

## The Interaction of Hydrophobic Ions with Lipid Bilayer Membranes

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*Summary.* Electrical relaxation studies have been made on lecithin bilayer membranes of varying chain length and degree of unsaturation, in the presence of dipicrylamine. Results obtained are generally consistent with a model for the transport of hydrophobic ions previously proposed by Ketterer, Neumcke, and Läger (*J. Membrane Biol.* 5:225, 1971). This model visualizes as three distinct steps the interfacial adsorption, translocation, and desorption of ions. Measurements at high electric field yield directly the density of ions adsorbed to the membrane-solution interface. Variation of temperature has permitted determination of activation enthalpies for the translocation step which are consistent with the assumption of an electrostatic barrier in the hydrocarbon core of the membrane. The change of enthalpy upon adsorption of ions is, however, found to be negligible, the process being driven instead by an increase of entropy. It is suggested that this increase may be due to the destruction, upon adsorption, of a highly ordered water structure which surrounds the hydrophobic ion in the aqueous phase. Finally, it is shown that a decrease of transient membrane conductance observed at high concentration of hydrophobic ions, previously interpreted in terms of interfacial saturation, must instead be attributed to a more complex effect equivalent to a reduction of membrane fluidity.

It has been known for a number of years that the stationary state conductance of synthetic lipid bilayer membranes can increase markedly in the presence of a variety of lipid-soluble organic anions (Bielawski, Thompson & Lehninger, 1966; Liberman & Topaly, 1968; Le Blanc, 1969, 1971; Hopfer, Lehninger & Lennarz, 1970). The work of Ketterer, Neumcke and Läger (1971) has demonstrated, in addition, the occurrence of transient conductance increases in the presence of tetraphenylboron ( $\text{TphB}^-$ ) and of dipicrylamine ( $\text{DpA}^-$ ). Reviews by Haydon and Hladky (1972) and by Läger and Neumcke (1973) consider the transport mechanisms responsible for both the

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stationary state and the transient conductance of bilayer membranes in the presence of lipid-soluble anions.

The work of Ketterer *et al.* (1971), hereafter referred to as KNL, provides the starting point for the present investigation. They interpret the transport of lipid-soluble anions in terms of a three-step process of: (1) adsorption of the ion to the membrane-solution interface; (2) translocation of the ion across an energy barrier to the other interface; and (3) desorption of the ion from the interface into the aqueous solution. A chemical-kinetic model, including appropriate rate constants for the processes described above, has been presented by KNL. Their model provides also for the possibility of interfacial saturation, by including as a parameter a maximum density of charge which can be adsorbed to the interfaces. As such, the model represents an extension of a mechanism proposed by Bruner (1970) for blocking of charge transport through membranes. With this refinement the model could explain the occurrence of maxima in transient membrane conductance, observed by KNL as a function of the concentration of lipid-soluble anion.

The objectives of the present work are threefold: (1) to obtain a direct measure of adsorbed surface charge by observing transient current response to high applied electric fields; (2) to measure relaxation time and transient membrane conductance as a function of temperature and thereby determine activation enthalpies for ion translocation; and (3) to investigate the influence of hydrocarbon chain length and degree of unsaturation upon the observed relaxation effects. All results to be reported here were obtained using phosphatidylcholine (lecithin) bilayers of specified hydrocarbon composition, formed in the presence of  $DpA^-$  as lipid-soluble anion.

### Theoretical and Experimental Background

The model presented by KNL predicts that, in the limit of low applied transmembrane potential difference, the time-dependent current density  $J(t)$  following application of a voltage step will be related to the step amplitude,  $U$ , by

$$\begin{aligned}\lambda_0(t) &= \left( \frac{J(t)}{U} \right)_{U \approx 0} \\ &= (\lambda_{00} - \lambda_{0\infty}) e^{-t/\tau_0} + \lambda_{0\infty}.\end{aligned}\tag{1}$$

The initial conductance ( $t = 0$ ) is equal to  $\lambda_{00}$ . It is dependent upon the concentration of lipid-soluble ions and upon fixed parameters of the model. In particular  $\lambda_{00}$  is proportional to  $k_{is}$ , the rate constant for translocation of

ions across the hydrocarbon interior of the membrane. The stationary-state conductance ( $t \rightarrow \infty$ ), given by  $\lambda_{0\infty}$ , also depends upon ion concentration and upon fixed parameters of the model. It is proportional to  $k$ , the rate constant for desorption of ions from the membrane-solution interface into the aqueous phase, provided that  $k \ll k_i$ . Under this condition desorption of ions from the interface is the rate-limiting step for stationary current flow. In the opposite limit,  $k \gg k_i$ , translocation of ions across the membrane interior becomes the rate-limiting step for stationary flow, and  $\lambda_{0\infty}$  becomes equal to  $\lambda_{00}$  according to the KNL model. Thus in this limit the transient part of the current response to a voltage step would vanish according to Eq. (1). It has been established by KNL that  $\lambda_{00} \gg \lambda_{0\infty}$  for both  $\text{DpA}^-$  and  $\text{TphB}^-$ , indicating that the inequality  $k \ll k_i$  is well satisfied in both cases. They conclude, however, that  $\lambda_{0\infty}$  cannot be evaluated experimentally because the current flowing at times  $t \gg \tau_0$  is limited by diffusion polarization of aqueous unstirred layers rather than by the rate of interfacial adsorption or desorption of ions.

