

Mechanical Calorimetry of Large Dimyristoylphosphatidylcholine Vesicles in the Phase Transition Region[†]

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ABSTRACT: Micromechanical tests have been used to determine the equilibrium changes in area of a dimyristoylphosphatidylcholine (DMPC) bilayer vesicle as produced by variable bilayer tensions at discrete temperatures in the range of 20–30 °C. The micromechanical tests involved aspiration of a large ($>2 \times 10^{-3}$ cm diameter) vesicle with a small suction pipet ($\sim 8 \times 10^{-4}$ cm diameter). Because the internal volume was held fixed by the 100 mM sucrose solution, the tension in the vesicle membrane (created by the pipet suction pressure) caused a displacement of the vesicle projection inside the pipet that was proportional to the increase in total area of the vesicle. From the tension–area isotherms, the elastic compliance (area compressibility) of the membrane was determined throughout the temperature range of the main liquid–crystal–crystalline phase transition of DMPC. Similarly, the area–temperature relations at constant membrane tension were obtained. These data yielded values for the elastic area compressibility in the liquid state (~ 30 °C) of 0.007–0.008 cm/dyn, in the solid state (~ 20 °C) of less than 0.001 cm/dyn, and in the coexistence region (~ 24.2 °C) of 0.03–0.05 cm/dyn. Likewise, the thermal area expansivity (i.e., fractional change in area

with change in temperature at constant membrane tension) values ranged from $(4\text{--}6) \times 10^{-3}/^\circ\text{C}$ in the liquid state to $0.1\text{--}0.2/^\circ\text{C}$ in the coexistence region to $(5\text{--}8) \times 10^{-4}/^\circ\text{C}$ in the solid state. A significant feature of the area–temperature observations was the *absence* of a pretransition area change in the range of 10–15 °C. The only area transition that was seen occurred at about 24 °C; the area increase from the solid to liquid state was 12–13% (comparable to the value deduced from X-ray diffraction measurements for the relative area change from the $L_{\beta'}$ to L_{α} phases). We have shown that a simple convolution of an idealized first-order phase transition with a Gaussian, transition temperature, dispersion function gives excellent correlation with the observed data. The convolution approach coupled with the “Clausius–Clapeyron” equation for the membrane surface provided the means to derive the thermal properties of the transition from the elastic compliance vs. temperature data; this is a form of mechanical calorimetry. The results of the analysis gave an expectation value for the transition temperature of 24.2 °C, the statistical width of the transition of 0.3 °C, and the heat of the transition of about 7 ± 0.7 kcal/mol.

Order–disorder phase transitions of the acyl chains of synthetic phospholipids in aqueous media have been studied with many techniques: X-ray diffraction (Tardieu et al., 1973; Janiak et al., 1976, 1979), volume dilatometry (Melchior & Morowitz, 1972; Nagle & Wilkinson, 1978), differential scanning calorimetry (Hinz & Sturtevant, 1972; Mabrey & Sturtevant, 1976), lateral diffusivity of molecular probes (Fahey & Webb, 1978; Derzko & Jacobson, 1980), etc. With the exception of diffusivity measurements on individual vesicles, all of these studies have dealt with lipid dispersions; there have been no direct observations on single bilayer vesicles. This distinction is important for two reasons: (1) the proximity of adjacent bilayer structures in multilamellar forms may alter the transition properties; (2) it is not possible to control the stresses in all of the bilayer surfaces in a dispersion. The latter is the consequence of the anisotropic structure of lipid bilayers. The bilayer is at best isotropic in its surface plane but anisotropic with respect to its thickness coordinate. As such, the smallest set of state variables that must be controlled includes the temperature, hydrostatic pressure, and bilayer tension. Hence, there are two equations of state that give the volume and area per molecule, respectively.

The significance of the surface equation of state¹ is readily apparent when we observe the liquid–crystal–crystalline phase transition in large single bilayer vesicles ($>10^{-3}$ cm in diameter). As is known from X-ray diffraction measurements, this transition is characterized by a relatively large (15–25%) lipid

surface area condensation in comparison to the lipid volume reduction (3–5%). Thus, a spherical bilayer vesicle must lose 20–30% of its internal volume in order to pass completely through the transition. If the aqueous solution contains salt or sugar to which the bilayer is impermeable, the bilayer becomes stressed when water is forced out of the vesicle interior as the lipids attempt to condense. The result is an incomplete area condensation. In fact, it is possible to prevent the transition if the osmotic strength of the aqueous solution is high; eventually, the vesicle will break when the temperature is lowered sufficiently. This observation is a surface “Clausius–Clapeyron” effect; i.e., the bilayer tension results in a lower freezing temperature with a proportionality factor determined by the properties of the transition. In this paper, we will demonstrate the dependence of the phase transition temperature on bilayer tension, evaluate the thermodynamic properties of the transition in a single bilayer structure (i.e., heat of transition, fractional area change, and transition temperature), and compare the results with published data on lipid multilayers.

