Heat Changes in Lipid Membranes under Sudden Osmotic Stress†

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ABSTRACT: Closed lipid vesicles act as osmometers increasing or decreasing their volume under the influence of osmotic gradients. The enthalpy changes accompanying membrane compression or expansion have not been measured yet, and first results obtained with high-sensitivity titration calorimetry are reported here. Phospholipid vesicles suspended in and in equilibrium with an electrolyte or nonelectrolyte with a defined initial concentration of c_i, were injected into a solution with a final concentration of c_f, and the heat changes were monitored with a titration microcalorimeter. Osmotic compression ($\Delta c = c_f - c_i > 0$) produced an exothermic heat change with $\Delta H \approx -500 \pm 100$ cal/mol and osmotic expansion ($\Delta c < 0$) an endothermic heat change with $\Delta H \approx 1000 \pm 200$ cal/mol; both results normalized to a concentration gradient of $|\Delta c| = 1$ M NaCl. The heats of compression and expansion varied linearly with the lipid content and the size of the osmotic gradient but were independent of the vesicle size. The cubic thermal expansion coefficient α_v which equals $(1/V)(\partial V/\partial T)_p$ could be derived and was found to be 1.25×10^{-3} and 2.5×10^{-3} K^{-1} for the compressed and expanded bilayer vesicles, respectively. The entropy changes associated with compression and expansion could be estimated. Compression of the membrane led to a negative entropy change and increased the hydrocarbon chain order. Expansion of the membrane was accompanied by a positive entropy change which can be explained, in part, by more disordered hydrocarbon chains. Vesicle expansion and compression thus appear to be asymmetric as far as the thermodynamic driving force is concerned.

Biological membranes are much more permeable for water than for most other molecules and are therefore highly sensitive to osmotic gradients. When exposed to a medium of lower osmolarity, cells start to swell, and when placed into a hyperosmotic environment, they shrink. Large volume changes are however intolerable for biological organisms, and additional mechanisms are immediately activated upon osmotic stress which dampen the effect of the osmotic gradient and bring back the cell volume close to its starting state. Inorganic ions and small organic molecules are used as compensatory osmolytes; i.e. the composition of the cytoplasm is changed by the removal or accumulation of ions (K^+, Cl^-, or HCO_3^-), organic polyols, or amino acids [cf. Häussinger (1996)].

The specific signals which trigger the cell volume response are not known. However, one may hypothesize that the mechanical disturbance of the cell membrane, i.e. the tension change in the plane of the membrane, could constitute the start signal proper. Pressure effects on biological systems are well-documented for excitable membranes, in particular the reversal of anesthesia by application of a sufficiently large hydrostatic pressure (Spyropoulos, 1957; Kendig & Cohen, 1977; Frank & Lieb, 1982). Another example is mechanosensitive ion channels, first discovered in specialized mechanoreceptor cells, where the open probability of the channel depends strongly on the stress at the membrane [cf. Morris (1990)].

A common model system for studying the elastic properties of membranes is phospholipid dispersions in buffer. The bilayer arrangement in such dispersions depends on the method of preparation. The lipid dispersions can be multilamellar or unilamellar, and they can also vary considerably in size. Under defined conditions, closed lipid vesicles may act as osmometers, changing their volume according to the osmotic gradient (Lichtenberg & Barenholz, 1988; de Gier, 1993). In early studies, only multilamellar lipid vesicles (MLVs)[1] were used, and the enclosed volume was estimated from the encapsulated amount of bilayer impermeable probes (Bangham et al., 1967). More recently, osmotic swelling and shrinking have also been observed for unilamellar lipid vesicles using phase contrast microscopy (Boroske et al., 1981) or dynamic light-scattering techniques (Sun et al., 1986; Rutkowski et al., 1991; Hallett et al., 1993). The analysis of osmotic swelling with dynamic light scattering has provided quantitative insight into the elastic properties of the lipid vesicle membrane (Rutkowski et al., 1991). The elastic area compressibility was in good agreement with that measured with the micropipette aspiration technique, commonly considered to be the standard method for determining membrane elastic properties (Kwok & Evans, 1981; Bloom et al., 1991). Since the elastic properties of the lipid membrane are intimately connected to the free energy, these measurements allow an estimate of the free energy change induced upon membrane expansion. However, knowledge of the elasticity parameters alone does not provide insight

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[1] Abbreviations: POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; MLV, multilamellar lipid vesicle; SUV, small unilamellar vesicle; NMR, nuclear magnetic resonance.

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into the thermodynamic basis of elasticity, i.e. whether membrane elasticity is enthalpic or entropic in origin. This can only be decided if the heat changes accompanying membrane expansion or compression can also be measured.

