Relationship between the unbinding and main transition temperatures of phospholipid bilayers under pressure

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Using neutron diffraction and a specially constructed high pressure cell suitable for aligned multibilayer systems, we have studied, as a function of pressure, the much observed anomalous swelling regime in dimyristoyl- and dilauroyl-phosphatidylcholine bilayers, DMPC and DLPC, respectively. We have also reanalyzed data from a number of previously published experiments and have arrived at the following conclusions. (a) The power law behavior describing anomalous swelling is preserved in all PC bilayers up to a hydrostatic pressure of 240 MPa. (b) As a function of increasing pressure there is a concomitant decrease in the anomalous swelling of DMPC bilayers. (c) For PC lipids with hydrocarbon chains $\geqslant 13$ carbons the theoretical unbinding transition temperature $T^{\star}$ is coupled to the main gel-to-liquid crystalline transition temperature T_M. (d) DLPC is intrinsically different from the other lipids studied in that its $T^{\star}$ is not coupled to T_M. (e) For DLPC bilayers we predict a hydrostatic pressure (>290 MPa) where unbinding may occur.

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I. INTRODUCTION

Over the past three decades, there has been a great deal of attention paid to the physics of the main gel-to-liquid crystalline transition of disaturated phosphatidylcholine (PC) lipids. This particular interest is due to the fact that many membrane parameters such as, bilayer permeability [1], heat capacities [2], fluorescence label lifetimes [3], NMR order parameters [4], ultrasound velocities [5], and multilamellar repeat distances [6] were found to exhibit pretransitional behavior typical for phase transitions of the second order, this despite the fact that the main transition itself is first order. On theoretical grounds, this can be understood by considering that the onset of a second-order transition is being intercepted by a first-order transition [7]. A more recent proposal [8], based on ultrasound velocity measurements and Frenkel's heterophase fluctuation theory [9], considers the main transition to be weakly first order, far from an unrealized critical point. However, as noted by Kharakoz and Shlyapnikova [8], while this may explain the observation of phenomena occurring within the plane of the bilayer, such as hydrocarbon chain packing, there may still be critical momentum out-of-plane fluctuations of the bilayers affecting the interbilayer water region to which ultrasound measurements are insensitive.

Neutron and x-ray diffraction measurements are sensitive to changes occurring to both the structure of the bilayer and the interstitial water. Of particular interest, and noted by diffraction, is the observation of the so called ''anomalous swelling'' phenomenon [6,10–17]. In this phenomenon, the lamellar repeat distance d, comprised of lipid bilayer stacks and interstitial water, increases nonlinearly as the temperature of the system is lowered towards the main liquid crystalline-to-gel transition or T_M. It should be noted that for a ''normal'' first-order phase transition exhibited by, for example, phosphatidylethanolamines, d increases linearly [18]. Early on, there was disagreement as to which part of d contributed to the anomalous swelling, i.e., whether the bilayer or water matrices, or both, were increasing anomalously [6,13,15,16,19]. After much debate on the proper analysis of diffraction of aligned lamellae and isotropic multilamellar vesicles, it seems that the lipid thickness increases, for the most part, linearly, while the water layer accounts for the majority of the nonlinear, anomalous swelling [10].

NMR is sensitive to the extension of the hydrocarbon chains through orientational order parameters of labeled chain segments. Data from Bonev and Morrow [20] and Nagle *et al.* [13] show only a small amount of nonlinearity of the acyl-chain order parameter near T_M. For dimyristoylphosphatidylcholine (DMPC) bilayers this contribution accounts for about 25% of the anomalous swelling [10]. Most recently, Pabst *et al.* [10] were further able to attribute the increase in interbilayer water to a drop of the bilayer modulus of bending rigidity K_c in the vicinity of T_M. This was earlier suggested, but also doubted [13,21]. Moreover, the functional form of K_c seems to follow a power-law dependence with temperature upon approaching T_M [8,10,22,23].

Independent of the observed phase transition phenomena in phosphatidylcholines, Lipowsky and Leibler [24] considered the critical unbinding of two interacting membranes due to steric repulsion. Presently, the only experimental results of a thermal unbinding transition were reported by Mutz and Helfrich [25] using digalactosyl diacylglycerol (DGDG) multilamellar vesicles (MLVs), and by Pozo-Navas *et al.* [26] using a mixture of phosphoethanolamine and phosphatidylglycerol with appropriate amounts of NaCl. One of the reasons mentioned by Lipowsky and Leibler [24] that leads to unbinding of membranes is a reduction in K_c, which, due to bilayer undulations enhances the steric repulsion of opposing bilayers [27].

It therefore seems that thermal unbinding and anomalous swelling, both suggested as being caused by a decrease in K_c, may be linked. Moreover, both of these phenomena exhibit mean membrane separations that diverge as $d \approx (y - y_c)^{-\psi}$ [15,24]. Lemmich *et al.* take the critical exponent ψ to be unity because this is consistent with the theoretical treatment of the thermal unbinding of membranes and because the accuracy of the data could not support an independent determination of ψ [24]. In addition, y is any modulus of a mechanical property contributing to the system's total free energy (e.g., K_c or the Hamaker constant), and y_c is the corresponding critical field value for that property. Changes in y as a function of temperature could drive the system into an unbound state at a temperature $T^\star$.

From theory which predicts the unbinding of lamellar stacks with the softening of the bilayer [22,24], we infer that although the mean value of K_c changes as a function of acyl-chain length [28], its functional form, with temperature, is always reflected in the functional form of the anomalous swelling. Therefore, as long as K_c follows the same temperature dependent power-law form in the vicinity of T_M, while varying either hydrocarbon chain length or hydrostatic pressure, we do not expect a relative difference between T_M and $T^\star$. More explicitly, $t_c = (T_M - T^\star)/T_M$ should remain constant.

