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THE INTERACTIONS OF L- α -DIPALMITOYL LECITHIN MONOLAYERS WITH Na⁺, K⁺ OR Li⁺, AND ITS POSSIBLE ROLE IN MEMBRANE PHENOMENA

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SUMMARY

1. The surface properties of L- α -dipalmitoyl lecithin monolayers spread on 0.15 M solutions of NaCl, KCl or LiCl were determined at 25°, 35° and 45°. Using as comparison the properties of dipalmitoyl lecithin monolayers at the air-water interface it was observed that the presence of ions in the subphase increases the area per molecule, the surface potential and the surface dipole moment per molecule in the order Na⁺ > K⁺ \approx Li⁺ > H₂O; the surface viscosity and the energy of activation of viscous flow decrease in the order H₂O > Na⁺ > K⁺ > Li⁺.

2. The different effects of these ions on the properties of dipalmitoyl lecithin monolayers could be interpreted as being due to the ability of this substance to discriminate between the alkali ions, and this is discussed in relation to the selective permeability of ions through cellular membranes and to Na⁺ movement through epithelial membranes.

INTRODUCTION

The phospholipids of cell membranes have been classically considered to be structural components which can act on the exchange of substances between the cell and its environment only indirectly, although the presence in these molecules of charged polar groups (with a net charge +, - or zero) suggests the possibility of an active role in the transport and exchange of ions in cell membranes¹. As models in which the permeating molecule interacts very specifically with the membrane replace the concept that the membrane behaves as a passive and homogeneous diffusion medium, it becomes clear that phospholipids may play a major role in membrane phenomena. Thus GOLDMAN² has derived a complete set of curves describing the ionic current elements directly in terms of membrane potential, time and ion concentration on the basis that the polar groups of the phospholipids control the ion flux through a gate mechanism in a very selective way. The specific interaction between monolayers and alkali metal ions was also recently used^{3,4} to explain the movement of Na⁺ through epithelial membranes. Some authors have proposed that the Na⁺-transporting compartment consists of a system of selective fixed charges^{5,6} constituted

by the lipid polar groups of the outer layer of the plasma membrane of the epithelial cells.

Previous studies^{7,8} carried out at ion concentrations between 0.01 and 0.1 M failed to show that the surface pressure and surface potential–area curves of synthetic dipalmitoyl lecithin are dependent on the presence of Na^+ , K^+ , or Li^+ in the subphase. However, it was shown more recently that a slight expansion of the phospholipid monolayer⁹, as well as other homologues of this substance^{10,11} spread at the air–NaCl interface can be obtained. The present study of the surface pressure, surface potential–area curves and surface viscosity of synthetic L- α -dipalmitoyl lecithin, spread at the interfaces of air and 0.15 M NaCl, KCl or LiCl was carried out on the assumption that the interaction of these cations with the polar group of dipalmitoyl lecithin monolayers might yield some useful information which would further the understanding of the role of electrically neutral phospholipids in biological membranes.

MATERIALS AND METHODS

Reagents

L- α -Dipalmitoyl lecithin (Mann Research Laboratories, New York, N.Y.) was analysed by thin-layer chromatography and by gas-liquid chromatography, consistently giving a single spot and a fatty acid composition of 96.4% palmitic acid together with 2.74% and 0.85% of C_{12} and C_{15} saturated fatty acids, respectively. A mixture of abs. ethanol and purified light petroleum (b.p., 35–65°) (2:8, v/v) was used for the preparation of the spreading solutions. Deionized water treated with Norit A was distilled with alkaline permanganate and redistilled in an all-glass apparatus. After air equilibration it gave a pH value of 5.6–5.8, a conductivity of $0.2 \cdot 10^{-6} \Omega \cdot \text{cm}^{-1}$ and a surface tension (drop volume method) of $71.98 \text{ dynes} \cdot \text{cm}^{-1}$ (± 0.02) at 25°. Reagent-grade inorganic chemicals were used without further purification for the preparation of saline solutions which always gave pH values between 5.6 and 5.8. The following criterion for surface purity was adopted: subphase saline solutions were left standing in the balance trough for 30 min; 0.2 ml of the ethanol–light petroleum mixture was then spread on the surface, and after 5 min the area of the subphase was reduced from 550 to 27.5 cm^2 . A surface pressure of less than 0.4 $\text{dyne} \cdot \text{cm}^{-1}$ was accepted as proof that no surface contaminants were present in the subphase. The volume of the spreading solutions used for the formation of the monolayers was always less than 0.1 ml (± 0.0001).

