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NON-ISOTHERMAL POTENTIAL OF PHOSPHOLIPID BILAYER FILMS

INFLUENCE OF CHOLESTEROL AND MACROCYCLIC CARRIER EFFECTS

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Summary

The effect of cholesterol on the ion selective behavior of phospholipid (phosphatidylcholine or phosphatidylethanolamine) bilayer films is studied through the measurement of the membrane non-isothermal potential.

It is shown how the mixed phosphatidylcholine-cholesterol membrane can be either cation of anion permselective according to the film composition (cationic behavior is met in the 0—10% cholesterol composition range while anionic selectivity appears in the 20—50% range).

On the contrary, mixed phosphatidylethanolamine-cholesterol membranes show the absence of ionic selectivity already met with pure phosphatidylethanolamine films.

The presence of a cationic carrier as Dibenzo-18-crown-6 in the film transforms all the studied films (cationic, anionic and no selective bilayers) into ideally cationic selective membranes.

These results are discussed on the basis of the current ideas on the charge distribution through the bilayer membranes. Moreover, the role of the permeating ions as potential determining species is stressed.

Introduction

Phospholipid bilayer films can be assimilated to a membrane separating two liquid phases and formed by a liquid-like hydrocarbon interior, 50 Å thick, with the polar groups at the membrane-solution interfaces. The nature and number of polar groups can determine the existence of a space charge in the interfacial region. In isothermal conditions an electrical potential difference between the bilayer bounding surfaces can be originated by a difference in the chemical potential of some mobile species present at the two sides of the membrane. In the case of a zero charged film (phosphatidylethanolamine bilayer for

example), where the liquid-like hydrocarbon interior is conceived as the essential barrier through which ions must move unaffected by the polar groups at the membrane-solution interfaces [1—3], the electrical potential difference has to be of the form of a diffusion potential

$$-\Delta\psi = (2t_+ - 1)(RT/F)\ln(a''_{\pm}/a'_{\pm}) + \tau_o \ln(a''_w/a'_w) \quad (1)$$

($a_{\pm}$ and a_w are the electrolyte and water activities at the two sides of the membrane; t_+, t_- and τ_o are the transport numbers across the membrane of cations, anions and water). For charged highly permselective films the electrical potential is given by

$$-z_k\Delta\psi = (RT/F)\ln(a''_k/a'_k) \quad (2)$$

(a_k is the activity of the kth ion with charge z_k). In the case of phosphatidylcholine films (from egg yolk or synthetic L-α-dipalmitoyl phosphatidylcholine in *n*-dodecane) a well defined anionic Nernstian potential (59 mV/decade) has been observed only with iodide solutions [4]. With NaCl, KCl and KIO_3 in the 10^{-2}—10^{-3} M range, potentials of about 30 mV/decade were obtained [4,5]. With films of phosphatidylethanolamine (from *Bacillus megaterium* and *Escherichia coli*) in presence of NaCl and KCl the potential measurement for a 10-fold increase in salt activity scatter [1] from −14 to 30 mV. From these data it is rather difficult to reach any conclusion on the isothermal electrochemical behaviour of the phospholipid films. Several causes can contribute to make unclear the isothermal electrical measurements and between them the transport of water due to different water activities at the two sides of the membrane should be mentioned. In a previous work [6] the non-isothermal ion transport across phospholipid bilayers has been studied in terms of thermal electrical potential originated by a difference in temperature at the two sides of the membrane. The thermal electrical potential is given by

$$-z_k(\Delta\psi/\Delta T) = 2.303(R/F)\log a''_k + (S^*_k/TF) \quad (3)$$

(S^*_k is a term accounting for the energy transport per unit mass flux, T is the temperature, F is the Faraday constant and a''_k the activity of the kth permeant ion in the solution at temperature $T + \Delta T$). The non-isothermal electrical potential equation has been derived from the existing theories on transport processes in solid [7] and liquid [8] ion exchange membranes. Eqn. 3 is valid under isochemical conditions (same electrolyte activity at the two sides of the membrane $a'' = a'$). In this case any transport process due to difference in the activities of the aqueous solution components vanishes. It is worth noting that Eqn. 3 has strict analogies with Eqn. 2. In fact, in the isothermal case the function $\Delta\psi$ vs. $\log a_k$ gives a slope of 59 mV/decade at 23°C, where the sign of the electrical potential depends on the charge of the transported ion and therefore, on the charge of the membrane. In the non-isothermal case (Eqn. 3) the function $(\Delta\psi/\Delta T)$ vs. $\log a''_k$ has a slope of $-2.303(R/z_kF) = \pm 200\,\mu V/°C$ where the minus sign refers to the cation (negative charged films) and the plus sign to the anionic (positive charged films) permselective membranes. Using Eqn. 3 it has been found [6] that the phosphatidylcholine (egg yolk and L-α-dipalmitoyl phosphatidylcholine in *n*-dodecane) films behave as a cationic membrane with respect to the Na^+ (NaCl as aqueous electrolyte solution). The phosphatidyl-

ethanolamine films, on the contrary, behave as zero-charged membranes. By comparing isothermal and non-isothermal electrical behaviour of phosphatidylcholine films it appears that, by removing uncertainities associated with water transport, as is the case in thermal isochemical potential measurements, the bilayer shows a clear permselective behaviour (cationic permselective membrane) with resepect to Na^+ in NaCl aqueous solution.

