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The dependence of the conductance of phosphatidylcholine bilayers upon the concentration and composition of the external electrolyte

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The area-specific conductance of egg phosphatidylcholine and egg phosphatidylcholine/cholesterol bimolecular lipid membranes formed with *n*-hexadecane solvent has been studied as a function of both the nature and concentration of the external electrolyte. For each individual membrane, the area-dependence of its conductance was studied, and in most cases the results indicated that the measured conductance could be primarily attributed to the bilayer region of the film rather than to its torus and surrounds. It was found that a 3-fold increase in the ionic radius of the cationic species present in the external electrolyte did not significantly alter the measured bilayer conductance. The presence of an electrolyte containing only divalent ions also did not significantly affect the conductance. However, an increase in pH from 4–10 was found to increase the bilayer conductance 3-fold. The bilayer conductance was also found to increase only slightly with increasing external electrolyte concentration. These results appear to dismiss the possibility that charge is translocated across the bilayer via the passage of ions through its hydrophobic interior. They are consistent, however, with (amongst other possibilities) the translocation of charge via the passage of ions through aqueous pores in the bilayer phase.

Introduction

Although the electrical properties of planar bimolecular lipid membranes have often been studied, emphasis has generally been placed upon either measuring their thickness via capacitance measurements, or measuring their conductance in the presence of additives which greatly increase the conductance. However, little appears to be known at present about the nature of the low intrinsic conductance of unmodified lipid membranes **. The measured area-specific conductances of unmodified bimolecular lipid membranes have been often described as 'irreproducible' and a wide range of values reported. This is shown by the reported values listed below for the area-specific conductances of phosphatidylcholine or phosphatidylcholine/cholesterol membranes formed with different *n*-alkane solvents in ionic solutions ranging from 1 mM to 1 M (in mS/m^2) 0.01–10 [1], 2.5–40 [2], 0.125–0.19 [3], 0.005–0.025 [4], 0.1–10 [5], 0.05 [6], 1–10 [7], 3–30 [8], 0.3–10 [9], 3–15 [10], 0.75–17 [11], 0.1–3 [12], 0.07–2.7 [13]. Comparable values have also been measured for other 'black' lipid membranes (the thickness of which were not measured, but were also presuma-

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** In this paper 'membrane' will refer to the entire area of the lipid film across the hole in the septum, including the torus, through which the applied current flows. 'Bilayer' will refer to regions of the membrane which are two lipid molecules thick. It is not assumed, however, that these latter regions necessarily contain only contiguous areas of bilayer leaflet.

bly bimolecular); e.g., 1.0 [14], 2.5–50 [15], 1.0 [16], 0.06 [17], 0.7 ± 0.5 [18], 0.12 ± 0.04 [19]. There appears to be no obvious correlation in the above data between the conductance and *n*-alkane solvent used, and a strong correlation was usually not apparent between the nature and concentration of electrolyte (an exception appears to be phosphatidylcholine/*n*-tetradecane membranes where a strong dependence of the conductance upon external concentration was reported [15,20]).

One difficulty with such measurements, however, is determining what fraction of the measured conductance is associated with the bilayer itself and what fraction is associated with 'leaks' in the surrounding torus. It has been proposed [4] that only the very lowest measured values of G (i.e., approx. 0.01 mS/m^2) reflect those intrinsic to the bilayer itself, and that any higher values are a consequence of 'leaks' associated with the torus and its interface with the septum. This conclusion was reached from the observed absence of any area-dependence of the conductance of phosphatidylcholine/*n*-decane membranes (except for membranes with a very low conductance) in experiments in which the membrane area was altered by varying the hydrostatic pressure difference across the membrane. However, Miyamoto and Thompson [15] found that the conductance of phosphatidylcholine/*n*-tetradecane membranes was appreciably higher and varied approximately linearly with the membrane area when membranes were formed on septa with holes of different areas. They therefore concluded that the measured conductance of phosphatidylcholine/*n*-tetradecane membranes was primarily attributable to the bilayer phase. Recent measurements [13] in this laboratory on the temperature-dependence of phosphatidylcholine/*n*-hexadecane membranes in 1 mM KCl also support this latter conclusion. There is thus disagreement not only about the magnitude of the measured conductance, but also about what fraction is attributable to the bilayer itself.

In this paper, we present the results of investigations into the area-dependence of the measured conductance of bimolecular lipid membranes formed with *n*-hexadecane solvent. Although such membranes are known to contain only small concentrations of alkane solvent in comparison with membranes formed with *n*-decane [12,21], there appear to be no published studies of the conductance of membranes with such very low solvent concentrations. From our measurements, it was possible to evaluate the fraction of the measured conductance which is attributable to the bilayer and also the fraction attributable to the torus and its surrounds. To investigate possible mechanisms whereby charge translocation might occur across the bilayer, a study was also made of the effects of both the concentration and nature of the external electrolyte upon the bilayer conductance.

