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Effect of acyl chain mismatch on the contact mechanics of two-component phospholipid vesicle during main phase transition

Ning Fang[a], Alvin Chi-Keung Lai[a], Kai-Tak Wan[b], 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 Mechanical and Aerospace Engineering, University of Missouri-Rolla, Rolla, MO 65409-0050, USA*

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Abstract

It has been recently demonstrated that acyl chain mismatch of phospholipid bilayer composed of a binary lipid mixture induces component formation on the lateral plane of the bilayer [Biophys. J. 83 (2002) 1820–1883]. In this report, the contact mechanics of unilamellar vesicles composed of binary dimyristoyl-phosphatidylcholine (DMPC)/dipalmitoyl-phosphocholine (DPPC) mixtures on fused silica and amino-modified substrates is simultaneously probed by confocal-reflectance interference contrast microscopy (C-RICM) and cross-polarized light microscopy during gel to liquid crystalline transition of the lipid bilayer. C-RICM results indicate that the average degree of vesicle deformation for DMPC-rich and DPPC-rich vesicles adhering on fused silica substrate is increased by 30% and 14%, respectively, in comparison with that in pure DMPC and DPPC vesicles. Also, lateral heterogeneity induced by acyl chain mismatch increases the average magnitude of adhesion energy in DMPC-rich and DPPC-rich vesicles of all sizes by 6.4 times and 2.3 times, respectively. Similar modulation of adhesion mechanics induced by carbon chain difference is obtained on amino-modified substrate. Most importantly, the thermotropic transition of the mixed bilayer from gel (below T_m) to fluid phase (above T_m) further exemplifies the effect of acyl chain mismatch on the increases of degree of vesicle deformation and adhesion energy.

Keywords: Acyl chain mismatch; Contact mechanics; Phospholipid vesicle

1. Introduction

Cell membrane is a supramolecular structure composed of proteins, carbohydrates, glycocalix, etc., embedded in a two-dimensional matrix of a lipid bilayer. Numerous studies demonstrate that various physiochemical properties of cell membrane directly affect major biological functions such as cell fusion, endocytosis and protein sorting [1]. Among all membrane constituents, the phospholipid bilayer provides the structural integrity of cells against external stimulations, e.g. mechanical stress. In general, the lipid composition of the cell membrane is highly dependent on cell types and is usually made up of multi-component mixtures. Therefore, the cell membrane has a complex phase behavior which provides the physical driving forc-

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

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

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es for performing and regulating numerous biological functions [2].

Recently, Korlach et al. demonstrated that there is a two-phase coexistence on the wall of a single unilamellar vesicle (ULV) composed of a binary lipid system [3]. The group demonstrates that the interesting micron-scale patterns of gel-rich and fluid-rich regions on the vesicle wall only emerge under two prescribed conditions. Firstly, the lipid bilayer contains two types of phospholipids with a significant difference in acyl chain length, e.g. dipalmitoyl-phosphatidylcholine (DPPC) and dilauroyl-phosphatidylcholine (DLPC). Secondly, the mole ratio of the two phospholipids in the binary mixture must fall within a certain range (e.g. $0.2 < \mathrm{DLPC}/\mathrm{DPPC} < 0.6$). Moreover, this group shows that the addition of cholesterol to the DLPC/DPPC mixture regulates the micro-domain formation of the vesicle wall [3]. However, there is still a missing link between the unique phase behavior of the binary phospholipid mixture and its underlying biological implications.

One critical role of cell membrane in biological functions is cell adhesion on the extracellular matrix. Recently, one group has shown that the thermal fluctuation of membrane–substrate distance within the adhesion contact for the bound vesicle composed of a highly heterogeneous lipid mixture (cholesterol, DEPC, PEG-DEPC), is different from that for vesicle composed of DEPC at constant temperature [4]. However, no quantitative information on the mechanical deformation and adhesion strength for the vesicle composed of highly miscible lipid mixtures during thermal transition has yet been provided. We have previously shown that the contact mechanics of the adherent vesicle composed of a single lipid is significantly modified during the gel to liquid crystalline transition of the bilayer [5]. Exploiting the fundamental interactions between a vesicle composed of thermodynamically well-characterized lipid mixtures and non-deformable substrates is critical for revealing the role of the multi-component lipid bilayer in cell adhesion. Recently, Muresan et al. illustrated that a change of composition in a binary lipid mixture (saturated phosphatidylcholines with different chain length) alters the rupture/fusion mechanism of small ULV on mica above the main phase transition temperature [6]. Fundamentally, the adhesion and deformation of vesicles on mica must occur before the fusion of the binary lipid bilayer on the substrate.

In this study, we demonstrate that the contact mechanics and adhesion strength of model vesicle composed of binary lipid mixture during the main phase transition are modulated on fused silica substrate compared to that in single-component vesicle. In the process, we measure the degree of vesicle deformation with confocal-reflectance interference contrast microcopy (C-RICM) in conjunction with cross-polarized light microscopy on both fused silica and amino-modified substrates, examine the effect of surface functionality, and determine the adhesion energy with a proven contact mechanics model at both gel and liquid crystalline phase co-existences.

