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Substrate-induced deformation and adhesion of phospholipid vesicles at the main phase transition

Alvin Chi-keung Lai[a], Kai-tak Wan[b,1], Vincent Chan[a,*]

[a]*Tissue Engineering Laboratory, School of Mechanical and Production Engineering, Nanyang Technological University, MPE, 50 Nanyang Avenue, Singapore 639798, Singapore*

[b]*Department of Engineering Sciences and Mechanics, Virginia Tech, Blacksburg, VA 24061, USA*

Abstract

The physiochemical properties of phospholipid vesicle, e.g. permeability, elasticity, etc., are directly modulated by the chain-melting transition of the lipid bilayer. Currently, there is a lack of understanding in the relationship between thermotropic transition, mechanical deformation and adhesion strength for an adherent vesicle at temperature close to main phase transition temperature T_m. In this study, the contact mechanics of dimyristoyl-phosphatidylcholine (DMPC) vesicle at the main phase transition are probed by confocal reflectance interference contrast microscopy in combination with phase contrast microscopy. It is shown that DMPC vesicles strongly adhere on pure fused silica substrate at T_m and the degree of deformation as well as the adhesion energy is a decreasing function against the mid-plane diameter of the vesicles. Furthermore, an increase of osmotic pressure at the gel/liquid crystalline phase co-existence imposes insignificant changes in both the degree of deformation and adhesion energy of adherent vesicles when the lipid bilayer permeability is maximized. With the reverse of substrate charge, the mechanical deformation and adhesion strength for larger vesicles (mid-plane diameter >18 μm) are significantly reduced. By monitoring the parametric response of substrate-induced vesicle adhesion during main phase transition, it is shown that the degree of deformation and adhesion energy of adhering vesicle is increased and unchanged, respectively, against the increase of temperature.

Keywords: Transition temperature; Osmotic pressure; Phospholipid

1. Introduction

Phospholipid is a major constituent of cell membrane and forms a two-dimensional matrix of fluid-like structure for hosting membrane-associated proteins and glycoproteins (e.g. transmembrane domain of cell receptor). At the same time, phospholipid bilayer directly triggers or co-regulates the signal transduction cascades between cell and external environment such as extracellular matrix. Recently, it has been demonstrated that the mechanical properties of biological membrane are key physical determinants of several biological functions including phosphatidylcholine synthesis, endocytosis, phagocytosis, cell fusion, etc. [1].

*Corresponding author. Tel.: +1-65-6790-4040; fax: +1-65-6397-1320.

E-mail address: mvchan@ntu.edu.sg (V. Chan).

[1] Present address: Department of Mechanical and Henspace Engineering and Engineering Mechanics, Univeristy of Missouri-Rolla, 1870 Winer Circle, Rolla Missouri, 65409-1060, USA.

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Thus understanding how the mechanical properties of phospholipid bilayer change against different environmental conditions is vital for understanding cellular behavior in a quantitative manner.

Among several environmental conditions, temperature plays a key role in driving all biochemical processes of cells and regulating the integrative physiological functions of all mammals [2]. Lately, several groups have demonstrated that the biomechanical properties of phospholipid bilayer locating on the wall of liposomes or vesicles are directly correlated with the change of temperature [3]. For instance, the bending modulus of dimyristoylphosphatidylcholine (DMPC) bilayer in a large unilamellar vesicle (ULV) was reduced by 50% when the bilayer undergoes gel to liquid crystalline transition (from 20 to 24 °C) as shown by optical dynamometry [4]. DMPC bilayer is an attractive candidate for thermal effect investigation because it provides an ideal model system with a convenient phase transition temperature ($T_m \sim 23$ °C). To date, there is a lack of understanding in the tightly coupled thermotropic and mechanical effects of DMPC bilayer on the adhesive interaction between a ULV and a non-deformable substrate. Furthermore, the permeability of ions across phospholipid bilayer is maximized at T_m during the thermotropic transition [5]. This interesting property provides an experimental system to access the effect of osmotic pressure and surface charge on the contact mechanics of adherent vesicles at the metastable thermodynamics state.

The elucidation of the complex bioadhesion phenomena under the influences of thermal and mechanical perturbations will shed light on the physical origin of cell adhesion on extracellular matrix which is central to cell growth, differentiation and migration, morphogenesis, integrity and repair, etc. [6]. In order to monitor the bioadhesive interaction as mentioned above, ultra-sensitive biophysical technique with the capability of resolving between adhesive contact (membrane–substrate separation: 10–30 nm) and cohesive zone (membrane–substrate separation: 50–250 nm) of an adherent vesicle is required. Recently, confocal reflectance interference contrast microscopy (C-RICM) has been proven as an effective analytical tool for elucidating the biophysical mechanisms of phospholipid vesicle or cell adhesion under physiological conditions [7]. Until now, relatively little is known on the effect of temperature change on the contact mechanics of adhering vesicles, particularly close to the phase transition temperature. In this study, C-RICM, phase contrast microscopy and contact mechanics modeling are used to probe the adhesive contact mechanics of DMPC-ULV on model substrates at main phase transition. Our main objective is to determine the effects of membrane permeability, osmotic pressure, substrate charge and thermal transition on critical bioadhesion parameters of DMPC vesicle including degree of vesicle deformation and adhesion energy.

2. Experimental

2.1. Materials

DMPC in powder form was obtained from Matryea Inc. (USA) and was used as received. Dibasic sodium phosphate (Na_2HPO_4); monobasic potassium phosphate (KH_2PO_4); dibasic potassium phosphate (K_2HPO_4); sodium chloride (NaCl); monobasic sodium phosphate (NaH_2PO_4); potassium chloride (KCl); 1 N hydrochloric acid (HCl); 3-amino-propyl-triethoxy-silane (APTES); acetic acid methanol and chloroform were obtained from Fisher Chemicals Inc. (USA) and used as received. 18.2 MΩ water was obtained from Maxima water purification system (Elga, USA) and was used in the preparations of all solutions. IX phosphate buffer saline (PBS) was prepared with 150 mM sodium chloride, 10 mM sodium phosphate, 50 mM potassium chloride and 80 mM potassium phosphate and was adjusted to pH 7.4 with 1 N hydrochloric acid.