In this paper the non-isothermal (isochemical) electrical potential of mixed phosphatidylcholine-cholesterol and phosphatidylethanolamine-cholesterol films is investigated. Moreover, the effect of the presence of "carriers" (dibenzo-18-crown-6) on the measured thermal potentials is also examined.

Experimental

Materials. L-α-Phosphatidylcholine from egg yolk and phosphatidylethanolamine from sheep brain have been obtained from Sigma (commercial grade) and have been used without further purification. L-β-γ-Dipalmitoyl-α-phosphatidylcholine puriss., cholesterol U.S.P. crystalline puriss grade and *n*-dodecane purum (99%) have been supplied by Fluka. Sodium chloride, chloroform, methanol, benzene and *n*-exane have been obtained from Carlo Erba and were analytical grade purity reagents. Dibenzo-18-crown-6 has been

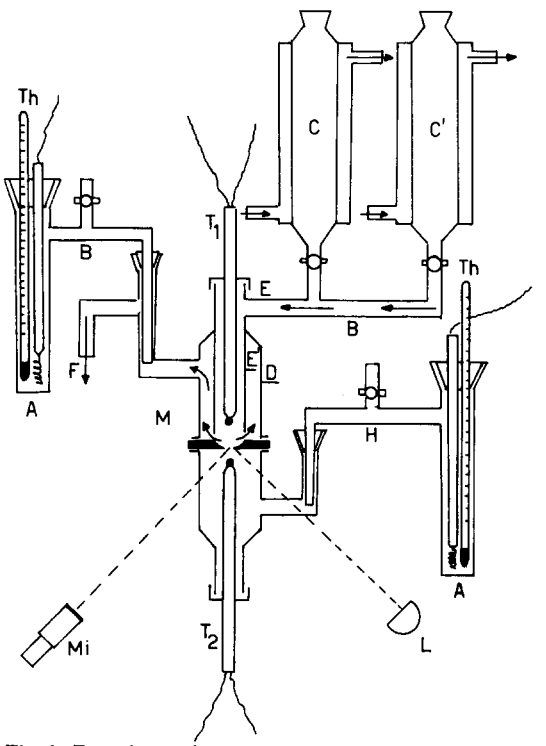

Fig. 1. Experimental apparatus used to measure the thermal potential of bilayer membranes.A, Ag/AgCl electrodes; B and H, NaCl solution bridges; C′ and C, NaCl solution at temperature $T + \Delta T$ and T, respectively (the temperature is obtained using a thermostat); D, pyrex glass cell; E, NaCl solution inlet; F, NaCl solution outlet; G, Teflon gasket with hole of 1.9 mm diameter; M, bilayer; T, thermistors (1 mm distance between the two heads); L, light source; Th, calibrated thermometers; Mi, stereomicroscope.

prepared according to ref. 9. Silver-silver chloride electrodes and standard calomel electrodes (S.C.E.) were prepared following ref. 10. Care was taken to use electrodes with an asymmetry less than 0.05 mV.

Membrane preparation. Bilayer membranes of the type of Mueller et al. [11] were formed from a solution of lipid (2%, w/v) and *n*-dodecane (18%, v/v) both dissolved in 3 : 2 (v/v) chloroform/methanol solvent. The solution were freshly prepared the same day of the measurements. The membranes so formed were usually stable for several hours.

Equipment. A Cary vibrating reed electrometer model 31V coupled with a Hewlett-Packard recorder (model 17501) was used to measure the potentials. To avoid stray e.m.f. values the measurements were performed in a Faraday cage. The temperature differences across the membrane were measured by means of two thermistors [12].

Thermal cell and procedure. Experimental values of the thermal e.m.f. for lipid films were obtained by using the apparatus schematically shown in Fig. 1. The cell shown in Fig. 1 can be schematized as follows:

$$\underset{T}{\text{Ag/AgCl}}\underset{T}{\text{/NaCl(M)}}\underset{T}{\text{/lipid bilayer}}\underset{T+\Delta T}{\text{/NaCl(M)}}\underset{T}{\text{/AgCl}}\underset{T}{\text{/Ag}} \qquad \text{(A)}$$

The measurements were performed according to the procedure described in ref. 6 with $T = 23°C$.

