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Osmoelastic Coupling in Biological Structures: A Comprehensive Thermodynamic Analysis of the Osmotic Response of Phospholipid Vesicles and a Reevaluation of the "Dehydration Force" Theory[†]

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ABSTRACT: A comprehensive thermodynamic analysis of the osmotic response of phospholipid vesicles is presented, using the Gibbs free energy of a vesicle suspension including the elastic contribution of the bilayer membrane. The results indicate that, in addition to the hydrostatic pressure difference across the membrane and the interbilayer pressure due to electrostatic repulsion, the elastic pressure arising from the coupling between the osmotic stress and the elasticity of the membrane (osmoelastic coupling) should participate in the osmotic response of phospholipid vesicles. The data of Cowley et al. [Cowley, A. C., Fuller, N. L., Rand, R. P., & Parsegian, V. A. (1978) *Biochemistry 17*, 3163–3168] and of Parsegian et al. [Parsegian, V. A., Fuller, N., & Rand, R. P. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2750–2754] on the osmotic shrinkage of multilayer vesicles are discussed in terms of the elastic pressure and the interbilayer pressure, and the proposed "dehydration force" theory is reevaluated from the viewpoint of the present analysis.

The heterogeneous distribution of cosolvent molecules in membrane suspensions can create local imbalances in osmolarity. Charged molecules, inorganic as well as organic ions, do not easily penetrate lipid bilayer membranes. The resulting imbalance of osmolarity exerts an osmotic stress across membranes, which causes, for example, swelling or lysis of cells. This effect has been well-known and analyzed in terms of van't Hoff's law. An additional osmotic perturbation arises from the preferential exclusion of macromolecules from the region adjacent to the membrane surface (the exclusion layer). In this case there occurs an imbalance of osmolarity between the outside bulk phase and the exclusion layer. This imbalance of osmolarity exerts an osmotic stress on the membrane. This type of osmotic stress has not been previously recognized. As shown in the present analysis, the osmotic stress may cause an elastic strain in the membrane to produce elastic pressure which counteracts the stress (osmoelastic coupling). When the osmotic stress increases above a certain threshold, the vesicles tend to aggregate tightly in order to avoid the free energy increase due to the elastic strain (osmophobic association) (Yamazaki et al., 1989).

There have been several studies of the behavior of multilayer vesicles in response to the osmotic stress arising from the exclusion of dextran from the vesicle interior (LeNeveu et al., 1976, 1977; Cowley et al., 1978; Lis et al., 1982a,b). In these studies a decrease in the bilayer distance with increased osmotic stress has been measured quantitatively. To analyze the results, it has been assumed that the osmotic stress acts on the phospholipid vesicles in the same way as a mechanical pressure. That is, the interbilayer force should counterbalance the "externally applied osmotic stress acting perpendicular to the bilayer surface", and the surface pressure that compresses the bilayer membrane laterally counterbalances the "osmotic stress acting parallel to the the membrane surface" [see eq 1 and 2 in Parsegian et al. (1979), for example]. However, no comprehensive thermodynamic analysis of the effects of osmotic stress has been carried out yet.

Not only the interbilayer interactions but also the membrane elasticity should participate in the osmotic response of phospholipid vesicles. We present in this paper a comprehensive thermodynamic analysis including the contribution of membrane elasticity to the Gibbs free energy. The results can explain well various types of osmotic response of lipid vesicles. In the case of multilayer vesicles, we show that the osmotic stress should be counteracted by both the pressure between bilayers due to the interbilayer interactions and the elastic pressure arising from the osmoelastic coupling in the membranes. The data of Cowley et al. (1978) and of Parsegian et al. (1979) can be satisfactorily analyzed by the present analysis; the observed small change of interbilayer distance with increased osmotic stress at small distances, <30 Å, can be reasonably explained by the contribution of elastic pressure without any ad hoc assumptions, though it has been previously explained to be due to a strong nonelectrostatic repulsion arising from the "dehydration force" (LeNeveu et al., 1976; Parsegian et al., 1979).