Materials and methods

Membranes were formed from solutions of egg phosphatidylcholine or egg phosphatidylcholine/cholesterol (2 : 1 molar ratio) in *n*-hexadecane solvent (some measurements with *n*-tetradecane or *n*-dodecane solvent are also reported). Each membrane thinned from a film placed (with a syringe) over a hole (approx. 1.5 mm diameter) in a polycarbonate septum which divided a chamber containing the desired electrolyte. The film was formed with the chambers and electrolyte at 40°C. The pH of the 1 mM KCl electrolyte was modified as desired by the addition of KOH or HCl. Whether or not each membrane was planar could be determined by observation with a stereo microscope, as well as by determining the minimum value of the measured capacitance when the hydrostatic pressure difference across the membrane was varied.

The membrane capacitance C and conductance G were simultaneously measured to a precision of better than 0.1% using a digital four-terminal impedance measuring technique described previously [11,12,20,22]. Measurements were made at an a.c. frequency of either 1 or 0.1 Hz, both of which were sufficiently low to ensure that the measured conductance substantially reflected the low frequency limit (e.g., see Fig. 5 [11], Fig. 1 [12]). Measurements at 1 Hz were usually preferred (although the measured G was higher) because more rapid (every 4 s) measurements were possible than at 0.1 Hz (every 30 s).

In an endeavour to determine the conductance of the bilayer itself (denoted by G_B), the measured conductance was examined as a function of the bilayer area by two methods. The first method involved measuring C and G of the membrane while it remained planar and the area occupied by the bilayer phase increased from approx. 50 to 100% of its final planar area (100% corresponding to when the bilayer occupied virtually the entire area of the hole in the septum). The second method involved introducing small amounts of electrolyte to one side of the experimental chamber via a syringe. The resultant difference in hydrostatic pressure across the membrane caused it to 'bow' and thus increase its area. This second method was originally used by Hanai et al. [4], who demonstrated that the measured membrane capacitance was linearly proportional to the membrane area. In both methods, it was assumed that the measured capacitance was identical to that of the bilayer phase.

Providing that the intrinsic area-specific properties of the bilayer remain invariant, extrapolation of the relationship between the measured G and C back to the limit of $C=0$ (i.e., zero bilayer area) should reveal the value of any conductance element which is independent of the bilayer area (henceforth referred to as the 'leak' conductance). Such elements are presumably not localised within the bilayer and are associated with the torus and its interface with the septum. Subtraction of this 'leak' conductance from the value of G measured when the membrane is planar (and approx. 100% bimolecular) should then yield G_B, the conductance of the bilayer itself.

Data presented is summarised in the form mean ± standard error (number of membranes).

Results

The area-specific conductance of membranes formed in solutions of low electrolyte concentration (1 mM) was found to vary only very slowly with time once the concentration of alkane solvent 'dissolved' in the bilayer approached equilibrium (as revealed by no detectable change in the measured electrical time-constant $= C/G$). Usually only small variations (both increases and decreases) in the magnitude of the conductance were seen over long periods. As an example of how stable the membrane conductance could be over a long period, the low frequency conductance of a phosphatidylcholine/cholesterol membrane formed with *n*-tetradecane in 1 mM KCl was found to decrease by an average value of less than 3% per h over the period from 4 to 30 h after formation, and then to decrease by approx. 1% per h until its ultimate demise 49 h after formation. For a given set of experimental conditions, the membrane conductance in 1 mM electrolyte was usually quite reproducible, as indicated by the standard errors in Tables I–III. Occasionally, however, a membrane was formed with a conductance distinctly different from the average. Usually it was found that lipid solutions made on different days gave similar results, but occasionally a particular solution was found to produce membranes with conductances significantly higher than those formed on previous days.

The variation of conductance with area

Fig. 1 shows some results for the relationship between G and C measured at a frequency of 1 Hz for different bilayer areas of phosphatidylcholine membranes formed with *n*-hexadecane in 1 mM

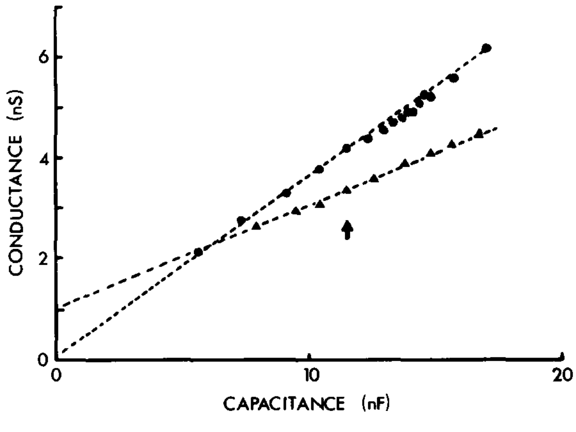

Fig. 1. The relationship between the capacitance and conductance of phosphatidylcholine membranes formed with *n*-hexadecane solvent and measured at a frequency of 1 Hz for different areas of the bilayer. The data points shown are from two membranes, one formed in 1 mM KCl (●) and the other in 1 mM NH_4Cl (▲). The vertical arrow indicates the measured value of the capacitance (11.4 nF) at which the membranes were planar with a bilayer area equal to that of the hole in the septum (area = 1.7 mm^2). At this stage, the area-specific capacitance was 6.7 mF/m^2 and the area-specific conductances were in the range 2–2.5 mS/m^2. The results to the left of the arrow are thus for different areas of planar bilayer, while those to the right are for different areas of a 'bowed' bilayer.