2.2. Substrate and liposome preparations

In brief, fused silica coverslips were cleaned in 1 N NaOH overnight, washed thoroughly in 18 MΩ water for three times, dried with a stream of N_2 and heated in vacuum oven at 120 °C for 1 h. In some experiments, pure fused silica substrates were used without further modification. Amine modified substrate that is positively charged are

prepared from fused silica substrates according to a well-established procedure [8]. In brief, cleaned fused silica substrates were dipped into a 1% (v/v) APTES in methanol/water mixture (95%:5% by volume) for 25 min and were subsequently cleaned in pure methanol for three times. After drying in a stream of nitrogen, the silanized surfaces were heated in vacuum oven at 120 °C for 1 h in order to cross-link the silane film.

Giant ULVs were synthesized by a well-established method [9]. One mg of DMPC was dissolved in methanol/chloroform co-solvent (2:1 by volume) and the mixture was subsequently added on the surface of a roughened Teflon disc. A thin film of DMPC was left on the Teflon surface following the evaporation of solvent and was dried in vacuum for 12 h. Then the Teflon disc was covered with 1× PBS buffer and was hydrated at 42 °C for 16 h. The detail of our Differential Scanning Calorimetry (DSC) measurement has been previously described [10]. The concentration of DMPC molecules in aqueous solution that is used in all DSC measurements was 20 mg/ml. Finally, opaque suspension of vesicles was dispersed in solution by gentle shaking of the sample. DSC were performed with a TA 2920 DSC calorimeter (TA Instrument Inc., DE) for measuring the phase transition temperature and enthalpy of these DMPC liposomes. All scans were recorded in the range of 20–35 °C at a scan rate of 0.5 °C/min.

2.3. Cross-polarized light microscopy

Pascal 5 confocal microscope system (Carl Zeiss, Germany) with a 63× oil-immersion objective, cross-polarizers and a transmitted light analyzer (Carl Zeiss) was used for imaging the adhering unilamellar liposomes. Dilution of the original vesicle solutions in 1× (isotonic) or 0.25× PBS buffer (osmotic pressure) was incubated on either pure fused silica or amine-modified coverslip for an hour and was loaded in a temperature controlling chamber (SEC Engineering, Korea) for subsequent imaging under the microscope. An image analysis software, ZSM5 (Carl Zeiss), was used for measuring the mid-plane diameter of adhering liposomes.

2.4. Confocal reflection interference contrast microscopy

The system was built on a laser scanning confocal microscope (Pascal 5, Carl Zeiss) and was integrated with a temperature controlling chamber (SEC Engineering). The detail of the instrument has been described elsewhere [7]. The illumination source was an Argon-ion laser with a maximum power of 1 mW and excitation wavelength of 488 nm 63× oil immersion objective (Neofluar, N.A.: 1.25) was used. Strong contact zone of the adhering liposome appears as dark region on the image. Dilution of the original liposome solutions in 1× (isotonic) or 0.25× PBS buffer (osmotic stress) was incubated on fused silica or amine modified coverslip for an hour and images were taken at temperatures ranging from 19 to 36 °C (at least 30 min of incubation time at each temperature). ZSM5 software (Carl Zeiss) was used for all image analysis. The degree of vesicle deformation is the ratio of the contact zone radius and the mid-plane radius (from phase contrast microscopy) of an adherent vesicle at a particular temperature.

2.5. Contact mechanics model

The detail of the contact mechanics modeling has been reported previously [11]. Briefly, the equilibrium geometry of a water-filled liposome adhering on non-deformable substrate is modeled as a truncated sphere with a mid-plane radius R and $\sin\theta = (a/R) = \alpha$ where a is the contact zone radius. The liposome wall is under a uniform equibiaxial stress, $\sigma = C\varepsilon$ where C is equivalent to $Eh/(1-\upsilon)$ in a linear system under small strain with E and υ the elastic modulus and the Poisson's ratio, respectively, and h the film thickness. Only stretching with negligible bending and rigidity is considered in our model. The average biaxial strain is given by

$$\varepsilon = \frac{1}{2}\left[\frac{2+2(1-\alpha^2)^{1/2}}{4/R^2-\alpha^2}-1\right] \quad (1)$$

In the absence of external influence, the capsule spontaneously adjusts its distance from the substrate until equilibrium is achieved. The adhesion

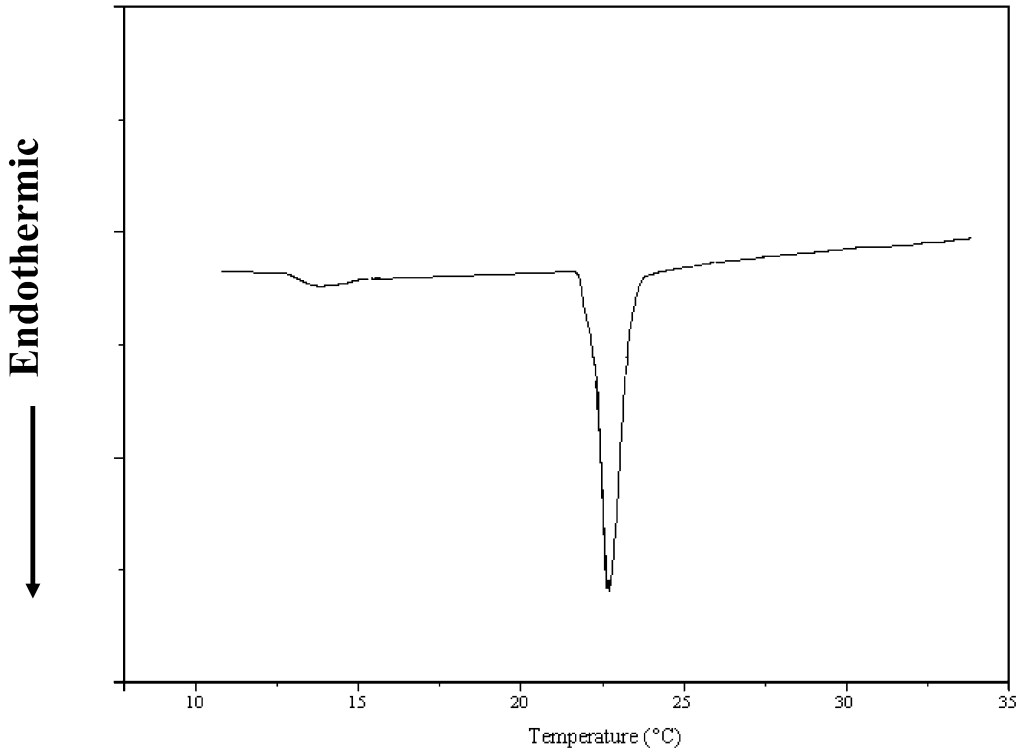

Fig. 1. The DSC thermogram of DMPC bilayer ranging from 10 to 35 °C in 1× PBS buffer.

energy is shown as

$$W=(1-\cos\theta)C\varepsilon+C\varepsilon^2 \quad (2)$$

based on the experimental measurements of the mid-plane diameter R (cross-polarized light microscopy) and the radius of contact zone, a (HR-RICM), W can be found by Eq. (1) and Eq. (2). E of DMPC bilayer of a ULV in gel and liquid crystalline phase is taken as 28 800 and 16 000 N/m^2, respectively, according to the experimental results obtained from optical dynamometry and micropipette aspiration [4,12].