The relaxation time is

$$\begin{aligned} \tau_0 &= \frac{1}{k + 2k_i} \\ &\approx 1/(2k_i) \end{aligned} \quad (2)$$

according to the KNL model, with the approximation following from the experimental evidence cited above. For dioleylecithin membranes the measured relaxation times at 25 °C are 1.3 msec for  $\text{DpA}^-$  and 55 msec for  $\text{TphB}^-$ .

For additional information on saturation effects upon initial conductance, and on the voltage dependence of both initial conductance and relaxation time, the paper by KNL should be consulted. For our purposes it is of primary importance that the validity of Eq. (1), with  $\lambda_{00} \gg \lambda_{0\infty}$ , is well established experimentally. This fact provides strong support for the three-step transport mechanism proposed by KNL.

## Materials and Methods

Black lipid membranes were formed from dipalmitoleyl-, dioleyl-, and dierucoyllecithin [di(16:1)-; di(18:1)-; and di(22:1)-PC], as well as egg lecithin. All lipids were prepared in this laboratory, the monounsaturated lecithins being synthesized by methods previously described (Benz, Stark, Janko & Lauser, 1973). The membrane-forming solvent was *n*-decane in all cases. Dipicrylamine (Fluka, puriss) was used without further purification. Unbuffered (pH  $\sim$  6) aqueous solutions containing 0.1 M KCl were employed throughout.

Mechanical and electrical arrangements for measurement were closely similar to those employed by KNL. Membranes were formed in a teflon cell, spanning a circular aperture of 3 mm diameter. Silver/silver chloride electrodes of 1 cm<sup>2</sup> surface area were used. The voltage pulses required for relaxation measurements were supplied by a Philips Model PM 5712 pulse generator. Current transients were photographed from a Tektronix Model 5103 N/D 15 storage oscilloscope. Temperature control of the cell was accomplished by means of a surrounding water jacket, thermostated by use of a Lauda Model K-2 circulating constant temperature bath.

## Results

### *High Field Measurements*

The KNL model assumes that only the rate constant  $k_i$  is modified by an applied electric field, i.e., the field is presumed to be confined to the hydrocarbon core of the membrane. This suggests immediately that if the reduced applied transmembrane potential difference  $u$  satisfies the inequality

$$u = \frac{U}{RT/F} \gg 1 \quad (3)$$

( $R$  = gas constant,  $T$  = absolute temperature,  $F$  = Faraday constant), then it should be possible to deplete the adsorbed charge at the interface of higher electrostatic potential energy, transferring virtually all of it to the lower potential interface. In fact the KNL model predicts a Boltzmann distribution of charge

$$\frac{\overline{N}'}{\overline{N}''} \approx e^{-zu} \quad (k \ll k_i) \quad (4)$$

in the nonequilibrium stationary state, where  $\overline{N}'$  is the density of charge adsorbed to the higher potential interface,  $\overline{N}''$  is the charge density on the lower potential interface, and  $z$  is the ion valence. Thus, provided inequality (3) is satisfied and desorption of charge during the transient can be neglected, it should be possible to measure directly the density of adsorbed surface charge. This quantity would be equal to the integral over time of the observed transient current density, following application of a high amplitude voltage step.

To test this prediction experimentally we have used di(22:1)-PC, a lipid not available at the time of the original work of KNL. This lipid forms bilayer membranes possessing remarkable stability and dielectric strength. Typical transient current responses to high amplitude voltage steps are shown in Fig. 1. The higher amplitude current decays are distinctly nonexponential.

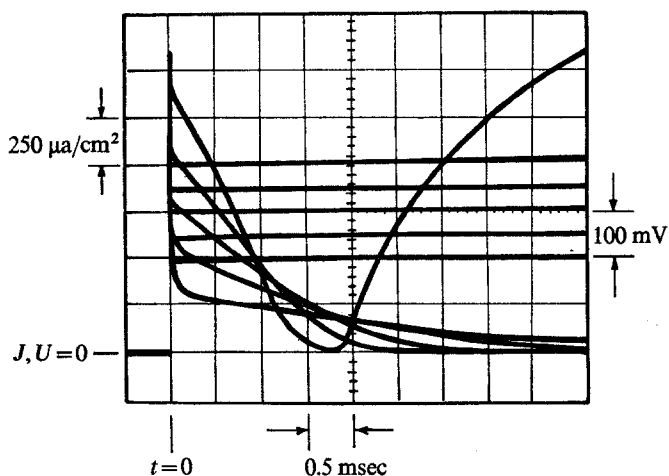


Fig. 1. The tracing, prepared from a typical photograph, illustrates applied voltage pulses ranging from 200 to 400 mV, and associated current transients, at a concentration of  $10^{-7}$  M  $\text{DpA}^-$ . The abrupt rise of current noted in the tail of the highest amplitude transient is due to rupture of the membrane

With each increase in step amplitude it is noted that the resulting current transient “crosses over” all transients arising from lower amplitude voltage steps. This is to be expected if initial current increases with voltage step amplitude while the total charge transported (area under each curve) remains approximately constant.

