Phase Transitions in Spherical Bilayer Membranes Prepared of Bulk Erythrocyte Membrane Lipids

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Abstract. The phase transitions of spherical lipid bilayers built of bovine red cell lipids have been studied. The electrical and ESR measurements revealed some conformational changes in lipid bilayers taking place at 36—38 °C.

Key words: Phase transition — Spherical bilayers — Lipids

Introduction

Lipids in biological membranes are generaly believed to exist in the bilayer conformation. Organization of the lipids into groups or clusters, i.e. phase separation, can be induced by various agents such as Ca^{2+} (van Dijck et al. 1978), proteins (Boggs et al. 1977) and lipids themselves (Lentz et al. 1982). Phase transitions in membranes reflect their specific structural properties. These can be most readily observed by calorimetric methods which allow to detect processes characterized by a significant energy change, e.g. thermally induced transitions in membranes of *Mycoplasma Laidlawii* (Melcher et al. 1970). However, conventional calorimeters are not sensitive enough to detect the thermally induced phase transitions in red cell ghosts which have recently been observed by means of a much more sensitive differential heat capacity calorimeter (Jackson et al. 1973). At least four endothermic transitions could be observed over the temperature range of 40—80 °C. In contrast to the data reported for *Mycoplasma Laidlawii* (Melcher et al. 1970), where the transitions occurred only in the lipid phase, the phase transitions observed in red blood cell ghosts were considered to be a result of protein denaturation and protein-lipid interaction (Jackson et al. 1973). Two major and one minor transitions have been observed by the same method in isolated human myelin (Moscarello et al. 1983). The nature of the transitions has not been clearly identified. The protein components of the membrane are certainly involved in a complex manner, probably in association with some lipids. Some pure lipids have recently been examined by different techniques and phase transitions

were found in a variety of lipids (Mitaku et al. 1981; Keough et al. 1979; Cuypers et al. 1980; Gliozzi et al. 1983; Jackson et al. 1978); however, in a mixture of lipids, the phase transitions usually disappear (Lentz et al. 1982; Petrov et al. 1982). In the present communication we report results of our studies on artificial bimolecular membranes prepared of lipids from bovine red cells. We could observe phase transitions using ESR methods and measurements of some electrical parameters of the membranes. It could be concluded that at least one phase transition is specific for lipids only.

Materials and Methods

Erythrocytes

Fresh heparinized bovine blood was used. Plasma and leukocytes were removed after centrifugation at $1000 \times g$ for 10 min. Erythrocytes were then washed four times with an isotonic phosphate buffer-NaCl solution, pH = 7.4, and suspended in the same solution.

Lipid extraction and preparation of liposomes

The lipids were extracted from erythrocyte ghosts with n-butanol at 0 °C according to Dodge et al. (1963), dried under vacuum in a glass tube, and resuspended in 25 mmol l Tris-HCl buffer, pH = 7.4. The mixture was mechanically shaken until a milky suspension was obtained. The concentration of lipids was 50 mg ml.

Formation of spherical black membranes

Spherical black membranes were formed at room temperature in 0.01 N KCl aqueous solution of lipids dispersed in n-decan and n-butyl at a ratio 1:1 (v/v) to obtain a concentration of 30 mg/ml. The membranes were prepared according to the technique of Schagina et al. (1976) on a teflon capillary tube (outer and inner diameters 5.0 and 0.5 mm, respectively). Membranes were stable for eight or more hours. A block circuit diagram of the apparatus used to measure the electrical parameters of the membranes is shown in Fig. 1. The temperature was kept constant in the range of 20—50 °C by a water

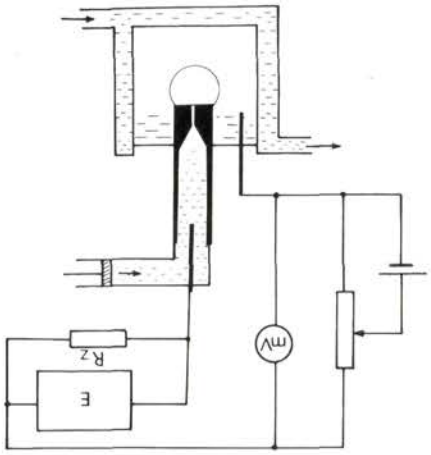

Fig. 1. A block circuit diagram of the apparatus used to study the electrical parameters of spherical bilayers. $R_z = 5 \times 10^7 \Omega$. E — electrometer. For further details, see text.

