ASYMMETRIC CHARGE DISTRIBUTIONS IN PLANAR BILAYER SYSTEMS

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ABSTRACT Using a simple argument based on irreversible thermodynamics and the Gouy-Chapman theory of the double layer, we show that the equilibrium distribution of charged lipid molecules between the two surfaces of a bilayer is asymmetric if the two solutions bathing the surfaces have the same ionic strength but contain ions of different valencies. For example, if one bathing solution contains 0.10 M NaCl and the other contains 0.70 M NaCl and 0.10 M $CaCl_2$, the ratio of charged lipid molecules of the two surfaces in a membrane that contains 50% total negative lipids is 1.46, leading to a transbilayer potential of 18 mV. A complete set of such numerical results is presented in four figures.

Biological membranes contain both charged and neutral lipids and separate solutions of different ionic composition. In recent years a number of papers dealt with the distribution of charged lipids between the two surfaces of membranes separating solutions of identical composition, and in this paper we shall extend some of these to include the effect of bathing solutions of different composition.

McLaughlin and Harary (1974) showed that the steady-state distribution of charged lipids between the two sides of a planar membrane separating solutions of similar composition is strongly affected by an imposed external electrical potential. Using the known values for the percentage of anionic phospholipids in squid axons (10–15%), a membrane potential of 50–100 mV, and an ionic strength of the bathing solution equal to 0.5 M, they showed that the charge density and double-layer potential at the outer surface of the nerve was substantially greater than the charge density and double-layer potential at the inner surface.

Although their paper is directed toward a discussion of flip-flop, i.e. the transbilayer migration of lipid molecules, the rate of flip-flop has been shown to be quite slow (cf. next paragraph). The results and conclusions of their paper, however, are of more general interest, possibly applying to the equilibrium or steady-state distribution of charged and neutral lipid molecules in self-assembling lipid systems as well.

Sherwood and Montal (1975) studied the kinetics of the migration of charged lipid molecules across planar asymmetric membranes formed by apposing a monolayer of the neutral lipid glyceroldioleate with one of the negatively charged lipid oleyl acid phosphate. They find that at 22° ± 2°C, in the absence of an externally applied

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electric field, the half-time of oleyl acid phosphate transbilayer migration is between 14.4 and 18.7 hr. This indicates that an asymmetric lipid distribution can be stable for a relatively long period. Ehrenstein, et al (1975) have obtained similar results for the squid axon membrane.

Using a simple model involving spherical capacitors, Israelachvili (1973) showed an asymmetric distribution of charged lipid molecules in curved bilayers such as vesicles, with more charged lipids at the outer surface than at the inner surface. In this case, however, there is no applied potential; the curvature itself induces the asymmetry in the distribution.

In all of the above studies the two solutions bathing the two sides of the bilayer were assumed to be identical. In this note we shall show that if the two bathing solutions are not the same, there results an asymmetry of the charged lipid molecules that leads to transbilayer potentials of the order of tens of millivolts in many cases. In particular, we shall consider the case in which the ionic strengths of the two solutions are the same but that one of them contains only a 1 – 1 electrolyte such as NaCl, whereas the other contains both a 1 – 1 and a 2 – 1 electrolyte. We shall use essentially the same approach as McLaughlin and Harary. Using a simple argument based on irreversible thermodynamics and a tightly coupled model of charged and neutral lipids, they show that if C_{tot} is the total concentration of lipids on each side of the bilayer and σ_1 and σ_2 the surface concentrations of charged lipid molecules on sides 1 and 2, respectively, then the steady-state or equilibrium distribution of charged and neutral lipid molecules is given by

$$\frac{\sigma_2}{\sigma_1}\frac{C_{tot} - \sigma_1}{C_{tot} - \sigma_2} = e^{-F\phi/RT} \tag{1}$$

where in their case

$$\phi = \psi_1 + V - \psi_2 \tag{2}$$

where ψ_j is the surface potential on side j and V is defined as the resting potential, or the potential that would be measured by two electrodes in the bulk aqueous solutions. In the cases studied here, $V = 0$. The tightly coupled model referred to above requires that the total number of lipids at each surface of the bilayer remain constant, i.e. that

$$\sigma_1 + C_1 = \sigma_2 + C_2 = C_{tot} \tag{3}$$

where C_j is the number of neutral lipids on the surface of side j. In addition, since the total number of charged lipid molecules is fixed, we can write

$$\sigma_1 + \sigma_2 = 2\sigma \tag{4}$$

where σ is a constant.

