Eur Biophys J (2003) 32: 55–59
DOI 10.1007/s00249-002-0266-7

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Electrical capacitance of lipid bilayer membranes of hydrogenated egg lecithin at the temperature phase transition

Received: 9 April 2002 / Revised: 15 October 2002 / Accepted: 28 October 2002 / Published online: 19 December 2002

Abstract Electrical capacitance of the planar bilayer lipid membrane (BLM) formed from hydrogenated egg lecithin (HEL) has been studied during many passages through the phase transition temperature. In contrast to the BLM from individual synthetic phospholipids, membranes from HEL did not demonstrate any capacitance change at the phase transition temperature maximum, as measured by differential scanning calorimeter at 52 °C. Instead, two temperatures have been discerned by capacitance records: thickening at 42–43 °C and thinning at 57–59 °C. The first temperature region is close to the transition temperature of dipalmitoyllecithin, whereas the second is close to that of distearoyllecithin, two main components of the HEL. It was suggested that capacitance measurements were able to reveal a phase separation in the BLM from HEL which was not detected by differential scanning calorimetry. The phase transition of the BLM from the liquid crystal state to the gel state is followed by thickening of the bilayer structure, partly due to a *gauche-trans* transition of lipid molecules but mainly due to redistribution of the solvent n-decane.

Keywords Planar bilayer lipid membrane · Capacitance measurements · Phase transition · Hydrogenated egg lecithin

Introduction

Phase transitions of membrane lipids play an important role in cryobiology, thermoadaptation, thermoreception and cell fusion. Study of the temperatures of the phase transition in the planar bilayer lipid membrane (BLM) formed from natural phospholipids seems to be rather difficult because of a number of unsaturated chemical bonds in the fatty acid residues, which result in temperatures below 0 °C for the L_α-L_β transition. Hydrogenation of natural phospholipids provides a useful method to convert the unsaturated lipids into saturated ones, retaining all other chemical components without changes. This procedure is followed by a significant rise in the phase transition temperature. An X-ray study (Gennis 1989) of lipid bilayers at the phase transition of the L_α-L_β type showed that the transition of the lipid bilayer from the liquid crystal state to the gel state is followed by thickening of both monolayers, due to a transition of the fatty acid residues of the lipid molecules into the all-*trans* configuration. To obtain additional data concerning the thickness of the lipid bilayer at the temperature of the phase transition, the method of electrical capacitance has been used in this study.

There are several papers aimed at describing the physical properties of the planar bilayer membranes formed from individual phospholipids at the temperature of the main phase transition. The first paper (Pagano et al. 1973) was based on optical reflectivity on membranes from monostearoylglycerol in n-hexadecane, which demonstrated a 70% increase in membrane thickness when the system was cooled below the transition temperature of 55 °C. On the other hand, White (1975) obtained quite the reverse result during investigation of the structure of planar bilayer membranes formed from glycerol monooleate dispersed in various n-alkanes, using precise measurements of specific membrane capacitance. One possibility for this contradiction is that the authors have used different solvents, which could be able to shift the temperature of the phase transition significantly (McIntosh et al. 1980). Here we report the results of an experimental investigation of the electrical capacitance of planar bilayer membranes prepared from hydrogenated egg lecithin (HEL) at the phase transition of the L_α-L_β type. We show that the phase transition is associated with

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a phase separation of the lipids and with complex behavior of the membrane thickness due to a phase rearrangement of phospholipid molecules and simultaneous solvent redistribution between the bilayer and the torus in the planar BLM.

Materials and methods

All experiments were performed with HEL, kindly supplied by Predvoditelev (Moscow Pedagogical Institute). The sample of HEL migrated as a single spot on a silica gel thin-layer plate. Other properties of the HEL were described earlier by Nuhn et al. (1985). The calorimetric study with the HEL was carried out using a differential scanning calorimeter of the adiabatic type, DASM-4. The dry lipid was suspended in unbuffered 0.1 M KCI solution. Sonication was used to prepare a lipid dispersion, which was incubated at 53 °C for 15 min. All experiments were repeated at least on 2–4 separate preparations. Synthetic dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids and used without additional purification.