jacket around the observation chamber. In all experiments, the spherical bilayers were observed through a microscope with a scale eyepiece. The membrane area was determined by an optical measurement of membrane dimensions. The estimated error did not exceed 3%. Ag/AgCl electrodes were employed to apply and record the electric potentials. Electrical resistance of the membrane was calculated from voltage-current characteristics. The external electric potential was not greater than 50 mV, which assured linearity of the characteristics (the correlation coefficient, r, was better than 0.99). The potentials were measured by an electrometer (Mera Elwro type 504.10) with an accuracy of ± 0.5 mV. Membrane capacitance was determined from a single impulse relaxation time. The signal was recorded. The cationic transference numbers t_+ were calculated from following equation (Ohki 1976):

$$V_{\text{diff}} = (2t_+ - 1)\frac{RT}{F}\ln\frac{C_2}{C_1}$$

where V_{diff} is the diffusion potential; C_1 and C_2 are the concentrations of KCl outside and inside the spherical bilayer, respectively; T is the temperature; R is the gas constant; and F is the Faraday constant. Concentration difference was obtained by the addition of several milliliters of 1N KCl to one side of the membrane (usually, $C_1/C_2 = 10$).

Spin-labelled preparates

The spin probe used in the experiments was 5-doxylpalmitate methyl ester I 10,3 incorporated into liposomes prepared of the lipids studied. An appropriate amount of 2×10^{-3} mol/l ethanolic solution I 10,3 was used to maintain the label within a lipid molecular ratio of 1:50. The above solutions were allowed to dry on test tube walls. Liposome suspensions were added and the tube was mechanically shaken for 20 min. This way, the lipids were sufficiently labelled for ESR experiments.

ESR experiments

All the spectra were recorded on a SE/X—28 spectrometer (Technical University of Wrocław). The temperature was varied within 16—50 °C. In order to estimate the mobility of the spin probe, an empirical motion parameter W was calculated: $W = T°/T$, where $T°$ is the outer hyperfine splitting at 24 °C, and T is the outer hyperfine splitting at the temperature of the probe.

Results and Discussion

Information on membrane structural changes induced by the increasing temperature could be obtained by measuring the membrane electrical properties. The results obtained on different membranes were qualitively identical. Figs. 2,3 and 4 show representive results obtained on a single membrane. Fig. 2 shows the logarithm of the resistance plotted against $1/T$. The straight line ($r > 0.99$) indicates an exponential behaviour of the resistance as a function of $1/T$, as expected for the Arrhenius plot. At temperatures around 37 °C, the activation energy increased from $(14.7 \pm 2.1) \times 10^3$ J/mol to $(25.5 \pm 2.5) \times 10^3$ J/mol. However, there was a large spread of these data for the membranes, the ratio of the activation energy above 37 °C to that below this temperature remaining constant (1.71 ± 0.15) and independent on the membrane. Membrane capacitance was determined simultaneously with the resistance at every temperature level. Data of a single membrane are shown in Fig. 3. The curve is bell-shaped with the maximal capacitance at 35—37 °C. The bulk cationic transference number t_+ is shown in Fig. 4. The maximum t_+ was observed at 24 °C. Fig. 5 illustrates the effect of temperature on the mobility of the spin label (expressed by the W parameter) incorporated into the

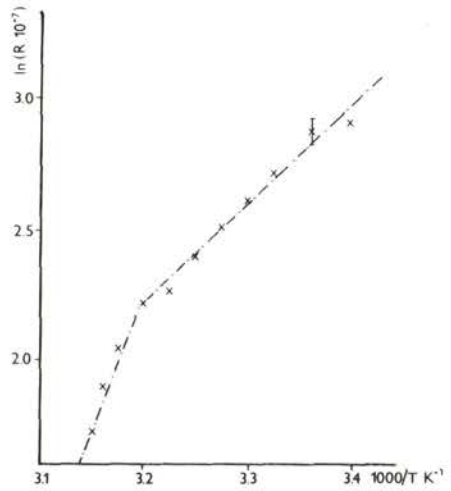

Fig. 2. The logarithm of the electrical resistance plotted against the reciprocal of the absolute temperature. The different slopes of the straight lines reflect a change in the activation energy at 37 °C. R was expressed in $\Omega = cm^2$.

