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Lipid Bilayer Phase Transformations Detected Using Microcantilevers

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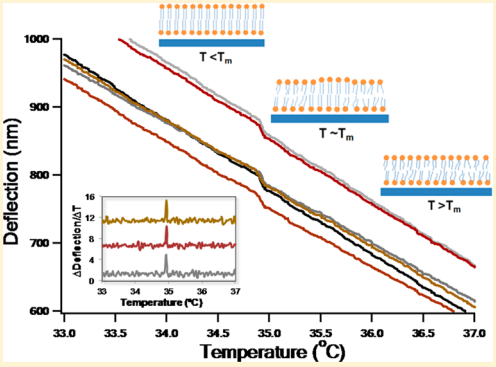
ABSTRACT: We report the use of microcantilevers to measure the phase transition temperature (T_m) of supported lipid bilayers or lipid monolayers. During the solid−liquid phase transition, a supported lipid bilayer or monolayer undergoes a conformational change in which the lipid acyl chains transition from an ordered state to a disordered state. This process is accompanied by a free energy change, which is coupled to changes in the surface stress in the underlying solid support layer. These surface stress changes can be readily detected using microcantilevers. The surface stress of the solid-like phase of 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (MPPC) decreases linearly as the temperature increases and abruptly jumps at the main phase transition temperature. This phase transition temperature corresponds well with that found for free membranes. For an MPPC monolayer, this phase transition temperature is shifted, indicating that the existence of the solid support affects the monolayer structure. The addition of cholesterol into the bilayer decreases the phase transition temperature by ~0.38 °C per mol % of cholesterol. Differences in MPPC stability when it is either a bilayer or a monolayer can be detected through these sensitive surface stress measurements.



INTRODUCTION

Lipid bilayers and monolayers are known to exhibit phase transitions as a function of temperature. The thermally induced solid−liquid transition is particularly important because of its role in membrane permeability and electrical conductivity.[1] Supported lipid bilayers (SLBs) have served as a useful model for complex biological membranes and have led to novel membrane-on-chip devices for biosensor design.[2−4] Attempts to understand the differences between supported and free-standing lipid membranes, and how a solid support interacts with and influences the stability of an SLB, are ongoing.[5−10] One important difference between supported and free-standing membranes is the phase transition temperature T_m. It is believed that the solid−liquid transition of a lipid membrane is a first-order process at T_m,[11,12] where the acyl chains in the lipid molecules are organized into a crystal-like lattice in the solid state and disordered in the liquid phase. However, in an SLB the two leaflets composing the bilayer face different environments: one leaflet faces an ambient aqueous solution, and the other leaflet faces a rigid solid. This results in membrane asymmetry, which is reported to affect the surface tension, the lipid lateral diffusion coefficient, and the phase transition temperature. Leonenko et al.[13] reported that mica-supported SLBs undergo a broader solid−liquid phase transition than do free-standing SLBs and that this transition is accompanied by observable structural changes around the main T_m. Feng et al.[14] reported that the solid−liquid phase transition for SLBs is a two-step process with a primary transition at T_m and a secondary transition at a temperature 5 °C above T_m. They proposed that the proximal leaflet is stabilized by the ordered thin water film beneath it, resulting in a secondary transition that occurs at a temperature higher than the primary T_m. Oncins et al.[15] confirmed the existence of the secondary T_m in the proximal leaflet by examining the phase transition of a Langmuir−Blodgett lipid monolayer. Seeger et al.[16,17] further investigated the factors (ionic strength, incubation temperature and solid support) that influence the SLB solid−liquid phase transition, which switches between a one-step and a two-step process. All of these studies involved applying external forces on the membrane using methods such as force spectroscopy or atomic force microscopy with their probe tips exerting a force on the soft films during scanning.[16] Other less intrusive methods to probe the order/disorder transition of lipids in monolayer, bilayer, or vesicle films include vibrational sum frequency spectroscopy (VSFS)[18,19] and quartz crystal microbalance with dissipation (QCM-D).[20]

More recently, microcantilevers have been able to provide a noninvasive method for measuring free energy changes of adsorbates.[21] Conformational changes in the adsorbed molecular films and temperature-induced melting of dsDNA are readily observed using microcantilevers.[22−24] The detection principle is based on measuring the mechanical bending of the cantilever as a result of changes in the surfaces stress on the cantilever surface. The source of the surface stress change is a change in surface free energy. Changes in the Gibbs free energy of surface-coated films, either physisorbed or chemisorbed on the cantilever surface, are coupled to changes in the surface free energy of the solid surface. These lead to changes in the surface stress, thus resulting in cantilever bending.[25] Microcantilevers also respond to temperature variations, which enable them to

