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Changes of structural and dynamic properties of model lipid membranes induced by α-tocopherol: implication to the membrane stabilization under external electric field

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Abstract

The effects of α-tocopherol on electric properties of bilayer lipid membranes were investigated. Planar bilayer membranes formed by the Mueller-Rudin method were used. Voltammetric and chronopotentiometric measurements were performed using a four-electrode potentiostat-galvanostat. It was demonstrated that registration of membrane capacitance, resistance, and voltammetric characteristics provided information about the change in the structure and permeability of bilayer lipid membranes. The results suggested that incorporation of α-tocopherol into lipid membrane destabilized its structure and facilitated the electrogeneration of pores. The possible role of observed changes in physiological functions of α-tocopherol was discussed.

Keywords: α-Tocopherol; Bilayer lipid membrane; Chronopotentiometry; Electroporation

1. Introduction

There are several tocopherols existing in living systems [1]. However, α-tocopherol (vitamin E) plays the most important role in human nutrition [2,3].

It is supposed that vitamin E functions as an efficient inhibitor of peroxidation of cell membrane lipids [4,5]. Phospholipids of mitochondrial and plasmatic membranes display affinity to α-tocopherol and it seems that this vitamin is located at high concentration in these structures. However, the concentration of α-tocopherol in biological membranes is rather low. The knowledge about the reason and how it may be active at very low concentrations is still limited [6,7].

Although there is some information about the biological function of α-tocopherol, the studies on its behaviour in the membrane at the molecular level are rather limited [8–10]. It was demonstrated that α-tocopherol affects the structure of membrane and its dynamic properties [11,12]. Several authors suggested that interaction between vitamin E and membrane plays an important role in the mechanisms of antioxidant action and membrane stabilization [7,13]. Due to this fact some attempts have been made to understand the interaction of α-tocopherol with natural membranes and biological model membranes [14,15]. In order to obtain this information, different forms of vitamin E were incorporated into model

Abbreviations: E, α-tocopherol; BLM, bilayer lipid membrane; BLM+E, bilayer lipid membrane with α-tocopherol

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lipid membranes [11,16–18]. Most of the experiments on model membranes with incorporated vitamin E were performed in model systems without external electric field applied to the membrane, although it is generally accepted that the electric properties of lipids and other constituent parts of membrane together with membrane interface influence strongly the biological function of the whole membrane system [19].

The aim of this work was to investigate using voltammetric and chronopotentiometric methods the effect of α-tocopherol on electric properties of model membranes and to provide information on how the membrane electric properties could reflect the changes in the structure and dynamic behaviour of model bilayer lipid membranes. It was to demonstrate that the effect of α-tocopherol on lipid membrane was dependent on the external electric conditions of the membrane. Under an external electric field applied to the membrane, α-tocopherol destabilized the bilayer lipid membrane.

2. Materials and methods

2.1. Chemicals

Egg yolk phosphatidylcholine was purchased from Fluka (Buchs, Switzerland). α-Tocopherol was from Sigma-Aldrich (Steinheim). Analytical-grade KCl was obtained from POCH (Gliwice, Poland). *n*-Decane (Sigma-Aldrich) was used as a solvent in a forming solution. The total concentration of the lipids and α-tocopherol in the forming solutions was 20 mg/ml. The content of α-tocopherol was 2.5, 5 or 10% of the total amount of lipids (w/w). The electrolytes (0.1 M KCl) were buffered with HEPES (Sigma-Aldrich) to pH 7.0. Ultrapure water was prepared with a Milli-Q system (Millipore).

2.2. Methods

Planar bilayer lipid membranes (BLM) formed by the Mueller-Rudin method were used [20]. The membranes were formed in a 1.0 mm diameter, round aperture in two Teflon hydrophobic septum separated cells filled with 10 ml of 0.1 M KCl each. The formation of the membranes was monitored visually and electrically by measuring the membrane capacitance. It was assumed that the membrane was complete when its capacitance was about 1 nF and the capacitance drift was less than 10 pF/min.

The membrane capacitance was measured directly using the capacitance-to-period conversion method [21,22]. The resistance of the membranes was calculated using cyclic voltammograms recorded in the range of potential −50 to +50 mV with a sweep speed of 10 mV/s, applying the least square method. The estimation of the electric parameters of electropores generated by a constant intensity current was performed according to [24]. Voltammetric and chronopotentiometric measurements were performed using a four-electrode potentiostat-galvanostat [23]. The electrodes were Ag/AgCl with an average area of 0.5 cm^2, immersed directly into the electrolyte solutions. The temperature was 22 ± 1°C.

