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Surface Charge and the Conductance of Phospholipid Membranes*

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Abstract. Bilayer membranes, formed from various phospholipids, were studied to assess the influence of the charge of the polar head groups on the membrane conductance mediated by neutral "carriers" of cations and anions. The surface charge of an amphoteric lipid, phosphatidyl ethanolamine, was altered by varying the pH, and the surface charge of several lipids was screened by increasing the ionic strength of the solution with impermeant monovalent and divalent electrolytes. The surface charge should be a key parameter in defining the membrane conductance for a variety of permeation mechanisms; conductance measurements in the presence of carriers may be used to estimate the potential difference, due to surface charge, between the interior of the bilayer and the bulk aqueous phase. The large changes in conductance observed upon varying the surface charge density and the ionic strength agree with those predicted by the Gouy-Chapman theory for an aqueous diffuse double layer. Explicit expressions for the dependence of the membrane conductance on the concentrations of the carrier, the permeant ion, the surface charge density, and the ionic strength are presented.

With the development of the artificial phospholipid bilayer membrane,[1,2] the relationship between phospholipid composition and permeability properties of the membrane has become accessible to experimental study. The significance of the fluidity of the membrane for solute permeation has been shown.[3] Moreover, surface potential measurements on phospholipid monolayers and studies of the electrophoretic mobility of phospholipid dispersions have demonstrated that the presence of a charged polar head group produces a substantial potential at the lipid–solution interface.[2] Such a potential should influence the concentration of ions at the interface, and hence, the permeability properties of the membrane. Studies on phospholipid vesicles[4,5] and bilayers[6,7] have indeed shown that the anion to cation permselectivity is influenced by the charge of the membrane. Furthermore, the conductance of a bilayer due to the iodide ion or certain weak acids is affected by the charge on the polar head group of the phospholipid.[8,9] The present study examines theoretically and experimentally how certain molecules which enhance the membrane conductance, probably by acting as carriers[10–12] of particular cations and anions, may be used as "probes" to distinguish between effects of charge and of other variables, such as the fluidity of the bilayer interior and the lipid solubility of the complex.

Materials and Methods. The experimental apparatus and procedures used to obtain conductance measurements on bilayers have been described previously.[12] Membranes were formed from mixtures of *n*-decane and the following lipids: (1) 7-dehydrocholesterol (DC) (Sigma), which is uncharged; (2) a cyclopropane-rich phosphatidyl ethanolamine (PE) extracted from *Escherichia coli* by J. Law,[13] which is amphoteric but nearly neutral at neutral pH, positively charged at acid pH, and negatively charged at alkaline pH; (3) the mixed plant lipid asolectin (A),[12] which is a mixture of neutral and negatively charged lipids; (4) phosphatidyl inositol (PI) from brain (Sigma), which bears one negative charge per lipid residue; (5) a cyclopropane-rich phosphatidyl glycerol (PG) from *E. coli* (Supelco), which bears the same net charge as phosphatidyl inositol; and (6) phosphatidyl glycerophosphate (PGP) extracted from *Halobacterium cutirubrum* by M. Kates,[14] which has three dissociable negative groups per lipid residue.

Theoretical Expectations. There exists strong evidence indicating that the neutral molecules used here act as carriers.[10-12,15,16] A Nernst-Planck treatment,[15,16] for a carrier mechanism of ion permeation, can be extended rather simply to include the effects of the potential difference developed between the bilayer and the bulk aqueous phase when the polar head groups bear a net charge. For such a treatment, the conductance, G_0, in the limit of zero applied voltage (or current) of a membrane separating identical solutions is given[15,16] by the expression

$$G_0 = F^2/\int_0^d [1/u^*C^*(x)]dx, \tag{1}$$

where $C^*(x)$ is the equilibrium concentration of the single permeant monovalent species at the point x in the membrane, u^* its mobility in the membrane (a constant for this model[15,16]), F the Faraday, and d the membrane thickness. Provided the permeant ions are present in the membrane at a sufficiently low concentration, the equilibrium profile of electric potential within the membrane is essentially constant. We may therefore assume that the concentration of the charged permeant species is also uniform, that is, $C^*(x) = C^*$, and Eq. (1) reduces to the simple form:

