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Small phospholipid vesicles: Internal pressure, surface tension, and surface free energy

(phospholipid bilayer)

STEPHEN H. WHITE

Department of Physiology and Biophysics, University of California, Irvine, California 92717

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ABSTRACT **Tanford [Tanford, C. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3318–3319] used thermodynamic arguments to show that the pressure difference across the bilayer of small phospholipid vesicles must be zero. This paper analyzes the implications of this conclusion in terms of Laplace's law and the basic thermodynamics of interfaces. In its usual form, Laplace's law is of questionable value for the vesicle. If the vesicle is in a state of metastable equilibrium, the surface free energy must be minimal with respect to several thermodynamic variables; the condition $(\partial F/\partial A) = 0$ is not adequate by itself.**

Tanford (1) has raised a question of fundamental importance to understanding the physical chemistry of small phospholipid vesicles of the type first described by Huang (2). He points out that Laplace's law requires the existence of a pressure difference $P_i - P_o$ across a curved surface given by

$$P_i - P_o = 2\gamma/R_s \qquad [1]$$

where γ is the surface tension and R_s is the radius of curvature of the surface. He further notes that the vesicle is permeable to water and resides in a huge excess of water. At equilibrium, the chemical potential of water in the interior cavity of the vesicle must, therefore, be the same as that outside the vesicle. From basic thermodynamics,

$$RT\ln a_w^i + \int_{P_o}^{P_i} \bar{v}\,dP = RT\ln a_w^o \qquad [2]$$

where a_w^i and a_w^o are the activities of water inside and outside the vesicle and $\bar{v}$ is the partial molar volume of water; a_w^i cannot be different from a_w^o in the absence of a solute. It follows that $P_i = P_o$ if the vesicle is permeable to water. Tanford attempted to reconcile this important conclusion with Laplace's law by making assumptions about the surface tensions of the monolayers of the vesicle bilayer. He concluded that either both of the monolayer surface tensions must be zero or that one must be negative while the other is positive. He further suggested that the dynamics of vesicle formation establish which situation prevails.

Tanford's analysis of the vesicle surface tension rests upon two assumptions given that $P_i = P_o$. The first is that Laplace's law in the form of Eq. 1 is applicable to the phospholipid vesicle. The second is that the forces stabilizing the vesicle act to minimize its surface tension (γ). This last assumption supposedly follows from the definition of γ as $(\partial F/\partial A)_{T,V}$, where F is the free energy and A is surface area. The purpose of this paper is to examine the validity of these two assumptions. I will first discuss the nature of surface tension and its relation to the mechanical equilibrium of a curved surface as stated by Laplace's law. I will then present a simple thermodynamic analysis of the vesicle.

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SURFACE TENSION AND LAPLACE'S LAW

Despite the fact that surfaces consist of thin layers a few molecules thick, surface tension is a macroscopic quantity because it is determined by some macroscopic means, such as a measurement of the force exerted by a surface phase on a wire frame or a platinum plate. Surface tension can be defined thermodynamically as $(\partial F/\partial A)_{T,V}$, but a more useful definition for my purposes is that of Bakker (3, 4), given by

$$\gamma = \int_{Z_1}^{Z_2} [\mathbf{P_N}(Z) - \mathbf{P_T}(Z)]dZ. \qquad [3]$$

In this equation, the Z axis is normal to the surface layer, and the integration limits Z_1 and Z_2 include the entire surface phase; γ arises because the layer is anisotropic. Consequently, the pressure within the bilayer is properly a tensor. The pressure at any point Z has been resolved in Eq. 3 into two components: $\mathbf{P_N}(Z)$ normal to the surface and $\mathbf{P_T}(Z)$ tangential to the surface. The molecular details of the surface tension are contained within $\mathbf{P_N}$ and $\mathbf{P_T}$; γ has units of force per unit length and represents an *integrated* property of the whole surface.

Now consider a planar bilayer. The pressure at each side of the surface will be P_o (atmospheric). If the surface is in mechanical equilibrium, $\mathbf{P_N}(Z) = \text{constant} = P_o$, and Eq. 3 becomes

$$\gamma = \int_{Z_1}^{Z_2} [P_o - \mathbf{P_T}(Z)]dZ. \qquad [4]$$

Several points should be made here. First, the surface can be in equilibrium without $\gamma = (\partial F/\partial A)_{T,V} = 0$. This will be shown later. Second, even if $\gamma = 0$, $\mathbf{P_T}(Z)$ could vary in many different ways, the simplest being $\mathbf{P_T} = P_o$. Eq. 4 simply demands that the integrand be zero *on the average* for $\gamma = 0$. Third, any attempt to attribute separate surface tensions to the monolayers of the bilayer as Tanford has done is purely arbitrary and not really meaningful, because γ is a macroscopic quantity.