The data were processed and analysed with the computer programme Excel (Microsoft) or Prism (GraphPad Software).

3. Results and discussion

3.1. Formation of bilayer lipid membrane

The electric measurements were performed on BLMs without and with the addition of α-tocopherol

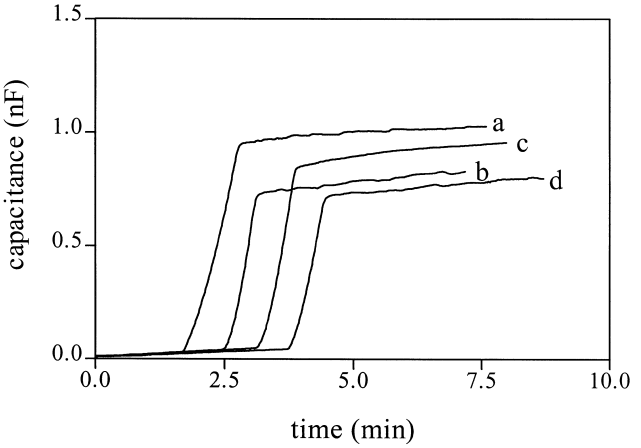

Fig. 1. Capacitance during the formation of membranes from phosphatidylcholine with α-tocopherol (w/w): (a) 0%, (b) 2.5%, (c) 5%, and (d) 10%. Electrolyte, 0.1 M KCl, HEPES pH 7.0. The membranes were formed by the Mueller-Rudin method in a hole of 1.0 mm diameter.

Table 1
Physical parameters of membranes made of phosphatidylcholine without (BLM) and with (BLM+E) the addition of different contents of α-tocopherol

Membrane composition	Time for the beginning of membrane formation (t, min)	Rate of capacitance increase during membrane formation (V, pF/s)	Time for reaching capacitance drift <10 pF/min (t, min)	Final membrane capacitance (C, nF)
BLM	1.5 ± 0.6	11 ± 4	4.0 ± 0.7	0.97 ± 0.11
BLM+E (2.5%)	1.8 ± 0.6	18 ± 2	3.9 ± 0.5	0.87 ± 0.07
BLM+E (5%)	2.7 ± 0.9	18 ± 2	5.7 ± 1.3	0.98 ± 0.10
BLM+E (10%)	3.9 ± 1.4	16 ± 4	8.0 ± 1.8	0.84 ± 0.12

The results were obtained from eight membranes for each composition.

(BLM+E). The content of α-tocopherol was 2.5, 5, or 10% of the total amount of lipids (w/w).

Fig. 1 demonstrates an example of the curves representing BLM formation from lipids (a) and from 2.5% (b), 5% (c), and 10% (d) α-tocopherol/lipid mixtures (w/w). The examples of chosen physical parameters registered during formation of membrane made of phosphatidylcholine without (BLM) and with the addition of different contents of α-tocopherol (BLM+E) are presented in Table 1. The time needed for starting the membrane formation increased with increasing content of α-tocopherol. The addition of α-tocopherol also influenced the increasing part of the curves obtained during membrane formation, which represents the process of lipid organization into the bilayer structure [25]. The rate of capacitance increase was higher for membranes containing α-tocopherol. These results suggest that α-tocopherol slightly facilitated the self-organization of lipids. The formation process was completed if membrane capacitance reached a stable level [25]. The stable capacitance recorded for unmodified membranes and membranes modified by α-tocopherol was very similar, about 1 nF. This may suggest that the presence of α-tocopherol does not affect significantly the final self-assembled bilayer lipid membrane.

3.2. Voltammetric characteristics

Fig. 2 shows typical voltammetric curves for BLM (a) and BLM+E (b). The smooth, linear part of the voltammetric curve can be used for the calculation of membrane resistance. Membrane resistance was calculated from the slope of the curves. The typical value of membrane resistance was about 20 GΩ for BLM and BLM+E. The results suggest that the amounts of α-tocopherol used in our experiments do not change significantly the hydrophobic part of the membranes and their permeability for ions at least for low transmembrane potentials.