Here we report on several small-angle neutron scattering (SANS) experiments, carried out as a function of hydrostatic pressure and temperature, using aligned, fully hydrated multibilayer stacks of dimyristoyl- and dilauroyl-phosphatidylcholine (14:0 PC, DMPC and 12:0 PC, DLPC, respectively) [29,30]. We are able to observe that the power law form of anomalous swelling in PC bilayers is preserved under conditions of high hydrostatic pressure, up to 240 MPa. Moreover, we observed, as a function of pressure, the systematic suppression of anomalous swelling in DMPC bilayers. We interpret this to mean that although the functional form of K_c is preserved, the function's amplitude is decreasing with increasing pressure. The extrapolated point of thermal unbinding $T^\star$ is coupled to T_M for lipids with hydrocarbon chains longer than those in DMPC (i.e., >14), including DMPC. We also observe that DLPC is intrinsically different from the other lipids studied. $T^\star$ for DLPC bilayers is no longer coupled to its transition into the gel state, but rather the transition into the L_X phase, and predict a point of high hydrostatic pressure where complete unbinding may occur. Finally, we have reanalyzed the work of Korreman and Posselt [11] and discovered that the same rules apply to bilayers of different hydrocarbon chain lengths at ambient pressure, leading to the observation that the effect of pressure is analogous to extending the hydrocarbon chains by the addition of methylene groups. The approximate relationship determined by applying pressure to DMPC is that, 100 MPa of hydrostatic pressure is analogous to extending the fatty acyl chain by 2 carbons. From the DMPC data we predict that for a PC lipid composed of two saturated 21 carbon acyl chains, any anomalous change of the bending rigidity with temperature will be completely suppressed. This result is qualitatively confirmed, by measuring d of dibehenoyl phosphorylcholine (DBPC) bilayers (two 22:0 hydrocarbon chains) as a function of temperature, at ambient pressure, and showing that the amount of anomalous swelling is greatly reduced.

II. MATERIALS AND EXPERIMENTAL METHODS

To determine the effect of pressure on the anomalous swelling behavior of phospholipids, we constructed a special pressure cell capable of withstanding 370 MPa of hydrostatic pressure at room temperature and reasonably transparent to neutrons [31]. Made of high yield strength 7075 aluminum alloy, the sample cell uniquely accommodates oriented lipid samples on a flat substrate. The cell uses less than 2 ml of water, such that the total stored energy under pressure is less than 100 J. Transmission of neutrons was measured to be slightly better than 70%. The cell, however, had a limited duty cycle before failure at combined high temperatures (>343 K) and high pressures (>240 MPa) [31].

Sample temperature was controlled using a circulating water bath and water jackets affixed to the sample cell block, to an accuracy of ± 0.2 K. High resolution neutron diffraction was carried out at the NRU reactor (Chalk River Laboratories), using the N5 and E3 triple-axis spectrometers. Monochromatic neutrons of suitable wavelengths ($\lambda \approx 1-3.5$ Å) were obtained using either a pyrolitic graphite or germanium single crystal monochromators.

The disaturated lipids 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC, 12:0), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC, 14:0), and 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine (DBPC, 22:0) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without any further purification. DLPC and DMPC samples were deposited on a clean Si substrate from a concentrated methanol solution, forming highly aligned bilayers in a standard manner. DBPC was dissolved in water and sonicated above the T_M to form unilamellar vesicles before being deposited on the Si substrate. After initial evaporation of the solvent, the samples were kept under vacuum for several hours. The DBPC samples were then annealed in a humid environment, above their T_M, for several hours to help in better aligning them, and then were dehydrated. The dry samples were then placed into the sample cell, slowly immersed in heavy water, and the pressure cell purged of air as described in Ref. [31]. Pressure was applied by a manually operated piston.

III. RESULTS AND DISCUSSION

The open symbols in Fig. 1(a) depict the d spacing of DMPC multibilayers as a function of temperature while cooling from the L_α phase at ambient pressure (0.1 MPa). The measured T_M occurs at 297 K, in agreement with the known main transition. The inset to Fig 1(a) shows a rocking curve, taken at $T = 320$ K, which measures the sample's mosaicity. A full width at half maximum of $\leqslant 0.2°$ is indicative of a sample that is highly aligned with respect to the Si substrate. The anomalous swelling is very clear, occurring in the vicinity of T_M and resulting in ~ 5 Å increase in d spacing. Figures 1(b) and 1(c) contain data from independent experiments performed at 100 MPa and 200 MPa pressure, respectively. The rocking curve shown in the inset of Fig.