RESULTS

Thermodynamic Formulation of Osmotic Response of Phospholipid Vesicles. In the thermodynamic analysis of a suspension of phospholipid vesicles, the system can be divided into two parts, i.e., the inside and outside of the vesicle, and the bilayer membrane can be treated as a two-dimensional elastic body. Let us consider the flow of water $\mathrm{d}n_w$ from the inside to the outside which is caused by the imbalance of water chemical potential between the two sides. This flow of water increases the Gibbs free energy of the inside by $\mathrm{d}G^i$ and that of the outside by $\mathrm{d}G^o$:

$$\begin{aligned}\mathrm{d}G^i &= n_l f \mathrm{d}S_l - \mu_w{}^i \mathrm{d}n_w \\ &= n_l f \mathrm{d}S_l + P_w{}^i \mathrm{d}V_w\end{aligned}$$

$$\mathrm{d}G^o = -P_w{}^o \mathrm{d}V_w$$

where μ_w is the chemical potential of water represented by P_w

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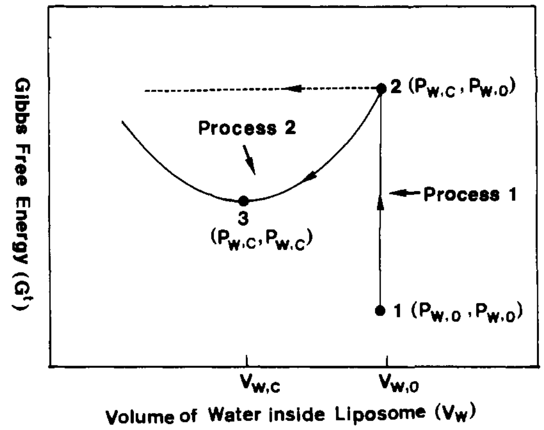

FIGURE 1: Change in Gibbs free energy of a suspension of phospholipid vesicles, G^t, caused by the addition of a cosolvent that does not enter the inside of the vesicles. State 1: Initial equilibrium state in the absence of cosolvent. State 2: State immediately after the addition of cosolvent. State 3: Final equilibrium state in the presence of cosolvent. Each state is characterized by the specific values of chemical potentials of water outside (left in the parentheses) and inside (right) of the vesicle and of the volume of water inside the vesicle (on the abscissa), respectively. The dotted line represents the process in which the vesicle shrinks without any dissipation of free energy.

in pressure units (using our sign convention for the pressure). The superscripts i and o denote the inside and the outside of the vesicle, respectively. n_w and V_w are the moles and the volume respectively, of water inside the vesicle, and n_l is the number of bilayers in the vesicle ($n_l = 1$ for a single-bilayer vesicle and $n_l \geq 2$ for a multilayer vesicle). f represents the surface pressure that compresses the bilayer laterally and S_l the surface area of the bilayer. $f\mathrm{d}S_l$ is thus the elastic contribution of the bilayer membrane to the free energy change. Note the case where the flow of water is induced by an imbalance of water chemical potential caused by preferential exclusion of macromolecules from the region adjacent to the membrane surface. In this case, the region (exclusion layer) should be included in the "inside" part, as discussed later. Then, the change in Gibbs free energy of the whole system $\mathrm{d}G^t$ is

$$\mathrm{d}G^t = n_l f \mathrm{d}S_l + \Delta P_w \mathrm{d}V_w \tag{1}$$

where $\Delta P_w = P_w^{\,i} - P_w^{\,o}$. Applying the Maxwell relation to eq 1

$$(\partial \Delta P_w/\partial S_l) = (\partial^2 G^t/\partial S_l \partial V_w) = n_l(\partial f/\partial V_w)$$

we obtain

$$\begin{aligned}\int \mathrm{d}\Delta P_w &= n_l \int \partial f/\partial V_w \,\mathrm{d}S_l \\ &= n_l(\partial/\partial V_w) \int f \,\mathrm{d}S_l\end{aligned}$$

