Biochimica et Biophysica Acta, 778 (1984) 602–611
Elsevier

BBA 72374

PHASE TRANSITION AND LATERAL PHASE SEPARATION IN PLANAR LIPID MEMBRANES AS SENSED BY THE LIPOPHILIC ION DIPICRYLAMINE

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(Received March 9th, 1984)
(Revised manuscript received September 10th, 1984)

Key words: Phase transition; Phase separation; Cholesterol; Dipicrylamine; (Lipid bilayer)

The permeation of the lipophilic ion dipicrylamine through planar lipid membranes formed from dipalmitoylphosphatidylcholine in *n*-decane shows an anomaly near the main phase transition of this system. Both the rate constant, k_i, of ion translocation across the membrane interior and the interfacial concentration, N, of this ion have a maximum at about 36°C. Analogous experiments were performed with tetraphenylborate. A considerably lesser effect of the phase transition was found. The addition of cholesterol leads to a broadening of the maxima for k_i and N. The time course of the current following a voltage jump shows a characteristic change below a temperature of about 45°C, if the molar ratio cholesterol/phosphatidylcholine in the membrane forming solution exceeds 1. While the current transient decays exponentially above 45°C, a sum of two exponential terms yields an adequate fit below that temperature. This is regarded as evidence for a lateral phase separation below 45°C into structurally different domains, which provide two different pathways for dipicrylamine.

Introduction

The influence of the physical state of the membrane lipids on functional properties of biomembranes is at present investigated by a variety of different methods in native membranes and model systems. Phase transition and phase separation phenomena have been detected in lipid/water systems by making use of differential scanning calorimetry, X-ray diffraction, electron microscopy and by optical-, ESR- and NMR-spectroscopy (see Refs. 1–3 for a review). Studies on planar (black) lipid membranes have contributed a great deal to the clarification of the molecular basis of ion transport phenomena across biomembranes. Cation transport mediated by macrocyclic ion carriers (e.g., valinomycin) has been reported to be strongly affected by the transition from the liquid-cristalline to the gel state of a lipid membrane [4–7], while ion transport through pore-like structures seems to be less influenced by a phase transition of the surrounding lipid molecules. While there is basically agreement that the translational motion of molecules inside the membrane is influenced by the physical state of the latter, there is a controversy about the extent of this influence. Krasne et al. [4] found a drastic reduction – by many orders of magnitude – of the translational mobility in the gel state of membranes formed from a 1:1 mixture of dipalmitoyl- and distearoylglycerol in *n*-decane ('freezing'). Similar results were obtained by Boheim et al. [7] on solvent-free membranes made from 1-stearoyl-3-myristoylglycero-2-phosphocholine. On the other hand our own studies on dipalmitoylphosphatidylcholine membranes doped with valinomycin showed a considerably lesser effect of the main phase transition of the membrane [5]. The only

evidence for a change in the behaviour of this ion carrier at the phase transition was a break in the slope of the Arrhenius plot of the conductance-temperature relationship. The mechanical stability of the membranes in the solid state was drastically impaired, however, so that the measurements were restricted to a relatively small temperature range (the lower limit was about 10°C below the break indicative of a change of the physical state of the membrane). We have recently examined the problem in greater detail by performing a kinetic analysis of the movement of hydrophobic ions across the membranes [8]. The interfacial concentration of dipicrylamine and its translocation rate across the membrane interior was determined by voltage-jump relaxation experiments. An increase of both quantities with decreasing temperature was found in the temperature range of the phase transition, with a relative maximum at about 36°C. The findings may be considered as an extension of previous studies on lipid vesicles, where maxima in the ion permeability of different ionic species at the phase transition of the vesicles were reported [9–14]. Our experiments on planar lipid membranes indicate that the observed anomaly of the ion permeability is a consequence of both an enhanced partition coefficient and an increased mobility of the ions inside the membrane.

The experiments presented below will show that hydrophobic ions may be also used as sensors of lateral phase separations in planar lipid membranes formed from lipid mixtures. The time course of the electric current following a voltage jump in the presence of these ions may – at least in many cases – well be approximated by a single exponential term. This is true for homogeneous membranes. It is shown that in the presence of cholesterol, and dependent on the temperature, more or less pronounced deviations from a simple exponential time behaviour occur. The data may be fitted by a sum of two exponential terms, which are interpreted on the basis of a heterogeneous membrane composed of domains of different compositions. Ca^{2+}-induced phase separation of lipid mixtures monitored by the ion carrier valinomycin has recently been reported by Schmidt et al. [15].

