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Effects of DNA Adsorption on the Phase Cycling of a Supported Mixed Phospholipid Bilayer

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ABSTRACT

In this paper we demonstrate a powerful method to visualize directly DNA on a bilayer during a lipid phase transition. Using temperature-dependent atomic force microscopy (AFM), we observed that DNA appears to induce lipid demixing during phase cycling.

The ability to observe the interactions of single DNA molecules with cationic phospholipid (CL) bilayer surfaces at nanoscale resolution will provide valuable information about CL-DNA complex formation. This process is of great interest because cationic liposome−DNA complexes have been shown to serve as viable, nontoxic gene delivery vehicles.[1] Because DNA is a polyanion, it binds to a cationic phospholipid bilayer due to an electrostatic interaction and forms a lipoplex. However, there is little understanding at the molecular level of how the mechanism of complex formation correlates with the efficiency of gene delivery. Empirically, it is known that neutral helper lipids are essential for fusion of DNA−CL with cell membranes.[2] Moreover, the helper lipids with the greatest effect are generally those that have a propensity to form inverted hexagonal phase (H_{II}) under physiological conditions.[3] Thus, systems such as DOTAP/DOPE were developed and have proven to be efficacious for cell transfection. These findings suggest that the best DNA−CL lipoplex for gene delivery should exist on the thermodynamic knife-edge between lamellar and hexagonal phase under appropriate conditions. It is possible that this situation may only exist when the lamellar phase is L_α (liquid) rather than L_β (gel).

Until recently, the effect of DNA complexation on CL phase was completely unknown. A series of papers by Rädler and co-workers described the structures of DNA−CL complexes using diffraction techniques.[4,5] It is generally accepted now that when the lipoplex is charge neutral, the greatest effects are observed and that DNA complexation can induce a $L_\alpha-H_{II}$ transition.[3] Significantly, Zantl et al.[6] have investigated the structure of DMTAP/DMPC at temperatures above and below ($L_\beta-L_\alpha$) phase transition using wide- and small-angle X-ray diffraction. They suggested that the presence of DNA might affect the tilting of lipids in the gel phase.

It is well documented that atomic force microscopy is a powerful tool to study DNA and DNA dependent processes.[7] Steps toward understanding DNA−CL complexes at the single molecule level were made using AFM to study DNA binding to gel phase bilayers.[8,9] In these studies, gel phase cationic bilayers provided good stable substrates for DNA binding, resulting in high contrast images. However, L_α CL bilayers may serve as a more realistic model to study DNA bilayer interaction with respect to gene delivery, but imaging DNA on a liquid-phase bilayer is a greater challenge for AFM at the present time.

In this work, we demonstrate that a supported cationic bilayer with adsorbed DNA can be transformed from the gel phase to liquid phase by heating the complex above the chain melting transition temperature. We used dipalmitoyl(trimethylamino)propane/dipalmitoylphosphatidylethanolamine (DPTAP/DPPE) as a room temperature gel phase analogue to DOTAP/DOPE and atomic force microscopy to investigate the phase behavior of a DPTAP/DPPE bilayer on a mica support in the presence and absence of complexed DNA. To our knowledge, this is the first temperature-dependent study where DNA has been directly visualized on a supported bilayer during a lipid phase transition.

CL liposomes were prepared by sonicating and stirring a solution of mixed (1:1) phospholipids in water. Supported bilayers were formed via vesicle fusion[10] on regular freshly cleaved mica by applying 50−70 μL of a 0.2−0.3 mg/mL vesicle solution in water at room temperature. After 20 min, the liquid cell was gently rinsed with water. AFM images[11] reveal that the supported bilayers formed from water solutions were planar and contained defects and regions of different elevation (a.k.a. domains), as displayed in Figure 1. The difference in the thickness of the two highest domains