Tanford defined the vesicle as a three-phase system consisting of (*a*) external solution, (*b*) phospholipid bilayer, and (*c*) internal solution, and he attributed surface tension γ_{ab} to the outer monolayer and γ_{bc} to the inner one. Tanford concluded from Laplace's law that two cases were possible: Either $\gamma_{ab} = \gamma_{bc} = 0$ or $\gamma_{ab}/R_o = -\gamma_{bc}/R_i$ where R_o and R_i are the outer and inner radii of the vesicle. What he has done in effect, is to specify two ways in which $\mathbf{P_T}(Z)$ may vary to cause the macroscopic γ to be zero. There are many other possibilities, however.

Tanford used Laplace's law to arrive at conclusions about γ_{ab} and γ_{bc}. However, Laplace's law is usually applied to macroscopic systems such as soap bubbles, air bubbles in liquids, and droplets of liquid in air. In all these systems, the radius of curvature of the surface is large compared to molecular dimensions. Whether or not Laplace's law can be applied as written in Eq. 1 to vesicles is problematical, since the radius of curvature is of molecular dimensions and about the same size as the thickness

of the surface. The thickness of the surface of a soap bubble is negligible compared to the radius; the surface can consequently be considered as two-dimensional with an easily measured and understood macroscopic surface tension γ.

The vesicle is a microscopic system; its macroscopic surface tension cannot be measured, even though it can be defined formally as in Eq. 3. That is, γ could be calculated if the pressure tensor for the bilayer of the vesicle were known. Laplace's law presents additional difficulties, because the thickness of the bilayer cannot be ignored. One approach to this problem is to divide the surface arbitrarily into a series of nested contiguous surfaces and ascribe a *microscopic* surface tension to each one. The condition of mechanical equilibrium for each microscopic surface is Laplace's law. Fig. 1 shows a schematic drawing of a vesicle in which a microscopic surface σ_{ij} of thickness $r_j - r_i$ is shown. r_j and r_i are chosen so that $r_j - r_i \ll r_i$. By applying Eq. 3, the microscopic surface tension γ_{ij} is defined as

$$\gamma_{ij} = \int_{r_i}^{r_j} [P_N(r) - P_T(r)]dr. \quad [5]$$

For mechanical equilibrium, Laplace's law requires

$$P_N(r_i) - P_N(r_j) = \frac{2\gamma_{ij}}{r_i}. \quad [6]$$

Eq. 6 is formally a complete solution, but without a detailed knowledge of P_N and P_T, it is useless. Tanford proposes that the pressure in the aqueous cavity of the vesicle is the same as outside the vesicle. This places no strictures upon the value of the *macroscopic* tension of the bilayer. The surface tension, γ, can be zero or nonzero as calculated from Eq. 3; Eqs. 5 and 6 could be satisfied in either case. Thus, in considering the vesicle, one has no way of knowing whether γ is zero or not. It would be useful and interesting to know the value of γ for vesicles, but its exact value is not necessary for understanding the vesicles' thermodynamics.

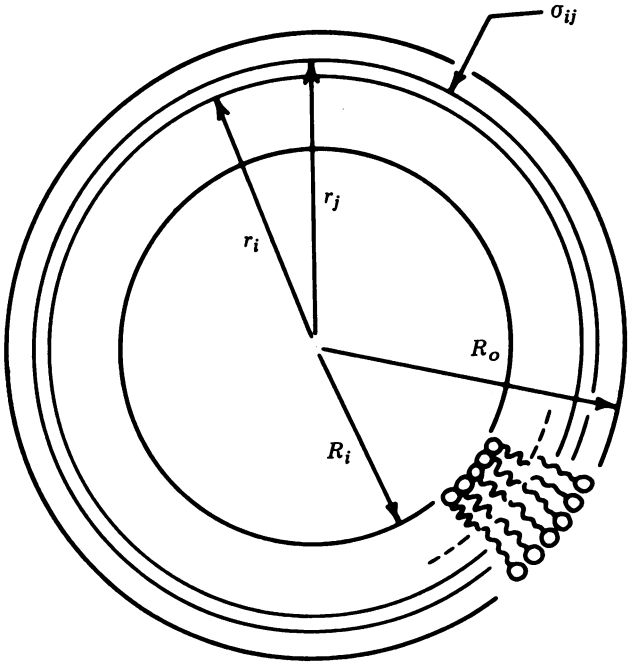

FIG. 1. Schematic drawing of a phospholipid vesicle. The macroscopic surface tension γ will be given by Eq. 5 in which $r_i = R_i$ and $r_j = R_o$. Laplace's law, as stated by Eq. 1, is of questionable validity, since $R_o - R_i$ is the same order of magnitude as R_i or R_o. A condition of mechanical equilibrium for the vesicle, Eq. 6, based on Laplace's law can be defined by considering a *microscopic* surface σ_{ij} whose tension is given by Eq. 5. The formulation does not allow one to draw conclusions about the value of γ without knowing the pressure tensor of the bilayer. It is possible for $\gamma \neq 0$, even though the hydrostatic pressure in the cavity of the vesicle may be the same as the pressure outside the vesicle.

