1989).
8. M. D. Barratt, R. A. Badley, and R. B. Leslie, Eur. J. Biochem., **48**, No. 2, 595 (1974).
9. A. N. Erin, N. V. Gorbunov, V. I. Brusanovic, et al., Brain Res., **398**, 85 (1986).
10. G. W. Grams, Tetrahedron Lett., **50**, 4823 (1971).