STRUCTURAL STABILIZATION OF LIPIDS AND THE VISUAL PIGMENT RHODOPSIN IN THE PHOTORECEPTOR MEMBRANE BY VITAMIN E

V. A. Tyurin, S. N. Korol'kov,
A. L. Berman, and V. E. Kagan

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Vitamin E (α-tocopherol — TP), present in the photoreceptor membranes (PRM) of the visual cells of the retina in a high concentration [8], protects them against oxidative destruction, thereby preventing the uncontrollable development of photosensitized oxidative reactions on account of physical and chemical quenching of singlet oxygen [10] and free-radical peroxidation reactions of polyunsaturated lipids due to interaction with alkoxyl and alkylperoxide radicals [14]. The view has recently been expressed that TP may exert a stabilizing action in biomembranes through a structural mechanism — on account of the ordering of the molecular mobility of polyunsaturated fatty acids, whether in the composition of membrane phospholipids or in the free state, formed as a result of the action of endogenous type A phospholipases [2]. This mechanism of stabilization may be of great importance in PRM, which contain exceptionally high concentrations of polyunsaturated phospholipids (up to 70 moles %) [5], whose structural and functional integrity is determined by a mechanism of "molecular replacement" of damaged phospholipid residues [7], functioning with the participation of the phospholipases A_2 [6].

To test this possibility of structural stabilization of PRM by vitamin E experimentally it was decided to study the action of TP on the molecular mobility of phospholipids in PRM, using the spin probe method, and on the thermostability of the visual pigment rhodopsin.

EXPERIMENTAL METHOD

PRM of the retina were obtained as described previously [1]. Protein was determined by a modified Lowry's method [12]. Thermodenaturation of rhodopsin in a suspension of PRM was carried out in a medium of the following composition: Tris-HCl 40 mM, carnosine 10 mM, NaCl 100 mM (pH 7.4). Absorption spectra were recorded on a Specord M-40 spectrophotometer (East Germany). The velocity constants of thermal denaturation of rhodopsin (K_{td}) were calculated as described in [9]. To insert TP into PRM, mild ultrasonic treatment in a cylindrical resonator was used, with no possibility of direct contact of the membrane suspension with the surface of the resonator, on the UZDN-2 apparatus (22 kHz, exposure for 20 × 15 sec, with an interval of 30 sec, at 0-4°C). The change in the microviscosity of the liquid bilayer of PRM was estimated from the rotary correction time (τ_s) [13] of the spin-labeled derivative of palmitic acid, the nitroxyl fragment of which is located at the first carbon atom from the carbonyl group (spin-probe I). Concentration of the specimen was 26 mg/ml. The spin-probe I was added to the PRM suspension at the rate of 1 molecule of probe to 100 molecules of phospholipids. EPR spectra were recorded on a compact EPR-spectrometer, produced by the Leningrad Electrotechnical Institute. The temperature of the specimens was kept constant with an accuracy of 0.5°C. Chromatographically pure oleic and arachidonic acids (from Koch-Light, England) and D,L-TP (Serva, West Germany) were introduced into the membrane suspension from ethanol solutions up to a final ethanol concentration of 0.1%.

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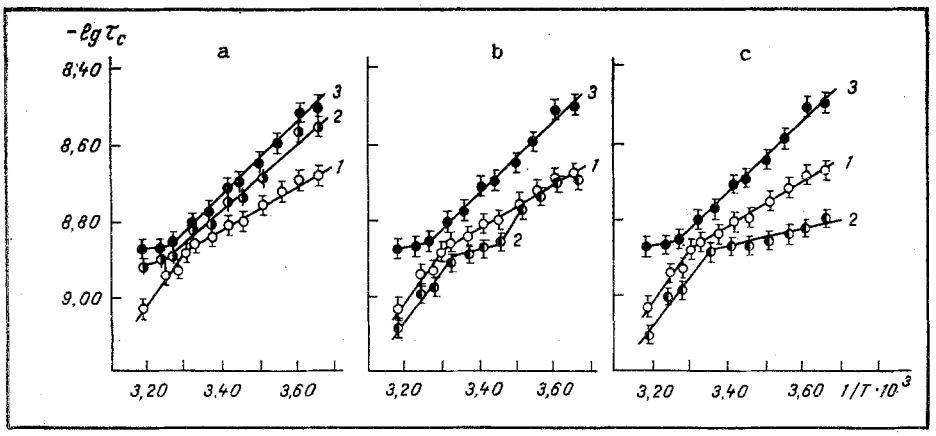