Instruments

Surface pressure (π) and surface potential (ΔV) area curves were determined at a constant compression rate of $8 \text{ \AA}^2 \cdot \text{molecule}^{-1} \cdot \text{min}^{-1}$ (approx. 9 min per curve) with an automatic recording film balance previously described¹² and a General Radio electrometer (1230 A) with an ^{241}Am air electrode located at 2.5 cm from the float and 2 mm above the water surface; the water in the trough was earthed with a platinum electrode.

Surface viscosities were determined¹³ using a Teflon compression barrier with a transverse slit (0.16 cm \times 2.56 cm). Monolayers were compressed to a particular surface pressure with the slit closed. The slit was opened, and the monolayer was allowed to flow onto the clean surface behind the barrier. The automatic constant

pressure device was connected, and the rate of compression was adjusted to maintain a constant surface pressure (± 0.2 dyne \cdot cm $^{-1}$). Thus rates of monolayer flow were obtained at different constant surface pressures. All measurements were performed in an air-conditioned room; the temperatures of the trough were maintained constant ($\pm 0.2^\circ$) by circulating water from a constant temperature bath and were measured with two thermometers located just below the surface of the solution.

RESULTS

Surface pressure-area isotherms (π - A)

Figs. 1 and 2 show the π - A isotherms obtained at 25° , 35° and 45° for dipalmitoyl lecithin monolayers spread on water, 0.15 M NaCl, 0.15 M KCl or 0.15 M LiCl. Each curve was drawn with the average of at least eight isolated experiments; the standard error of the mean, represented by the small horizontal segment, was always between ± 0.25 and ± 0.50 Å 2 ·molecule $^{-1}$ for the interval 1 to 40 dynes \cdot cm $^{-1}$. Fig. 3 represents the compressibilities

$$C_s = -\frac{1}{A} \left(\frac{\partial A}{\partial \pi} \right)_T$$

as a function of the area per molecule at 25° and 35° . The thermodynamic functions for the process:

$$\text{Initial state } (\pi = 0, P, T) \rightarrow \text{final state } (\pi = 40, P, T)$$

are represented in Table I and were calculated from the compression curves using the following equations^{14,15}

$$(\Delta G)_{PT} = \int_{\pi=0}^{\pi=40} A d\pi; (\Delta S)_{P,\pi} = \left(\frac{\partial \gamma}{\partial T} \right)_{P,\pi} \cdot (A_0 - A_{40}) - \left(\frac{\partial \Delta G}{\partial T} \right)_{P,\pi}; \Delta H = \Delta G + T \Delta S$$

in which γ is the surface tension of the subphase, A_0 is the area per mole at $\pi = 0$ and A_{40} the area per mole at $\pi = 40$. G and H are defined¹⁶ by the equations:

$$G = E - TS + PV - \gamma A \text{ and } H = E + PV - \gamma A$$

The integrals $\int_{\pi=0}^{\pi=40} A d\pi$ were separately evaluated from the corresponding compression curves; the standard error of the mean for ΔG values was always between ± 0.017 and ± 0.030 kcal·mole $^{-1}$. The values of γ for the calculation of $(\partial \gamma / \partial T)_P$ were obtained using the equation¹⁷:

$$\gamma - \gamma_0 = \frac{79.517}{D} \cdot c \cdot \log \frac{1.143 \cdot 10^{-13} (DT)^3}{c}$$

in which γ is the surface tension of the 1:1 electrolyte solution, γ_0 and D the surface tension and the dielectric constant, respectively, of water, and c the molarity of the solution.

It can be observed that the general appearance of the isotherms obtained at 25° , 35° and 45° is not dependent on the composition of the subphase and that the phase transition previously reported¹⁸ for dipalmitoyl lecithin monolayers at the air-water interface at 25° is only quantitatively affected by the presence of cations in the subphase. The shift towards larger areas per molecule in the compressibility curves

is in the order $\text{Na}^+ > \text{K}^+ > \text{Li}^+ > \text{H}_2\text{O}$. When spread on 0.15 M saline subphases, the dipalmitoyl lecithin monolayers show an expansion, which is reflected by the increasing ΔG values for the compression process in the order: $\text{Na}^+ > \text{K}^+ \approx \text{Li}^+ > \text{H}_2\text{O}$; it can also be observed that the ΔS value for the compression process at 25–35° on