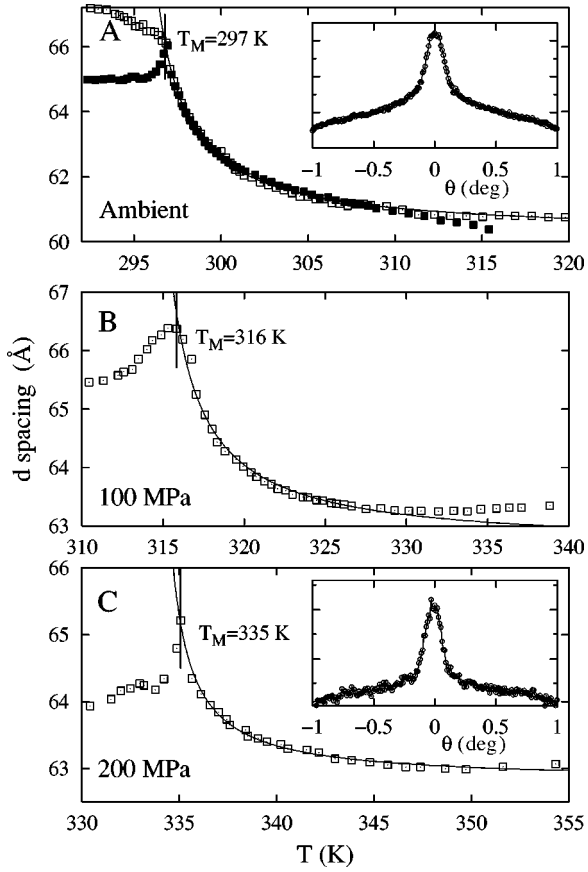

FIG. 1. DMPC lamellar repeat spacings as a function of temperature for (a) ambient pressure (0.1 MPa), (b) 100 MPa, and (c) 200 MPa. The open symbols indicate cooling from the L_α phase, while the closed symbols represent d spacings obtained during a heating cycle. The solid lines are the best fits of Eq. (1). The insets show the θ rocking curve of the first Bragg reflection, taken at $T=320$ K (a) and $T=350$ K (c).

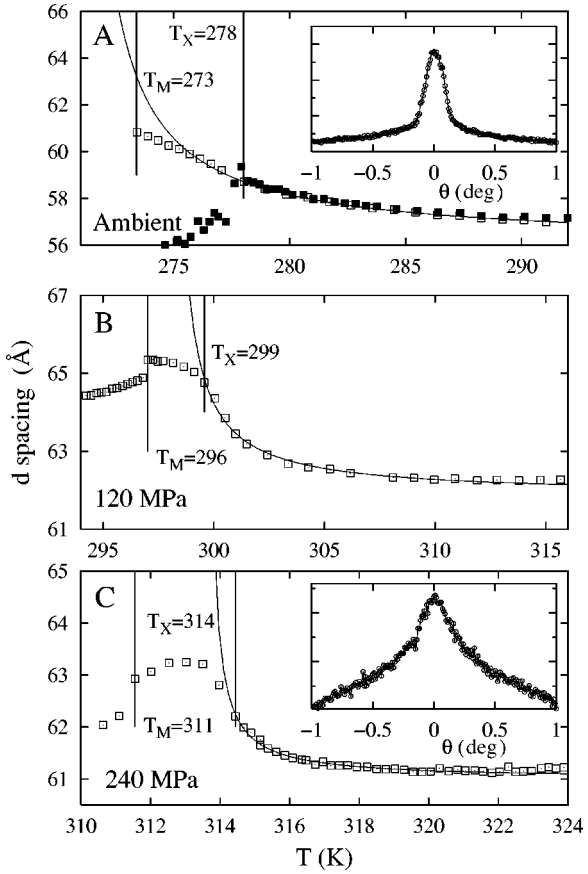

FIG. 2. DLPC lamellar repeat spacings as a function of temperature for (a) ambient pressure (0.1 MPa), (b) 120 MPa, and (c) 240 MPa. The open symbols indicate cooling from the L_α phase, while the closed symbols in (a) represent a heating cycle. The solid lines are the best fits of Eq. (1). It is interesting to note that the temperature range occupied by the L_X phase decreases as a function of hydrostatic pressure. The insets show the θ rocking curve of the first Bragg reflection, taken at $T=295$ K (a) and $T=325$ K (c).

1(c) indicates that even at this high pressure the sample remained highly aligned. The effect of pressure is that the main transition temperature has increased to 316 K at 100 MPa and 335 K at 200 MPa, while the amount of swelling at 200 MPa has been reduced to ~ 2 Å.

Figure 2 shows similar data for the shorter chain DLPC, at ambient, 120 MPa and 240 MPa hydrostatic pressures. In these samples, there is an ordered L_X phase occurring between the transition from the L_α-to-gel phase [32], and observable by a change in the slope of the anomalous swelling. At the highest pressure the swelling is arrested and reversed before the sudden onset of the gel phase and the concomitant, discontinuous drop in d.

The functional form of the swelling occurring in L_α bilayers has been proposed to be a power law [15] of the form

$$d - d_0 \propto (T - T^\star)^{-\psi}, \tag{1}$$

where d_0 is the d spacing well into the L_α phase, and ψ, the critical exponent, is 1 regardless of the number of interacting layers [33]. This is a crucial point for our analysis since the data available in the asymptotic region are too few to allow for an analysis of the critical exponent. Only by having ψ fixed to 1 it is possible to determine $T^\star$ accurately.

Richter *et al.* [34] have attempted to determine ψ from similar data at ambient pressures. However, for reasons which we will outline, we do not believe that their analysis is correct. First, for the same homogeneous composition, swelling is expected to be caused by the same mechanism at all pressures, therefore there is no *a priori* reason for the critical exponent to be a function of pressure. In other words, we would expect the universality class to be preserved [35]. Second, as we have mentioned, their data suffer from not having enough ordinate data in the critical (asymptotic) region to justify a fit capable of accurately obtaining ψ. Third, and perhaps most importantly, the power-law behavior in the critical region is applicable only to the nonanalytical part of $d(T)$ [i.e., $d(T)-d_0$] and not to $d(T)$ as a whole. However, in Ref. [34] the critical exponent has been obtained by fitting the power-law model to the $d(T)$ data, without subtracting an analytical part. Finally, they erroneously discredit the value of $\psi=1$, by using an incorrect value of T_c (our $T^\star$), since a different exponent would necessarily yield a different critical temperature.