Results and Discussion

In Fig. 2 the experimental $\Delta\psi$ (mV) vs. ΔT (°C) values at different NaCl concentrations (in the range $5 \cdot 10^{-3}$—1 M) for phosphatidylcholine (from ref. 6) phosphatidylcholine-cholesterol (1 : 1), phosphatidylethanolamine and phosphatidylethanolamine-cholesterol (1 : 1) films are reported. In Fig. 3 the experimental $\Delta\psi$ (mV) vs. ΔT (°C) values at different NaCl concentrations for L-β,γ-dipalmitoyl-α-phosphatidylcholine and L-β,γ-dipalmitoyl α phosphatidylcholine-cholesterol (1 : 1) films are reported. The corresponding $\Delta\psi/\Delta T$ vs. $\log a_{NaCl}$ plots are shown in Fig. 4. Let us first consider the phosphatidylcholine case. Phosphatidylcholine and phosphatidylcholine-cholesterol (1 : 1) bilayers behave like charged permselective membranes of opposite sign (curves a and f). The potential values for phosphatidylcholine films fit the equation $(\Delta\psi/\Delta T) = -200 \log a - \bar{S}$ (cationic permselective membrane; Eqn. 3 with $z_k = +1$; $\bar{S} = S^*/TF$) with $-\bar{S} = 400\,\mu V/°C$ [6]; those for phosphatidylcholine-cholesterol bilayers are fitted by the equation $(\Delta\psi/\Delta T) = +200 \log a + \bar{S}$ (anionic permselective membrane; Eqn. 3 with $z_k = -1$) being $-\bar{S} = -40\,\mu V/°C$. The same change in selectivity is observed with films prepared from synthetic phosphatidylcholine and synthetic phosphatidylcholine-cholesterol (Fig. 4, curves b, d). However, as expected [6] the $-\bar{S}$ is now different: $-\bar{S} = +180\,\mu V/°C$ for synthetic phosphatidylcholine and $-\bar{S} = +200\,\mu V/°C$ for mixed synthetic phosphatidylcholine-cholesterol films. Therefore, the phosphatidylcholine film behaves as a charged membrane highly selective to cations while the mixed phosphatidylcholine-cholesterol films are seen to be highly selective to anions. It is useful to note that the value of the derivative $d(\Delta\psi/\Delta T)/d\log a_k = \pm 200\,\mu V/°C$ (+ for anion and — for cation selective membranes) has

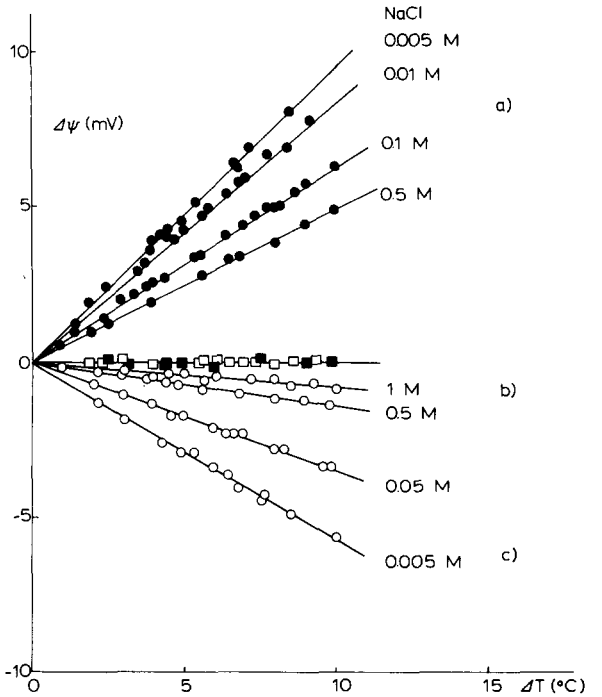

Fig. 2. Electrical thermal membrane potential ($\Delta\psi$ mV) vs. temperature difference (ΔT°C) plots at different NaCl aqueous concentrations for (a) egg yolk phosphatidylcholine (●); (b) sheep brain phosphatidylethanolamine (□) phosphatidylethanolamine-cholesterol (1 : 1) (■); (c) phosphatidylcholine-cholesterol (1 : 1) (○).

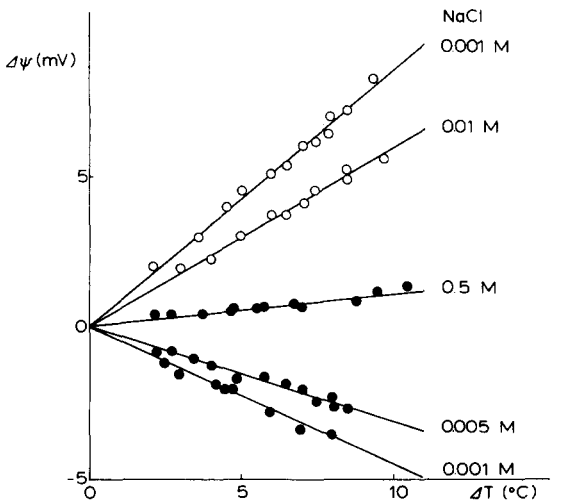

Fig. 3. Electrical thermal membrane potential ($\Delta\psi$ mV) vs. temperature difference (ΔT°C) plots at different NaCl aqueous concentrations for L-β-γ-dipalmitoyl-α-phosphatidylcholine (○) and phosphatidylcholine-cholesterol (1 : 1) (●) films.