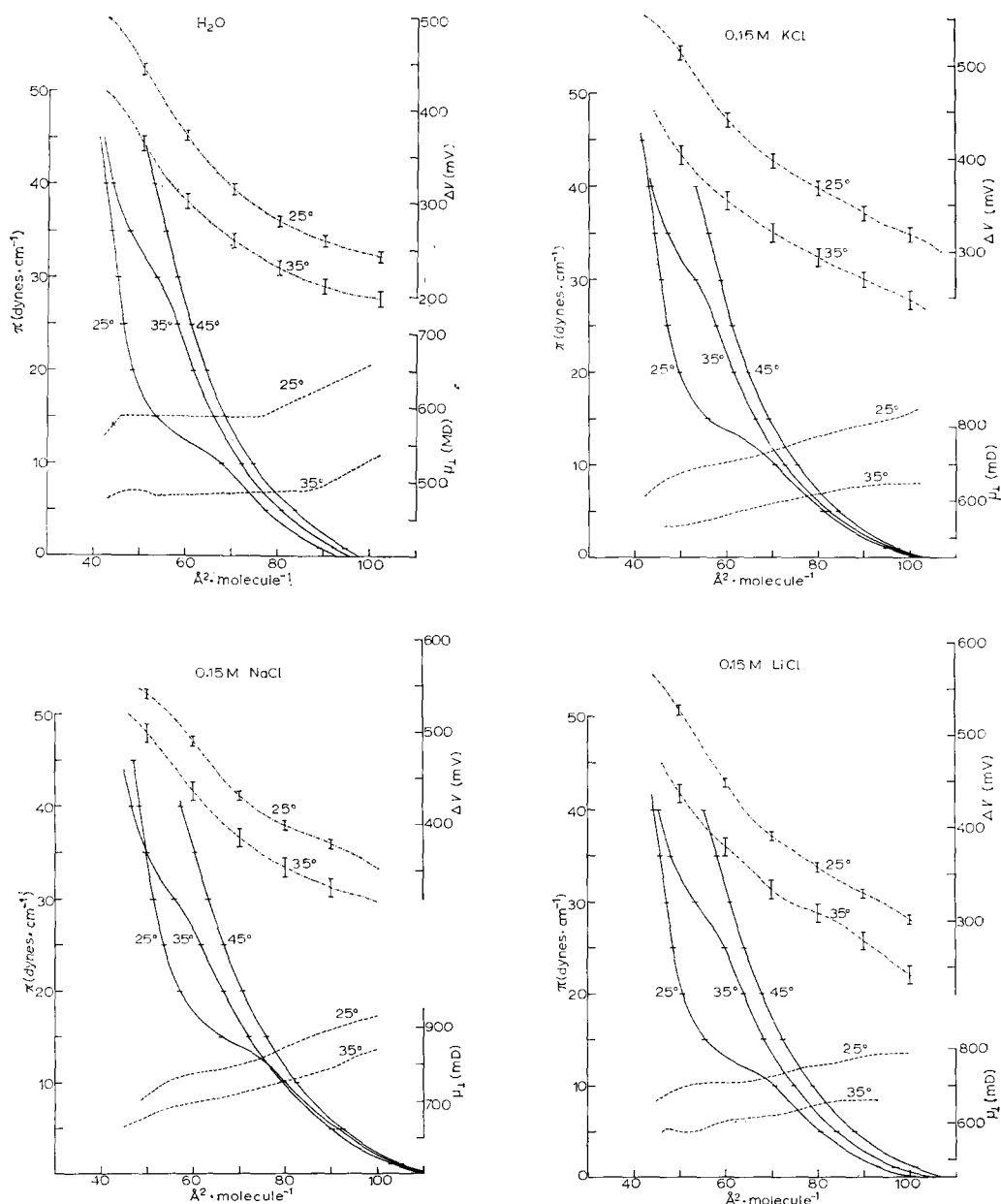


Fig. 1. Surface pressure (—), surface potential (---) and surface dipole moment per molecule (····) area curves for dipalmitoyl lecithin at the air-water, air-0.15 M KCl, air-0.15 M NaCl and air-0.15 M LiCl interfaces.

0.15 M NaCl is the least negative and that its negative enthalpy is about one half that of the water, 0.15 M LiCl or 0.15 M KCl subphases.