Planar BLMs were formed over a circular hole in a vertical wall of a Teflon pot as described by Mueller et al. (1962). The membrane-forming solution contained 25 mg of HEL dissolved in 1 mL of a mixture of n-decane/chloroform/methanol (7:2:1, by vol). All experiments were performed in unbuffered 0.1 KCI (pH 6.0). Before each experiment the vertical wall of the Teflon pot was covered with a thin layer of dried membrane-forming solution.

To carry out experiments at constant temperature, the measuring cell was placed in a water jacket connected with a thermostat. The temperature in the cell was maintained with an accuracy 0.5 °C. Scanning of the temperature was provided by a programmable semiconductor heater. The temperature near the BLM was measured with high accuracy by a sensitive thermocouple.

The capacitance changes were measured by applying a charge pulse of triangular form (in the voltage range ±30 mV) to the BLM and registration of the resulting cyclic current-voltage curves. It is known that the measured current is the sum of the ohmic and capacitive currents. The theoretical cyclic current-voltage curve of the planar BLM is shown in Fig. 1. The capacitance of the BLM (C_M) has been calculated using the well-known formula:

$$C_M = I_C / 4fU_{max} \tag{1}$$

where I_C is the capacitive current (see Fig. 1), f is the frequency of the applied voltage pulse and U_{max} is the amplitude of the applied voltage.

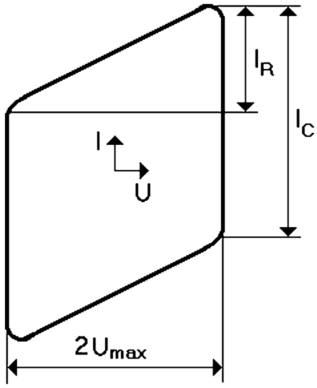

Fig. 1 Measurement scheme of cyclic current-voltage characteristics of a planar bilayer lipid membrane obtained by application of triangular pulses. U_{max}, amplitude of the applied pulse; I_R and I_C, ohmic and capacitance components of the transmembrane current, respectively

Results

Before proceeding to study the electric capacitance of the BLM formed from the HEL, the thermogram of the lipid was investigated (see Fig. 2). The thermogram in Fig. 2a reveals an endothermic comparatively wide transition with a single peak near 52 °C. It should be noted that the pretransition peak revealed for synthetic DPPC (Fig. 2b) also escaped detection. These data were in agreement with the results obtained earlier by Nuhn et al. (1985). Taking into account that the HEL was a mixture of lecithins enriched with palmitoyl and stearoyl residues, experiments with lecithin mixtures were carried out. As shown in Fig. 2c, experiments with HEL enriched with synthetic DPPC did not show any splitting of the peak or pretransition peak appearance. One can see only shifting and broadening of the peak in the thermograms. Therefore the presence of the HEL in the mixture of phospholipids makes elucidation of the phase separation by scanning calorimetry more difficult.