Our study deals with the area–temperature relation for dimyristoylphosphatidylcholine (DMPC, Avanti Biochemicals) at different states of bilayer tension over a temperature range of 20–30 °C. For fully hydrated systems, X-ray diffraction (Janiak et al., 1976, 1979) and calorimetry (Mabrey & Sturtevant, 1976) studies have shown that the main liquid–crystal–crystalline transition occurs in the range of 23–25 °C with a secondary (pre-) transition near 13 °C. The latter is ascribed to a change in angle of the frozen acyl chains with

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¹ The significance of the surface equation of state has long been recognized by theorists. Selected references to theory are discussed later.

respect to the bilayer surface, accompanied by a change from planar to "rippled" surface structure. The X-ray data of Janiak et al. (1979) show about a 6% decrease in projected area as the temperature is raised above the pretransition (13 °C) and about a 20% increase in projected surface area as the temperature is raised above the main transition at 23–25 °C. Thus, the net increase in lipid surface area would be about 12% if the temperature was raised from below 10 to above 25 °C.

Our approach has been to use micromechanical tests to determine the equilibrium change in vesicle area produced by a change in bilayer tension at discrete temperatures concentrated in the range of 20–30 °C. From this data, we derive the isothermal elastic compliance of the bilayer surface as a function of temperature. Correlation of the compliance vs. temperature measurement with an analysis based on a Clausius–Clapeyron equation provides the heat of the main transition at about 24 °C. The method as such is a form of mechanical calorimetry.

Experimental Procedures

The procedure for preparation of large phospholipid vesicles was adapted from that of Reeves & Dowben (1969). First, 0.1 mL of DMPC (dissolved in 10:1 chloroform–methanol at a concentration of 100 mg/mL) was added to a small glass tube. The solvent was evaporated by passing argon gas over the sample. Residual solvent was removed by placing the sample in vacuo for 24 h. A total of 15 mL of buffer (0.1 M sucrose) was then added to the sample, and the sample was allowed to swell at 45 °C for 1 h. Finally, the dispersion was swirled gently and centrifuged at 10 000 rpm for 15 min to remove undispersed lipids. The supernatant was used for the vesicle experiments.

Vesicles were injected (in very small concentration) into a microchamber that was mounted on the stage of an inverted microscope. The chamber temperature was controlled to better than 0.1 °C over a range of 5–50 °C and was monitored by a small thermocouple in the microchamber itself. A long working length objective was used to avoid heat conduction from the chamber. Temperature, pipet suction pressure, and time were simultaneously recorded on video tape with video multiplexing. A micrometer eyepiece was always in the video microscope image in order that the system could be calibrated at the time of recording. A Hoffman phase optical system was employed to enhance the vesicle image. Glass micropipets [(1–10) $\times 10^{-4}$ cm inner diameter] were pulled from 0.1-cm glass tubing to a needle point and then broken by quick fracture to the desired tip diameter. Accurate measurement of the tip inner diameter was obtained from the insertion depth of a tapered microneedle. The tapered microneedle was calibrated with a scanning electron microscope.

The micromechanical test involved aspiration of a large vesicle ($>2 \times 10^{-3}$ cm diameter) with a small pipet [(~ 8 – 10) $\times 10^{-4}$ cm diameter]. The suction pressures ranged from 10 to 10^4 dyn/cm². Because of the osmotic strength of the suspending buffer, the internal volume of a vesicle remained essentially constant when the vesicle was aspirated. Hence, the displacement of the vesicle projection inside the pipet was proportional to the change in area of the vesicle [see Kwok & Evans (1981) for details of the vesicle mechanical test].