Surface potential-area isotherms (ΔV - A)

In Figs. 1 and 2 the ΔV - A curves at 25° and 35° are given. The standard error of the mean, represented by the vertical segments, was always between ± 4 and ± 8

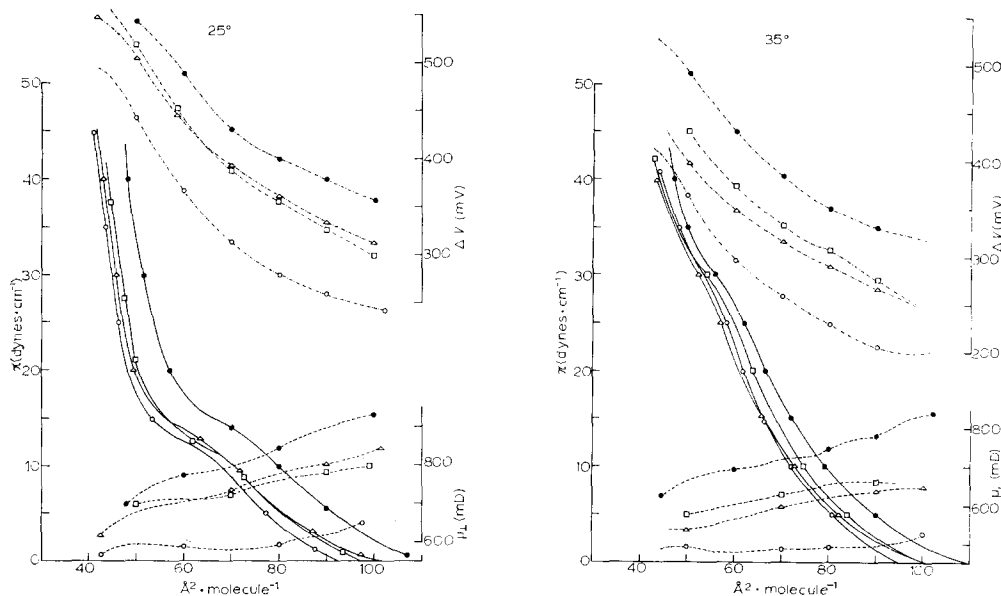


Fig. 2. Surface pressure (—), surface potential (---) and surface dipole moment per molecule (— · —) area curves at 25° and 35° for dipalmitoyl lecithin spread at the air-water (○), air-0.15 M NaCl (●), air-0.15 M KCl (△) and air-0.15 M LiCl (□) interfaces.

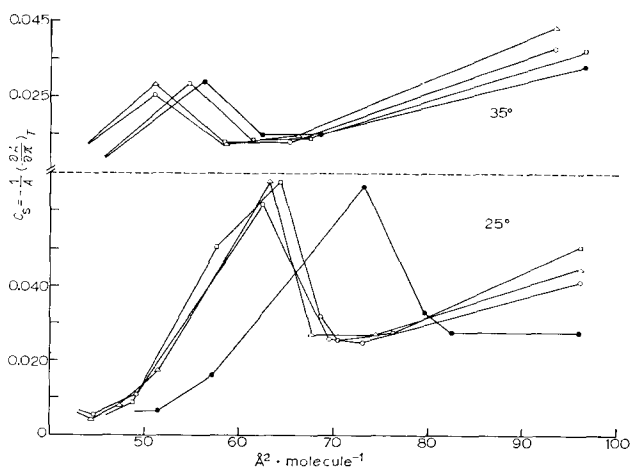


Fig. 3. Compressibility of dipalmitoyl lecithin monolayers spread at the air-water (○—○), air-0.15 M NaCl (●—●), air-0.15 M KCl (△—△) and air-0.15 M LiCl (□—□) interfaces at 25° and 35°.

TABLE I

THERMODYNAMIC FUNCTION OF THE COMPRESSION PROCESS:

Initial state ($\pi = 0, P, T$) \rightarrow final state ($\pi = 40, P, T$)

Subphase	ΔG (kcal \cdot mole $^{-1}$) 25 $^{\circ}$	$-\Delta H$ (kcal \cdot mole $^{-1}$) 25-35 $^{\circ}$	$-\Delta S$ (cal \cdot degree $^{-1} \cdot$ mole $^{-1}$) 25-35 $^{\circ}$
H ₂ O	3.22 \pm 0.02	12.57	53
K ⁺	3.50 \pm 0.03	10.07	45
Li ⁺	3.56 \pm 0.02	12.93	54
Na ⁺	3.68 \pm 0.02	5.98	32

mV for the curves at 25 $^{\circ}$ and between ± 4 and ± 12 mV for the curves at 35 $^{\circ}$. The study of the surface potential was not performed at 45 $^{\circ}$ because the irregular condensation of water on the air electrode at this temperature produces erratic values. It can be seen that the shape of the curves is not affected by variation of the temperature or by the composition of the subphase, but there is an increase in the surface potential values, at A and T constants, when 0.15 M electrolytes are present in the subphase. The order of this increment is Na⁺ > K⁺ \approx Li⁺ > H₂O. This sequence of the effect of the cations is one of the orders of selectivity reported by EISENMAN¹⁹ (Sequence IX).