We have therefore decided to monitor osmotic swelling or shrinking with a recently developed high-sensitivity titration calorimeter (Wiseman et al., 1989). Vesicles were prepared at a defined osmolyte concentration and were injected into a solution with a different concentration. The concentration gradient induced either vesicle compression or vesicle expansion, and the associated heats of reaction were detected in the calorimeter cell. Both exothermic and endothermic heat changes were measured depending on the experimental conditions. They were studied systematically as a function of the lipid concentration, the magnitude and direction of the osmotic gradient, the size of the lipid vesicles, and the chemical nature of the osmolytes. The experimental results could then be analyzed in terms of thermodynamic models providing (i) the thermal expansion coefficients of membrane volume, area, and thickness and (ii) the enthalpy and entropy changes associated with expansion and compression.

MATERIALS AND METHODS

Materials. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids (Birmingham, AL) and was used without further purification. All other chemicals were purchased at highest purity from commercial sources.

Preparation and Characterization of Lipid Vesicles. For vesicle preparation, 100 μL of a chloroform solution of POPC ($\sim$10 mg/mL) was filled into a 5 mL round-bottomed flask and was dried down into a thin film under a stream of nitrogen gas. Residual organic solvent was removed by applying high vacuum for at least 12 h. Typically, 100 μL of osmolyte solution of the desired concentration was added, and the lipid suspension was vortexed and subjected to at least six freeze−thaw cycles in order to establish an equilibrium distribution of osmolyte solution in the multilamellar vesicles.

Small unilamellar vesicles (SUVs) were prepared by sonication of the lipid suspension for 20−40 min until an almost clear solution was obtained (at 4 °C under a nitrogen atmosphere). Metal debris of the titanium tip was removed by centrifugation in an Eppendorf centrifuge (10 min at 3000 rpm). Sonication produces vesicles with an average diameter d of $\sim$30 nm (cf. below).

Unilamellar vesicles with a diameter d of $\sim$100, $\sim$200, and $\sim$400 nm were prepared by extrusion of multilamellar phospholipid dispersions through polycarbonate filters (Mayer et al., 1986).

The phospholipid concentration in the vesicle preparations was determined by measuring the phosphorus content according to Böttcher (1961).

The vesicle size was characterized with electron microscopy. Vesicle suspensions were absorbed onto electron microscopy grids covered with carbon-coated collodium films after treatment by glow discharge at low pressure in air. The grids were washed with water and negatively stained with 0.75% uranyl formate. Images were recorded on Agfa Scientia EM R 200 Film at a magnification of 8000 (400 and 200 nm vesicles), 12 500 (100 nm vesicles), and 31 500 (sonified vesicles) using a Zeiss EM 910 transmission electron microscope operated at 80 kV. Vesicle diameters were measured by hand using the NIH Image 1.59b1 program running on a Macintosh Centris 650. Sonicated vesicles exhibited a non-Gaussian size distribution. The major fraction had an average diameter $d \approx 25 \pm 11$ nm. However, vesicles with a diameter larger than 150 nm were also found.

Unilamellar vesicles prepared by the extrusion method also showed a non-Gaussian distribution of sizes. The distribution was rather homogeneous for 100 and 200 nm vesicles with average diameters of 150 $\pm$ 50 and 220 $\pm$ 80 nm, respectively. The 400 nm vesicles were characterized by a very heterogeneous size distribution, with a large fraction having a d of 250 $\pm$ 150 nm and additional vesicles with a d of $\geq$1000 nm.

The lipid concentration (c_{lipid}) varied between $\sim$7 and $\sim$44 mM (6−35 mg/mL).

High-Sensitivity Titration Calorimeter. Heats of reaction were measured with a Microcal MC-2 high-sensitivity titration calorimeter (MicroCal, Northampton, MA; Wiseman et al., 1989) as described previously. Solutions were degassed under vacuum prior to use. The calorimeter was calibrated electrically. The data were acquired by computer using the Origin software developed by MicroCal.

RESULTS

Lipid vesicles were prepared in three different osmolytes, namely NaCl, urea, and saccharose. As an example, Figure 1 shows titration experiments with sonified lipid vesicles in different NaCl gradients. In all three experiments, the lipid vesicles were composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and were sonified in 100 mM NaCl. Figure 1A−C summarizes control experiments. Ten microliters of a 100 mM NaCl solution is injected into a 10, 100, or 900 mM NaCl solution contained in the calorimetric cell. The injection of 100 mM NaCl into 10 mM NaCl gives rise to an exothermic reaction; the same injection into 900 mM NaCl produces an endothermic reaction. Integration of the areas under the titration peaks yields the heat of dilution. The latter is not exactly constant throughout a titration experiment but varies slightly with consecutive injections since the NaCl concentration in the calorimeter cell is continuously increased or decreased. Figure 1D−F displays the corresponding osmotic experiments. Sonified POPC lipid vesicles, prepared in 100 mM NaCl, were injected into the same three NaCl concentrations mentioned above. For an isotonic injection, the heats of mixing with and without lipid were virtually identical within 2 μcal/injection. However, under conditions of osmotic swelling, i.e. injection into 10 mM NaCl, the measured heat was less exothermic with lipid than without it, i.e. $h_i = -10$ μcal with lipid vs $h_i = -27$ μcal without lipid. Hence, lipid swelling produced an endothermic enthalpy Δh of +17.5 μcal. In contrast, if 10 μL of a 100 mM NaCl solution were injected into 900 mM NaCl, the heat of mixing was endothermic with an h_i of +1000 μcal (Figure 1C). The same experiment performed with lipid vesicles yielded an h_i of 915 μcal (Figure 1F), and the difference, $\Delta h = -85$ μcal, was exothermic. Lipid compression thus leads to an exothermic reaction enthalpy.