Materials and Methods

Planar lipid membranes were formed from 1% solutions of lipid in *n*-decane (Fluka, puriss.). The lipids were obtained from the following sources: dipalmitoylphosphatidylcholine (Fluka, Sigma), dioleoylphosphatidylcholine (Sigma), cholesterol (Eastman). 1-Stearoyl-3-myristoylglycero-2-phosphocholine was prepared by K. Janko in our laboratory.

The technique of the voltage-jump experiments was described in earlier publications [16,17]. A battery-driven pulse generator supplied the voltage pulses to the membrane. A Tektronix oscilloscope 5115 in combination with a waveform recorder (Biomation, type 805) and a signal averager (Nicolet, model 1072) was used for the current measurements. The digitized data were finally transferred into a computer (Hewlett Packard, model HP 85) for further analysis. The temperature in the cuvette was measured via a miniature thermocouple (Philips thermocoax 2 ABAC 05, diameter 0.5 mm) which was mounted to the membrane as closely as possible and used in combination with a digital thermometer (Technoterm 9500). The cuvette used for bilayer formation was attached to a metal block. Its temperature could be changed through two thermostats held at different temperatures. The heating and cooling rate was about 1 K/min. There was a temperature difference between the place of the thermocouple and the membrane of up to 3 K throughout a heating and cooling cycle. This was corrected, so that the accuracy of the temperature measurement was better than 1 K. Partition equilibrium of the hydrophobic ions between membrane and water was not fully adjusted throughout the heating and cooling cycle, because of the comparatively fast change of temperature.

The size of the hole across which the membrane was formed, was relatively small (0.5–1 mm) to improve the membrane stability in the gel state of the membrane. Measurements were usually started 30 min after the transition of the membrane into the black state. This time interval was chosen to minimize aging effects on the relaxation data [17]. The measurement uncertainty of the electronic circuit was largely determined by the resolution of the analogue-digital converter (8 bit). Current noise

was effectively reduced by signal averaging. The overall accuracy of the current measurement was about 1% of the initial current I_0. The initial slope of the current relaxation was obtained by a least-square fit to the current data between I_0 and about 0.7 I_0. Eq. 1 was fitted to the experimental data using a PDP11/40 computer.

Theory

The permeation of hydrophobic ions across lipid membranes has been found to agree sufficiently well with a model essentially comprising the following kinetic steps [16–19]:

(a) diffusion through the aqueous phase to the membrane (diffusion coefficient, D);

(b) adsorption at the interface (rate constant, βk; β = partition coefficient);

(c) translocation across the membrane interior (rate constant, k_i);

(d) desorption into the aqueous phase (rate constant, k);

(e) diffusion though the unstirred aqueous layer (diffusion coefficient, D).

A test of this model on the basis of voltage-jump experiments yields the following general result for the relaxation of the electrical current, $I(t)$ [17]:

$$I(t) = I_0 \frac{2\sqrt{\omega}}{\pi} \int_0^\infty \frac{\exp(-\nu^2 t/\tau)\, d\nu}{\omega\nu^2\left[g(\nu^2-1)-1\right]^2+(\nu^2-1)^2} \quad (1)$$

$$I_0 = I(t=0) = \frac{N}{\tau} zFA \tanh(zu/2),\ 1/\tau = 2k_i \cosh zu/2 \quad (2)$$

$$\omega = \tau D/\beta^2,\ g = 1/k\tau \quad (3)$$

where z is the valency; F, the Faraday constant; A, the membrane area; N, the interfacial concentration of the lipophilic ions at a concentration c in water ($N = \beta c$); u, the reduced voltage ($u = VF/RT$); V, the voltage; R, the gas constant, and T the temperature.

The slope of the initial current is obtained as:

$$(d\ln I/dt)_{t=0} = -1/\tau \quad (4)$$

The determination of the model parameters k_i, β and k proceeds as follows. k_i and β are easily determined from the initial current I_0 and the initial slope $d\ln I/dt$ (Eqns. 2 and 4). The rate constant, k, of the desorption from the membrane into water at given values of k_i, β and of the diffusion coefficient, D, specifies the shape of the function $I(t)$ according to Eqn. 1. k may be obtained by a computer fit of this equation to the experimental data under favourable conditions, as discussed in Ref. 17.