As noted previously, a spherical vesicle with sufficient osmotic strength in the aqueous environment is prevented from reducing its surface area in order to pass through the transition. Thus, it was necessary to remove volume from the vesicle interior to provide enough excess area (over that of a sphere of equivalent volume) to accommodate the area condensation

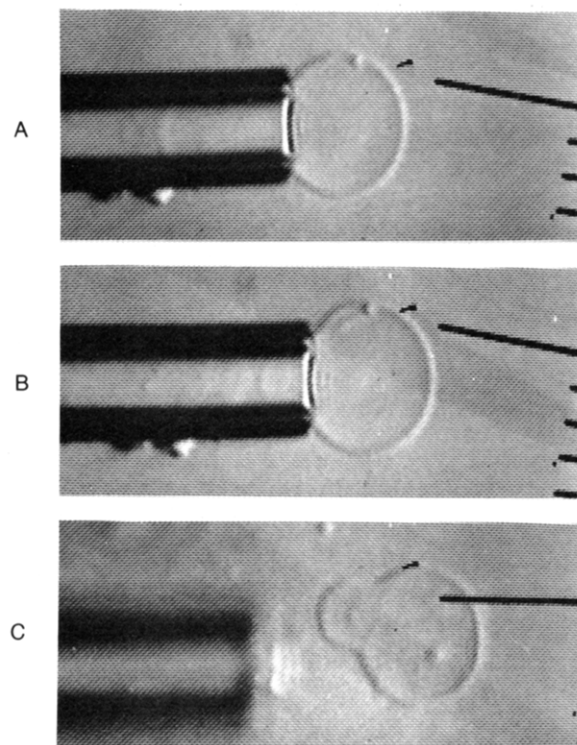


FIGURE 1: Video recordings of micropipet aspiration of a large DMPC bilayer vesicle. The projection of the vesicle inside the pipet is proportional to the excess surface area over that of a sphere with the same internal volume. The pipet suction pressure was constant and the vesicle was recorded (A) at a temperature (~ 26 °C) above the phase transition and (B) at a temperature (~ 21 °C) below the transition. Note the reduction in projection length that represents the area condensation (since the volume was held constant by the 100 mM sucrose solution). For demonstration of the rigidity of the bilayer below the transition temperature, the frozen vesicle was expelled from the pipet (C). Each division on the micrometer scale is equal to about 4×10^{-4} cm. The image was enhanced by a Hoffman interference contrast optical system.

without membrane stress buildup. This was accomplished by segmenting the microchamber into two regions separated by a small air gap. One side contained the vesicle suspension at 0.1 M sucrose and the other side contained 0.13 M sucrose solution (30% higher osmotic strength). A single vesicle was selected with one micropipet and inserted into a larger caliber pipet; then, the stage was translated to transfer the pipet assembly to the second side, and the vesicle was withdrawn from the larger (shielding) pipet. With the vesicle held by the small pipet, the volume was reduced by the osmotic transfer of water until equilibrium was established as evidenced by the stationary position of the vesicle projection inside the pipet. If the vesicle was expelled from the pipet above the transition temperature (with the excess area), it broke up immediately because the surface was in the liquid state and lacked rigidity. However, when the vesicle was held against the pipet wall by a slight pressure, the vesicle could be observed for hours without breakup. An example of an initially dehydrated vesicle is shown in Figure 1A; the long projection inside the pipet indicates the excess area. Subsequent to freezing the acyl chains of the vesicle membrane, the vesicle projection retracted in proportion to the area condensation as is shown in Figure 1B. In this state, the vesicle membrane was solid (rigid), and the vesicle could be expelled from the pipet without breakup as shown in Figure 1C.

Finally, single bilayer vesicles were selected by the procedure described by Kwok & Evans (1981). First, the most transparent (thinnest wall appearance in the interference contrast

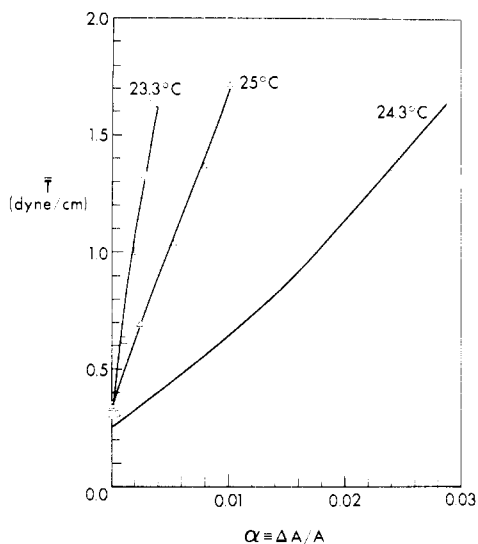


FIGURE 2: Vesicle bilayer tension vs. fractional increase in surface area at three fixed temperatures: above, below, and within the phase transition region. The curves are theoretical correlations derived with the analytical procedure described below and illustrated in Figure 4. The area dilation was normalized by the vesicle area at a tension level of 0.3 dyn/cm.

microscope system) vesicles were chosen to be tested. Then, the elastic compliance was measured above the transition temperature. As shown previously, the compliance measurements group with discrete values indicative of the number of bilayer walls. The most compliant vesicles were chosen as single walled, that is, those vesicles whose elastic compliance was in the 0.01 cm/dyn (elastic modulus of 100 dyn/cm) group.