Fig. 1. Temperature dependence of molecular mobility of spin-probe I in intact PRM (a) and after incorporation of oleic (b) and arachidonic (c) FFA into membranes. Abscissa, temperature (1/T, °K); ordinate, rotary correlation time τ_s. a: 1) Intact PRM, 2) PRM + TP (0.5 mole %), 3) PRM + TP (5 moles %); b: 1) intact PRM, 2) PRM + oleic acid (2 moles %), 3) PRM + TP (5 moles %) + oleic acid (2 moles %); c: 1) intact PRM, 2) PRM + arachidonic acid (2 moles %), 3) PRM + TP (5 moles %) + arachidonic acid (2 moles %).

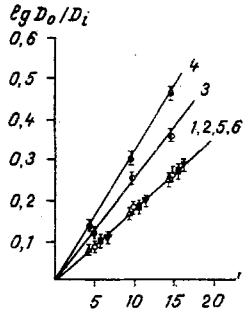

Fig. 2

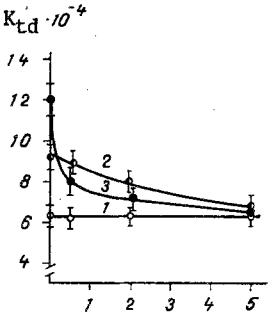

Fig. 3

Fig. 2. Kinetics of thermal denaturation of rhodopsin in PRM at 69°C. Abscissa, time (in min); ordinate, change in optical density of absorption of rhodopsin at 500 nm: value of optical density at $t = t_0$, $t = t_1$. 1) Intact PRM, 2) PRM + TP (5 moles %); 3) PRM + oleic acid (2 moles %), 4) PRM + arachidonic acid (2 moles %), 5) PRM + TP (5 moles %) + oleic acid (2 moles %), 6) PRM + TP (5 moles %) + arachidonic acid (2 moles %).

Fig. 3. Effect of different concentrations of TP on K_{td} of thermal denaturation of rhodopsin in intact PRM and FFA-modified PRM at 69°C. Abscissa, concentration of TP (in moles %). Ordinate, values of K_{td} (in sec^{-1}). 1) Intact PRM, 2) PRM + oleic acid (2 moles %), 3) PRM + arachidonic acid (2 moles %).

EXPERIMENTAL RESULTS

Graphs showing temperature dependence (Arrhenius plot) of the molecular mobility of spin-probe I in intact PRM, and also in PRM into which free fatty acids (FFA) — oleic and arachidonic — had been inserted beforehand, are given in Fig. 1. Incorporation of TP into the intact membranes led to ordering of the molecular movements of the phospholipids in the membrane, as shown by an increase in the rotary correlation time of spin-probe I. This effect was more marked in the presence of a higher TP concentration (5 moles %) than a lower concentration. The stabilizing action of TP also was expressed by the fact that the activation energy (E_a) of the mobility of the probe was 2.3 kcal/mole in the control within the temperature range from 0-30°C, but increased after incorporation of TP up to 4.5 kcal/mole. Thus insertion of TP into bovine PRM, containing 70 moles % of polyunsaturated fatty acids, up to a

molar concentration of 5 moles % had stabilizing action, manifested as the more compact packing of the fatty-acid residues of the phospholipids, with the result that their molecular mobility was reduced.

FFA are known to have a disorganizing action on the structure of the lipid bilayer [3]. In fact, as will be clear from Fig. 1b, c, introduction of fatty acids into PRM in a concentration of 2 moles % caused an increase in molecular mobility of the phospholipids (a decrease of the rotary correlation time and a fall of E_a to 1.6 kcal/mole within the temperature region 0-30°C). This effect was distinctly visible for arachidonic acid over the whole temperature range studied, and for oleic acid from 16°C and above, i.e., in the region of temperature above the melting temperature for oleic acid. When TP was added up to a concentration of 5 moles %, i.e., until the ratio of TP to fatty acid was 5:2, a stabilizing effect was observed, quantitatively equal to the action of TP on intact PRM.

Thus TP behaves as a structural stabilizer both in PRM and in FFA-modified PRM.