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function as temperature or heat flow sensors.[26] In this paper, we use microcantilevers to probe the solid−liquid phase transition temperature T_m of phospholipid bilayers and monolayers supported on a silicon dioxide surface. We also report how the microcantilevers can be used to examine the effect of cholesterol, which is known to regulate the physical properties of the lipid membrane. A model considering the bimetallic bending of microcantilevers was developed to characterize the lipid properties in the solid and liquid phases and to interpret the phase transition of lipid bilayers in terms of thermal expansion coefficients. The recent success of microcantilevers over other biosensing techniques is due to their ability to sensitively measure surface stress changes associated with liquid−solid interfacial behavior. Furthermore, microcantilevers have the advantage of being a sensitive, real-time, and label-free technique.

EXPERIMENTAL TECHNIQUES

The 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (MPPC) (Avanti Polar Lipids, Alabaster, AL, U.S.A.) and cholesterol (Sigma Aldrich, U.S.A.) were used as received. MPPC was chosen because its transition temperature ($T_m \sim 35$ °C) is within the working temperature range of the equipment. The inner (surface-facing) and outer (solution-facing) leaflets in MPPC bilayers have different structures, leading to an asymmetrical lipid bilayer structure.[27,28] Lipid vesicles were prepared by the standard extrusion method and were kept in a hot water bath at least 15 °C above their phase transition temperature at all times.[29] Briefly, the lipid was dissolved in 0.25 mL of chloroform at a concentration of 5 mg/mL in a glass vial. For lipid-cholesterol mixtures, a specific amount of cholesterol was added to the chloroform solution. The chloroform was evaporated and dried under a gentle ultrapure nitrogen stream. The resulting lipid film was desiccated in a vacuum chamber for at least two hours and then hydrated in 0.25 mL of a pH 7.4 phosphate-buffered saline (PBS) buffer solution (Sigma-Aldrich, U.S.A.) at 50 °C, followed by vortexing the solution. The solution was then extruded 40 times through a polycarbonate membrane with 100 nm pore-size using a mini-extruder (Avanti Polar Lipids), resulting in a translucent solution of large unilamellar vesicles (LUVs) approximately 100 nm in diameter in PBS. The vesicle solution was further diluted with nine parts of PBS to 1 part of the freshly extruded vesicle solution and stored in a warm water bath at 50 °C. The estimated lipid concentration was 0.5 mg/mL.

The cantilevers were 500 μm in length, 100 μm in width, and 1 μm in thickness (Concentris GmbH, Basel, Switzerland). Each chip contained eight rectangular cantilevers in which one surface was silicon and the other was coated with a 3 nm titanium layer followed by a 20 nm gold layer. Before functionalization, the cantilever arrays were placed in a UV ozone cleaner for 5 min under oxygen at 5 psi to generate a silicon dioxide surface. The gold surface of the cantilever was coated with a dithiolaromatic-PEG ($C_{25}H_{44}O_6S_2$, MW = 504.74 g/mol, Sensopath Technologies, Bozeman, MT, U.S.A.) monolayer to prevent vesicle binding. The surface functionalization typically proceeded for two hours. To generate a reference cantilever, a PEG-silane, 2-[methoxy-(polyethyleneoxy)propyl]-trimethoxysilane ($C_{13}H_{30}O_7Si$, MW = 326.46 g/mol, Gelest Inc. Morrisville, PA, U.S.A.) was used to prevent vesicles from adsorbing onto the silicon dioxide surface of the cantilever. The cantilever deflection is a result of the changes in the surface free energy associated with physical or chemical adsorption of molecules to the cantilever surface. Preferential molecular adsorption on either side of the cantilever surface induces a mismatch in the surface stress between the two surfaces of the cantilever, causing the cantilever to bend.[30] The relationship between cantilever deflection, Δz (m), and the change in surface stress, $\Delta\sigma$ (N/m), is described by Stoney's equation[31]