Results and Analysis

In the micropipet suction test, the projection of the vesicle inside the pipet could be increased by two independent processes: (1) the temperature increased with the suction pressure held constant; (2) the suction pressure increased with the temperature held constant. Both processes were observed to be reversible. From simple geometry, the increase in projection length, ΔL , is simply proportional to a small increase in vesicle surface area, ΔA , since the change in volume is negligible:

$$\Delta A \approx 2\pi R_p \Delta L (1 - R_p/R_o)$$

where R_p and R_o are the radii of the pipet and outer spherical portion of the vesicle, respectively.

At mechanical equilibrium (no movement), the bilayer tension, \bar{T} , can be considered constant over the entire surface and simply proportional to the pipet suction pressure, ΔP :

$$\bar{T} = \Delta P R_p / [2(1 - R_p/R_o)]$$

In our experiments, the procedure was to measure the tension vs. area relation (isotherm) at specific temperatures for both descending and ascending temperature processes (each temperature was held constant for over 1 min to assure equilibrium before the tests were performed). No hysteresis was observed limited by the 0.1 °C control of the measurements. Figure 2 presents the tension in the vesicle bilayer vs. the fractional change in surface area, $\alpha = \Delta A/A$, measured on a same vesicle at three temperatures: above, within, and below the transition at 24 °C. The isothermal elastic compliance is the derivative of the fractional change in surface area with respect to membrane tension at constant temperature, τ :

$$C_e \equiv \frac{1}{A} \left(\frac{\partial A}{\partial \bar{T}} \right)_\tau = \left(\frac{\partial \alpha}{\partial \bar{T}} \right)_\tau$$

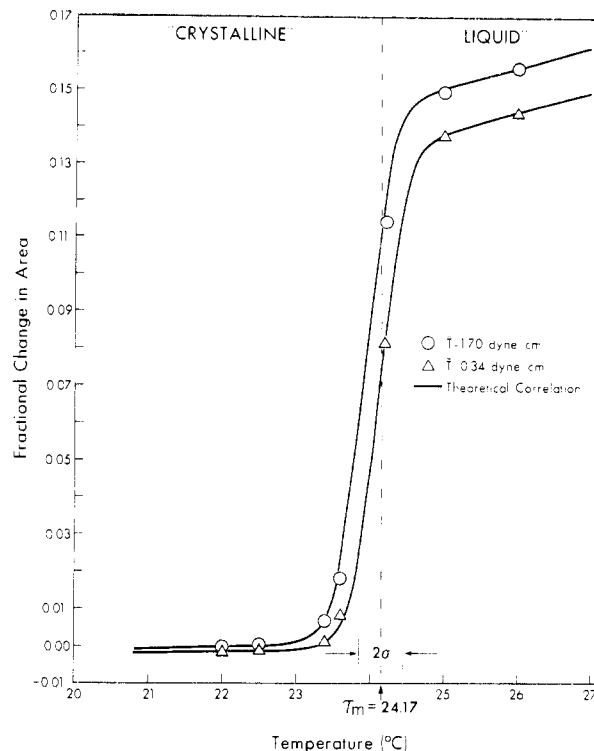


FIGURE 3: Fractional change in area of a single bilayer vesicle (relative to area at a temperature in crystalline phase) vs. temperature at two levels of membrane tension (0.34 and 1.7 dyn/cm). Note the shift of the area-temperature curve to a lower temperature for the higher membrane tension; this is the Clausius-Clapeyron effect (i.e., the thermoelastic shift). The curves are theoretical correlations based on the convolution of an idealized first-order phase transition with a freezing-point dispersion function (illustrated in Figure 4). The theoretical correlation yielded a value of 13% for the area increase at the transition from the crystalline to liquid state.

The compliance is the inverse of the elastic area compressibility modulus: $C_e = K_e^{-1}$.

