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Phase Transitions in Dipalmitoylphosphatidylcholine Monolayers

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Abstract

A self-assembled phospholipid monolayer at an air–water interface is a well-defined model system for studying surface thermodynamics, membrane biophysics, thin-film materials, and colloidal soft matter. Here we report a study of two-dimensional phase transitions in the dipalmitoylphosphatidylcholine (DPPC) monolayer at the air–water interface using a newly developed methodology called constrained drop surfactometry (CDS). CDS is superior to the classical Langmuir balance in its capacity for rigorous temperature control and leak-proof environments, thus making it an ideal alternative to the Langmuir balance for studying lipid polymorphism. In addition, we have developed a novel Langmuir–Blodgett (LB) transfer technique that allows the direct transfer of lipid monolayers from the droplet surface under well-controlled conditions. This LB transfer technique permits the direct visualization of phase coexistence in the DPPC monolayer. With these technological advances, we found that the two-dimensional phase behavior of the DPPC monolayer is analogous to the three-dimensional phase transition of a pure substance. This study has implications in the fundamental understanding of surface thermodynamics as well as applications such as self-assembled monolayers and pulmonary surfactant biophysics.

Abstract

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ASSOCIATED CONTENT

Supporting Information

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Typical results of surface tension–surface area–drop volume–drop curvature vs time for quasi-equilibrium compression of a DPPC monolayer on a 3 mm droplet with the CDS. Reproducibility of compression isotherms. Thermodynamic quantities about LE–TC phase transitions derived from the compression isotherms. (PDF)

The authors declare no competing financial interest.

INTRODUCTION

A self-assembled phospholipid monolayer at an air–water interface is a well-defined model system for studying surface thermodynamics, membrane biophysics, thin-film materials, and colloidal soft matter.^{1–4} The popularity of this model system lies in its unique advantages.¹ First, the air–water interface provides an ideally smooth substrate to ensure excellent two-dimensional ordering. Second, intensive properties of surface thermodynamics, including the temperature, surface pressure, and surface area per molecule, can be readily manipulated and directly controlled. Third, not only can the monolayer be studied in situ with various microscopic and spectroscopic techniques, but transferring the monolayer from the air–water interface to a solid substrate using the Langmuir–Blodgett (LB) technique also permits high-resolution submicrometer imaging of the lateral structure and molecular organization using atomic force microscopy (AFM).^{4,5}

A long-standing objective of study for phospholipid monolayers at the air–water interface is the polymorphism phenomenon.^{6,7} Two-dimensional phase transitions can be thermodynamically studied by analyzing compression isotherms and the compressibility of monolayers.^{8,9} Upon lateral compression (i.e., increasing molecular density), phospholipid monolayers, with dipalmitoylphosphatidylcholine (DPPC) being the most studied species,^{10,11} at room temperature undergo two first-order phase transitions. The first transition usually takes place at a very low near-zero surface pressure and a large molecular area of around 90 Å²/molecule. In this transition, the monolayer is condensed from a two-dimensional gaseous phase (G) to a liquid-expanded (LE) phase that nevertheless preserves the conformational disorder of individual molecules.¹ With further compression of the monolayer, the surface pressure starts lifting off to a certain value at which the disordered LE phase is transformed into an ordered tilted-condensed (TC) phase, formerly known as the liquid-condensed (LC) phase.¹

The first-order phase transition is indicated by a non-horizontal rising plateau in the compression isotherms.¹ The nonzero slope of the phase-transition plateau was usually attributed to impurities or nonequilibrium effects such as the finite rate of film compression.^{6,7} Present structural evidence has proven that the nonzero slope is most likely

caused by the formation of small molecular aggregates or domains.¹ This view is consistent with experimental evidence that increasing the molar mass of film spreading helped reduce the slope of the phase-transition plateau.¹² Along the lines of this experimental evidence, theoretical treatments by considering a two-dimensional mixing entropy of the coexisting phase domains show good agreement with the experimentally obtained isotherms.^{13,14}