Regardless of the value of ψ, the amplitude of the divergence of d was theoretically found to scale with the number of layers, decreasing progressively the range where critical swelling can be observed [33]. For a stack of many interacting membranes the unbinding transition would then appear discontinuous. One possible reason why we observe continuous swelling, even though our samples are made up of hundreds of layers, may be that the amplitude of the critical swelling may also depend on the cause of the swelling (e.g., reduction in the bending rigidity). Thus, critical swelling induced by a reduction of K_c may depend less on the number of interacting layers than believed previously on the basis of theoretical arguments.

In applying Eq. (1) to the DMPC data, we start the fit from the highest measured d, just before the transition, to the lowest d in the L_α phase, using a total of three variables; d_0, $T^\star$, and a proportionality constant. The transition from the L_α phase in DLPC is less clear, and in this case we continue to take points in the upward swelling d until the fit no longer improves. The solid lines in Figs. 1 and 2 are the fits to the data and show that Eq. (1) describes, more than adequately, the entire region of interest in the L_α phase.

The variable d_0 has been described as the repeat spacing well into the fluid phase [11]. The fit is reasonably sensitive to the value of d_0, however, it is difficult to assign it a physical meaning since the d spacing in the L_α phase begins to increase at higher T [Fig. 1(b)], as noted in Ref. [36]. Nevertheless, we take d_0 to represent an ideal bilayer with homogeneous melting of the acyl chains and with minimal density fluctuations and thermal undulations. The swelling at higher T values is likely caused by a similar softening of K_c as is the anomalous swelling near T_M [36]. However, for the purposes of this discussion we restrict ourselves to the region in the vicinity of T_M. The standard error in the measurement of T_M is determined from the size of the temperature step during scanning or ~ 0.5 K. The fitted parameter $T^\star$ is sensitive to the fitting with an accuracy of ~ 0.2 K. For d_0 the standard error is ~ 1 Å and for the proportionality constant less than 1%.

Using only Eq. (1), the relative change in the swelling of the d spacing is defined as $d_{swell}=d_{max}-d_0$, where d_{max} is the maximum d spacing just before the main transition. However, this method of fitting cannot distinguish the thickening of the lipid bilayer only—due to a decrease in *trans-gauche* isomerizations—from the intake of water between the bilayers as both contributions to the d spacing are folded into Eq. (1). On the other hand, neutron diffraction of DMPC unilamellar vesicles at ambient pressures is sensitive only to the bilayer thickness [12]. From such measurements Mason *et al.* [12] determined the contribution of bilayer thickening to the total d_{swell} to be ~ 2.3 Å. Interestingly, we have inspected the data presented by Mason *et al.* [12] and find that the increase also follows the power-law form of Eq. (1). Since we cannot distinguish between the bilayer and water contributions and since Eq. (1) is the correct theoretical description of the separation of bilayers, there is an ~ 2 Å overestimate in our d_{swell} values and will be discussed in detail later on.

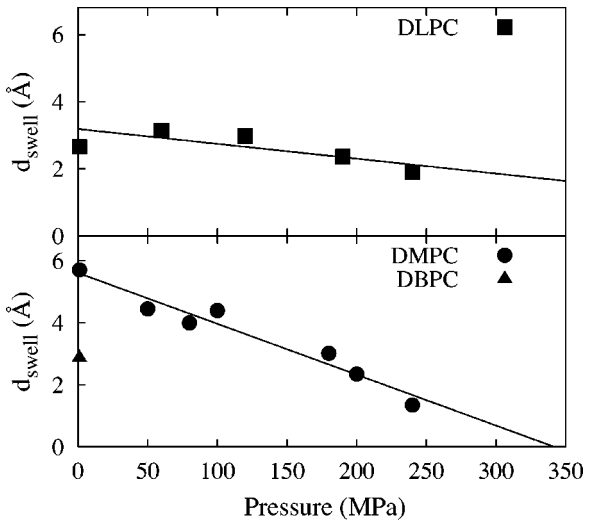

FIG. 3. The amount of anomalous swelling d_{swell} as a function of pressure, as determined by Eq. (1), for DLPC (■), DMPC (●), and DBPC (▲). The solid line is a linear fit. The error bars are contained within the solid symbols.

The uncertainty in the changing bilayer thickness led us to attempt to account for it by reanalyzing the data. This was done by fitting and subtracting a linear component to the data (i.e., nonanomalous contribution to d). From T_M+10 to T_M+3 the change in d was found to be directly related to the change in bilayer thickness at ambient pressure [10,12]. The best fit line in this region, however, was never very satisfactory, but it was still possible to proceed by subtracting it from our data before subsequently fitting with Eq. (1). Most importantly for our current study, the results between the two fitting procedures yielded only small differences in $T^\star$. Ultimately, this method proved unsatisfactory because we cannot differentiate, with confidence, the bilayer and water matrices, nor is it clear that we should. The following discussion does not assume anything about the thickness of the bilayer. Instead, the values reported here are from the application of Eq. (1) only.

Figure 3 shows the amount of swelling, as determined from Eq. (1), for DLPC and DMPC bilayers as a function of pressure. The data for DMPC show that the amount of anomalous swelling decreases monotonically with pressure at a rate of -1.6 Å/100 MPa. On the other hand, the trend is less pronounced for DLPC, occurring at a rate of -0.4 Å/100 MPa. The data therefore indicate that anomalous swelling will be eliminated in DMPC at a pressure of 340 MPa. By definition, this means there will no longer be any changes either to the water or bilayer thickness. The amount of swelling in DLPC is only slightly less than previously reported [13,14].