at V_w. Therefore

$$\begin{aligned}\Delta P_w(S_l,V_w) &= \Delta P_w(S_{l,0},V_w) + \\ &\qquad n_l(\partial/\partial V_w)[F_e(S_l,V_w) - F_e(S_{l,0},V_w)] \\ &= \Delta P_w(S_{l,0},V_w) + n_l \partial \Delta F_e/\partial V_w\end{aligned} \tag{2}$$

where $F_e = \int f\,\mathrm{d}S_l$ and $\Delta F_e = F_e(S_l,V_w) - F_e(S_{l,0},V_w)$.

Initially the system is in equilibrium at state 1 with the equilibrium values of the surface area $S_{l,0}$, the volume of water inside the vesicle $V_{w,0}$, and the chemical potential of water outside the vesicle $P_{w,0}$ (see Figure 1). Addition of a membrane-impermeable compound or a macromolecule excluded from the exclusion layer brings the system to a new equilibrium state (state 3) with the values of $S_{l,c}$, $V_{w,c}$, and $P_{w,c}$. Let us imagine a pathway to reach state 3 via state 2 as shown in Figure 1. Immediately after the addition of the cosolvent, the system moves to state 2 through process 1 without changing V_w and then gradually to state 3 through process 2 with a decrease in V_w. At state 3, the following relation should hold from the equilibrium condition and eq 2:

$$\begin{aligned}\Delta P_w(S_{l,c},V_{w,c}) &= P_w^{\,i}(S_{l,c},V_{w,c}) - P_{w,c} \\ &= \Delta P_w(S_{l,0},V_{w,c}) + n_l \partial \Delta F_e/\partial V_w \\ &= 0\end{aligned} \tag{3}$$

The first term on the right can be written as

$$\begin{aligned}\Delta P_w(S_{l,0},V_{w,c}) &= \Delta P_w(S_{l,0},V_{w,0}) + \\ &\qquad [\Delta P_w(S_{l,0},V_{w,c}) - \Delta P_w(S_{l,0},V_{w,0})] \\ &= P_w^{\,i}(S_{l,0},V_{w,0}) - P_{w,c} + P_w^{\,i}(S_{l,0},V_{w,c}) - \\ &\qquad P_w^{\,i}(S_{l,0},V_{w,0}) \\ &= P_{w,0} - P_{w,c} + P_w^{\,i}(S_{l,0},V_{w,c}) - P_w^{\,i}(S_{l,0},V_{w,0})\end{aligned}$$

since $P_w^{\,i}(S_{l,0},V_{w,0}) = P_{w,0}$. We set

$$C_{osm} = P_{w,0} - P_{w,c} \tag{4}$$

C_{osm} represents the osmolarity of the cosolvent in units of pressure. Substituting eq 4 into eq 3 gives

$$C_{osm} = -n_l \partial \Delta F_e/\partial V_w - [P_w^{\,i}(S_{l,0},V_{w,c}) - P_w^{\,i}(S_{l,0},V_{w,0})] \tag{5}$$

The first term in eq 5 is related to the free energy increase to the elastic deformation caused by the osmotic shrinkage and can be represented as the increase in the elastic pressure ΔP_E ($=P_{E,c} - P_{E,0}$), where the elastic pressure P_E is defined as $-\partial F_e/\partial V_w$ (Flory, 1953). Note that $F_e(S_{l,0},V_{w,c}) \simeq F_e(S_{l,0},V_{w,0})$.