At sufficiently small values of k (i.e., $g \to \infty$, cf. Eqn. 3) the current decays exponentially

$$I(t) = I_0 e^{-t/\tau} \quad (5)$$

The same result is obtained in case of comparatively slow aqueous diffusion ($\omega \to 0$). In both cases, the membrane appears isolated from the aqueous phase, i.e., the exchange of ions between membrane and water is negligible. The transient electrical current arises from the voltage-induced redistribution of ions between the two membrane/water interfaces and is – under the conditions of Eqn. 5 – completely determined by the translocation rate constant, k_i, and the interfacial ion concentration, N (cf. Eqn. 2).

Eqns. 1–5 hold for a homogeneous membrane. The problem is now generalized to membranes composed of domains of different composition. The behaviour of hydrophobic ions as well as that of macrocyclic ion carriers has been found to dependend on the structure of a lipid membrane (see Ref. 19 for a review). The parameters k_i and β (or N) respond to a change in the polar region of the membrane (variation of the surface potential and/or the dipole potential at the interface) and also to a change in the properties of the membrane interior (thickness or microviscosity). The problem is further illustrated on the basis of a membrane composed of two kind of domains different in structure, which act in parallel, and independently from one another, with respect to the transport of a lipophilic species. The two domains are characterized by different model parameters $k_i(1)$, $\beta(1)$ and $k_i(2)$, $\beta(2)$, respectively. It is further assumed that the exchange of ions between membrane and water is sufficiently slow for both kinds of domains so that the prerequisites of Eqn. 5 are met. Then, the time dependence, $I(t)$, instead by Eqn. 5, is given by the sum of two exponentials:

$$I(t) = I_0(1)e^{-t/\tau_1} + I_0(2)e^{-t/\tau_2}, \quad (6)$$

with $I_0(1)$, $I_0(2)$, τ_1 and τ_2 determined through a corresponding change of Eqn. 2. The ratio $\tau_1 I_0(1)/\tau_2 I_0(2)$ is equal to n_1/n_2, the ratio of the number of moles of lipophilic ions in the two different phases.

In the following section, experiments are presented showing a transition from a single time dependence according to Eqn. 5 to that according to Eqn. 6. The transition is induced by a variation of the temperature and is regarded as an evidence for a lateral phase separation in a planar lipid membrane.

Results and Discussion

Our evidence of a lateral phase separation is based on deviations of the current relaxation from a single exponential. We have to test therefore, whether Eqn. 5, as a special case of Eqn. 1, is indeed sufficient to describe the behaviour of a homogeneous membrane. Fig. 1 illustrates a typical current relaxation observed in the fluid state of a dipicrylamine-doped neutral dioleoyllecithin membrane. The solid curve represents a fit of Eqn. 1 to the initial time dependence of the current.

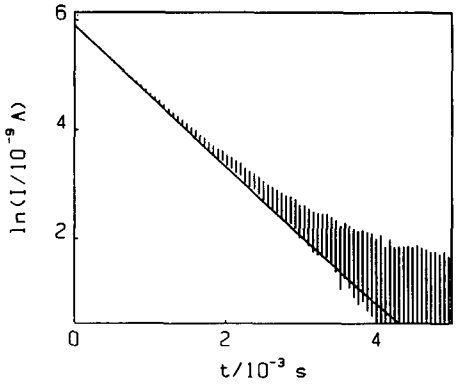

Fig. 1. Current relaxation observed with a dioleoyllecithin membrane in the presence of $2 \cdot 10^{-8}$ M dipicrylamine and 1 M NaCl in water, 90 min after the blackening process (area of the aperture $8 \cdot 10^{-3}$ cm^2, temperature 25°C, average of 32 pulses of 10 mV amplitude, measurement resistance 10^3 Ω). The bars indicate twice the measurement uncertainty (data ±1% of the initial current J_0; the resolution of the transient recorder was 0.4%). The solid curve was calculated from Eqn. 1 assuming $D = 4.7 \cdot 10^{-6}$ cm^2/s and using the experimental values $I_0 = 369$ nA and $\tau = 7.69 \cdot 10^{-4}$ s. From these values one finds $\omega = 2.8 \cdot 10^{-6}$. Any value of $g \leq 100$ is consistent with the solid curve.