$$G_0 = (F^2/d)u^*C^*. \tag{2}$$

As the system is at equilibrium, the concentration of the permeant ion in the membrane, C^*, is related to its bulk concentration in the aqueous phase, C, by the expression:[15]

$$C^* = k\,C \exp(\pm F\psi^*/RT) \tag{3}$$

where ψ^*, called hereafter the "surface potential," is the absolute value of the potential difference between the interior of the membrane and the bulk aqueous solution and k is a constant (partition coefficient), independent of the potential. (The sign is (+) if the potential difference is of opposite sign to the charge of the permeant species, (−) if it is the same). Eq. (2) and (3) illustrate that positive and negative permeant species can be used as "probes" to distinguish between the effects of surface potential, ψ^*, and the effects of fluidity, dielectric constant, or more specific factors. A change of ψ^* will alter the conductance of positive and negative species in opposite directions, whereas a change of fluidity or dielectric constant should affect u^* and k, and hence the conductances of both species, in the same direction.

It should be noted that the use of ions as "probes" of the surface potential is not contingent upon their passage through the membrane by a carrier mechanism. The results obtained here apply whenever the membrane conductance can be described by an expression equivalent in form to the combination of Eq. (2) and (3) (e.g., as found for an Eyring treatment of a "jump" mechanism[17]).

Provided the net charge of the polar head groups is the sole cause of the surface potential (i.e., ignoring dipoles), the electric potential will be continuous across the membrane-solution interface. The Gouy-Chapman theory[18,19] for the diffuse double layer then yields the desired relation between ψ^* and our experimental variables, the surface charge density (σ) and the total molar concentration ($\Sigma_i C_i$) of 1-1 electrolytes in the aqueous phase:

$$\exp(\pm F\psi^*/RT) = (\alpha + \sqrt{\alpha^2 + 1})^{\pm 2}, \quad \text{where } \alpha = \sqrt{\sigma^2\pi/2RTD\Sigma_i C_i} \qquad (4)$$

and D is the dielectric constant of water (at 20°C, $D = 1.113 \times 10^{-12}$ coulomb/volt per cm, and $\alpha = 136\sqrt{\sigma^2/\Sigma_i C_i}$ for σ in units of electronic charge per Å^2 and $\Sigma_i C_i$ in moles/liter).

Eq. (2), (3), and (4) may now be combined to give the expression

$$G_0 = (F^2/d)u^*kC[\alpha + \sqrt{\alpha^2 + 1}]^{\pm 2} \qquad (5)$$

(The sign in Eq. (5) is (+) if the charge of the membrane is opposite to that of the permeant species, (−) if it is the same.)

To use Eq. (5) for neutral carriers of ions, it is only necessary to express the bulk aqueous concentration of the permeant species, C, in terms of the bulk aqueous concentrations of the carrier and carried-ion. As the system is in equilibrium, Eq. (6) applies for the I_5^- complex, Eq. (7) for the K^+·nonactin (or the K^+·valinomycin) complex, and Eq. (8) applies for the Cs^+(polyether)$_3$ complex:

$$C = K_{I_5}[I^-][I_2]^2 \qquad (6)$$

$$C = K_{K\text{-nonactin}}[K^+](\text{nonactin}] \qquad (7)$$

$$C = K_{Cs(\text{polyether})_3}[Cs^+][\text{polyether}]^3, \qquad (8)$$

where K_{I_5}, $K_{K\text{-nonactin}}$, and $K_{Cs(\text{polyether})_3}$ are the equilibrium constants for the formation of the complexes in the aqueous phase.