3. Results and discussions

The thermotropic property of DMPC bilayer in solution directly dictates the biomechanical response of DMPC vesicle against the change of temperature [4]. Fig. 1 shows the DSC thermogram of DMPC bilayer ranging from 10 to 35 °C in 1× PBS buffer. The result indicates that DMPC bilayer has a main phase transition temperature (T_m) at 22.5 °C when it transforms from rippled gel ($P_{\beta'}$) to liquid crystalline (L_α) phase. The thermotropic transition is endothermic during sample heating as shown by the direction of the DSC peak. By determining the area under the DSC thermograph, the enthalpy involved in the endothermic transition is determined as 6.3 kcal/mole and agrees well with the reported values in the literature [13]. Only the heating scan of the DSC thermogram of DMPC vesicles is presented because all our temperature dependency results in vesicle adhesion focus at rising temperature in this study.

At the mid-point of the main phase transition (T_m), there is a co-existence of rippled gel and liquid crystalline phases within the two-dimensional DMPC bilayer matrix [14]. Thus it is interesting to probe the effect of the phase co-existence in DMPC bilayer on the contact mechanics of adherent DMPC vesicle. Fig. 2 shows the cross-polarized light micrograph (A) and C-RICM image (B)

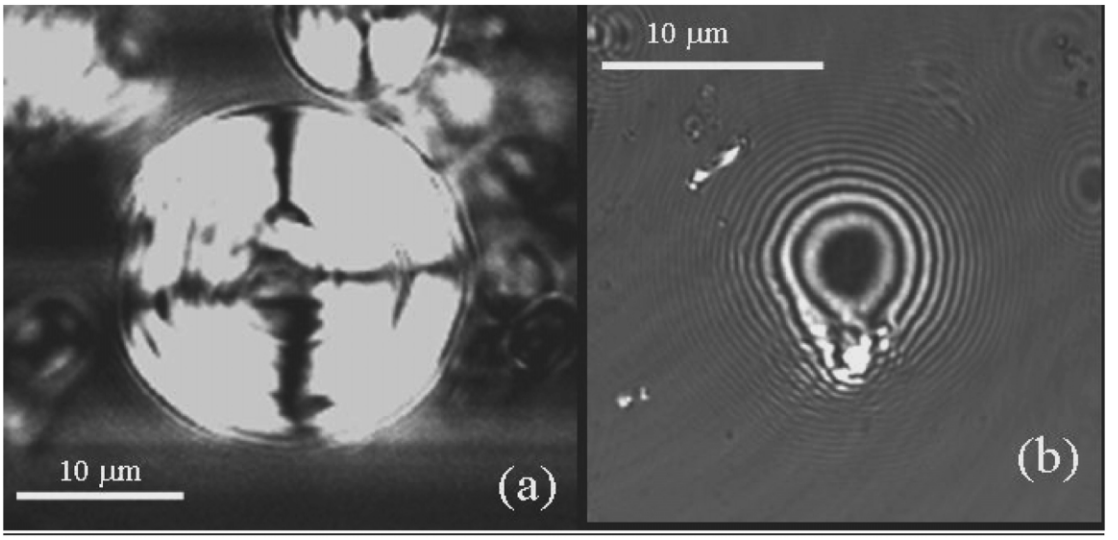

Fig. 2. The cross-polarized light micrograph (a) and C-RICM image (b) of a typical DMPC-ULV adhering on pure fused silica substrate at T_m in isotonic condition 1× PBS.

of a typical DMPC-ULV adhering on pure fused silica substrate at T_m in isotonic condition (1× PBS). From cross-polarized light microscopy, it is shown that the DMPC vesicle has a mid-plane diameter of 22 μm and is strongly bound on the substrate since no drifting in vesicle's position has been detected against time. Once the geometry of the adhering vesicle is determined, C-RICM directly probes the adhesive and cohesive zones at the membrane-substrate interface. The dark region in the center of the C-RICM image (Fig. 2b) corresponds to the strong adhesive contact where the membrane bilayer at the bottom part of the adhering vesicle is within 30 nm from the non-deformable substrate. Furthermore, a series of Newtonian rings propagating from the edge of strong contact zone represents the start of cohesive zone as DMPC membrane bilayer starts to bend away from the non-deformable surface. The lateral movement from any maxima (minima) of a fringe to the successive maxima (minima) can be directly converted to a vertical displacement of $\lambda/4$ (122 nm for our experiments) with optical interference theory [15]. We have previously shown that our contact mechanics model as described above precisely models the vesicle profile (membrane-substrate distance vs. lateral distance) by inputting the adhesion contact radius (a) and mid-plane radius (R) of adherent vesicles to another sub-solution of our model [24]. Thus, the application our final solution for calculating adhesion energy of vesicle in this study has already taken the vesicle profile into account [9].

In order to quantify the contact area for adhering vesicles, the contact zone is defined as the region that starts at the center of the adhesive contact (center of dark circle in Fig. 2b) and extrapolates to the edge of the first Newton's ring using the truncated sphere model [11]. It is determined that the contact area and contact zone diameter of the adherent vesicle is 20.5 μm^2 and 5.1 μm, respectively. Although the adhesion contact zone of DMPC vesicle appears quasi-circular in shape, the average contact diameter ($2a$) with statistical accuracy can be calculated from the contact area (measured from software) with area $= \pi a^2$. Since the mid-plane geometry of the vesicle remains spherical in shape in all data, the approach mentioned above should impose insignificant variance in the calculated adhesion energy as shown in our previous study [24]. Most important, the quasi-circular contact zone is not directly related to the

surface roughness of the substrate because perfectly circular contact zones of relatively rigid polymer microcapsule have been detected on the same types of substrates used in this study [25].