$$\Delta\sigma = \frac{Et^2}{3(1-\nu)L^2}\Delta z \tag{1}$$

SLBs were formed on the silicon dioxide surfaces of microcantilevers through vesicle fusion at 10 °C above the primary T_m. A solution of MPPC vesicles was injected at 0.42 μL/sec into the measurement chamber to form the SLB on the silicon dioxide surface of the microcantilever. The supported lipid monolayer was prepared outside the measurement chamber. A lipid monolayer was deposited on the silicon dioxide surface using the Langmuir−Blodgett transfer method (KSV 2000 series, KSV instruments Ltd., Helsinki, Finland). The cantilever was first prefunctionalized with dithiolaromatic-PEG to prevent lipid adsorption to the gold surface. The cantilever was then held on a Teflon clip and immersed in a DI water-filled Langmuir trough. Lipid molecules were dispersed onto the air−water interface with the total surface area and surface pressure controlled by two barriers. The lipid monolayer was transferred onto the cantilever at room temperature and a surface pressure of 30 mN/m (surface area of 50 Å^2 per MPPC molecule). The monolayer was dried in a desiccation chamber, and cantilever measurements were performed within 20 min in PBS. A commercial system (Concentris GmbH, Basel, Switzerland) was used to obtain real-time deflection positions of the microcantilevers via a scanning laser diode aligned to the tip of the microcantilevers. The position of the reflected laser beam was captured using a position-sensitive-detector (PSD) with a sampling frequency of 1 Hz. Phase transition curves were acquired by slowly ramping the temperature from 45 to 31 °C for the bilayer or from 31 to 45 °C for the monolayer at a rate of ±0.8 °C/min in PBS buffer.

THERMAL EXPANSION COEFFICIENT ANALYSIS

The solid−liquid phase transition of lipid bilayers is studied by altering the temperature. The mechanism of microcantilever bending with changing temperature is based on the bimetallic effect, that is, the difference in the thermal expansion between the gold film and a silicon substrate. The deflection-versus-temperature relation is derived as follows[32,33]

$$\Delta z = 3(\alpha_1 - \alpha_2)L^2\frac{t_1 + t_2}{t_2^2 K}\Delta T \tag{2}$$

$$K = 4 + 6\left(\frac{t_1}{t_2}\right) + 4\left(\frac{t_1}{t_2}\right)^2 + \frac{E_1}{E_2}\left(\frac{t_1}{t_2}\right)^3 + \frac{E_2}{E_1}\left(\frac{t_2}{t_1}\right)$$

where Δz is the change in vertical deflection of cantilever, t is the thickness of the layer, α is the thermal expansion coefficient, E is the Young's modulus, L is the cantilever length, and ΔT is the temperature change. The two layers made of different materials are referred to by the subscripts 1 and 2. The values of the parameters for the gold and silicon layers are listed in Table 1. The thermosensitivity is defined as the deflection per unit

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Table 1. Physical Properties of the Microcantilever Layers: Gold, Silicon, and Lipid Bilayer

layer in cantilever		thickness t, nm	Young's modulus E, MPa	thermal expansion coefficient α, K^{-1}	relative deflection per T unit $\Delta z_{Si\text{-}SLB}$ / ΔT, nm/K
gold		20	7.9×10^4	1.42×10^{-5}	N/A
silicon		1×10^3	1.69×10^5	2.59×0^{-6}	N/A
lipid bilayer, solid phase	0 mol % chol	4.7[b13,14]	28.1[b50,51]	0.0068 ± 0.0008[a]	4.0 ± 0.5[a]
	2 mol % chol	4.72[b43,52]	28.19[b44,53]	0.0031 ± 0.0009[a]	1.8 ± 0.6[a]
	5 mol % chol	4.76[b43,52]	28.32[b44,53]	0.0009 ± 0.0006[a]	0.6 ± 0.4[a]
lipid bilayer, liquid phase	0 mol % chol	3.6[b13,14]	19.3[b50,51]	0.0236 ± 0.0029[a]	7.7 ± 1.2[a]
	2 mol % chol	3.64[b43,52]	19.36[b44,53]	0.0187 ± 0.0018[a]	5.9 ± 0.6[a]
	5 mol % chol	3.70[b43,52]	19.54[b44,53]	0.0135 ± 0.0025[a]	4.4 ± 0.8[a]

[a]Calculated values: average ± standard deviation, obtained from at least three independent experiments. [b]Estimated values from the literature with references below values, considering both lipid phase and cholesterol content.

