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Dynamic heat capacity at the gel to liquid-crystalline phase transition in large unilamellar vesicles of dimyristoylphosphatidylcholine in the ultralow frequency region

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Dynamic heat capacity has been measured near the gel to liquid-crystalline phase transition of large unilamellar vesicle (LUV) samples of dimyristoylphosphatidylcholine with a vesicle size of 100 nm using a heat capacity spectroscopic technique. A large broad heat capacity peak was observed at 24.35 °C. Almost no frequency dependence of the excess heat capacity was seen in the frequency region of 2–50 mHz. The transition enthalpy obtained by integrating the excess heat capacity was 22 kJ/mol, which roughly agrees with the static transition enthalpy of 18.9–26.4 kJ/mol for LUV samples obtained by differential scanning calorimetry. These facts indicate that there is no relaxation process below the frequency region of the present measurement. The heat capacity peak showed a significant rounding over a 200 mK range, which has been ascribed to the intrinsic nature of the transition. As a possible origin of this rounding, the external-field effect and the finite-size effect have been discussed. In particular, the result of the data fitting with a parametric representation of the equation of state suggests a possibility that the gel phase of LUV is a hexatic phase.

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I. INTRODUCTION

In a preceding paper [1], referred to as I hereafter, we studied the slow relaxation process in multilamellar vesicles (MLVs) of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) in the ultralow frequency range of 0.5–50 mHz using a heat capacity spectrometer developed by the present authors. In both DMPC and DPPC, extremely slow relaxation processes with mean relaxation times of more than 100 s were found at the main transition. On the other hand, it has been reported [2–4] that relaxation processes observed at the main transition for large unilamellar vesicle (LUV) samples and small unilamellar vesicle (SUV) samples differ from those seen in MLV samples. According to Tsong and Kanehisa [2], the relaxations for SUVs of 25 nm diameter are an order of magnitude faster than those for MLVs both in DMPC and DPPC. Van Osdol *et al.* [3] reported that the relaxation time for 120 nm LUVs of DPPC is about 80 ms over most of the transition range, while the relaxation time for MLVs varies from about 50 ms to 4 s in the transition range. They also reported that similar results were obtained with MLVs and LUVs of DMPC. Mayorga *et al.* [4] used somewhat larger (400 nm) LUV samples of DMPC and found relaxation processes with time constants on the order of 3.8 s and 80 ms. These facts suggest that the bilayer-bilayer interaction plays an important role for the relaxation process. However, only few measurements have been done so far on LUV samples in the millihertz frequency region. The lowest frequency used was 0.01 Hz in the work by Van Osdol *et al.* [3] and 0.04 Hz by Mayorga *et al.* [4]. Thus the whole relaxation spectrum has not yet been clarified. Besides, not only the dynamic nature, but also the static behavior shows a difference for MLV and LUV samples. Judging from differential scanning calorimetry (DSC) results, the heat capacity of MLVs shows a very sharp peak at the transition, while that for LUVs is much more broad, as seen in Fig. 1 of Ref. [3], for example. Relaxation amplitudes [3] and turbidity data [2] also show a similar tendency.

In the present work we have carried out dynamic heat capacity measurements on LUV samples of DMPC with a diameter of 100 nm in an ultralow frequency range similar to that used for MLV samples in I. Since the observed dynamic heat capacity showed almost no frequency dependence in this frequency region and the integrated dynamic heat capacity anomaly roughly agrees with the static transition enthalpy, it is expected that the heat capacity obtained in the present measurement represents the static behavior of the heat capacity. Hence the heat capacity data have been analyzed in detail to elucidate the thermodynamic nature of the transition.

II. EXPERIMENT

Synthetic 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine was purchased from Avanti Polar Lipids Inc. (Birmingham, AL). *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) was a product of Dojin Chemical Ltd. (Kumamoto, Japan). Sodium azide from Fluka (Neu-Ulm, Germany, MicroSelect grade) was used as a bacteriostat. Water was obtained from a Milli-Q-labo water purification system (Millipore Corp., Bedford, MA).

First, MLVs were prepared by adding appropriate amounts of 50 mM HEPES buffer (*p*H 7.0) containing 3 mM of sodium azide to weighed dry lipid. Subsequently,

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the mixture was vortexed for 1–2 min at a temperature 10 K above the gel to liquid-crystalline transition and incubated at this temperature for 1 h. During the incubation the dispersion was additionally vortexed for 30 s several times.