The surface dipole moment per molecule (μ_{\perp}) is defined²⁰ by the equation

$$\mu_{\perp} = 2.65 \cdot 10^{-2} \cdot A \cdot \Delta V$$

in which A is the area (\AA^2) per molecule; it is represented in Figs. 1 and 2 in milli Debyes (1 Debye = 10^{-18} e.s.u.cm). At the air-water interface μ_{\perp} is temperature dependent, but it is independent of the area per molecule between 80 and 46 $\text{\AA}^2 \cdot \text{molecule}^{-1}$. For the air-electrolyte solution interfaces, μ_{\perp} depends on the temperature and also on the area per molecule. The fact that the effect of the ions on the dipalmitoyl lecithin monolayer is not independent of the distance between phospholipid molecules suggests that the interaction between the free cations with the phosphate groups could be modified by the presence of the trimethylammonium group in the vicinity of a dipalmitoyl lecithin molecule.

Surface viscosity

Fig. 4 represents the logarithm of the surface viscosity (η_s) of dipalmitoyl lecithin monolayers at the air-water, air-0.15 M KCl, air-0.15 M NaCl or air-0.15 M LiCl interfaces as a function of the surface pressure at 25 $^{\circ}$ obtained with the average of at least six isolated experiments; the standard error of the mean was below 0.03 mP. The surface viscosity was calculated using the equations²¹:

$$Q = \frac{\Delta\pi}{l(c\eta_0)} \left[W - 2 \left(\frac{\eta_0}{c\eta_0} \right)^{\frac{1}{2}} \tanh \left\{ \left(\frac{c\eta_0}{\eta_s} \right)^{\frac{1}{2}} \cdot \frac{W}{2} \right\} \right]$$

in which Q is the rate of flow in cm² of a monolayer through a slit l centimeters long and W centimeters wide by the action of a surface pressure gradient, $\Delta\pi$, dynes \cdot cm $^{-1}$; η_s and η_0 are, respectively, the surface viscosity of the monolayer and the bulk viscosity of the subphase. The values of c were calculated using the table given by

JOLY²¹, assuming that c is independent of the ionic strength of the subphase. Bulk viscosities of the electrolyte solutions were obtained using the equation²²:

$$\eta/\eta_0 = 1 + A\sqrt{c} + Bc$$

in which η and η_0 are, respectively, the viscosities of the electrolyte solution and of water, and c is the molarity of the solution. Values for the parameter A were taken from HARNED AND OWEN²³, and for parameter B from KAMINSKY²⁴.

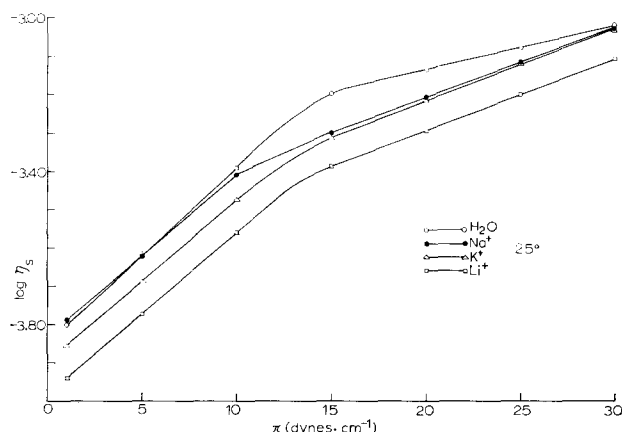


Fig. 4. The logarithm of the surface viscosity of dipalmitoyl lecithin monolayers as a function of the surface pressure when spread at air-water (O—O), air-0.15 M NaCl (●—●), air-0.15 M KCl (△—△) and air-0.15 M LiCl (□—□) at 25°.