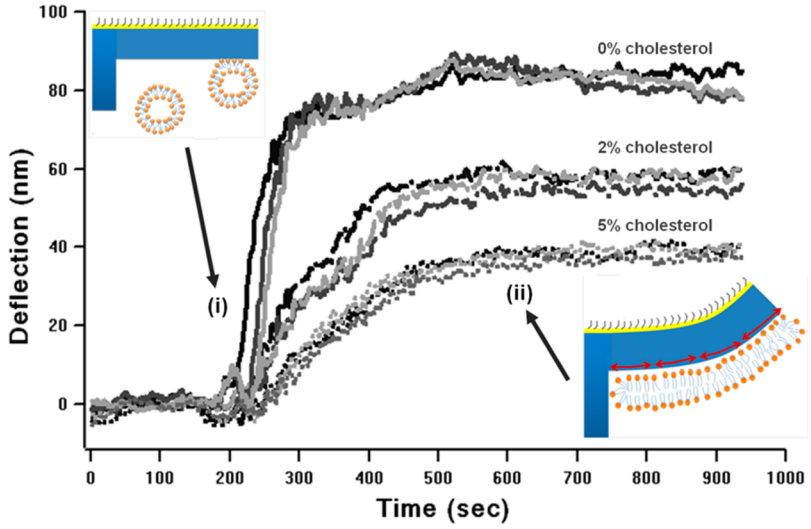

Figure 1. Real-time deflection measurement of the cantilever as an MPPC supported lipid bilayer with 0 mol % (solid lines), 2 mol % (dot-dash lines), and 5 mol % cholesterol (dash lines) forms on the SiO_2 surface of the cantilever at 45 °C. Arrow (i) indicates the injection of MPPC vesicles into the measurement chamber. SLB forms on SiO_2, causing a deflection toward the gold side. Arrow (ii) indicates that the flow is switched from vesicle solution to PBS buffer. Three separate cantilevers on the same array are shown to confirm the formation of SLBs under each condition. For the solid lines, one cantilever (black curve) responds prior to other two (gray curves) because the vesicles reach it earlier in the measurement chamber.

change in temperature. From eq 2, the thermosensitivity of the cantilever used is 79.95 nm/K, which agrees very well with our experimental result (80.6 ± 6.3 nm/K), demonstrating the accuracy of eq 2 in describing the bimetallic effect.

To include a lipid bilayer film on the silicon side, the total deflection of the cantilever, $\Delta z_{Au-Si-SLB}$, is divided into two parts: the deflection due to the bimetallic effect between gold and silicon, Δz_{Au-Si}, and the relative deflection due to the bimetallic effect between silicon and the lipid bilayer, $\Delta z_{Si\text{-}SLB}$. This assumption relies on the fact that the thickness of either the gold layer or the lipid bilayer is much smaller than that of the silicon substrate. The relative deflection due to the bimetallic effect between silicon and the lipid bilayer $\Delta z_{Si\text{-}SLB}$ is obtained by subtracting the deflection of the reference cantilever Δz_{Au-Si} (blank experiment) from the deflection of the lipid bilayer-coated cantilever $\Delta z_{Au-Si-SLB}$. The thermal expansion coefficient α of lipid bilayers can be calculated using eq 3.

$$\alpha_1 = \frac{t_2^2 K}{3(t_1 + t_2)L^2}\frac{\Delta z_{Si-SLB}}{\Delta T} + \alpha_2 = \frac{t_2^2 K}{3(t_1 + t_2)L^2}\frac{(\Delta z_{Au-Si-SLB} - \Delta z_{Au-Si})}{\Delta T} + \alpha_2 \quad (3)$$

RESULTS AND DISCUSSION

Solid−Liquid Phase Transition of Supported Lipid Bilayers and Monolayers. Figure 1 shows the equilibrium deflection of microcantilevers upon the formation of an MPPC SLB with varying cholesterol concentrations at 45 °C. The vesicle solution flows through the measurement chamber from 240 to 720 s. As vesicles fuse onto the SiO_2 surface and rupture to form a planar lipid bilayer, a compressive surface stress is induced, causing the cantilever to bend toward the gold side. The free energy of adsorption is transferred to the solid as a surface free energy at the solid−water−lipid interface.[25] This surface free energy disturbs the atoms of the solid surface, leading to a surface stress in the solid that deforms the material laterally. Because of a surface stress mismatch between bulk and surface, the cantilever bends toward the gold surface. After switching back to a vesicle-free buffer, the microcantilever

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remains deflected, confirming that a stable SLB has formed. As the cholesterol percentage increases, the equilibrium deflection value is reduced, corresponding to a decrease in surface stress that is consistent with previously reported data.[34]

After the formation of the pure MPPC SLB, the temperature is lowered from 45 to 31 °C, and the resulting cantilever responses are shown in Figure 2A. Because of the mismatch in

