Critical Temperature for Unilamellar Vesicle Formation in Dimyristoylphosphatidylcholine Dispersions from Specific Heat Measurements

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ABSTRACT Using a heat conduction calorimeter with very high resolution (± 0.00005 J/°C·cm^3), we have measured the specific heat Cp_L between 25 and 35°C of dimyristoylphosphatidylcholine (DMPC) in aqueous dispersions. Previous studies of the temperature dependence of the chemical potential of DMPC in the Lα phase (lamellar, liquid crystalline) indicated that a dispersion consisting only of unilamellar vesicles forms spontaneously at a critical temperature T^* of 29.0°C. Our present measurements show an anomaly in Cp_L between 28.70 and 29.50°C: the curve for Cp_L versus T first decreases and then exhibits an inflection point at 28.96°C before it flattens. This anomaly is attributed to the transformation from multilamellar dispersion to unilamellar vesicles at $T^* = 28.96$°C. Two independent properties of the Cp_L data also indicate T^* is a critical point for the formation of unilamellar vesicles: (*a*) the time to reach equilibrium upon changing temperature increased dramatically between 28.7 and 28.96°C, increasing as $(T^* - T)^{-1}$; at $T > T^*$ the dramatic "slowing-down" phenomenon was not observed. This slowing-down near T^* is a general characteristic of critical phenomena. (*b*) The free energy change for the multilamellar-unilamellar transformation was obtained from the Cp_L-T data over this temperature interval and found to be 3.2 J/mol or 0.016 ergs/cm^2 of bilayer, in agreement with other estimates of the interaction energy between neutral bilayers. We conclude with a discussion of the implications for membrane bilayer stability of these newly identified dynamic properties of the transformation.

INTRODUCTION

When dispersed in water, membrane phospholipids generally form the Lα phase, a water-swollen, multilamellar, liquid crystalline array of stacked bilayers where the intrabilayer spacing is of the order of 30 Å. Although considered as a model for the single bilayer of cell membranes, it is only recently that the significance of the distinction between the multilamellar state in the dispersion and the unilamellar or single bilayer state of the cell has been recognized (Gershfeld et al., 1986; Gershfeld, 1989a, 1989b). Thermodynamic studies of membrane lipids dispersed in water indicate that at a critical temperature, T^*, they spontaneously transform to a state that is structurally equivalent to the membrane bilayer: the dispersions consist solely of unilamellar vesicles; at temperatures below and above T^* only the multilamellar state is present. This critical temperature depends on lipid composition (Tajima and Gershfeld, 1985; Gershfeld, 1989a, 1989b). For the *total* lipid extracts of a large variety of cell types (e.g., bacteria (Gershfeld, 1986), squid axons (Ginsberg et al., 1991), erythrocytes (Gershfeld, 1986), rat brain tissue (Ginsberg et al., 1991), human brain tissue (Ginsberg and Gershfeld, 1991)), T^* equals the physiological temperature of the cell from which the lipids were extracted. These critical temperatures range from 15 to 60°C, encompassing a major portion of the physiological conditions for animal life. The striking correspondence between T^* and physiological temperatures has been taken to mean that equivalent assembly processes for the unilamellar state occur in the cell and in vitro (Gershfeld, 1986, 1989a). When lipid composition or ambient temperature do not correspond to the critical conditions, membranes become unstable and predictably degenerate with accompanying cell death (Gershfeld and Murayama, 1988; Ginsberg and Gershfeld, 1991).

The properties of this critical unilamellar state have been largely inferred from a thermodynamic analysis of the air-water surface films that form in equilibrium with the dispersions (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985; Gershfeld, 1989a, 1989b). Since this analysis predicts the existence of a critical phenomenon in the dispersion, a more direct, and perhaps conceptually simpler description of the transformation involved in the formation of the critical unilamellar state may be obtained by measuring the temperature dependence of the heat capacity of the lipid dispersions. We have developed a differential heat conduction microcalorimeter for this purpose (Mudd et al., 1993), and now report the results of heat capacity-temperature measurements for dispersions of dimyristoylphosphatidylcholine (DMPC) for which a considerable body of experimental data of the critical state is available (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985; Gershfeld, 1989a, 1989b). For DMPC, T^* is 29°C. Consequently we anticipated that an anomaly in specific heat (Cp) of the dispersion would appear at or very near this temperature. This expectation has now been confirmed. Moreover, from the variation of Cp with

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temperature in the region of the anomaly, the work of converting the multilamellar state to unilamellar vesicles may be obtained and is shown to be consistent with the intrabilayer cohesive energy measured by other methods (Evans and Metcalf, 1984; Rand and Parsegian, 1989).