Results. Our first object was to establish for each of the above lipids the experimental range over which molecular "carriers" of cations (valinomycin,[20,21] nonactin[12] and the cyclic polyether XXXII[22]) and of an anion (I_2 in the presence of I^-)[11,23] act to increase the membrane conductance in a regular manner. Fig. 1 illustrates that the log conductance versus log carrier concentration curves have slopes of the same characteristic integers for each carrier in the different lipids, with the exception of the I_2–I^- system in the case of the lipids PE and DC (see Fig. 1, lower left). For these lipids easily interpretable data for the I_2–I^- system can be obtained only over a restricted range of pH and concentration. Since the membrane conductance was always proportional to the first power of the concentration of the carried-ion (provided the ionic strength was maintained constant with an impermeant salt), the data of Fig. 1 imply that the permeant species

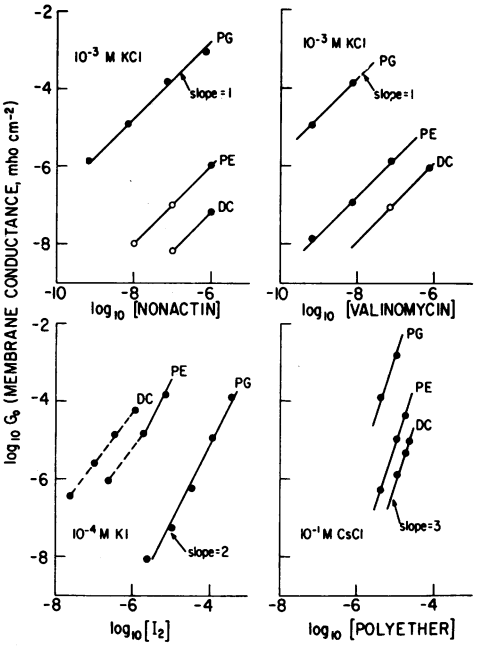

FIG. 1. The dependence of membrane conductance on carrier concentration for the lipids phosphatidylglycerol (PG), phosphatidyl ethanolamine (PE) and 7-dehydrocholesterol (DC). The open circles indicate values extrapolated from measurements made at higher KCl concentrations, while dashed lines indicate nonintegral slopes.

in the presence of K^+ and nonactin (or valinomycin) is the 1:1 complex, that for Cs^+ and the polyether XXXII it is the 1:3 complex, and that for I^- and I_2 it is the 1:2 complex (i.e. the species I_5^-), in accord with the expectations of Eq. (5) on substitution of Eq. (6) to (8).

We next examined the dependence of conductance on membrane charge, as illustrated in Fig. 2, where lipids of different charge have been ranked along the abscissa according to their relative negativity. Conductance measurements in both the upper (10^{-6} M nonactin, 10^{-3} M KCl) and lower (10^{-6} M I_2, 10^{-3} M KI) portion of Fig. 2 are represented by heavy horizontal lines while the height

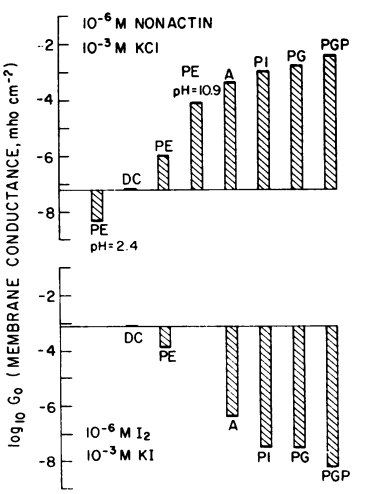

FIG. 2. The effect of lipid composition on the conductance induced by the positive nonactin–potassium complex (*upper*) and negative polyiodide complex (*lower*). The very low bilayer conductances for nonactin with PE at pH 2.4 (upper) and for I_2 with PGP (*lower*) are not significantly larger than the conductance in the absence of carriers. All other conductances are.

of the shaded bars represents the conductances relative to the neutral lipid, DC. By using the neutral lipid as a reference, we normalize for the difference in the effectiveness of the carriers. Note the large and opposite changes of conductance for positive and negative permeant species on varying the charge of the lipid. The increases and decreases would be exactly symmetrical if the surface potential were the only variable. Deviations from exact symmetry are seen in Fig. 2 to be small, and could be interpreted in terms of fluidity and dielectric constant, as discussed in relation to Eq. (2) and (3). Note that similar conductance values are observed for PI and PG in spite of the difference in the hydrocarbon tails of these lipids—the fatty acids of PI are unsaturated, those of PG contain cyclopropyl groups.