A planimeter has been used to measure the area under current density transient curves such as those shown in Fig. 1. Plots of charge density transported versus voltage applied are shown in Fig. 2. As  $U$  is increased the transported charge density approaches a limiting value at each concentration of  $\text{DpA}^-$  shown. However, this saturating charge density,  $Q_s$ , does not itself approach a well-defined limiting value as the concentration of  $\text{DpA}^-$  is increased. This point is illustrated in Fig. 3. The initial conductance at an applied voltage of 400 mV,  $\lambda_{16,0}$ , is also shown on this Figure. This high field initial conductance appears to be remarkably insensitive to the concentration of  $\text{DpA}^-$ . The low field initial conductance  $\lambda_{0,0}$ , on the other hand, shows a well-defined maximum resembling that reported by KNL for di(18:1)-PC. The fact that  $Q_s$  decreases with decreasing  $\text{DpA}^-$  concentration while  $\lambda_{16,0}$  remains virtually constant implies that the high field current density transients decay more rapidly as the  $\text{DpA}^-$  concentration is decreased. This is in fact observed.

At the end of an applied voltage step or pulse a reverse current density transient is observed, associated with a redistribution of charge back to the

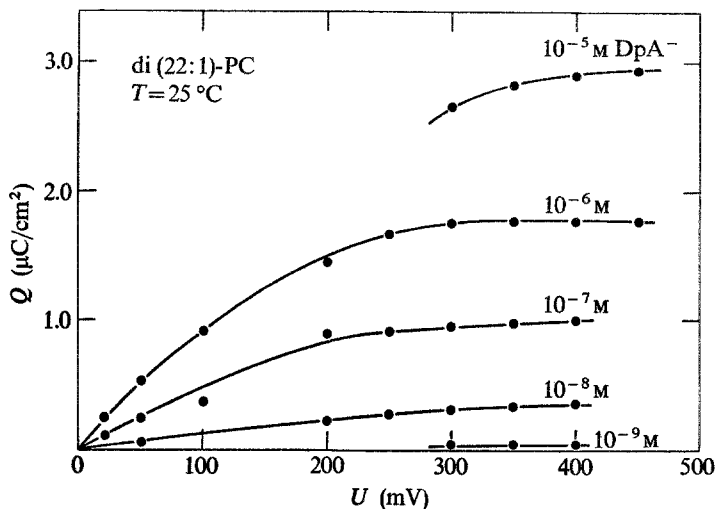


Fig. 2. The charge density translocated across membranes is plotted versus voltage applied. In the case of  $10^{-5}\text{ M DpA}^-$  the curve could not be extended to low voltage because of uncertainties associated with the long relaxation times encountered

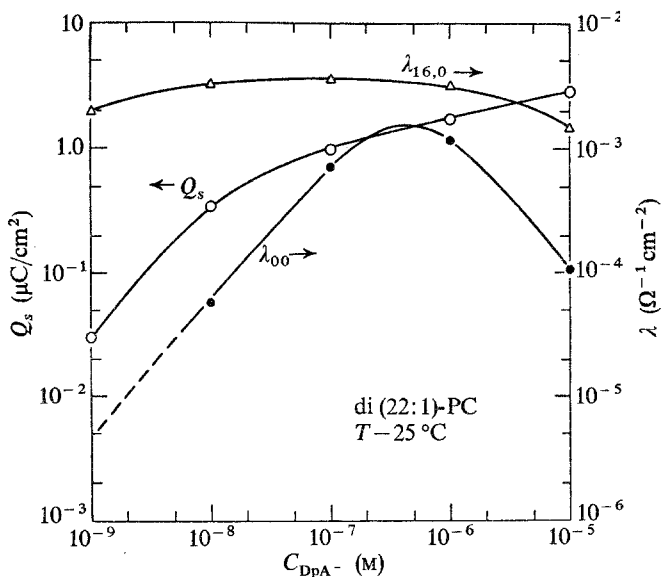


Fig. 3. Log-log plots of saturating charge density  $Q_s$ , low field initial conductance  $\lambda_{00}$ , and high field initial conductance  $\lambda_{16,0}$ , all versus  $\text{DpA}^-$  concentration, are shown. Arrows indicate location of the appropriate ordinate scale. The high field conductance is measured at an applied voltage  $U = 400\text{ mV}$ , for which the reduced voltage, given by Eq. (3), is  $u = 16$  at  $25^\circ\text{C}$

symmetric equilibrium state of the membrane. We expect the charge transported during this "field-free" decay to be equal to that transported during the initial field-driven transient, provided no desorption of charge from the