MATERIALS AND SAMPLE PREPARATION

DMPC (Avanti Polar Lipids, Birmingham, AL) was routinely tested by thin-layer chromatography; only those samples which gave a single spot (maximum contamination from lysolecithin of approximately 0.2 mol%, by phosphorus analysis) were used. Previous studies (Tajima and Gershfeld, 1981) indicated that the critical temperature T^* was not significantly affected by contaminations of this magnitude. Aqueous dispersions of DMPC were prepared by weighing both the lipid and water (triple distilled, the last two times from a quartz distillation apparatus) into screw-capped test tubes; sodium azide (1.5×10^{-5} M) was added to retard bacterial growth. The tubes were thoroughly vortexed at 35°C and then alternately heated to 50°C and cooled to 0°C for a total of five cycles. At the end of the last heating cycle particular care was taken to be sure that dissolved air released as bubbles at 50°C was removed by centrifugation before final cooling was begun. After cooling, approximately 3 ml were removed in a gas-tight syringe for loading the calorimeter cell. This procedure was followed for two objectives: (*a*) the heating and cooling cycling was necessary to anneal the lipid phase; if this was omitted *Cp* measurements vary unpredictably with time for many days. (*b*) Heating above 50°C eliminated the formation of air bubbles at lower temperatures; when air bubbles form they displace solution and concomitantly yield low values of *Cp*. This procedure for removing dissolved air was applied routinely to all solutions and dispersions used in the calorimeter.

METHODS

Measurement of specific heat

A heat conduction calorimeter developed for the measurement of small changes in heat capacity of milligram samples of lipid dispersed in water has been described in complete detail elsewhere (Mudd et al., 1993). Briefly, the calorimeter consists of two, 0.6-ml tantalum cells, each fitted with internal solution heaters and wired in series with each other. One of the cells is generally filled with water as a reference, and the other is filled with the sample. Each cell is sandwiched between two sensor assemblies, each consisting of four thermopiles; the assemblies from the two cells are wired in opposition so that only the difference between cell responses is measured. The cells are permanently mounted in a heat sink. The filling of each cell is achieved by tantalum tubing (0.5-mm ID, 0.4-mm wall) which is connected to each cell and extends outside the body of the heat sink. The temperature of the heat sink is set to an accuracy of 0.01° in the range of 0–50°C with a proportional plus integral temperature controller (Mudd et al., 1982). After the set temperature is reached, the controller stability is approximately ±0.001°.

In operation, the large mass of the heat sink and air space between the controlled shell and heat sink combine to create a low pass filter which reduces the temperature perturbations in the tantalum cells by several orders of magnitude. Based on a thermocouple sensitivity of 400 μV/°C per junction, 144 junctions per channel, and sensor noise level of ±40 nV (peak to peak), the average baseline temperature perturbation in each channel is $\pm 7 \times 10^{-7}$°C (peak to peak).

The data acquisition cycle consists of a period of 400 s; baseline data are taken for the first 20 s then the heaters are turned on for 10 s. The remainder of the time is used to record the differential peak response and to allow the system to return to thermal equilibrium before the next pulse is applied. The peak height of the pulse response is calculated and stored. A computer repeats this cycle until a peak response is registered that is independent of time, at which time a new temperature is set for the calorimeter and the data acquisition cycle is begun again.

The difference between the heat capacity of the sample and of the water reference is obtained from the peak voltage output via the relation $\Delta V = \rho CpK + K^*$, where K is the instrument constant, ρ the sample density, and K^* is an offset constant that depends on the choice of the reference solution, typically water. K^* arises because of small structural differences that exist between the reference and sample cells and is evaluated by measuring ΔV with water in both cells. We have calibrated the instrument (Mudd et al., 1993) using solutions of known density and specific heat and have found the instrument constant $K = -1.3 \times 10^{-3}$ J/(°C·cm^3·μV). With an electronic noise level of ± 40 nanovolts in our system, the equivalent resolution for *Cp* is $\pm 5 \times 10^{-5}$ J/°C·cm^3. The instrument stability was obtained by placing water in both cells and monitoring the peak voltage at constant temperature over a 36-h period; the resulting baseline stability is 10^{-5}J/°C·cm^3 for 36 h.