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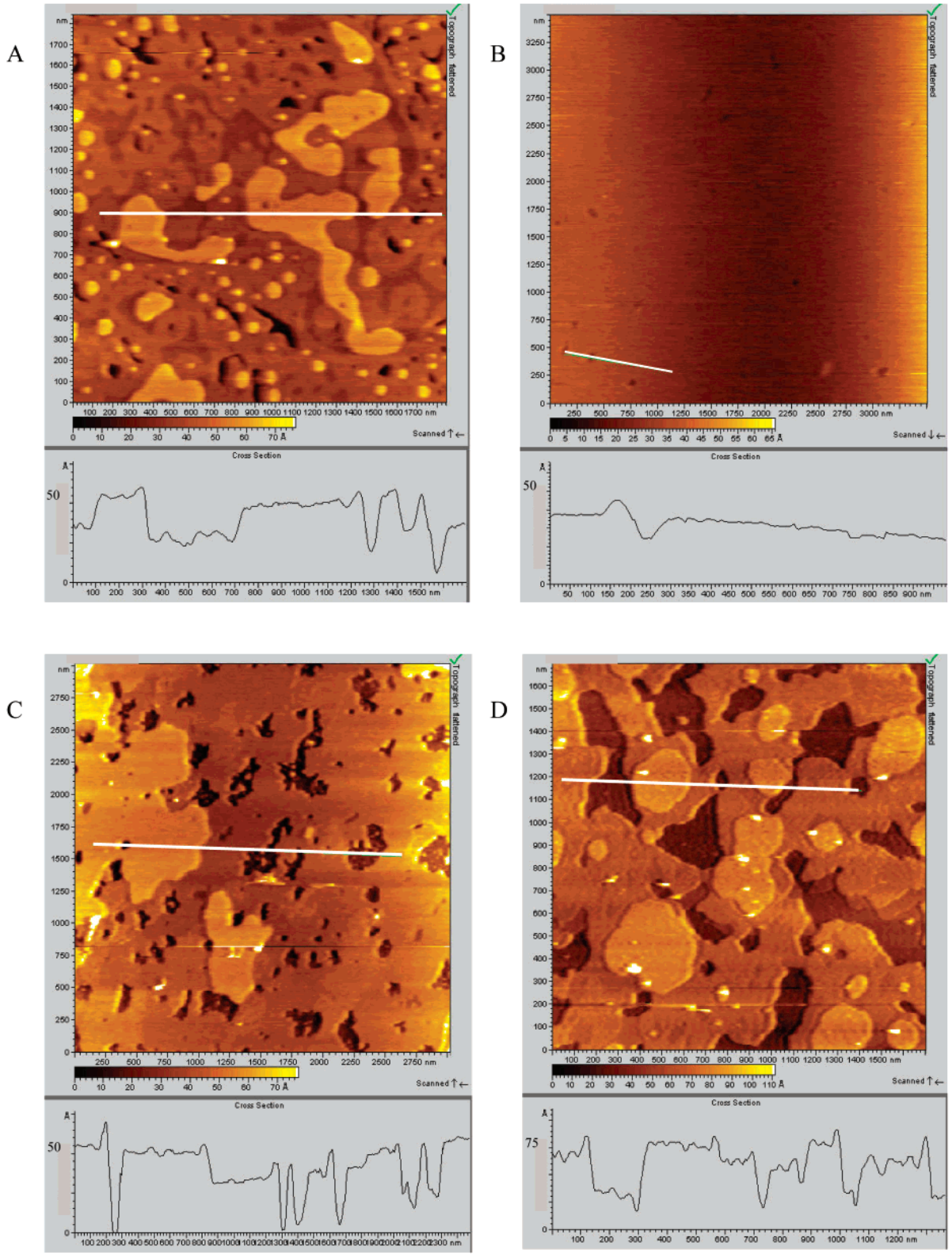