electrolyte. It is apparent in each of these examples that the relationship between C and G is reasonably linear, and that the slope is similar for increases in the area of both the planar and bowed bilayer. This suggests that areas of the membrane which are not yet part of the bilayer phase make a negligible contribution towards the measured capacitance. Thus, for the results shown in Fig. 1, extrapolation of the measured relationship back to the limit of zero bilayer area (i.e., C=0) should yield an unequivocal result for the conductance of any element with a value that was independent of the bilayer area. In the two examples in Fig. 1, the percentage contributions of the conductance of this 'leak' (defined as the conductance of elements other than the bilayer), relative to the conductance of the membrane when planar and 100% black, were 1.5 and 30% for 1 mM KCl and NH_4Cl, respectively. For membranes formed in 1mM electrolyte it was found that the contribution of this 'leak' was usually less than 5%. Indeed, for 105 membranes formed with *n*-hexadecane in 1 mM electrolyte, it was found that the 'leak' conductance exceeded 5% for only 11 membranes and in these cases the average value of the leak was 21 ± 5%. Similar results were generally found when the area of a bowed membrane was increased or decreased.

On several occasions, it was found that the relationship between C and G for different bilayer areas was different for planar and bowed membranes (see Fig. 2), i.e., the conductance of bowed bilayers sometimes increased more rapidly than would be expected from the measured increase in capacitance. For membranes with behaviour similar to that shown in Fig. 2, when extrapolation back to zero area would suggest a negative value for the leak conductance, the leak conductance was assumed to be zero. For these membranes, however, the area-specific conductance was still found to be steady when the membrane was maintained planar.

When membranes were formed in solutions with a higher ionic concentration (e.g., 100 mM), the experiments were complicated by the occasional occurrence of abrupt jumps in the measured conductance (see also Ref. 1, 4). These sudden transitions in conductance could either increase or decrease the measured conductance. In between any such jumps, the relationship between C and G usually remained effectively linear, and the presence or absence of an appreciable 'leak' was still obvious upon extrapolation back to zero area (e.g., see Fig. 3). Thus, it was found that most small jumps in the measured conductance were intrinsic to the bilayer, whereas larger conductance jumps (e.g., greater than 100%) were associated with jumps in border leakage. Fig. 4 shows some results for membranes in 100 mM electrolyte which did not exhibit any major conductance jumps, but which did exhibit the previously mentioned differences in the slope of the area-dependent relationship between C and G for planar and bowed membranes. These results suggest that it is possible for the bilayer region of an unmodified lipid membrane to be quite conductive (e.g., greater than 10 mS/m^2).

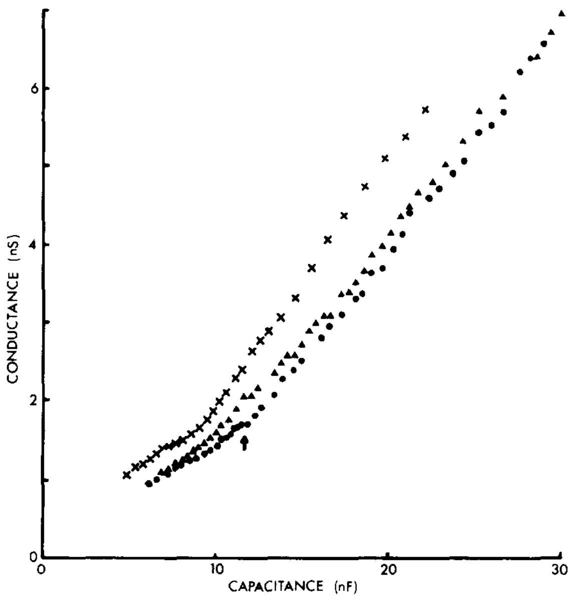

Fig. 2. The relationship between the capacitance and conductance of three different phosphatidylcholine membranes formed with *n*-hexadecane in 1 mM tetraethylammonium chloride electrolyte and measured for different areas at a frequency of 1 Hz. Each different symbol refers to a different membrane. When each membrane was planar with a bilayer area equal to that of the hole in the septum (1.76 mm^2) as indicated by the vertical arrow, the measured capacitance (11.6 nF) corresponded to approx. 6.6 mF/m^2. At this point, the area-specific conductance was in the range 1–1.4 mS/m^2. Otherwise, the legend to Fig. 1 applies.