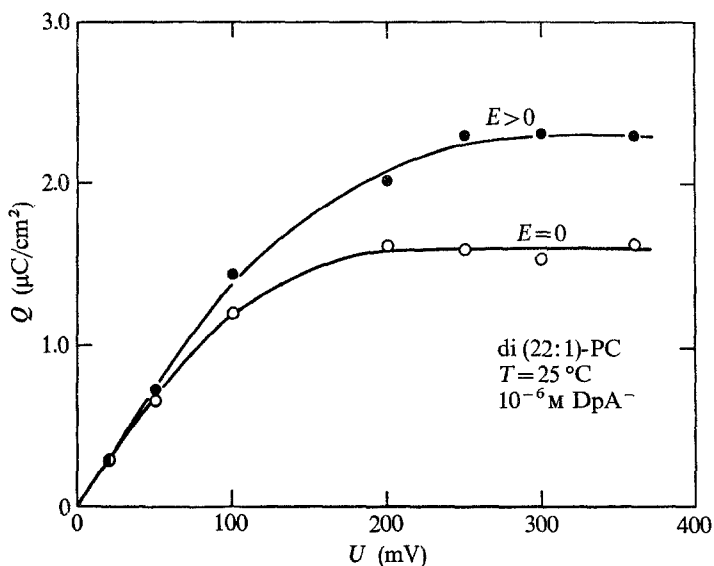


Fig. 4. The curves shown compare charge density transferred under applied electric field ( $E > 0$ ) with that which flows back across the membrane after the field is removed ( $E = 0$ )

membrane occurs during the latter. Results of an experimental test of this point are shown in Fig. 4. About one-third of the charge transported under high field is seen to be unaccounted for in the subsequent field-free decay. This discrepancy becomes negligible for applied pulses of less than 50 mV amplitude. The desorption of charge under high applied field must itself be a transient process, however, since no increase of stationary conductance with applied field is observed. This observation may, however, be attributable to current-limiting by diffusion polarization. Experiments using double pulses are planned to further study this matter.

#### *Temperature-Dependence Studies*

As part of their analysis, KNL present estimates of activation energies for adsorption, desorption, and translocation of  $\text{DpA}^-$  and of  $\text{TphB}^-$  ions. The estimates are based upon the Eyring (Glasstone, Laidler & Eyring, 1941) transition state theory of chemical reaction, in which a rate constant is equated to a frequency factor,  $f$ , times an exponential term incorporating the Gibbs free energy,  $\Delta F$ , of activation. Thus KNL formulate the rate

constants

$$k_i = f_i e^{-\Delta F_i/RT} \quad (5)$$

$$k = f e^{-\Delta F/RT} \quad (6)$$

$$\beta k = f_a I_a e^{-\Delta F_a/RT} \quad (7)$$

for translocation, desorption, and adsorption, respectively, with  $I_a$  being a jump length for transition from the aqueous phase into the interface. In this formulation  $\beta$  has the physical significance of a partition coefficient, being equal to  $N/c$ , where  $N$  is the equilibrium density of adsorbed ions and  $c$  is their concentration in the aqueous phase. We decompose  $\Delta F$  into enthalpy and entropy components, rewriting Eq. (5), for example, as

$$k_i = f_i e^{AS_i/R} e^{-\Delta H_i/RT} \quad (8)$$

with corresponding modification of Eqs. (6) and (7) as well. Thus, the measurement of any variable proportional to a rate constant, when carried out as a function of temperature and plotted on a log scale versus  $1/T$ , should yield a straight line with slope of magnitude  $\Delta H/R$ . This will be the case if  $\Delta H$  and  $\Delta S$  are independent of temperature or weakly dependent upon it.

Using di(22:1)-PC, we have measured the low field transient relaxation time  $\tau_0$ , over the temperature interval 15–40 °C. Plots of  $\log \tau_0$  versus  $1/T$  for various concentrations of  $\text{DpA}^-$  are presented in Fig. 5. Measured activation enthalpies are shown on the graph. Though the data show considerable scatter, a trend toward increasing activation enthalpy with increasing  $\text{DpA}^-$  concentration is clearly evident. From Eqs. (2) and (8) it follows that the measured values of  $\Delta H$  pertain to translocation of  $\text{DpA}^-$  across the membrane. Fig. 5 also shows that  $\tau_0$  is dependent upon  $\text{DpA}^-$  concentration, increasing sharply above  $10^{-6}$  M. This dependence, which is even more pronounced for di(18:1)-PC, cannot be accounted for by the KNL model. This point will be discussed further below.

In Figs. (6) and (7), respectively, we present Arrhenius plots of  $\lambda_{00}$  and of the product  $\lambda_{00} \tau_0$ , again for di(22:1)-PC. The latter quantity is of particular interest since, upon integration of Eq. (1) subject to the condition  $\lambda_{00} \gg \lambda_{0\infty}$ , we find that  $\lambda_{00} \tau_0$  is equal to the charge density transported during the transient per unit of voltage applied in the low field limit. The quantity is dimensionally equivalent to a capacitance per unit area. One expects that, in the absence of saturation effects,  $\lambda_{00} \tau_0$  would be proportional to  $N$ , the equilibrium density of adsorbed ions, and hence to  $\beta$ , the partition