The second term is related to the interbilayer pressure P_R which is defined as the force per unit area acting between bilayer membranes and also to the hydrostatic pressure inside the vesicle P_H, as follows. Using the Gibbs–Duhem equation under the condition that the surface pressure f and temperature T are constant, we can get

$$V_w \mathrm{d}P_w^{\,i} = -V\mathrm{d}P$$

where V is the volume of the vesicle and P is the internal pressure which is conjugated with the change in V. Since the pressure should be represented by the sum of the interbilayer pressure P_R and the hydrostatic pressure P_H

$$\int \mathrm{d}P_w^{\,i} = -\int 1/\phi_w \,\mathrm{d}P = -\int 1/\phi_w \,\mathrm{d}(P_R + P_H)$$

where ϕ_w is the volume fraction of water, V_w/V. Approximating ϕ_w as a constant

$$P_w^{\,i}(S_{l,0},V_{w,c}) - P_w^{\,i}(S_{l,0},V_{w,0}) = -(1/\phi_w)(\Delta P_R + \Delta P_H) \tag{6}$$

where ΔP_R ($=P_{R,c} - P_{R,0}$) and ΔP_H ($=P_{H,C} - P_{H,0}$) represent the individual changes in P_R and P_H caused by the addition of the cosolvent. ΔP_H corresponds to the change in the hydrostatic pressure difference through the membrane induced by the cosolvent. Equation 6 is valid only in the case that the change in ϕ_w is small, but it will be used in the general case for simplicity. The essential points of our arguments in this paper are not affected by the approximation. Finally, eq 5 can be rewritten by using eq 6:

$$C_{osm} = P_E + (1/\phi_w)(P_R + P_H) \tag{7}$$

where the individual values of ΔP_E, ΔP_R, and ΔP_H are represented as P_E, P_R, and P_H by setting those values in the absence of cosolvent as zero, for simplicity.

Response of Phospholipid Vesicles to Various Types of Osmotic Stress. The above thermodynamic analysis leads to a conclusion that the osmotic stress on phospholipid vesicles can be counterbalanced by the elastic pressure P_E, the inter-

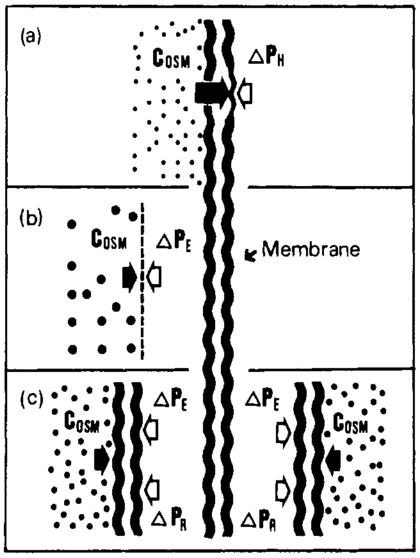

FIGURE 2: Schematic drawing of the osmotic response of phospholipid vesicles. The drawing in each column represents the individual response of the vesicles to the various types of osmotic stress: (a) osmotic stress across membrane, (b) osmotic stress on exclusion layer, and (c) osmotic stress between membranes.

bilayer pressure P_R, and the hydrostatic pressure difference P_H. We discuss the following three particular cases in which one or two of those terms are predominant.

(*1*) *Osmotic Stress Across a Bilayer Membrane.* When a membrane-impermeable small molecule, such as an inorganic ion, is added to a suspension of single-bilayer vesicles, the osmotic stress acts on the interior of the vesicle across the membrane (Figure 2a). There should be no contribution of either elastic pressure or interbilayer pressure in this case. Therefore, the osmotic stress has to be counterbalanced only by the hydrostatic pressure difference (van't Hoff's law):

$$C_{\mathrm{osm}} = (1/\phi_w)P_H = P_H$$

since $\phi_w = 1$.

(*2*) *Osmotic Stress on the Exclusion Layer.* Large molecules such as high molecular weight poly(ethylene glycol) (PEG) should be preferentially excluded from the region adjacent to the membrane surface (exclusion layer) by steric hindrance (Arakawa & Timasheff, 1985; Yamazaki et al., 1989). When such a large molecule is added to a suspension of single-bilayer vesicles, the osmotic stress acts on the exclusion layer (Figure 2b). In this case the osmotic stress should be compensated with the elastic pressure, since a hydrostatic pressure difference cannot be maintained between the exclusion layer and the bulk phase:

$$C_{\mathrm{osm}} = P_E$$

The elastic deformation of the membrane that gives the elastic pressure increases the free energy of the vesicles in suspensions and, above a critical intensity, causes aggregation of vesicles to avoid the free energy increase (osmophobio association) (Yamazaki et al., 1989).