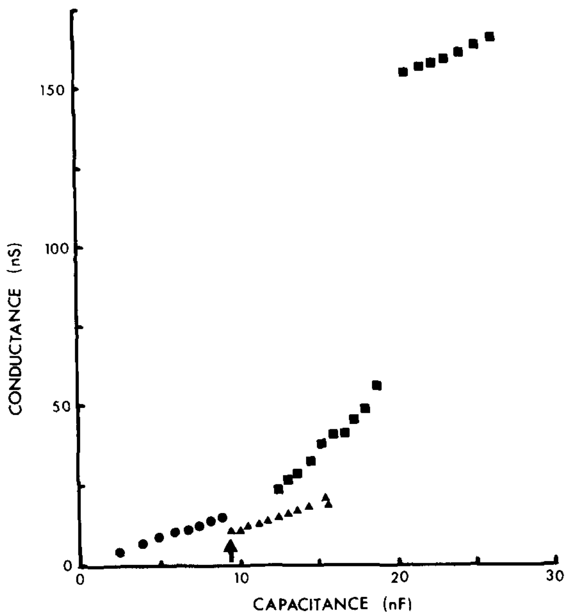

Fig. 3. The relationship between the capacitance and conductance measured at different areas and at a frequency of 1 Hz of a single phosphatidylcholine membrane formed with *n*-hexadecane in 100 mM KCl. The vertical arrow indicates when the bilayer regon was planar with an area equal to that of the hole in the septum (1.43 mm^2). At this point, the area-specific capacitance and conductance were approx. 6.7 mF/m^2 and 10 mS/m^2, respectively. Each different symbol refers to a series of measurements performed at different times in the order (●), (▲), (■). A jump in the measured conductance is apparent in the last series of measurements denoted by (■). Extrapolation of the relationship between C and G back to zero area (i.e., at $C=0$) indicated that any 'leak' conductance was small for series (●) and (▲), but for series (■) a very large 'leak' conductance became evident.

The effects of different external electrolytes

To investigate some possible methods of charge translocation across bimolecular lipid membranes, we measured the conductance of membranes formed in 1 mM electrolyte solutions containing ions of differing radius and valence. In the results presented in Table I (also II and III), the magnitude of any 'leak' conductance was estimated for each membrane by extrapolation of the measured relationship between C and G at different areas back to zero area. This value was then subtracted from the value of G determined when the membrane was planar to yield G_B. It is apparent that G_B does not vary strongly with the radius or valence of the ions in the external electrolyte.

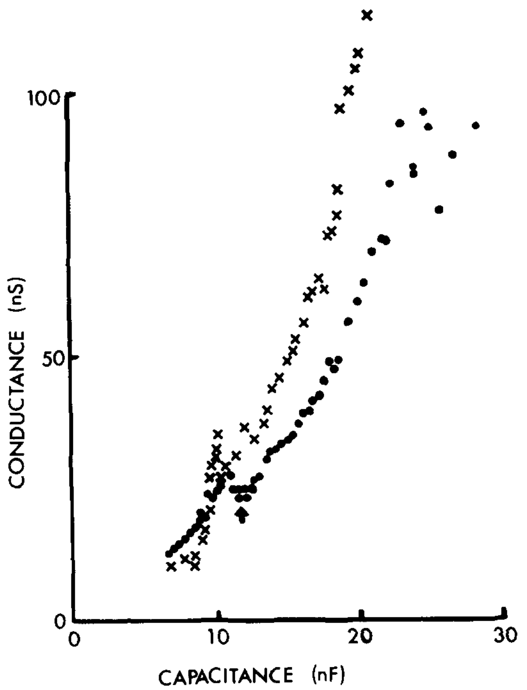

Fig. 4. The relationship between the capacitance and conductance measured at different areas and at a frequency of 1 Hz of two phosphatidylcholine membranes formed with *n*-hexadecane in 100 mM tetraethylammonium chloride. The symbols (●) and (×) refer to different membranes. As in Figs. 1 and 2, the vertical arrow indicates when the bilayer regions were planar with areas equal to that of the hole in the septum (1.76 mm^2). At this point, the capacitance was approx. 7 mF/m^2 and the conductance was in the range 14–19 mS/m^2.

The effect of increasing external concentration

Table II summarises how G_B was found to vary with different concentrations of the external electrolyte *. For those membranes in which conduc-

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* The concentration dependence of the conductance reported herein is smaller than reported previously by us [11,12,20]. There are five possible reasons for this difference: (i) in some earlier measurements, the conductance of membranes was not examined as a function of area and so a contribution from border 'leaks' could have been present, particularly at higher electrolyte concentrations; (ii) the investigations of the very small frequency dependence of the membrane impedance required membranes of exceptional stability and so the conductances reported previously were from a different membrane population; (iii) the very low frequency conductances reported previously are lower than those reported herein, all of which were measured at 1 Hz; (iv) previous measurements were at 20°C rather than 40°C; (v) previous membranes were formed with *n*-tetradecane solvent rather than *n*-hexadecane.

TABLE I

THE EFFECT OF DIFFERENT ELECTROLYTES UPON BILAYER CONDUCTANCE

Egg-phosphatidylcholine membranes were formed with *n*-hexadecane in 1 mM electrolyte and their area-specific conductance at 40°C was measured at 1 Hz. For each membrane, the area-dependence of the conductance was examined and any 'leak' conductance so determined was subtracted to yield the bilayer conductance G_B. The numbers presented are the means ± S.E. for (n) different membranes. Theoretical values for the partition coefficients (γ_P and γ_N) for ions partitioning into a hydrophobic region with $\epsilon_H = 2.1$ were calculated from their crystal radii via Eqns. 4 and 5. The theoretical conductance G_H for 'naked' ions passing through the hydrophobic region was then calculated via Eqn. 1. TMA, tetramethylammonium; TEA, tetraethylammonium.

