

Effect of chain length on the heat-capacity anomaly at the gel to liquid-crystalline phase transition in unilamellar vesicles of phosphatidylcholines

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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 dilauroylphosphatidylcholine (DC₁₂PC, or DLPC), ditridecanoylphosphatidylcholine (DC₁₃PC) and dipentadecanoylphosphatidylcholine (DC₁₅PC) with a vesicle size of 100 nm. The heat capacity showed a broad peak, which is qualitatively similar to that observed in LUV sample with the same vesicle size of dimyristoylphosphatidylcholine (DC₁₄PC, or DMPC). It was found that the nature of the transition depends on the chain length of the lipid molecules. The observed half-widths of the anomalies are 2.4 K in DC₁₂PC and 0.7 K in DC₁₃PC, which are both larger than that in DC₁₄PC. The transition is first order in DC₁₅PC. It was shown that the results for DC₁₂PC, DC₁₃PC, DC₁₄PC, and DC₁₅PC can be described in a semiquantitative way with the theoretical calculation, which takes into account the curvature of the vesicle surface. [S1063-651X(97)01607-3]

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I. INTRODUCTION

There have been intensive theoretical and experimental efforts to describe the behaviors of lipid bilayers, especially focusing on the gel to liquid-crystalline phase transitions. In our recent work (referred to as I hereafter) [1], we measured the heat-capacity anomaly accompanying the gel to liquid-crystalline phase transition of large unilamellar vesicles (LUV's) and small unilamellar vesicles (SUV's) of dimyristoylphosphatidylcholine (DC₁₄PC, also called DMPC in some literature). It was found that the heat-capacity anomaly depends significantly on the vesicle size. To explain this result, we modified the statistical theory proposed by Izuyama and Akutsu [2] so that we can apply it to the case of curved bilayers. Hereafter, we call the original theory by Izuyama and Akutsu the original IA theory or just IA theory, and our modified version as the modified IA theory. It was found that the modified IA theory can reproduce the observed size dependence of the heat-capacity anomaly.

On the other hand, the present authors [3,4] pointed out that interlayer interaction plays an important role in multilamellar vesicles (MLV's), and the dynamic character of the transition in MLV's differs significantly from that in LUV's (or SUV's). In particular, the existence of a very slow relaxation process in MLV's makes the precise evaluation of the static properties difficult. Further, it is probable that the static characters of MLV's are also affected by the interlayer interaction. Most theories for lipid bilayers including the IA theory treat a single bilayer, and therefore the above situation makes the application of such theories to MLV's unrealistic. Because of this, we see that LUV's and SUV's are the best candidates for comparing the theories and experiments on lipid bilayers. Earlier, precise measurements on unilamellar vesicles were quite limited. This is mainly because unilamellar vesicles prepared by ultrasonic irradiation are generally unstable [5]. However, the present authors showed that unilamellar vesicles prepared by the extrusion method are quite stable, and therefore enable us to obtain their detailed physical properties [1,4].

In I, the effect of the vesicle size on the heat-capacity anomaly was studied. Another important parameter of the system is the chain length of the lipid. Quite interestingly, the vesicle size and the chain length both act in a similar way in the sense that they drive the transition from first order to critical, and then to supercritical. Therefore, studying the effect of the chain length on the transition is expected to provide further information on the validity of the modified IA theory. It is to be noted that the Monte Carlo simulation [6] based on the Pink model [7] also predicted that the peaks in the susceptibility and the heat capacity become broadened as the chain length decreases, which agrees with the expectation of the IA theory.

In the present work we have carried out heat-capacity measurements on LUV samples of dilauroylphosphatidylcholine (DC₁₂PC), ditridecanoylphosphatidylcholine (DC₁₃PC), and dipentadecanoylphosphatidylcholine (DC₁₅PC). The results described below show that the heat-capacity anomaly depends significantly on the chain length. It is found that the modified IA theory can reproduce the chain-length dependence of the heat-capacity anomaly.

