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ANOMALY OF TRANSPORT OF DIPICRYLAMINE ANIONS ACROSS THE CENTRAL BARRIER OF PLANAR DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYER MEMBRANES AT THE PHASE TRANSITION

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The rate of translocation of the hydrophobic ion dipicrylamine across planar lipid membranes formed from dipalmitoyllecithin in *n*-decane was determined by voltage jump relaxation experiments. The activation energy of the rate constant shows a change from a positive to a negative value at about 42°C near the main phase transition temperature of this lipid. Below this temperature, the rate constant was found to increase with decreasing temperature. This anomalous behaviour extends over a temperature range of at least 10 K and may be formally interpreted as an enhanced mobility of dipicrylamine in the solid state of the membrane.

Phase transition and phase separation phenomena in lipids have been examined by a variety of different physical methods [1]. Most of these studies were performed with lipid/water mixtures or with aqueous suspensions of lipid vesicles. There are only few reports on thermotropic phase changes at planar (black) lipid membranes, this situation being mainly due to the mechanical instability of these membranes, when the lipids are in the solid state. On the other hand, planar lipid membranes are very appropriate to the study of the influence of structural changes on functional membrane properties such as ion transport. Krasne et al. [2] have reported that the membrane conductance induced by mobile ion carriers of the valinomycin type is reduced to virtually zero in the solid state of the membrane, while the conductance induced by the pore former gramicidin A is scarcely affected. Their result has been considered as a general criterion to distinguish mobile carriers from immobile pore formers. The experiments of Krasne et al. [2] were carried out on membranes formed from a 1:1 mixture of dipalmitoyl- and distearoylglycerol in *n*-decane. Our own parallel investigations of these problems were performed on dipalmitoylphosphatidylcholine membranes and have raised some doubts as to whether the results of Krasne et al., namely a freezing of translational motion below the main phase transition of the lipids, can be generalized. We found that the valinomycin-induced K^+ conductance is still present 10 K below the well established transition temperature of 41°C of this lipid [3]. The only evidence for a change in the behaviour of this ion carrier was a break in the slope of the Arrhenius plot of the conductance–temperature relationship. While our previous study was based on a global membrane property, the conductance, we have now examined the problem in more detail by measurement of the essential elementary step, namely the rate of movement across the central barrier of the membrane. Our result is in clear contradiction to the 'freezing hypothesis': we found that the rate of ion translocation across the membrane even increased with decreasing temperature below 42°C.

As a probe of the central membrane barrier we used the lipophilic ion dipicrylamine. Its rate of

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translocation across the central barrier is readily determined by measurement of the electric current relaxation following the application of a voltage jump to the membrane. The details of this technique, as well as the interpretation of the experimental data in terms of a simple mechanism, have been presented in earlier publications [4,5]. In brief, the electric current response following a voltage jump mirrors the voltage-dependent redistribution of the lipophilic ions between the two membrane interfaces. The concentration of the anion dipicrylamine decreases at the electrically negative and increases at the electrically positive interface of the membrane. As a consequence, a transient current is observed. From the initial slope $(\mathrm{d}\ln I/\mathrm{d}t)_{t=0}$ the rate constant k_i of ion translocation across the inner membrane barrier is obtained according to Eqn. 1 below (see also Eqns. 30a–32b of Ref. 5}.

$$k_i = 1/(2\tau\cosh(zu/2)) \qquad (1)$$

with $1/\tau = -(\mathrm{d}\ln I/\mathrm{d}t)_{t=0}$, I = current, $u = FU/RT$, F = Faraday constant, R = gas constant, U = voltage, z = valency of the ion.

At the limit of very slow exchange of the ions between membrane and water, the current decays exponentially according to $I = I_0 \exp(-t/\tau)$. The interfacial concentration N of the lipophilic ion may be calculated from the absolute value of the current I at zero time:

$$N = I_0/(2zFA_m\tanh(zu/2)) \qquad (2)$$

A_m = membrane area.