From Fig. 2 it may be seen that the conductance produced by the K^+·nonactin complex on the PG membrane is enhanced, at 10^{-3} M ionic strength, by a factor of 30,000 relative to the neutral DC membrane, whereas the conductance produced by the negatively charged I_5^- complex is suppressed to the identical degree. The potential in the PG membrane (relative to the DC membrane) necessary to cause these conductance differences is calculated to be about −250 mV from Eq. (2) and (3), taking u^*, k, and d to have the same values for each lipid. According to the Gouy-Chapman theory (see Eq. 4), this potential corresponds to a physically reasonable value for the PG surface charge density of one charge per 50 Å^2. Practically identical values can be deduced from measurements with the Cs^+–(polyether)$_3$ and K^+·valinomycin complexes (see Fig. 1).

It is possible, by varying the pH, to produce either a positive or negative surface charge on a bilayer formed from an amphoteric lipid such as PE. This is illustrated in Fig. 3, where large changes in the nonactin-induced conductance

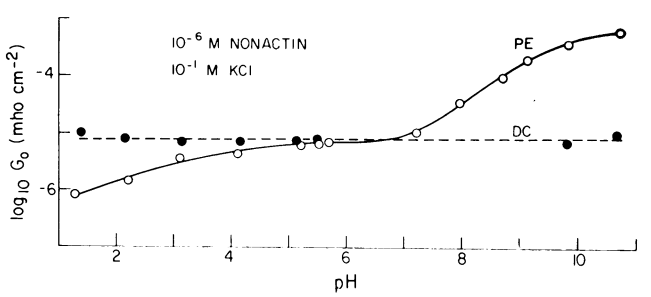

FIG. 3. The effect of pH on the nonactin-induced conductance of amphoteric PE (*open circles, solid line*) and neutral DC (*filled circles, dashed line*) membranes.

of the membrane are observed even at the relatively high ionic strength of 0.1 M (note the absence of any effect of pH on the conductance of the neutral DC membrane under identical conditions). The surface potential changes inferred for PE bilayers from these conductance changes parallel those observed for PE monolayers at an air–water interface.[2]

An increase in the ionic strength should reduce the surface potential by screening the surface charge (Eq. 4), and should therefore increase the membrane conductance (Eq. 5) if the permeant ions are of the same sign as the surface charge and decrease it in the opposite case. Fig. 4 illustrates for a positive permeant species that increasing the ionic strength by adding the impermeant electrolyte

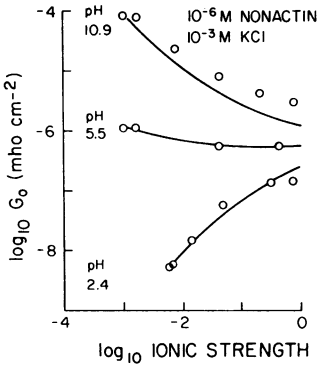

FIG. 4. The effect of ionic strength, which was increased by the addition of LiCl, on the conductance of the amphoteric membrane, PE, at different values of the pH. The solid lines are theoretical curves drawn according to Eq. (5) with the following values of σ chosen to fit the initial points: σ (pH 10.9) = 1 per 700 Å^2; σ (pH 5.5) = 1 per 12,000 Å^2; σ (pH 2.4) = 1 per 370 Å^2.

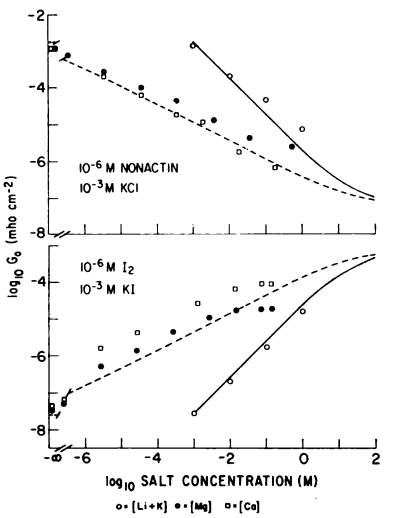

FIG. 5. The effect of impermeant monovalent (LiCl) and divalent ($MgCl_2$, $CaCl_2$) cations on the conductance induced in the negatively charged lipid, PG, by the K^+·nonactin complex (*upper*) and the polyiodide complex (*lower*).

LiCl increases the conductance of the positive membrane (pH 2.4), decreases the conductance of the negative membrane (pH 10.9), but has little effect when the membrane is essentially neutral (pH 5.5), as expected from Eq. (5) (according to which the solid lines were drawn[24]).