This determines the values of I_0 and ω. The value of the parameter g was chosen in such a way to achieve an optimal agreement between theory and experiments for the time $t > \tau$. The curve – a consequence of the small value of ω – is almost exponential irrespective of the value of g (or k). This indicates that – due to the large values of the rate constant, k_i, of ion translocation across the membrane and of the partition coefficient β – the membrane appears effectively isolated from the aqueous phase, i.e., aqueous diffusion is comparatively slow. For membranes, where the partition coefficient β is smaller (e.g., negatively charged membranes) the exchange of ions between membrane and water cannot be neglected. A pronounced deviation from an exponential time course is observed in this case, in good agreement with Eqn. 1 [17]. Though theory, with the experimental conditions of Fig. 1, supports the validity of Eqn. 5, there is a small discrepancy for $t \gg \tau$. The deviations become more significant if the experiments are performed with larger membranes to improve the signal-to-noise ratio [17]. (We used comparatively small membranes in the present study to improve membrane stability in the gel state of the membrane.) We believe that this discrepancy arises from the inherent heterogeneity of a planar lipid membrane. The thickness of a bilayer will – possibly induced through an uneven distribution of the solvent decane – vary within certain limits. In consequence, the rate constant, k_i (i.e., also the relaxation time τ in Eqn. 5), will show a certain distribution around a mean value leading to the observed deviations between theory and experiment. As a result, evidence for a lateral phase separation must be based on deviations from Eqn. 5 which are considerably larger than those from the inherent heterogeneity of the membrane.

Our next aim is an extension of our previous study on the transport behaviour of lipophilic ions at a phase transition of the membrane [8]. In Fig. 2, the temperature dependence of the relaxation data of two membranes formed from dioleoyl- and dipalmitoylphosphatidylcholine are compared. The initial slope, τ^{-1}, and the product $I_0\tau$ are proportional to the rate constant, k_i, and to the interfacial concentration, N, respectively (see Eqn. 2). The dioleoylphosphatidylcholine membrane is in

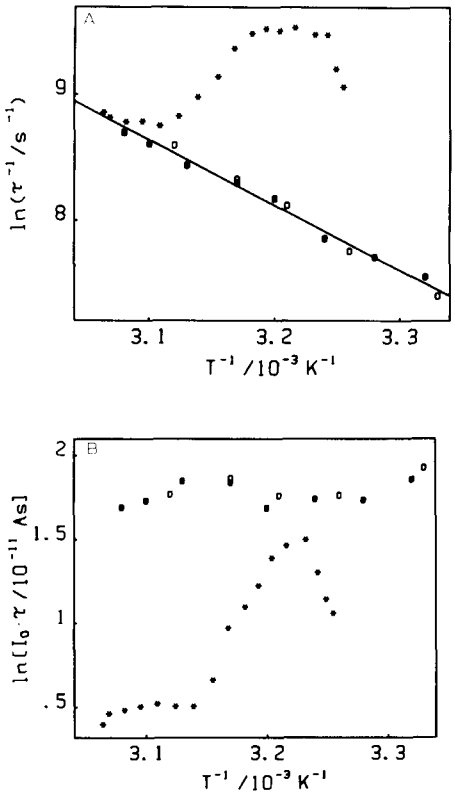

Fig. 2. Temperature dependence of the relaxation data obtained from dioleoyl- and dipalmitoylphosphatidylcholine membranes in the presence of dipicrylamine. (*) Cooling of a dipalmitoylphosphatidylcholine membrane 30 min after the blackening process in an aqueous solution containing $1 \cdot 10^{-7}$ M dipicrylamine and 0.1 M NaCl (membrane aperture $1.2 \cdot 10^{-3}$ cm^2). Heating (●) and cooling (○) of a dioleoylphosphatidylcholine membrane ($5 \cdot 10^{-8}$ M dipicrylamine and 1 NaCl in water; membrane aperture, $5 \cdot 10^{-3}$ cm^2). The voltage jump was from 0 to 30 mV in both cases. (A) The initial slope $1/\tau$ of the current relaxation corresponds to the translocation rate constant, k_i. (B) The product $I_0\tau$ is proportional to the interfacial concentration, N (cf. Eqn. 2).

the fluid crystalline state within the applied temperature range and shows a normal Arrhenius behaviour for the rate constant, k_i, and a fairly temperature-independent interfacial concentration, N. The dipalmitoylphosphatidylcholine membrane on the other hand exhibits an anomaly near the main phase transition temperature of this lipid at about 41°C. The data in Fig. 2 represent an extension of a previous study [8] to slightly lower temperatures. For both experimental quantities, k_i and N, maxima in the temperature range 35–40°C were found. This is in line with previous studies on the ion permeability of different ionic species at the phase transition of lipid vesicles [9–14]. The transition temperature is shifted to slightly lower temperatures due to the solvent decane [20,21]. Since the ion permeability is proportional to the product $k_i N$, our results indicate that both quantities, k_i and N, contribute to the previously reported maximum of the ion permeability. The following results will, however, show that the behaviour of the ion permeability at the phase transition is strongly dependent on the kind of the ionic probe and also on the particular nature of the lipid component of the membrane.