Together, C-RICM in combination with cross-polarized light microscopy illustrates the presence of strong physical interactions between the adherent DMPC vesicle and pure fused silica substrate at the main phase transition of DMPC bilayer. Generally, the surface of pure fused silica substrate is composed of silanol group (–SiOH) and carries a net negative charge at pH 7.4 with the hydrolysis of silanol to $–SiO^-$ groups. The headgroup of DMPC lipid that is in short range from the fused silica substrate contains both a quaternary amine with a permanent positive charge and phosphate group with a negative charge. First, electrostatic interactions between DMPC bilayer and other charged molecules have been proven to be mediated by the positively charged quaternary amine in the presence of partial neutralization of the phosphate groups on the lipid headgroup [16]. Second, only van der Waals force was sufficient to mediate the colloidal adhesion of PC based vesicles on non-deformable substrate in relatively high ionic strength [17]. Third, a repulsive interaction known as undulation force is resulted from the thermal fluctuation of membrane away from the fused silica substrate. Overall, the firm adhesion of DMPC-ULV at the chain meting transition (T_m) indicates that the electrostatic attraction and van der Waals force suppress the repulsive undulation force.

The spreading kinetics of phospholipid vesicle adhering on polylysine coated glasses has been shown as a function of vesicle size [18]. The equilibrium contact mechanics at the end of vesicle spreading can be quantified by the degree of vesicle deformation (the ratio of contact zone diameter and mid-plane diameter). Fig. 3a shows the degree of DMPC vesicle deformation (a/R) against vesicle diameter on pure fused silica substrate at DMPCs T_m of 22.5 °C in both isotonic and hypotonic conditions. The two curves are the best fits for the two sets of result. It is shown that the degree of vesicle deformation is highest ($a/R \sim 0.41$) at the lowest mid-plane diameter of 7.3 μm in isotonic condition. When the mid-plane diameter increases from 7.3 to 41 μm, the degree of vesicle deformation is reduced by 52%. Interestingly, the slope of the a/R vs. vesicle diameter curve is a reducing function against the increase of vesicle diameter until it approaches zero at the highest vesicle diameter. Basically, these results support that smaller vesicles are more deformable upon forming adhesive contact on a fused silica substrate due to their larger surface area to volume ratio. When the size of vesicle ($R < 8$ μm) becomes comparable with the range of attractive surface force (e.g. long-range electrostatic interaction), it will indeed become more 'deformable' as observed experimentally. Similar trend has been observed in the adhesion of elastic or viscoelastic microcapsule on non-deformable substrate [19]. In hypotonic condition, the increase of osmotic pressure has no apparent effect on the degree of vesicle deformation as shown by the similar values of a/R for all vesicle diameters (Fig. 3a). It is known that the co-existence of gel and liquid crystalline phases within the lipid bilayer matrix at T_m enhances the ions transport across the bilayer membrane [20]. Our adhesion study indicates the increase of lipid bilayer permeability at the main phase transition significantly dampens the effect of osmotic pressure on the contact mechanics of adherent vesicle. It is because the concentration gradient of ionic species from the vesicle interior ($1\times$ PBS) to the surrounding medium ($0.25\times$ PBS) is vanished by the rapid release of ions through the permeable lipid bilayer [21]. Once the concentrations of ionic species inside the vesicle approach those of the external medium, the influx of water from the medium to the vesicle is minimized.

Based on a contact mechanics model of adhering thin-walled microcapsule, the adhesion energy of the vesicle can be calculated from experimental parameters [11]. Fig. 3b shows the average adhesion energy of DMPC-ULV against vesicle diameter on pure fused silica substrate in isotonic and hypotonic conditions at the phase transition temperature (T_m) of 22.5 °C. The two curves are the best fits for the two sets of data. In general, the average adhesion energy spans two orders of magnitude ranging from 1.4×10^{-8} to 9.9×10^{-11} J/m^2 when vesicle diameter increases from 7.25 to