Figure 1. Heating a supported mixed DPTAP/DPPE bilayer, formed from an aqueous vesicle solution, 0.3 mg/mL, by pipetting 50 μL; time of incubation on mica: 10 min. Key: (A) room temperature, 22 °C; (B) heated to 70 °C; (C) cooled back to room temperature, 23 °C; (D) cooling to room temperature after heating to 50 °C.

was 1.7 nm (Figure 1A), with the higher being 6.0 nm thick and the lower 4.3 nm. Although the difference is comparable to that measured for pure DPTAP by Longo and co-workers[12] (1.4 nm) and by Cramb and co-workers[13] (1.7 nm), the difference here is more likely due to domains rich in DPPE versus domains rich in DPTAP. DPPE is known to have a thickness[14] of 6.0 nm, whereas an annealed DPTAP supported bilayer has been observed to have a thickness[10,13] between 4 and 5 nm. The two lowest domains in Figure 1A could be domains in the enriched DPTAP bilayer similar to

those observed by Longo and co-workers. We observe a difference in two lower domains as 1.2−1.4 nm. We assume that they were present in the vesicles and could remain after bilayer formation by vesicle fusion (the vesicle solution was heated as high as 45 °C during sonication).

For a supported bilayer made from a single lipid below the ($L_\beta - L_\alpha$) phase transition temperature, the equilibrium gel phase structure is stable. Within the gel phase regime stable, metastable, and transient lamellar structures may be present. These may result from perpendicular or tilted chains and/or interdigitated or partially interdigitated chains. In a mixed bilayer, the structures may be even more complicated. We assume that our two lower domains correspond to the presence of some of these structures.

After heating the mixed bilayer to 70 °C, we observe that domains and defects disappear and the surface was covered with a flat bilayer with only a few small defects (holes); see Figure 1B. The $L_\beta - L_\alpha$ phase transitions for DPTAP[12] and DPPE[15] are 45 and 65 °C, respectively. DPPE is believed to have a higher melting point because of hydrogen-bonding between headgroups. After cooling the system back to room temperature (Figure 1C), we observed the formation of only two domains (5.5 and 4.0 nm) in addition to defect formation. This is suggestive again of DPPE rich and DPTAP rich bilayer areas, respectively. Unfortunately, it is not always possible to follow the same imaging area as the temperature is ramped up and down, because force readjustment during heating may preclude imaging the same spot. Two domains also re-formed in the instance where the mixed bilayer was heated only to 50 °C and then allowed to cool, Figure 1D. Here the domains have the same thickness as after heating to 70 °C, but the continuous regions are smaller. This suggests that with a lower fluidity, there was less opportunity for the DPPE rich regions to coalesce. These images are reminiscent of recent findings by Giocondi et al.,[16] who reported the observation of domain growth in dioleoylphosphatidylcholine/dipalmitoylphosphatidylcholine (DOPC/DPPC) bilayers using AFM. Moreover, the fact that we do not observe complete coverage after heating suggests that the image in Figure 1A does not represent multibilayers. Further experiments are planned to confirm the bilayer demixing.

Next, DNA was adsorbed onto a freshly prepared gel phase DPTAP/DPPE bilayer at room temperature. We used linearized 3000 base pair B-Gal DNA, which was a gift from the Biotechnology Center, University of Calgary. DNA solutions in Tris−EDTA buffer (100−200 μL), at 10 μg/mL, were pipetted onto the hydrated phospholipid bilayer and were left to incubate at room temperature for 1−4 h. Excess DNA was gently rinsed away and the surface was imaged under water in the liquid cell. DNA molecules were randomly distributed, well resolved on the surface and adsorbed preferentially on the surface of the higher domains, as shown in Figure 2A. This implies that the higher domains contain cationic lipid, although they may be richer in DPPE than the lower domains. The height of DNA on the bilayer, measured at room temperature, was 1.7−1.8 nm. The system was then heated slowly to 50 °C, with a ramp of 1 °C/min, while imaging. At 50 °C, the contrast and resolution of DNA adsorbed on the bilayer were lower than at room temperature; see inset of Figure 2B. Heating a pure DPTAP bilayer with DNA above the melting transition point shows low contrast and lower DNA height as well.[13] After slow cooling, the contrast and resolution of DNA were restored, Figure 2C. The height of DNA measured changed from 1.7 nm at room temperature, 0.7 nm at 50 °C, and 1.6 nm at room temperature after cooling.[10] We assume that low contrast and low DNA height at 50 °C could be due to partial penetration of DNA into the fluid bilayer.