Fig. 5 presents similar data for a PG membrane, which bears a high negative charge. The theoretical curves for the effects of adding impermeant monovalent (*solid curves*) and divalent (*dashed curves*) ions are drawn according to Eq. (5) for monovalent ions and according to an extended equation[25] for divalent ions, using the same value of surface charge density (σ = 1 per 50 Å^2) in all cases and taking the limiting value of the conductance at high ionic strength to be that of the neutral DC membrane (see Fig. 2). When the ionic strength is increased from 10^{-3} to 1 M with LiCl, Eq. (5) predicts for this highly charged lipid (where $\alpha \gg 1$) that the conductance due to the K^+·nonactin complex (*upper portion*, Fig. 5) should be inversely proportional to the ionic strength, whereas the conductance due to the I_5^- complex (*lower portion*, Fig. 5) should be directly proportional to it. The different effects of monovalent versus divalent impermeant ions (compare the slope of 1 vs. $^1/_2$) are also seen to be predicted by the Gouy-Chapman theory without invoking specific binding.

The impermeant electrolytes LiCl, $CaCl_2$, and $MgCl_2$ were found to have no effect on the "carrier-induced" conductances of the neutral DC membrane, as expected from Eq. (5) when $\alpha = 0$.

Discussion of Assumptions. To derive Eq. 1, we assumed diffusion in the membrane interior to be rate-limiting. Other processes could be rate-limiting

instead (e.g., complexing kinetics, diffusion to and across the interface). The rates of formation and dissociation of monactin complexes, however, are sufficiently rapid[26] that complexing kinetics need not be rate-limiting. Furthermore, for the present "carriers," the current–voltage characteristics are time-independent and not current-limited over a wide concentration range, indicating that diffusion in the aqueous solution to and from the interface is not rate-limiting.

To obtain Eq. (2) from Eq. (1), the potential was assumed to be constant within the membrane. Within the framework of our model, the proportionality observed between membrane conductance and concentration of the permeant species (see Fig. 1) supports this assumption.[16] We also assumed that the mobility was constant within the membrane phase and that image charge effects[27] could be ignored. It is irrelevant if these assumptions are invalid, because one could include these effects merely by defining an average mobility and partition coefficient.

In obtaining Eq. (4), possible effects of discontinuities of electric potential at the membrane–solution interface (e.g., due to dipoles) were ignored. A dipole layer which gives rise to such a discontinuity would affect positive and negative probes in the same manner as the surface charges here considered, but it would not be modified by ionic strength. We cannot exclude the possibility that part of the probe effect is due to such dipoles, but the fact the amphoteric lipid PE and the neutral lipid DC, which are unlikely to have the same dipole terms, have the same conductance at neutral pH and high ionic strength (see Fig. 3) suggests that the dipole effect is small.

Lastly, to obtain Eq. (4), the Gouy-Chapman theory, with all of its assumptions (e.g., smeared surface charge, negligible size of ions, uniform dielectric constant with a sharp discontinuity at the interface), has been used. Some justification for the validity of these assumptions at a hydrocarbon-water interface has been given by Haydon.[19]

Conclusion. In the presence of a variety of neutral molecules that cause phospholipid bilayer membranes to become electrically conducting, the membrane conductance is strongly dependent on the net charge of the polar head groups of the lipid. This dependence must be taken into account when characterizing any effects of antibiotics on the electrical properties of membranes and can be quantitatively described by representing the polar head groups as a uniformly distributed surface charge whose effect is simply to give rise to a diffuse double-layer. Such an effect of surface charge has previously been proposed to account for the changes observed on varying the ionic strength in the interior of the squid giant axon.[28]

We thank Drs. Max Delbrück and Robert Eisenberg for perceptive comments.

Abbreviations: DC, 7-dehydrocholesterol; PE, phosphatidyl ethanolamine; A, asolectin; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PGS, phosphatidyl glycerophosphate.

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[1] Mueller, P., D. O. Rudin, H. T. Tien, and W. C. Wescott, *Circulation*, **26**, 1167 (1962).