Bonev and Morrow [20] have studied the phase behavior of DLPC and DMPC under pressure using NMR, a technique, however, that cannot measure the d spacing of bilayer stacks. Their method of measurement obtains an order parameter from the splitting of the deuterium resonance that can then be related to a projection of the acyl-chain segment to the bilayer normal. The measured sudden jump of this order parameter at the main transition was attributed to the

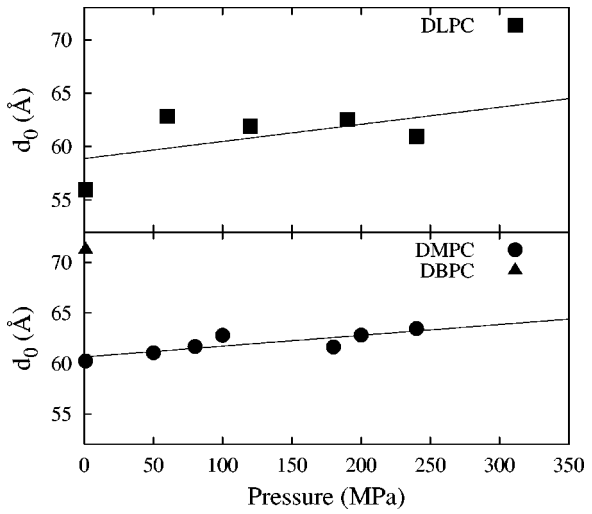

FIG. 4. The baseline value of d_0 as a function of pressure for DLPC (■), DMPC (●), and DBPC (▲). The solid line is a linear fit. The error bars are contained within the solid symbols.

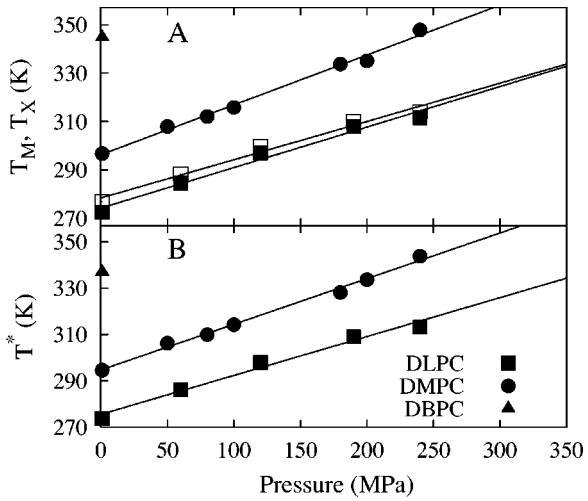

FIG. 5. The measured and calculated transition temperatures for DLPC (■), DMPC (●), and DBPC (▲). (a) The measured thermodynamic transition temperatures include the main transition temperature T_M (closed symbols) and the $L_\alpha \rightarrow L_X$ transition temperature T_X (open symbols). (b) The calculated unbinding temperature $T^\star$.

expected thickening of the bilayer as the acyl chains adopt the gel state or nearly all-*trans* configuration. The validity of measuring the bilayer thickness from this order parameter was discussed by Bonev and Morrow, nevertheless, their data indicated that under pressure, there is a 0.96 Å/100 MPa change in the thickness of L_α DMPC bilayers. This is in agreement with the present data, where there was an increase in the d_0 of DMPC bilayers of 1.0 Å/100 MPa (shown in Fig. 4).

DLPC, on the other hand, shows little trend above ambient pressures with respect to d_0. Further, the DLPC data of Bonev and Morrow indicate that the change in bilayer thickness across the temperature range of the L_X phase closely resembles that of the change in d seen in Fig. 2. This may indicate the cessation of swelling in the water regime and the change in d is entirely due to changes in the L_X bilayers.

The main transition temperature of both lipids increases with pressure and can clearly be seen from Fig. 5(a). The slope of the straight line fit is 16.7 ± 1.4 K/100 MPa for DLPC and 20.5 ± 0.8 K/100 MPa for DMPC. Using optical methods, Ichimori *et al.* report 20.0 and 21.2 K/100 MPa for DLPC and DMPC, respectively [37], although Bonev and Morrow reported values of 15.0 and 19.1 K/100 MPa, respectively [20,38]. In addition, the intermediate transition to the L_X phase in DLPC also changes with pressure at a similar rate of 15.8 ± 1.1 K/100 MPa. This was also present in the data of Bonev and Morrow [38], although it is difficult to be quantitative about the rate from their data.

Here, we make the observation that the fitted transition point $T^\star$ also changes with pressure at a rate similar to that of T_M; 16.7 ± 1.0 K/100 MPa for DLPC and 19.7 ± 0.7 K/100 MPa for DMPC [Fig. 5(b)]. It therefore seems that $T^\star$ and T_M for DMPC bilayers are coupled as a function of pressure, indicating that there is no possibility of pressure facilitated unbinding of the DMPC multilamellar stacks. For DLPC bilayers, on the other hand, the fitted unbinding transition temperature $T^\star$ approaches the L_α-to-L_X transition temperature, holding out the possibility that membrane unbinding may take place at a hydrostatic pressure above 290 MPa.

Besides the recent attempts of explaining pretransitional effects in terms of a weak first-order transition [8], it has long been supposed in the literature that there is a critical point of chain melting in the phase diagram of PC lipids near T_M. One clue as to its existence is the prediction that for phosphocholines at ambient pressure and hydrocarbon chains $\leqslant 8$ carbons, the thermodynamic discontinuity of chain ordering disappears [39]. It is believed that for a bilayer of that thickness, there is little ''freezing'' of the hydrocarbon chains across the main transition. Concomitantly, the latent heat released is negligible. At this point, the pseudocritical character of the phase transition is expected to be more pronounced due to the overlap of the critical point and the main transition. However, the main transition of such short chain phospholipds is <233 K [40], and difficult to observe experimentally. As such, it may be that either the application of pressure, or additional acyl-chain carbons, can separate the main transition from this first critical point [20].