Qualitatively similar results were observed when the vesicles were prepared with other concentrations of NaCl or with urea or saccharose; i.e. in all cases studied, osmotic

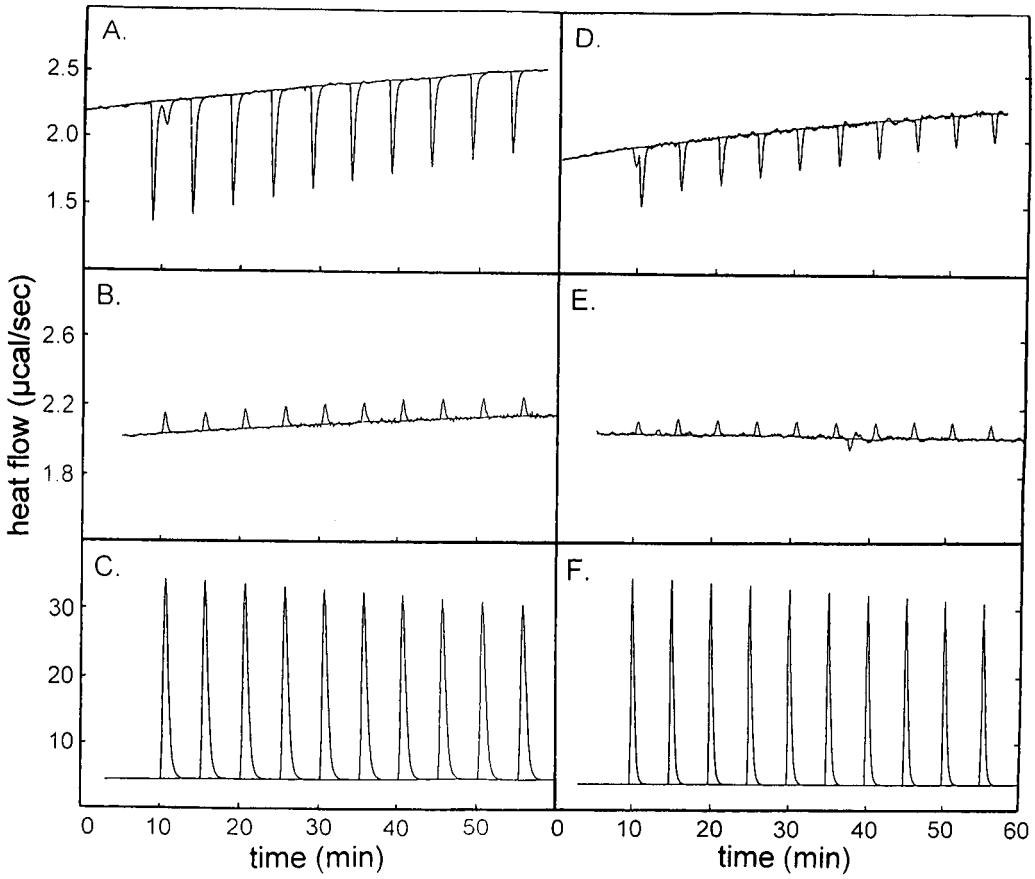

FIGURE 1: Titration calorimetry of vesicle expansion and vesicle compression. Control measurements were made as follows. Ten microliters of 100 mM NaCl was injected into 1.2788 mL of (A) 10 mM NaCl, (B) 100 mM NaCl, and (C) 900 mM NaCl. Lipid experiments were carried out as follows. Sonified lipid vesicles were prepared by sonication in 100 mM NaCl. Ten microliters of the phospholipid dispersion was injected into 1.2788 mL of (D) 10 mM NaCl, (E) 100 mM NaCl, and (F) 900 mM NaCl. The area under the titration peaks yields the heats of dilution/reaction. The difference of D − A is Δh_i which equals 17.5 μcal and corresponds to the heat of expansion. The difference of F − C yields $\Delta h_i = -85$ μcal and is the heat of compression.

shrinking was associated with an *exothermic* heat change whereas osmotic *swelling* was accompanied by an *endothermic* heat change.