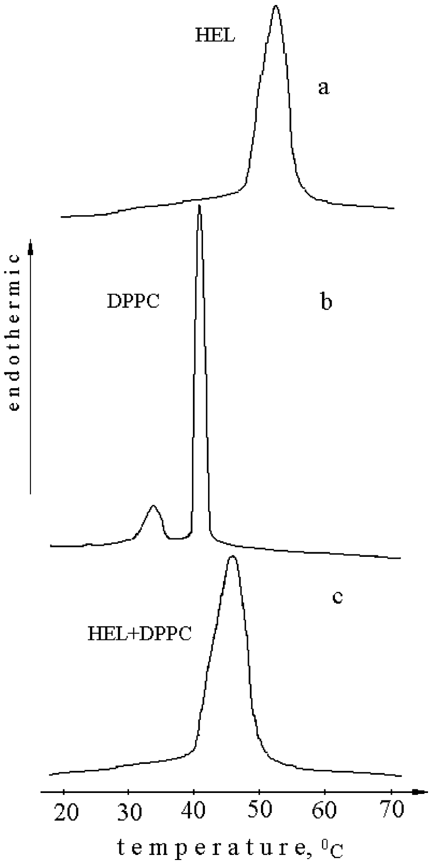

Fig. 2 Differential scanning calorimetry thermograms of (**a**) HEL; (**b**) DPPC; (**c**) a mixture of HEL plus DPPC (1:1 mol/mol); all in excess water at pH 6.0. Lipid concentration in the sample was 1 mg/mL

Based on the thermogram of the HEL (Fig. 2a), we attempted to test the BLM capacitance at the temperature of the phase transition (52 °C). It is well known that spreading and thinning of the lipid film precede the formation of a stable lipid membrane. Electrical capacitance measurements were made 15–20 min after the black film was formed in order to ensure that equilibrium had been attained. Figure 3 demonstrates a sequence of current-voltage curves recorded at a constant temperature of 52 °C. The time interval between the Fig. 3a and Fig. 3e recordings was 35 min. The sequence of the curves exhibits the very high stability of the BLM with a slight tendency of the capacitance to raise. Calculations using Eq. 1 gave the average specific capacitance of the BLM as 0.73 μF/cm^2, which is nearly twice as much as that for BLM formed from native egg lecithin (Antonov et al. 1992). The rather high conductance seen in Fig. 3a disappeared at a later time. It should be noted that all other sequences obtained at different constant temperatures in the range 40–60 °C (not shown) demonstrate the same behavior except for an instability of the BLM at temperatures well above 58 °C.

The next set of experiments was aimed at studying capacitance changes of the BLM during scanning of the temperature in the interval 40–60 °C. Figure 4 shows the capacitance changes in the BLM during the slow cooling at a constant rate of 1 °C/min (a) and fast heating at a rate of 40 °C/min (b). Preliminary experiments had revealed that the stability of the BLM in the gel state was less than the stability in the liquid crystal state. Thus during many passages through the phase transition it was significant to have the BLM move out of the gel state quickly. For this purpose, a heating rate of 40 °C/min was used. For thermal gradients to decrease, a magnetic mixer was used. The arrows in Fig. 4 show the jump of the capacitance. All experiments were carried out in a regime of automatic registration of the current-voltage characteristics of the BLM at a frequency of 0.25 Hz. One can see that there is no significant change in the membrane capacitance down to 43 °C. A sharp decrease in the membrane capacitance could be detected within narrow limits of 43–42 °C. Subsequent cooling of the BLM was not followed by any additional change of the capacitance. The rapid heating of the BLM after exposure at 40 °C is followed by complete restoration of the membrane capacitance (Fig. 4b). This process occurs in a wide temperature range, from 52 °C to 57 °C. As one can see, exposure of the BLM at the temperatures in the vicinity of the phase transition is accompanied by a rise in membrane conductance. It correlates with data described earlier (Antonov et al. 1980) and confirmed more recently (Anosov et al. 2000; Antonov et al. 1992; Bogatyreva et al. 1998) about ionic conductance appearing in planar BLMs at the phase transition temperature.

The absence of visible changes in the BLM capacitance in the vicinity of the phase transition temperature at 52 °C and the dramatic decrease of the capacitance at 42 °C by cooling indicates a shift of the phase transition toward the lower temperature. Seemingly this result could be easily resolved by taking into account the ability of n-decane to shift the phase transition temperature (Gennis 1989). It is known that n-decane, among other short-chain solvents, shifts the transition temperature toward a lower value, but this effect is rather modest (not more 6 °C) and insufficient to explain the obtained data (more than 10 °C). Furthermore, it should be noted that the melting temperature is also