The other important relation is the area vs. temperature at constant bilayer tension. Figure 3 shows this data for a single vesicle at two levels of bilayer tension (0.34 and 1.7 dyn/cm). The area data is represented by the fractional change in area measured relative to the crystalline state. Four significant observations were made: (1) the area expanded slightly with temperature in the liquid state for the acyl chains above the transition and exhibited a much lower expansivity in the crystalline state; (2) the area exhibited a uniform rate of decrease from the transition at 24 °C down to 5 °C (the lowest temperature observed); (3) the transition was not sharp; and (4) the transition was shifted to lower temperatures as the bilayer tension was increased. The first observation represents the thermal area expansivity in the liquid and crystalline states:

$$C_\tau \equiv \frac{1}{A} \left(\frac{\partial A}{\partial \tau} \right)_T = \left(\frac{\partial \alpha}{\partial \tau} \right)_T$$

This was observed and measured previously for egg yolk lecithin (Kwok & Evans, 1981) to be $2.4 \times 10^{-3}/^\circ\text{C}$. For DMPC, the value is higher in the liquid state (especially near the transition) but significantly lower in the crystalline state. Of special note was the second observation of a continuous decrease in area down to 5 °C, which showed an absence of the pretransition observed in X-ray diffraction and calorimetry studies on dispersions. If a comparable pretransition had occurred, there would have been a significant area increase as the temperature went below 13 °C. The third observation was a transition broadening. This broadening complicates the evaluation of the transition properties, especially the transition

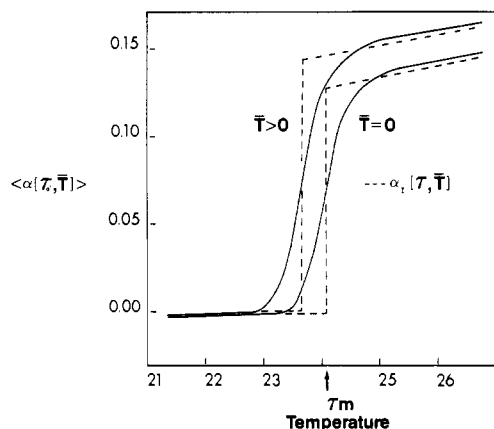


FIGURE 4: Schematic illustration of method of analysis. The idealized area-temperature relation at two levels of membrane tension is shown as dashed lines where the shift in the transition temperature would be given by the Clausius-Clapeyron equation. These relations were convolved with a Gaussian freezing-point dispersion function to give the "observed" area-temperature relations shown as the solid curves.

shift. The fourth observation, the transition shift, is the thermoelastic Clausius-Clapeyron effect.

The Clausius-Clapeyron equation for the surface transition can be derived with the use of thermodynamic state variables defined for a membrane surface as (Evans & Waugh, 1977; Marcelja & Wolfe, 1979; a brief development is in the Appendix)

$$\left(\frac{\partial \bar{T}}{\partial \tau_m} \right) = - \frac{\bar{Q}_m}{\tau_m \Delta \alpha_m}$$

where τ_m is the transition temperature, $\Delta \alpha_m$ is the fractional increase in area at the transition, and \bar{Q}_m is the heat of melting per unit area (i.e., the area measured just below the transition). For an ideal first-order phase transition, the area-temperature relation at two states of bilayer tension would appear as illustrated in Figure 4 (the area is represented by the fractional change relative to the crystalline area just below the transition temperature). Even though the ideal and observed area-temperature relations are similar, they differ significantly in one aspect: the elastic compliance of the surface is infinite for an ideal transition but remains finite in the actual case.

In order to analyze the broadened transition, we have modeled the bilayer transition as a superposition of ideal, first-order phase transitions that are normally distributed about an expected melting temperature. This model is equivalent to the summation of transitions of microregions in the surface, each with a slightly different transition temperature [perhaps due to impurities or cooperative limitations (Albon & Sturtevant, 1978)]. Using a Gaussian dispersion function, we have formed the convolution with the ideal representation of the area equation of state. Hence, the fractional change in area that would be observed at different temperatures is given by

$$\langle \alpha(\tau_o, \bar{T}) \rangle = \frac{1}{\sigma(2\pi)^{1/2}} \int_{-\infty}^{\infty} \alpha_1(\tau', \bar{T}) \exp[-(\tau' - \Delta\tau)^2 / (2\sigma^2)] d\tau' \quad (1)$$

where $\alpha_1(\tau', \bar{T})$ is the ideal relation for a first-order transition as shown in Figure 4, σ is the statistical width of the transition, and $\Delta\tau$ is the temperature difference between the phase transition and the observation temperature, τ_o . In the linear thermodynamic approximation, the difference between the observation temperature and the tension-dependent transition temperature is given by

$$\Delta\tau \equiv \tau_o - \tau_m + \chi \bar{T} \quad (2)$$

where χ is the inverse of the Clausius-Clapeyron equation

$$\chi \equiv \frac{\tau_m \Delta \alpha_m}{\bar{Q}_m} \quad (3)$$

Similarly, the ideal equation of state for the fractional change in area with respect to the crystalline state is given by the summation of a step change at the transition, $\Delta \alpha_m$, plus an (isothermal) elastic contribution, $C_e \bar{T}$, plus a (thermal) expansivity contribution, $C_\tau(\tau - \tau_m)$; thus