For a systematic investigation of the osmotically induced heat changes, we have first measured the influence of lipid concentration. Sonified lipid vesicles were prepared in either 10 or 100 mM NaCl and injected into 100 or 10 mM NaCl solutions, respectively. The lipid concentration was varied by about a factor of 3. Figure 2 shows the measured excess enthalpy as a function of the lipid concentration. Inspection of Figure 2 allows two conclusions. (1) For both types of osmotic gradients, the measured excess heat depends linearly on the total lipid concentration; (2) the exothermic excess heat of shrinking is smaller by about a factor 2 than the endothermic heat of swelling. The enthalpies shown in Figure 2 correspond to 10 μL injections. From the slopes of Figure 2, a molar excess heat of −40.6 cal/(mol of lipid) is evaluated for osmotic shrinking and +81.3 cal/(mol of lipid) for osmotic swelling with a gradient Δc of 0.09 M NaCl. Referred to an osmotic gradient Δc of 1 M NaCl, the corresponding enthalpy changes are $\Delta H = -451$ cal/(mol of lipid) and $\Delta H = +903$ cal/(mol of lipid) for shrinking and swelling, respectively. This normalization neglects osmotic coefficients.

In a third series of experiments, the size of the osmotic gradient was systematically varied. The results obtained with sonified POPC vesicles and NaCl gradients are summarized in Figure 3. The excess heat per mole of lipid is

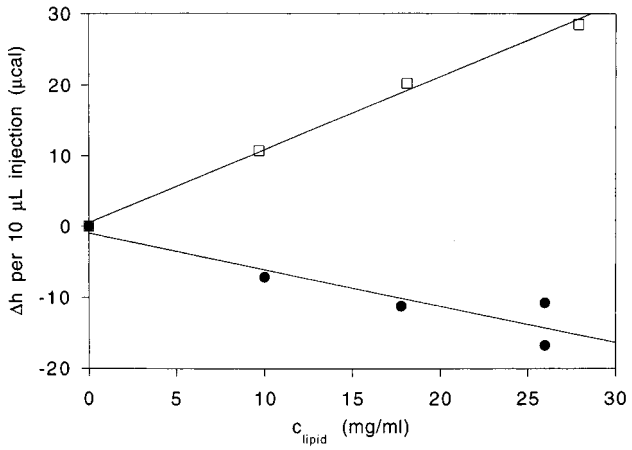

FIGURE 2: Heats of compression or expansion as a function of lipid concentration. (□) Vesicle expansion. Vesicles were prepared by sonication in 100 mM NaCl. Ten microliters of the vesicle suspension was injected into 1.2788 mL of 10 mM NaCl. (●) Vesicle compression. SUVs were prepared in 10 mM NaCl. Ten microliters of the vesicle suspension was injected into 1.2788 mL of 100 mM NaCl.

plotted vs the concentration difference, $\Delta c = c_f - c_i$, where c_f is the final osmolyte concentration in the calorimeter cell and c_i the initial concentration inside the lipid vesicle. Negative Δc values induce a volume increase of the vesicles through the uptake of water; positive Δc values induce a decrease due to loss of water. Vesicles with a c_i of 100 mM NaCl (c_i = 900 mM NaCl) were used for osmotic

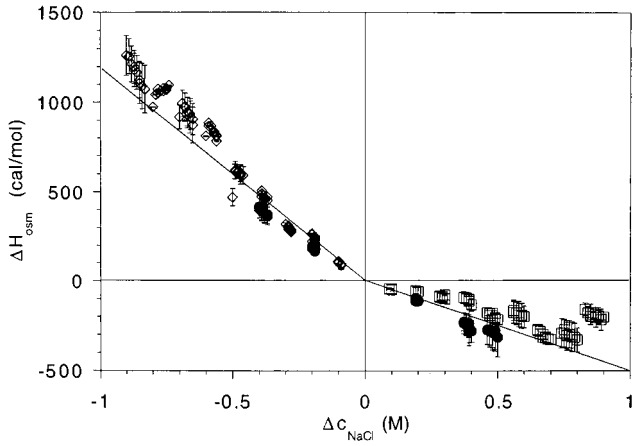

FIGURE 3: Heats of vesicle compression or expansion as a function of osmotic gradient. $\Delta c_{NaCl} = c_f - c_i$. The case where $\Delta c_{NaCl} > 0$ leads to compression and is associated with exothermic ΔH values. The case where $\Delta c_{NaCl} < 0$ produces osmotic swelling and leads to endothermic ΔH values. Vesicles were prepared in (◇) 900 mM NaCl, (●) 500 mM NaCl, and (□) 100 mM NaCl.