The surface potentials, ψ_1 and ψ_2 in Eq. 2, are produced by the charged lipids at the surfaces of the bilayer. We shall calculate the surface potentials using the standard, but nevertheless approximate (Olivares and McQuarrie [1975]), Gouy-Chapman theory of the diffuse double layer. In the case of both 1 – 1 and 2 – 1

electrolytes in the bathing solution, Abraham-Shrauner (1975) has recently given a simple relationship between the surface charge density and the surface potential. From above, σ_j is the surface concentration of charged lipids, and so if q is the net charge on the lipid head group, then the surface charge density is $q\sigma_j$. Abraham-Shrauner has shown that

$$q\sigma_j = -\left(\frac{\epsilon kTn_j}{2\pi}\right)^{1/2} \cdot (e^{-\phi_j} - 1) \cdot [K_j + (1 + 2K_j)e^{\phi_j}]^{1/2} \tag{5}$$

where in cgs units ϵ, the dielectric constant of water, = 79.1, k, the Boltzmann constant, = 1.3805×10^{-16} ergs/°K, T is the temperature in degrees Kelvin (296°K here), n is the number of univalent cations per cubic centimeter, K_j is the ratio of the number density of divalent cation to univalent cation, and ϕ is a reduced surface potential, $F\psi/RT$, where RT/F = 25.52 mV. When K = 0, Eq. 5 reduces to the 1 – 1 electrolyte equation used by McLaughlin and Harary.

Nelson et al. (1975) have shown in some detail that the direct electrostatic coupling between the two double layers of surface potentials is negligible and so we shall ignore this effect and use Eq. 5 with j = 1 and 2 to calculate ψ_1 and ψ_2.

The procedure now is to solve Eqs. 1, 2, 4, and 5 simultaneously. Eq. 1 is the central equation. By using Eq. 4, the left-hand side of Eq. 1 can be written as a func-

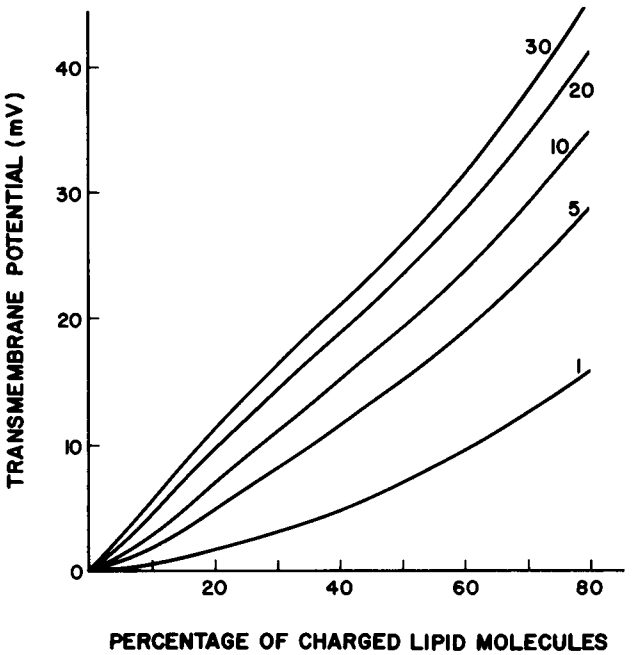

FIGURE 1 The transmembrane potential vs. the percentage of lipids that carry a net charge. The two bathing solutions have ionic strengths equal to 0.10; one contains only a 1 – 1 electrolyte and the other contains divalent ions in millimolar quantities indicated on each curve and the necessary concentration of 1 – 1 electrolyte to give the ionic strength of 0.10.

tion of σ_1. Eqs. 5 and 4 give ψ_1 and ψ_2 as implicit functions of σ_1, and so the right-hand side of Eq. 1 can also be considered a function of σ_1. Thus Eq. 1 is a transcendental equation in σ_1, which can be solved by standard numerical methods. For the results obtained here, Eq. 1 was solved by the Newton-Raphson method with the necessary values of $\psi_1(\sigma_1)$ and $\psi_2(\sigma_2)$ called in a Newton-Raphson subroutine.