2. Experimental

2.1. Materials

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. Dimyristoyl-phosphatidylcholine (DMPC) and dipalmitoylphosphocholine (DPPC) in powder form were obtained from Matryea Inc. (USA) and were 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. 1X 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

Pure fused silica coverslips were cleaned as described previously [7]. Amine modified substrate is prepared from fused silica substrate according

to a well-established silanization procedure [8]. Giant unilamellar vesicles (ULV) were synthesized by a well-established method and the unilamellar structure was verified by phase contrast microscope [9]. The ratio of two lipids in any binary lipid mixture is controlled by dissolving the prescribed amounts of lipids in a chloroform/methanol (2:1) solvent before loading on a Teflon disc.

2.3. Cross-polarized light microscopy

The detail of the instrument has been previously described [5]. Vesicle solutions in 1X (isotonic) was incubated on either pure fused silica or amine-modified coverslip for 1 h and was loaded in a temperature controlling chamber for subsequent imaging under the microscope. An image analysis software, ZSM5 (Carl Zeiss, Germany), was used for measuring the mid-plane diameter of adherent vesicles. All experiments for each lipid mixture were carried in sets of triplicate.

2.4. Confocal reflection interference contrast microscopy (C-RICM)

The detail of the instrument that is based on a laser scanning confocal microscope has been described elsewhere [7]. The illumination source was an Argon-ion laser with an excitation wavelength of 488 nm. A 63× oil immersion objective (Neofluar, N.A.: 1.25) was used. A strong contact zone of the adhering liposome appears as a dark region on the image. Vesicle solution in 1×PBS buffer was incubated on pure fused silica or amine modified substrate for 1 h and images were taken at temperatures ranging from 19 to 49 °C (at least 30 min of incubation time at each temperature). All experiments for each lipid mixture were carried in sets of triplicate. ZSM5 software (Carl Zeiss, Germany) 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 cross-polarized light microscopy) of an adherent vesicle at each temperature.

2.5. Contact mechanics model

The contact mechanics model for an adherent vesicle has been reported in detail [11]. A truncated spherical geometry, i.e. a sphere with a planar contact area of radius a, is assumed. The membrane–substrate profile $y(x)$ beyond the contact area is shown as

$$y = R\cos\theta - \sqrt{R^2 - (r + R\sin\theta)^2} \quad \text{for } r > a \tag{1}$$

with r the radial displacement from the contact center, R is the mid-plane radius and degree of vesicle deformation $\sin\theta = (a/R) = \alpha$. The adhesion energy when the vesicle wall is under a uniform equi-biaxial stress, $\sigma = C\varepsilon$ is shown as

$$W = (1 - \cos\theta)C\varepsilon + C\varepsilon^2 \tag{2}$$

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. The parameter ε is the average biaxial strain [11]. Based on the experimental measurements of the mid-plane diameter R (cross-polarized light microscopy) and the radius of contact zone, a (C-RICM), W can be found by Eq. (2). E of phosphocholine bilayer of a ULV composed of phosphatidylcholines in gel and liquid crystalline phase is taken as 28 800 N/m^2 and 16 000 N/m^2, respectively, according to the experimental results obtained from optical dynamometry, membrane bending spectroscopy and micropipette aspiration [12–14]. For each figure, the line is the best fit that statistically describes the trend of the experimental data.

2.6. Differential scanning calorimetry (DSC)

TA 2920 DSC calorimeter (TA Instrument Inc., DE, USA) was used for measuring the phase transition temperature and enthalpy of vesicle made of either lipid mixture or a single lipid. The sample preparation and experimental procedure has been described in detail [10]. All scans were recorded in the temperature range of 15–55 °C at a scan rate of 0.5 °C/min. One milligram of binary lipid mixture was dissolved in chloroform/methanol mixture in a glass vial, dried under a stream of N_2 and dried overnight in a vacuum oven. Each lipid sample in a set of triplicate was tested.