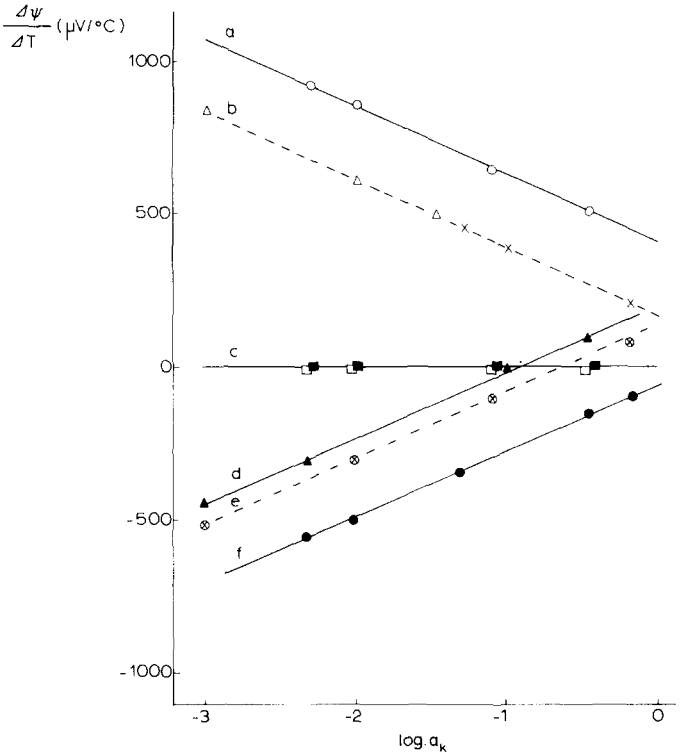

Fig. 4. Thermal coefficient of bilayer membrane potential ($\Delta\psi/\Delta T$ μV/°C) vs. logarithm of aqueous electrolyte activity (log a_{NaCl}) for (a) egg yolk phosphatidylcholine (○); (b) dipalmitoyl phosphatidylcholine (△) and dinonylnaphtalen sulfonic acid in *o*-dichlorobenzene (X); (c) sheep brain phosphatidylethanolamine (□) and phosphatidylethanolamine-cholesterol (1 : 1) (■); (d) phosphatidylcholine-cholesterol (1 : 1) (▲); (e) THACl in *o*-dichlorobenzene (⊗); (f) egg yolk phosphatidylcholine-cholesterol (1 : 1) (●).

been verified for the thick liquid anionic and cationic permselective ion exchange membranes [6,13]. For comparison purposes in Fig. 4 the data for both a liquid anion selective (from ref. 13; curve e) and cation selective (from ref. 6; crosses on curve b) membranes are also reported. In order to avoid any possible mistake about the sign of the measured potential the same experimental electrical connection arrangement has been used in all experiments (the electrode of the half cell at temperature $T + \Delta T$ was inserted in the positive electrometer inlet). From the information obtainable from Eqn. 4, the addition of cholesterol induces a drastic change on the electrochemical answer of the film. Not only changes in the $\bar{S}$ value, but also in the membrane selectivity, are observed. Moreover, the use of NaCl as aqueous electrolyte and the experimental arrangement make any aqueous ion thermal diffusion process negligible and, therefore, the observed changes of both the $\bar{S}$ values and the selectivity are intrinsic characteristics of the bilayer. In order to explore the role of cholesterol on the electrochemical behaviour of mixed films we have also measured the $\Delta\psi/\Delta T$ (μV/°C) coefficient for films with different phosphatidylcholine/cholesterol ratios at a constant NaCl concentration (0.017 M). The results, reported in Table I, show that in the 0—10% cholesterol composition range films behave as cationic membranes while in the range 20—50% they

TABLE I

THERMAL POTENTIAL COEFFICIENTS OF MIXED PHOSPHATIDYLCHOLINE-CHOLESTEROL BILAYER MEMBRANES IN CONTACT WITH AQUEOUS 0.01 M NaCl SOLUTIONS

a, $-500\ \mu V/°C$ corresponds to the experimental $\Delta\psi/\Delta T$ value at 10^{-2} M for the anionic membrane bilayer (see Fig. 3); b, $+800\ \mu V/°C$ corresponds to the experimental $\Delta\psi/\Delta T$ value at 10^{-2} M for the cationic membrane bilayer (see Fig. 3); c, not well defined potential values (see Fig. 5).

Weights % cholesterol	$\Delta\psi/\Delta T$ ($\mu V/°C$)
50	−500 (a)
36	−510 (a)
29	−500 (a)
21	(c)
18	(c)
14.6	(c)
10	+800 (b)
5	+860 (b)
0	+860 (b)

behave as anionic membranes. At intermediate composition values (12—18%) the sign of the thermal potential is not well defined. As an example the thermal potentials for a film with 15% of cholesterol is reported in Fig. 5. From these data it follows that the composition range 12—20% in cholesterol is a transition region.