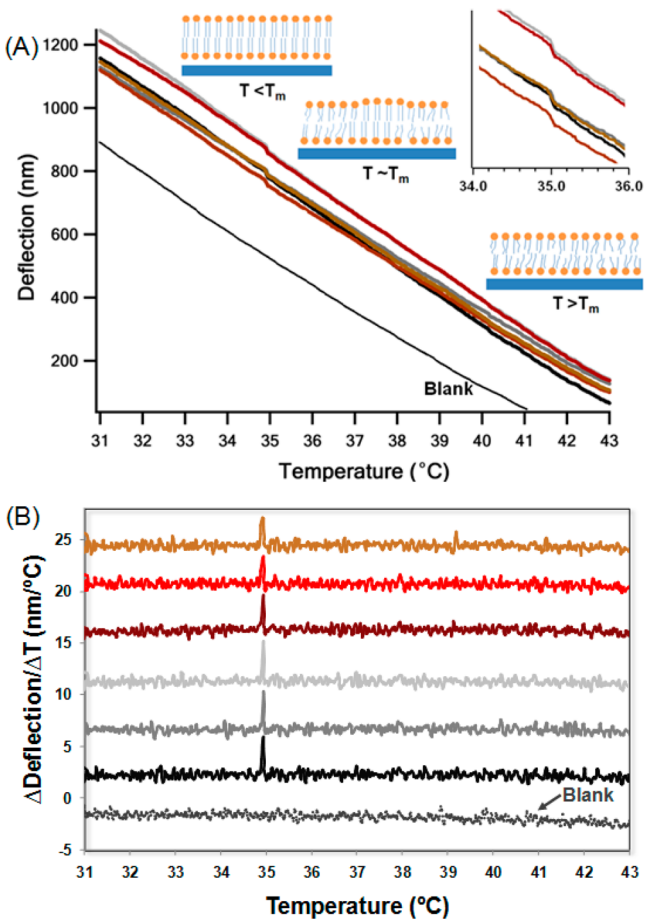

Figure 2. Phase transition of a pure MPPC supported lipid bilayer. (A) Deflection of microcantilevers versus temperature. Inset figure provides finer resolution between 34 and 36 °C. (B) The derivative of the deflection with respect to temperature is shown. The T_m value can be determined from the position of the peaks. The derivative plots are offset for clarity. The blank in both A and B represents the reference cantilever without coating of lipid bilayers. Different colored lines represent different cantilevers on a single sensor chip.

the thermal expansion coefficients between gold and silicon, the reference cantilever (blank) deflection decreases linearly with increasing temperature. For lipid bilayer coated cantilevers, a discontinuity in the linear bending profile is observed at ~35 °C. This discontinuity, an abrupt step of ~20 nm, can be attributed to the solid–liquid phase transition of the lipid bilayer. By plotting the derivative of the deflection with respect to temperature (d(Deflection)/dT), as in Figure 2B, a distinct peak can be observed that corresponds to the phase transition temperature, T_m. The T_m value for the MPPC SLB measured with microcantilevers (at 34.9 ± 0.1 °C) agrees well with the value reported for MPPC lipids in the literature (35 °C).[35] This value corresponds to the temperature where the fluid phospholipid membrane undergoes a liquid-disordered to solid-ordered phase transition. An additional compressive surface stress of ~4.7 mN/m is exerted on the solid support at T_m. It is well-known that this phase transition is an exothermic process that results in an abrupt rise in conformational order and reduced mobility of the lipid molecules.[36] A single phase transition is observed for a SiO_2 supported MPPC bilayer compared with a two-step phase transition previously observed for a mica-supported lipid bilayer.[14,15] The difference in surface roughness between silicon dioxide and mica may change the interactions between the SLB and the solid support.[17] AFM studies report that the solid support significantly broadens the transition temperature range,[13] but as shown in Figure 2A the phase transition is relatively narrow when measured using microcantilevers.

We compare the supported MPPC bilayer phase transition with that of a supported MPPC monolayer. The MPPC monolayer prepared on the SiO_2 surface of microcantilevers using the Langmuir–Blodgett transfer method is initially kept at 31 °C. As the temperature is slowly increased to 45 °C, there is a change in the slope of the deflection curve at ~41 °C, as shown in Figure 3A. In contrast to the MPPC SLB, the monolayer phase transition results in a slope change rather than an abrupt step. The gradual change of the cantilever deflection