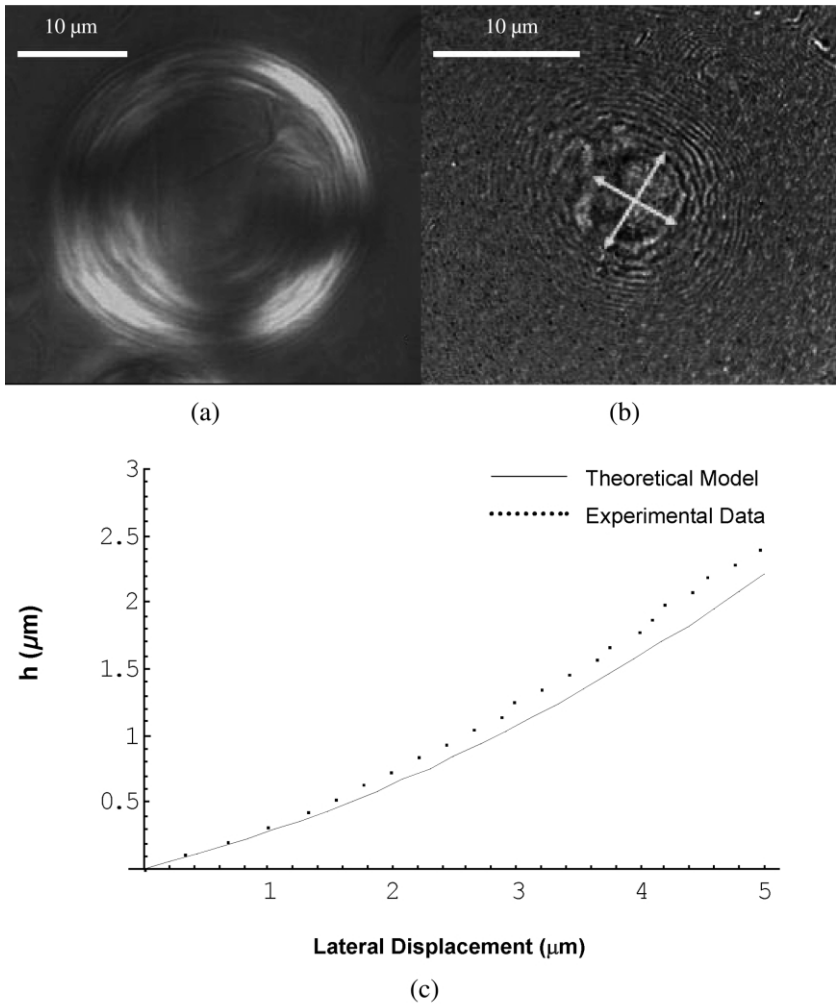


Fig. 1. A cross-polarized light image (a) and a C-RICM image (b) of a typical unilamellar vesicle (ULV) made of a binary DMPC/DPPC (ratio: 9/1) mixture on a fused silica substrate at 20 °C. (c) The profile of membrane–substrate separation (h) against the lateral distance away from the boundary of the contact zone on fused silica substrate.

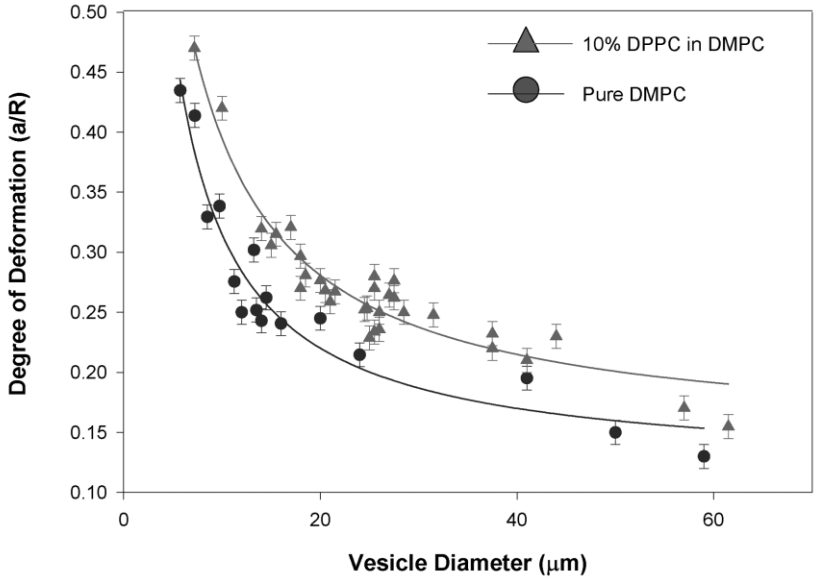
3. Result and discussions

Confocal-reflectance interference contrast microscopy (C-RICM) has been proven as an effective biophysical probe for adherent vesicle composed of a single phospholipid on non-deformable substrates [5]. Fig. 1 shows a cross-polarized light image (a) and a C-RICM image (b) of a typical unilamellar vesicle (ULV) composed of a binary DMPC/DPPC (ratio = 9:1) mixture on a fused silica substrate at 20 °C. DMPC/DPPC binary lipid bilayer is a well-characterized two-component biological membrane [20]. From DSC measurement, the gel to fluid phase transition of DMPC bilayer is detected at 21.5 °C (main transition temperature, T_m). In the presence of 10% DPPC molecules in the binary lipid mixture, T_m is shifted upward to 23 °C. From cross-polarized light microscopy, the adherent vesicle has a mid-plane diameter ($2R$) of 31 μm. From C-RICM, it is shown that the DMPC-rich vesicle develops a strong contact zone in the center of the image (the

cross on Fig. 1b). The dark appearance of the contact zone results from the interference of the reflected beams from two opposing surfaces (vesicle wall and reflective substrate) at low membrane–substrate separation (2–30 nm) [7]. The area and diameter ($2a$) of the contact zone is measured as 44.2 μm^2 and 7.5 μm, respectively. Generally, the geometry of the contact zone of the two-component vesicle is similar to that of vesicle composed of a single phosphocholine [5]. Fig. 1c shows the profile of membrane–substrate separation (h) against the lateral distance extending from the edge of the contact zone on fused silica substrate. The profile of h is directly determined from the inverse cosine transformation of the light intensity profile [7]. The dotted line is the experimental data from C-RICM and the solid line is the calculated membrane–substrate profile by inputting a and R into Eq. (1) of our contact mechanics model. The result indicates that our contact mechanics model is valid for adherent two-component vesicles.