$$\alpha_1(\tau', \bar{T}) \equiv \Delta \alpha_m(\tau') + C_e(\tau') \bar{T} + C_\tau(\tau')(\tau' - \chi \bar{T}) \quad (4)$$

$$\Delta \alpha_m(\tau') \equiv \Delta \alpha_m, \tau' \geq 0 \\ 0, \tau' < 0$$

(Note that the thermoelastic coefficients are discontinuous between crystalline and liquid phases. Also, τ' is the temperature relative to the transition temperature.) The convolution integral, eq 1, is expressed in terms of error functions and is illustrated in Figure 4.

The theoretical result depends on the expectation value of the transition temperature, τ_m , the statistical width, σ , the thermoelastic shift, χ , and the fractional change in area at the transition, $\Delta \alpha_m$. A sensitive method was found to evaluate the transition temperature, the statistical width, and the thermoelastic shift. The approach is to correlate the elastic compliance measured at specific temperatures with the compliance derived from the convolution integral, eq 1. The theoretical relation for the elastic compliance is obtained by differentiating eq 1 with respect to the bilayer tension at constant temperature, i.e.

$$\langle C_e \rangle \equiv \lim_{\bar{T} \rightarrow 0} \left(\frac{\partial \langle \alpha \rangle}{\partial \bar{T}} \right) \quad (5)$$

$$\langle C_e \rangle = \frac{1}{\sigma(2\pi)^{1/2}} \int_{-\infty}^{\infty} [\alpha_1(\tau', 0)(\tau' - \Delta\tau)\chi / \sigma^2 + C_e(\tau') - \chi C_\tau(\tau')] \exp[-(\tau' - \Delta\tau)^2 / (2\sigma^2)] d\tau'$$

Figure 5 is an example of the correlation of eq 5 with the elastic compliance measured on a single vesicle at several temperatures in the neighborhood of the phase transition. The compliance below 22 °C was less than 0.001 cm/dyn (i.e., an elastic modulus of 1000 dyn/cm); above 26 °C, the compliance was 0.0075 cm/dyn (i.e., an elastic modulus of 133 dyn/cm). The expectation value of the transition temperature was 24.2 °C. The thermoelastic shift was found to be 0.18 °C-cm/dyn; that is, each dyne per centimeter increment in bilayer tension lowers the transition temperature about 0.2 °C.

Finally, this data was used with eq 1 to determine the fractional change in area at the transition by correlation with data such as shown in Figure 3. The theoretical curves in Figure 3 are the predicted relations for the above parameters and an area increase of about 13% where the acyl chains melt. Also, the thermal area expansivity was determined for the liquid phase to be about $4 \times 10^{-3}/^\circ\text{C}$; in the crystalline phase, the value was found to be approximately $(5-6) \times 10^{-4}/^\circ\text{C}$. With the values of the thermoelastic shift and the fractional change in area, the heat of melting per unit area was calculated to be 210 ergs/cm². Since the area per molecule in the crystalline state has been determined from X-ray diffraction studies (Janiak et al., 1979) to be about $(47-50) \times 10^{-16}$ cm², each square centimeter of bilayer contains about 4×10^{14} molecules. Thus, the heat per molecule would be about 5.3×10^{-13} erg/molecule or 7.5 kcal/mol for the data shown in Figures 3 and 5.