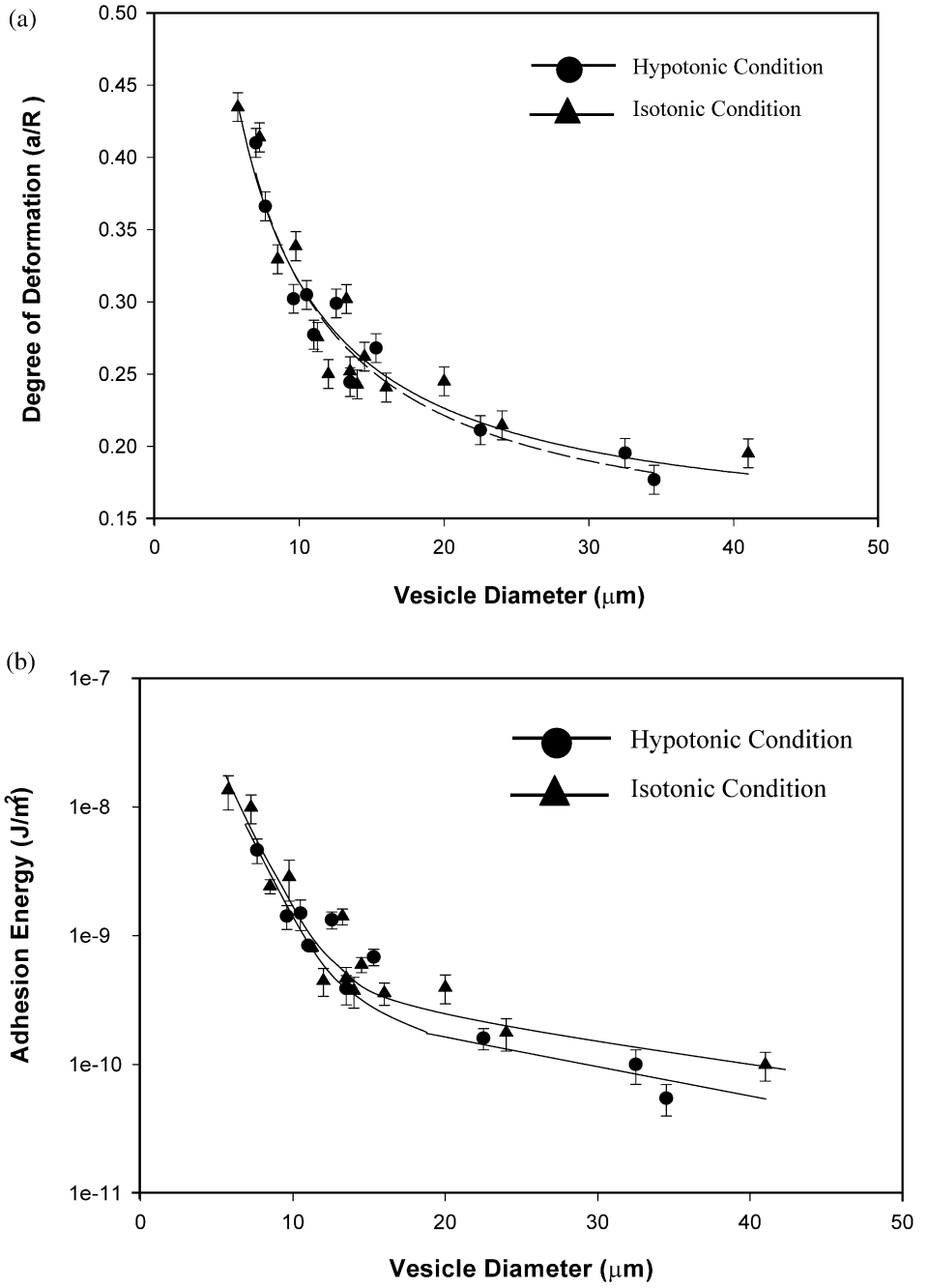

Fig. 3. (a) The degree of DMPC vesicle deformation (a/R) against vesicle diameter on pure fused silica substrate at DMPC'S T_m of 22.5 °C in both isotonic and hypotonic conditions. (b) The average adhesion energy of DMPC-ULV against vesicle diameter on pure fused silica substrate in isotonic and hypotonic conditions at the phase transition temperature of 22.5 °C.

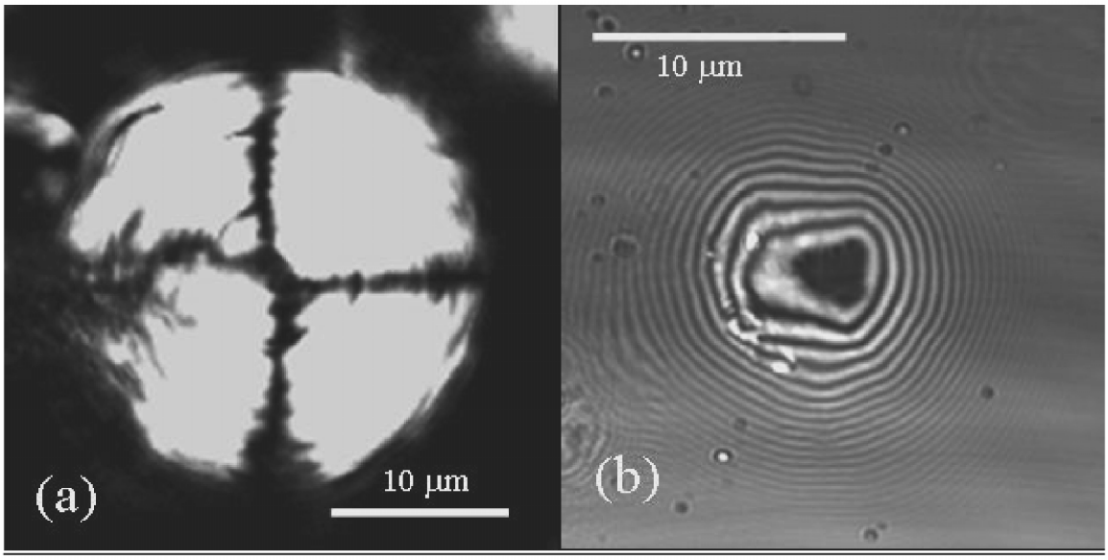

Fig. 4. The cross-polarized light image (a) and C-RICM image (b) of a DMPC-ULV adhering on a APTES coated glass in isotonic condition at T_m.

41 μm under isotonic condition. Moreover, the adhesion energy is dramatically reduced against the increase of vesicle diameter for smaller vesicle ($2R < 15$ μm) in comparison with larger vesicles. The general trend as mentioned above is supported by the stronger adsorption affinity of smaller phospholipid vesicle on charged substrate [22]. At T_m, the increase of osmotic pressure has no apparent effect on the adhesion energy for vesicles of all sizes as shown by the overlap of the two adhesion energy vs. vesicle diameter curves in isotonic and hypotonic conditions. This result is caused by the dramatic increase of membrane permeability of DMPC vesicle at T_m [21]. The change of DMPC bilayer property leads to the instant release of ionic species from vesicle interior to external medium and the disappearance of concentration gradient for ionic species Subsequently, the osmotic pressure stemmed from the difference in ionic concentration between vesicle interior and external solution is canceled out and the volume of the vesicle remains unchanged as confirmed by cross-polarized light microscopy.

Relatively little is known about the effect of surface charges on the contact mechanics of adhering vesicle in the middle of the main phase transition of lipid bilayer. Fig. 4 shows the cross-polarized light image (a) and C-RICM image (b) of a DMPC-ULV adhering on a APTES coated glass in isotonic condition at T_m. The results indicate that the mid-plane diameter and the adhesive contact area of the adhering ULV is 25 μm and 27 μm^2, respectively. Also, firm adhesion of the vesicle is detected despite primary amine group is introduced to the fused silica surface by silanization. Fundamentally, there is a co-existence of amino and silanol groups on the APTES coated substrate [23]. At neutral pH, significant number of amino groups are protonated with a net positive charge ($-NH_3^+$) and most silanol groups are deprotonated with a net negative charge ($–SiO^-$). Thus, an electrostatic repulsion is induced between the quaternary amine on DMPC headgroup and the primary amine on APTES modified glass in comparison with bare silica substrate. At the same time, there is an electrostatic attraction between the quaternary amine and the deprotonated silanol group on the substrate in addition to the van der Waals attraction. The result shows that the two attractive forces mentioned above suppress the