In our data for DMPC we see no indication of approaching a critical point or unbinding with the application of pressure. As shown in Fig. 6, the relative distance between $T^\star$ and T_M is, within experimental error, a constant, and averaged across all pressures we obtain $t_c \equiv (T_M - T^\star)/T_M = 0.0084 \pm 0.004$. Previously, the relation of $T^\star$ to T_M has only been reported as a function of the acyl-chain length using two different species of lipid. Lemmich *et al.* [15] reported that the difference between these temperatures normalized to T_M was 0.008 for DMPC and 0.01 for 16:0 PC (DPPC). As the authors were not capable of distinguishing between these two values they claimed that t_c might in fact be the same. This result by Lemmich *et al.* seems to be supported by our reexamination of the data of Korreman and Posselt [11]. Although they did not determine t_c, from their

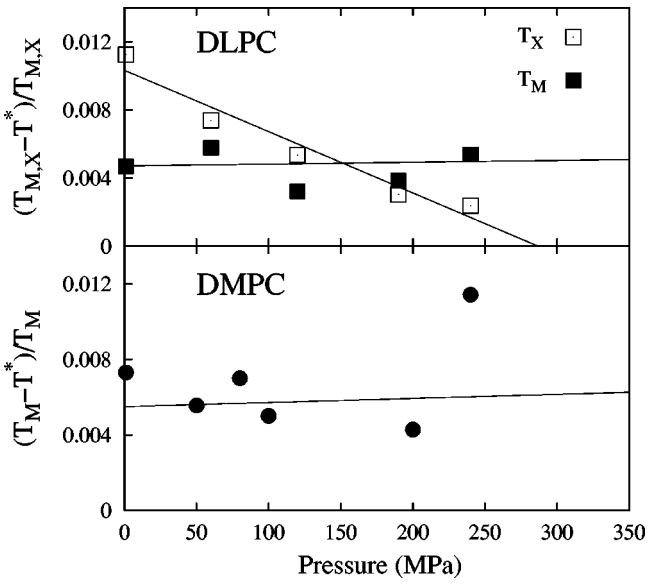

FIG. 6. The relative distance between the thermodynamic and unbinding transition temperatures for DLPC (top) and DMPC (bottom). For DLPC, the differences between $T^\star$ and both T_M and T_X are plotted.

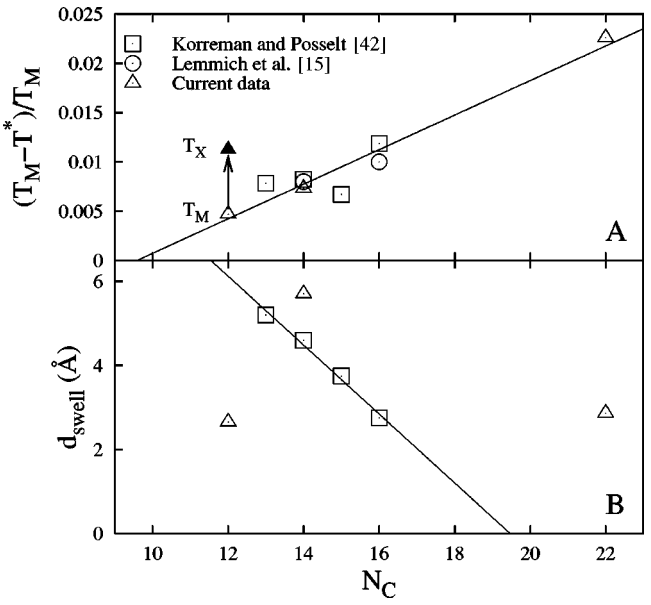

FIG. 7. (a) The relative difference between transition and unbinding temperatures and (b) the amount of anomalous swelling at ambient pressure as a function of hydrocarbon chain length N_C. Included are data from the literature; Korreman and Posselt [42]: (□), Lemmich *et al.* [15] (○), and the current data (△). All the data were treated with Eq. (1) for consistency. The solid lines are linear fits to (a) all of the data and (b) the data of Korreman and Posselt.

reported data our calculations indicate $t_c = 0.0086 \pm 0.002$ for phosphocholines with chain lengths 13 through 16 carbons. This is in excellent agreement with our result for t_c.

The data of Korreman and Posselt [11] indicate that d_{swell} increases with decreasing chain length, while the authors point out that they believe the trend of t_c is to increase with longer chain lipids. This seems to lend support to the idea that shorter chain lipids should have greater anomalous swelling, an idea that has been proposed in the past [14]. However, our data on DLPC support the observation of differential scanning calorimetry measurements which show a fundamental shift in thermodynamic behavior scaling with chain length $\leqslant 12$ carbons [41].

For DLPC bilayers the relative difference between T_M and $T^\star$ is a constant with value of 0.05. However, it is in fact the L_X phase which interrupts the L_α phase, and $t_c = (T_X - T^\star)/T_X$ is no longer constant with pressure (see Fig. 6). It should be noted that T_X is the transition temperature between L_α and L_X bilayers, while T_M is the transition temperature between the L_X and gel phase. The fact that DLPC breaks the trend toward unbinding can be seen in Fig. 7, which combines the data at ambient pressures from the literature and the current data. In Fig. 7(a), the trend of the difference between T_M and $T^\star$ as a function of chain length shows that at ambient pressures there may be complete unbinding for PCs with hydrocarbon chains of 9 carbons or fewer, if the value of T_M is considered for DLPC. This is true in as much Eq. (1) remains a predictor of the swelling of the water matrix, and the effect of acyl-chain thickness changes are small. This is completely analogous to the hypothesis of a critical point occurring in short chain length lipids. However, if instead of T_M we consider T_X, then the trend is broken and unbinding will in fact not be seen [Fig. 7(b)] in shorter chain lipids at ambient pressure.