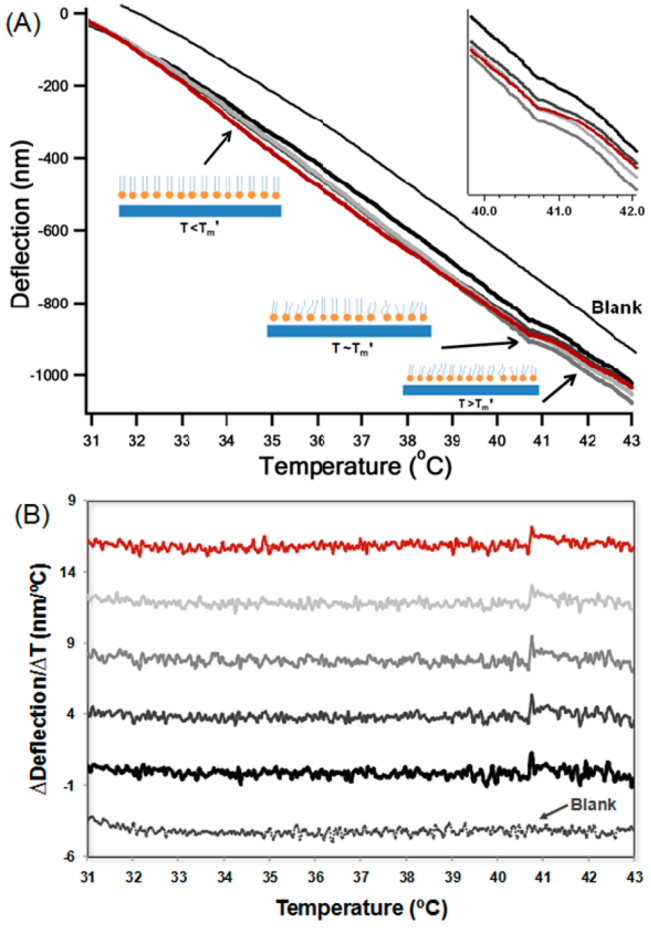

Figure 3. Phase transition of pure MPPC supported lipid (Langmuir–Blodgett) monolayer. (A) Deflection of microcantilevers versus temperature. Inset figure provides finer resolution between 40 to 42 °C. (B) The derivative of the deflection with respect to temperature is shown. The derivative plots are offset for clarity. The blank in both A and B represents the reference cantilever without coating of lipid bilayers. Different colored lines represent different cantilevers on a single sensor chip.

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suggests a broader phase transition of the lipid monolayer due to its interaction with the solid support.[13] The plot of d(Deflection)/dT (Figure 3B) shows a clear change at 40.8 ± 0.10 °C for all monolayer coated cantilevers, which is ~6 °C above the bilayer T_m. This corresponds to the secondary phase transition temperature for the lower leaflet observed in mica supported bilayers, which is due to the decoupling of the two leaflets.[15] The slope of the deflection with respect to temperature increases by 0.3 ± 0.1 nm/°C when going from the ordered to the disordered phase. The increased T_m for the monolayer is related to the interactions of an MPPC monolayer with the solid support. The slope change for the MPPC monolayer is smaller than that observed for the MPPC bilayer. Because of the interleaflet coupling of the SLB, the monolayer appears to adhere more rigidly to the silicon dioxide surface. This is likely due to the strong electrostatic attraction of the polar lipid headgroups (positively charged choline groups) to the negatively charged silicon dioxide surface[18] and to the crystalline-like water beneath the lipid layer.[14] Thus, T_m shifts to a higher temperature from 34.9 ± 0.1 to 40.8 ± 0.10 °C.

Effects of Cholesterol on the Phase Behavior of an MPPC Supported Lipid Bilayer. Cholesterol alters the physical properties of a lipid bilayer, including the phase transition behavior. The effect of cholesterol on the lipid phase transition is largely related to the symmetry of the two acyl chains. The heat-capacity curves of the symmetric lipids are normally resolved into a sharp and a broad component for analysis,[37] while the phase transitions of bilayers containing asymmetric (chain mismatched) lipids are simpler and the effects of cholesterol more obvious.[38] Thus, MPPC is used here to probe the effect of cholesterol on the phase transition measured with microcantilevers. After the formation of MPPC SLBs in the presence of cholesterol, the temperature is decreased, and the abrupt step size, as well as additional compressive surface stress, is obtained from the discontinuity in the linear cantilever responses at T_m (Table 2). Both the size of the abrupt step and the surface stress are reduced by the addition of cholesterol. Thus, the phase transition of lipid bilayers depends upon the concentration of cholesterol.[38−40]

Table 2. Abrupt Steps in Discontinuity and Corresponding Surface Stresses in the Lipid Bilayer Obtained from Cantilever Responses at T_m

cholesterol content, mol %	abrupt step, nm[a]	surface stress, mN/m[a]
0	22.4 ± 2.1	5.2 ± 0.5
2	13.3 ± 1.5	3.1 ± 0.4
5	7.1 ± 1.2	1.7 ± 0.3

[a]Average value ± standard deviation, obtained from at least three independent experiments.