Phosphatidylethanolamine case. With phosphatidylethanolamine films the temperature gradient does not originate any significant electrical potential both in simple phosphatidylethanolamine [6] and mixed phosphatidylethanolamine-cholesterol films (Fig. 2). All the phosphatidylethanolamine film thermal

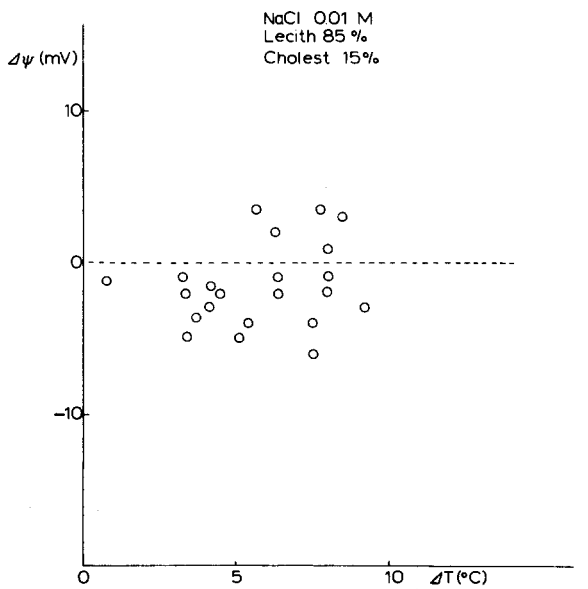

Fig. 5. Electrical thermal membrane potential ($\Delta\psi$ mV) vs. temperature difference (ΔT°C) for phosphatidylcholine (85%) cholesterol (15%) bilayer in NaCl 0.01 M.

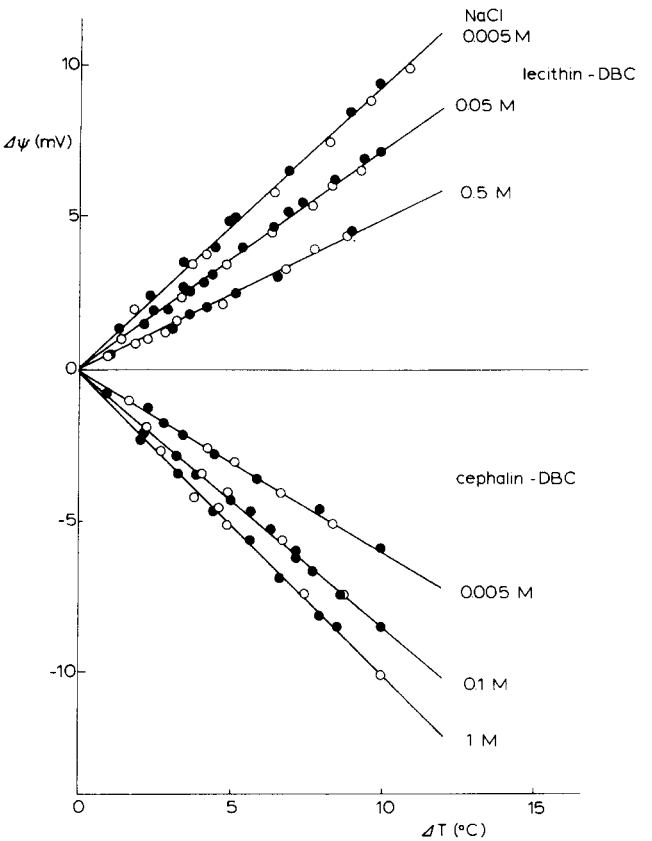

Fig. 6. Electrical thermal membrane potential ($\Delta\psi$ mV) vs. temperature difference (ΔT°C) for phosphatidylcholine (egg yolk)-DBC (○) and phosphatidylcholine (egg yolk)-cholesterol (1 : 1)-DBC (●) (upper part of the plot); phosphatidylethanolamine (sheep brain)-DBC (○) and sheep brain phosphatidylethanolamine-cholesterol (1 : 1)-DBC (●) (lower part of the plot).

potential values seem to be consistent with a zero charge membrane model in which the main ion transport barrier is due to the interior hydrocarbon region [6].