At elevated temperatures the bilayer fluidity increases and we observed an interesting behavior of the DNA-complexed bilayer during this cycle. If one compares Figures 1 and 2, one notices that domains and defects disappear or are greatly reduced at elevated temperatures and then restored after cooling. Remarkably, when the DNA−DPTAP/DPPE system is cooled back to room temperature, areas around adsorbed DNA are restored to higher domains, while areas with DNA adsorbed remain at low domains. Recall that initially the DNA were adsorbed onto higher domains. Interestingly, in a parallel study we found that DNA on a pure DPTAP bilayer remained on top of the higher domains after slow heating and cooling.[13] In the present work, after heating and cooling, the domains were found to have thicknesses of 6−7 and 4−5 nm for the DNA-free and DNA-containing regions, respectively. Elevated regions at the bilayer edges appear even higher (Figure 2C). This effect was observed only in the presence of DNA and was a stable feature of the image for up to 1 h of scanning. Nevertheless, these features could represent a metastable state of regions high in DPPE content, where tip interactions have induced a folding of the bilayer upon itself. Further speculation is beyond the scope of the present letter.

With respect to the domain containing DNA, we assume that the presence of DNA adsorbed on the bilayer surface must somehow constrain the domain restoration observed for the DNA-free gel phase bilayer observed in Figure 1. Presumably, this occurs due to a demixing process, which is possible in a more fluid phase. Furthermore, it is compelling that the DNA appears to be more condensed after the phase transition cycle. This is consistent with a degree of demixing, which would promote a higher concentration of DPTAP in DNA-containing regions. It is noteworthy that we observed no evidence of hexagonal phase induction during heating. This could be compared with the $L_\beta - H_{II}$ transition temperature at 56 °C for α-tocopherol in pure DPPE multilamellar bilayers, examined used wide-angle X-ray scattering.[17] Unfortunately, it was impossible to operate on DNA-containing samples at temperatures much greater than 55 °C, because of image instability. This is possibly due to the onset of DNA melting.

We report the first direct observations of DNA on a supported bilayer while temperature is altered. We have shown the utility of temperature dependent AFM imaging to directly observe lipid phase transitions. Indeed, we provide compelling evidence of lipid demixing when a DNA−CL complex is heated above the $L_\beta - L_\alpha$ phase transition tem-

