Proc. Natl. Acad. Sci. USA
Vol. 76, No. 7, pp. 3318–3319, July 1979
Biophysics

Hydrostatic pressure in small phospholipid vesicles

(surface tension/phospholipid bilayer)

CHARLES TANFORD

Whitehead Medical Research Institute and Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Contributed by Charles Tanford, April 18, 1979

ABSTRACT **The internal solvent-filled cavity of a single-walled spherical phospholipid vesicle must be at essentially the same pressure as the aqueous medium outside the vesicle. Whether or not the bilayer itself is under elevated pressure cannot at present be determined.**

It is a fundamental principle of surface chemistry (law of Laplace) that the hydrostatic pressure on the two sides of a curved surface between two homogeneous fluids is different (see ref. 1, for example). For a spherical surface of radius R, the relation between the equilibrium pressure p_1 on the concave side and the pressure p_2 on the convex side is given by

$$p_1 - p_2 = 2\gamma/R, \qquad [1]$$

in which γ is the surface tension. Surface tensions being ordinarily positive, the pressure on the concave side is ordinarily higher, and it has therefore been argued that the hydrostatic pressure in the internal space of a small spherical phospholipid vesicle (with radii of order of 100 Å) must be much higher than the external pressure (2, 3).

In fact, the pressures must be the same or nearly so. For phospholipid vesicles formed from neutral lipid molecules in pure water, the pressures must be exactly equal because the vesicle walls are permeable to water (4, 5) and the chemical potential of intravesicular water must therefore be the same at equilibrium as the chemical potential of external water. This condition clearly cannot be satisfied unless the pressures are equal. For phospholipid vesicles formed in salt solutions, the internal and external water potentials must also be the same at equilibrium, but in this case it is conceivable that the vesicles may become sealed in the course of their formation before exact osmotic equilibrium between internal and external space can be established. Because the vesicle wall is essentially impermeable to many ions (6, 7), a small hydrostatic pressure difference may then arise by influx of water to compensate for the osmotic imbalance, but a difference of significant magnitude from this source would seem to be unlikely. Additional factors might alter this conclusion when one is dealing with vesicles made from lipid molecules carrying a net charge.

The condition of equal chemical potential for water does not abolish the validity of the law of Laplace. How can the two conditions be satisfied simultaneously? This question is conveniently examined by considering the system as a system of three phases: (*a*) external solution, (*b*) phospholipid bilayer, and (*c*) internal solution, with two interfaces at the two surfaces of the bilayer. Treatment of the system in this way involves the assumption that there can be no pressure difference at the hydrocarbon/hydrocarbon boundary between the two halves of the bilayer (8, 9). Pressure differences obeying the law of Laplace can exist at the two phase boundaries, but to satisfy the condition that phases *a* and *c* be at the same pressure requires (Eq. 1) that the interfacial tensions γ_{ab} and γ_{bc} be related as

$$\gamma_{ab}/R_o = -\gamma_{bc}/R_i, \qquad [2]$$

in which R_o and R_i are the external and internal radii of the vesicle, respectively. If the surface tensions are not zero, one of the surface tensions must therefore be negative. Alternatively, however, the entire system, including the bilayer phase *b*, can be at the same pressure, with γ_{ab} and γ_{bc} both equal to zero.

Negative surface tensions cannot exist at the interface between two simple homogeneous fluids because the interface disappears when $\gamma = 0$ and the two fluids become miscible (1). This restriction does not apply to the interface between an amphiphile and water because this interface is microscopically inhomogeneous, being composed of regions where hydrocarbon and water are in contact and regions where the contact is between amphiphile head groups and water. There is a variable present here that does not exist at a homogeneous interface—namely, the variable that describes the relative proportions of the hydrophobic and hydrophilic parts of the amphiphile in contact with water, subject to the constraints of the molecular structure of the amphiphile. Minimization of free energy with respect to this variable prevents interpenetration of the adhering fluids. With mixing prevented, thermodynamics of interaction at the surface is governed by two opposing forces (10). One is the result of the hydrophobic effect, which favors minimization of the contact between hydrocarbon and water and thus leads to an increase in surface free energy with *increasing* area. The second force results from repulsion between amphiphile head groups and makes an increasingly positive contribution to the free energy with *decreasing* area. As a result of the interplay between these forces there is an optimal surface area where the overall surface free energy is a minimum. As has been demonstrated (11–13), the drive to attain this optimal surface area dictates the size and shape of micellar aggregates of amphiphiles and is responsible for the fact that diacyl phospholipids exist in aqueous solution in bilayer form rather than as small micelles. Since surface tension is defined as $(\partial F/\partial A)_{T,V}$, in which F is free energy and A is surface area, it is evident that γ will be positive when A exceeds its optimal value and negative when A is less than its optimal value.