RESULTS

As an example of a typical measurement we first present the data for DMPC dispersions at 25.0°C. Fig. 1 shows the heat capacity of the dispersion as a function of the concentration of DMPC (wt%). Since the peak voltage is a function of ρCp, to obtain *Cp* requires an independent measurement of ρ. For this value we use the published data of the buoyant density of DMPC (Gershfeld, 1978) and the density of water (Weast, 1966) at 25°C. For the dispersion

$$\rho_{\text{Disp}}^{-1} = W_w \rho_w^{-1} + W_L \rho_L^{-1}, \quad (1)$$

where W is the weight fraction of water (w) and lipid (L), and ρ is the density of the dispersion (Disp), water (w), and lipid (L), respectively. The plot of heat capacity of the dispersion is a linear function of the weight fraction of the lipid in the dispersion. Since the dispersion is a two phase system, calculation of the specific heat of the lipid for each composition may be obtained from the relation

$$Cp_{\text{Disp}} = W_w Cp_w + W_L Cp_L, \quad (2)$$

where Cp_{Disp} denotes the heat capacity of the total dispersion. The mean Cp_L for the four concentrations is 4.384 ± 0.014 J/°C·g; the relatively large error is due primarily to contributions from weighing and pipetting in the preparation of the individual dispersions in addition to the instrumental errors of the heat capacity measurement. For measurements of the

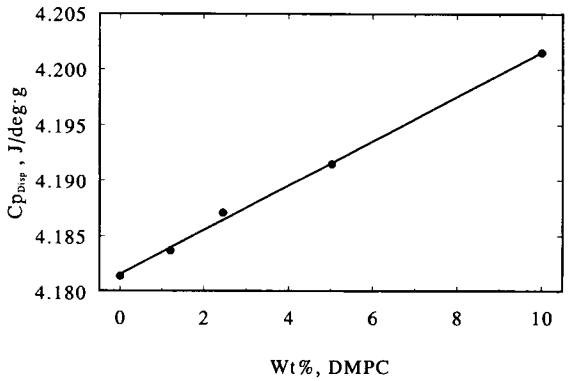

FIGURE 1 Heat capacity of aqueous dispersions of DMPC, Cp_{Disp}, at 25°C as a function of the concentration of the lipid (weight %). The line is drawn from a linear regression analysis of the individual measurements.

temperature dependence of Cp_{Disp}, a single sample is used for all temperatures and the error is considerably smaller ($\pm 5 \times 10^{-5}$ J/°C·g).

Specific heat anomaly of DMPC at T^*

The specific heat for DMPC, Cp_L, using a 10 weight% DMPC dispersion, was measured over a temperature range of 25–35°C; Fig. 2 presents only the data for 28–31°C, temperatures that bracket the critical temperature, $T^* = 29$°C, previously observed for this lipid by the surface film thermodynamic studies (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985; Gershfeld, 1989b). With increasing temperatures from 25°C (*open symbols*) Cp_L decreases continuously from a value of 4.3823 J/°C·g at 25°C to 3.3061 J/°C·g at 35°C. An anomaly in Cp_L commences at 28.70°C, where Cp_L decreases more rapidly then flattens out with the curve showing an inflection point at 28.96°C. At still higher temperatures the slope of the Cp_L-T curve decreases further.

Heating was continued to 31°C where the transformation represented by the heat capacity anomaly appears to be concluded. At this point we tested the reversibility of the anomaly by cooling the sample progressively back to 28°C. The heat capacities obtained upon cooling are also given in Fig. 2 (*filled symbols*). Until 29.5°C the cooling curve retraces the heating curve; but at temperatures lower than 29.5°C the curves diverge, the cooling curve falls below the heating curve with the apparent loss of the heat capacity anomaly. Upon reheating this sample from 28 to 31°C the specific heat retraces the cooling curve. That is, heating and cooling of the dispersion are now completely reversible, but along a curve (*filled symbols*) that lies below the initial heating curve. It was possible to retrace the initial heating curve (*open symbols*) and recover the heat capacity anomaly, but only after the sample had been cooled below the main transition temperature of DMPC, i.e., 24°C.