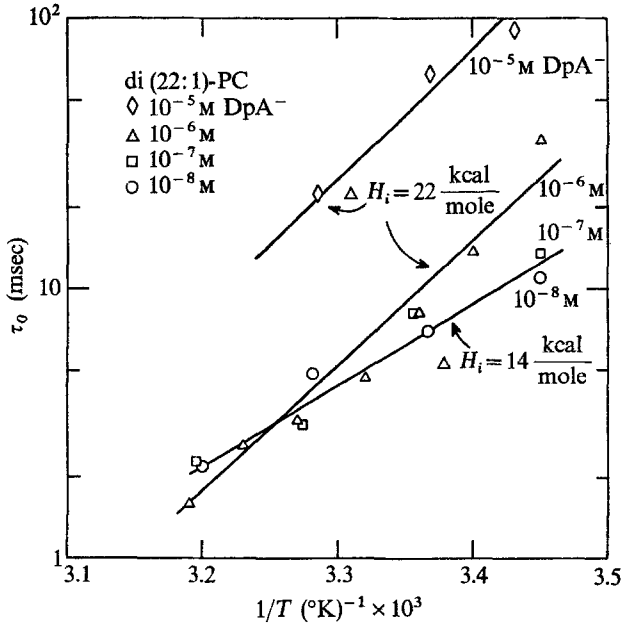


Fig. 5. Plots of  $\log \tau_0$  versus  $1/T$  for di(22:1)-PC at various concentrations of DpA $^{-}$

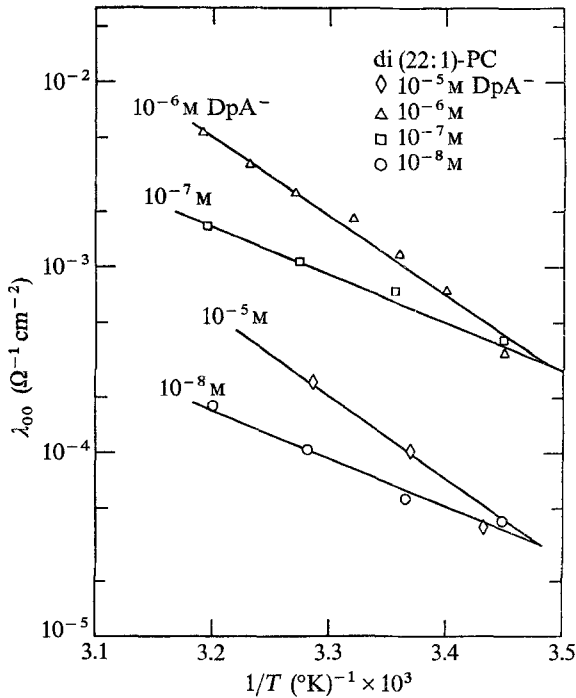


Fig. 6. Arrhenius plots of  $\lambda_{00}$  for di(22:1)-PC at various concentrations of DpA $^{-}$

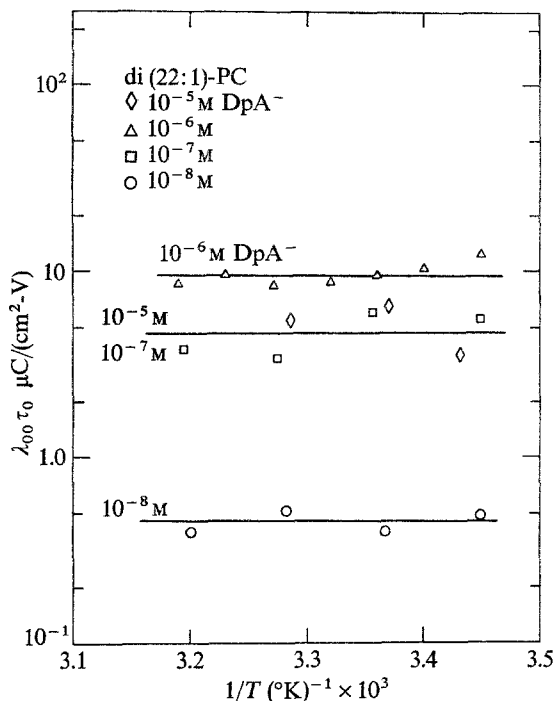


Fig. 7. Arrhenius plots of  $\lambda_{00} \tau_0$  for di(22:1)-PC at various concentrations of  $\text{DpA}^-$ . Note that  $\lambda_{00} \tau_0$  is maximal at  $10^{-6}\text{ M DpA}^-$ .

coefficient introduced in Eq. (7). These expectations are borne out by the KNL model. The insensitivity of the product  $\lambda_{00} \tau_0$  to change of temperature is therefore a point of considerable interest which will be discussed further below.

#### *Effects of Hydrocarbon Composition*

In Fig. 8 we present plots of  $\log \tau_0$  versus  $1/T$  for di(22:1)-, di(18:1)-, di(16:1)-, and egg PC, all for a fixed concentration of  $10^{-6}\text{ M DpA}^-$ . Activation enthalpies are shown. Corresponding plots of  $\log \lambda_{00}$  versus  $1/T$  are shown in Fig. 9. Plots of the product  $\lambda_{00} \tau_0$  (not shown) display the same insensitivity to temperature noted in Fig. 7. The hydrocarbon composition of the egg PC used is shown in Table 1.