II. EXPERIMENT

1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DC₁₂PC), 1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine (DC₁₃PC), and 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine (DC₁₅PC) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). LUV's of 100 nm diameter were prepared with a similar extrusion procedure on MLV's as described in our previous works [1,4]. Two stacked polycarbonate filters (100 nm pore size, Nuclepore 110405, Costar Corp., Cambridge, MA) were used for the extrusion, employing a nitrogen pressure of 20 atm. Then residual MLV's were removed by a centrifugation under 20 000g for 30 min, which was repeated two times. The extrusion and the centrifugation procedures were carried out at temperatures as shown in Table I. The phospholipid concentrations were assayed by phosphate analysis [8]. The vesicle size of the LUV's thus prepared was mea-

TABLE I. Temperature of the extrusion T_{ext} and centrifugation T_{cent} procedures, and the effective vesicle diameter D_{eff} . The range in D_{eff} shows the standard deviation. The *effective* diameter is the averaged value weighted with the squares of the vesicle size (see Ref. [1]).

System	T_{ext} ($^{\circ}\text{C}$)	T_{cent} ($^{\circ}\text{C}$)	D_{eff} (nm)
DC ₁₂ PC	21	5	88 \pm 25
DC ₁₃ PC	26	10	92 \pm 24
DC ₁₅ PC	42	30	97 \pm 33

sured by dynamic light scattering (DLS) using an Ohtsuka Electronics model DLS600 multiangle particle analyzer system (Makikata, Japan) with a 5-mW helium-neon laser. The samples were diluted about 20 times with the same buffer solution as used in preparing MLV's. The sample temperature was kept 10 K above the gel to liquid-crystalline transition temperature and DLS data were collected for 20–90 min at a scattering angle of 90° . The results are also shown in Table I.

The dynamic heat-capacity has been measured with the heat capacity spectrometer used in I. About 60 mg of sample was hermetically sealed into a sample cell made of gold. The bath temperature was scanned very slowly, with scan rates of 0.04–0.15 K/h. In the results shown below, the accuracy of the absolute values of the obtained heat-capacity anomaly is about 5%, which is mainly due to the uncertainty in the phosphate analysis. On the other hand, the accuracy of the absolute values of the temperature is believed to be better than 0.2 K. The measuring frequency was set to 0.01 Hz in DC₁₂PC and DC₁₃PC, and 0.01 Hz and 0.0033 Hz in DC₁₅PC. The LUV samples prepared by the extrusion method were quite stable, just as in the LUV and SUV samples of DC₁₄PC reported earlier [1,4].

III. RESULTS

A. Results for DC₁₃PC

We first describe the results obtained for DC₁₃PC samples, because this system has been studied in most detail among the three. Figure 1 shows the temperature dependence of the anomalous heat capacity ΔC_p obtained on heating [Fig. 1(a)] and on cooling [Fig. 1(b)] near the gel to liquid-crystalline phase transition of a DC₁₃PC LUV sample. The measuring frequency was 0.01 Hz. The concentration of the lipid was 1.59 wt. % for this sample. The data show a main peak located at 14.2 $^{\circ}\text{C}$, accompanied by a small peak at 13.3 $^{\circ}\text{C}$. The main peak shows a similar feature to that of DC₁₄PC LUV samples reported earlier [4] in the sense that the data on heating and on cooling are almost identical, and that the heat-capacity anomaly shows a noticeable rounding. The full width at half maximum $\Delta T_{1/2}$ in this result is about 0.7 K, which is about twice that in LUV's of DC₁₄PC. This fact shows that the transition is supercritical in DC₁₃PC, and further away from the critical point than in DC₁₄PC. The peak at 13.3 $^{\circ}\text{C}$ is ascribed to the small amount of residual MLV component because it coincides with the main transition temperature in MLV, which is located about 0.9 K lower than the main peak of LUV's [9]. In addition to the peak at

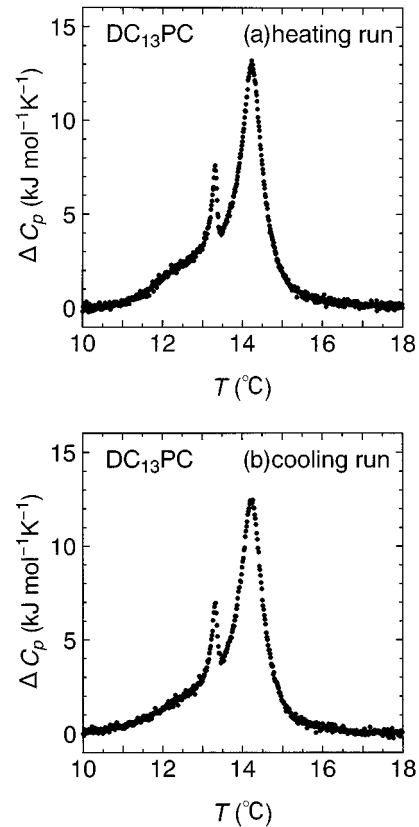


FIG. 1. Temperature dependence of the anomalous heat capacity ΔC_p of LUV sample of DC₁₃PC obtained (a) in a heating run and (b) in a cooling run.