Carrier effects. In Fig. 6 the experimental $\Delta\psi$ (mV) vs. ΔT (°C) values at different NaCl concentrations ($5 \cdot 10^{-3}$—1 M) for phosphatidylcholine-dibenzo-18-crown-6; phosphatidylcholine-cholesterol (1 : 1)-dibenzo-18-crown-6; phosphatidylethanolamine-dibenzo-18-crown-6; phosphatidylethanolamine-cholesterol (1 : 1)-dibenzo-18 crown-6 are reported. The $(\Delta\psi/\Delta T)$ vs. $\log a_{NaCl}$ values for the same films are shown in Fig. 7. All the films behave as membranes highly selective to the cations. The potential values (Fig. 7) are fitted by the equation $(\Delta\psi/\Delta T) = -200 \log a - \bar{S}$ (cationic membranes; Eqn. 3 with $z_k = +1$). To understand these results it has to be remembered that the dibenzo-18-crown-6 can form [5,14] both positively charged complexes, dibenzo-18-crown-6 Na^+, and ion pairs dibenzo-18-crown-6 NaCl, in the aqueous phase according to the reactions

$$Na^+ + D \overset{K_{id}}{\rightleftharpoons} DNa^+ \qquad (4)$$

$$DNa^+ + Cl \rightleftharpoons DNaCl\,. \qquad (5)$$

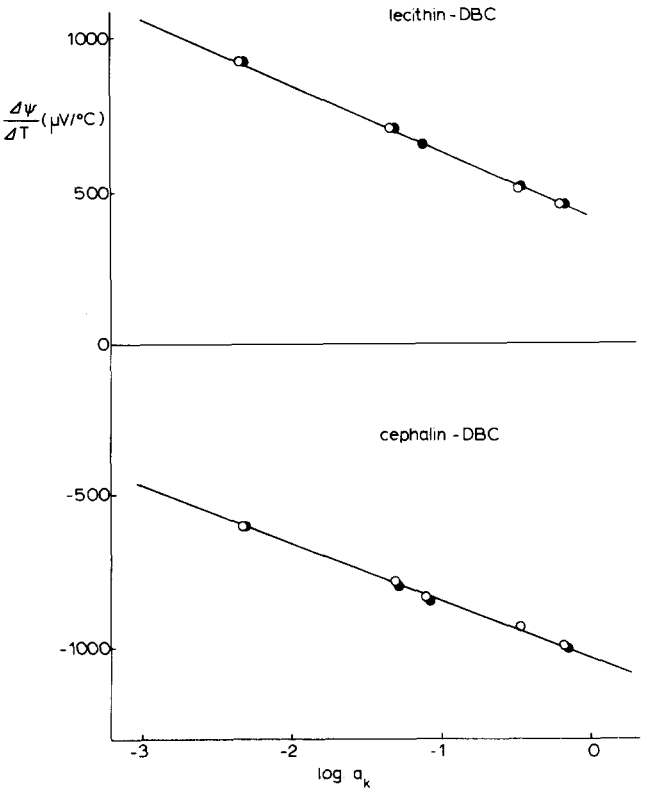

Fig. 7. Thermal coefficient of bilayer membrane potential ($\Delta\psi/\Delta T$ $\mu V/°C$) vs. log a_{NaCl} for: phosphatidylcholine-DBC (○); phosphatidylcholine-cholesterol (1 : 1)-DBC (●) (upper part of the plot); phosphatidylethanolamine-DBC (○) phosphatidylethanolamine-cholesterol (1 : 1)-DBC (●) films (lower part of the plot).

D,dibenzo-18-crown-6. All the species participating in these reactions can permeate the films. For isothermal condition, in absence of dibenzo-18-crown-6 (DBC), a slightly greater transference number for cations than for anions in pure lipids is observed [2,4]. In the presence of 10^{-4} M dibenzo-18-crown-6 considerably larger changes in favour of cations occur. For KCl a slope of 50 mV/decade is obtained in presence of dibenzo-18-crown-6, 10^{-4} M, in the membrane potential plot. In presence of nonactin and Nernstian slope is obtained (58.5 mV/decade). The potential difference across the bilayer is originated by a selective diffusion of the permeant complex DK^+. The potential is given by Eqn. 1 with $t_+ = 1$ and $\tau_o = 0$. Following this point of view the liquid-like hydrocarbon interior is conceived as the essential barrier through which the ions must move in the case of zero charged films. For charged membranes the surface charge will affect the aqueous-membrane distribution of the permeant species but not the diffusion across the membrane and the related potential. In non-isothermal conditions the electrical potential is given by

[7,8]:

$$-\Delta\psi = (2t_+ - 1)(RT/F)\ln(a''_\pm/a'_\pm) + (2t_+ - 1)(R\Delta T/F)\ln a''_\pm + \\ + (t_+\alpha_+ + t_-\alpha_-)\Delta T + G \quad (6)$$

$$G = \tau_o RT \ln(a''_w/a'_w) + \tau_o R\Delta T \ln a''_w$$

with

$$\alpha_+ = \eta - (S_+/F) \text{ and } \alpha_- = \eta - (S_-/F)$$

All quantities but α and $t(\pm)$ refer to the external aqueous phases: phase ($'$) at temperature T and phase ($''$) at temperature $T + \Delta T$; $a_\pm$ and a_w are the mean aqueous electrolyte and water activities; S_+,S_- and t_+,t_- are the cation and the anion aqueous entropies and membrane transport numbers respectively; η is assumed constant (see ref. 7); τ_o is the water reduced transport number. For highly selective membranes ($t_\pm = 1$) and under isochemical condition ($a'' = a'$) Eqn. 4 reduces to Eqn. 3. According to these arguments the preferential diffusion of DNa^+ across the film can be assumed as the thermal potential determining process. Consequently, all the films either charged (phosphatidylcholine and phosphatidylcholine-cholesterol) or uncharged (phosphatidylethanolamine and phosphatidylethanolamine-cholesterol) behave as membrane highly selective to the cation DBC-Na^+ and the function $(\Delta\psi/\Delta T)$ vs. $\log a_{DBCNa^+}$ has a slope of $-200\,\mu V/°C$. In Figs. 6 and 7 we have used a_{NaCl} instead of a_{DBCNa^+}. However, it has to be noted that following reaction 4 it is