Fig. 3 illustrates the results obtained with dipicrylamine in a membrane which was formed from 1-stearoyl-3-myristoylglycero-2-phosphocholine in *n*-decane. The temperature, t_c, of the main transition for lipid/water emulsions of this mixed-chain lipid was reported to be in the range of 27–34°C [7]. While there is a maximum of the interfacial concentration, N, in this temperature range, no maximum is found for the rate constant, k_i, of ion translocation. In some experiments a complete absence of the anomaly was found, i.e., the rate constant, k_i, showed a normal Arrhenius behaviour ($k_i \approx \exp(-\Delta E_i/RT$, ΔE_i being temperature independent). In other experiments (see Fig. 3) deviations from the normal behaviour at

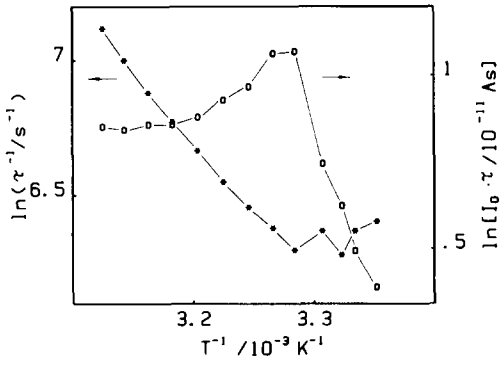

Fig. 3. Temperature dependence of the relaxation data obtained from a membrane which was formed from a 1% solution of 1-stearoyl-3-myristoylglycero-2-phosphocholine in *n*-decane. The area of the membrane aperture was $1.2 \cdot 10^{-3}$ cm^2. The other experimental data correspond to those of dipalmitoylphosphotidylcholine (see legend to Fig. 2).

about 30°C were observed, which were, however, substantially smaller than those found for dipalmitoylphosphatidylcholine (Fig. 2).

Fig. 4 (in comparison to Fig. 2) shows that different ionic probes respond differently to a change of the physical state of the membrane. The lipophilic ions dipicrylamine and tetraphenylborate have been found to behave qualitatively identically, when studied by voltage-jump relaxation experiments [16]. The partition coefficient of the ions is very similar, the rate constant, k_i, is one to two orders of magnitude smaller for tetraphenylborate. Though both lipophilic ions are of comparable size and 'lipophilicity' (partition coefficient), tetraphenylborate is less influenced by the physical state of the membrane. There is no maximum for k_i and only a weakly pronounced maximum for N.

As to the nature of the transition sensed by the lipophilic ions, at least two alternative interpretations may be envisaged [8]:

(1) The structure of the microenvironment of a lipophilic ion differs considerably from the structure of an unperturbed lipid bilayer. At a phase transition of the unperturbed lipid membrane, the microenvironment of the ion remains 'fluid' and allows a passage of the ion across the membrane interior. But the 'local fluidity' of the membrane around a lipophilic ion – responsible for the magnitude of its translocation rate k_i – is affected by

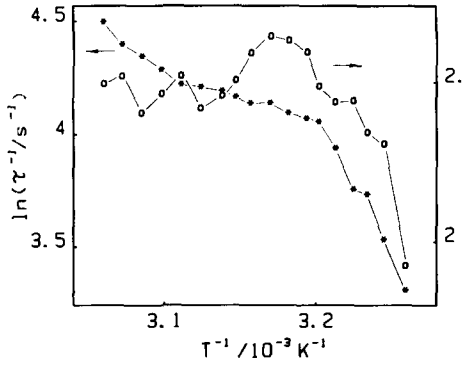

Fig. 4. Temperature dependence of the relaxation data found in the presence of the lipophilic ion tetraphenylborate. The membrane was formed from dipalmitoylphosphatidylcholine in the presence of $1 \cdot 10^{-6}$ M tetraphenylborate. The other experimental conditions correspond with those for dipicrylamine (see legend to Fig. 2).