To date, nearly all studies of lipid polymorphism exclusively relied on the classical Langmuir film balance.^{6,7,15,16} However, the Langmuir balance suffers from two intrinsic limitations for such studies. First, because of its relatively large size (usually >100 cm² in surface area), precise control of the environmental temperature in a Langmuir balance is difficult. A typical commercial Langmuir balance depends on external water circulation to maintain the trough temperature. However, the temperature of the air–water interface can deviate from that of the subphase as a result of evaporation. Second, the Langmuir balance suffers from film leakage. In spite of various techniques developed to reduce leakage, it cannot be removed completely as indicated by the fact that large area reductions are needed to increase the surface pressure.¹⁷ Both temperature control and film leakage in the Langmuir balance become more problematic with increasing temperature.

Here we report the study of phase transitions in DPPC monolayers using a new methodology called constrained drop surfactometry (CDS).¹⁷ By shrinking the air–water interface of a Langmuir balance to a single droplet, we will show that CDS permits rigorous temperature control with a leak-proof environment, thus making it ideal for studying lipid polymorphism. In addition to compression isotherms produced at various well-controlled temperatures, we have developed a novel LB transfer technique that allows the direct transfer of lipid monolayers from the drop surface under well-controlled conditions. This LB transfer technique permits the direct visualization of phase coexistence in the DPPC monolayer, thus complementing thermodynamic analysis. With CDS and the integrated LB transfer technique, we found that the two-dimensional phase behavior of the DPPC monolayer is analogous to the three-dimensional phase transition of a pure substance. This study has implications for the fundamental understanding of surface thermodynamics as well as applications such as self-assembled monolayers and pulmonary surfactant biophysics.

EXPERIMENTAL SECTION

Constrained drop surfactometry (CDS) was developed from an experimental approach called the constrained sessile drop for studying adsorbed¹⁸ and spread films.¹⁹ In comparison to the classical Langmuir balance that holds a water reservoir commonly larger than 50 mL, CDS uses the air–water interface of a sessile drop (~3 mm in diameter, ~0.2 cm² in surface area, and ~10 μ L in volume) to accommodate the spread monolayer. As shown in Figure 1, the sessile drop is constrained on a carefully machined pedestal that uses its knife-sharp edge to maintain the droplet integrity and to prevent film leakage even at low surface tensions. The system miniaturization of CDS facilitates rigorous control of the experimental conditions with an environmental control chamber. The monolayer can be compressed or expanded by controlling the surface area of the droplet through manipulation of the liquid out of or into the droplet with a motorized syringe. The surface tension and surface area of the monolayer are determined photographically from the shape of the droplet using axisymmetric drop

shape analysis (ADSA).²⁰ Compared to the Wilhelmy plate method used in the Langmuir balance, ADSA measures surface tension accurately and remotely, thus minimizing potential sample contamination. Compared to other droplet-based surface tensiometry techniques, such as the well-developed pendant drop method,²¹ CDS is superb in its capacity to study very low surface tension, owing to its leak-proof environment and mechanical stability.²² Compared to the planar air–water surface in the Langmuir balance, the droplet surface in CDS has a curvature of 1 to 6 cm⁻¹. (See Figure S1 in the Supporting Information for typical results of the surface tension–surface area–drop volume–drop curvature vs time determined by ADSA.) This macroscopic curvature is 7 orders of magnitude smaller than the microscopic curvature of DPPC molecules. Therefore, it is safe to ignore the curvature of the droplet and assume a flat air–water surface for studying DPPC monolayers with CDS.