$$\log a_{DNa+} = \log a_{NaCl} + \log(K_{id} \times a_{DBC}) \quad (7)$$

Neglecting reaction 5 and for small values of K_{id} the activity of DBC, a_D, can be assumed constant. Therefore, using relation 7, Eqn. 3, with $z_k = +1$, becomes

$$(\Delta\psi/\Delta T) = -200 \log a_{NaCl} - S^\star ; \quad S^\star = -200 \log(K_{id} \times a_D) + S \quad (8)$$

$$d(\Delta\psi/\Delta T)/d \log a_{DNa+} = d(\Delta\psi/\Delta T)/d \log a_{NaCl} = -200\,\mu V/°C \quad (9)$$

In the literature, values of $K_{id} = 1$ have been reported, and with such a value the constancy of a_D is obtained and relation 9 is satisfied. Alternatively we can assume that the dibenzo-18-crown-6 dissolves in the bilayer film. The presence of dibenzo-18-crown-6 in the film enhances the permeation of the membrane to the sodium cation through the formation of the complex dibenzo-18-crown-6 · Na^+ in the bilayer phase. This point of view is supported by kinetics studies on biphasic extraction [14]. From this point of view the thermal potential will depend on the aqueous activity of the NaCl a''_{NaCl}. Whichever approach we follow it is evident that the film behaves as a cationic selective membrane as a consequence of the preferential solubility of the sodium cation, with respect to the anion, in the films.

Comments

From electrokinetic potential measurements on phosphatidylcholine bilayer leaflets [3], and from surface potential and force area measurements of mono-

layers of phosphatidylcholine and phosphatidylethanolamine at the air-water interfaces [15] it has been concluded that the polar groups of phospholipid molecules lie in a plane parallel to the surface of the monolayer and normal to the direction of the close packed hydrocarbon chains. The normal displacement of positive and negative charges in the phospholipid zwitterions from the plane has been calculated as less than 0.1 Å on a time average. More generally it is though that arrangments of phospholipids in which the positive and negative charges are not coplanar are energetically unstable except for the binding of other ions or interdigitation of zwitterions [15]. In the case of mixed phosphatidylethanolamine-cholesterol monolayers at the air-water interfaces surface potential measurements show that the surface potential values are very close to the proportionate mean of the surface potentials of the monolayers of the pure components [15]. It has to be inferred that the phospholipid zwitterions and the cholesterol dipoles are not mutually distorted in the mixed monolayer. On this basis the presence in the mixed monolayer of either Finean's "walking stick" configurations [16] for phosphatidylcholine-cholesterol or the Vandenheuvel's [17] arrangement for phospholipid-cholesterol layers has been criticized. In fact, both these models imply a large surface dipole moment as a consequence of the fact that the cationic and anionic centers of the phospholipid zwitterions are not tangentially coplanar. The thermal potential measurements with phosphatidylethanolamine [6] and phosphatidylethanolamine-cholesterol films agree with these views. In fact, the thermal potential values can be coherently interpreted with an electrolyte diffusion process across a film with zero charge. A different situation arises with phosphatidylcholine [6] and phosphatidylcholine-cholesterol films. As already stressed, the $\pm 200\,\mu V/^{\circ}C$ slope for the derivative $d(\Delta\psi/\Delta T)/d\log a''_k$ is a result obtainable only with a membrane selective to one ion species (−200 for cation and +200 for anion transport). Since in the system there is not any other process which can give rise to these values of the slope (ion thermal diffusion in the aqueous layers can be neglected as a consequence of the use of NaCl as electrolyte) the conclusion is drawn that the measured thermal potential is proof that the phosphatidylcholine and phosphatidylcholine-cholesterol films act like a permselective ion membrane. Further, the phosphatidylcholine-cholesterol interaction is such that for cholesterol above 20% the film becomes an anionic membrane. From the above arguments it appears that the alternative model of phosphatidylcholine bilayer with the two charged groups in different planes is highly improbable. However, from NMR studies [18] on the conformation of molecules of phosphatidylcholine and lysophosphatidylcholine in aqueous micelles in the presence or absence of lanthanide ions the following suggestions have been made; (i) in the absence of lanthanide cations the molecules are staggered with neighboring phosphorus and nitrogen atoms in the same plane thus allowing maximum electrostatic interaction between oppositely charged groups; (ii) in the presence of lanthanide cations the arrangement of the molecules is typical of the lipid bilayers (extended and not staggered positions) with the two charged groups in different planes; (iii) the metal ion phospholipid interaction of the type M + PL → PL — M (M metal, PL phospholipid) is obtained through metal phosphodiester group interaction (the minimum requirement for the interaction is that the metal is in contact with at least one oxygen