The phase transition temperatures of SLBs containing 0−5 mol % cholesterol in MPPC are shown in Figure 4. Similar to Figure 2B, the phase transition temperature T_m is determined by the position of the peak in the plot of d(Deflection)/dT versus temperature. T_m decreases with increasing cholesterol content at a rate of ~0.38 °C per mol %. The reduced T_m may be explained by the local disordering effects in the lipid solid phase caused by the bulky tetracyclic ring of cholesterol.[38] Moreover, increasing cholesterol content leads to a smaller change in surface stress, as shown in Table 2. As described earlier, changes in surface stress are readily detectable in the coupled lipid leaflets of the SLB. Increasing the cholesterol content stiffens the membrane, which makes it more difficult to detect changes in surface stress. The peak in the d(Deflection)/dT plot disappears at 10 mol % cholesterol, indicating the complete removal of the lipid phase transition. Higher cholesterol content results in less deflection of the microcantilevers, as shown in Figure 1. Thus, the surface stress of the microcantilevers is smaller as a result of the smaller free energy changes due to SLB adsorption,[34] which indicates that the interactions among lipid molecules also become smaller. Therefore, less energy needed for the phase transition, and the transition temperature is lower. In short, the increasing cholesterol content induces a decrease in the microcantilevers' deflection via lipid physisorption, corresponding to a reduced "melting" energy, so the transition temperature T_m is also decreased.

Physical Interpretation of the Temperature-Induced Phase Transition in Microcantilevers Using Thermal Expansion Coefficients. To understand the mechanism by which cholesterol alters the membrane's phase transition, we consider the bimetallic effect of the microcantilevers. The difference in deflection between the coated and reference cantilevers ($\Delta z_{Au-Si-SLB} - \Delta z_{Au-Si}$) represents the effect of lipid bilayers on cantilever bending. The relative change in cantilever deflection per unit temperature, $\Delta z_{Si-SLB}/\Delta T$, is then used to calculate the thermal expansion coefficient α (eq 3), which has been reported for different lipid phases.[41] The change in thermal expansion coefficient with respect to temperature has been reported to be small in the absence of a phase transition (~5×10^{-6} K^{-1})[42] and was thus considered to be constant within the temperature range considered in the calculation (<7 K). Likewise, for a particular phase the thickness t[43] and Young's modulus E[44] are also assumed to be constant with respect to temperature. The values of these parameters are estimated from the literature and are reported in Table 1. We can consider the SLB to be an elastic thin film and Young's modulus to be the elastic modulus parallel to the SLB plane that is related to the lateral elasticity of the bilayer. Although we hypothesize that the temperature does not significantly affect the thickness t and Young's modulus E of lipid bilayer outside the phase transition, the phase of lipids and the content of cholesterol do affect these values. To improve the accuracy and reliability of our calculations, both lipid phase and cholesterol content are considered in the estimation of lipid thickness and Young's modulus (Table 1). Using the relative deflection per unit temperature, together with the estimated parameters, the thermal expansion coefficients α of lipid bilayers under different conditions are calculated and shown in Figure 5. The values of α here compare well with previously reported measurements.[43] However, they are relatively large compared to other measurements[45,46] because α increases significantly at temperatures near a phase transition.[43]

Two conclusions are evident from Figure 5. First, the thermal expansion coefficient α of lipid bilayers is larger in liquid phase than in solid phase, reminiscent of the change in thermal expansion coefficient α reported previously or after the main phase transition.[41,47] Apparently, the lipids in the solid phase are more rigid, so they are less able to expand. Moreover, the small value of α in the solid phase is explained by the combined effects of chain packing and chain tilting.[48] Briefly, the lipids in the solid phase are ordered and tilted, and the increase in temperature reduces both the ordering and tilting of the lipids. The reduced chain tilting thus offsets the expansion of the chain lattice, and α becomes much smaller. In contrast, there is

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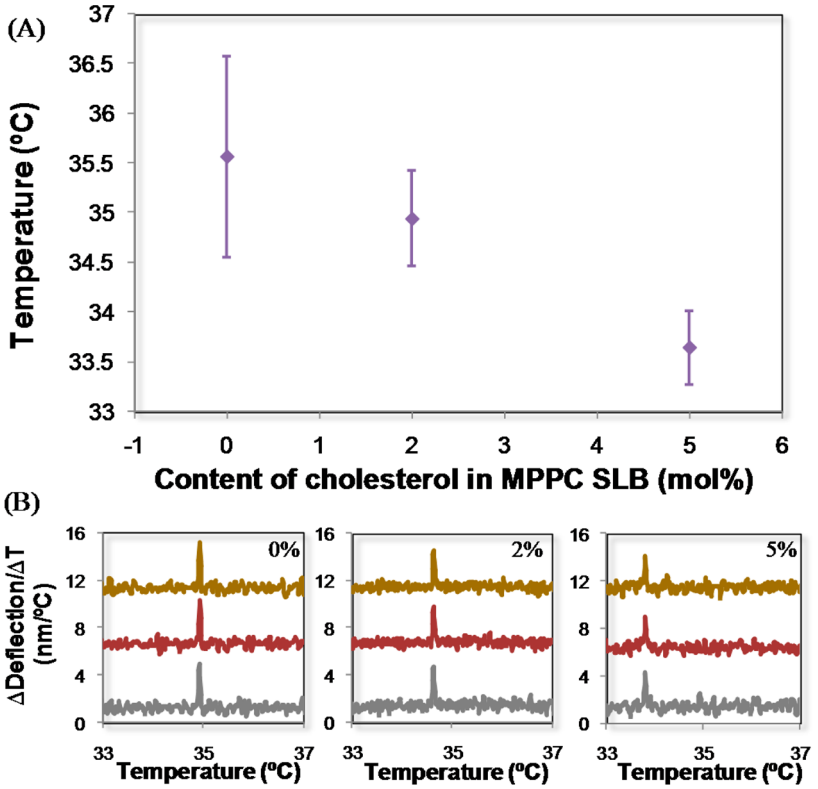