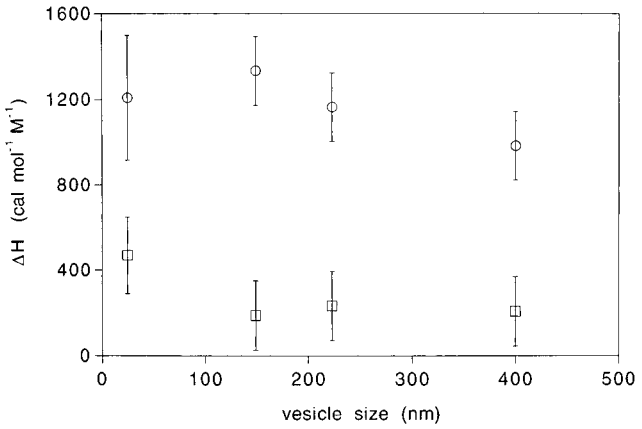

FIGURE 4: Osmotic compression and osmotic shrinking as a function of vesicle size. All enthalpy values (ΔH) are extrapolated to an osmotic gradient of 1.0 M NaCl and refer to 1 mol of POPC. (○) osmotic expansion and (□) osmotic compression.

shrinking (swelling). Lipid vesicles with a c_i of 500 mM NaCl were employed in both shrinking and swelling experiments. The following conclusions can be derived from Figure 3. (i) The heat of expansion is endothermic and that of compression exothermic. (ii) The heat of expansion (compression) increases (decreases) with the concentration gradient $|\Delta c|$ across the membrane. (iii) The heat of expansion is larger than the heat of compression. (iv) Limiting the discussion to the concentration range between −0.5 and 0.5 M NaCl, we approximate the experimental data by straight lines with slopes of −1200 and −510 cal M^{-1} per mole of lipid for osmotic swelling and shrinking, respectively. The discontinuity of the slopes at the origin has no theoretical meaning.

Analogous experiments were performed with lipid vesicles prepared by extrusion through polycarbonate filters with 100, 200, and 400 nm pore sizes (Figure 4). The results obtained with extruded vesicles were qualitatively similar to those found for vesicles prepared by sonication; quantitatively, the heats of swelling and shrinking were slightly reduced with slopes of −930 and −300 cal M^{-1} (per mole of lipid), respectively. Within the accuracy of the measurement, no systematic differences were detected between the three types of extruded vesicles.

Osmotic expansion and compression experiments were also performed with nonelectrolytes such as urea and saccharose. For compression experiments, POPC was sonified either in pure water or in 0.2 M urea. Aliquots (10 μL) of the vesicle suspension were injected into 0.2 or 0.5 M urea solutions. After the correction for dilution effects, an exothermic heat of compression was measured which varied linearly with the magnitude of the urea gradient (data not shown). The heat of compression was evaluated as $\Delta H = -200$ cal/mol normalized to a gradient Δc of 1 M urea. This result is comparable to $\Delta H = -255$ cal/mol observed for a NaCl gradient of the same osmolarity, i.e. 0.5 M NaCl (cf. Figure 3). (For a given concentration gradient, Δc, the osmotic pressure produced by a nonelectrolyte is half of that of a 1-1 electrolyte.) The analogous compression experiment performed with saccharose yielded a ΔH of −140 cal/(mol of POPC) for a Δc of 1 M saccharose.

Expansion experiments were performed with POPC vesicles prepared by sonication of POPC in 0.2 M saccharose. Injection of these vesicles into pure water caused vesicle expansion. The measured heats of expansion were endothermic. Different lipid concentrations were employed, and the heat of expansion was found to be proportional to the lipid content (data not shown). The heat of expansion was calculated as $\Delta H = +360$ cal/(mol of POPC) for a saccharose gradient Δc of 1 M. This value is distinctly lower than $\Delta H = +600$ cal/(mol of POPC) measured for a NaCl gradient of the same osmolarity ($\Delta c = 0.5$ M) (cf. Figure 3) and demonstrates that the measured heats of reaction are dependent not only on the size of the gradient but also on the chemical nature of the osmolyte involved.

DISCUSSION

The permeability of lipid membranes to water is high with a permeability coefficient $P_{H_2O} \approx 3 \times 10^{-3}$ cm/s (cf. Finkelstein (1987) and Jansen and Blume (1995)]. For a vesicle with a diameter of 30 nm, an osmotic equilibrium is established within less than 1 ms. The osmotic pressure across the membrane, Δp, is related to the concentration gradient, Δc, according to

$$\Delta p = 2\Phi_{osm} RT \Delta c \qquad (1)$$

The factor 2 accounts for the dissociation of NaCl, and Φ_{osm} is the osmotic coefficient, describing the deviation from ideality.[2] The efflux or influx of water changes the volume of the lipid vesicles and, simultaneously, the dimensions of the lipid bilayer proper. An increase in the vesicle volume requires a larger lipid surface area which, in turn, reduces the membrane thickness. Depending on the extent of the area change, ΔA, and thickness change, Δl, the total volume, V, of a single lipid molecule may also vary, since

$$\frac{\Delta V}{V} = \frac{\Delta A}{A} + \frac{\Delta l}{l} \qquad (2)$$

Thermodynamic Analysis. The lipid bilayer together with its hydration sphere can be considered a homogeneous physical phase, the state of which is completely determined by the two intensive variables p and T. The titration

[2] For the nonelectrolytes saccharose and urea, the osmotic pressure is given by $\Delta p = \Phi_{osm} RT \Delta c$.