13.3 $^{\circ}\text{C}$, a very slight shoulderlike anomaly is seen around 12.1 $^{\circ}\text{C}$. The origin of this anomaly is not clear.

The transition enthalpy obtained by integrating the present heat-capacity anomaly is $\Delta H = 18$ kJ/mol. Because of the uncertainty in estimating the normal heat capacity, this ΔH value might have an error of ± 2 kJ/mol or so. The ΔH obtained here almost agrees with $\Delta H = 18.4$ kJ/mol obtained by differential scanning calorimetry (DSC) [10]. This fact ensures that the frequency of the present measurement is low enough to obtain the quasistatic value of the heat capacity.

The values of the temperature of the main peak T_m , the transition enthalpy ΔH , and the half-width of the main peak

TABLE II. Comparison of the heat-capacity anomaly obtained for LUV samples of DC₁₂PC, DC₁₃PC, DC₁₄PC, and DC₁₅PC. Here T_m is the temperature of heat capacity maximum, ΔH is the transition enthalpy, and $\Delta T_{1/2}$ is the half-width of the heat-capacity peak. The value of M has been estimated in the way described in the text.

System	T_m ($^{\circ}\text{C}$)	ΔH (kJ/mol)	$\Delta T_{1/2}$ (K)	M
DC ₁₂ PC	-2	5.5	2.4	16.8
DC ₁₃ PC	14.2	18	0.7	20.2
DC ₁₄ PC ^a	24.4	22	0.36	21.5
DC ₁₅ PC	34.1	17.5 ^b	- ^b	> 23.6

^aThe values for DC₁₄PC have been taken from Ref. [4].

^bCannot be accurately estimated because of first-order nature of the transition. See text.

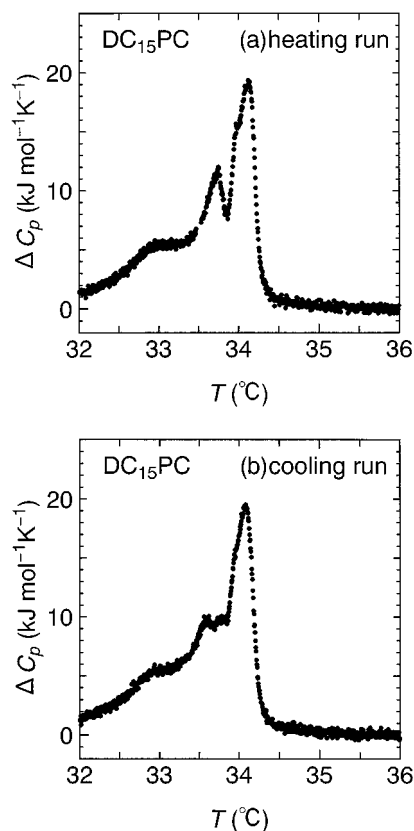


FIG. 2. Temperature dependence of the anomalous heat capacity ΔC_p of LUV sample of DC₁₅PC obtained (a) in a heating run and (b) in a cooling run.

$\Delta T_{1/2}$ are summarized in Table II. The values for DC₁₃PC and DC₁₅PC obtained in the present measurement, which are described below, and the values for DC₁₄PC obtained in our previous work [4] are also shown.

B. Results for DC₁₅PC

Figure 2 shows the temperature dependence of the anomalous heat capacity ΔC_p obtained on heating [Fig. 2(a)] and on cooling [Fig. 2(b)] near the gel to liquid-crystalline phase transition of a DC₁₅PC LUV sample measured at 0.01 Hz. The concentration of the lipid was 1.17 wt. % for this sample. The data show a somewhat rounded main peak located at 34.1 °C, accompanied by small peaks at 33.7 °C and 32.9 °C. It is quite probable that these small peaks are due to MLV residuals as mentioned in the result for DC₁₃PC in the previous subsection. We here do not discuss this point because they are small in magnitude and therefore do not affect the main feature of the data.

