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Membrane conductance and surface potential

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SUMMARY

The specific conductances of black lipid membranes of lecithin, glycerylmonooleate and lecithin and cholesterol in aqueous solutions of KCl + nonactin have been measured. The relative conductances of the lecithin and the glycerylmonooleate membranes are accurately accounted for by the difference in their surface potentials. The large effect of cholesterol in depressing the conductance of the lecithin membranes is, however, not accounted for by changes in the surface potential and it is necessary to invoke either changes in the membrane solubility of the nonactin–K^+ complex, or changes in the membrane viscosity (as experienced by the ionophore in transit), or both.

The surface potentials which, in all the present membranes, arise solely from layers of oriented molecular dipoles, are not affected by the KCl concentration.

The influence of the interfacial potentials on the conductance of lipid bilayer membranes has recently received much attention[1–7]. It has been pointed out that the potential jump originating from oriented dipoles in the polar group region is likely to be as important, at least in carrier processes, as that arising from the ionic double layer[4], and a quantitative investigation of this proposition has now been carried out[7]. The results show that for bilayers formed from glycerylmonooleate and *n*-decane, rendered conducting by the presence of nonactin, the conductance changes which occur on the addition of positively and negatively charged and zwitterionic surfactants are accurately accounted for by the changes in the total (or Galvani) potential across the membrane interfaces. This total potential change includes the dipole and ionic double layer potentials but, unlike its components, which cannot be readily separated, it is unambiguously measurable. (While this is well known to be so for a single interface it has been argued that the data thus obtained may be applied also to a lipid membrane in equilibrium with the single interface[7].)

The successful prediction of the conductance changes from surface potential measurements in the systems already examined was not especially surprising as there was no reason to suppose that other relevant factors, such as the composition of the hydrocarbon interior of the membranes, varied appreciably. In the present investigation, however, membranes of quite different composition have been compared and it will be shown that conductance differences are not necessarily accounted for by the surface potentials.

The membranes examined were of egg yolk lecithin (10 mg/ml) in *n*-decane, lecithin (10 mg/ml) + cholesterol (20 mg/ml) in *n*-decane and glycerylmonooleate (7 mM) in *n*-decane and were made conducting by the addition of nonactin. The method of Szabo *et al.*[8] was used to form the membranes; the cell was as described previously[9]. In each experiment a membrane was formed, the background conductance was noted to ensure that it was negligible, the nonactin in ethanol was added to the stirred outer aqueous phase (10^{-7} M nonactin and 0.1% (v/v) ethanol for lecithin; 10^{-9} M and 0.01% (v/v) for glycerylmonooleate) and the new conductance was then measured (at 25 mV applied potential). For lecithin + decane, large membranes ($\gtrsim 5 \cdot 10^{-3}$ cm^2) could be reformed repeatedly from the lipid initially added to the cell and, after about 2 h, the conductances ceased to depend on time, or on stirring. Provided that the membranes were large and the system was continuously stirred, however, the steady-state conductance of the first membrane did not differ significantly from the final equilibrium conductance. For the glycerylmonooleate systems membranes could not be repeatedly reformed until equilibrium was reached. Nevertheless, for glycerylmonooleate + hexadecane membranes it has been found that, as for the lecithin systems, the steady-state conductance of the initial membrane is within 10–20% of the equilibrium value[9]. A conservative estimate of the accuracy of both the lecithin and glycerylmonooleate membrane conductances is thus 20%. Comparably detailed studies have not been carried out for the lecithin–cholesterol membranes and in these systems it is possible that the nonactin did not reach its equilibrium concentration. The range of conductances given in Table III should, however, cover the maximum possible discrepancy. Surface (compensation) potentials were measured for spread monolayers of the membrane-forming lipids on the appropriate aqueous phases and were reproducible to within 2% (5% when cholesterol was present). Details of the technique and the justification for supposing that such measurements hold for black film interfaces have been given previously[7].

TABLE I

LECITHIN–DECANE BLACK FILMS

Specific capacitance 0.39 $\mu F \cdot cm^{-2}$; lecithin area/molecule 61 Å^2; volume fraction of decane 0.31 (ref. 11).

KCl concn ($mole \cdot l^{-1}$)	$\frac{G(0)}{c_N}$ ($\Omega^{-1} \cdot cm^{-2} \cdot l \cdot mole^{-1}$)	*Compensation potential change,* $\Delta(\Delta\varphi)$ *(mV)*
0.01	10	441
0.10	90	447
1.0	900	441

TABLE II

GLYCERYLMONOOLEATE–DECANE BLACK FILMS

Specific capacitance 0.39 $\mu F \cdot cm^{-2}$; glycerylmonooleate area/molecule 39 $Å^2$; volume fraction of decane 0.47 (ref. 11).