experiment proceeds essentially under adiabatic conditions. We therefore consider the variation of the entropy, S, with temperature T and pressure p [cf. Landau and Lifshitz, (1991)]:

$$dS = \left(\frac{\partial S}{\partial T}\right)_p dT + \left(\frac{\partial S}{\partial p}\right)_T dp = \frac{C_p}{T} dT - \alpha_V V_0 dp \quad (3)$$

Here C_p is the molar heat capacity of the bilayer phase, V_0 its molar volume, and α_V is the cubic thermal expansion coefficient defined as

$$\alpha_V = \frac{1}{V_0}\left(\frac{\partial V}{\partial T}\right)_p \quad (4)$$

In an adiabatic experiment, no heat exchange occurs with the environment. With the adiabatic condition $dS = 0$, eq 3 leads to

$$C_p \Delta T = \alpha_V V_0 T \Delta p \quad (5a)$$

$$\Delta Q = \alpha_V V_0 T \Delta p \quad (5b)$$

Combining eq 5 with eq 1 yields the final result

$$\Delta Q = 2\alpha_V V_0 R T^2 \Phi_{osm} \Delta c \quad (6)$$

(if the International System of Units units are used, Δc must be measured in moles per cubic meter). ΔQ is the heat change measured in the titration calorimeter (normalized to 1 mol of lipid). The osmotic gradient $\Delta c > 0$ for osmotic compression and $\Delta c < 0$ for expansion.

Volume Expansion Coefficient α_V. The effect of osmotic shrinking is moving of the lipid molecules closer together and induction of a tighter membrane packing. The titration experiments demonstrate that osmotic shrinking is accompanied by an exothermic reaction, i.e. an increase in temperature, and the thermal expansion coefficient must be positive. For the reverse experiment, i.e. osmotic swelling, the effect of a negative osmotic gradient is expansion of the vesicle surface and weakening of the lipid packing. Under these conditions, an endothermic reaction, i.e. a decrease in temperature, is observed. A negative osmotic gradient, $-\Delta p$, is associated with a negative temperature change, $-\Delta T$, and α_V is again positive.

Equation 6 can be used to evaluate the thermal expansion coefficient α_V of the lipid phase from the measured ΔQ. The lipid volume, V_0, was estimated as follows. POPC has a molecular mass of 760 Da. About 10−20 water molecules constitute the hydration sphere. Taking an upper limit of 20 H_2O, the effective molecular mass is 1120 Da. With a lipid density ρ of 0.987 mL/g, the final lipid volume $V_0 \approx 1.105 \times 10^{-3}$ m^3/(mol of POPC).

In evaluation of Figure 2, it should be noted that the osmotic coefficient of a 0.1 M NaCl solution is $\Phi_{osm} = 0.93$, and that of 0.01 M NaCl is $\Phi_{osm} = 1.0$. For osmotic shrinking, the slope of Figure 2 yields a ΔQ of −40.6 cal/(mol of lipid) at 293 K, and application of eq 6 leads to an α_V of 1.27×10^{-3} K^{-1}. For osmotic swelling, the corresponding analysis results in an α_V of 2.54×10^{-3} K^{-1}. Likewise, Figure 3 yields an α_V of 1.25×10^{-3} K^{-1} for the osmotic compression of 30 nm vesicles and an α_V of 3.0×10^{-3} K^{-1} for their osmotic expansion.

Dilatometric measurements of multilamellar lipid dispersions composed of DPPC or related lipids with saturated hydrocarbon chains have led to a thermal expansion coefficient α_V of $\sim 1.0 \times 10^{-3}$ K^{-1} for the liquid-crystalline phase (Nagle & Wilkinson, 1978). A more recent measurement of multilamellar vesicles composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine reports an α_V of 1.2 ($\pm$0.2) $\times 10^{-3}$ K^{-1} (Böttner & Winter, 1993). The calorimetric α_V value derived from osmotic compression is in excellent agreement with these results.

Unexpectedly, osmotic expansion produces α_V values which are larger by a factor 2−3. Obviously, osmotic shrinking and expansion of unilamellar vesicles are not symmetric since osmotic expansion is accompanied by a distinctly larger heat change than osmotic shrinking. Molecular models for this divergence will be discussed below.

Linear Expansion Coefficient α_l *and Area Expansivity* α_A. The linear thermal expansion coefficient α_l describes the variation of the membrane thickness, l, with temperature and is defined as $\alpha_l = (1/l)\,(\partial l/\partial T)_p$. It has been measured with high accuracy for a number of different bilayers using deuterium NMR (Seelig & Seelig, 1974; Schindler & Seelig, 1975; Lafleur et al., 1990) and X-ray diffraction (Luzzati & Husson, 1962). α_l was found to vary between zero and approximately -3.0×10^{-3} K^{-1} depending on the system and the temperature range investigated. A typical value for a fluid lipid bilayer 20 °C above its phase transition is $\alpha_l \approx -1.8 \times 10^{-3}$ K^{-1}. α_l is negative since the fatty acyl chains contract with increasing temperature, a phenomenon known from rubber elasticity.