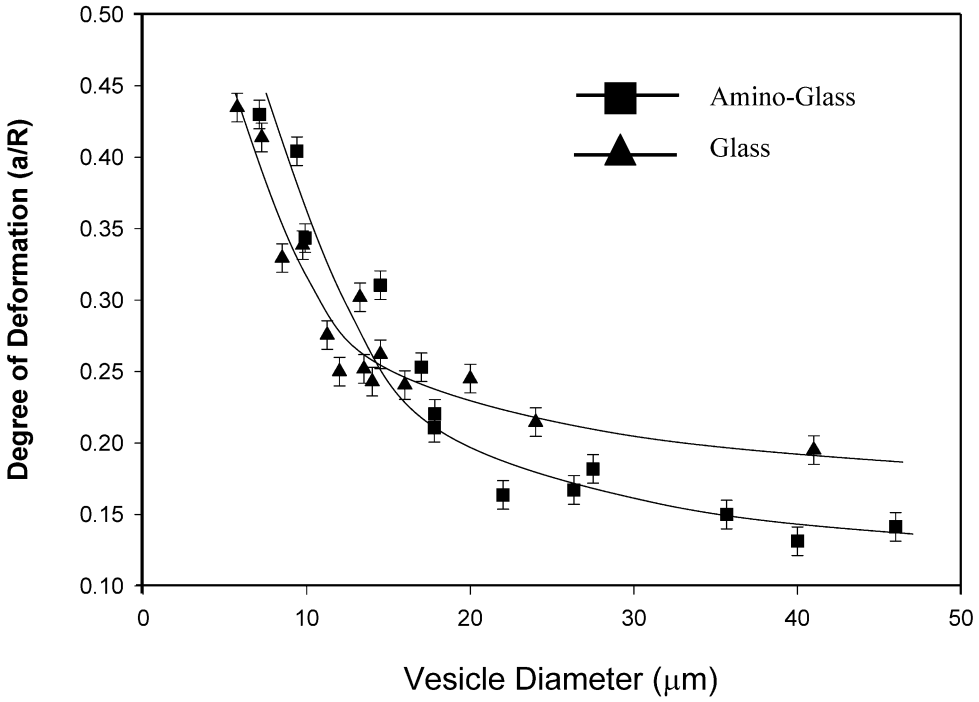

Fig. 5. The degree of vesicle deformation against mid-plane diameter for DMPC-ULV on APTES modified glass in isotonic condition at T_m.

repulsive forces generated from both electrostatic interaction and membrane undulation and prevent the vesicle detachment from the substrate.

Fig. 5 shows the degree of vesicle deformation against mid-plane diameter for DMPC-ULV on APTES modified glass in isotonic condition at T_m. The result indicates that vesicles with mid-plane diameter smaller than 18 μm have similar degree of deformation in comparison with those on pure fused silica substrate. When the vesicle diameter goes beyond 18 μm, the degree of vesicle deformation on APTES coated glass is smaller than that on pure fused silica substrate. In other words, the effect of surface modification with amino group on the contact mechanics of DMPC vesicle is more pronounced in larger vesicles. This trend is likely caused by the higher probability for smaller vesicle with larger surface area to volume ratio in forming effective adhesive contact with the amine modified substrate in comparison with larger vesicles [22]. Fig. 6 shows the average adhesion energy of DMPC-ULV against the mid-plane diameter on APTES modified substrate at the chain melting transition (T_m). On APTES modified substrate, the average adhesion energy spans two orders of magnitude ranging from 9×10^{-9} to 1.41×10^{-11} J/m^2 when vesicle diameter increases from 9.4 to 46 μm in the middle of thermotropic transition under isotonic condition. When vesicle diameter is between 9.4 and 18 μm, the adhesion energy for the vesicle on APTES modified substrate falls within the same range as that on pure fused silica substrate. On the other hand, the adhesion energy on APTES modified substrate is significantly reduced in comparison with that on pure fused silica substrate as vesicle diameter is larger than 18 μm. At the largest diameter of 42 μm, the adhesion energy on modified substrate is one order of magnitude smaller than that on unmodified substrate. Again, the result indicates that the contact mechanics and adhesion strength for DMPC vesicles at T_m are strongly

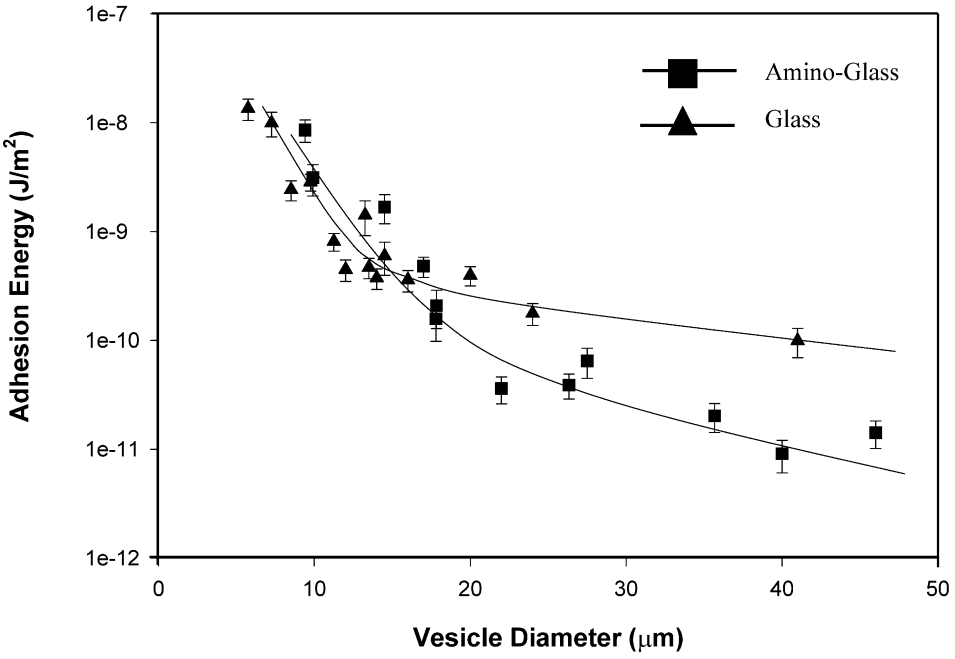

Fig. 6. The average adhesion energy of DMPC-ULV against the mid-plane diameter on APTES modified substrate at the chain melting transition (T_m).

dependent of the surface functionality of the apposing substrate and the vesicle size.