[2] Bangham, A. D., in *Progress in Biophysics and Molecular Biology* (New York: Pergamon Press, 1968), p. 29.

[3] De Gier, J., J. G. Mandersloot, and L. L. M. Van Deenen, *Biochim. Biophys. Acta*, **150**, 666 (1968).
[4] Bangham, A. D., M. M. Standish, and J. C. Watkins, *J. Mol. Biol.*, **13**, 238 (1965).
[5] Papahadjopoulos, D., and J. C. Watkins, *Biochim. Biophys. Acta*, **135**, 624 (1967).
[6] Andreoli, T. E., J. A. Bangham, and D. C. Tosteson, *J. Gen. Physiol.*, **50**, 1729 (1967).
[7] Hopfer, U., A. L. Lehninger, and W. J. Lennarz, *J. Mem. Biol.*, **2**, 41 (1970).
[8] Lesslauer, W., J. Richter, and P. Läuger, *Nature*, **213**, 1224 (1967).
[9] Hopfer, U., A. L. Lehninger, and W. J. Lennarz, *Fed. Proc.*, **29**, 549 Abs. (1970).
[10] Pressman, B. C., E. J. Harris, W. S. Jagger, and J. H. Johnson, *Proc. Nat. Acad. Sci. USA* **58**, 1949 (1967).
[11] Finkelstein, A., and A. Cass, *J. Gen. Physiol.*, **52**, 1455 (1968).
[12] Szabo, G., G. Eisenman, and S. M. Ciani, *J. Mem. Biol.*, **1**, 346 (1969).
[13] Chung, A. E., and J. H. Law, *Biochemistry*, **3**, 967 (1964).
[14] Kates, M., L. S. Yengoyan, and P. S. Sastry, *Biochim. Biophys. Acta*, **98**, 252 (1965).
[15] Eisenman, G., S. M. Ciani, and G. Szabo, *Fed. Proc.*, **27**, 1289 (1968).
[16] Ciani, S. M., G. Eisenman, and G. Szabo, *J. Mem. Biol.*, **1**, 1 (1969).
[17] Läuger, P., and G. Stark, *Biochim. Biophys. Acta*, in press.
[18] Delahay, P., *Double Layer and Electrode Kinetics* (New York: Interscience, 1965), chap. 3.
[19] Haydon, D. A., in *Recent Progress in Surface Science* (New York: Academic Press, 1964) vol. 1, p. 94.
[20] Lev, A. A., and E. P. Buzhinski, *Cytology (U.S.S.R.)*, **9**, 102 (1967).
[21] Mueller, P., and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26**, 398 (1967).
[22] McLaughlin, S. G. A., G. Szabo, G. Eisenman, and S. M. Ciani, *Biophysical Society Abstracts*, 96a (1970).
[23] Läuger, P., W. Lesslauer, E. Marti, and J. Richter, *Biochim. Biophys. Acta*, **135**, 20 (1967).
[24] σ should not remain exactly constant because an increase in the ionic strength, through its effects on ψ^*, should alter the interfacial pH, and hence alter the degree of dissociation.
[25] The Gouy-Chapman equation for unsymmetrical electrolytes (see Eq. 9, page 35 of ref. 18) was solved to yield an expression for the interfacial potential appropriate for the addition of a 2-1 electrolyte to a 10^{-3} M 1-1 electrolyte for $\sigma = 1$ charge per 50 Å^2. For a concentration of 2-1 electrolyte greater than 10^{-6} M, this expression differs negligibly from that obtained using the simple Gouy-Chapman equation for a symmetrical z-z electrolyte (which is obtained by extending Eq. 4 to include a factor $1/z$ in the exponent of the right-hand side). The dashed conductance curves in Fig. 5 were calculated by inserting the extended Eq. 4 with $z = 2$ directly in Eqs. (2) and (3).
[26] Diebler, H., M. Eigen, G. Ilgenfritz, G. Maass, and R. Winkler, *Pure Appl. Chem.*, **20**, 93 (1969).
[27] Neumcke, B., and P. Läuger, *Biophys. J.*, **9**, 1160 (1969).
[28] Chandler, W. K., A. L. Hodgkin, and H. Meves, *J. Physiol.*, **180**, 821 (1965).