Typical results are summarized in the four figures. In Figs. 1 and 2 the transbilayer potential, $\psi_1 - \psi_2$, is plotted vs. the percentage of the total number of negatively charged lipids in the bilayer (both sides) that are charged, i.e. $(\sigma_1 + \sigma_2)/2C_{tot}$. In Fig. 1 one bathing solution contains 0.100 M NaCl, for example, and the other contains various millimolar concentrations of Ca^{+2} (as indicated on the various curves) along with the necessary concentrations of NaCl to maintain the ionic strength equal to that of the 0.100 NaCl solution on the other side of the bilayer. For example, the curve labeled with a 10 shows results in which one solution contains 70 mM NaCl and 10 mM Ca^{+2} and the other solution contains only 0.100 M NaCl. The ionic strengths of the two bathing solutions were made equal for convenience in the presentation of the numerical results. Fig. 2 displays similar results for the case in which one solution is 0.500 M NaCl and the other various millimolar concentrations of $CaCl_2$ and enough NaCl so that the ionic strength is equal to that of a 0.500 M NaCl solution. Fig. 3 shows the transbilayer potential vs. the millimolar concentration

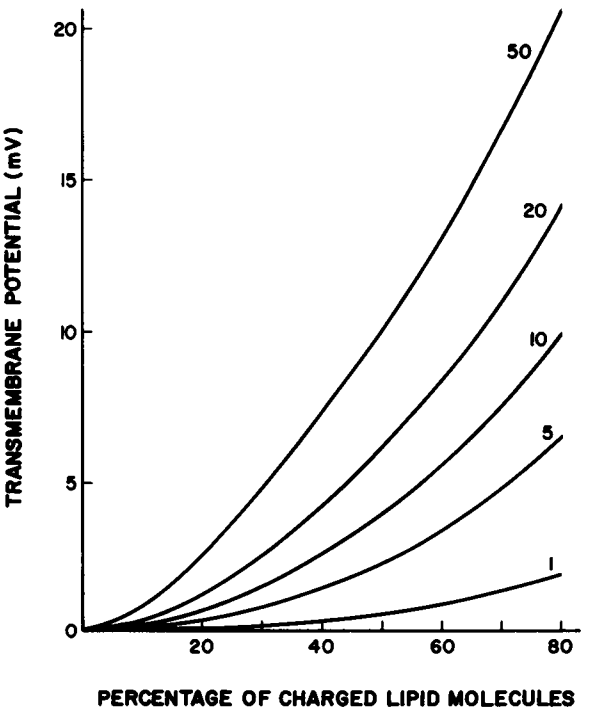

FIGURE 2 Same as Fig. 1 but with the ionic strengths of the two bathing solutions equal to 0.50.

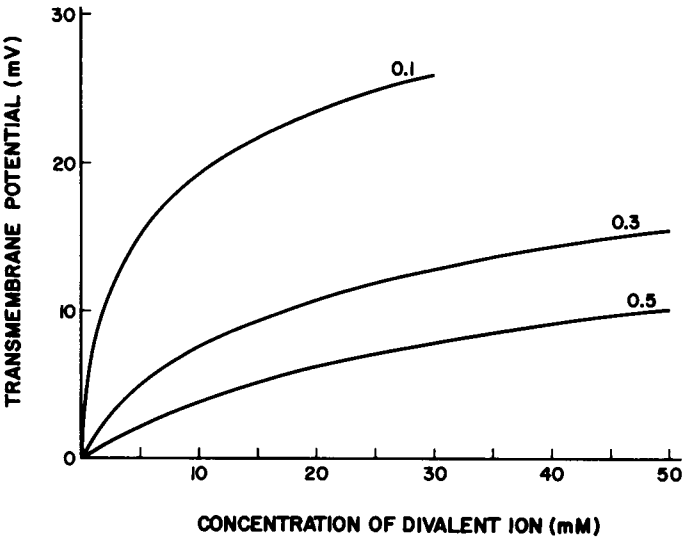

FIGURE 3 The transmembrane potential vs. the concentration of divalent ions for the cases in which one bathing solution contains only 1 – 1 electrolyte at the ionic strength labeling each curve and the other contains the millimolar concentration of divalent ion indicated on the abscissa and the necessary concentration of 1 – 1 electrolyte to give the same ionic strength as the other solution. For each of the curves, the percentage of charged lipids is 50%.