These results indicate that the kinetics of both adsorption and translocation of hydrophobic ions are insensitive to hydrocarbon chain length and degree of unsaturation. Only for the longer chain di(22:1)-PC are substantial deviations observed.

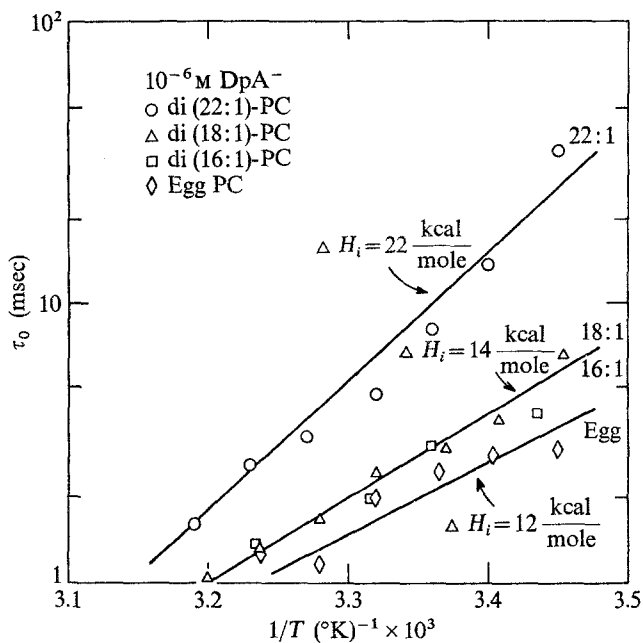


Fig. 8. Arrhenius plots of  $\tau_0$  for several lecithins, all at a fixed concentration of  $10^{-6} \text{ M DpA}^-$

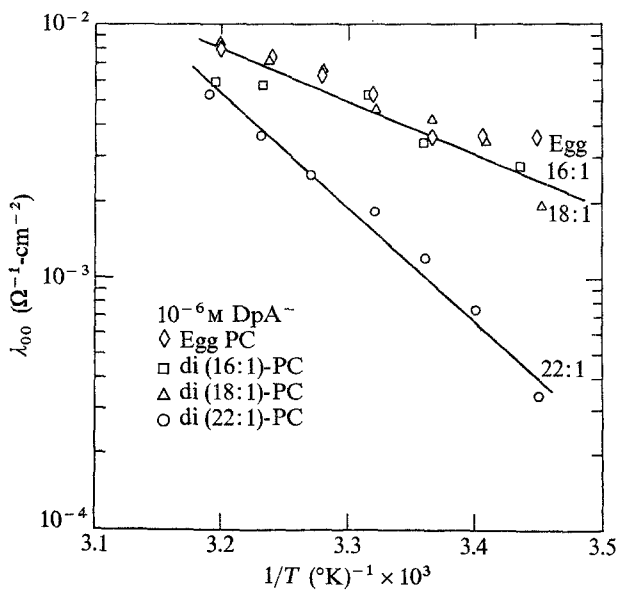


Fig. 9. Arrhenius plots of  $\lambda_{00}$  for several lecithins, all at a fixed concentration of  $10^{-6} \text{ M DpA}^-$

Table 1. Hydrocarbon composition of egg phosphatidylcholine

Residue	Percentage
(16:0)	25.9
(16:1)	2.1
(18:0)	15.5
(18:1)	42.8
(18:2)	13.6

## Discussion

### *High Field Measurements*

The saturating charge density observed at  $10^{-6}$  M  $\text{DpA}^-$  (Fig. 3) implies a surface density of adsorbed ions ( $Q_s/e$ ) of  $1.1 \times 10^{13} \text{ cm}^{-2}$ . This agrees roughly with the value  $N_s = 4 \times 10^{12} \text{ cm}^{-2}$  estimated by KNL to be the maximum surface density of  $\text{DpA}^-$  ion which could be absorbed. Though the increase of  $Q_s$  is slowed at higher concentrations of  $\text{DpA}^-$ , the data presented in Fig. 3 do not provide unequivocal evidence for the attainment of such a maximum surface density in the concentration range covered.

Inspection of Fig. 2 indicates that the charge density transferred at high field does not approach its limiting value until the applied potential difference exceeds 200 mV. Reference to Eq. (4), on the other hand, indicates that a potential difference of 100 mV should suffice to bring the transferred density of charge to within 2% of its saturation value. This discrepancy suggests that adsorbed hydrophobic ions are embedded in the membrane to a sufficient extent that they do not "see" the full applied potential difference upon translocation.