It is critical to quantify the change of vesicle adhesion towards the introduction of acyl chain mismatch. Fig. 2a shows the degree of vesicle deformation (a/R) against the mid-plane diameter ($2R$) of DMPC-rich (DMPC/DPPC = 9/1) and pure DMPC vesicles on fused silica substrate in 1×PBS at 20 °C. The error bars represent the standard deviation of three measurements of vesicle of the same size. The degree of vesicle deformation provides a quantitative measure of the geometry of a deformed vesicle under the physical interactions between substrate surface and phospholipid bilayer [11]. For both types of vesicle, a/R reduces against the increase of vesicle diameter from 7.2 to 61 μm. At the same time, the slope of a/R vs. vesicle diameter for both vesicles approaches zero at the largest vesicle diameter (61 μm). This general trend of a/R is originated from the higher effective surface area (or area/volume ratio) of smaller vesicles for forming effective adhesion contact as the dimension of smaller vesicle approaches the effective distance of long-range intermolecular forces, e.g. electrostatic interaction [21]. For both one-component and two-component vesicles, the physical driving force of strong adhesion is stemmed from a combination of electrostatic attraction and van der Waals force at the vesicle–substrate interface [5]. Interestingly, the average magnitude of a/R for DMPC-rich vesicles of all sizes is 30% higher than that of pure DMPC vesicles as shown by the shift upwards of a/R vs. vesicle diameter curve. The enhanced deformation of DMPC-rich vesicle is directly correlated with the 2-methylene group mismatch on the vesicle wall. This result is supported by the nanoscale domain formation on the vesicle wall as shown by Monte Carlo simulation and neutron scattering of binary lipid mixture [10,23].

Adhesion energy of the vesicle is determined from Eq. (2) after a and R are experimentally measured as mentioned above. Fig. 2b shows the adhesion energy of DMPC-rich vesicle (DMPC/DPPC ratio = 9:1) and pure DMPC vesicle against vesicle diameter on fused silica substrate at 20 °C. Overall, the adhesion energies for both types of vesicle span more than two orders of magnitude against the increase of vesicle diameter from 7 to 61 μm. Most importantly, the average magnitude of adhesion energy for DMPC-rich vesicle of all sizes is 6.4 times higher than that of pure DPPC vesicle as shown by the shift up of the adhesion energy vs. vesicle diameter curve. Direct comparison of adhesion energy for the two types of vesicles is valid since the mechanical properties (e.g. volume compressibility) of DMPC-rich vesicles is similar to that of pure DPPC vesicles as shown by ultrasonic velocity measurement [20]. The smaller vesicles have larger adhesion energy because of their larger surface area to volume ratio for forming more physical bonds per unit area on the substrate. A similar trend has been reported in adhesion of vesicles composed of only DMPC or DPPC on various substrates [18]. Our result shows that the formation of a lateral domain originated from the binary lipid mixture significantly enhances the adhesion strength of vesicles on fused silica substrate. Our result is supported by the role of acyl chain mismatch in altering the rupture mechanism (involves adhesion and deformation) of small unilamellar vesicles composed of binary lipid mixture on mica [6]. This group demonstrates that acyl chain mismatch (e.g. difference of CH_2 group between the two lipids, mole fraction of lipid, etc.) leads to the formation of a unique nano-



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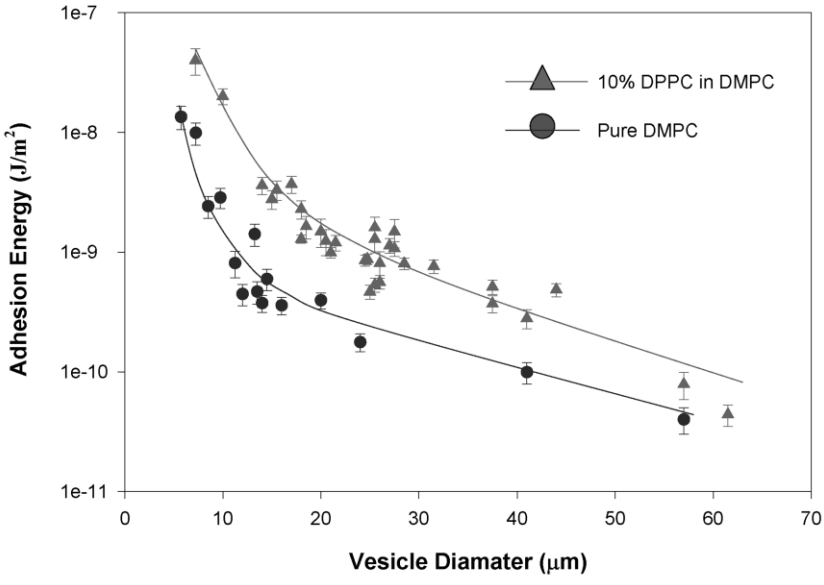


(b)

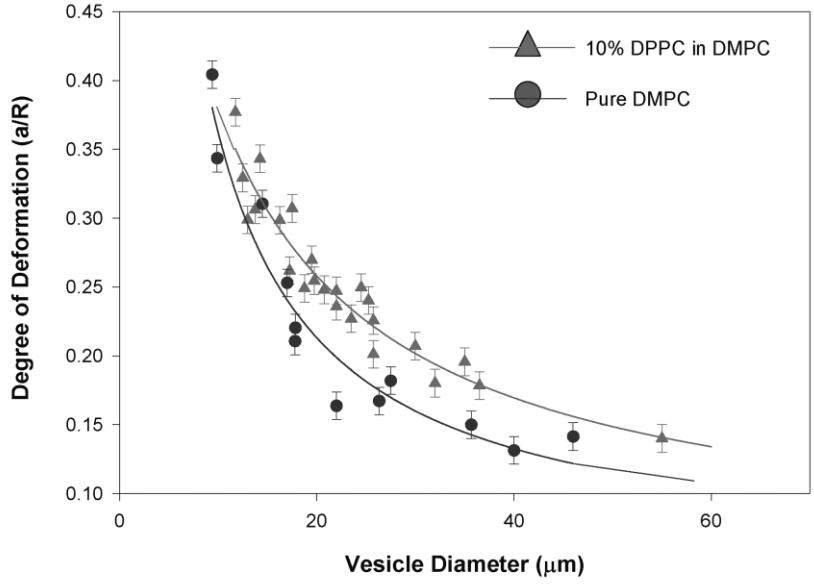
Fig. 2. (a) The degree of vesicle deformation (a/R) against the mid-plane diameter ($2R$) of DMPC-rich (DMPC/DPPC = 9/1) and pure DMPC vesicles on fused silica substrate in 1×PBS at 20 °C. (b) The adhesion energy of DMPC-rich vesicle (DMPC/DPPC ratio: 9/1) and pure DMPC vesicle against vesicle diameter on fused silica substrate at 20 °C.