Currently, there is a lack of understanding in the parametric response of an adherent DMPC vesicle during the thermotropic transition of DMPC bilayer. Fig. 7a shows a series of C-RICM images of a typical DMPC-ULV at different temperatures during sample heating (at a rate of 0.5 °C/min) around T_m on pure fused silica substrate. Cross-polarization light microscopy demonstrates that the mid-plane diameter of the adherent vesicle is 15 μm. The center of each RICM image is the contact zone of the adhering liposome at the corresponding temperature. The contact zone is defined as the region that starts at the center of the adhesive contact (center of those crosses in Fig. 7i and v) and extrapolates to the edge of the first Newton's ring using the truncated sphere model (the ends of the arrows on Fig. 7i and v). The result indicates that the contact zone of the vesicle is significantly modified by the increase of temperature when DMPC bilayer is transformed from complete gel (i) to liquid crystalline state (v). In addition, our preliminary result indicates that the thermal induced response of the contact zone of adherent DMPC vesicle on pure fused silica substrate against decreasing temperature is not directly opposite to that during increasing temperature. However, an independent investigation will be necessary in order to systematically address the observed hysteresis of contact zone structure which can be substrate-dependent during a temperature cycle. Indeed, it is well known that the shape a vesicle may change against a change of temperature. Fig. 7b shows the cross-polarization light images of the 15 μm adherent vesicle (Fig. 7a) on pure fused silica substrate during sample heating at 20 °C (i) and 36 °C (ii). The result indicates that the vesicle remains spherical in shape and the cross-sectional area at the mid-plane of the adherent DMPC vesicle is unchanged (within 4%) during the increase of temperature. This verification supports our assumption of constant vesicle volume in the calculation of adhesion energy during sample heating. Of course, the determination of the exact three-dimensional geometry of

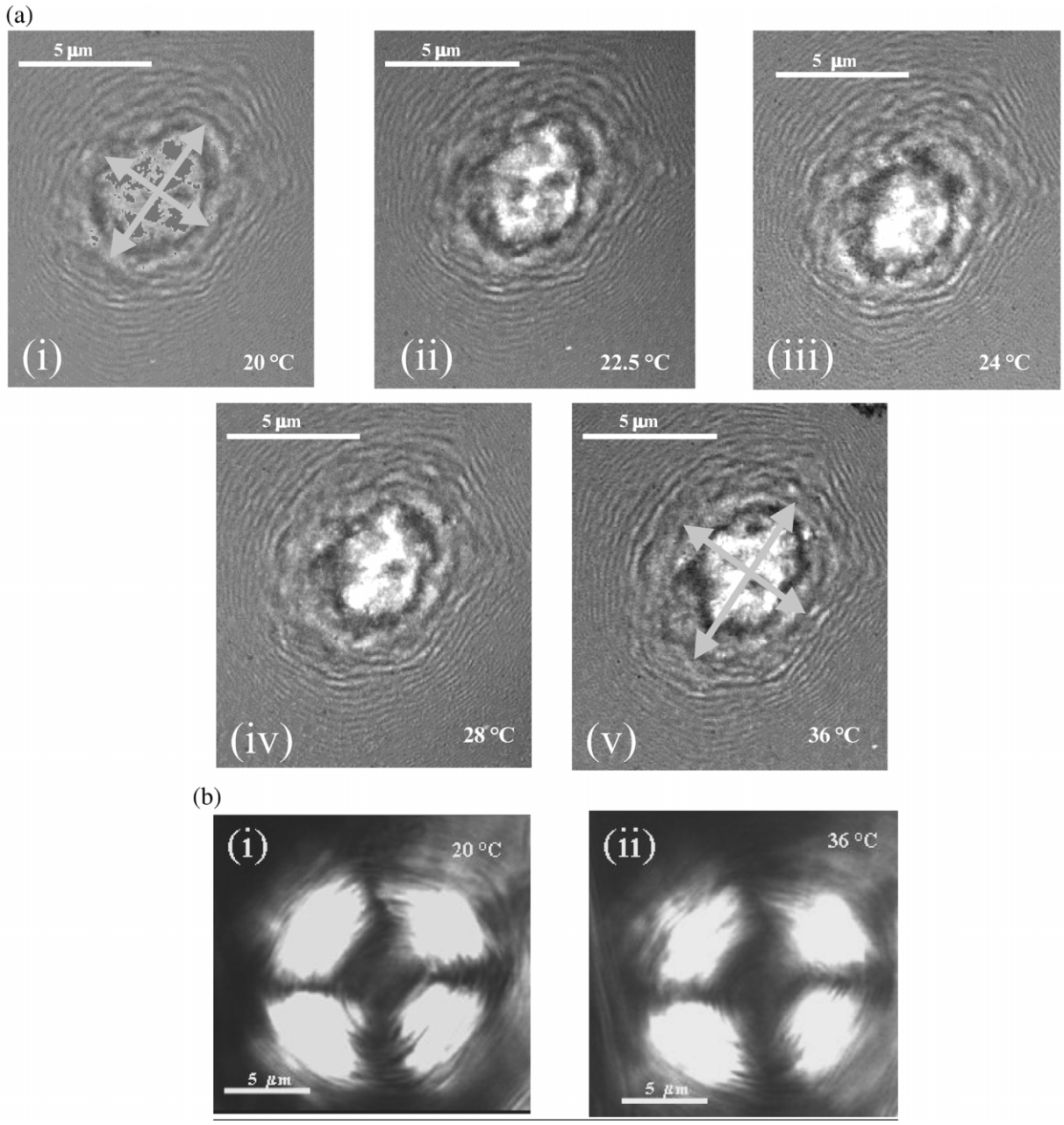

Fig. 7. (a) A series of C-RICM images of a typical DMPC-ULV at different temperatures during sample heating (at a rate of 0.5 °C/min) around T_m on pure fused silica substrate. (b) The cross-polarization like images of the 15 μm adherent vesicle (a) on pure fused silica during sample heating at 20 °C (i) and 36 °C (ii).