To record the compression isotherm, the system temperature was maintained at the target value of ± 0.1 °C using an electrothermostatic controller. DPPC was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. DPPC was dissolved in chloroform to form a 1 mg/mL stock solution. The DPPC monolayer was spread onto a droplet of pure water of which the surface tension prior to spreading was recorded as γ_0 , which was within ± 0.5 mN/m compared to literature values of pure water at the controlled temperature.²³ The droplet was then slowly expanded to increase the surface tension (γ) until the corresponding surface pressure ($\pi = \gamma_0 - \gamma$) was reduced to ~ 1 mN/m. The droplet was left undisturbed for an additional 1 min to allow solvent evaporation. The spread DPPC monolayer was subsequently compressed at a quasi-equilibrium rate of 0.05 cm²/min. The surface pressure and surface area were analyzed with ADSA in real time.

LB transfer from the droplet was implemented by lifting a small piece of a freshly peeled mica sheet at a speed of 1 mm/min. During LB transfer, the surface pressure of the DPPC monolayer was rigorously maintained at a constant value using a newly developed droplet manipulation technique based on closed-loop ADSA.²⁴ Topographical images were obtained using an Innova AFM (Bruker, Santa Barbara, CA). Samples were scanned in air in contact mode with a silicon nitride cantilever with a spring constant of 0.12 N/m and a tip radius of 2 nm. Lateral structures were analyzed using Nanoscope Analysis (version 1.5).

RESULTS AND DISCUSSION

To demonstrate the accuracy of CDS, Figure 2 compares the compression isotherms of the DPPC monolayer produced by CDS with isotherms obtained by established methods. The literature values at room temperature (20 °C) were produced with the classical Langmuir balance.^{5,25} The literature values at body temperature (37 °C) were produced with the captive bubble surfactometer (CBS).^{3,26} It appears that the compression isotherms obtained with CDS at both temperatures are in excellent agreement with the isotherms obtained using the Langmuir balance and CBS.

Figure 3 shows the compression isotherms of the DPPC monolayer at increasing temperatures of 10, 20, 30, 40, and 45 °C. (The reproducibility of these compression isotherms and additional isotherms at 25 and 35 °C can be found in Figure S2 of the Supporting Information.) With exact temperature control and a leak-proof environment,

CDS produces complete compression isotherms from nearly zero to the collapse pressures (π_c) at various temperatures. The collapse pressure of the DPPC monolayer matches the surface tension of pure water at the corresponding temperature,²³ which is much higher than the equilibrium spreading pressure (π_e) of fully hydrated DPPC bilayers at the same temperature,²⁷ thus indicating that the DPPC monolayer maintains excellent metastability in CDS.

Between 20 and 40 °C, the compression isotherm shows a clear LE–TC phase-transition plateau. Outside this temperature range (e.g., at 10 and 45 °C), the LE–TC phase-transition plateau disappears. Within this temperature range, the surface pressure at which phase transitions occur increases with increasing temperature. The length of this phase-transition plateau (i.e., the change in the molecular area across the main transition) decreases with increasing temperature. Lining up the starting and ending points of phase-transition plateaus in the compression isotherms forms a dome that is analogous to the saturation dome of three-dimensional phase transitions of a pure substance compressed isothermally in a piston–cylinder device. Surface pressures at which phase transitions occur at corresponding temperatures can be determined from the isothermal film compressibility (κ_T):

$$\kappa_T = -\frac{1}{A} \left(\frac{\partial A}{\partial \pi} \right)_T \quad (1)$$

As shown in Figure 4, the surface pressure for the phase transition (π_{tr}) is indicated by a peak in the isothermal film compressibility. At surface pressures lower than π_{tr} (i.e., to the left of the κ_T peak shown in Figure 4 or to the right of the phase-saturation dome shown in Figure 3), the DPPC monolayer is in a single disordered LE phase that features a relatively high film compressibility. At surface pressures higher than π_{tr} (i.e., to the right of the κ_T peak shown in Figure 4 or to the left of the phase-saturation dome shown in Figure 3), the DPPC monolayer is in a single ordered TC phase that features a low compressibility of $0.004 \text{ (mN/m)}^{-1}$. Within the phase-saturation dome, the LE and TC phases coexist in the DPPC monolayer.