the physical state of the unperturbed membrane. Jähnig and Bramhall [22] suggested that the permeation of relatively small molecules through a lipid membrane requires a local compression of the microenvironment of the particles. Since the lateral compressibility is maximal at the phase transition – a consequence of the cooperative nature of this phenomenon – a maximum of k_i should be observed at the temperature t_c. The permeation of larger molecules according to the view of these authors requires a local melting of lipid molecules in the microenvironment (boundary lipids). There is no maximum of the rate of permeation in this case, but a drastic change of the activation energy of the phase transition is expected, i.e., a break in the Arrhenius plot of the permeation rate. The two hydrophobic ions, dipicrylamine and tetraphenylborate, both belong to the class of relatively small molecules. The differences of their behaviour as expressed in Figs. 2–4 show the limitations of the ideas of Jähnig and Bramhall [22], if applied to our results.

(2) Alternatively, the change of the physical state of the membrane, as sensed by the hydrophobic ions, might indicate a lateral phase separation, involving the membrane constituents dipalmitoylphosphatidylcholine and the solvent decane, into a decane-enriched fluid and a decane-poor solid phase. In this case, dipicrylamine would be restricted to only one (presumably the fluid phase) of the two phases. This may be concluded from the fact that the shape of the current relaxation is temperature independent and is similar to a membrane in the fluid crystalline state, as shown in Fig. 1.

There is no unequivocal evidence for one of the two alternatives at present. Unfortunately, planar membranes formed from solvents other than decane (e.g., hexadecane) have not proved stable enough. We favour, however, alternative 1, since maxima in the permeation rate of small molecules have been observed also with other artificial lipid membrane systems, such as vesicles. These measurements were performed in the absence of decane [9–14].

Evidence for the existence of a lateral phase separation was obtained in case of the ternary system dipalmitoylphosphatidylcholine, cholesterol and decane. The addition of relatively small

amounts of cholesterol ($\alpha \leq 0.5$, where α is the molar ratio cholesterol/phosholipid) to the membrane forming solution leads to a broadening of the maxima in the transition region. Simultaneously, the onset of the region of negative activation energy is shifted to a higher temperature. At $\alpha = 0.5$ (cf. Fig. 5), the experimental parameters τ and $I_0\tau$ (data not shown) were found to be largely temperature independent in part of the experiments (open circles). Other experiments (crosses) showed an increase of τ below 63°C and a relatively constant value below 53°C. For a $\alpha \leq 0.5$, the time course of the current – apart from minor deviations probably caused by the inherent heterogeneity of the membrane (cf. Fig. 1 and its interpretation) – was found to be largely exponential (Eqn. 5) within the temperature range applied. At higher cholesterol concentrations ($\alpha = 1$ and $\alpha = 2$), an exponential behaviour was found at high temperatures, whereas a pronounced deviation from Eqn. 5 was obtained at lower temperatures. Eqn. 1 predicts an exponential time dependence according to Eqn. 5 at both temperatures (not shown). The data illustrated in Fig. 6 were obtained from the same membrane within a time interval of about 10 min. At low temperatures, the time course of the current is in good agreement with Eqn. 6. The temperature-induced transition of the current shape may be interpreted – as outlined in the theoretical section – on the basis of a lateral phase separation of the membrane. Below about 45°C, the movement across the membrane of the lipophilic ion dipicrylamine proceeds via two independent paths, which differ with respect to the magnitude of the model parameters k_i and β. In consequence, the relaxation of the electric current is the sum of two exponential terms. Fig. 7 shows the complete set of data obtained from a single membrane.

The coexistence of more than one phase in binary mixtures of cholesterol and phosphatidyl-

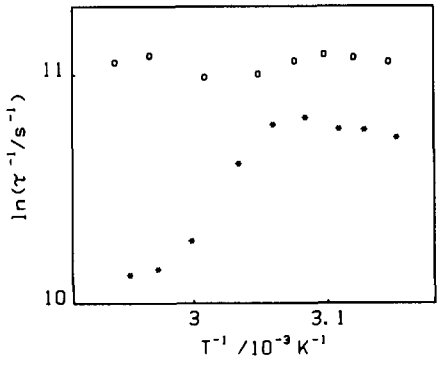

Fig. 5. Temperature dependence of the initial slope $1/\tau$ of the current response following a voltage jump of 30 mV for membranes formed from a mixture of dipalmitoylphosphatidylcholine and cholesterol (molar ratio, 2:1) in *n*-decane. The aqueous solution contained $5 \cdot 10^{-8}$ M dipicrylamine and 1 M NaCl. The data of two separate experiments are shown.