The gel to liquid-crystalline transition in this case is believed to be first order from the following reasons. Figure 3 shows the behavior of the phase lag ϕ of the ac temperature response to the ac heating. It is seen that ϕ shows an abrupt increase around 34.1 °C, where the heat capacity peak is located. Such an increase in ϕ is usually understood to indicate the first-order nature of the transition [11]. Figure 4 shows a comparison of the data obtained on heating measured at 0.01 Hz (open circles) and at 0.0033 Hz (crosses). It is seen that the peak becomes larger for lower measuring

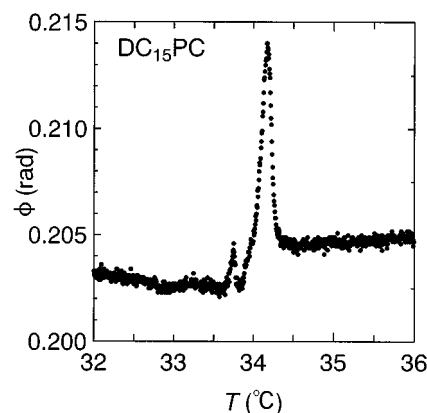


FIG. 3. Temperature dependence of the phase lag ϕ of LUV sample of DC₁₅PC.

frequency. Such a frequency dependence of the anomaly is qualitatively similar to what was observed in MLV sample [3], which is ascribed to the motion of the two-phase coexistence boundary. Therefore, this result is another support that the transition is first order in DC₁₅PC.

The transition enthalpy obtained by integrating the present heat-capacity anomaly measured at 0.0033 Hz is $\Delta H = 17.5$ kJ/mol. In addition to the error of about ± 2 kJ/mol due to the uncertainty of the normal heat capacity mentioned above, this ΔH value is expected to be smaller than the real value because some part of the latent heat is not included in the measured heat anomaly [12]. Therefore, the above value seems reasonable because it is about 61% of the value obtained by DSC, $\Delta H = 28.9$ kJ/mol [10].

C. Results for DC₁₂PC

Figure 5 shows the temperature dependence of the anomalous heat capacity ΔC_p obtained on heating [Fig. 5(a)] and on cooling [Fig. 5(b)] near the gel to liquid-crystalline phase transition of a DC₁₂PC LUV sample. The measuring frequency was 0.01 Hz. The concentration of the lipid was 1.49 wt. % for this sample. The data show two broad peaks located around -2 °C, and 4 °C. It has been reported that DC₁₂PC MLV's undergo two successive transitions [13,14].

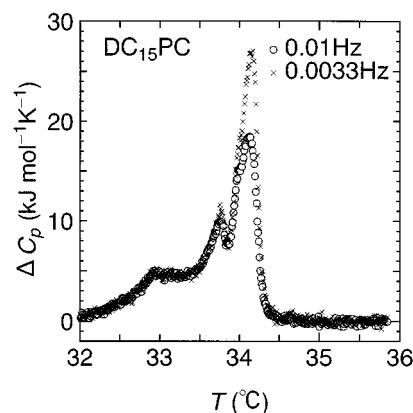


FIG. 4. Comparison of the anomalous heat capacity ΔC_p of LUV sample of DC₁₅PC measured at 0.01 Hz (open circles) and 0.0033 Hz (crosses).

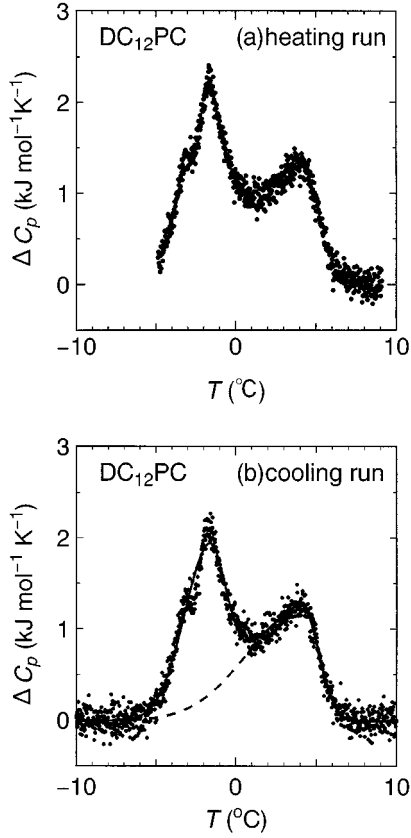


FIG. 5. Temperature dependence of the anomalous heat capacity ΔC_p of LUV sample of DC₁₂PC obtained (a) in a heating run and (b) in a cooling run. In (b), the solid line shows a fit to the data assuming a form of two asymmetric Gaussian components. The dashed line shows the higher-temperature side peak thus obtained.