Knowledge of the temperature coefficients of both bilayer volume and bilayer thickness finally allows the calculation of the area expansivity $\alpha_A = (1/A)(\partial A/\partial T)_p$ since the three thermal expansion coefficients are related to each other according to

$$\frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p = \frac{1}{A}\left(\frac{\partial A}{\partial T}\right)_p + \frac{1}{l}\left(\frac{\partial l}{\partial T}\right)_p \quad (7a)$$

$$\alpha_V = \alpha_A + \alpha_l \quad (7b)$$

Insertion of $\alpha_V = 1.2 \times 10^{-3}$ K^{-1} (osmotic shrinking) and $\alpha_l = -1.8 \times 10^{-3}$ K^{-1} (deuterium NMR) into eq 7 leads to an area expansivity α_A of $+3.0 \times 10^{-3}$ K^{-1}. The thermal expansion coefficient of large unilamellar vesicles composed of egg lecithin has been measured previously with the pipette aspiration technique (Kwok & Evans, 1981). The method allows a direct observation of the area increase as a function of temperature at a constant lateral tension σ. An expansion coefficient $\alpha_{A,\sigma}$ of 2.4×10^{-3} K^{-1}was observed which is in broad agreement with the above results.

Under conditions of osmotic expansion, the cubic thermal expansion coefficient of lipid vesicles is distinctly larger, i.e. $\alpha_V = 2.5 \times 10^{-3}$ K^{-1}. Together with $\alpha_l = -1.8 \times 10^{-3}$ K^{-1}, this leads to $\alpha_A = 4.3 \times 10^{-3}$ K^{-1}. For a critical appraisal of this result, it should be noted that the linear expansion coefficient α_l is known only for multilamellar lipid dispersions but not for single-walled vesicles. It can be expected that $|\alpha_l|$ is smaller for an expanded lipid vesicle than for a flat membrane. In the extreme, assuming $\alpha_l = 0$ for expanded vesicles, this leads to $\alpha_A = \alpha_V \approx 2.5 \times 10^{-3}$ K^{-1}, predicting an increase in the volume and the surface area without a corresponding thinning of the membrane.

Energy Considerations. The interpretation of thermodynamic measurement in terms of molecular mechanisms is difficult. In the following, two models are suggested which shed light on different molecular aspects of the measured ΔH values.

The change in the *free energy*, ΔG, of a compressible fluid or solid under the influence of a pressure Δp is given by

$$\Delta G = {}^1/_2 \chi_V (\Delta p)^2 V_0 \tag{8}$$

Here χ_V is the isothermal volume compressibility defined as

$$\chi_V = -\frac{1}{V}\left(\frac{\partial V}{\partial p}\right)_T \tag{9}$$

The isothermal compressibility of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) has been determined with volumetric measurements using a high-pressure cell (Böttner & Winter, 1993). For multilamellar dispersions of DMPC in the liquid-crystalline phase at 30 °C, χ_V was found to be 14 ($\pm$2) $\times$ 10^{-5} bar^{-1} [=1.4 $\pm$ (0.2) $\times$ 10^{-9} m^2/N]. This value is similar to the compressibility of organic fluids such as dodecane ($\chi_V = 1.0 \times 10^{-9}$ m^2/N; Cutler et al., 1958).[3] An isotropic *compression* of lipid vesicles in an osmotic gradient of $\Delta c = 1$ M NaCl (corresponding to $\Delta p = 4.95 \times 10^6$ N/m^2 $\approx$ 50 atm, assuming ideal behavior) leads to a change in the free energy ΔG of only 11 J/mol ($\approx$3 cal/mol). Very little elastic energy can be stored in the lipid vesicle upon compression. With ΔG close to zero, it follows that $\Delta H \approx T\Delta S$. Since vesicle compression is exothermic ($\Delta H < 0$), the entropy term is negative. Vesicle compression is thus associated with a decrease in entropy. This could be explained by a more ordered conformation of the hydrocarbon chains in the compressed state. The influence of pressure on hydrocarbon chain ordering has been measured recently with deuterium magnetic resonance by Peng et al. (1995) and by Bonev and Morrow (1995). Both studies demonstrate an increase in chain order and a lengthening of the hydrocarbon chains. An increase in membrane thickness upon application of hydrostatic pressure was also deduced from measurements of the membrane capacitance (Aldrige & Bruner, 1985) and from X-ray diffraction studies (Braganza & Worcester, 1986).

A different model is suggested for vesicle *expansion*. Dynamic light scattering spectroscopy has been applied to monitor size changes in vesicles exposed to transmembrane gradients (Rutowski et al., 1991; Ertel et al., 1993; Hallett et al., 1993). Only a limited swelling of phospholipid vesicles was observed with an increase in radius of no more than 6%. For large concentration differences Δc, osmotic swelling was accompanied by solute leakage, shown by a release of fluorescent dye from the vesicle interior (Ertel et al., 1993). Dye leakage began when the extravesicular medium had an osmolarity approximately 300 mosM lower than the initial osmolarity of the vesicle lumen (1500 mosM) and was linearly proportional to the osmotic gradient. These measurements indicate that the expansion of lipid vesicles beyond a certain threshold is accompanied by pore formation or related processes which make the membrane leaky for medium-sized molecules.