Salt:	KCl	NH_4Cl	TMA-Cl	TEA-Cl	$MgSO_4$	KI
G_B (mS/m^2) measured	1.7 ± 0.4(18)	5.0 ± 1.3(5)	2.7 ± 0.2(5)	1.5 ± 0.1(5)	2.2 ± 0.4(6)	6 ± 1.5(5)
γ_P-cation	$2 \cdot 10^{-41}$	$1.4 \cdot 10^{-38}$	$9 \cdot 10^{-19}$	$9.3 \cdot 10^{-13}$	$3.4 \cdot 10^{-252}$	$2 \cdot 10^{-41}$
γ_N-anion	$1.2 \cdot 10^{-30}$	$1.2 \cdot 10^{-30}$	$1.2 \cdot 10^{-30}$	$1.2 \cdot 10^{-30}$	$2.4 \cdot 10^{-69}$	$6.6 \cdot 10^{-26}$
G_H (mS/m^2) theory	$3 \cdot 10^{-21}$	$3 \cdot 10^{-21}$	$2 \cdot 10^{-9}$	$2 \cdot 10^{-3}$	$6 \cdot 10^{-60}$	$2 \cdot 10^{-16}$

TABLE II

THE EFFECT OF IONIC CONCENTRATION UPON BILAYER CONDUCTANCE

Membranes were formed with *n*-hexadecane in different concentrations of KCl and the bilayer conductance G_B (tabulated in units of mS/m^2) was measured at a frequency of 1 Hz and at 40°C.

[KCl] (mM):	0.1	1	10	100	300
G_B phosphatidylcholine	1.4 ± 0.2(7)	1.7 ± 0.4(18)	3.5 ± 1(7)	2.5 ± 0.3(17)	5.5 ± 1(3)
G_B phosphatidylcholine/ cholesterol	–	1.8 ± 0.5(26)	2.7 ± 0.2(5)	4.2 ± 1.2(12)	–

TABLE III

THE EFFECT OF pH UPON BILAYER CONDUCTANCE

Egg-phosphatidylcholine/cholesterol membranes were formed from *n*-hexadecane in 1 mM KCl and the bilayer conductance G_B (tabulated in mS/m^2) measured at a frequency of 1 Hz and at 40°C. Data is from an average of five membranes at each pH.

pH:	4.3	5.2	6.0	7.0	8.0	9.0	9.8
G_B	1.1 ± 0.1	1.7 ± 0.1	1.5 ± 0.4	1.6 ± 0.5	2.2 ± 0.4	3.0 ± 0.1	3.0 ± 0.3

tance jumps occurred, the values presented are for the lowest stable conductance measured for each bilayer. It is apparent that G_B increases slightly with increasing external electrolyte concentration. The conductances of phosphatidylcholine and phosphatidylcholine/cholesterol bilayers are also seen to be similar.

The effect of variation of external pH

Table III summarises how the bilayer conductance of phosphatidylcholine/cholesterol membranes formed with *n*-hexadecane was found to vary in solutions of 1 mM KCl at different pH. It is apparent that G_B varied only slightly while [H$^+$] and [OH$^-$] varied by approx. 10^6.

Discussion

Does the n-alkane solvent influence the bilayer conductance?

It was generally found that individual membranes formed with *n*-hexadecane solvent in 1 mM electrolyte had reproducible values for their area-specific conductance. For each membrane, G_B was found to remain reasonably constant once the *n*-alkane solvent within the bilayer had approached a low equilibrium concentration (which occurred relatively quickly [12,21]). In this regard, it is interesting to note that reports of irreproducibility or low membrane conductance (e.g., Refs. 1, 3, 4, 17), are often associated with membranes formed with *n*-decane solvent which, because of large amounts of *n*-decane retained within the bilayer, are known to possess a hydrophobic interior of thickness almost twice that of bilayers formed with *n*-hexadecane [12,21]. Capacitance measurements suggest that an equilibrium concentration of *n*-decane is usually not reached for several hours, if at all [23,24]. It is therefore plausible that differences between our measured conductances for bilayers formed with *n*-hexadecane and reported values for bilayers formed with *n*-decane, might to some extent reflect the large differences in composition and structure between these bilayers. The reported conductances of phosphatidylcholine membranes formed with *n*-tetradecane are often in excess of 0.1 mS/m^2 (e.g., Refs. 2, 8, 11, 18, 19) and are thus closer to those of bilayers formed with *n*-hexadecane. The influence of solvent is also illustrated by measurements performed in this laboratory (Karolis, C., unpublished data), which show that although the measured conductance of phosphatidylcholine membranes (i.e., bilayer + torus) formed with *n*-dodecane (which, unlike *n*-decane, does appear to attain an equilibrium concentration), is similar to those formed with *n*-hexadecane, the intrinsic bilayer conductance, G_B, is typically only 40% of the measured conductance.