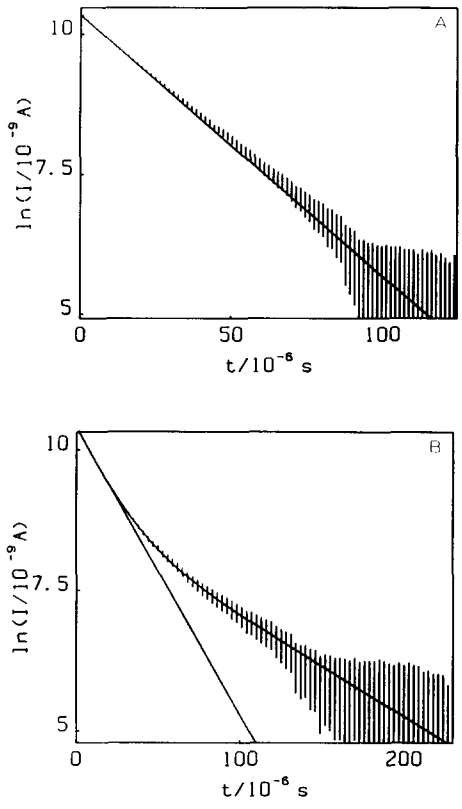

Fig. 6. Current relaxation for a membrane formed from a mixture of dipalmitoylphosphatidylcholine and cholesterol (molar ratio, 1:1) in *n*-decane in the presence of $1 \cdot 10^{-6}$ M dipicrylamine and 1 M NaCl in water (area of the aperture, $8 \cdot 10^{-3}$ cm^2; amplitude of the voltage jump, 30 mV, average of 32 current transients; measurements resistance, 33 Ω). The bars indicate the measurement uncertainty (data ±1% of the initial current I_0). (A) Measurement performed at 50.5°C. The solid curve was drawn according to Eqns. 4 and 5. (B) Measurement performed at 40.7°C. The two solid curves correspond to Eqns. 4 and 5 (assuming a single exponential fitted to the initial slope of the current) and to Eqn. 6 (sum of two exponentials).

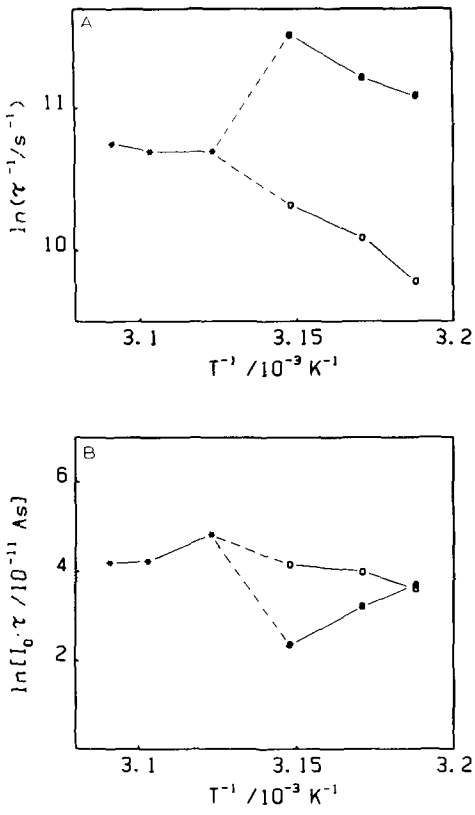

Fig. 7. Temperature dependence of the relaxation data obtained from a membrane which was formed from a mixture of dipalmitoylphosphatidylcholine and cholesterol (molar ratio, 1 : 1) in *n*-decane. For further experimental details, see legend to Fig. 6. The experiment started at a temperature of 50.5°C. Subsequently, the temperature was lowered until the membrane broke at about 40°C. Above about 45°C the shape of the current response could be fitted by a single exponential term (cf. Fig. 6A and Eqns. 4 and 5). Below that temperature, strong deviations were observed (cf. Fig. 6B), and the data were fitted using Eqn. 6, i.e., a sum of two exponential terms. The change of the fitting function is indicated by using different symbols.