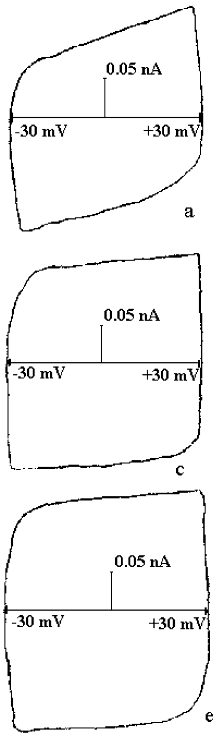

Fig. 3a–e A set of current-voltage curves of the BLM from HEL at the temperature of the phase transition (52 °C) maintained by thermostat with an accuracy within 0.5 °C. Duration of **a**–**e** interval, about 35 min. The area of the BLM minus torus is about 1 mm^2

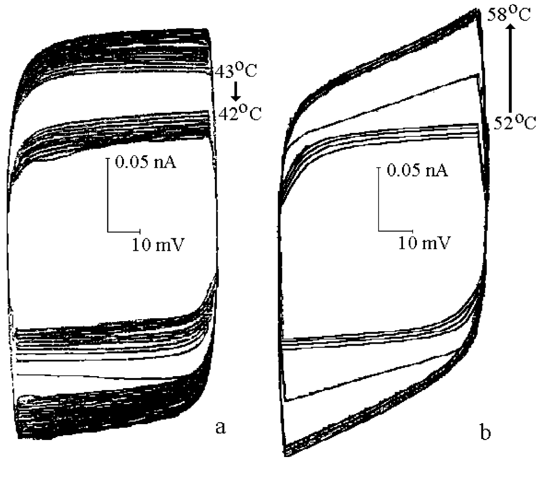

Fig. 4 A set of current-voltage curves of the BLM from HEL recorded by a two-coordinate recorder in automatic regime with a frequency 0.25 Hz: (**a**) by cooling from 43 °C to 40 °C; (**b**) by heating from 52 °C to 58 °C of the BLM previously cooled to 40 °C. *Arrows* show the direction of the temperature changes

shifted toward a temperature well above 52 °C (see Table 1). This process is more in the nature of a phase separation. This suggestion is based on data accumulated by Gennis (1989) about nonideality of a mixture of natural phospholipids, especially in the gel state.

White (1975) first observed a weak hysteresis between cooling and heating curves for membrane capacitance in the vicinity of the phase transition temperature. As one can see in Fig. 5, our data verify that result.

The decrease of the BLM capacitance indicates an increase in the thickness of the BLM. X-ray diffraction data (Gennis 1989) revealed the growth of BLM thickness at the phase transition of the L_α-L_β type by 17%. Our data shown in Fig. 5 indicate that a deeper decrease of capacitance, up to 65%, that could be explained exclusively by reverse movement of the solvent from the torus to the bilayer. As indicated in Fig. 5, subsequent heating of the same BLM is followed by an increase of capacitance up to complete restoration of the original values. Obviously it includes two phenomena: rapid repeat thinning of the BLM and melting of the phospholipid molecules. We suggest that phase transitions in the phospholipid moiety of the BLM play the key role, triggering the solvent redistribution between the torus and bilayer. Actually, the thinning of the BLM occurs under the influence of pressure derived from the van der Waals attraction between two water layers on both sides of the lipid bilayer (Gennis 1989). This pressure is strongly dependent on the thickness of the bilayer in a cubic manner. Therefore a slight change in the thickness of the bilayer at the phase transition must be followed by strong changes in pressure, which in turn provides the redistribution of the solvent.

It seemed to be reasonable to study the electrical capacitance of the BLM through many passages of the same BLM at the phase transition temperature. The data obtained are summarized in Table 1. It shows that a threefold passage is followed by some changes in low and high transition temperatures, revealed by capacitance jumps. Initially the wide transition temperature range becomes narrower on the second and third passages. It means that an excess of solvent in the first passage influences the width of the transition revealed by the capacitance jump. Surprisingly, a very narrow temperature range has been revealed for melting after many passages of the BLM through the transition. It should be noticed that this temperature range of 57–59 °C is well above the temperature of the main phase transition detected by differential scanning calorimetry for the HEL (Fig. 2).