The data of Fig. 2 indicates that DMPC forms two distinct multilamellar states, each characterized by a different Cp_L dependence on temperature: state I exists below 28.7°C and state II above 29.5°C, with a transition region between these two temperatures, the region of the anomaly in Cp_L. The heating and cooling response suggests that when temperatures exceed T^* the bilayers are trapped in multilamellar state II and, when cooled, cannot revert to multilamellar state I until nucleation of the process is triggered with formation of the gel state at $T < 24$°C. This phenomenon is the basis for the annealing method which we use to prepare the dispersions (see Methods); the samples must be preconditioned by a series of cycles of heating above T^* and cooling below T_m before reproducible measurements of heat capacity can be obtained.

Preliminary studies of the cooling behavior of DMPC starting at different temperatures within the interval of 28.7–29.5°C indicate that the cooling curve always falls below the initial heating curve. This behavior suggests that within the transition region a continuum of lamellar liquid crystalline states form; at any point within this region lowering the temperature results in supercooling of that particular state. The phase rule indicates that at each and every temperature between 25 and 35°C only one bilayer state can exist at equilibrium.[1] Thus, we may conclude that bilayer states I and II (both multilamellar) cannot coexist at equilibrium, and that the transformation between states I and II must occur through an intermediary state. The temperature where the intermediary state forms is at the inflection point of the curve in Fig. 2, 28.96°C, the temperature that has been identified as the critical temperature T^* for the formation of the unilamellar state (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985; Gershfeld, 1989b). The uniqueness of this temperature becomes more obvious when we examine dynamic properties of the dispersions as reflected in the time required for DMPC dispersions to reach equilibrium.

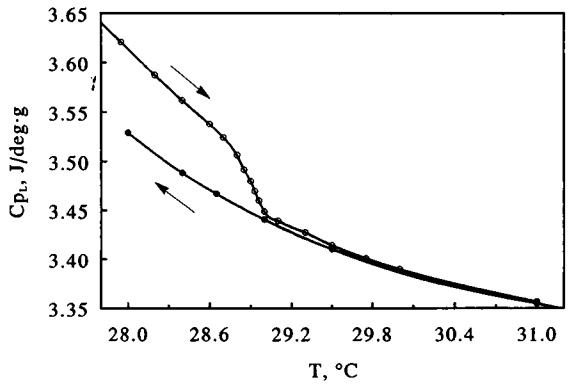

FIGURE 2 Specific heat of DMPC, Cp_L, in the 10 wt% dispersion as a function of temperature. Heating curve from 25°C, open points; cooling from 31°C to 28°C and reheating to 31°C, filled points. Point of inflection is $T^* = 28.96$°C. Error in measurement is smaller than the size of each data point.

Kinetics of the transformation from multilamellar to unilamellar vesicles

Our calorimeter has been designed to permit acquisition of heat capacity data during the time that sample equilibrating temperatures are changed (see Methods). In our system when a new temperature is set, a finite time is required for thermal equilibrium to be attained. For simple liquids the heat capacity follows virtually the same time course as the temperature change, and when thermal equilibrium has been reached heat capacities remain constant with time. However, with DMPC dispersions, we have observed that the time to reach equilibrium (when Cp_L becomes independent of time) increases dramatically as the set temperature approaches 28.96°C. That is, long after thermal equilibrium has been reached, heat capacity of the dispersion continues to change.

[1] Since Cp_L varies continuously with temperature, at constant atmospheric pressure, temperature is the only independent intensive variable; $F = C - P + 2$, $C = 2$ (lipid, water), and $P = 2$ (water, bilayer).