In a symmetrical planar bilayer bounded on both sides by water, the surface area is simply a function of the bilayer thickness and will adjust itself to its optimal value, at which point $(\partial F/\partial A)_{T,V} = 0$ and the surface tension therefore vanishes. For egg yolk phosphatidylcholine multilayers separated by layers of intervening water optimal areas of 63–68 Å^2 per molecule have been reported by x-ray diffraction (14, 15), and it has been assumed (8, 9) that a value in the same range applies to an isolated bilayer as well. Chrzeszczyk *et al.* (8) have recently used a combination of NMR and hydrodynamic measurements to determine the exact geometry of small vesicles formed by dipalmitoyl phosphatidylcholine above its phase transition temperature and have observed a large difference

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. § 1734 solely to indicate this fact.

between the area per molecule on the internal and external surfaces. The same method has been applied to egg yolk phosphatidylcholine vesicles by Huang and Mason (9) with similar results: they have reported areas of 74 Å^2 and 61 Å^2 per molecule, respectively, at the outer and inner surface. If one could assume that the relation between surface free energy and area is unaffected by curvature, this result would mean that the surface tension is positive at the outer surface of the vesicle and negative at the inner surface, and by Eq. 1 this would mean that the vesicle bilayer has an elevated internal hydrostatic pressure.

It is, however, unreasonable to assume that curvature and the attendant distortion of lipid hydrocarbon chains have no effect on the relation between surface free energy and surface area. To determine the true surface tensions of the vesicle on the basis of geometry alone would require a sophisticated statistical mechanical analysis that takes into consideration the effect of geometrical packing on hydrocarbon chain conformation and on both polar and nonpolar interactions (16).

From a purely thermodynamic point of view, it would seem that the expected surface tensions must depend on the dynamics of vesicle formation. Vesicles are formed under environmental conditions quite different from those under which they are ultimately stored and characterized—i.e., under high fluctuating pressure if formed by sonication (17) or with other amphiphiles incorporated into the bilayer if formed by other available techniques (18, 19). The expected surface tensions will depend on whether the surface areas reach their effective final values while exchange of phospholipid between the outer and inner surface is still possible. If exchange is possible, each surface is capable of independent free energy minimization and the expected final result would be $\gamma_{ab} = \gamma_{bc} = 0$. The different optimal areas at the two surfaces would then be interpreted as ascribable to the effect of packing geometry on local interactions. If, on the other hand, exchange is impossible, the areas per molecule at the two surfaces are no longer independent because $R_o^3 - R_i^3$ is fixed by the volume of the system. Free energy minimization in the course of pressure relaxation or removal of detergent or alcohol would then be likely to result in different values for γ_{ab} and γ_{bc}, subject to the condition given by Eq. 2.

This work has been aided by discussion with Prof. G. D. Halsey and has been supported by Grant PCM-7615240 from the National Science Foundation. C.T. is the recipient of a Research Career Award of the National Institutes of Health.

1. Adam, N. K. (1941) *The Physics and Chemistry of Surfaces* 3rd ed. (Oxford Univ. Press, New York), pp. 8–9.
2. Sheetz, M. P. & Chan, S. I. (1972) *Biochemistry* **11**, 4573–4581.
3. Lee, Y. & Chan, S. I. (1977) *Biochemistry* **16**, 1303–1309.
4. Finkelstein, A. (1976) *J. Gen. Physiol.* **68**, 127–135.
5. Haran, N. & Shporer, M. (1976) *Biochim. Biophys. Acta* **426**, 638–646.
6. Hauser, H., Oldani, D. & Phillips, M. C. (1973) *Biochemistry* **12**, 4507–4517.
7. Toyoshima, Y. & Thompson, T. E. (1975) *Biochemistry* **14**, 1518–1531.
8. Chrzeszczyk, A., Wishnia, A. & Springer, C. S., Jr. (1977) *Biochim. Biophys. Acta* **470**, 161–169.
9. Huang, C. & Mason, J. T. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 308–310.
10. Tanford, C. (1973) *The Hydrophobic Effect* (Wiley, New York).
11. Tanford, C. (1974) *J. Phys. Chem.* **78**, 2469–2479.
12. Tanford, C. (1978) *Science* **200**, 1012–1018.
13. Israelachvili, J. N., Mitchell, D. J. & Ninham, B. W. (1976) *J. Chem. Soc. Faraday Trans. 2*, **72**, 1525–1568.
14. Reiss-Husson, F. (1967) *J. Mol. Biol.* **25**, 363–382.
15. Levine, Y. K. & Wilkins, M. F. H. (1971) *Nature (London) New Biol.* **230**, 69–72.
16. Nagle, J. F. (1976) *J. Membr. Biol.* **27**, 233–250.
17. Huang, C. (1969) *Biochemistry* **8**, 344–351.
18. Batzri, S. & Korn, E. D. (1973) *Biochim. Biophys. Acta* **298**, 1015–1019.
19. Brunner, J., Skrabal, P. & Hauser, H. (1976) *Biochim. Biophys. Acta* **455**, 322–331.