The relationship between conductance and bilayer area

In most cases, the measured membrane conductance, G, was found to increase approximately linearly with increasing capacitance, C, even for reasonably conductive membranes (e.g., greater than 10 mS/m^2, see Fig. 4). To determine the location of the measured conductance within the experimental system of the membrane and septum, we now consider several possibilities. It is assumed that only the bilayer region of the membrane contributes to C, and that membranes were only bowed after each membrane had become almost entirely bimolecular. As a first approximation, it is also assumed that the thickness of the bilayer region does not vary significantly with time. The possible locations of G and the expected relationships between C and G are as follows. (i) If G was associated only with the bilayer region of the membrane, then G should increase linearly with increasing C for both planar and bowed membranes. (ii) If G was distributed over the entire membrane (i.e., bilayer + thick-film regions), then G should be independent of C for planar membranes and increase linearly with C for bowed membranes. (iii) If G was associated with the border between the membrane and the septum, then G should be independent of C for both planar and bowed membranes. (iv) If G was associated with the torus and regions of the membrane which were not bimolecular, then G would decrease with increasing C for planar membranes and remain relatively independent of C for bowed membranes. (v) If G was associated with the interface between the bilayer and membrane (i.e., the transition region where the membrane becomes bimolecular), then G would increase as $C^{1/2}$ for planar membranes and be independent of C for bowed membranes. (vi) Another possibility is that G might be associated with lipid aggregates present in the initial thick film. As the area of the bilayer increases for both planar and bowed membranes, these aggregates might be swept into the bilayer/membrane interface and G would then increase linearly with C.

On most occasions we found that G varied linearly with C for both planar and bowed membranes. This provides strong evidence that, in these experiments at least, the measured conductance is predominantly determined by the area of the bilayer region. This is consistent with both possibilities (i) and (vi) listed previously. This conclusion is similar to that reached by us from studies of the temperature dependence of G_B in these membranes [13], in which it was found that the measured activation energy appeared to be independent of the magnitude of G and was significantly larger than would be expected if current flowed through large aqueous channels at the interface between the membrane and septum. Leakages external to the bilayer appeared to be readily detectable, when they occurred, by the relative independence of G upon C (e.g., see Fig. 3). The overall nature of these results for *n*-hexadecane membranes is, however, quite different from that found by Hanai et al. [4] for membranes formed with *n*-decane, where a dependence of G upon C for bowed membranes was only observed at very low conductances (e.g., 0.01 mS/m^2).

The occasional situation whereby extrapolation of the measured relationship between C and G for bowed bilayers back to zero capacitance yielded a negative value for G (and thus a negative 'leak' conductance), was only evident in relatively 'young' bilayers (less than 1 h since formation). The rate of increase in bilayer area in these experiments was typically of the order of 0.1% per s. One possible explanation is that the additional areas of bilayer phase formed during bowing have not yet had sufficient time to become quite as thin as the older regions of bilayer. The measured change in capacitance would then slightly underestimate the change in bilayer area. If the conductance of the bilayer region is not significantly different in these slightly thicker regions, this could explain how a negative value of G can occur upon extrapolation back to zero capacitance *.

The effect of different electrolytes

One method of investigating the mechanism(s) responsible for charge translocation through the bilayer is to examine how G_B depends upon the radius and charge of the ionic species present in the external electrolyte. The data presented in Table I indicates that a 3-fold increase in cationic radius (from potassium to tetraethylammonium) has only a slight effect upon G_B. Similarly replacing the electrolyte with one that contained only divalent ions ($MgSO_4$) had little effect. An increase in anionic radius (from Cl^- to SO_4^{2-}) also had little effect. The slightly higher conductance observed for KI electrolyte could be a consequence of the presence of trace amounts of I_2 [5]. If the conductances presented in Table I were a consequence of ions passing through the hydrophobic interior of the bilayer, a first approximation for its area-specific conductance G_H is given by

$$G_H = q^2(D_P C_P + D_N C_N)/kTL \qquad (1)$$

Here q is the magnitude of the electronic charge, k is the Boltzmann constant, T is the absolute temperature and L is the thickness of the hydrophobic region. C_P and C_N are the concentrations of cations and anions, respectively, in the hydrophobic phase, where they are assumed to be independent of position. D_P and D_N are their appropriate diffusion constants. If γ_P and γ_N are their partition coefficients into the hydrophobic phase, then

$$C_P = \gamma_P C_P^o \qquad (2)$$

$$C_N = \gamma_N C_N^o \qquad (3)$$

where C_P^o and C_N^o are the concentrations of cations and anions, respectively, in the external electrolyte. If E_A is the activation energy for the translocation of an ion into the hydrophobic interior and R is the universal gas constant, then

$$\gamma = \exp(-E_A/RT) \qquad (4)$$

For a dehydrated ion of radius r partitioning from an aqueous phase into a region of dielectric constant ϵ_H, E_A is approximately equal to the Born energy (neglecting the finite bilayer thickness)

$$E_A = z^2q^2/8\pi\epsilon_o\epsilon_H r \qquad (5)$$

where ϵ_o is the permittivity of free space, and z is the ionic valence.