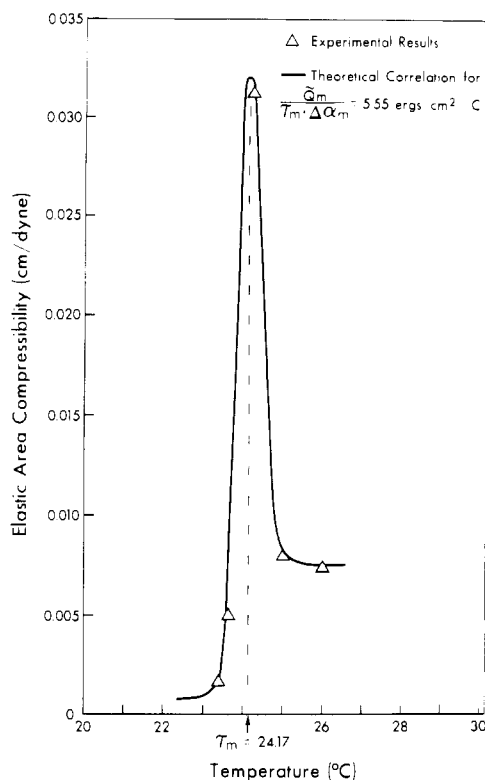


FIGURE 5: Elastic area compressibility of DMPC bilayer vesicle vs. temperature in the vicinity of phase transition. The data were obtained from the reciprocal tangent to the tension–area dilation isotherms (e.g., Figure 2) in the limit of zero area dilation. The curve is the theoretical correlation derived from the area convolution equation. This correlation provided the thermoelastic shift (Clausius–Clapeyron effect), 5.5 dyn/(cm²·°C), the expectation value for the transition temperature, ~24.2 °C, and the statistical width of the transition, ~0.3 °C. With the increase change in area at the transition (~13%), the transition temperature (~24.2 °C), and the thermoelastic shift, we calculated the heat of the transition to be about 210 ergs/cm² or approximately 7.5 kcal/mol of DMPC.

Conclusions and Discussion

In summary, we have measured the area, tension, and temperature relation throughout the phase transition region for a DMPC bilayer. We have shown that simple convolution of an ideal first-order phase transition with a transition temperature dispersion function gives excellent correlation with the observed data. In addition, the convolution provides good analytical means to derive the transition properties from the data with use of the elastic compliance function. With this approach, we have determined the fractional change in area for the transition, $\Delta\alpha_m = 0.12$ – 0.13 , the transition temperature, $\tau_m = 24.2$ °C, the statistical width of the transition, $\sigma = 0.3$ °C [note, this is peculiar to the lipid sample as has been discussed by Albon & Sturtevant (1978)], and the heat of transition, $Q_m = 7 \pm 0.7$ kcal/mol. The measurements yielded the elastic compliance in the liquid state (~30 °C) as 0.007–0.008 cm/dyn ($K_e = 120$ – 140 dyn/cm) and showed that the compliance in the solid state was less than 0.001 cm/dyn ($K_e \sim 1000$ dyn/cm). Near the transition, the thermal area expansivity in the liquid state was $(4$ – $6) \times 10^{-3}$ /°C and much lower in the solid state, $\sim(5$ – $8) \times 10^{-4}$ /°C. It was significant that no pretransition area change was observed in the 20 °C range below the main transition. Another interesting observation was the modest elastic compliance, ~0.03–0.05 cm/dyn ($K_e \sim 20$ – 30 dyn/cm), in the transition and the associated structural viability of the bilayer at the transition. The implication is that this is the result of the broadened transition, perhaps due to slight impurities. If the transition was sharp,

the compliance would diverge, and the bilayer would breakup.

How do these results compare with data from X-ray diffraction and calorimetry studies? As noted, no pretransition was observed that has been associated with a transition from the planar $L_{\beta'}$ phase to the rippled $P_{\beta'}$ phase (Janiak et al., 1976). Next, the change in area at the transition that we observed was much less than that calculated from X-ray diffraction and gravimetric measurements, i.e., 12% compared with 20%. However, if the transition was from the liquid L_{α} phase directly to the $L_{\beta'}$ phase, the change expected from the X-ray data would be consistent, that is, ~12%. A similar deduction results from comparison of the heat of melting that we have calculated from the thermoelastic shift with that measured calorimetrically. The heat of melting has been measured to be about 6 kcal/mol at the main transition [6.6 kcal/mol by Hinz & Sturtevant (1972); 5.4 kcal/mol by Mabrey & Sturtevant (1976)], but there is an additional kcal/mol for the pretransition (Mabrey & Sturtevant, 1976). If these heats are cumulated as would be expected for a direct transition from the $L_{\beta'}$ to L_{α} phase, then the value is comparable to the heat derived from the thermoelastic shift, i.e., 7 kcal/mol.

There have been several theoretical developments of surface equations of state for bilayer membranes [e.g., Marcelja (1974) and Jahnig (1981)] with two recent reviews (Caille et al., 1980; Nagle, 1980). In the vicinity (± 5 °C) of the transition, the statistical mechanical models indicate that (1) there would only be a moderate decrease in elastic compliance (factor of 2–3) from the liquid to solid phase and (2) the thermal area expansivity would be comparable in both phases. These particular aspects are singled out because they are in contradiction with our experimental observation. First of all, we observed 1 order of magnitude reduction in elastic compliance from the liquid to solid state. Second, the thermal area expansivity was nearly 1 order of magnitude lower in the crystalline state than in the liquid state. Finally, the observed fractional area increase is much smaller than that considered in the models. In short, it appears that the lipid bilayer is more compliant and more expandable above the transition but less compliant and less expandable below the transition than the theoretical models predict.²