Then LUV samples were obtained by extruding MLVs using a stainless steel extrusion device (1.5 ml TBX, Lipex Biomembranes Inc., Vancouver, Canada) [5]. The temperature of the extruder was controlled at the same incubation temperature during the extrusion. Each extrusion cycle was performed through two stacked polycarbonate filters (100 nm pore size, Nuclepore 110405, Costar Corp., Cambridge, MA) employing a nitrogen pressure of 30 atm. This cycle was repeated ten times. According to Nayar *et al.* [5], LUVs 100±23 nm in diameter were obtained with this procedure. We have checked our LUV samples by a freeze-fracture electron microscopy and obtained a similar result. It has been reported that the SUVs prepared by ultrasonication are generally unstable, especially at a gel phase temperature, and aggregate to transform into vesicles of MLV packing [6]. As described later, our LUV samples prepared by the extrusion method turned out to be extremely stable. Nevertheless, to avoid any possibility of aggregation and to ensure high homogeneity of the sample, the LUV dispersions were preserved at a temperature 1 K above the transition until the measurements.

The frequency-dependent dynamic heat capacity has been measured with a fully automated heat capacity spectrometer. The details of this calorimeter have been given in I. About 30 mg of the sample was hermetically sealed into a sample cell made of gold. The sample cell was loosely coupled to a thermal bath. The bath temperature was changed stepwise, each step being 20 mK in the vicinity of the transition temperature, and the measurement was done after the bath temperature was stabilized within ±0.15 mK. At each temperature, the dynamic heat capacity was measured at seven frequencies ranging from 50 to 2 mHz.

III. RESULTS AND DISCUSSION

Figure 1 shows the temperature dependence of the absolute value of the dynamic heat capacity obtained on heating [Fig. 1(a)] and on cooling [Fig. 1(b)] near the gel to liquid-crystalline phase transition of LUVs. The concentration of the lipid was 15 wt. % for this sample. The heat capacities of the gold cell and the water contained in the cell have been subtracted. The temperature of the heat capacity maximum is located at 24.35 °C, which is about 0.5 K higher than the main transition temperature of MLVs [1]. Because the accuracy of the absolute temperature value is about 0.1 K in our calorimeter, this increase of 0.5 K is clearly beyond the experimental error. A small hump around 23.9 °C may be due to small residual MLV components. A small shoulder is also seen around 22.5 °C, but the origin is not clear at present.

It should be stressed that the stability of the sample was excellent. Including the results shown here, three heating runs and three cooling runs were made on the same sample, which needed about 6 months. The peak height agreed within ±5% and the peak temperature remained unchanged during this whole period. Two more samples with reduced lipid concentrations (1.8 wt. % and 0.9 wt. %) were also measured, which gave essentially the same results. For example, the peak height agreed within ±10% in these three samples and this discrepancy is comparable with the present error in estimating the lipid concentration.

The heat capacity anomaly is quite different from that of MLVs reported in I. First of all, it is noticed from Fig. 1 that the anomalies on heating and on cooling are almost identical, while the anomalies of MLVs exhibited considerable irreversibility.

It is also seen that the heat capacity anomaly accompanying the transition is very broad. The full width at half maximum is about 360 mK, which is larger than that in our MLV data almost by an order, but agrees with the value 0.4 K reported for 400 nm LUVs by Mayorga *et*

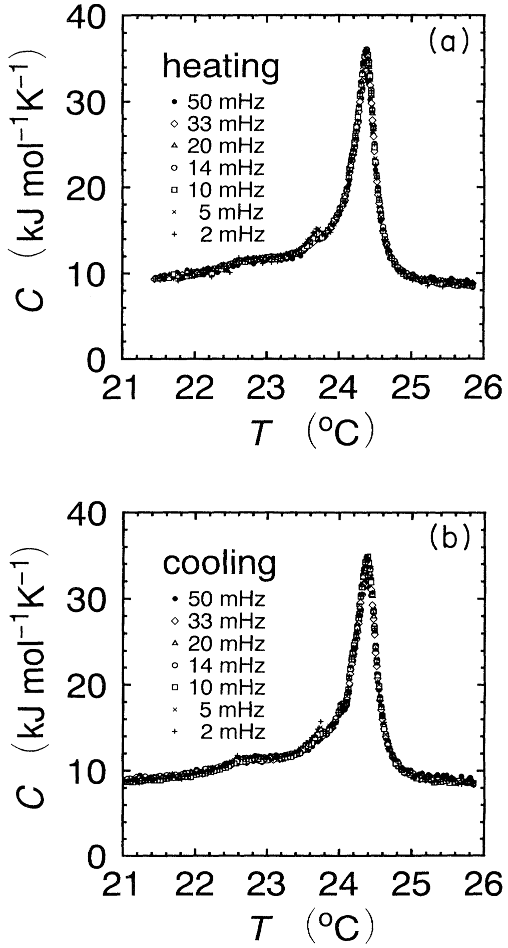

FIG. 1. Temperature dependence of the absolute value of the complex heat capacity of the LUV sample of DMPC measured in a frequency region of 2–50 mHz obtained (a) in a heating run and (b) in a cooling run.

al. [4]. We will discuss the reason for this broadening in detail later.