In Table I, theoretical values for γ_P, γ_N and G_H are given which have been calculated on the basis of the above considerations. As Parsegian [25] has suggested, the discrepancy between the theoretical value for G_H and the measured magnitude of G_B is so massive as to immediately discount the possibility of translocation of charge through the hydrophobic interior of the bilayer via 'naked' ions. This is further supported by the absence in our (and other) measurements of the predicted strong variations in G_B with different ionic radii and valences.

To explain the absence of a significant dependence of the bilayer conductance upon different

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* This explanation is supported by recent measurements (Karolis, C., unpublished data) on significantly 'older' (more than 4 h since formation) phosphatidylcholine/cholesterol membranes formed with n-hexadecane. When the bilayer area of these membranes was increased very slowly by bowing (by typically approx. 0.01% per s), it was found that extrapolation of the measured relationship between G and C back to zero capacitance never yielded negative values for G. It thus appears that this rate of area increase was sufficiently slow for the thickness of the newly formed bilayer regions to approach its limiting value.

ionic radii, Macdonald [26] suggested that when inside the bilayer different ionic species will be surrounded by spherical water 'bubbles' of a similar size. If these bubbles were sufficiently large (diameter greater than 1 nm), this might explain the lack of an observed variation of G_B with different ionic radii. However, the presence of such bubbles cannot explain why G_B is not very much smaller when only divalent ions rather than monovalent ions are present, as would be predicted from Eqn. 5 (e.g., compare the values of G_B for $MgSO_4$ and KCl in Table I). The minimum value for E_A will occur for monovalent ions when the bubble radius is approx. 0.35 nm and then E_A is approx. 140 kJ/mol [13]. The predicted value for G_H would then be approx. $2 \cdot 10^{-14}$ mS/m^2. Thus, mechanisms involving the passage of hydrated ions (or ions in water 'bubbles') through the hydrophobic interior will also not predict (by many orders of magnitude) a sufficiently high bilayer conductance.

It thus appears from the above considerations that the experimental data is inconsistent with the notion that charge is translocated by ions that actually travel through the hydrophobic interior of the bilayer. A possible explanation is that ions travel through aqueous (hydrophilic) pores across the bilayer that are an intrinsic, if perhaps fluctuating, part of the bilayer structure. It is possible to show [13] that the conductance, G_P, of each individual pore, would be approximately given by

$$G_P = \left[q^2 (D_P + D_N) C^o \pi b^2 / kTL \right] \times \exp\left(-z^2 q^2 \alpha / 4\pi b \epsilon_o \epsilon_H RT \right) \tag{6}$$

where b is the pore radius, C^o is the external electrolyte concentration and α is a constant which depends upon the pore geometry and dielectric constants (see Ref. 25). From our measurements of the temperature dependence of G_B it was found that a minimum value of $b \approx 1$ nm would be consistent with the observed activation energy of approx. 35 kJ/mol for 1 mM KCl [13]. Only a relatively small number of pores (approx. $6 \cdot 10^{10}$ pores/m^2) would then be needed to account for the measured value of G_B. It can be seen that the conductance of the individual pores should not then depend upon the ionic radius. Eqn. 6 does predict, however, that G_P should still depend upon the ionic valence.

The dependence of conductance upon pH

One possibility not considered so far is that charge is not translocated across the bilayer by the major ions present in the external electrolyte, but is translocated by protons (or hydroxyl ions). The results presented (Table III) indicate that G_B increased only 3-fold while $[H^+]$ decreased from approx. 10^{-4} to 10^{-10} M and $[OH^-]$ increased from approx. 10^{-10} to 10^{-4} M. This small variation is not inconsistent with one prior report for *n*-decane membranes [1], but is significantly less than the magnitude of some other reports [9,19,27]. From our measurements, it does appear unlikely that either H^+ or OH^- translocate charge across the bilayer, unless the mechanism responsible begins to saturate at quite low concentrations (less than 10^{-12} M). One possible mechanism involves protons being translocated through channels of ordered water via an exchange mechanism similar to that occurring in water or ice, e.g., see review in Ref. 28.

The dependence of conductance upon ionic concentration

For [KCl] less than 100 mM, G_B for both phosphatidylcholine and phosphatidylcholine/cholesterol bilayers was found to vary approx. as $[KCl]^{0.2}$. This result appears to be in reasonable agreement with the small concentration dependences reported elsewhere (e.g., Refs. 1, 4) for phosphatidylcholine/*n*-decane membranes, but is different from the linear dependence reported for phosphatidylcholine/*n*-tetradecane membranes [15] and also the strong dependence reported for cholesterol/HDTAB membranes [29]. These results also support our conclusions that the measured G is predominantly associated with the bilayer region because, if G was associated with large aqueous channels at the interface between the membrane and septum, it might be expected to increase linearly with [KCl].