The apex and baseline of the phase-saturation dome can be determined by analyzing the entropy (S_{tr}) and enthalpy (H_{tr}) of the LE–TC phase transition using the two-dimensional Clausius–Clapeyron equation

$$\Delta S_{tr} = \frac{d\pi_{tr}}{dT} (A_{LE} - A_{TC}) \quad (2)$$

$$\Delta H_{tr} = T \Delta S_{tr} = T \frac{d\pi_{tr}}{dT} (A_{LE} - A_{TC}) \quad (3)$$

where π_{tr} is the surface pressure for the LE–TC phase transition and A_{LE} and A_{TC} are the beginning and ending molecular areas of the LE–TC phase transition.

Figure 5 shows plots of the surface pressure (π_{tr}), entropy (S_{tr}), and enthalpy (H_{tr}) of the main phase transition as a function of temperature. (Detailed statistical data of the thermodynamic quantities about the main phase transition at different temperatures can be found in Table S1 of the Supporting Information.) It appears that all of these thermodynamic properties are linearly correlated with temperature, indicating a first-order phase transition. Two characteristic temperatures can be determined from these plots.

First, the LE–TC phase transition occurs only for sufficiently flexible molecules. At a low enough temperature, the increased molecular rigidity causes the monolayer to be transformed directly from the gaseous phase to the condensed phase without passing through the LE phase.^{1,6} This temperature is defined as the triple-point temperature (T_0) at which the gaseous, LE, and TC phases of the DPPC monolayer coexist in equilibrium.^{1,6} Hence, T_0 defines the lowest temperature at which the LE–TC phase-transition plateau can be observed. It can be determined from the linear extrapolation of the phase-transition pressure toward zero.^{16,28,29} Here, T_0 is determined to be 17.5 °C, which defines the triple line of the saturation dome shown in Figure 3.

Second, when the temperature is increased to a critical value (T_c), the phase-transition entropy and enthalpy must vanish. At this point, the differences between the LE and TC phases disappear. Hence, T_c can be determined from the linear extrapolation of the phase-transition enthalpy toward zero.^{16,28–30} Here, T_c is determined to be 44.1 °C, which defines the highest temperature at which the LE–TC phase transition plateau can be observed (i.e., the apex of the saturation dome shown in Figure 3).

It should be noted that the critical temperature (T_c) of the DPPC monolayer is different from the melting temperature of either DPPC bilayers (T_m) or monolayers (T_M). First, T_c determined here is higher than the gel (L_β) to liquid-crystalline (L_α) melting temperature (T_m) of fully hydrated DPPC bilayers (i.e., 41.5 °C).³¹ Second, T_c determined here is lower than T_M at which the DPPC monolayer irreversibly collapses at a surface pressure lower than its normal collapse pressure (π_c) (i.e., the value equivalent to the surface tension of pure water (γ_0) at the corresponding temperature).³² Hall and co-workers determined T_M of the DPPC monolayer in the range of 48–55 °C.^{3,33} Hence, the three characteristic temperatures pertinent to DPPC monolayers and bilayers rank as $T_m < T_c < T_M$. These differences suggest the importance of molecular packing in affecting phospholipid phase behavior. Although the surface pressure of phospholipid bilayers is largely conserved at around 30 mN/m,³⁴ the monolayer before melting has to be compressed to a higher pressure, which increases the melting temperature of monolayers beyond that of bilayers. At temperatures between T_c and T_M , the DPPC monolayer can still reach π_c without early collapse. (See the compression isotherm at 45 °C in Figure 3 as an example.) This observation suggests that under certain conditions the supercritical DPPC monolayer is capable of sustaining high surface pressures, similar to the behavior of the DPPC monolayer in a pure TC phase. This finding may add new insight to the understanding of how pulmonary surfactant films reach low surface tensions.^{4,22}