THERMODYNAMIC ANALYSIS OF THE VESICLE

I assume that the phospholipid vesicle is at least in a metastable state of equilibrium in the sense used by Guggenheim (5). Since vesicles have long life times, this is a reasonable assumption. In the following analysis, one should keep clearly in mind that the bilayer has a definite thickness and is not two dimensional. The bilayer is perhaps best described as a *molecular bulk phase*. A statement by Guggenheim (5) should also be kept in mind: "Since the surface layer σ is a material system with a well-defined volume and material content, its thermodynamic properties require no special definition. We may speak of its temperature, Helmholtz function, composition, and so on just as for a homogeneous bulk phase. The only functions that call for special comment are the pressure and the interfacial tension."

Consider the Helmholtz surface free energy (F^σ) of the "surface" which must be distinguished from the surface tension γ. The differential of F^σ is given by Guggenheim (5) as

$$dF^\sigma = -S^\sigma dT - PdV^\sigma + \gamma dA + \sum_i \mu_i^\sigma dn_i^\sigma \quad [7]$$

where S^σ is the surface entropy, A is the area, and V^σ is the volume of the surface. The "surface" should be considered as consisting of the phospholipid plus whatever water (6) associates with it. Since the surface has two components, water (w) and lipid (ℓ), Eq. 7 may be written at constant temperature as

$$dF^\sigma = -PdV^\sigma + \gamma dA + \mu_\ell^\sigma dn_\ell^\sigma + \mu_w^\sigma dn_w^\sigma. \quad [8]$$

At equilibrium, $dF^\sigma = 0$. Therefore, V^σ, γ, A, μ_w^σ, n_w^σ, and n_ℓ^σ are interrelated and must adjust themselves in such a way that $dF^\sigma = 0$. This explains the differences in packing and areas per molecule observed in vesicles (7, 8). The particular size of the vesicle, no doubt, is also a result of this fact. P and μ_w^σ are constants, because the external pressure can be fixed and μ_w^σ must have the value of bulk water. Thus, if the vesicle is at equilibrium, the composition of the bilayer and the molecular interactions of the components must be such that $dF^\sigma = 0$.

Tanford (1) assumes that F^σ minimizes itself only with respect to changes in A. This leads to the conclusion that the surface tension of a planar bilayer must always be zero, since $\gamma = (\partial F/\partial A)_{T,V}$. This is clearly unjustified by Eq. 8 and is not observed experimentally (9). Tanford used the principle of opposing forces (10), which requires an optimum area per molecule in bilayers. He concluded that γ would be positive when A exceeds its optimum value and negative when A is less than its optimum value. This would be reasonable if γ represented the total free energy of the surface and A were the only variable to be adjusted in minimizing free energy. It is clear that γdA represents only part of the free energy, and it adjusts itself relative to other terms to help $dF^\sigma = 0$. Taking $(\partial F/\partial A)_{T,V} = 0$ as the statement of equilibrium is incorrect.

Eq. 8 under equilibrium conditions does *not* require the total surface free energy F^σ to be zero; rather, it requires only that it be minimized. Indeed, with the vesicle being spherical, F^σ is probably not zero because the sphere is the geometric shape that encloses a given volume with the smallest surface area. The vesicle is spherical to insure the lowest possible value of total free energy given by F^σ.

DISCUSSION

The validity of Laplace's law as stated in Eq. 1 is questionable for phospholipid vesicles. Eqs. 5 and 6 are more appropriate; but without knowing the pressure tensor of the bilayer, we can come to no conclusions about the value of γ. We cannot rule out the possibility that $\gamma \neq 0$, even though the hydrostatic pressures of the water inside and outside the vesicle are equal. On the other hand, we cannot rule out the possibility that γ is zero.

Extreme care must be exercised in using the principle of opposing forces (10), which focuses on free energy changes with respect to changes in area per molecule. Many other variables are important in determining the free energy of the system. Eq. 8 includes all of the relevant thermodynamic variables—not the least of which is the chemical potential of the lipid, which must also be considered in the principle of opposing forces. Even though γ has units of energy per unit area, it is best not to view it solely from the point of view of surface free energy. Rather, it should be treated as a force making a special addition to the free energy of the "PdV type". Guggenheim (5) notes that all of the equations of thermodynamics for bulk systems can be applied to surface systems if the PdV term is replaced by $PdV - \gamma dA$; γ is often equated to surface free energy, but this is properly done only for pure liquids (11).

Vesicles have been assumed to be metastable in this paper, because they apparently must be produced by sonication (2) or some other energy-requiring procedure (12–14). An analysis similar to that presented here can also be applied to the multilamellar liposomes that form spontaneously when phospholipids are placed in water above their gel-to-liquid crystal transition temperature. Values of μ_ℓ^σ, V^σ, n_ℓ^σ, n_w^σ, F^σ, and γ can be different from the values for vesicles, even though both structures are acting to minimize F^σ. If F^σ for the vesicle is greater than F^σ for the liposomes, then eventually the vesicles must condense into liposomes. The work of Suurkuusk *et al.* (15) suggests that this is the case.

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