If the inner membrane barrier is considered as temperature independent, then the temperature dependence of the translocation rate constant k_i should follow the usual Arrhenius equation

$$k_i = B \cdot \exp(-\Delta E_i/RT) \qquad (3)$$

with the activation energy ΔE_i being temperature independent. This has in fact been found for pure lipids of varying chain length of their unsaturated fatty acid residues [6–8] and is illustrated for lipid mixtures in Fig. 1. For membranes formed from dioleoylphosphatidylcholine in *n*-decane, from a 1:1 mixture of dioleoyl- and distearoylphosphatidylcholine, or from a natural lipid mixture (ox brain), the data may be well described by Eqn. 3. For membranes formed from the saturated phospholipid dipalmitoylphosphatidylcholine in *n*-decane, however, a clear deviation from Eqn. 3 is apparent (Fig. 2). Starting at a temperature of about 42–44°C, decreasing temperature leads to an increase of $1/\tau$ (equivalent to k_i). This behaviour formally corresponds to a negative activation energy and may be considered as evidence for a temperature-dependent change of the inner membrane barrier. There is a transition of the

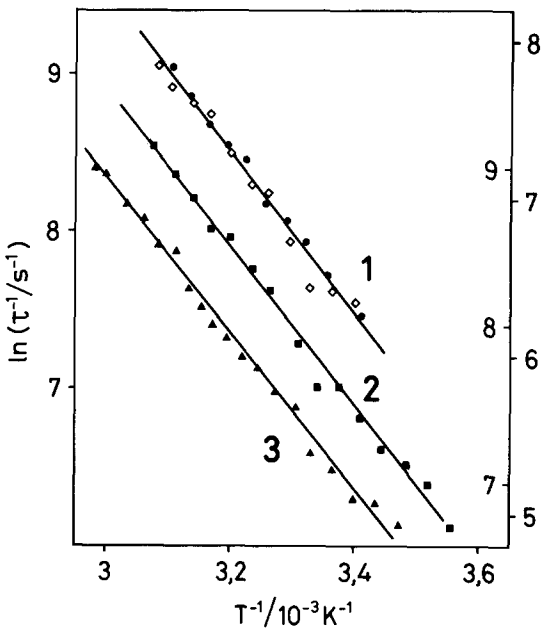

Fig. 1. Temperature dependence of the rate constant k_i (inverse relaxation time τ, see Eqn. 1) for different lipids. (1) Dioleoylphosphatidylcholine: open squares, heating; ●, cooling (ordinate on the left side). (2) Brain lipid: ■ (right ordinate on the right side). (3) 1:1 mixture of distearoyl- and dioleoylphosphatidylcholine: ▲ (left ordinate on the right side). The membranes were formed from 1–2% (w/v) solutions of lipid in *n*-decane. The diameter of the hole was 1 mm. The aqueous phase contained 1 M NaCl (unbuffered, pH≈5.6) and the following concentration of dipicrylamine: $5\cdot10^{-8}$ (dioleoylphosphatidylcholine), $5\cdot10^{-7}$ M (brain lipid) and 10^{-7} M (mixture). The measurements were performed on aged membranes (at least 30 min after the blackening process). The voltage jump was from 0 to 30 mV. The lines represent least-squares fits to the respective data, which were obtained from a single membrane. The cooling and heating rate was about 1 K/min. The activation energies ΔE_i calculated from Eqn. 3 closely agree (41–43 kJ/mol).

physical state of the membrane extending from about 44°C to at least 34°C. The translocation rate of dipicrylamine is larger in the low-temperature state ($T \leqslant 34$°C) than in the high-temperature state ($T \geqslant 44$°C). As to the nature of the transition, two alternative interpretations are suggested:

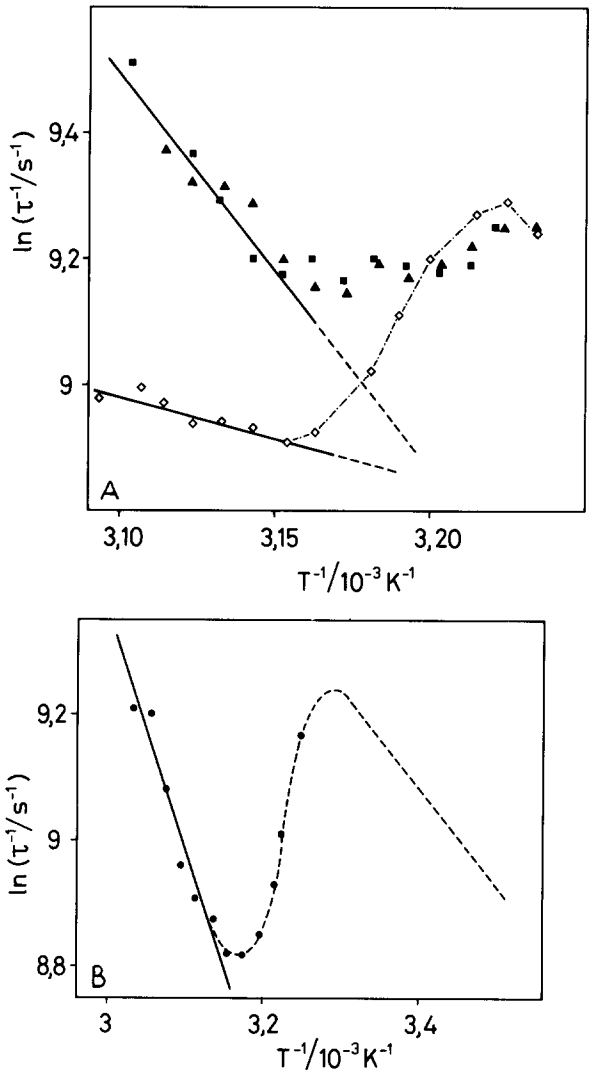

Fig. 2. Temperature dependence of the rate constant k_i (inverse relaxation time τ, compare Eqn. 1) for membranes formed from dipalmitoylphosphatidylcholine in *n*-decane in the presence of 0.1 M NaCl and 10^{-7} M dipicrylamine in the aqueous solution. The solid lines represent least-square fits to the data above 45°C. The data of Fig. 2A (and those of Fig. 2B) were obtained from a single membrane: open squares, first cooling; ■, heating; ▲, second cooling. The broken line (Fig. 2B) is a hypothetical extrapolation (see text). The cooling and heating rate was about 1 K/min. The voltage jump was from 0 to 30 mV, the membrane area was $5 \cdot 10^{-3}$ cm^2. There was no obvious change in membrane capacity (about 0.37 μF/cm^2, see Ref. 3 throughout the heating cycle.

(1) The presence of the solvent decane lowers the main transition temperature of dipalmitoylphosphatidylcholine from 41°C to several degrees below 40°C. This is in line with the findings of McIntosh et al., [9], who studied the effect of *n*-alkanes on the phase behaviour of lipids in multilamellar arrays of lipid-alkane bilayers by X-ray diffraction and differential scanning calorimetry. They reported a lowering of the main transition temperature of dipalmitoylphosphatidylcholine to 36°C induced by the presence of decane and a concomitant broadening of the transition. If we accept their interpretation, the consequence is a larger value of the rate constant k_i in the solid state of the membrane in comparison to the fluid state. This is in sharp contrast with the general view on the fluidity dependence of diffusion processes. The discrepancy may be resolved if the microenvironment of a hydrophobic ion differs from the rest of the lipid bilayer. There is evidence from other experimental approaches that, on insertion of a foreign molecule, the bilayer structure may be considerably perturbed [10]. One could imagine that at the transition of the bilayer from the fluid to the solid state, the microenvironment of dipicrylamine is changed to another 'fluid' structural state, which allows a faster membrane translocation of this ion. Below the transition, a bilayer doped with dipicrylamine might thus be imagined as an area of unperturbed solid structure, in which small domains of fluid, centered around the sites of the ions, are dispersed. The area occupied by the fluid regions could be comparatively small. An estimate on the basis of Eqn. 2 and Fig. 3 shows that less than 1% of the total area of the bilayer surface is occupied by dipicrylamine ions under the experimental conditions of Fig. 2. However, the size of a domain could exceed considerably the geometric area of the inducing ion [10]. Though our data favour this interpretation, the following alternative cannot be completely excluded at present.