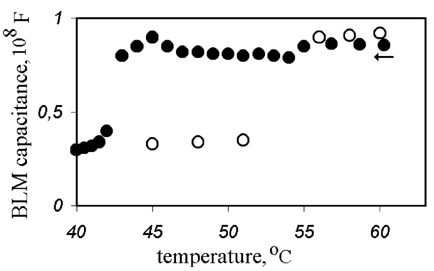

Fig. 5 Dependence of the electrical capacitance of the BLM from HEL on the temperature scanned in the range 40–60 °C with different scanning rates: cooling at 1 °C/min (*closed circles*), heating at 40 °C/min (*open circles*). *Arrow* shows the start of the experiment

Discussion

Lipid bilayers composed of two phospholipids with distinct acyl-chain mismatch behave as nonideal mixtures capable of phase separation. Data obtained in our experiments with the BLM capacitance measurements testify that HEL could be assigned as such mixtures, despite the single unresolved curve revealed in the thermogram by the differential scanning calorimeter (Fig. 2). According to Nuhn et al. (1985), chemical analysis of the fatty acid residues of HEL showed that phospholipids contained largely stearate (58.4%) and palmitate (33.4%). One can expect therefore that HEL should contain largely mixed-chain lecithin and distearoyllecithin in variable proportions. As was shown by Keough and Davis (1979), values of the transition temperatures for water dispersions of samples of distearoylphosphatidylcholine (DSPC) and DPPC have been observed to be 55 °C and 44 °C, respectively. As illustrated in Fig. 5 and in Table 1, both values are very close to transition temperatures revealed by the capacitance jump. It seems to be the first indication of the phase separation of phospholipids seen immediately in the planar BLM. From a biological point of view it seems to be very important that the rather wide hysteresis seen in Fig. 5 has been provided by weak modifications in fatty acid residues of the lecithin molecule by two CH_2 groups.

Our data conform the results obtained early by Pagano et al (1973) and data of the X-ray examination of lipid bilayer dispersion (Gennis 1989) that revealed thickening of the lipid bilayer structure at the phase transition of phospholipids from the liquid crystal state to the gel state. Additional data concern the behavior of

Table 1 Temperatures of electric capacitance jumps in the BLM from HEL by threefold passage of a single BLM through the transition temperature. Temperature determined with accuracy ±0.5 °C

Scan number	Jump down, start (°C)	Jump down, finish (°C)	Jump up, start (°C)	Jump up, finish (°C)
First scan	48	44	57	59
Second scan	44	42	57	59
Third scan	44	42	–	–

n-decane as a solvent. Previously, White (1974) discovered temperature-dependent structural changes in planar BLMs which he called "solvent freeze-out". In his experiments the structure of planar BLMs from glyceryl monooleate in n-hexadecane was investigated using precise measurements of specific capacitance. At the freezing point of the solvent (about 16 °C) the torus froze but the film did not rupture. The freezing was accompanied by a large increase in capacitance due to freeze-out of the solvent. In our experiments the temperature range of 40–60 °C was well above the freezing point of the solvent (decane) and thus we could not observe the "freeze-out" effect. Instead, dealing with the transition of the phospholipids from the liquid crystal state to the gel state, we suggest that the decrease of capacitance of the BLM followed "freeze-in" of the solvent. It is well known that, as with the flow of fluids in channels, a pressure gradient is required to maintain the BLM thinning process. We suggest that freezing of phospholipid monolayers in the BLM is followed by a decrease of the pressure gradient (see above) that allows the solvent to "freeze-in".

Acknowledgements The research was supported in part by a grant (01-04-48611) from the Russian Foundation for Fundamental Research.

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