The increase of the external potential above 100

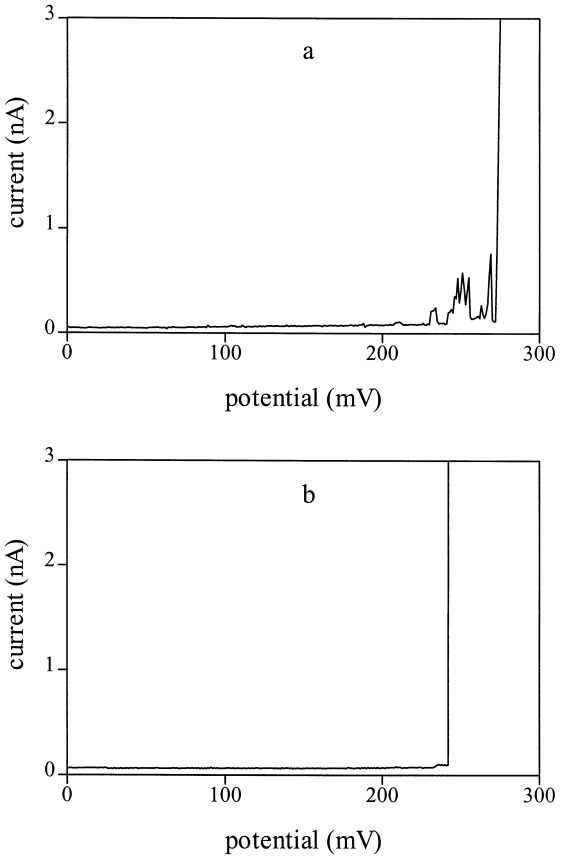

Fig. 2. Voltammetric curves of BLM (a) and BLM+E (b). The content of α-tocopherol in the forming solution was 10% of the total amount of lipids (w/w). Potential sweep speed, 10 mV/s. Electrolyte, 0.1 M KCl, HEPES pH 7.0.

mV caused an increase in current flowing through the membranes. However, the current did not rise smoothly and gradually: starting from a certain potential, the current intensity 'jumped up' and then 'jumped down'. This picture was repeated several times with increased potential and was manifested as consecutive current peaks appearing on voltammetric curves (Fig. 2a). This means that the membrane permeability consecutively increased and decreased. The fluctuating disruptions of the membrane structure may be interpreted as the generation of fluctuating pores in bilayer lipid membrane [23]. The incorporation of α-tocopherol decreased the potential at which pores can be generated from about 230 mV for BLM to about 210 for BLM+E with 5% of α-tocopherol (data not shown). A further increase of the external potential did not allow to keep fluctuating pores. At a certain potential, irreversible membrane breakdown was observed (Fig. 2). This was accompanied by a rapid increase in current. The membranes were destroyed.

The dynamic properties of membrane (generation of fluctuating pores) and characteristic potential at which irreversible breakdown started were dependent on the membrane used (BLM or BLMs+E) and the concentration of α-tocopherol (Figs. 2 and 3). The action of α-tocopherol on decreasing the irreversible breakdown was statistically significant: the higher the α-tocopherol concentration the lower the irreversible breakdown potential (Fig. 3). However, the presence of higher concentrations of α-tocopherol (10%, for example) in lipid membrane led to direct irreversible breakdown of membrane without earlier fluctuations of the current (Fig. 2b). Hence, in this case irreversible breakdown was not proceeded by the generation of fluctuating pores in the bilayer lipid membrane.

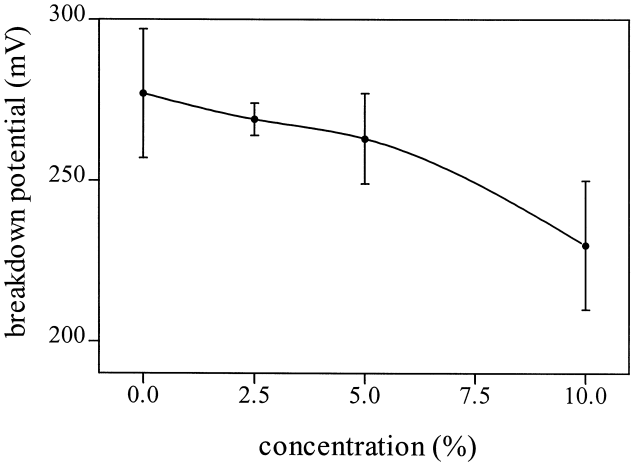

Fig. 3. Dependence of irreversible breakdown voltage of BLMs on the concentration of α-tocopherol. Each result is the mean value of eight measurements. Bars indicate the standard deviation.

The incorporation of α-tocopherol into the BLM structure increased the membrane suppleness to irreversible breakdown. This result does not confirm the suggestion by several authors [16,30,31] that vitamin E can act as a structural component which stabilizes biomembranes. These authors performed their experiments on α-tocopherol without an external electric potential. It seems that the model membranes in which the membrane potential is formed are closer to those of living organisms. That is why it cannot be excluded that the 'electric environment of living organism' may cause biomembranes with α-tocopherol to be less stable, although α-tocopherol can stabilize membranes in studies without an external potential.