The writers showed previously that the thermal stability of rhodopsin depends to a marked degree on the composition and properties of its lipid microenvironment. In particular, it was demonstrated that one of the hydrolysis products of phospholipids by phospholipase A_2, namely FFA, significantly reduce the thermal stability of the visual pigment, rhodopsin [11]. It was therefore considered important to test to what extent the structural stabilization of lipids of PRM by TP is reflected in the thermal stability of the principal integral protein of that membrane, namely rhodopsin. Both fatty acids were found to increase the velocity of thermal denaturation (thermal decolorization) of rhodopsin (Fig. 2). Incorporation of TP in a concentration of 5 moles % restored the thermal stability of the visual pigment so that curves 5 and 6 in Fig. 2 appear indistinguishable from the control. It will be noted that despite the fact that TP, incorporated into PRM, causes limitation of the molecular mobility of the lipids, the thermodenaturation process of rhodopsin in PRM took place at the same velocity as in the absence of TP. The stabilizing action of TP against the background of preliminary addition of fatty acids also was manifested to the level recorded in intact membranes (Fig. 2: 1, 5, 6). It can be concluded that the thermal stability of rhodopsin is maximal in intact membranes of bovine PRM. This conclusion is also confirmed by experiments to study the effect of different TP concentrations on K_{td} for the velocity of thermal denaturation of rhodopsin in intact and FFA-modified PRM (Fig. 3). In this case also, up to a concentration of 5 moles %, K_{td} of rhodopsin in PRM was minimal and was equal to K_{td} of rhodopsin in the absence of TP. This was perhaps due to the presence of a high concentration of endogenous TP in the intact PRM. On incorporation of 2 moles % of oleic or arachidonic acid into the composition of the membranes the value of K_{td} rose by 1.5 or 2 times, respectively. As TP was incorporated into the PRM membranes the velocity of thermal denaturation of rhodopsin fell, and when the TP concentration was 5 moles %, it became indistinguishable from the control values.

It can be concluded from these results as a whole that TP can give rise to structural stabilization of lipids and of the visual pigment rhodopsin in PRM, and this can be regarded as a physiologically important function of this lipid component in PRM, as was shown previously for membranes of synaptosomes and the sarcoplasmic reticulum [2, 4].

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COMPARATIVE ANALYSIS OF THE ROLE OF β-ENDORPHIN SYSTEMS IN MECHANISMS OF DIFFERENT TYPES OF ANALGESIA

E. O. Bragin, N. N. Batueva, A. V. Kotov, S. M. Tolpygo, and G. Sh. Burbaeva

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Experiments using the opiate antagonist naloxone have shown that the development of analgesia under the influence of stress stimuli, acupuncture, and electrical stimulation of brain structures may arise through the intervention of opioid and other neurochemical mechanisms [1-10]. However, there is evidence that opioid systems are not always involved in the mechanisms of analgesia [6, 10], evidently because of selective activation of neurochemical components during exposure to certain types of action.

Accordingly, in the investigation described below, which was conducted on animals immunized with a conjugate of β-endorphin (EN) and bovine serum albumin (BSA), in order to inhibit activity of the EN-system, a comparative analysis was made of the role of this system in mechanisms of analgesia in different situations.

EXPERIMENTAL METHOD

Experiments were carried out on 36 albino rats. To depress activity of the EN system 18 rats were immunized by two injections (at an interval of 7 days) of 0.1 ml of conjugate, mixed in the ratio of 1:1 with Freund's adjuvant, into the upper third of the hind limb. The conjugate was prepared in a reaction mixture of EN—BSA—bis-diazotized benzidine (10:1:10), and the efficiency of the conjugation reaction was 60% (i.e., EN:BSA = 6:1). The final concentration of EN was 75 μg in 0.1 ml. Rats of the control group (n = 18) were given an injection of 0.1 ml of an unconjugated mixture (UCM) of EN—BSA—Freund's adjuvant in the same proportions.

Experiments were carried out 8 days after the second immunization. Nociceptive sensitivity was assessed by studying latent periods (LP) of paw licking responses (PLR) to a hot plate at 55°C and the tail withdrawal response (TWR) to the same thermal stimulation. Anesthesia was induced by intraperitoneal injection of morphine (5 mg/kg), by swimming in cold water at 4°C for 3 min, or by unavoidable electric shock stress (ESS). The animals were exposed to ESS in a chamber with an electrically conducting floor, through which a continuous pulsed current was passed (2.5 mA, 8 pulses/min, 2 sec, 10 min).

The rats were decapitated 3-4 days after the experiment. To determine the EN concentration the pituitary and hypothalamus were isolated from the brain and kept at −30°C. EN was extracted by the method in [8]. The EN concentration was determined by radioimmunoassay, using reagents from Immuno Nuclear Inc. (USA).

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