KCl concn $(mole \cdot l^{-1})$	$\frac{G(0)}{c_N}$ $(\Omega^{-1} \cdot cm^{-2} \cdot l \cdot mole^{-1})$	*Compensation potential change,* $\Delta(\Delta\varphi)$ *(mV)*
0.01	1000	321
0.10	10000	319
1.0	80000	321

TABLE III

LECITHIN–CHOLESTEROL–DECANE BLACK FILMS

Specific capacitance ≈ 0.6 $\mu F \cdot cm^{-2}$; lecithin area/molecule ≈ 66 $Å^2$; cholesterol area/molecule ≈ 170 $Å^2$; volume fraction of decane $\lesssim 0.1$ (ref. 12).

KCl concn $(mole \cdot l^{-1})$	$\frac{G(0)}{c_N}$ $(\Omega^{-1} \cdot cm^{-2} \cdot l \cdot mole^{-1})$	*Compensation potential change,* $\Delta(\Delta\varphi)$ *(mV)*
0.01	< 0.02	420
1.0	2–5	420

The results are shown in Tables I–III. The electrolyte solutions, which were all buffered to pH ≈ 7 with $NaHCO_3$ ($\lesssim 10^{-3}$ M), are described in the first column, specific membrane conductances ($G(0)$) divided by the nonactin concentration in the aqueous phase (c_N) are given in the second column and, in the third column, are the changes in compensation potential, relative to the interior of the aqueous solution, which are found when the lipid is spread at the aqueous solution–air interface. These potentials are obviously insensitive to the electrolyte concentration, as might have been anticipated from the fact that at pH ≈ 7 neither the lecithin nor the glycerylmonooleate carries a nett charge[7]

It has been shown that if interfacial potentials only need be considered, the specific conductances (in the limit of zero applied potential) $G(0)$ of two membranes are related to the difference in their surface potentials $\Delta(\Delta\varphi)$ by the expression

$$-\frac{RT}{zF} \ln \frac{G_2(0)}{G_1(0)} = \Delta(\Delta\varphi) \qquad (1)$$

where z is the valence of the current carrying ion[4,7]. The consequences of applying this equation to the present systems are shown in Table IV. (The differences in surface potentials $\Delta(\Delta\varphi)$ are here given by the differences between the potentials relative to the clean aqueous solution–air interfaces shown in Tables I–III.)

TABLE IV

COMPARISON OF POTENTIALS CALCULATED FROM CONDUCTANCE RATIOS (Eqn 1) WITH THOSE OBSERVED

gmo = glycerylmonooleate.

Electrolyte concn (mole · l^{-1})	*Potentials (mV)*			
	$-\frac{RT}{F} ln \frac{(G(0)/c_N)_{lecithin}}{(G(0)/c_N)_{gmo}}$	$\Delta(\Delta\varphi)_{lecithin} - \Delta(\Delta\varphi)_{gmo}$	$-\frac{RT}{F} ln \frac{(G(0)/c_N)_{lec.-chol.}}{(G(0)/c_N)_{gmo}}$	$\Delta(\Delta\varphi)_{lec.-chol.} - \Delta(\Delta\varphi)_{gmo}$
0.01	116	120	> 270	99
0.10	118	128	–	–
1.0	113	120	244–266	99

As can be seen, the differences between the specific conductances of the lecithin and glycerylmonooleate membranes are accounted for, to within experimental error, by their differences in surface potential. (It has recently been stated that a similar relationship holds for lecithin and glyceryldioleate membranes[13], but no details of this work have yet been published.) It follows from the fact that diffuse ionic double layers are absent, that the potentials in question must arise from oriented molecular dipoles. These dipoles thus generate a potential inside the lecithin membranes more positive than that inside the glycerylmonooleate membranes. The fact that the data in Table IV are independent of electrolyte concentration shows that, barring a fortuitous cancellation of effects, the dipole potentials at the interfaces of both lecithin and glycerylmonooleate black films must individually be independent of electrolyte concentration. The success of Eqn 1 in the lecithin and glycerylmonooleate systems implies that the compositional differences between the two types of membrane are insufficient to affect appreciably the solubility of the nonactin–K^+ complex in the membrane hydrocarbon, or the "viscosity" of the membrane, in so much as it affects the ion transport process.

In contrast, Eqn 1 clearly does not account for the large decreases in conductance observed when cholesterol is added to the lecithin membranes. Szabo *et al.*[6,8] have discussed the possible influence of cholesterol in black films and have noted the difficulties of separating the electrostatic potential, "viscosity" and ionophore solubility effects. In the present investigation the electrostatic potential has been estimated and shown to be unimportant. It has not been possible, however, to distinguish between the effects of "viscosity" and of changes in the solubility of the nonactin–K^+ complex. There is some evidence that the latter acts in the right direction and is important[10], but it is not clear that changes in solubility account for the whole effect of the cholesterol.

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