Figure 4. (A) Phase transition temperatures of MPPC supported lipid bilayers in the presence of cholesterol. (B) The phase transition temperature, T_m, is determined by the position of the peak in the d(Deflection)/dT plot at the corresponding cholesterol content (from left to right: 0, 2, and 5% cholesterol). The derivative plots are offset for clarity. The data points reported were obtained from at least three independent experiments.

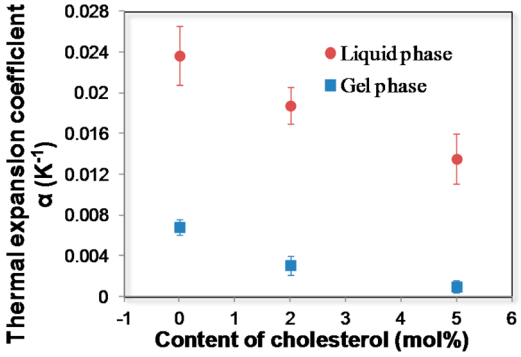

Figure 5. The cholesterol dependence of the thermal expansion coefficient α of lipid bilayers in both liquid (red circles) and solid (blue squares) phases. The values are all calculated from eq 3. The data points reported were obtained from at least three independent experiments.

no tilting effect in the liquid phase, so the thermal expansion coefficient is higher.[45] Second, the thermal expansion coefficient α decreases with increasing cholesterol content in both the liquid and solid phases, which is similar to the reported trends.[43,47] The reason might be that the incorporation of cholesterol fills in the free space among lipids and decreases the flexibility of the lipids, so the thermal expansion coefficient is reduced. Detection of the phase transition on the cantilever is attenuated at higher cholesterol content and disappears at 10% cholesterol content. This is related to the stiffening and reduction in α with increasing cholesterol content. The comparison of values in Table 1 further illustrates that the variation of thermal expansion coefficient with cholesterol is more apparent than the variation of other parameters (e.g., thickness and Young's modulus). In addition, the thermal expansion coefficient of the carbon chain has been reported not to vary significantly with temperature,[49] suggesting that the change in α results from changes in the lipid interactions between phases. Therefore, thermal expansion coefficient can be used to characterize lipid phase behaviors. Some of the parameters used in our calculations are from data involving lipid vesicles, which may differ from SLBs. More precise values of SLB properties (e.g., thickness and Young's modulus) under various conditions will lead to more accurate results.

CONCLUSION

In summary, we have demonstrated the use of microcantilevers to probe the solid–liquid phase transitions of supported lipid bilayers and monolayers. The phase transition temperature T_m can be accurately measured for the MPPC SLB, and the T_m for a MPPC supported lipid monolayer can be successfully detected at ~6 °C above the bilayer T_m. Cholesterol is found to decrease the phase transition temperature at a rate of ~0.38 °C per mol %. By considering the bimetallic effect in the microcantilever, the thermal expansion coefficients α of lipid bilayers under various conditions are obtained and used to explain the cantilever deflection. This coefficient is found to be smaller in the solid phase than in the liquid phase and decreases with increasing concentrations of cholesterol. Therefore, the stiffening effect of cholesterol on lipid membranes is reflected in the thermal expansion coefficient α, suggesting that α can describe the physical structure of lipid bilayer. Generally speaking, treating the SLB as a thin film is a reasonable way to

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characterize the bilayer. This technique has been shown to accurately probe the conformational changes due to phase transitions in macromolecular assemblies confined on surfaces. We offer this technique as a promising tool for future studies on the phase transition temperature of supported model lipid membranes.

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Notes

The authors declare no competing financial interest.

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