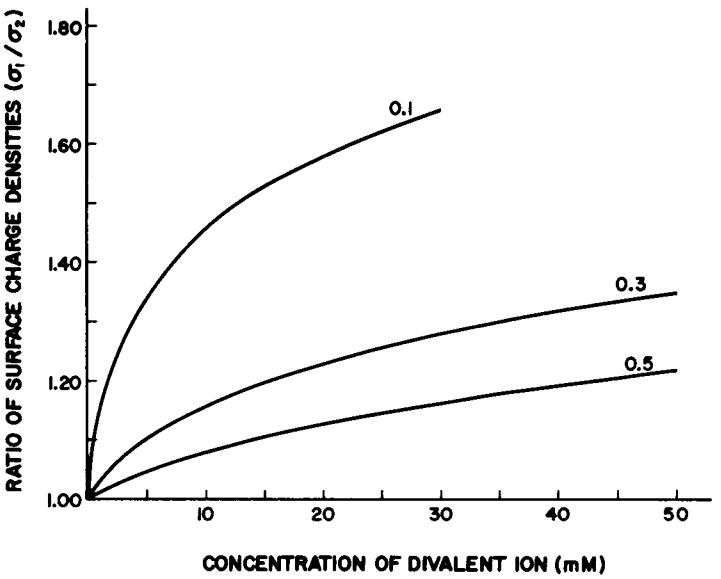

FIGURE 4 Same as Fig. 3 but with the ordinate being the ratio of the density of charged lipid molecules on the two surfaces instead of the transmembrane potential.

of Ca^{+2} for the case in which one half of the lipid molecules are charged. The labels on the three curves indicate the concentration of NaCl in the solution of fixed composition. It can be seen from the figure that as little as 10 mM Ca^{+2}, in a solution of total ionic strength equal to 0.10 separated from a solution of the same ionic strength but containing no Ca^{+2}, can generate a transmembrane potential of nearly 20 mV. As the ionic strength increases to 0.50, for instance, the effect of Ca^{+2} is seen to be less, as expected. Fig. 4 is similar to Fig. 3, but shows the asymmetry in the distribution of charged lipids vs. the millimolar concentration of Ca^{+2}, again for the case in which one half of the lipid molecules are charged. The asymmetry is measured by σ_1/σ_2 where side 1 is the side containing the Ca^{+2}.

Figs. 1 through 4 show that it is possible to generate quite large transbilayer potentials and surface charge asymmetries when the solution bathing one side of a planar bilayer contains divalent cations. Since we assume that these membranes are impermeable to ions, macroscopic diffusion potentials were not taken into account in these calculations. These results may have interesting implications in the assembly of lipid bilayer systems, since there are a number of biological systems that consist of saclike membranes enclosing solutions of the ionic strengths considered here and also containing Ca^{+2}. All of these systems, e.g. retinal disks, chloroplasts, photosynthetic bacteria, and mitochondria, have small internal volumes and so the enclosed Ca^{+2} can be of significant millimolar concentrations. In addition, the potentials developed across the membrane surfaces are involved in the regulatory processes of these systems, leading to the speculation that one role of the internal Ca^{+2} is to produce the asymmetry in the distribution of the charged lipids that help maintain this potential.

A similar situation might occur in mitochondria, where a gradient in the concentration of hydrogen ions may play the same role as Ca^{+2} here. This calculation is not possible within the standard Gouy-Chapman theory, since screening effects are included only through the charge on the ion, i.e. no specific interactions are included. By modifying the theory, however, to include the presence of titratable surface groups, specific interactions of hydrogen ions with the surface can be included.

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