Our present result indicates that LUV's of DC₁₂PC also show the same phase sequence. Because of large overlap of the two peaks, it is difficult to estimate $\Delta T_{1/2}$ and the transition enthalpy directly from the data. For this reason, we fitted the data assuming that the observed anomaly can be deconvoluted as a sum of two Gaussian components. It is seen from the figure that the higher-temperature side peak is clearly asymmetric. Therefore, we allowed the widths above and below the peak to be different from each other [15]. The result of the fit is shown as the solid line in Fig. 5(b). After subtracting the higher-temperature side component, also shown as the dashed line in Fig. 5(b), the lower-temperature side peak is shown in enlarged scales in Fig. 6. Assuming that this lower peak corresponds to the gel to liquid-crystalline phase transition as is in MLV's, and excluding the shoulder just below the peak, we obtain the half-width $\Delta T_{1/2}$ to be about 2.4 K. The transition enthalpy becomes $\Delta H = 5.5$ kJ/mol. This value is slightly smaller than the value $\Delta H = 7.6$ kJ/mol obtained by DSC measurement [13]. Another comparison can be made using the total transition enthalpy for the two phase transitions altogether, which gives $\Delta H = 12$ kJ/mol from the data before the deconvolution. This value roughly agrees with $\Delta H = 16$ kJ/mol obtained by DSC measurement [13]. A somewhat large discrepancy can be understood because the heat anomaly is broad and small in this case so that the uncertainty in the normal part affected more seriously.

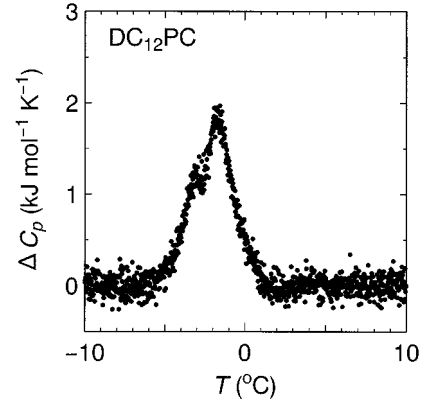


FIG. 6. Temperature dependence of the lower temperature side peak of the anomalous heat capacity ΔC_p of LUV sample of DC₁₂PC obtained by a deconvolution.

IV. DATA ANALYSIS

A. Modified IA theory

In I, we evaluated the effect of the finite curvature of the vesicle surface in the first approximation starting from the calculation made by Izuyama and Akutsu for a flat bilayer [2]. We here summarize the main results. The free energy obtained in the original IA theory is given as

$$G = G^{\text{chain}} + G^{\text{elas}}, \quad (1)$$

where

$$G^{\text{chain}} = \epsilon \rho + k_B T \left[\frac{\rho \ln \rho}{M} - (\ln 4) \rho + \frac{\pi}{2} \rho^2 + \frac{\pi^2}{12} \rho^3 + \frac{5\pi^3}{192} \rho^4 \right] \quad (2)$$

is the contribution from the cooperative conformation of hydrocarbon chains, and

$$G^{\text{elas}} = \frac{1}{2} \omega Q^2 + P(1+Q)^2 \quad (3)$$

is the elastic energy coming from the dilatation in the spacing of the lipid molecules [2]. Here, ρ is the ‘‘disorder’’ parameter, which becomes zero in the completely ordered state, Q is a normalized increase in the chain spacing from the value in the ground state for zero pressure, $P=0$, and M is twice the chain length. The excitation energy ϵ diminishes as Q increases because the steric hindrance of the CH₂ groups against the chain bending is reduced, and therefore we have

$$\epsilon = \epsilon_0 - \alpha Q, \quad (4)$$

with positive constants ϵ_0 and α .