A factor (F) thus calculated corrects for the lack of slippage of the monolayer on the underlying water and was subtracted from the uncorrected viscosity. The final equation used was:

$$\eta_s = \frac{A\pi W^3}{12Ql} - F$$

Energy (ΔE_{vis}) and free energy (ΔG_{vis}) of viscous flow were calculated using the equations^{25,26}:

$$\eta_s = C \exp. (\Delta E_{vis}/RT); \quad \eta_s = h/A \exp. (\Delta G/RT)$$

where C is a numerical constant, h Planck's constant, and A the cross-sectional area of the flow unit. R and T are, respectively, the gas constant per mole and the absolute temperature.

In Fig. 4 the linear relationship of the plots $\log \eta_s - \pi$ can be observed, with a change in the slope between 11 and 14 dynes·cm⁻¹. This change in the slope is related to the existence of different flow units below and above 12 dynes·cm⁻¹; consequently it can be concluded that this peculiarity of the dipalmitoyl lecithin monolayer is not affected by the presence of 0.15 M NaCl, KCl or LiCl in the subphase. The surface viscosity is in general decreased by the presence of electrolytes in the subphase.

The correction factor included in the calculation of surface viscosity allows for the lack of slippage between the flowing monolayer and the subphase. In other words, corrected surface viscosities would represent the true or absolute viscosities of the

monolayer-forming molecules with severe restrictions because of the particular characteristics of the flowing system²⁷. Considering only the interactions of dipalmitoyl lecithin monolayer with the subphase components, the uncorrected surface viscosity represents the rheological properties of a complex flow unit composed of the dipalmitoyl lecithin molecules and eventually the ions fixed on it, *plus* the layer of subphase which drags. Uncorrected relative surface viscosity (η_{rel}) is defined by the equation:

$$\eta_{rel} = \eta_{s, \text{elect}} / \eta_{s, w}$$

in which $\eta_{s, \text{elect}}$ and $\eta_{s, w}$ are the uncorrected surface viscosities of the dipalmitoyl lecithin monolayer spread on 0.15 M electrolyte solution and on water, respectively. Fig. 5 represents the plot of the uncorrected relative surface viscosity against surface pressure. Fig. 6 represents the values of the energy functions of viscous flow calculated using the uncorrected viscosity.

As can be seen from Figs. 4 and 5, the surface viscosity is affected by the presence of ions in the subphase. It would be difficult to draw any conclusions about the intrinsic mechanism by which a given ion modifies the surface viscosity, since, among other factors, the viscosity depends on: (1) the attraction between alkyl chains, (2) the energy of the attraction or repulsion between the polar groups of different molecules, (3) the relative affinity of the phosphate groups for the free cation and the trimethylammonium group of neighbouring molecules, (4) the amount of water involved in each ion-polar group interaction (see ref. 28), (5) the effect that the dipalmitoyl

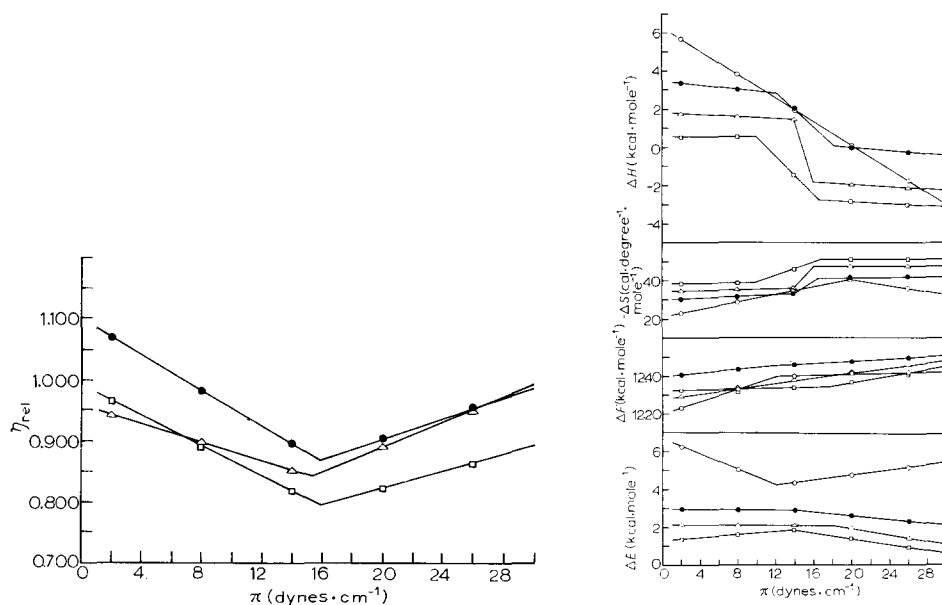


Fig. 5. Relative surface viscosities of dipalmitoyl lecithin monolayers at the air-0.15 M NaCl (●—●), air-0.15 M KCl (△—△) and air-0.15 M LiCl (□—□) interfaces at 25°.