(*3*) *Osmotic Stress Between Bilayer Membranes.* Small molecules, such as glucose or sucrose, can enter the aqueous region between bilayers of multilayer vesicles made by suspending dried phospholipids in an aqueous solution, owing to incomplete vesicular structures; but large molecules, such as dextran or PEG, cannot enter there, because of steric hindrance (LeNeveu et al., 1977). When such a large molecule is added to a suspension of multilayer vesicles, the osmotic stress acts between the bilayer membranes (Figure 2c). It should be compensated by the interbilayer pressure and/or the elastic pressure, since the vesicle cannot hold the hydrostatic pressure difference owing to the incomplete vesicular structure:

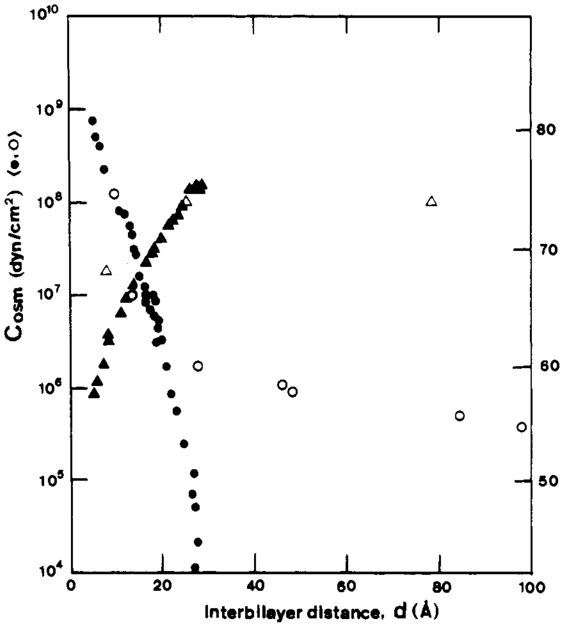

FIGURE 3: Osmotic stress C_{osm} and surface area per phospholipid molecule S_l as functions of interbilayer distance d of multilayer vesicles. Data for multilayer vesicles of egg yolk phosphatidylcholine (●, ▲) and of egg yolk phosphatidylcholine containing 10 mol % phosphatidylglycerol (○, △) were taken from Parsegian et al. (1979) and Cowley et al. (1978), respectively.

$$C_{\mathrm{osm}} = P_E + (1/\phi_w)P_R \qquad (8)$$

There have been several studies of the response of multilayer vesicles to the osmotic stress induced by the addition of dextran (LeNeveu et al., 1977, 1978; Cowley et al., 1978; Parsegian et al., 1979; Lis et al., 1982a,b). In these studies the interbilayer distance d and the surface area per phospholipid molecule S_l have been determined as a function of the osmotic stresses. Their data for neutral (Parsegian et al., 1979) and charged phospholipids (Cowley et al., 1978) are redrawn in Figure 3. In the absence of osmotic stress, the interbilayer distance for charged vesicles ($d > 200$ Å) was much larger than that for a neutral one ($d \simeq 30$ Å), owing to the electrostatic repulsion. The osmotic stress caused a decrease in d. For the charged vesicle, the decrease was very rapid until $d \simeq 30$ Å without changing S_l; then, it became much slower with a concomitant decrease in S_l. For the neutral vesicle, the osmotic stress caused only a small decrease in d, accompanied by a significant decrease in S_l, as observed for the charged vesicles at $d < 30$ Å.