scale compartment upon vesicle rupture on mica, which is otherwise absence in one-component vesicles (e.g. DMPC) [6]. Interestingly, the difference of acyl chain length between the two lipids is positively correlated with the adhesion energy (data not shown). Furthermore, small-angle neutron scattering demonstrates the existence of surface fractals at the nano-scale regime in binary DMPC/DPPC bilayer at the regime of gel phase co-existence [17]. We demonstrate that the evolution of lateral heterogeneity on the binary lipid mixture enhances the adhesion of the two-component vesicle under constant mechanical properties.

A change of surface chemistry is known to modulate the contact mechanics of the one-component vesicle [18]. Fig. 3a shows the degree of vesicle deformation against the vesicle diameter for DMPC-rich and pure DMPC unilamellar vesicles on APTES coated substrate at 20 °C. The result shows that the general trend of reducing the degree of vesicle deformation against the increase of vesicle size on the amino-modified substrate is similar to that on pure fused silica. However, the average magnitude of a/R ($7\ \mu m < a < 61\ \mu m$) for DMPC-rich and pure DMPC vesicles is reduced by 12 and 20%, respectively, compared to that on fused silica substrate (Fig. 2a). This trend is found in both one- and two-component vesicle and is driven by the electrostatic repulsion at vesicle–substrate interface following the introduction of amine on fused silica substrate [18]. Most importantly, DMPC-rich vesicles are more deformable than pure DMPC vesicles as shown by a 21% increase in the average magnitude of a/R for vesicle of all sizes (the shift up of a/R vs. R curve for DMPC-rich vesicles). Fig. 3b shows the adhesion energy against vesicle diameter for DMPC-rich and pure DMPC vesicles on APTES-modified substrate at 20 °C. The adhesion energy vs. vesicle diameter curve is shifted upward by doping pure DMPC vesicle with 10% DPPC, and the average magnitude of adhesion energy for DMPC-rich vesicles with size ranging from 7 to 61 μm is increased by three times in comparison with that for pure DMPC vesicles. The result indicates that lateral heterogeneity on the binary lipid bilayer enhances phospholipids–substrate interaction on amino-modified substrate at gel phase coexistence, and is supported by the role of acyl chain mismatch in modulating peptide–lipid bilayer interaction [24].

It is important to examine the effect of acyl chain mismatch on the adhesion of a binary lipid bilayer with a different composition at the regime of liquid crystalline phase coexistence. Fig. 4 shows the cross-polarized images [i (20 °C) and iii (49 °C)] and C-RICM images [ii (20 °C) and iv (49 °C)] for a typical DPPC-rich vesicle composed of a DMPC/DPPC (mole ratio = 1:9) binary mixture on fused silica substrate. The cross-polarized light image indicates that the DPPC-rich vesicle has a mid-plane diameter of 20 μm. Also, the thermal transition from 20 to 49 °C does not cause significant shape transformation of the adherent vesicle as shown by the less than 4% variation in the mid-plane area of the adhered vesicle during sample heating. From C-RICM, it is shown that the increase of DPPC mole fraction in the binary phospholipid mixture leads to similar geometry of contact zone in comparison with that of DMPC-rich vesicles. Moreover, the single contact zone supports the absence of micron-scale phase separation on vesicle wall induced by substrate-induced deformation which is theoretically illustrated in vesicle composed of highly heterogeneous lipid mixtures [22]. Interestingly, the contact area of the DPPC-rich vesicle is increased from 24.6 to 38.3 μm^2 during sample heating. The thermal-induced modification of the contact zone is directly correlated with the change of mechanical properties of phospholipid bilayer during the gel to liquid crystalline transition as shown in the case of single-component vesicle [5,14].

Fig. 5a shows the degree of vesicle deformation (a/R) against vesicle diameter for DPPC-rich and pure DPPC vesicle on pure fused silica substrate in 1×PBS at 20 °C [13]. In general, a/R is a reducing function of vesicle diameter for both types of vesicles. The average magnitude of a/R for DPPC-rich vesicle with diameter ranging from 7 to 60 μm is 14% higher than that in pure DPPC vesicles as shown by the shift-up of the a/R vs. vesicle diameter curve. The incorporation of DMPC (with two CH_2 groups mismatch) into the two-dimensional matrix of DPPC bilayer results in the reduction of T_m from 41 to 37 °C. There is an