Fig. 6. Thermodynamic functions of activation of viscous flow for dipalmitoyl lecithin monolayers spread at the air-water (○—○), air-0.15 M NaCl (●—●), air-0.15 M KCl (△—△) and air-0.15 M LiCl (□—□) interfaces (25–35°).

lecithin monolayer and the interacting ions may have on the structure of the water (see ref. 29), and (6) the effect of the ions on the viscosity of the subphase. However, from the data in Figs. 4, 5 and 6 it can be concluded that this interaction reveals that the dipalmitoyl lecithin monolayer discriminates between different alkali metal ions.

DISCUSSION

The present study shows that the state of dipalmitoyl lecithin monolayers depends on the nature of the ions in the subphase. In order to understand the mechanism of the interactions between free ions and dipalmitoyl lecithin monolayers, a model must be adopted. The interpretation of the position of the saturated hydrophobic chains seems to offer no difficulty: as the surface is decreased the alkyl chains tend to arrange themselves so that they are parallel to each other and perpendicular to the surface.

With respect to the polar group, which is fully charged at the pH used, the situation is somewhat less clear. The phosphatidyl choline groups could have two extreme positions: (a) the trimethylammonium group is perpendicular to the surface and extended below the phosphate, or (b) the trimethylammonium is situated in the same plane occupied by the phosphate, so that the axis of the zwitterion is parallel to the plane of the interface. On the basis of electrostatic and electrokinetic studies carried out on monolayers³⁰ and bilayers³¹, it was suggested that the phosphatidyl choline group of the lecithin is very probably oriented in a plane parallel to the interface. However, a statistical thermodynamic analysis of the long-range repulsion observed between leaflets of lamellar liquid-crystals of pure lecithin in water carried out by PARSEGIAN (see ref. 32) suggested that the leaflet surface is better described as a diffuse charge layer, the positively charged group acting as an independent counterion. In view of the elaborate manner in which the orientation of the dipoles of phospholipids influence the field and potential values³³, a great deal more of information is necessary before any conclusions can be reached. For the purpose of discussion it is assumed that the positive trimethylammonium group acts as a 'free' counterion of the comparatively fixed phosphate group of the nearest neighbour molecules. This interaction would give rise to a two-dimensional arrangement of the type proposed by PETHICA (see ref. 30) which satisfactorily explains the pressure-area relationship of dipalmitoyl lecithin on pure water at 25° observed in Fig. 1. The decrease of the slope between 68 and 52 Å²·molecule⁻¹ corresponds to the distance between dipalmitoyl lecithin molecules at which the van der Waals forces between the alkyl chains and the attraction between opposite charges in neighbouring molecules reach a maximum value, and the lattice has its lowest energy level. At 35° the increased thermal fluctuations necessitate compression of the monolayer to an area smaller than 60 Å²·molecule⁻¹ before the change in slope is reached. Finally at 45° the thermal energy is so high that there is no surface pressure (in the range studied) at which the lattice could be stabilized. It is rather difficult to make an exact calculation of the changes in the energy state of each phospholipid molecule at the new temperature and the new distance, since the configuration of the molecule does not seem to remain constant (as indicated by changes in the surface dipole moment per molecule). The decrease in surface potential with temperature would indicate that in a

large fraction of the dipalmitoyl lecithin molecules the positive choline groups involved become loose and sink into the aqueous phase.

As the molecules become closely packed and the alkyl chains reach a vertical position, the contribution of the dipoles formed by the carbonyl of the ester links to the total dipole moment of the dipalmitoyl lecithin molecule decreases. Since most of the total normal dipole moment could be attributed to the two fatty acid ester linkages alone (see ref. 11), it would be expected to decrease at small surface areas. This view is in agreement with the observed decrease of the dipole moment until an area of about $80 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ is reached, after which it remains at a steady level as the area is reduced further (Fig. 1).