According to eq 8, the osmotic stress on multilayer vesicles should be compensated by the elastic pressure P_E and/or the interbilayer pressure P_R. The latter is due to the increase in the interbilayer forces such as electrostatic interaction, and the former is due to the increase in the free energy of deformation of the membrane. Hence, the observed rapid decrease in the interbilayer distance without a change in the surface area S_l should increase P_R without any significant change in P_E, and the remarkable decrease in S_l with a negligible change in d should cause an increase predominantly in P_E. Therefore, the following relations should hold in the osmotic response of multilayer vesicles shown in Figure 3:

$$C_{\mathrm{osm}} = (1/\phi_w)P_R \text{ at } d > 30 \text{ Å}$$

$$C_{\mathrm{osm}} = P_E \text{ at } d < 30 \text{ Å}$$

the charged vesicle and

$$P_R = 0$$

$$C_{osm} = P_E$$

for the neutral vesicle. The interbilayer pressure P_R thus acts only in the case of the charged vesicles. It indicates that only the electrostatic repulsive force, which is due to the surface charge of vesicle membranes, should contribute to P_R.

The values of S_l vs d for egg yolk phosphatidylcholine multilayer vesicles shown in Figure 3 enable us to estimate the surface pressure f. From the relations that

$$\begin{aligned} C_{osm} &= P_E \\ &= n_l \partial \Delta F_e / \partial V_w = n_l(\partial \Delta F_e / \partial S_l)(\partial S_l / \partial V_w) = \\ &\qquad n_l f(\partial S_l / \partial V_w) \end{aligned}$$

$$f = (1/n_l)(\partial V_w / \partial S_l) C_{osm} \tag{9}$$

f can be approximated as

$$f \simeq (1/n_l)(\Delta V_w / \Delta S_l) C_{osm} \simeq (S_l \Delta d / \Delta S_l + d) C_{osm} \tag{10}$$

since $\Delta V_w \simeq n_l(S_l \Delta d + d \Delta S_l)$. The calculated value of f is 10–12 dyn/cm in the presence of C_{osm} of 10^7 dyn/cm^2. The f value should correspond to the surface pressure which decreases the surface area of egg yolk phosphatidylcholine *bilayer* membrane from 75 to 69 Å^2/molecule. We can compare the f value to the surface pressure of a *monolayer* at the oil–water interface, the surface pressure of which should be half of that bilayer. As expected, the calculated surface pressure of the bilayer is nearly twice the reported value of ~6 dyn/cm for the surface pressure in a monolayer of dioleoylphosphatidylcholine, which is needed to compress the surface area in the same range (Yue et al., 1976).

DISCUSSION

A comprehensive thermodynamic analysis of the osmotic behavior of phospholipid vesicles has been presented in this paper. The contribution of membrane elasticity is explicitly considered, adding the elastic term of $f\mathrm{d}S_l$ to the Gibbs free energy. To be more exact, the elastic term should be represented as $[f - (l_z/S_l)f_p]\mathrm{d}S_l$, where f and f_p are the elastic forces acting parallel and perpendicular to the membrane surface, respectively, and l_z is the thickness of the membrane. However, the contribution of f_p is neglected here, since the bilayer membrane should be treated as a two-dimensional elastic body. The present analysis indicates that three kinds of pressures may participate in the osmotic response: elastic pressure arising from the elastic deformation of the bilayer, interbilayer pressure arising from interbilayer interactions, and a hydrostatic pressure difference across the bilayer membrane.

The participation of hydrostatic pressure difference in the swelling or shrinkage of cells or lipid vesicles in hyper- or hypotonic solutions, respectively, has been well recognized. The hydrostatic pressure difference, which counteracts the osmotic stress across the membrane, produces a mechanical imbalance on the membrane, and it should be compensated by a change in the surface pressure of the membrane. The cells or lipid vesicles, therefore, swell or shrink to change the surface pressure by increasing or decreasing the surface area of membranes. The vesicular structure of the membrane will be mechanically broken when the change in the surface area of the membrane exceeds a threshold above a critical osmotic stress (osmotic lysis).