Osmotically expanded membranes not only are characterized by a large thermal expansion coefficient α_V but also exhibit a high compressibility χ_V. In fact, light scattering measurements yield bulk moduli of $K \approx 10^7 - 10^8$ N m^{-2} (Rutkowski et al., 1991). By definition, $K = \chi_V^{-1}$ and the isothermal compressibility observed by vesicle *expansion* is thus $\chi_V \approx 10^{-7} - 10^{-8}$ m^2 N^{-1}, i.e. 1$-$2 orders of magnitude larger than discussed above for vesicle *compression*. A similar conclusion is reached from an analysis of the bilayer expansivity as measured with the micropipette aspiration technique (Kwok & Evans, 1981; Evans & Kwok, 1982; Needham & Nunn, 1990; Bloom et al., 1991).

Assuming a compressibility of $\chi_V = 10^{-7}$ m^2 N^{-1}, the change in free energy can be estimated with eq 8 as $\Delta G \sim$ 330 cal/mol.[4] The corresponding enthalpy change in vesicle expansion is *endothermic* with $\Delta H \sim 1000$ cal/mol, yielding $T\Delta S \sim 670$ cal/mol. This crude calculation suggests that vesicle expansion is accompanied by an increase in entropy. Since the vesicle area increases, the hydrocarbon chains must adopt a more disordered conformation which, in part, could explain the gain in vesicle entropy.

The thermodynamic driving force for membrane elasticity thus appears to be different for compressed and expanded lipid vesicles. Compressed vesicles relax to equilibrium due to a gain in entropy and expanded vesicles due to a gain in enthalpy. Hence, membrane elasticity is an asymmetric process as far as the thermodynamics of compression and expansion are concerned.

The molecular interpretation of the entropy changes exclusively in terms of hydrocarbon chain order or disorder must be considered a first approximation only. For this model to be correct, identical ΔH values are to be expected for a given osmotic gradient, independent of the chemical nature of the osmolyte. However, quantitative differences in the heat of expansion and compression were observed for NaCl, urea, and saccharose gradients, suggesting that a change in the water structure at the lipid$-$water interface could also be involved in the elastic process. Moreover, the lipid composition of the bilayer membrane may be equally important. The role of hydrocarbon chain order/disorder could be investigated, for example, by incorporating cholesterol into the lipid membrane since this molecule is known to stiffen the hydrocarbon chains of a fluid membrane. Even more important would be osmotic experiments in the

[3] The adiabatic compressibility, χ_s, is defined according to $\chi_s = -(1/V)(\partial V/\partial p)_s$. The relation between χ_s and the isothermal compressibility, χ_v, is $\chi_s/\chi_v = C_v/C_p$, where C_v and C_p are the heat capacities at constant volume and pressure, respectively (Guggenheim, 1967). Since $C_v \approx C_p$ for fluids and solids, the two compressibities are virtually identical. χ_s has been derived from ultrasonic studies of lipid bilayers (Mitaku et al., 1978; Aruga et al., 1985). The authors report a bulk modulus of $K = 2.2 \times 10^{10}$ dyn/cm^2 for unilamellar DPPC vesicles at 50 °C. By definition, the bulk modulus, K, is χ^{-1}, yielding $\chi_s = 0.45 \times 10^{-10}$ m^2/N. This value is smaller by a factor of 2 than the compressibility determined by the volumetric method.

[4] An even higher estimate of ΔG can be derived directly from the area compressibility χ_A. In this two-dimensional model, the elastic energy, ΔG, can be estimated according to $\Delta G = {}^1/_2\, \chi_A (\Delta\sigma)^2 A_0$ [cf. Evans and Waugh (1977), Evans and Hochmuth (1978), and Bloom et al. (1991)]. χ_A is the area compressibility, $\Delta\sigma$ the surface tension produced by the osmotic gradient, and A_0 the molar bilayer area. For DOPC vesicles, χ_A was determined by light scattering as $\chi_A \approx 2$ m/N (Rutkowski et al., 1991). $\Delta\sigma$ depends on the vesicle radius R according to $\Delta\sigma = \Delta pR/2$. With an osmotic gradient of $\Delta c = 1$ M NaCl, the elastic energy of 100 nm vesicles is estimated to be $\Delta F = 780$ cal/mol.

presence of membrane-bound proteins since these could provide insight into pressure-induced trigger mechanisms.

In conclusion, membrane compression is accompanied by an entropy decrease, an exothermic heat change, and a small thermal expansion coefficient, whereas membrane expansion leads to an entropy increase, an endothermic heat change, and a large thermal expansion coefficient.

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