(2) There is a lateral phase separation below 44°C, involving the membrane constituents dipalmitoylphosphatidylcholine and the solvent decane, into a decane-enriched fluid phase and a

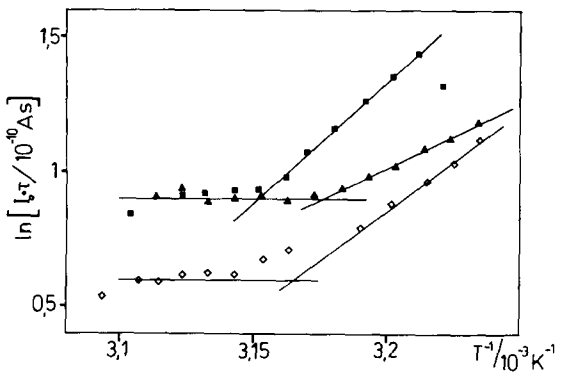

Fig. 3. Temperature dependence of $I_0 \cdot \tau$, i.e. of the interfacial concentration N of dipicrylamine (compare Eqn. 2). The data correspond to those of Fig. 2A (see legend of this figure for further experimental details). The full lines were drawn to indicate a transition at about 42–44°C. The partition equilibrium between membrane and water was not fully adjusted throughout the cycle, because the heating and cooling rate had to be chosen relatively fast.

decane-poor solid phase. The conductance probe dipicrylamine is removed from the solid to the fluid phase. There, the translocation rate constant is larger in comparison to the fluid phase in the high temperature state of the membrane.

A distinction between the two alternatives should be possible by measurement of k_i in the pure low temperature state of the membrane ($T <$ 30°C). Fig. 2B illustrates the prediction of alternative 1 (together with the data of another experiment). Normal Arrhenius behaviour (Eqn. 3) is to be expected for the pure low temperature phase. An initial decrease of k_i at the low temperature end of the transition is indeed indicated in Fig. 2A. This figure also shows the reversibility of the effect. The data from the first cooling of a relatively fresh membrane (20 min after the blackening process) show greater deviations from the data of the subsequent heating and second cooling, which agree within experimental accuracy.

For alternative 2, a steep decrease of k_i is to be expected at low temperatures (not shown in Fig. 2B), since dipicrylamine is assumed to be excluded from the solid phase. Therefore, the two alternatives should differ in the temperature dependence of k_i below the transition. This criterion could not, however, be strictly applied in view of the poor membrane stability at sufficiently low temperatures. The following experimental finding is in favour of alternative 1. The density N of ions at the bilayer surface, calculated from Eqn. 2 was found to increase with decreasing temperature at the onset of the transition, while it was relatively constant above the transition (Fig. 3). This is difficult to reconcile with alternative 2, since the latter rather predicts a decrease of N with decreasing fraction of the fluid phase.

Our present and previous [3] results are at variance with the findings of Krasne et al. [2]. The latter were, however, obtained with a different lipid. The same is true for a recent report by Boheim et al. [11] on solvent-free membranes made from 1-stearoyl-3-myristoyl-glycero-2-phosphocholine. Our preliminary results on decane-containing membranes of this lipid show a complete absence of the anomaly found for dipalmitoylphosphatidylcholine. Further evidence for a free mobility below the phase transition was provided by Pohl [12] who studied the ion carrier valinomycin in solvent-free dipalmitoylphosphatidylcholine vesicles. We cannot interprete the contradictory reports mentioned above at present. The question of whether the motion of mobile molecules is frozen if the surrounding membrane is transferred into the solid state could depend on the particular nature of the molecule and also on the membrane structure. The phenomenon described in this report has interesting parallels in biological systems [13,14].

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