Our interpretation of the high field data must take cognizance of possible contributions to charge transfer arising from field-induced membrane compression. The change of membrane capacitance  $C_m$  due to such compression would contribute a transient current equal to  $U(dC_m/dt)$ . Such transients have been observed (E. Bamberg, *personal communication*). This effect provides the basis for the study of the kinetics of pore formation in bilayers by gramicidin A, using the field jump technique (Bamberg & Lauser, 1973). The charge transferred by compression is equal to  $U(\Delta C_m)$ , where  $\Delta C_m$  is the associated change in capacitance. Assuming  $C_m \approx 0.5 \mu\text{F}/\text{cm}^2$ , with a 5% increase of this quantity under an applied voltage of 400 mV, we calculate a charge transfer of  $10^{-2} \mu\text{C}/\text{cm}^2$ . A charge transfer of this order was observed in di(22:1)-PC bilayers under these conditions in the absence of  $\text{DpA}^-$ , being carried by a transient of approximately 50  $\mu\text{sec}$

duration. Appropriate corrections to the observed charge transfer have been made in the determination of  $Q_s$  at  $10^{-9}$  M DpA<sup>-</sup>. The correction is negligible at higher DpA<sup>-</sup> concentrations.

### Temperature-Dependence Studies

Measured activation enthalpies  $\Delta H_i$  (see Eqs. (2) and (5), also Figs. 5 and 8) agree well at low DpA<sup>-</sup> concentration with the estimate of activation energy  $\Delta F_i$  (14 kcal/mole) given by KNL. This suggests that the activation entropy  $\Delta S_i$  [Eq. (8)] for ion translocation is relatively small. Such a result is consistent with the conclusion reached by KNL that electrostatic interaction between the ion and the hydrocarbon core of the membrane makes the major contribution to the free energy of activation for the translocation step. Ginsburg and Noble (1974) have reached the same conclusion for the case of ion transport through bilayers by diffusible carriers such as valinomycin and monactin.

In presenting the results of the temperature dependence studies we pointed out that the product  $\lambda_{00} \tau_0$  should be proportional to  $\beta$ , the partition coefficient introduced in Eq. (7). From Eqs. (6) and (7) and the discussion following we may write

$$\beta = \frac{f_a}{f} I_a e^{(S-S_a)/R} e^{-(H-H_a)/RT} \quad (9)$$

where  $S_a$  and  $H_a$  refer to the molar entropy and enthalpy of ions in the aqueous phase, while  $S$  and  $H$  are the corresponding quantities for ions adsorbed at the membrane-solution interface.

From the data presented in Fig. 7 we conclude that the enthalpy difference ( $H - H_a$ ) is equal to zero within the limits of error of our experiment ( $\pm 2$  kcal/mole). Using the experimental value of  $\lambda_{00} \tau_0$  at a concentration of  $10^{-8}$  M DpA<sup>-</sup>, and the KNL model, we calculate a value of  $\beta$  of  $2.5 \times 10^{-2}$  cm. This value for di(22:1)-PC is in good agreement with the value of  $2 \times 10^{-2}$  cm obtained by KNL for di(18:1)-PC. A more nearly model-independent estimate of  $\beta$  may be made from the saturating charge density data of Fig. 3. Using

$$\beta = \frac{(Q_s/e)}{c} \quad (10)$$

and the data for  $10^{-8}$  M DpA<sup>-</sup> we obtain  $\beta = 6 \times 10^{-2}$  cm, in reasonable agreement with the preceding result. To evaluate the entropy difference from our data and Eq. (9) we make the further assumptions that  $f_a/f \approx 1$  and  $I_a \approx 10^{-7}$  cm. The latter estimate, given by KNL, probably represents an

upper limit for the jump length in a condensed phase. The thermal de Broglie wavelength of the hydrophobic ion ( $\approx 10^{-9}$  cm) would certainly represent a lower limit. Using the upper limiting value of  $I_a$ , we obtain  $S - S_a \approx 25$  cal/mole-deg as a lower limiting value for the molar entropy difference. Thus, we conclude that the entropy of the membrane-solution system *increases* when  $\text{DpA}^-$  ions are adsorbed and that it is this increase of entropy, rather than a decrease of enthalpy, which is primarily responsible for the adsorption of hydrophobic ions to the interface. The corresponding change of free energy at 25 °C is  $F - F_a \approx -7.5$  kcal/mole, in good agreement with the value of  $-7.3$  kcal/mole given by KNL for  $\text{DpA}^-$  and di(18:1)-PC. We note that their quoted value is positive, being for  $\Delta F - \Delta F_a$ , which is equal to  $-(F - F_a)$ .

The contribution of the entropy term to the free energy of solution of nonpolar solvents in water was first recognized by Frank and Evans (1945) who inferred the existence of regions of highly ordered water ("icebergs") surrounding nonpolar solute molecules. More recently Tanford (1973) evaluated thermodynamic parameters for transfer of hydrocarbons from organic solvents to water. He emphasizes the importance of the entropic contribution to the hydrophobic effect, i.e., to the free energy change accompanying the transfer of a nonpolar molecule from an organic phase into water.

#### *Effects of Hydrocarbon Composition*

The most striking feature of the data in Figs. 8 and 9 is the lack of variation of  $\tau_0$  and  $\lambda_{00}$  with chain length and degree of unsaturation. Fettiplace, Andrews and Haydon (1971) have determined on the basis of capacitance measurements that membranes formed using shorter chain solvents, such as the *n*-decane employed here, retain a significant amount of solvent after thinning to the black state. Stark, Benz, Pohl, and Janko (1972) have measured bilayer membrane capacitance as a function of hydrocarbon chain length of the lipid used, and have also concluded that solvent content of the membranes is significant. We therefore suspect that residual solvent makes a major contribution to the barrier to the translocation of  $\text{DpA}^-$ , particularly for the shorter 16 and 18 carbon chain lipids. In the case of di(22:1)-PC, however, the longer chains do appear to be assuming more importance in determining the height of the barrier to ion translocation.