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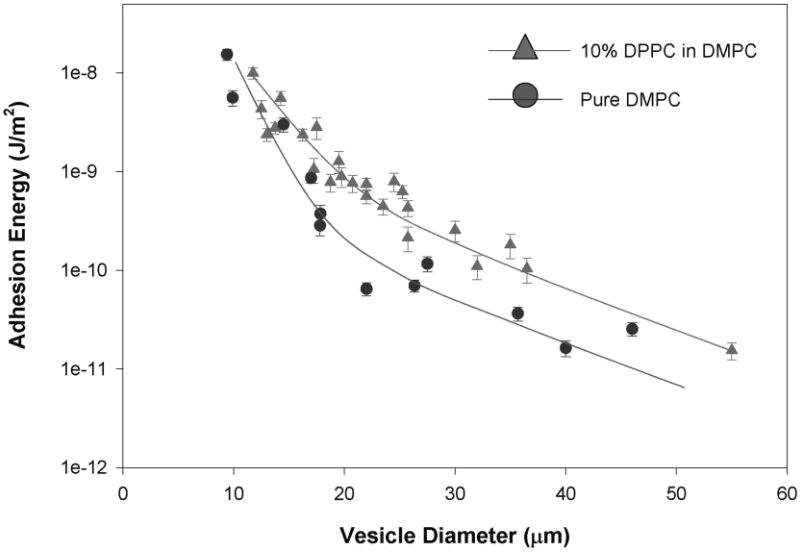


(b)

Fig. 3. (a) The degree of vesicle deformation against the vesicle diameter for DMPC-rich and pure DMPC unilamellar vesicles on APTES coated substrate at 20 °C. (b) Adhesion energy against vesicle diameter for DMPC-rich and pure DMPC vesicles on APTES-modified substrate in 1×PBS at 20 °C.

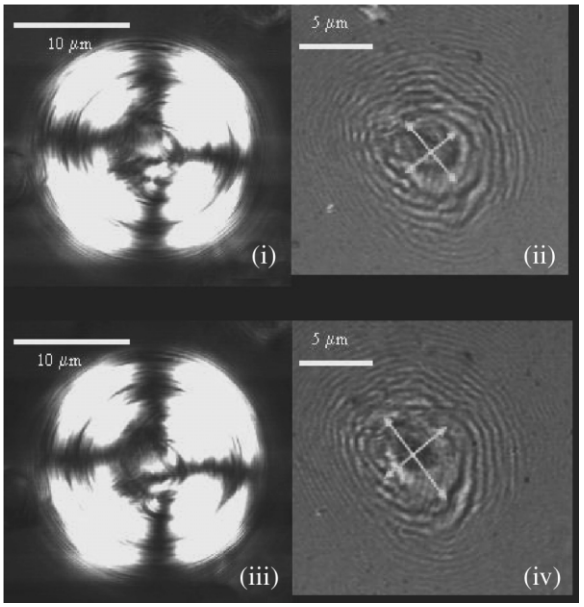
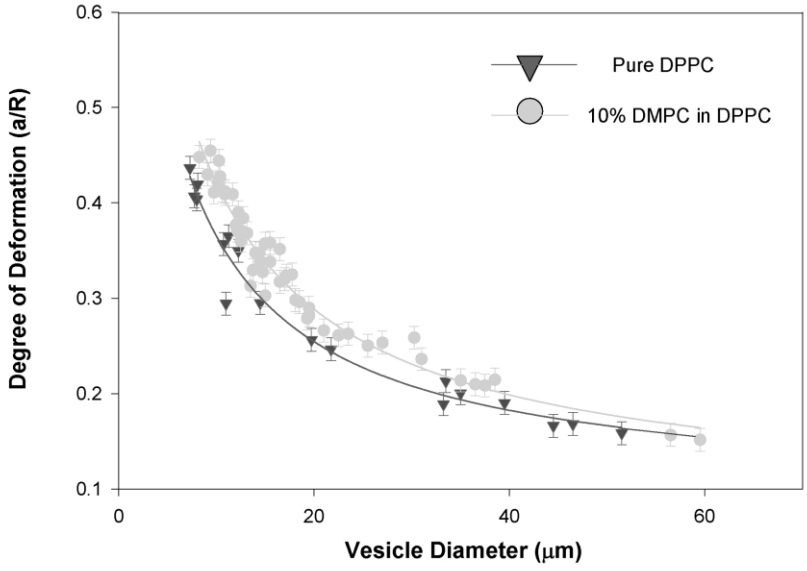


Fig. 4. The cross-polarized images [i (20 °C) and iii (49 °C)] and C-RICM images [ii (20 °C) and iv (49 °C)] for a typical DPPC-rich vesicle composed of a DMPC/DPPC (mole ratio = 1:9) binary mixture on fused silica substrate.

absence of micron-scale phase separation on the vesicle wall under the relatively low concentration of DMPC molecules in the binary lipid mixture at gel phase co-existence regime [15,19]. Compared with Fig. 2a, the average magnitude of a/R of DPPC-rich vesicle is similar to that of DMPC-rich vesicle. Therefore, the enhancement in the substrate-induced deformation is solely caused by the nano-scale domain formation on the binary lipid bilayer and is independent of the composition of the DMPC/DPPC mixture [24]. This result is further supported by the Monte Carlo simulation of the binary lipid mixture [23] and the change of rupture mechanism against the composition change of small ULV made of binary lipid mixture (with different chain length) upon deformation and adhesion on mica [6].