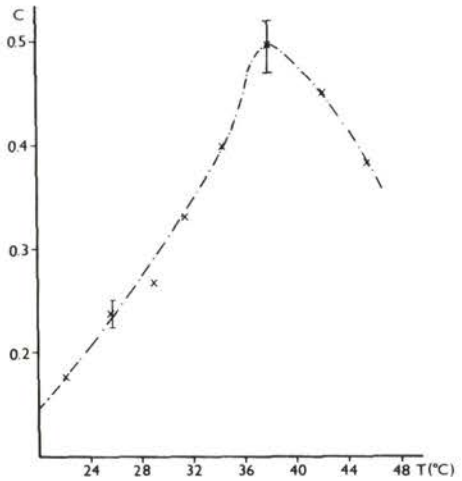

Fig. 3. Specific geometric capacitance plotted against the temperature. The approximate error of the measurements (±15%) is indicated. C was expressed in $\mu F = cm^{-2}$.

liposomes. It demonstrates the discontinuity in the Arrhenius plot, which, according to Schreier et al. (1978), probably indicates a lipid phase transition. The data from ESR spectra agree with the presented behaviour of the investigated electrical properties. The results reported here have suggested that, at 36—37 °C, a phase

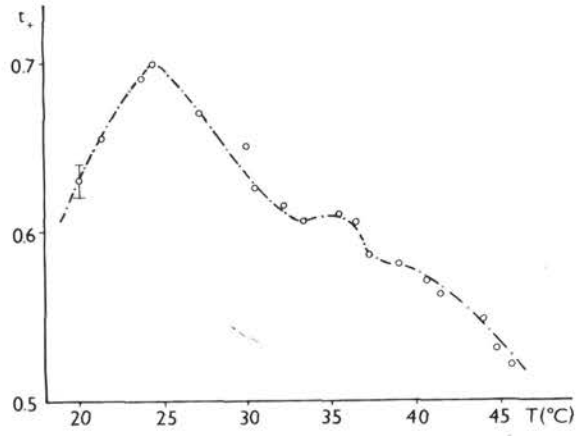

Fig. 4. The bulk cationic transference number plotted against the temperature. The approximate error (±3%) is indicated.

transition occurs in lipids. This effect has not been observed by calorimetry techniques (Jackson et al. 1973). The change in the activation energy is by one or two orders of magnitude smaller than reported by Jähnig and Bramhall (1982). This may explain why this transition has not been observed previously. We have not yet been able to explain the maximum seen in Fig. 4, corresponding to the temperature of 24 °C, since at this temperature other parameters studied have not revealed any peculiarities in their behaviour.

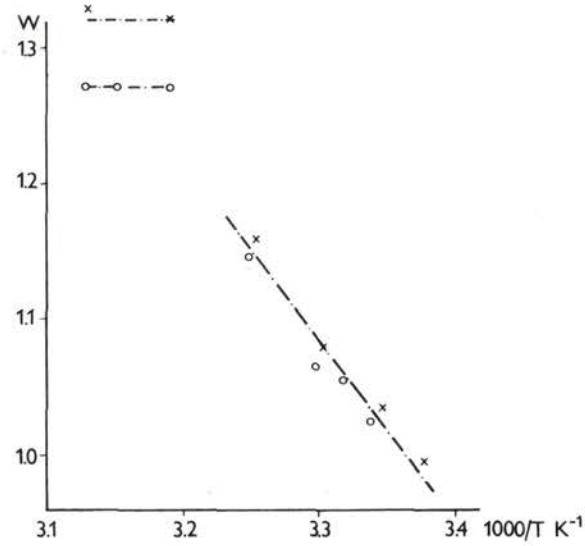

Fig. 5. The Arrhenius plot of the *W* parameter. x- and o- correspond to different runs.

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