The effect of ions

At the pH used the trimethylammonium and phosphate groups neutralize each other, and the dipalmitoyl lecithin monolayer does not have a net charge. As discussed above, the lack of net charge on the dipalmitoyl lecithin molecule, does not prevent strong interaction with its neighbours. Free ions in the subphase interact with the polar groups of the dipalmitoyl lecithin molecule and modify the monolayer as evidenced by changes in the pressure, surface potential, dipole moment (Figs. 1 and 2), and surface viscosity (Figs. 4, 5 and 6). When a mobile cation is adsorbed on the phosphate group of a dipalmitoyl lecithin molecule, the lattice may be disrupted by two main mechanisms: (1) the cation will compete with the trimethylammonium group for the phosphate groups of neighbouring molecules, and (2) the displaced trimethylammonium group will become an interstitial component in the lattice with an unusual property: because of the chemical attachment to the rest of the dipalmitoyl lecithin molecule, it will not migrate but will remain as a 'localized interstitial component', thus helping to disrupt the trimethylammonium-phosphate bridges between the nearest neighbour molecules, and thus making more polar groups available for interaction with ions in the subphase. This mechanism would constitute a positive feedback for cation binding, and the process will be repeated continuously throughout the surface.

In the present study an electrolyte concentration of 150 mM was chosen because it is approximately the concentration of Na^+ outside, and the concentration of K^+ inside mammalian cell membranes. For the sake of comparison one may calculate the concentration of the free trimethylammonium in contact with the phosphate groups. Considering that the trimethylammonium group can vary its distance from the line of phosphate groups to a maximum of about 5 \AA , when the dipalmitoyl lecithin molecule occupies 60 \AA^2 the concentration of trimethylammonium in the 5-\AA deep layer of water in contact with the phosphate interphase is about 5 M, *i.e.*, more than 30 times the concentration of any of the free cations studied. This overwhelming concentration of trimethylammonium groups with respect to free cations enables this group to compete favourably for the phosphate groups and may explain why the free cations tested produce quantitative modifications of the dipalmitoyl lecithin monolayer but do not impair its basic structure. Temperature tends to disrupt the lattice and lowers both the surface potential and dipole moment. Cations also disrupt the lattice, but they increase the surface potential and the dipole moment. This could be explained by assuming that the trimethylammonium group, besides

being displaced from the phosphate group by the free cations from the subphase, is repelled upwards.

Biophysical implications

The concept of a cellular membrane as a simple diffusion medium no longer affords a useful approach to the study of membrane phenomena. Current models involve charged channels, saltatory migration through specific binding sites^{34,35}, carrier molecules³⁶ and even phase transitions of the membrane itself³⁷. The common feature of these mechanisms is that the permeating substance interacts specifically with the membrane. It is often assumed that only phospholipids with a net charge will play a role in ion movement. However, the effect of ions on monolayers of neutral lipids of the sort studied in the present work suggests that neutral lipid may also play a major role in ion movement both across and along the membrane. The large movement of flexible dipoles at one end of the long hydrocarbon chains of phospholipids has been used by GOLDMAN² as a means of providing control of ion flow by acting as a gate operated by changes in the electrical field. The observation reported here that at a given pressure ions can modify the area occupied by the phospholipids, also suggests that a pore through a lipid bilayer can modify both its length and diameter, depending on which ion is flowing through its lumen. This effect is still more remarkable if one considers that, as evidenced by the changes in surface potential and dipole moment of dipalmitoyl lecithin molecules, the position of the positive group can bend toward or away from the lumen, hence varying the charge of the pore. Therefore the present results could offer a new clue in the understanding of selective permeability of ions through membranes.

When an ion from the bathing solution approaches a cellular membrane, it will tend to migrate by jumping between fixed polar groups rather than by crossing the hydrophobic component of the membrane, and penetrating the cell³. This tendency, which does not seem to play an important role in the penetration of ions into individual cells, may constitute the basis of Na⁺ movement through epithelial membranes. Studies of the mechanism of Na⁺ fluxes and distribution carried out by electrical and isotopic^{4,38} methods, indicate that Na⁺ migrates through a very ordered transporting compartment, probably constituted by the lipid polar groups of the outer layer of the cellular membrane of the epithelial cells.

The highly specific Na⁺ binding involved in this mechanism agrees with the results of nuclear magnetic resonance analysis of the state of Na⁺ carried out in the same biological preparation (abdominal skin of *Leptodactylus ocellatus*), which indicated that a large fraction of the Na⁺ content was not present as free Na⁺ (ref. 39). The fact that, as observed in the present study, a phospholipid-water interface reacts differently with different cations, together with the effect discussed above, *i.e.*, that the ion absorbed on the phosphate group promotes further ion binding (or vacancies to where it could jump) in the nearest neighbouring phospholipids, offers some understanding of the basic mechanism of the process.

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