Another striking feature is that the heat capacity anomaly shows almost no frequency dependence, while that of MLVs at the main transition significantly depends on the measuring frequency. This means that there exists no relaxation process in this frequency region in the case of LUVs. Moreover, the transition enthalpy calculated from the present data is 22 kJ/mol, which roughly agrees with the static transition enthalpy obtained by DSC: 18.9–26.4 kJ/mol [4,7–9]. Since this fact indicates that the frequency region studied in the present work is low enough compared to all the relaxation processes accompanying this phase transition, there exists no relaxation process in lower frequency regions. Therefore, it is concluded that the heat capacity obtained here represents the static one and the longest relaxation time is far shorter than $(2\pi \times 50\ \text{mHz})^{-1} \cong 3$ s, which is consistent with the result by Van Osdol *et al.* [3]. Although Mayorga *et al.* [4] found a relaxation time of 3.8 s on their 400 nm LUV sample, the quantitative comparison of their data and ours seems difficult because of the difference in the vesicle size.

Figure 2 shows a detailed view of the heat capacity near the transition obtained at 5 mHz on heating. The peak shows a significant rounding, having points of inflection separated by about 200 mK. Although rounding is often caused by impurities in a sample, it is quite unlikely that this is the case in the present LUV data. Our LUV samples were prepared from the MLV samples, for which the high quality was guaranteed by a very sharp heat capacity anomaly at the main transition [1]. Another concern is that the rounding might be caused by the distribution of the vesicle size. However, our LUV samples are quite homogeneously sized because they have been prepared by the extrusion method as described above. Hence we conclude that the rounding seen in the present LUV data is not due to impurity in the samples or the vesicle size distribution, but reflects the intrinsic nature of the transition.

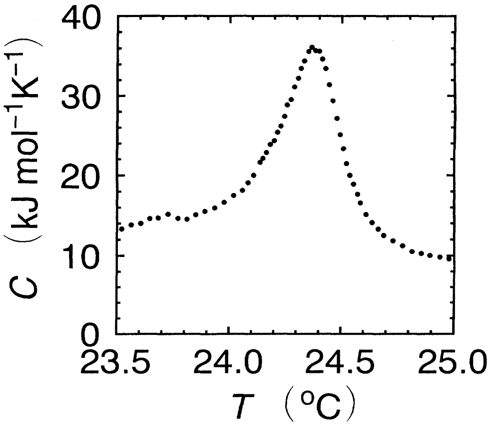

FIG. 2. Detailed view of the heat capacity peak for the LUV sample of DMPC obtained on heating at 5 mHz.

It is well known that a rounding of heat capacity peak is caused intrinsically when an external field conjugate to the order parameter is applied to the system in the vicinity of a critical point. In this case, the phase transition which accompanies a singularity in the free energy or one of its derivatives does not occur any more. Instead, the system continuously changes from one phase to another, showing somewhat broadened anomalies in the heat capacity.

Here we analyzed the present LUV data with the parametric representation of the equation of state proposed by Ho and Litster [10] to describe the behavior of thermodynamic quantities near a critical point with nonzero external field. The basic idea of the parametric approach is that in the temperature T and field H space near a critical point any thermodynamic quantity $X(T,H)$ with a critical exponent x can be transformed to $X(r,\theta) = f_x(\theta)\, r^{-x}$, where r is the distance to the critical point and θ defines the path of approach: $\theta = 0$ on the critical isochore, $\theta = \pm b^{-1}$ on the critical isotherm, and $\theta = \pm 1$ on the coexistence curve. To be more specific, we have utilized a linear parametric model [10] with

$$T(r,\theta) = (1 - b^2\theta^2)\, r\ , \tag{1}$$

$$H(r,\theta) = \theta(1 - \theta^2)\, r^{2-\alpha-\beta}\ , \tag{2}$$

$$m(r,\theta) = K\theta\, r^{\beta}\ , \tag{3}$$

where m is the order parameter, α and β are the critical exponents of the heat capacity and the order parameter, and $b^2 = (2-\alpha-4\beta)(2-\alpha-2\beta)^{-1}\,(1-2\beta)^{-1}$. Then the excess heat capacity at constant field is calculated as

$$\Delta C_H(r,\theta) = A\, c(\theta)\, r^{-\alpha} + B_C\ , \tag{4}$$

where B_C is a small critical contribution to the nonsingular part of the heat capacity [11]. In the present fitting we have assumed a temperature-independent normal heat capacity with a value of B_N. Hence the total heat capacity is written as

$$C_H(r,\theta) = A\, c(\theta)\, r^{-\alpha} + B\ , \tag{5}$$

where $B = B_C + B_N$. We also assumed that the field remains constant throughout the whole temperature region of the fitting $H = H_0 =$ const.