3.3. *Chronopotentiometric studies of BLM electroporation*

In order to observe the dynamic behaviours of BLM and BLM+E the chronopotentiometric method [24] was applied. Fig. 4 (solid line) shows typical chronopotentiometric curves for BLM (a) and BLM+E (b), for a constant current $i = 0.2$ nA. The shape of these curves is very similar for all membranes investigated and is characteristic of pore formation [24], i.e. a rapid increase in membrane voltage and then a sudden decrease after reaching a certain critical value. The membrane voltage did not reach the 0 value, indicating that the membrane was not destroyed. Instead, the drop in the membrane voltage reached the specific level and the voltage fluctuated around this value. Similar fluctuations have already been observed and described by other authors [26–29].

The fluctuations of potential are attributed to the changes in pore diameter. On the other hand, a fluctuating pore causes the fluctuation of membrane electric conductance [24]. The calculated conductance is presented in Fig. 4 (dashed line). The time evolution

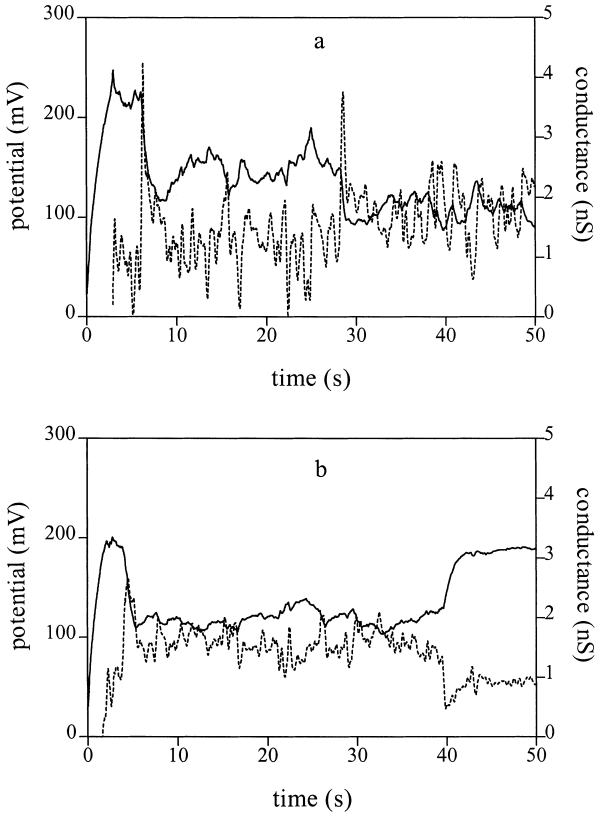

Fig. 4. Chronopotentiometric characteristics (solid line, left axis) of BLM (a) and BLM+E (b). The content of α-tocopherol in the forming solution was 10% of the total amount of lipids (w/w). Electrolyte, 0.1 M KCl, HEPES pH 7.0. After formation of the BLM, a constant intensity current (0.2 nA) was applied to the membrane. The conductance of the created single pore was calculated from the proper chronopotentiometric curve (dashed line, right axis).

of pores can be calculated from the membrane conductance. The value of mean conductance (the level around which the conductance fluctuates) informs about a mean pore diameter [24]. Surprisingly, the mean diameter of pores generated in different membranes was very similar, about 4 nm. The amplitude of pore fluctuations was, however, smaller in the case of the membrane with α-tocopherol, i.e. from about 2.3 nm to about 6.3 nm for BLM+E as compared with that for BLM (from 0 nm to about 6.1 nm). The important result is that the conductance of membranes with α-tocopherol very rarely reached the zero level, indicating that pores during fluctuation were not fully closed. This suggests that α-tocopherol impedes the recovery of a continuous structure of the bilayer lipid membrane and that membranes with incorporated α-tocopherol can be more permeable under external electric potential.

The results demonstrate that the dynamic properties of BLM and BLM+E are different when an external potential was applied to the membranes. This finding may be important respecting the fact that under normal physiological conditions the electric field in a cell membrane is approx. 10^5 V/cm, which is very close to the dielectric breakdown of liquid hydrocarbons [29].