The participation of the elastic pressure has been documented for the first time in our study of the mechanism of the PEG-induced aggregation of phospholipid vesicles (Yamazaki et al., 1989). The osmotic stress which arises from the preferential exclusion of large molecules such as PEG from the region adjacent to the membrane surface is not compensated by the hydrostatic pressure difference, since the pressure difference cannot be held mechanically. It is compensated by the elastic pressure that arises from the elastic strain in the membranes by the osmotic stress (osmoelastic coupling). The elastic strain has been observed as a decrease in the membrane fluidity of the phospholipid vesicle caused by the addition of a high molecule weight PEG (Yamazaki et al., 1989). This strain causes an increase in the free energy of the vesicle in the suspended state, and when the free energy exceeds a threshold, the vesicles tend to aggregate tightly (osmophobic association). The osmoelastic coupling and osmophobic association have been observed in other biological systems such as globular proteins, actin filaments, and red blood cells (Ito et al., 1987; Ito, Yamazaki, and Ohnishi, unpublished results).

The participation of the interbilayer pressure has been emphasized by Parsegian and his co-workers in their analysis of the shrinkage of multilayer liposomes caused by high molecular weight polymer, dextran (LeNeveu et al., 1976, 1978; Lis et al., 1982). In their analysis "osmotic pressure" P is defined as the force corresponding to the osmotic stress C_{osm} in our analysis, and the free energy increase g due to the shrinkage of liposomes is assumed to be equal to the work of transfer of water ΔV_w from the bulk phase to the multilayer lattice against osmotic pressure P; that is, $\Delta g = -P\Delta V_w$. This equation implies that the osmotic shrinkage of lipid vesicles is a reversible process that is not accompanied with dissipation of free energy of the system (dotted line in Figure 1) (Parsegian et al., 1979). To discuss it more quantitatively, let us consider the change in the Gibbs free energy of the total system $\mathrm{d}G^t$ caused by the movement of water from the inside to the outside $\mathrm{d}V_w$, using the "free energy of vesicles" g and the osmotic pressure P. The change in the Gibbs free energy at *state 2* in Figure 1 can be represented by

$$\mathrm{d}G^t = \mathrm{d}g + P\mathrm{d}V_w$$

since P is equal to the chemical potential difference of water between the inside and outside, ΔP_w, at that state. Their assumption that $\Delta g = -P\Delta V_w$ gives

$$\partial G^t / \partial V_w = \partial g / \partial V_w + P = 0$$

Therefore, the movement of water caused by the imbalance of the chemical potential of water (osmotic shrinkage) is not accompanied by dissipation of the free energy of the total system. This is quite unrealistic and might violate the second law of thermodynamics. The osmotic shrinkage should be accompanied by dissipation of free energy (solid line in Figure 1), since it is an irreversible diffusion process which gives rise to entropy production in the system.

From their treatments as mentioned above, LeNeveu et al. (1976, 1977), Cowley et al. (1978), and Parsegian et al. (1979) came to the conclusion that the osmotic stress is counterbalanced by the interbilayer pressure, i.e., $C_{osm} = P_R$, in contrast to our conclusion that it should be counterbalanced by the interbilayer pressure and also the elastic pressure, i.e., $C_{osm} = P_E + (1/\phi_w)P_R$. To explain the results shown in Figure 3, they introduced the two forces which contribute to the interbilayer pressure P_R, i.e., electrostatic repulsive force P_{elec} and the hypothetical "dehydration force" P_{dh}:

$$P_R = P_{elec} \text{ at the interbilayer distance } d > 30 \text{ Å}$$

$$P_R = P_{elec} + P_{dh} \simeq P_{dh} \text{ at } d < 30 \text{ Å}$$

P_{elec} is the force due to the surface charge of the vesicle membrane. Dehydration force P_{dh} is defined as the force to transfer 1 mol of water from the "hydration layer" near the membrane surface to the bulk phase. It is assumed to act at

$d < 30$ Å and to be much stronger than P_{elec} at these distances. On the other hand, our analysis can reasonably explain the results without any hypothetical force. That is, P_{elec} is the only force to contribute to P_{R}, and the observed small change in the interbilayer distance against the osmotic stress at $d < 30$ Å is due to the contribution of the elastic pressure P_{E}, not due to P_{R}. Furthermore, using the relation that $C_{\mathrm{osm}} = P_{\mathrm{E}}$ for the data of egg yolk phosphatidylcholine vesicles at $d < 30$ Å, we could estimated the value of the surface pressure of the bilayer, which agreed with that obtained from the monolayer experiments. This result strongly suggests that the dehydration force does not contribute to the interbilayer pressure at all.