Figure 3 shows the observed heat capacity data (dots) and theoretical fitting curve (solid line), also with the underlying critical behavior expected in the absense of a field (dashed line). The agreement between the observed and calculated values is considerably good. The values of the parameters are listed in Table I. As far as the authors

TABLE I. Parameter values for the fit with the parametric equation of state. Units for A and B are kJ/mol K and that for T_C is °C. Without losing any generality, the value of K has been set to unity.

10^3A	α	β	T_C	10^5H_0	B
2.12	0.51	0.10	24.29	2.8	5.73

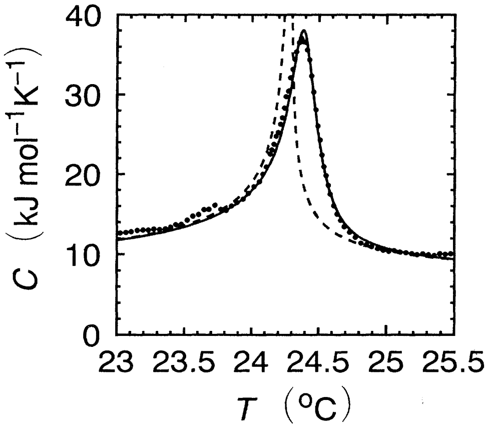

FIG. 3. Comparison of the observed data and theoretical fit. The solid curve is the parametric equation fit and the dashed curve represents the equation's prediction for the underlying critical behavior in the absence of a field.

are aware, there is no universality class that has the exponent values obtained here. The value of α obtained is very close to that for a tricritical point $\alpha = 0.5$, while the value of β is definitely smaller than the tricritical value $\beta = 0.25$. In particular, fixing the values of α and β to those for any known universality class resulted in clearly poor fits.

Significantly, there exists a phase transition that shows exponents α and β very close to those obtained here. Garland *et al.* [11] analyzed their data on the smectic-*I* (Sm-*I*)–smectic-*C* (Sm-*C*) transition of a liquid-crystal methylbutylphenyloctyloxybiphenyl carboxylate (8OSI) with the same approach used here and obtained $\alpha = 0.47$ and $\beta = 0.077$. Moreover, their heat capacity data show quite similar features seen in the present LUV data: a clear rounding near the maximum of the anomaly. This fact suggests that the gel to liquid-crystalline transition in LUVs has a common nature with the Sm-*I*–Sm-*C* transition.

It is worthwhile to consider to what extent the similarlity between these two systems holds. The Sm-*I* phase is one of the hexatic phases, in which there exists a long range sixfold order in the direction of local crystalline axes (bond-orientational order) in each smectic layer, while the two-dimensional translational order is only short range. In the Sm-*C* phase, there exists no long range order with respect to the arrangement of the molecules in layers. As for the LUV, its liquid crystalline phase is similar to the Sm-*C* phase in the sense that it has no long range order in lamellars. Although the structure in the gel phase of the LUV is not clearly known, Smith *et al.* [12] performed x-ray scattering studies of freely suspended multilamellar films of DMPC and suggested a possibility of a hexatic order in the $L_{\beta'}$ phase. Moreover, Nelson and Peliti [13] predicted that many two-dimensional membranes that are crystalline, when confined to a plane, will become hexatic when allowed to fluctuate into the third dimension. Therefore, it may be not so surprising if the gel phase of LUVs has the hexatic order. For further comparison, a detailed structural analysis on LUVs is needed.

The origin of the existence of the nonzero field is another issue. In the case of the tilted hexatic phases such as that of 8OSI, theory [14] predicts that a field due to the tilt of molecules induces finite bond-orientational order in the Sm-*C* phase. On the other hand, the origin of the field is not clear in the transition of LUVs. As for MLVs, the chains are tilted in the gel phase, while they are normal to the layer surface in the liquid-crystalline phase. In the case of LUVs, however, whether the chains are tilted is not known. It is also possible that the large curvature of the vesicle surface serves as the external field.

So far we have considered the field effect as the origin of the rounding of the heat capacity data. However, since the vesicle size of the present LUV samples, about 100 nm, is small enough to be regarded as semimicroscopic, it is possible that the rounding is caused by the finite-size effect. Because the growth of the correlation length in the vicinity of a critical point is limited by the system size, the divergence of the heat capacity is suppressed and results in a somewhat rounded peak in a finite-size system [15].

It is difficult to decide at this moment which of the two mechanisms discussed above (or both ?) is effective as the origin of the rounding observed in the transition of LUVs. In any case, it is of interest to make systematic measurements on LUVs of various vesicle sizes. Such an effort is now in progress and the result should be reported shortly.

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