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Theoretical considerations on the asymmetric distribution of charged phospholipid molecules on the inner and outer layers of curved bilayer membranes

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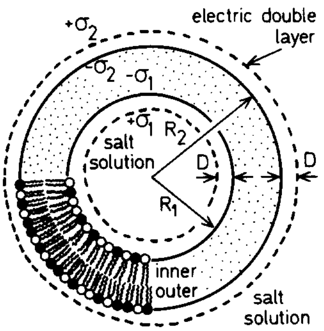
SUMMARY

Theoretical considerations based on electrostatic energy minimization predict an asymmetric distribution of charged phospholipid molecules on the outer and inner layers of a curved bilayer membrane. The results indicate that the outer layer should have a higher density of charged lipids than the inner layer, in quantitative agreement with a recent experimental result on vesicles.

Michaelson *et al.*[1] have found that cosonication of equimolar quantities of phosphatidylglycerol and phosphatidylcholine results in bilayer vesicles whose outer layers contain about 80% of the phosphatidylglycerol and 40% of the phosphatidylcholine, while the inner layers contain about 20% of the phosphatidylglycerol and 60% of the phosphatidylcholine. These investigators attributed this asymmetry in the phospholipid distribution to the reduced electrostatic repulsion between the negatively charged phosphatidylglycerol molecules when these are concentrated more on the outer layers. I should like to present a simple theoretical treatment of this matter, the results of which show how charged lipid molecules may be expected to distribute themselves on the inner and outer layers of bilayered vesicles and, in general, any curved bilayer membrane. The results of this analysis are in agreement with the experimental results of Michaelson *et al.*[1] and further predict that the asymmetric distribution of charged lipid molecules should increase with decreasing radius of curvature and decreasing ionic strength of the bathing solution.

At physiological pH the head groups of acidic lipids dissociate or ionize, leaving a



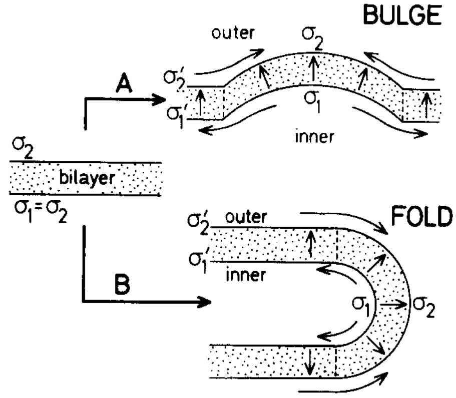


Fig. 1. Bilayer vesicle composed of charged (black circles) and uncharged (white circles) lipids in salt solution of Debye length D. The equilibrium density of charged lipids in the outer layer will be greater than that in the inner layer.

Fig. 2. When a flat bilayer becomes curved (bulged or folded) charged lipid molecules will tend to diffuse towards the outer curved layer and away from the inner curved layer by lateral diffusion (curved arrows) and flip-flop (straight arrows). At equilibrium the surface charge densities will be in the order $\sigma_2 > \sigma_2' = \sigma_1' > \sigma_1$.

net negative surface charge. This surface charge is balanced by an equal but opposite charged region in the aqueous phase consisting of free cations in rapid thermal motion. The distance that this ionic atmosphere of counter charges (known as the "electric double layer") extends into the aqueous phase is of the order of the Debye screening length D given by

$$D = (kT\epsilon/4\pi \sum_i n_i Q_i^2)^{1/2} \tag{1}$$

where k is Boltzmann's constant, T the absolute temperature, ϵ the dielectric constant of water ($\epsilon \approx 80$), and where n_i is the number of ions of charge Q_i per cm^3 of the bathing solution. For a 150 mM monovalent salt solution in water, $D = 8$ Å at 300°K.

The electric double layer may be treated as a capacitor whose plates are separated by a distance D (see, for example, Bull[2]). The electrostatic energy of a capacitor made up of two concentric spherical shells of inner and outer radii R and $R + D$, carrying charges $+Q$ and $-Q$, is

$$E = Q^2D/2\epsilon R(R + D) \tag{2}$$

where ϵ is the dielectric constant of the medium between the shells.