choline has been repeatedly proposed [23–25]. Phase diagrams from those studies can, however, not be applied to our system due to the presence of the solvent decane. The use of solvent-free planar lipid membranes would in principle circumvent the difficulty. The comparatively small area of the membranes formed by the use of that technique, however, impairs the accuracy of the analysis of the relaxation curve considerably. A further disadvantage of planar lipid membranes as compared to the other lipid model system consists in the fact that the actual concentrations of the membrane components are not known. The ratio cholesterol/phospholipid in the membrane may be different from that of the membrane-forming solution. If we accept the interpretation outlined above, membranes with a molar ratio $\alpha = 1$ or $\alpha = 2$ exhibit at least two different coexisting structural states below 45°C, which may be monitored via the relaxation behaviour of dipicrylamine. The concentration of these sensor molecules is comparatively low (about 200 lipid molecules per dipicrylamine under the experimental conditions of Figs. 6 and 7). A reduction of the dipicrylamine concentration by one order of magnitude has no influence on the reported effect. We therefore believe that the observed phase separation is not induced by the presence of the sensor molecules, but is an inherent property of the lipid phase. Boheim et al. [26] reported alamethicin-induced phase separation in membranes formed from mixtures of 1-stearoyl-3-myristoyl-glycero-2-phosphocholine and cholesterol. But the pore former alamethicin is, in contrast to the lipophilic ion dipicrylamine, a highly cooperative system, which promotes interaction with the surrounding lipid phase. As to the structure of the coexisting phases one could imagine that there are regions of relatively pure lecithin, and others composed of lecithin and cholesterol. Cholesterol has been found to influence the permeation of dipicrylamine through lipid membranes by a change of the dipole potential at the membrane/water interface and by a change of the membrane thickness [27–29]. As a results, the rate constant, k_i, is enhanced. Therefore, we suggest that the fast relaxation process is due to the permeation of dipicrylamine through regions containing phosphatidylcholine and cholesterol, while the slow relaxation process mirrors the permeation of this ion through phospholipid regions. In addition, both regions contain a certain amount of solvent.

Phase separation has also been observed in other lipid mixtures such as the binary systems dimyristoylphosphatidylcholine/distearoylphosphatidylcholine [30] and dipalmitoylphosphatidylcholine/dioleoylphosphatidylcholine [31]. We studied the relaxation behaviour of dipicrylamine in membranes formed from these mixtures and we

found that the deviations of the current from a purely exponential time course was considerably smaller than those shown in Fig. 6B. We cannot exclude in these cases that the observed effect is due to the inherent heterogeneity of planar lipid membranes, as discussed above and illustrated in Fig. 1. The non-homogeneous distribution of the solvent decane in a planar bilayer is responsible for the formation of 'solvent lenses'. The spatial distribution of the membrane thickness resulting from that could give rise to a distribution of k_i values leading to non-ideal behaviour of our system. This interpretation is supported by the experimental finding of an increased non-exponential behaviour for 'aged' membranes [17]. As the membrane ages, solvent decane is removed from the membrane and accumulated in solvent lenses (and the torus) leading to the well-known increase in membrane capacity, and presumably also to a broadening of the distribution of local membrane thickness. It is this inherent heterogeneity of solvent containing planar lipid bilayers which limits the study of phase separation phenomena in this system.

The main advantage of black lipid membranes for the study of phase transitions is the comparatively easy way to determine the single transport parameters of the sensor ion by voltage jump relaxation experiments. As a result, it was found that both quantities, ion mobility and partition coefficient, contribute to the previously observed maxima of the ion permeability at a phase transition. We think that this result can be extended also to other membrane systems such as vesicles, where similar maxima have been observed [9–14]. On the other hand, comparatively large differences were found in the behaviour of similar ionic probes such as dipicrylamine and tetraphenylborate. Moreover, the extent of the influence of a phase transition on the transport properties depends strongly on the kind of the lipid. These findings are difficult to reconcile with presently existing general theories of the effects of phase transitions on membrane transport. The effect of a phase transition seems to depend on the particular nature of the transported molecule and its interaction with the lipid forming the membrane.

Acknowledgement

We wish to thank Dr. G. Adam for many helpful discussions, K. Janko for a sample of 1-stearoyl-3-myristoylglycero-2-phosphatidylcholine, Dr. P.C. Jordan for leaving us a computer programme for the calculation of Eqn. 1 and the Deutsche Forschungsgemeinschaft for financial support.

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