Appendix

For the time scale of mechanical experiments on large vesicles (1 h), the bilayer membrane behaves as a thermodynamically closed (fixed mass) system. Hence, we can define state variables intensively per unit area of a reference state (say at some temperature in the crystalline phase); this is equivalent to a per molecule basis. As such, the Gibbs free energy density, \tilde{G} , the entropy density, \tilde{S} , and membrane tension, \tilde{T} , are related by

$$d\tilde{G} = -\tilde{S} d\tau - (1 + \alpha) d\tilde{T} \quad (\text{A1})$$

where τ is the temperature and α is the fractional change in surface area relative to the reference area [see Evans & Waugh (1977) for details]. Since variations in the Gibbs free energy density are the same for both phases at the transition, we obtain the Clausius–Clapeyron equation by taking the

² Note, as a reviewer has pointed out, this behavior is correct for mean-field, Ising-model, and Landau theories; however, other theories (Nagle, 1980; O'Leary, 1981) do behave as we have observed. This reviewer also noted that our observation of the same transition temperature for a single bilayer as in multilamellar dispersions supports the assumption used by theorists; i.e., interactions between bilayers are inconsequential.

difference in eq A1 between the two phases:

$$0 = -\Delta\tilde{S}_m d\tau_m - \Delta\alpha_m d\tilde{T}$$

or

$$\left(\frac{\partial\tilde{T}}{\partial\tau_m}\right) = -\frac{\Delta\tilde{S}_m}{\Delta\alpha_m}$$

where the variations are taken along the locus of transitions; $\Delta\tilde{S}_m$ is the difference in entropy density between the two phases and for a reversible process is equal to the heat of melting per unit area divided by the melting temperature, τ_m ; $\Delta\alpha_m$ is the fractional change in surface area between the liquid and solid phases. Therefore

$$\left(\frac{\partial\tilde{T}}{\partial\tau_m}\right) = -\frac{Q_m}{\tau_m\Delta\alpha_m}$$

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Phosphorus-31 and Carbon-13 Nuclear Magnetic Resonance Studies of Divalent Cation Binding to Phosphatidylserine Membranes: Use of Cobalt as a Paramagnetic Probe[†]

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ABSTRACT: The paramagnetic divalent cation cobalt has large and well-understood effects on NMR signals from ligands bound in the first coordination sphere, i.e., inner-sphere ligands, and we have used these effects to identify divalent cation binding sites at the surface of phosphatidylserine membranes. ³¹P NMR results show that 13% of the bound cobalt ions are involved in inner-sphere complexes with the phosphodiester group, while ¹³C NMR results show that 54% of the bound cobalt ions are involved in unidentate inner sphere complexes with the carboxyl group. No evidence is found for cobalt binding to the carbonyl groups, but proton release studies

suggest that 32% of the bound cobalt ions are involved in chelate complexes that contain both the carboxyl and the amine groups. All (i.e., 13% + 54% + 32% = 99%) of the bound cobalt ions can thus be accounted for in terms of inner sphere complexes with the phosphodiester group or the carboxyl group. We suggest that the unidentate inner-sphere complex between cobalt and the carboxyl group of phosphatidylserine and the inner-sphere complex between cobalt and the phosphodiester group of phosphatidylserine provide reasonable models for complexes between alkaline earth cations and phosphatidylserine membranes.

Thermodynamic aspects of the interaction between calcium and bilayer membranes containing phosphatidylserine can be explained in terms of the Gouy-Chapman-Stern theory (McLaughlin et al., 1981), but the functional groups that form ligands for calcium have not been identified. Some information has been obtained from the effects of calcium on the NMR signals from phosphatidylserine membranes (Kurland et al., 1979a,b), but these effects are small and difficult to interpret theoretically because calcium is diamagnetic. In contrast,

paramagnetic divalent transition metal cations have large and well-understood effects on the NMR signals from ligands bound in the first coordination sphere, i.e., inner-sphere ligands (Shulman et al., 1965; McDonald & Phillips, 1963; Strouse & Matwiyoff, 1970; Swift & Connick, 1962) and can be used as "probes" for the divalent cation binding site at the surface of phospholipid membranes. Cobalt is the most useful paramagnetic divalent cation for this purpose because its effects on the NMR signals of inner-sphere ligands are easiest to quantitate (Shulman et al., 1965).

Sonicated phospholipid vesicles show "high-resolution" ³¹P NMR spectra (Borden et al., 1974) and ¹³C NMR spectra (Metcalfe et al., 1971; Batchelor et al., 1972), and distinct signals can be observed from most of the potential ligands in

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