3.4. Studies of pore resealing using programmable chronopotentiometry

Our measurement system allows to perform chronopotentiometric experiments with programmable current and controlling the short circuit of electrode connections. We present some results of experiments with a short circuit of the current electrodes. The short circuit forced the membrane potential equal to zero. The examples of the results are presented in Fig. 5. The dashed lines show the intensity of current flowing through the membrane and the solid lines between them represent the short circuit of the current electrodes. When the current was flowing an increase in potential was observed. The potential reached the value of the breakdown. This part of the curve is similar to the typical chronopotentiometric curves presented in Fig. 4. Next, after forcing the potential equal to zero for 50 s and subsequent breaking of the electrodes' short circuit, the current flowing through the membrane caused a reincrease of the potential. The reversible breakdown potential for membranes without α-tocopherol was 251 ± 23 mV. Subsequent switching 'on' and 'off' the external electric field on the same membranes changed the reversible breakdown potential. It dropped to the value of 155 ± 40, 154 ± 42, 160 ± 46, 156 ± 46 mV for the first, second, third and fourth cycle of registration, respectively. Analogous values for membranes with 10% (w/w) α-tocopherol were 228 ± 18 mV and 173 ± 32, 182 ± 34, 175 ± 34, 170 ± 36 mV. The presented values were calculated for ten membranes. However, the same tendency was clearly observed in most performed experiments.

The BLMs did not usually recover the continuous structure of the bilayer after 50 s of short circuit (Fig. 5a). The pores were not completely closed,

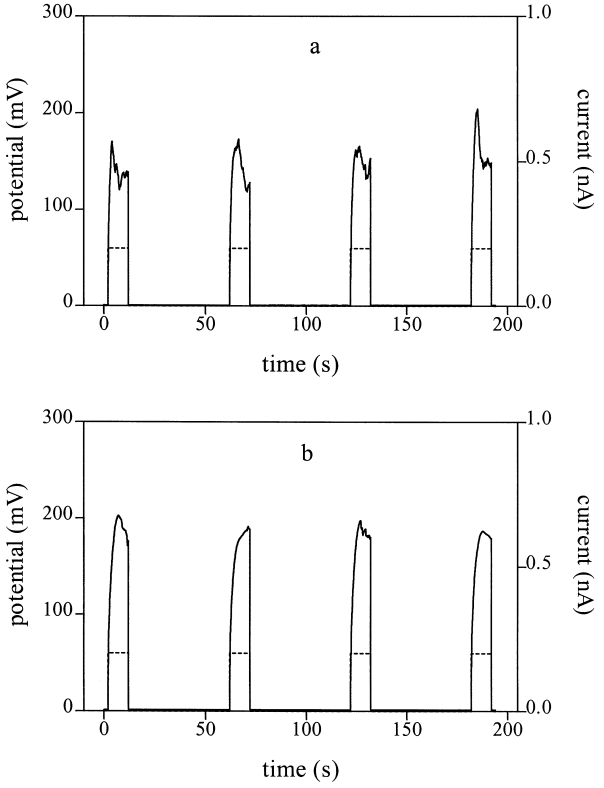

Fig. 5. Chronopotentiometric characteristics of BLM (a) and BLM+E (b) with forcing membrane potential equal to 0 mV. The content of α-tocopherol in the forming solution was 10% of the total amount of lipids (w/w). Changes of potential: solid line, left axis. Current intensity: dashed line, right axis. Repeatable sequence of measurements: 10 s of 0.2 nA, 50 s of current shortening.

the membranes had some defects in their structure. The pores were opened again by a potential lower than the breakdown potential. The same experiments performed on BLM+E (Fig. 5b) demonstrate that the membrane potential reached values closer to those characteristic for membrane reversible breakdown. Hence, these results suggest that α-tocopherol facilitated the recovery of a continuous membrane structure in the absence of an external electric field.

The resealing of pores under an external electric field and without field was different. Under an external electric field, the pores formed in BLM could be temporarily closed. This state was, however, not reached in BLM+E (Fig. 4). The disappearance of the external electric field changes the action of α-tocopherol: the resealing of pores in BLM+E is facilitated (Fig. 5).

The most important result is that we have demonstrated that α-tocopherol in high concentration can act destructively on membrane: under an external electric field (mimicking the electric environment of membranes of the living cell), α-tocopherol led to the destabilization of model bilayer lipid membranes. This may suggest that the physiological function of α-tocopherol (including antioxidative potency) can be strongly influenced by the electric potential of the membrane in the living cells.

It is, moreover, tempting to speculate why the concentration of α-tocopherol in biological membranes is very low (in the range 0.1–1.0 mole% of lipid [32]). One of the reasons could be associated with the fact that α-tocopherol may destabilize the membrane under an electric field. Hence, the danger of membrane destabilization may force the living organism to keep α-tocopherol at a very low concentration in biological membranes.

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