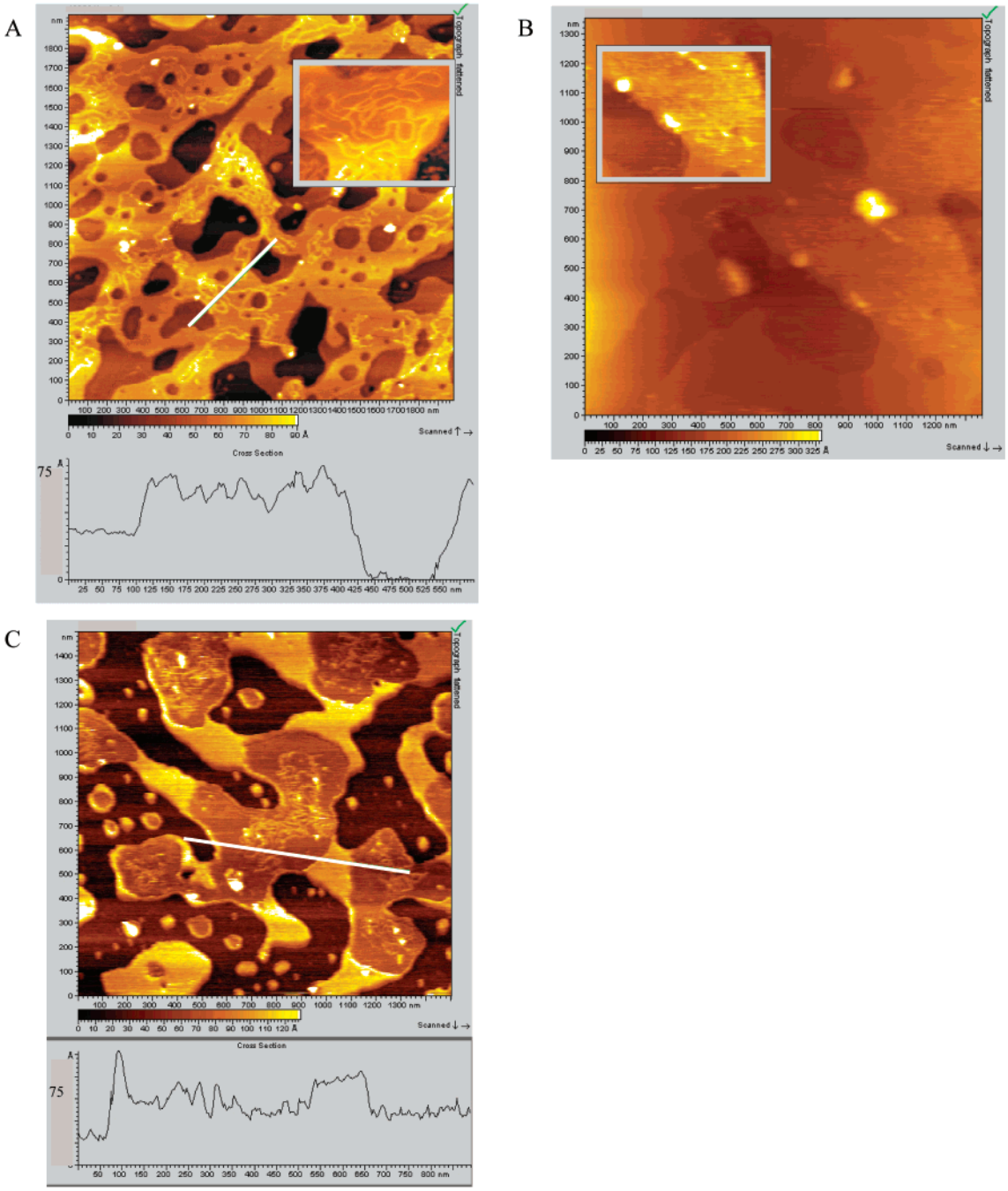

Figure 2. Heating a supported mixed DPTAP/DPPE bilayer with adsorbed DNA, formed from an aqueous vesicle solution, 0.2 mg/mL, by pipetting 60 μL; time of incubation on mica: 10 min. DNA was added to the wet bilayer, 200 μL, 10 μg/mL; time of incubation: 2 h at room temperature. Key: (A) room temperature, 22 °C; (B) heated to 70 °C; (C) cooled back to room temperature, 23 °C. Insets are digital enlargements of selected regions.

perature. Our work opens the possibility of using AFM to determine the effect of a solid support on bilayer phase transition thermodynamics and to use DNA in controlling the formation of lipid rafts.[18] Moreover, these observations will undoubtedly lead to a deeper understanding of DNA−liposome behavior and thus help to improve the efficacy of liposomal gene delivery. Finally, our evidence suggests that by choosing appropriate phospholipids, it will be possible to control the dynamics of lipoplex structure as a function of temperature.

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