atom of the phosphodiester group). The surface electrostatic implications of the two possible models (charged groups coplanar or in different planes) are depicted in Fig. 8. When the two charged groups are located in different planes (Fig. 8a) and there is sufficient space between the two polar groups to allow water and electrolyte ions to penetrate at least as far as the glycerol residue, we can have a diffuse space charge distributed over the two planes of positive and negative fixed charges. In the d space we have a positive space charge while a negative space charge will be present outside d (a positive potential is expected in this condition, Fig. 8b). When the two polar groups are in the same plane no diffuse space charged is expected and the potential should be zero (Fig. 8c).

Let us now consider the thermal potential values of this paper. These potential values are determined by the permeating ion diffusion process and are linked to the electrostatic surface conditions through the permeating ion distribution at the membrane-solution interfaces. In fact, surface potential affects the ion distribution at the interface. The thermal potential with phosphatidylethanolamine films [6] indicates that both sodium and chloride are permeating ions and that their transport numbers through the hydrocarbon-like interior determine the thermal potential value. In the case of phosphatidylcholine films [6] the thermal potential indicates that the sodium cation with NaCl as aqueous electrolyte is selectively permeating the film. The bilayer in this case acts as a cationic membrane. The reasons for this behaviour are outside the information obtainable with thermal potential experiments. We can only deduce that if an interaction exists between the cation and the phosphate diester group, as for lanthanides [18], this interaction could help the cations to overpass the negative potential barrier at the membrane-solution interface for conditions as depicted in Fig. 8a, or alternatively this interaction could favour a selective solubility of the sodium cation in the film side of the interface for the condition of Fig. 8c. Once the cation is preferentially solubilized in the film it can move through as the potential determining ion. Of course such a picture leaves unsolved the question why the same interaction is not working in the phosphatidylethanolamine case. In the presence of cholesterol the mixed phosphatidylcholine-cholesterol films behave as an anionic mem-

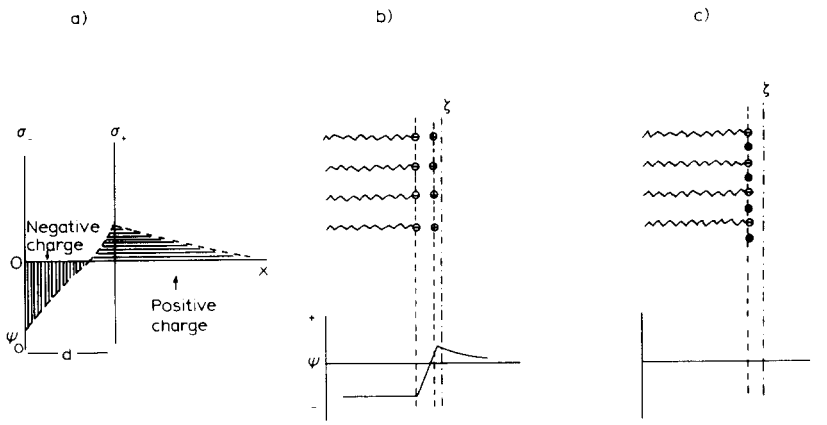

Fig. 8. Model proposed by T. Hanai et al. [3] for the charge distribution at the water-bilayer interfaces.

brane indicating that the chloride ion is selectively solubilized in the films. We have also no sound suggestions to explain this behaviour. In fact, both phosphatidylethanolamine and phosphatidylethanolamine-cholesterol films behave as membranes without specific electrochemical selectivity with respect to either cations or anions. In presence of dibenzo-18-crown-6 all films (phosphatidylcholine, phosphatidylethanolamine and mixed phospholipid-cholesterol) behave as cationic membranes. These results agree with the suggestion [19,20] that the macrocyclic antibiotic type molecules solubilize cations in the form of positive charged complexes with the solution-membrane partition coefficient of the complex much higher than those of the anions, as well as of those of the uncomplexed cations. This selective solubility has to be so high as to induce the phosphatidylethanolamine film to behave as a cationic to anionic membrane behaviour.

Before concluding let us note that for all films the lipid packing, the film thickness and the film composition could depend on the temperature. Therefore, some asymmetry could be introduced into the films by the temperature gradients. However, any lipid packing dependence on the temperature will be mainly reflected into the interfacial characteristics more than into the thermal potential. Any eventual change of the film thickness and composition under a temperature gradient will be associated to motion of chemical species with consequent possible influence on the electrical potential if these mobile species are interacting with the ion inducing potential. However, both theoretical consideration and experimental evidence [6] show that these effects are not present in monoionic thermal potential measurements.

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