The amount of the anomalous swelling is unrelated to the phase just below the L_α phase, as shown by the appearance of the L_X phase in the DLPC data. The solid symbols in Fig. 2 are from heating the DLPC sample through the various transitions where no L_X phase occurs [41]. The amount of anomalous swelling is the same, regardless. This observation confirms the result of Mason *et al.* [18] where anomalous swelling is seen regardless of the nature of the sub-L_α phase in methylated phosphatidylethanolamines.

The present data seem to indicate that the main transition becomes increasingly characteristic of a first-order transition with longer chain length PCs or higher hydrostatic pressures. It has been argued that the excess compressibility, measured as a change in molecular volume across the main transition, is proportional to the temperature and the excess heat capacity [22]. Our data suggest that this is no longer the case at high pressures or for very long chain lipids (i.e., $\geqslant 21$ carbons), since the amount of swelling—which we have argued is proportional to the compressibility—is being suppressed. It could be inferred that as a result, the modulus of bending rigidity is maximized across the transition and shows no critical behavior. This may be due, in part, to suppressed density fluctuations and thermal undulations, and indicates that K_c may be decoupled from changes in the total enthalpy at high pressures, a concept not previously explored.

Further to the DMPC data, the similarity between increasing chain length and pressure on T_X, T_M, as well as d_0, indicates that they have a similar effect on bilayer "softness." We hypothesize that hydrostatic pressure has a comparable effect on the order and disorder of the hydrocarbon matrix as the addition of methylene groups to the acyl

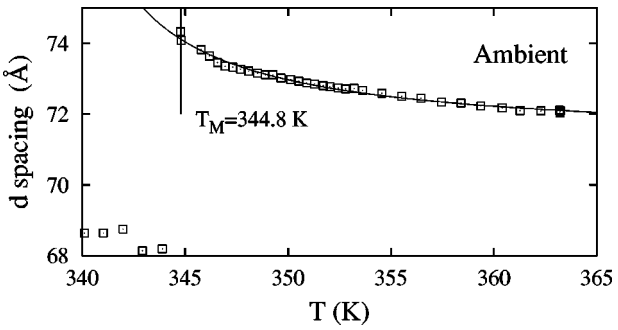

FIG. 8. DBPC lamellar repeat spacings as a function of temperature for ambient pressure (0.1 MPa). The solid lines are the best fits of Eq. (1).

chains. From the DMPC data, we derive the relationship that the addition of two carbons is equivalent to $\approx$100 MPa of hydrostatic pressure. From the present data, the anomalous swelling in DMPC bilayers is predicted to be eliminated around 340 MPa. We were not able to reach that pressure in this experiment, however, we are continuing those efforts. Nevertheless, using this relationship between pressure and additional carbons, the data predict that PCs with chain lengths of $\sim$21 carbons the anomalous behavior should be eliminated. Comparisons between high-pressure DMPC and ambient pressure DBPC are complicated by the fact that high pressure causes an extension of DMPC acyl chains through fewer *trans-gauche* isomerizations. For DBPC at ambient pressure, d_{swell} might come to be dominated by more acyl-chain isomerizations, and an increase in d will result in an overestimate in the amount of swelling. Figure 8 shows the d spacing with respect to temperature for DBPC bilayers at ambient pressure. Despite the large linear increase in d, we find only a small amount of anomalous swelling near T_M of 345 K. The fitted curve, using Eq. (1), is depicted by a solid line and shows a total swelling of 2.4 Å. This is remarkably similar to the ambient pressure data for DLPC (in Fig. 2) and DPPC (inspection of data from Ref. [15]) bilayers. As discussed earlier, the overestimation of d_{swell} for DMPC due to acyl-chain extension alone is $\sim$2 Å [12]. This being the case, the swelling observed for DBPC and DLPC can possibly be solely attributed to swelling of the bilayer only. The expansion of the water layer, being the major contribution to anomalous swelling in all of the other lipid bilayers studied, is essentially abolished.

In this report we describe the anomalous behavior of aligned, fully hydrated multilamellar stacks of DLPC, DMPC, and DBPC in the vicinity of T_M. For DLPC and DMPC bilayers, experiments were also carried out as a function of applied hydrostatic pressure. The major findings of this report can be summarized as follows: (a) For DMPC bilayers the main transition temperature T_M as a function of increasing hydrostatic pressure, changes in step with the critical unbinding temperature $T^\star$. That is, for every pressure $(T_M - T^\star)/T_M$ is a constant of value 0.08. (b) The amount of anomalous swelling d_{swell} in DMPC bilayers is reduced as a function of increasing hydrostatic pressure. This decrease is linear and from it we extrapolate that $d_{swell} = 0$ Å should occur at hydrostatic pressures in the vicinity of 340 MPa. (c) From the pressure data we also predict that d_{swell} should equal zero for a PC lipid with 21:0 hydrocarbon chains. Although not totally absent, DBPC (22:0) bilayers at ambient pressure did show much reduced amounts of anomalous swelling compared to DMPC. (d) Reanalyzing the recent results of Korreman and Posselt [11] shows that their data of 13:0–16:0 PCs are consistent with our conclusions (a) and (b). (e) We predict a hydrostatic pressure for DLPC bilayers ($\sim$290 MPa) where unbinding may occur.