Fig. 5b shows the adhesion energy against vesicle diameter for DPPC-rich and pure DPPC vesicles on fused silica substrate at 20 °C. From ultrasonic velocity measurement, the mechanical properties (e.g. volume compressibility) of DPPC-rich vesicles approach those of pure DPPC vesicles [20]. Similar to the DMPC-rich vesicle at the opposite end of the DMPC/DPPC phase diagram, adhesion energy is negatively correlated with vesicle diameter and spans three orders of magnitude against the increase of vesicle diameter. The average magnitude of adhesion energy for DPPC-rich vesicle of all sizes is 2.3 times higher than that of pure DPPC vesicles as shown by the shift up of adhesion energy vs. vesicle diameter curve, and is the same as that of the DMPC-rich vesicle (Fig. 2b). The enhancement in adhesion energy induced by the acyl chain mismatch on the binary lipid bilayer is directly related to the lateral heterogeneity on binary lipid bilayer. The thermotropic transformation of phospholipid bilayer from gel to liquid crystalline phase has been shown to directly influence the contact mechanics of adherent vesicle composed of a single phospholipid [5]. Fig. 6a shows the degree of vesicle deformation against vesicle diameter for DPPC-rich and pure DPPC vesicles on fused silica substrate at 49 °C. At temperatures well above T_m, DPPC-rich vesicles are in a liquid crystalline phase co-existence [20]. The average degree of vesicle deformation for DPPC-rich vesicles with diameter ranging from 7 to 59 μm is 19% higher than that of pure DPPC vesicles at gel state. Interestingly, the effect of lateral heterogeneity induced by acyl chain mismatch on a/R is intensified at liquid crystalline states (Fig. 6a vs. Fig. 5a). It is demonstrated that the progression of phase transition across T_m during sample heating directly lowers the elastic modulus of lipid bilayer by 50% [12,14]. Intuitively, the reduction of elastic modulus of adherent vesicle at liquid crystalline state partially accounts for the observed increases in the degree of vesicle deformation as shown in classical contact mechanics model [21].

Fig. 6b shows the adhesion energy of DPPC-rich and pure DPPC vesicles against vesicle diameter on fused silica substrate at 49 °C. Generally, the trend of reducing adhesion energy against the increase of vesicle size is found in both two- and single-component vesicles at liquid crystalline phase. The average magnitude of adhesion energy for DPPC-rich and pure DPPC vesicles of all sizes is increased by 82% and 118%, respectively, during



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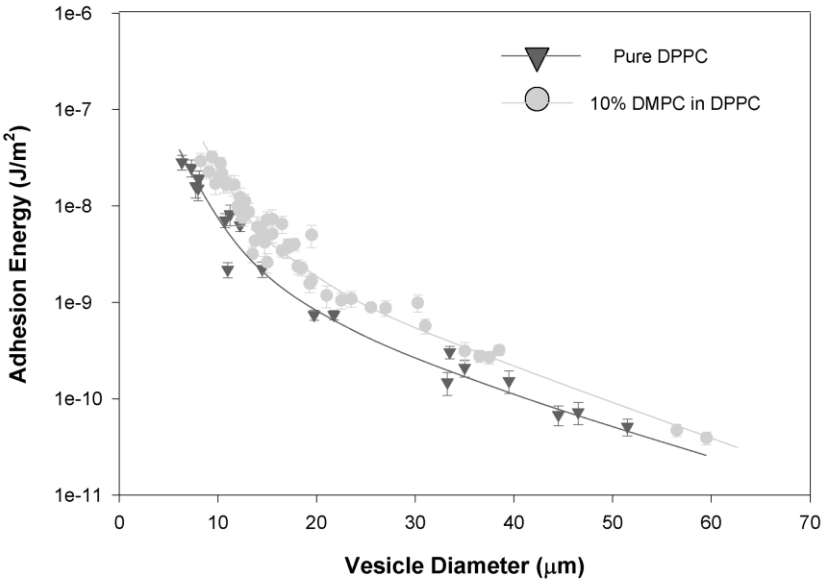
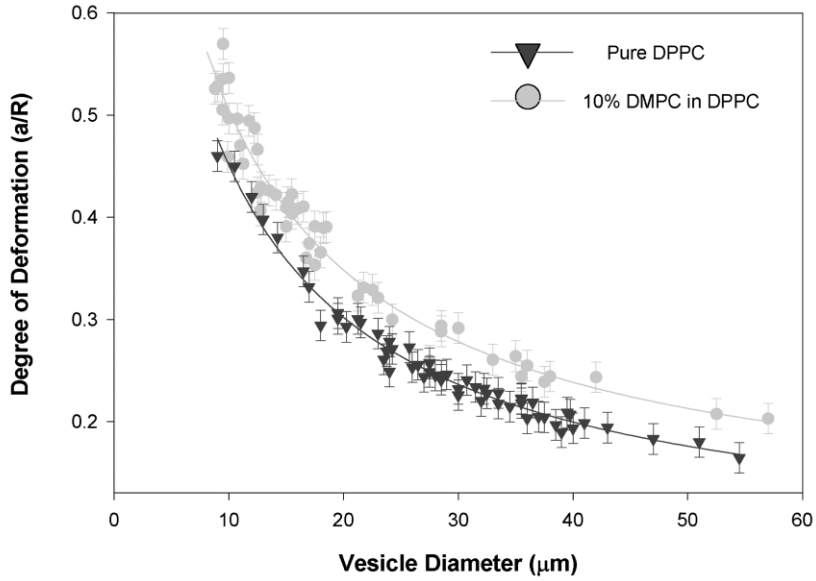


(b)

Fig. 5. (a) The degree of vesicle deformation (a/R) against vesicle diameter for DPPC-rich and pure DPPC vesicle on pure fused silica substrate in 1×PBS at 20 °C. (b) The adhesion energy against vesicle diameter for DPPC-rich and pure DPPC vesicles on fused silica substrate at 20 °C.