In addition to the interbilayer pressure, the modulus of compressibility of bilayer membrane has also been studied by the osmotic stress methods. Assuming that the surface pressure f should counterbalance the "osmotic stress acting parallel to the bilayer surface", Parsegian and his co-workers derived the relation that $f = C_{\mathrm{osm}}d/2$ and estimated the modulus of compressibility from the relation that $C = f(S_{\mathrm{l},0}/\Delta S_{\mathrm{l}})$, where C is the modulus of compressibility, $S_{\mathrm{l},0}$ is the surface area per lipid molecule in the absence of the osmotic stress, and ΔS_{l} is the osmotic stress induced change in the surface area (Parsegian et al., 1979; Lis et al., 1982). However, according to the present analysis, the relation $f = C_{\mathrm{osm}}d/2$ cannot hold, and instead, eq 9 that $f = (1/n_{\mathrm{l}})(\partial V_{\mathrm{w}}/\partial S_{\mathrm{l}})C_{\mathrm{osm}}$ should hold in neutral vesicles. To determine the value of f by eq 9, we need the values of S_{l} as a function of the interbilayer distance d (see eq 10). As estimated above, the f value needed to change S_{l} from 75 to 69 Å^2 in egg yolk phosphatidylcholine membranes is 10–12 dyn/cm, and so, the modulus of compressibility of the membranes is calculated to be 130–150 dyn/cm. The value is coincident quite well with that obtained by Kwok and Evans (1981), 140 dyn/cm for egg yolk phosphatidylcholine vesicles with the radius > 10 μm.

McIntosh and Simon (1986) reported that the lateral compression of bilayer membrane estimated by the "Fourier synthetic method" was more insensitive to the osmotic stress than those estimated by the "gravimetric method" by Parsegian et al. (1979). They suggested that the largest source of the discrepancy may be attributed to the inaccuracy of the gravimetric estimation of the volume fraction of phospholipids in the membranes. However, the reevaluation of the data of Parsegian et al. in the paper of McIntosh and Simon, in which the surface pressure f was estimated according to $f = C_{\mathrm{osm}}d/2$, should be inaccurate, since $f = (1/n_{\mathrm{l}})(\partial V_{\mathrm{w}}/\partial S_{\mathrm{l}})C_{\mathrm{osm}}$ holds in neutral phospholipid vesicles as discussed above. This equation indicates that the compression of membranes of multilayer vesicles with larger n_{l} should be more insensitive to the osmotic stress, and hence in order to compare the osmotic compressibility of membranes of multilayer vesicles prepared separately, the bilayer number n_{l} should be taken into consideration. In fact, using a spin-labeling method, we have detected much larger membrane compression caused by the osmotic stress in single-bilayer vesicles than in multilayer ones (Yamazaki et al., 1989).

Marra and Israelachvili (1985) have estimated interbilayer interactions mechanically, using bilayers adsorbed to mica plates. Their data have been considered complementary to those estimated by the osmotic stress methods. However, as analyzed in this paper, the applied osmotic stress does not counterbalance interbilayer forces directly, whereas the applied mechanical force should. Hence, the experimental results obtained by each individual method cannot be directly compared to each other in principle.

The osmotic stress method has been applied in other systems as well as phospholipid vesicles, and it has been suggested that the dehydration force might act between parallel DNA double helices or muscle fibers, etc. (Rau et al., 1984; Millman et al., 1983). However, our results show that almost all the data should be reexamined according to the present analysis, and the rationality of the dehydration force concept should be fundamentally reevaluated.

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