We may treat a bilayered vesicle as composed of two such capacitors (Fig. 1), one at the inner surface and one at the outer surface of the vesicle. If the vesicle contains, in addition to uncharged lipids, N charged lipid molecules each carrying a charge e, and if n of these lie in the outer layer, then from Eqn 2 the total electrostatic energy of the system is

$$E = \frac{e^2D}{2\epsilon}\left[\frac{n^2}{R_2(R_2 + D)} + \frac{(N - n)^2}{R_1(R_1 - D)}\right] \tag{3}$$

where R_1 and R_2 are the inner and outer vesicle radii, and where D is the thickness of each capacitor assumed to equal the Debye length as given by Eqn 1.

From Eqn 3 we have

$$\partial E/\partial n = \frac{e^2 D}{\epsilon}\left[\frac{n}{R_2(R_2+D)} - \frac{(N-n)}{R_1(R_1-D)}\right] \tag{4}$$

and

$$\partial^2 E/\partial n^2 = \frac{e^2 D}{\epsilon}\left[\frac{1}{R_2(R_2+D)} + \frac{1}{R_1(R_1-D)}\right] \tag{5}$$

Thus $\partial^2 E/\partial n^2 > 0$, so that when $\partial E/\partial n = 0$ the energy E is minimum, and corresponds to the most stable value of n. Putting Eqn 4 equal to zero we obtain the equilibrium condition (at which $n = n_0$)

$$\frac{n_0}{(N-n_0)} = \frac{R_2(R_2+D)}{R_1(R_1-D)} \tag{6}$$

and the minimum energy

$$E = E_0 = \frac{N^2 e^2 D}{2\epsilon}\left[\frac{1}{R_2(R_2+D)+R_1(R_1-D)}\right] \tag{7}$$

The ratio of the surface charge densities on the outer and inner surfaces at equilibrium is

$$\left(\frac{\sigma_2}{\sigma_1}\right)_0 = \frac{n_0}{(N-n_0)}\left(\frac{R_1}{R_2}\right)^2 = \frac{R_1(R_2+D)}{R_2(R_1-D)} \tag{8}$$

Eqn 8 shows that at equilibrium the outer surface will always have a greater charge density (*i.e.* a greater density of charged lipids) than the inner surface. The asymmetry increases with decreasing vesicle size and decreasing ionic strength (*i.e.* increasing D). For a flat surface $(\sigma_2/\sigma_1)_0 = 1$.

It is also of interest to know how "stable" is the equilibrium distribution. For a small change in n about the equilibrium value n_0 the energy E may be expressed as a Taylor's expansion

$$E = E_0 + (n-n_0)(\partial E/\partial n)_{n_0} + ½(n-n_0)^2(\partial^2 E/\partial n^2)_{n_0} + \ldots \tag{9}$$

As $(\partial E/\partial n)_{n_0} = 0$, we have, using Eqn 5

$$\Delta E = E - E_0 = (\Delta n)^2\,\frac{e^2 D}{2\epsilon}\left[\frac{1}{R_2(R_2+D)} + \frac{1}{R_1(R_1-D)}\right] \tag{10}$$

where $\Delta n = (n - n_0)$. Thus a change in n_0 by $\pm\Delta n$ will be accompanied by an increase in the electrostatic energy of the vesicle by ΔE.

As an example we may consider vesicles in 150 mM NaCl solution for which the Debye length is $D = 8$ Å at 300 °K. Assuming a bilayer thickness of 45 Å, then for $R_1 = 55$ Å, $R_2 = 100$ Å, we have from Eqn 6: $n_0/(N - n_0) = 4.2$. For $R_1 = 105$ Å, $R_2 = 150$ Å, we have $n_0/(N - n_0) = 2.3$. Thus for vesicles whose outer radii are in the range 100–150 Å we expect that the ratio of the total charges on the outer and inner surfaces to lie in the range 2.3–4.2. This may be compared with the experimental value of approx. 4 of Michaelson *et al.*[1] on vesicles in 150 mM NaCl solution whose outer radii were in the range 100–150 Å. While these authors stressed the relative concentrations of charged phosphatidylglycerol and uncharged phosphatidylcholine molecules on the outer layers, the present analysis suggests that the important ratio is the relative concentrations of charged molecules on the outer and inner layers.