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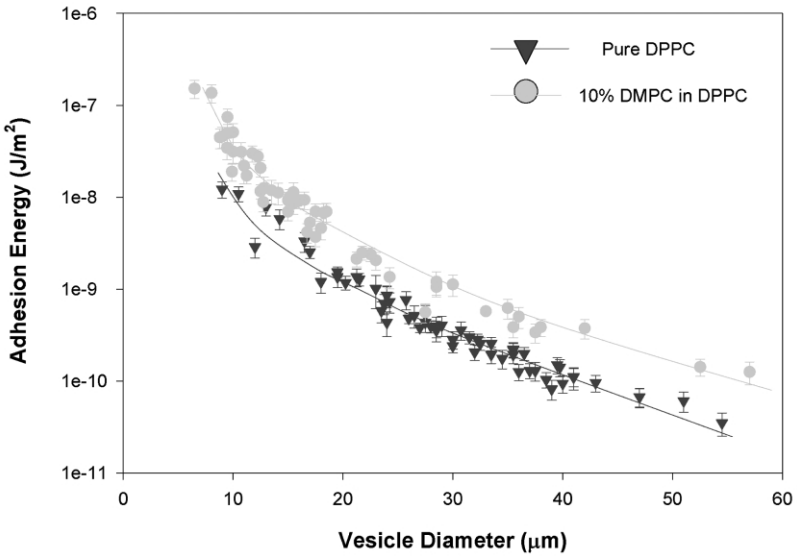

(b)

Fig. 6. (a) The degree of vesicle deformation against vesicle diameter for DPPC-rich and pure DPPC vesicles on fused silica substrate at 49 °C. (b) The adhesion energy of DPPC-rich and pure DPPC vesicles against vesicle diameter on fused silica substrate at 49 °C.

the gel to liquid crystalline transition (Fig. 5a). The increase of adhesion energy for DPPC-rich vesicle during sample heating is originated from the complex interplay of elastic modulus variation and vesicle–substrate interaction. It is known that the reduction of elastic modulus of adherent vesicle suppresses the adhesion energy if a/R remains constant during the thermal transition according to our contact mechanics model [11]. Under strong physical interaction (electrostatics and van der Waals attractions) between the two-component vesicle and fused silica substrate, the extent of a/R increase during sample heating, overcomes the effect of elastic modulus reduction and leads to higher adhesion energy above T_m as shown in single-component vesicle [5]. Also, the average magnitude of adhesion energy for DPPC-rich vesicles (with size ranging from 7 to 60 μm) is 2.8 times higher than that for pure DPPC vesicles as the adhesion energy vs. vesicle diameter curve is shifted upwards. Recently, Weikl and Lipowsky have provided a theoretical basis for the adhesion of vesicle composed of multi-component bilayer membrane [22]. In this study, they prove that adhesion-induced phase separation on the vesicle wall is originated from the existences of membrane components with different binding affinities towards a non-deformable substrate. In our experimental system, the binding affinity of DPPC bilayer against fused silica substrate is similar to that of DMPC bilayer, and micron-scale domain formation is unobservable on adherent DPPC-rich vesicle during the thermal transition [7,10]. Thus, the enhancement in adhesion strength of the DPPC-rich vesicle is directly correlated to the carbon chain mismatch that leads to lateral heterogeneity on the two-component bilayer [16]. This result is further supported by the insignificant deviation of the mechanical properties between slightly doped DPPC vesicle and pure DPPC vesicles [20]. Most importantly, the increase in average magnitude of adhesion energy induced by nano-domain formation [23] at liquid crystalline phase co-existence is higher than that of the gel state. It is also known that the inclusion of cholesterol into a binary phospholipid mixture leads to significant changes in the phase boundary shape [3]. At this stage, there is no information concerning the effect of cholesterol on the adhesion of the two-component vesicle. This interesting phenomenon will be focused in our future studies.

4. Conclusion

In summary, this study provides new evidence that acyl chain mismatch and lateral heterogeneity directly modulates the contact mechanics of two-component vesicles. First, our contact mechanics model has been validated for application to adhering vesicle composed of binary phospholipid mixtures on non-deformable substrate. Our C-RICM results demonstrate the lack of large domain formation (micron-scale) on the wall of two-component vesicle upon adhesion on fused silica substrate. The introduction of 10% DPPC molecules in pure DMPC bilayer leads to a significant increase in the degree of vesicle deformation and adhesion energy at gel phase co-existence in comparison with pure DMPC vesicle. The enhancement of vesicle contact mechanics induced by acyl chain mismatch is directly correlated with the nano-scale domain formation and is also observed on primary amine modified substrate. When the mole fraction of DPPC increases from 0.1 to 0.9, the two-component vesicles remain more deformable than pure DPPC vesicle at the temperature of gel phase co-existence. Last, thermal transformation of adherent vesicle from the gel to liquid crystalline phase further exemplifies the effect of lateral heterogeneity on the adhesion strength and substrate-induced deformation.

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