To gain a structural understanding of the phase transition, Figure 6 shows the AFM topographic images of the DPPC monolayer during phase transitions at various controlled

temperatures. At 10 °C (i.e., below the triple point), the topographic images show no phase separation, which is consistent with the lack of a phase-transition plateau in the isotherm and the thermodynamic prediction of three-phase coexistence. At 20 °C (i.e., between T_0 and T_c), the AFM images show the coexistence of the LE phase and the signature kidney-shaped TC domains that are well documented in the literature.^{5,25,27} These topographic images clearly demonstrate the nature of the first-order main phase transition. With increasing temperature to 30 °C, phase coexistence persists but the domains become significantly smaller (diameters of $13.4 \pm 5.0 \mu\text{m}$ at 20 °C vs $4.1 \pm 1.0 \mu\text{m}$ at 30 °C), indicating decreases in line tension with increasing temperature. The decrease in domain size and therefore increase in mixing entropy also explains the increase in the slope of the phase-transition plateau with increasing temperature.¹² At 40 °C, which is close to the critical point, the domains show fractured, irregular, and ramified morphology. This morphology is in good agreement with the thermodynamic prediction of critical phenomena. The ramified domain morphology is due to vanishing line tension near criticality, at which differences between phases disappear and hence the domain morphology is largely affected by system fluctuation.^{35,36}

CONCLUSIONS

Using a new experimental methodology called constrained drop surfactometry (CDS), we have studied the detailed polymorphism phenomenon in DPPC monolayers. CDS is superior to the classical Langmuir film balance in its capacity for rigorous temperature control and a leak-proof environment. We have determined the two characteristic temperatures that define the range of first-order phase transitions in DPPC monolayers (i.e., the triple-point temperature T_0 at 17.5 °C and the critical temperature T_c at 44.1 °C). T_0 and T_c define the lowest and highest possible temperatures for the LE–TC phase transition to take place in the DPPC monolayer. The isothermal analysis and thermodynamic interpretation of phase transitions in the DPPC monolayer are supported by structural evidence obtained with a novel LB transfer technique performed directly at the droplet surface under controlled conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic of the constrained drop surfactometry (CDS) with the integrated Langmuir–Blodgett (LB) transfer mechanism.

Figure 2.

Comparison of compression isotherms of the DPPC monolayer produced by CDS with isotherms obtained by established methods. Literature values at room temperature (20 °C) were obtained with the classical Langmuir balance. Literature values at body temperature (37 °C) were obtained with the captive bubble surfactometer (CBS). Insets are images of the constrained sessile drop that demonstrate correlations between the drop shape and the corresponding surface pressure determined with ADSA.

Figure 3.

Compression isotherms of the DPPC monolayer at temperatures of 10, 20, 30, 40, and 45 °C obtained with CDS. Lining up the starting and ending points of phase-transition plateaus in the compression isotherms forms a red-colored phase-saturation dome. To the right of the dome, the DPPC monolayer is in a single disordered liquid-expanded (LE) phase. To the left of the dome, the DPPC monolayer is in a single ordered tilted-condensed (TC) phase. Within the dome, the LE and TC phases coexist in the DPPC monolayer. The apex of the dome defines a critical point, and the baseline of the dome defines a triple point.

Figure 4. Film compressibility of the DPPC monolayer between 20 and 40 °C. The location of the peak compressibility defines the surface pressure for phase transition at the corresponding temperature.

Figure 5.

Surface pressure (π_{tr}), entropy (ΔS_{tr}), and enthalpy (ΔH_{tr}) of the main phase transition in the DPPC monolayer as a function of temperature. Linear extrapolations of π_{tr} and ΔH_{tr} toward zero determine the triple-point temperature (T_0) and the critical temperature (T_c), respectively.

Figure 6.

AFM topographic images of the DPPC monolayer at temperatures of 10, 20, 30, and 40 °C. All AFM images in the first row have the same scanning size of $20 \times 20 \mu\text{m}$ and a z range of 5 nm. Images in the second row show the zoom in and zoom out of the monolayer morphology as indicated by the white boxes. Domain structures of the DPPC monolayer at different temperatures support the isothermal analysis and thermodynamic interpretation in Figures 3–5