The above free energy is for a flat bilayer, and therefore should be modified for curved bilayers. For the outer layer, ϵ_0 , α , and P are replaced by $\epsilon_0 + \Delta\alpha$, $\alpha - \Delta\alpha$, and $P - \Delta P$, respectively. Here $\Delta\alpha$ and ΔP are both positive and proportional to the curvature of the bilayer. The result for the inner layer is obtained just reversing the signs of the corrections. These corrections make the values of ρ and Q become generally different for the outer and inner layers. We therefore write them ρ^+ and Q^+ for the outer, and ρ^- and Q^- for

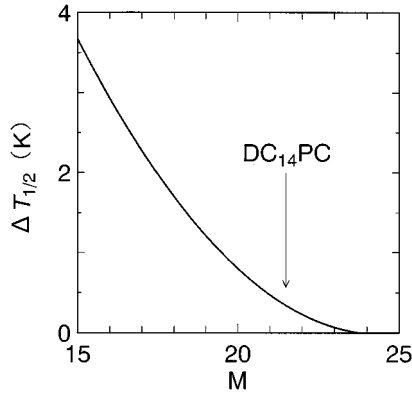


FIG. 7. Dependence of half-width of the heat-capacity anomaly on the parameter M calculated with the modified IA theory. Other parameter values are $\alpha = 1125$ K, $\omega = 985$ K, $\epsilon_0 = 408$ K, $P = 20$ K, $\Delta\alpha = 0.8$ K, $\Delta P = 1.1$ K, $\mu = 0.91$, and $\lambda = 0.07$.

the inner layer. It is also to be noted that Q^+ and Q^- are not independent in curved bilayers. In the first approximation we have

$$Q^+ = \mu Q^- - \lambda(\rho^+ + \rho^-), \quad (5)$$

where $\mu \equiv R^-/R^+$, with R^+ and R^- being the radii of outer and inner layers, and λ is a constant inversely proportional to the vesicle radius.

The total free energy per molecule is given as

$$G^{\text{total}} = \frac{1}{1 + \mu^2} G^{\text{outer}} + \frac{\mu^2}{1 + \mu^2} G^{\text{inner}}. \quad (6)$$

In Eq. (6), G^{outer} is given by Eqs. (1)–(4) using ρ^+ and Q^+ , and G^{inner} using ρ^- and Q^- , with appropriately corrected values of ϵ_0 , α , and P . Then G^{total} is minimized with respect to ρ^\pm and Q^\pm under the constraint Eq. (5).

B. Comparison with observed data

In this subsection we try to reproduce the observed chain-length dependence of the heat anomaly in DC₁₂PC, DC₁₃PC, DC₁₄PC, and DC₁₅PC LUV samples with the modified IA theory. The parameters contained in the theory are α , ω , ϵ_0 , P , M , $\Delta\alpha$, ΔP , μ , and λ . To be strict, all these parameter values should be determined separately for each lipid system. We can expect, however, that $\Delta\alpha$, ΔP , and μ , which reflect the vesicle curvature, are more or less alike for the four systems having equal vesicle size [16,17]. The parameter ϵ_0 , on the other hand, can be different for each system, but probably its main effect is to shift the overall temperature scale. Parameters α , P , and λ are expected to be more or less alike for the four systems. Based on such considerations, we here choose to change only M systematically, while other parameters are fixed to the values that were used in our former analyses on DC₁₄PC data [1]. Figure 7 shows the dependence of the half-width $\Delta T_{1/2}$ of the calculated heat-capacity peak on the parameter M . As expected, $\Delta T_{1/2}$ decreases monotonously with increasing M , showing that the transition becomes less supercritical with increasing M . At $M \approx 23.6$, the transition becomes critical and the heat capac-

ity diverges, and therefore $\Delta T_{1/2}$ becomes zero. The transition is first order for $M > 23.6$.

In I, we showed that the experimental data for DC₁₄PC LUV sample were described well with $M = 21.5$, which is indicated by an arrow in Fig. 7. Since we have fixed ϵ_0 , the absolute temperature scale of the calculated heat anomaly cannot be compared with the observed data. On the other hand, the relative temperature dependence of the heat anomaly, and therefore the half-width $\Delta T_{1/2}$, can be directly compared with the data. For example, we find from Fig. 7 that the observed value $\Delta T_{1/2} = 0.7$ K for DC₁₃PC corresponds to $M = 20.2$. The M values for DC₁₂PC and DC₁₃PC thus obtained are shown in the last column of Table II. We have seen that the transition for DC₁₅PC is first order. From Fig. 7, this corresponds to the region $M > 23.6$. Thus we estimate that $M > 23.6$ for DC₁₅PC. Theoretically, it is expected that M increases by 2 as the chain length increases by 1. From Table II, the increase in M determined from the comparison of the calculation and experimental data has values between 1.3 and 3.4. Therefore, we can say that the estimated M values for DC₁₂PC, DC₁₃PC, DC₁₄PC, and DC₁₅PC compare moderately well with this theoretical expectation.