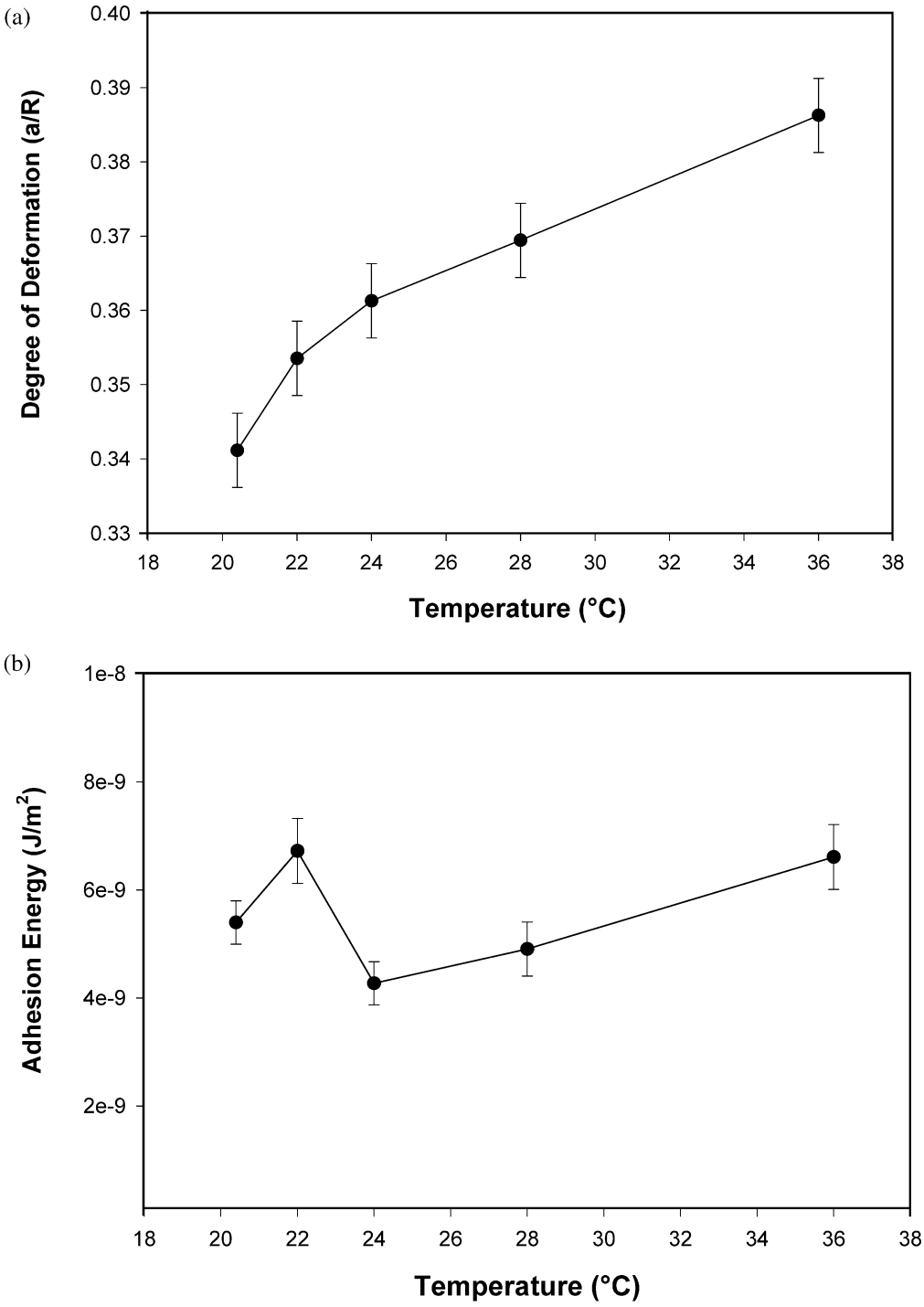

Fig. 8. (a) The degree of vesicle deformation (a/R) against the increase of temperature for adherent DMPC-ULV (averaged from 10 vesicles with same mid-plane radius) on pure fused silica substrate. (b) The adhesion energy against temperature for adherent DMPC vesicle on pure fused silica substrate (averaged from 10 vesicles with same mid-plane radius).

the adherent vesicle with confocal microscopy will help our contact mechanics study. However, this method suffers from the requirement of fluorescent labeling of the phospholipid before vesicle formation and the labeled vesicle may have different physicochemical properties in comparison to the non-labeled vesicles used in our study.

Fig. 8a shows the degree of vesicle deformation (a/R) against the increase of temperature for adherent DMPC-ULV (averaged from 10 vesicles with same mid-plane radius) on pure fused silica substrate. The degree of vesicle deformation is increased by 15% when the environmental temperature crosses over the phase transition of DMPC bilayer from gel to liquid crystalline state. This result is caused by the expansion of adhesion contact against the increase of temperature under constant vesicle volume as shown by the unchanged geometry of adherent vesicles under phase contrast microscopy during thermal transition. This result provides new implication in the use of temperature change in controlling the contact mechanics of adhering DMPC vesicle.

Fig. 8b shows the adhesion energy against temperature for adherent DMPC vesicle on pure fused silica substrate (averaged from 10 vesicles with same mid-plane radius). When DMPC bilayer is transformed from pre-dominant gel phase (20 °C) to co-existing gel/liquid crystalline phase (22 °C), the average adhesion energy is increased by 24% (from 5.4×10^{-9} to 6.7×10^{-9} J/m^2) as a result of the increased degree of vesicle deformation under constant elastic modulus. As the gel to liquid crystalline transition moves towards its completion (24 °C), the average adhesion is slightly dipped by 36% (from 6.7×10^{-9} to 4.3×10^{-9} J/m^2) compared with that at T_m. This reduction is caused by the step decrease of elastic modulus during the gel–liquid crystalline phase transition which is directly correlated to the adhesion energy [7,4]. When DMPC bilayer is converted to liquid crystalline phase, the average adhesion energy approaches the value of 6.6×10^{-9} J/m^2 before sample heating (20 °C). Overall, the average adhesion energy is maintained in the same level between the start and end of the phase transition of DMPC bilayer by the complex interplay between degree of vesicle deformation and elastic modulus. This result demonstrates that temperature is a critical parameter in dictating the adhesion strength of biomembrane vesicle and provides new insights into the origin of cell adhesion.

4. Conclusion

This study provides new insights into the biophysical response of biomembrane vesicle adhering on a non-deformable substrate at the middle of gel to liquid crystalline transition of DMPC bilayer. It is shown that the increase of membrane permeability at T_m leads to the dampening of osmotic effect on the contact mechanics and adhesion energy of DMPC vesicle on pure fused silica substrate. However, a change of surface chemistry from silanol to amino group results in significant decreases of degree of vesicle deformation and adhesion energy for larger vesicles. Last, the biophysical response of an adherent vesicle during temperature ramping is elucidated. This study provides new insights into the thermotropic and physiochemical effects of lipid bilayer on bioadhesion which can be instrumental in understanding the physical driving force of cell adhesion.

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