To illustrate this phenomenon we have calculated τ, the ratio of the time for the lipid dispersion to reach equilibrium upon changing temperature, Δt_L,to the time required for water to reach equilibrium, Δt_W, for the same increase in temperature. This ratio, τ, is plotted as a function of the equilibrium temperature in Fig. 3. For the temperature interval 28.4–28.96°C, T^*, the data obey the following relation

$$\tau \sim (T^* - T)^{-1}, \quad (3)$$

where the value of the exponent was obtained from a linear regression analysis of the log-log plot of τ vs. $(T^* - T)$ which gave a slope of −1.00 and correlation coefficient of 0.977. Equation 3 was used to draw the curve between 28.4 and 28.96°C. At T^* the time to reach equilibrium is about 140 times longer than a sample of water under equivalent conditions. For $T > T^*$, τ drops precipitously and approaches the value for water. Below 28.2°C and above 29.8°C the time required for the lipid dispersion to reach equilibrium is approximately the same as for water. The interval between these two temperatures corresponds to the region of the *Cp* anomaly (Fig. 2), with $T^* = 28.96$°C as an obvious endpoint. This slowing down has been observed for a number of liquid systems which exhibit a critical temperature (Stanley, 1987a), and the critical exponent in our system (1.00) is of comparable magnitude with those reported for these liquids.

Since this critical exponent has been associated with diffusion processes occurring in the region of the critical point (Stanley, 1987b), the time required to attain equilibrium, Δt, should scale with the diffusion coefficients of the medium, i.e., $\Delta t \sim D^{-1}$. Thus, when $\tau = 1$, as is the case for temperatures that are above and below the region of the heat capacity anomaly (Fig. 3), the processes observed are likely to involve the movement of water into and out of the multibilayers. However, as T^* is approached, the time for the dispersion to reach equilibrium is approximately 140 times longer than for water; the processes involved now are likely also to include movement within the lipid bilayers themselves since diffusion coefficients there are two orders of magnitude smaller than in aqueous solutions (Fahey and Webb, 1978).

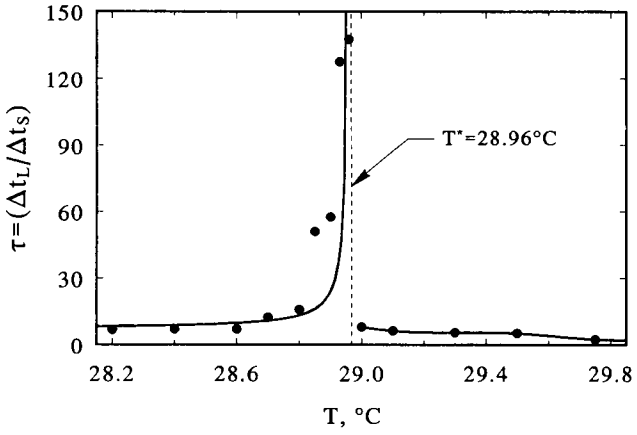

FIGURE 3 Dynamic properties of DMPC in the region of the critical point T^* as a function of temperature. The experiment entails measuring heat capacity as a function of time when changing equilibration temperatures; Δt_L and Δt_W are the elapsed times required for the heat capacity of the lipid dispersion (L) and water (w), respectively, to reach equilibrium for the same increase in temperature. For $T < T^*$ line drawn to fit relation $\tau \sim (T^* - T)^{-1.00}$; exponent obtained from slope of log(τ) vs log($T^* - T$).

The dynamic processes that are entailed in the transformation from state I to state II indicate that T^* is a critical point in which the multilamellar state I is transformed with rising temperature to a unique state that exists only at T^*. We attribute this process to the gradual disaggregation of the multilamellar state I to unilamellar vesicles. A further elevation in temperature above T^* results in another process that leads to multilamellar state II at 29.5°C; this process is the gradual reaggregation of unilamellar vesicles to form the multilamellar state II. At each and every temperature only one bilayer state is present, in agreement with the necessary conditions imposed by the phase rule.

DISCUSSION

The free energy change for the multilamellar-unilamellar transformation: intrabilayer interactions from *Cp* measurements

The transformation from multilamellar state I to unilamellar vesicles at T^* occurs over a range of temperatures, and therefore it is not a first order phase transition. Thus, the latent heat of the transition, ΔH_{tr}, is zero, and the driving force for the transition is entropic. Evaluation of the free energy change for the phenomenon is consequently a problem of measuring the entropy of the transformation ΔS_{tr}. We employ the concept of expressing the thermodynamic properties which describe the transformation in terms of a set of excess properties each of which is a function of temperature: $G^{xs}(T)$, $S^{xs}(T)$, and $Cp^{xs}(T)$. These excess properties represent the difference between the actual lamellar liquid crystal system and a reference system which is chemically identical to the actual system, but has not undergone the transition to unilamellar vesicles at T^*. Thus, the excess thermodynamic properties encompass only those energy changes that contribute to the transformation. The following equations give the thermodynamic properties of the transition in terms of the excess functions as the difference between the excess property at T^* and the initial temperature at the beginning of the transition T_i:

$$\Delta G_{tr} = G^{xs}(T^*) - G^{xs}(T_i) = G^{xs}(T^*) \quad (4)$$

$$\Delta S_{tr} = S^{xs}(T^*) = \int_{T_i}^{T^*} \left(\frac{Cp^{xs}(T)}{T} \right) dT, \quad (5)$$

where $Cp^{xs}(T) = (Cp_L(T) - Cp_L{}^{REF}(T))$ is the difference between the temperature-dependent specific heat of the lipid in the actual system and in the reference system. Equation 4 expresses the fact that all excess properties at T_i equal zero. Equation 5 provides the basis for our method of obtaining the

entropy change for the transformation from Cp measurements. Given these properties of the excess functions, Eq. 4 becomes

$$\Delta G_{tr} = -T^*\Delta S_{tr}. \quad (6)$$

Fig. 4 shows a plot of Cp_L/T vs. T along the line AC. Line AB gives Cp^{REF}/T for the reference state. To better understand the significance of these terms, we describe in detail the method for evaluating the entropy of the transformation. The total entropy change associated with this transformation may be obtained by graphical integration of the Cp_L/T vs. T curve, i.e., the area under the curve AC in Fig. 4. The total entropy change includes many contributions from sources such as modification of bilayer structure and changes in the amount of water in the bilayer, among many other possibilities. Some of these contributions would have been present even if the transformation to the vesicle state had not occurred simply because the temperature has increased. To obtain the contributions to the entropy change for the transformation that is related only to the process of disaggregating bilayers in multilamellar state I to unilamellar vesicles it is necessary to subtract the entropy change that would have been observed if this transformation had not occurred. This is the entropy change in our reference state, and it is obtained from the area under the curve AB, Fig. 4; AB is a linear extrapolation from the line which describes the change in Cp_L/T for the lipid at temperatures below T_i. The graphical evaluation of the integral in Eq. 5 is given by the shaded area in Fig. 4.

When Eq. 6 is evaluated using the data shown in Fig. 4, we find the work of forming unilamellar vesicles from multilamellar state I is $\Delta G_{tr} = 3.20 \pm 0.03$ J/mol DMPC. This energy may be converted to the work per unit bilayer area, Δg, by using the area/molecule of DMPC in the bilayer (Rand and Parsegian, 1989), and by assuming that the multilamellar state I contains $n > 50$ bilayers.[2] The resulting value is $\Delta g = 0.016$ ergs/cm^2 bilayer. The energy is positive and therefore indicates that work must be expended against an attractive force that exists between the bilayers that maintains the multilamellar structure in state I. Since DMPC is electrically neutral this must be primarily the van der Waals force. We compare this measure of the energy due to the van der Waals force to estimates obtained by two other methods.

In the osmotic stress method (Rand and Parsegian, 1989) the decay length for the repulsive force between neutral bilayers is extrapolated to the intrabilayer distance where the repulsive and attractive forces are equal; this leads to an estimate of 0.007–0.028 ergs/cm^2 for a variety of PC bilayers for the van der Waals attractive energy. In the pipette aspiration method, the work required to separate two vesicles that had been allowed to make stable contact was found for PC to be 0.01–0.015 erg/cm^2 (Evans and Metcalf, 1984). The estimate we have obtained for the work of disaggregating neutral bilayers (0.016 erg/cm^2) is in sensible agreement with the other two independent methods. Thus, Cp measurements in the region of T^* represents a new and independent method for evaluating intrabilayer interactions.