#### *Comments on the KNL Model*

The results presented are believed to strongly support the key assumptions of the KNL model, namely, that transport of hydrophobic ions across

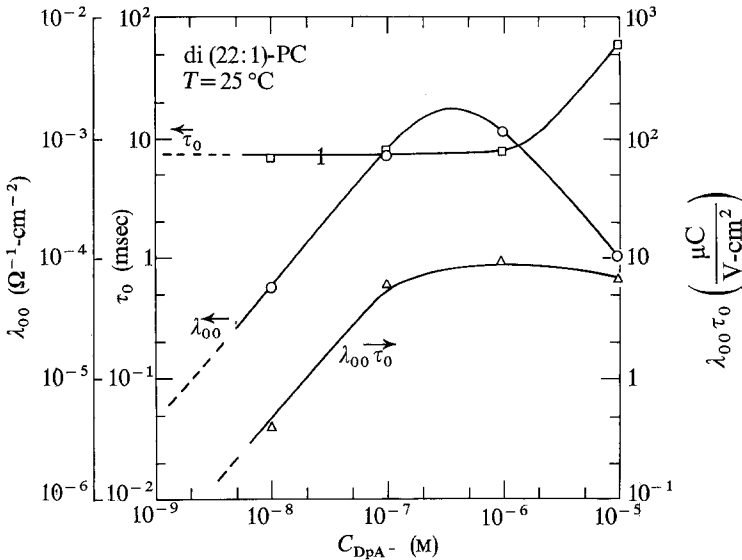


Fig. 10. Log-log plots of low field initial conductance  $\lambda_{00}$ , relaxation time  $\tau_0$ , and the product  $\lambda_{00}\tau_0$ , all versus  $DpA^-$  concentration, are shown. Arrows indicate location of the appropriate ordinate scale

bilayer membranes is a three-step process of adsorption at one interface between membrane and aqueous phase, translocation to the other interface, and desorption into the adjacent aqueous phase. The high field measurements have provided a direct, model-independent measure of the density of adsorbed ions. The temperature dependence studies have shown that the enthalpic contribution to the free energy of activation for ion translocation is consistent with the view that the electrostatic energy of the ion in the low dielectric constant hydrocarbon core constitutes the principal barrier to translocation. The same studies have also established that an increase of entropy provides the driving force for the adsorption of hydrophobic ions. This increase is most likely due to the destruction, upon adsorption, of a highly ordered water structure which surrounds the hydrophobic ion in the aqueous phase. Studies of the influence of lipid hydrocarbon composition, though probably complicated by residual solvent effects, indicate that increasing acyl chain length increases the height of the barrier to ion translocation.

Our results do not, however, provide clear support for the fixed sites hypothesis (Bruner, 1970) as extended by KNL to account for observed maxima in the initial conductance  $\lambda_{00}$ . To illustrate this point we replot, in Fig. 10, cross-sections at  $25^\circ\text{C}$  of the data presented in Figs. 5, 6 and 7.

Though  $\lambda_{00}$  shows a definite maximum when plotted versus concentration of  $\text{DpA}^-$ , the product  $\lambda_{00}\tau_0$  does not, as it should if  $\tau_0$  were independent of  $\text{DpA}^-$  concentration. This product, equal to the charge density moved across the membrane by the transient per unit of applied voltage ( $U \approx 0$ ), would be expected to show an unambiguous maximum corresponding to that of  $\lambda_{00}$  if saturation effects at high  $\text{DpA}^-$  concentration were limiting charge transfer. The continuing increase of  $Q_s$  with  $\text{DpA}^-$  concentration (Fig. 3), when contrasted with the limiting form of the plot of  $\lambda_{00}\tau_0$ , suggests that saturation effects may indeed limit charge transfer to some extent at low applied field. We conclude, however, that an explanation of the maximum in  $\lambda_{00}$  must be based primarily upon an understanding of the observed sharp increase of  $\tau_0$  at high  $\text{DpA}^-$  concentration.

We suggest that this increase of  $\tau_0$  may reflect a decrease of membrane fluidity at high  $\text{DpA}^-$  concentration. A more specific example of an associative effect of hydrophobic molecules upon the dynamics of their interaction with membranes is provided by the valinomycin carrier system. In this case it has been observed (G. Stark, *personal communication*) that the rate constant for the association of valinomycin with potassium ions is decreased at high concentration of the carrier. In our case we suggest that, since a lateral displacement of lipid molecules presumably must occur to facilitate ion translocation, an increase of membrane viscosity could contribute to the barrier and account for the increase of  $\tau_0$  at elevated  $\text{DpA}^-$  concentration. Studies of the surface viscosity of condensed monolayers, measured as a function of subphase concentration of hydrophobic ions such as  $\text{DPA}^-$ , might offer a more direct approach to this problem.

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