If charge is indeed translocated by ions passing through aqueous channels in the bilayer phase, it appears that the product of the number and conductivity of these channels is only weakly dependent upon the external electrolyte concentration. Indeed, it might be possible that the close approach of an ion can enhance the probability of channel formation via an electrostrictive process, as suggested by Parsegian [25,30]. If this were the case, then G_B might be expected to vary as $[KCl]^{1/3}$. This might also explain why G_B for divalent and monovalent ions are similar, in that although that it is energetically more difficult for the divalent ion to travel through an aqueous pore, the higher valence might increase the possibility of pore formation. However, unless measurements are made of the activation energy involved, it will be difficult to be certain of this proposal.

Possible mechanisms for pore formation

We now examine some possible mechanisms for the formation of conducting pores across the bilayer. One possibility is that micelles (or other lipid aggregates) might be trapped in the bilayer as the solvent drains from the thick film. However, it has been reported [31] that the conductance of bilayers made by monolayer apposition, where micelles should not be present, is similar to that of drained films and so this mechanism does not appear likely. This also seems to rule out the possibility (vi) discussed earlier, whereby the observed linear variation of G upon C is due to a sweeping of conductive lipid aggregates into the bilayer/membrane interface.

Another possibility is that lysophosphatidylcholine, which has been demonstrated to drastically increase membrane conductance [32], could be present within the bilayer and might aggregate to produce pores [33]. However, we found that the addition of cholesterol, which would be expected to restrict such lysophosphatidylcholine pore formation [34], did not reduce the conductance (see Table II) and this appears to make this mechanism less likely.

The pores might not even be permanent structures, but could be transient phenomena which arise from statistical fluctuations in the lipid organisation. Indeed, a detailed statistical-mechanical analysis of the formation of such pores and their relation to membrane stability has already been published [35,36]. On this basis, the probability of pore formation might decrease if the bilayer thickness increases significantly and this could account for the lower conductance of bilayers made with *n*-decane solvent. The probability of pore formation might also be enhanced by the presence of various impurities.

The pores, if they exist, presumably are lined with the polar-heads of the lipid molecules surrounding the pore, and the central core of water might be very small. The surface charge on the lipids could further buffer the concentration of ions within the pore itself and this would tend to reduce the dependence of G_P upon the concentration of ions in the bulk electrolyte.

Are impurities responsible?

One possibility that should be considered is whether the bilayer conductance is due to trace amounts of impurity. These impurities could be responsible for charge translocation directly through the impurities themselves (perhaps by electronic or proton conduction), or produce changes in the organisation of the bilayer lipids which enhance the probability of pore formation. To test whether impurities in the water were responsible, triply-distilled water from two different sources was used, and no difference in the results was apparent. Impurities could be present in the various salts used to make up the electrolyte solutions. However, similar values for G_B were found for six different salts (see Table I), and it seems unlikely that a common impurity (responsible for G_B) would be present. Trace amounts of impurity are likely to be present in the lipid/*n*-alkane mixture. However, a major difficulty arises because the final mass of the bilayer is very much smaller than that of the original lipid film. Thus, it is possible that some impurity could be concentrated by a very large factor from the bulk solution as the bilayer forms, and consequently it might be virtually impossible to make the original mixture sufficiently pure to ensure that the concentration of impurity in the bilayer is negligible.

In this light, it is interesting that the measured values of the conductance of glycerolmonooleate membranes [37] are similar to those reported herein for phosphatidylcholine bilayers. Providing that the measured conductance of glycerolmonooleate membranes is primarily attributable to the bilayer regions, it is possible that a common conduction mechanism might be shared by many bilayers of different composition.

If ions do traverse the bilayer via aqueous pores, then the structure of bimolecular lipid membranes is more complicated than simply an ultrathin sheet of homogeneous hydrocarbon liquid, and this could have implications for the interpretation of other experiments. The refractive index within these pores will also differ from that of the hydrocarbon phase and might need to be considered in the interpretation of some optical measurements.

If the probability of pore formation depends upon the voltage across the bilayer (as might occur if an electrostrictive process is involved), then the measured $I-V$ characteristics will be nonlinear (as has indeed been shown by experiments in this and other laboratories). The pores considered herein could also provide a permeation pathway for neutral polar molecules and, if the formation of these pores is voltage-dependent, the permeability of the membrane to such neutral molecules would then also be voltage dependent.

The additional conductive pathway through the pores will appear in parallel with that of the bilayer leaflet itself and should be considered when fitting the measured frequency dependence of the bilayer admittance to obtain information on the dielectric substructure [11,12,37]. Fortunately, the presence of this additional element makes only very minor differences to the derived parameters if the conductance of the hydrophobic region (G_H) is now attributed primarily to the conductance of these pores.

Conclusions

It was found that the conductance of our phosphatidylcholine bilayers with a low concentration of *n*-hexadecane solvent could be primarily attributed to the bilayer region rather than to the torus region (this is contrary to conclusions reached elsewhere, based upon measurements on membranes made with *n*-decane solvent). The results are consistent with the bilayer conductance being a consequence of ion permeation through (transient) aqueous pores. It is possible, however, that conduction also occurs via the translocation of electrons or protons through the bilayer.

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