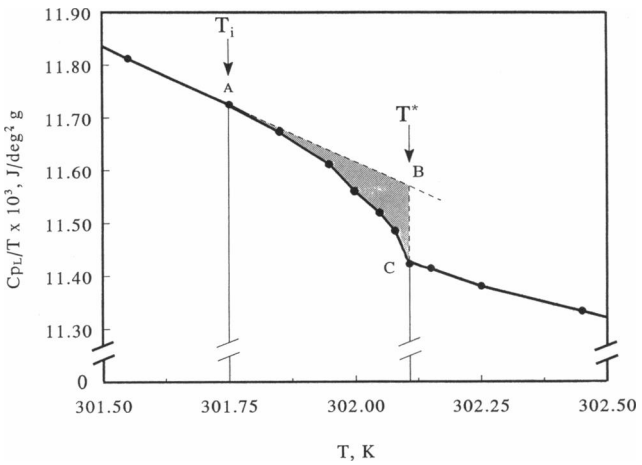

FIGURE 4 Specific heat of DMPC, Cp_L, divided by the absolute temperature as a function of temperature. Shaded area is the entropy (negative) for the transformation from multilamellar state I to unilamellar vesicles at T^*.

Characteristics of the unilamellar critical state

The Cp_L-T characterization of the transformation between the two liquid-crystalline multilamellar states I and II with an intermediate unilamellar state for DMPC represents a new phenomenon. The characteristics of this transformation are in general agreement with the deductions from our previous studies in which the properties of the equilibrium surface films were used to deduce the formation of the unilamellar state. Thus, the same critical unilamellar temperature T^* for DMPC has been observed with "surface bilayer" formation (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985; Gershfeld, 1989a, 1989b), water permeability (Ginsberg and Gershfeld, 1985), x-ray diffraction (Cevc et al., 1990), and confirmed by a systematic thermodynamic analysis of the surface films in equilibrium with the dispersion (Gershfeld, 1989b). It is anticipated that heat capacity studies of more complex membrane lipid mixtures will also exhibit the correspondence of T^* and the physiological temperature for membranes (Gershfeld, 1986, 1989a; Ginsberg et al., 1991; Ginsberg and Gershfeld, 1991).

In addition to confirming our deductions of the existence of a critical unilamellar state, our present studies also provide some perspectives on the stability of the unilamellar state. Although both of the liquid-crystalline states I and II are lamellar, there are barriers for conversion of one state into the other. We have observed that for temperatures outside the region of the transformation (28.7°–29.5°C) heating and cooling is reversible. But, as we note in Fig. 2, cooling from

[2] For a liposome containing n bilayers, complete separation of all bilayers to vesicles involves forming $n - 1$ new bilayer surfaces. This should be considered when calculating the energy/bilayer. For example, if liposomes contain an average of 50 bilayers the error in calculating the energy per unit bilayer area is only 2%, but for ten bilayers per liposome, the energy calculation will be 10% low.

state II does not retrace the heating curve. It is only by cooling below T_m, the main transition temperature of DMPC, 24°C, that it is possible to retrace the heating curve from state I to the unilamellar state at T^*. These properties are manifested in the following stability characteristics of the unilamellar membrane at T^*. A slight elevation of the temperature will result in the formation of a multilamellar state. However, a decrease in temperature will result in the supercooling of the unilamellar state.

We believe that these dynamic properties have important consequences for the stability of mammalian membranes. Consider a biological membrane which is stable at its physiological temperature (T^*) where a slight increase in temperature would be expected to result in a transformation to a multilamellar state; if the formation of multilayer was at the expense of normal (unilamellar) bilayer, the interior of the cell would be exposed with potentially catastrophic results. A decrease in its ambient temperature (below T^*) would lead to supercooling of the unilamellar structure of the membrane. We have observed both heating and cooling behavior with human erythrocytes by examining the rate of hemolysis as a function of temperature (Gershfeld and Murayama, 1988). At 37°C (the critical temperature T^*) erythrocytes are stable showing no hemolysis for many hours, nor do they hemolyze when cooled below this temperature. However, upon raising the ambient temperature even 1°C a significant amount of hemolysis occurs (Karle, 1969; Gershfeld and Murayama, 1988). We have recently applied these concepts of the critical unilamellar state for predicting the influence of membrane lipid defects on neurodegeneration (Ginsberg and Gershfeld, 1991; Ginsberg et al., 1993a, 1993b).

These studies illustrate the equivalence of thermodynamic measurements made by examining either the surface films or the lipid bilayers in the equilibrium dispersions. In both approaches the presence of a critical temperature for forming unilamellar vesicles has been demonstrated. Future specific heat studies will examine the conditions that influence the critical temperature T^*, including the contribution of bilayer charge, and lipid composition to the work of forming unilamellar vesicles.

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