V. DISCUSSION

In the present study we have seen that the gel to liquid-crystalline phase transition is supercritical in DC₁₂PC, DC₁₃PC, and DC₁₄PC. The fact that $\Delta T_{1/2}$ decreases in the order of DC₁₂PC, DC₁₃PC, and DC₁₄PC indicates that the transition gets closer to the critical point in this order. Finally, the transition becomes first order in DC₁₅PC. Such a trend is in agreement with the prediction of the (modified) IA theory and also with that of the Pink Model [7]. Moreover, as shown in Sec. IV, these results can be roughly explained also in a quantitative manner with the modified IA theory in the sense that the M values for the four systems differ approximately by 2. The slight discrepancy between the observed M values and the theoretical expectation should not be viewed too seriously. The comparison with the data has been made in a rather simplified way, changing only M systematically, while other parameters were fixed. Therefore, the difference in the parameters for the different lipid systems might have been renormalized into the observed M values. In addition, the comparison of theory and experiment is not straightforward for DC₁₂PC. One reason is that an experimentally obtained $\Delta T_{1/2}$ value is less accurate since the anomaly is small and broad. Further, the existence of another phase transition in DC₁₂PC might reduce the applicability of the theory at least in a quantitative sense. Taking such situations into account, we can conclude that the modified IA theory can explain both the vesicle-size and the chain-length dependence of the gel to liquid-crystalline phase transition.

The effect of the critical fluctuation is neglected in the original and also in the modified IA theory. In I, we argued that the use of the IA theory is justified as long as the transition is not too close to the critical point. However, it is expected that the fluctuation effect becomes significant in the immediate vicinity of the critical point. In that case, the heat capacity is expected to show a divergent character with some critical exponent α . It is therefore of special interest to check

whether the experimentally observed value of α agrees with the value $\alpha=2/3$ predicted by the IA theory [18]. The critical point can be reached by appropriately choosing the vesicle size and the chain length. From the present result, the critical point is located in a range $14 < x < 15$ for 100 nm LUV's of DC_xPC. Therefore, there are three candidates that we can reach the critical point: (A) 100 nm LUV's of a mixture of DC₁₄PC and DC₁₅PC, (B) DC₁₄PC LUV's with a diameter larger than 100 nm, or (C) DC₁₅PC LUV's with a diameter smaller than 100 nm. An approach on this line is planned in the near future.

As seen in the present work and also in our previous works [1,4], unilamellar vesicle samples prepared by the extrusion method are fairly stable over a period of more than several weeks. On the other hand, it is known that unilamellar vesicles prepared by ultrasonic irradiation are generally unstable [5]. It has been also reported that there exist relatively stable sonicated large vesicles as an intermediate state between MLV's and the sonicated SUV's [19,20]. According to Kodama *et al.* [20], these vesicles are about 200 nm in mean diameter, and composed of mostly four or so lamellae. The LUV samples prepared in the present study are believed to be different from these sonicated large vesicles. This is first because the sonicated large vesicles have transition temperature slightly lower than that of MLV's, while the transition temperature in our LUV's is slightly higher than that. Further, the vesicle size of LUV's determined by dynamic light scattering turned out to have a sharp distribution with the average value of about 100 nm [1]. A recent observation

by a transmission electron microscopy [21] has revealed that most of the vesicles prepared by an extrusion method are unilamellar vesicles, while some amount of oligolamellar vesicles also coexist (see Fig. 3 of Ref. [21]). This is consistent with our result since a very small amount of MLV-like component are seen in ours, although its fraction has been reduced significantly by centrifugation. Very recently, Brumm *et al.* [22] studied the effect of membrane curvature on the phase transition for DC₁₄PC and distearoylphosphatidylcholine (DC₁₈PC). They observed broadening of the heat-capacity peak as the curvature increases, in agreement with our result reported in I. However, they also found that the transition temperature decreases significantly with increasing curvature, which they ascribed to the reduction of lateral pressure. They showed that such a decrease in T_c is reproduced by a computer simulation based on the Pink model. Their interpretation seems reasonable because they used single bilayers on a spherical support of a silica and are likely to be in a mechanically clamped condition. It is conceivable that our unilamellar vesicles prepared with the extrusion method is close to stress-free condition, while the sonicated vesicles are in mechanically excited state.

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