As regards the stability of the equilibrium distribution, putting $R_1 = 55$ Å, $R_2 = 100$ Å, $D = 8$ Å, $\epsilon = 80$, $e = 4.8 \cdot 10^{-10}$ e.s.u. (unit electronic charge) into Eqn 10 we find that when more than 8 charged lipid molecules are transferred from one of the layers to the other the energy involved is in excess of $kT = 4.2 \cdot 10^{-14}$ ergs at 300 °K. Thus if the vesicles consist of 1600 charged phosphatidylglycerol molecules and 1600 phosphatidylcholine molecules (typical values), the equilibrium distribution, including thermal fluctuations, will be 1291 ± 8 charged phosphatidylglycerol molecules on the outside and 309 ± 8 charged phosphatidylglycerol molecules on the inside, while the equilibrium ratio of the surface charge densities on the outer and inner surfaces will be 1.26 ± 0.04. The equilibrium distribution is not affected by a change in the number N or charge e of the charged molecules, but the stability of the equilibrium distribution will rise with increasing e.

The present analysis applies not only to vesicles, but in general to any curved bilayer. Thus if a flat bilayer that initially contains an equal distribution of charged and uncharged lipid molecules on both its layers becomes bulged at some point (Fig. 2A), charged lipid molecules will move towards the outer layer and away from the inner layer of the bulge until an equilibrium is established where the outer and inner surface charge densities on the curved surfaces σ_2 and σ_1, and on the uncurved surfaces σ'_2 and σ'_1, are related by

$$\left(\frac{\sigma_2}{\sigma_1}\right)_0 = \frac{R_1(R_2 + D)}{R_2(R_1 - D)} > 1, \qquad \left(\frac{\sigma'_2}{\sigma'_1}\right)_0 = 1,$$
$$\left(\frac{\sigma_2}{\sigma'_2}\right)_0 = \frac{R_2 + D}{R_2} > 1, \qquad \left(\frac{\sigma_1}{\sigma'_1}\right)_0 = \frac{R_1 - D}{R_1} < 1 \tag{11}$$

i.e. the outer curved surface has the highest charge density, σ_2, and the inner curved surface has the lowest charge density, σ_1, while the two uncurved surfaces have the same charge density, $\sigma'_2 = \sigma'_1$. Such an equilibrium will be established only if the charged lipid molecules can diffuse laterally as well as traverse (flip-flop) from the inner to the outer layer. If there is no flip-flop, but only lateral diffusion within each layer, the equilibrium values of $(\sigma_2/\sigma_1)_0$ and $(\sigma'_2/\sigma'_1)_0$ will be slightly less than those given by Eqn 11. The exact distribution of charge when there is no flip-flop depends on the ratio of the surface areas of the curved and uncurved regions.

In the case of folded bilayers (as in the mitochondrial inner membrane) where the geometry at the fold is cylindrical rather than spherical (Fig. 2B), it may be shown that at equilibrium

$$\left(\frac{\sigma_2}{\sigma_1}\right)_0 = \frac{R_1 \ln[R_1/(R_1 - D)]}{R_2 \ln[(R_2 + D)/R_2]} > 1, \quad \left(\frac{\sigma_2'}{\sigma_1'}\right)_0 = 1,$$
$$\left(\frac{\sigma_2}{\sigma_2'}\right)_0 = \frac{D}{R_2 \ln[(R_2 + D)/R_2]} > 1, \quad \left(\frac{\sigma_1}{\sigma_1'}\right)_0 = \frac{D}{R_1 \ln[R_1/(R_1 - D)]} < 1, \tag{12}$$

where R_1 and R_2 are the inner and outer radii at the fold. For example, when R_1 = 55 Å, R_2 = 100 Å, D = 8 Å, we obtain for the cylindrical geometry $(\sigma_2/\sigma_1)_0$ = 1.12, $(\sigma_2/\sigma_2')_0$ = 1.04, compared with 1.26 and 1.08, respectively, for the spherical cases.

One consequence of the outer layer of a curved bilayer containing a higher surface charge density than the other layers is that we may expect the lipid molecules to be less tightly packed in this layer; this is in addition to molecular packing considerations where the outer curved layer molecules are again expected to be less tightly packed than in the other layers.

The present analysis may be expected to hold whenever $R_1 \gg D$. At low ionic strengths (*i.e.* large D) the electric double layer on the inner aqueous compartment will begin to overlap with itself and a more rigorous theoretical treatment is required.

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