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[1] J. Nagle and H. Scott, Biochim. Biophys. Acta **513**, 236 (1978).
[2] I. Hatta, K. Suzuki, and S. Imaizumi, J. Phys. Soc. Jpn. **52**, 2790 (1983).
[3] A. Ruggerio and B. Hudson, Biophys. J. **55**, 1111 (1989).
[4] M.R. Morrow, J.P. Whitehead, and D. Lu, Biophys. J. **68**, 18 (1992).
[5] D.P. Kharakoz, A. Colotto, K. Lohner, and P. Laggner, J. Phys. Chem. **97**, 9844 (1993).
[6] T. Hønger, K. Mortensen, J.H. Ipsen, J. Lemmich, R. Bauer, and O.G. Mouritsen, Phys. Rev. Lett. **72**, 3911 (1994).
[7] J. Nagle, Proc. Natl. Acad. Sci. U.S.A. **70**, 3443 (1973).
[8] D. Kharakoz and E. Shlyapnikova, J. Phys. Chem. B **104**, 10 368 (2000).
[9] J. Frenkel, *Kinetic Theory of Liquids* (Dover, New York, 1946).
[10] G. Pabst, J. Katsaras, V.A. Raghunathan, and M. Rappolt, Langmuir **19**, 1716 (2002).
[11] S.S. Korreman and D. Posselt, Eur. Phys. J. E **1**, 87 (2000).
[12] P.C. Mason, B.D. Gaulin, R.M. Epand, and J. Katsaras, Phys. Rev. E **61**, 5634 (2000).
[13] J. Nagle, H. Petrache, N. Gouliaev, S. Tristram-Nagle, Y. Liu, R. Suter, and K. Gawrisch, Phys. Rev. E **58**, 7769 (1998).
[14] F.Y. Chen, W.C. Hung, and H.W. Huang, Phys. Rev. Lett. **79**, 4026 (1997).
[15] J. Lemmich, K. Mortensen, J.H. Ipsen, T. Hønger, R. Bauer, and O.G. Mouritsen, Phys. Rev. Lett. **75**, 3958 (1995).
[16] R. Zhang, W. Sun, S. Tristam-Nagle, R.L. Headrick, R.M. Suter, and J.F. Nagle, Phys. Rev. Lett. **74**, 2832 (1995).
[17] S. Kirchner and G. Cevc, Europhys. Lett. **23**, 229 (1993).
[18] P.C. Mason, J.F. Nagle, R.M. Epand, and J. Katsaras, Phys. Rev. E **63**, 030902 (2001).
[19] J. Lemmich, K. Mortensen, J.H. Ipsen, T. Hønger, R. Bauer, and O.G. Mouritsen, Phys. Rev. E **53**, 5169 (1996).
[20] B.B. Bonev and M.R. Morrow, Phys. Rev. E **55**, 5825 (1997).
[21] L. Fernandez-Puente, I. Bivas, M. Mitov, and P. Méléard, Europhys. Lett. **28**, 181 (1994).
[22] T. Heimburg, Biochim. Biophys. Acta **1415**, 147 (1998).

[23] C.-H. Lee, W.-C. Lin, and J. Wang, Phys. Rev. E **64**, 020901 (2001).
[24] R. Lipowsky and S. Leibler, Phys. Rev. Lett. **56**, 2541 (1986).
[25] M. Mutz and W. Helfrich, Phys. Rev. Lett. **62**, 2881 (1989).
[26] B. Pozo-Navas, V.A. Raghunathan, J. Katsaras, M. Rappolt, K. Lohner, and G. Pabst, Phys. Rev. Lett. **91**, 028101 (2003).
[27] W. Helfrich, Z. Naturforsch. A **33A**, 305 (1978).
[28] W. Rawicz, K. Olbrich, T. McIntosh, D. Needham, and E. Evans, Biophys. J. **79**, 328 (2000).
[29] J. Katsaras, Biophys. J. **73**, 2924 (1997).
[30] J. Katsaras, Biophys. J. **75**, 2157 (1998).
[31] M.J. Watson, M.-P. Nieh, T.A. Harroun, and J. Katsaras, Rev. Sci. Instrum. **74**, 2778 (2003).
[32] I. Hatta, S. Matouka, M.A. Singer, and L. Finegold, Chem. Phys. Lipids **69**, 129 (1994).
[33] R. Lipowsky, Z. Phys. B: Condens. Matter **97**, 193 (1995).
[34] F. Richter, L. Finegold, and G. Rapp, Phys. Rev. E **59**, 3483 (1999).
[35] P. M. Chaikin and T. M. Lubensky, *Principles of Condensed Matter Physics* (Cambridge University Press, Cambridge, 1995).
[36] G. Pabst, J. Katsaras, and V.A. Raghunathan, Phys. Rev. Lett. **88**, 128101 (2002).
[37] H. Ichimori, T. Hata, T. Yoshioka, H. Matsuki, and S. Kaneshina, Chem. Phys. Lipids **89**, 97 (1997).
[38] B.B. Bonev and M.R. Morrow, Biophys. J. **70**, 2727 (1996).
[39] D.C. Wack and W.W. Webb, Phys. Rev. A **40**, 2712 (1989).
[40] C. Huang, Biochemistry **30**, 26 (1991).
[41] R.N.A.H. Lewis, N. Mak, and R.N. McElhaney, Biochemistry **26**, 6118 (1987).
[42] S.S. Korreman and D. Posselt, Eur. Biophys. J. **30**, 121 (2000).