Aggregation and Self-Organization of a Chromophore-Labeled Double-Chain Amphiphile

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The membranes of a double-chain amphiphile dioctadecyl-*N*-[4-[[*p*-(*N*,*N*-dimethylamino)phenyl]azo]-pyridiniumbutyryl]-L-aspartate bromide (DDPA) were investigated by atomic force microscopy (AFM) and UV−vis absorption spectroscopy. The line-shaped aggregates of the molecules were found in the Langmuir−Blodgett (LB) monolayers of DDPA. The widths of these lines were about twice the length of the molecule. A semicylindrical structure was proposed to account for the observation. It was found that the DDPA molecules self-organized into bilayers and vesicles when the LB film was immersed in aqueous solutions. The bilayer showed good compressibility. The mixed DDPA and α-L-dipalmitoylphosphatidylcholine LB films were also examined, and the phase separation was observed. From the in situ AFM observation, the self-organization process was found to occur in the DDPA region. The mechanism of self-organization in aqueous solutions was also discussed.

Introduction

Phospholipid films on solid supports have been paid increasing attentions as model systems for biological membranes as well as for application in biosensor devices.[1] Advanced surface-specific techniques such as atomic force microscopy (AFM),[2] scanning near-field optical microscopy (SNOM),[3] second harmonic generation (SHG),[4] and total internal reflection fluorescence (TIRF)[5] have provided great knowledge of the interfacial properties of the biomaterials. Because the SHG signal of natural phospholipids is very weak, a double-chain amphiphile dioctadecyl-*N*-[4-[[p-(*N*,*N*-dimethylamino)phenyl]azo]pyridiniumbutyryl]-L-aspartate bromide (DDPA), whose structure is similar to that of phospholipids, has been introduced as a nonlinear optical probe. The acid−base reaction and phase transition of DDPA thin film have already been characterized by SHG.[6,7]

Amphiphilic molecules, including phospholipids, often organize themselves into aggregates in a variety of conditions. Hydrogen bond,[8] hydrophobic interaction,[9] and $\pi-\pi$ interaction[10] may play roles in the formation of aggregates. The structure of the amphiphile has a strong influence on the superstructure of the aggregates,[11] while environmental conditions can also affect the superstructure. To provide information for the design of novel membrane materials, it is important to gain full understanding of the superstructure of aggregates formed by amphiphiles. For surface aggregates, scanning probe microscopy (SPM) can provide direct information of their structures. Spectroscopic technique is also an appropriate tool to study the aggregates if the amphiphilic molecules bear chromophores.[10,12,13]

In the past few years, AFM[14,15] and molecular dynamics simulation[16] studies about surfactant aggregates on the solid−water interface have been carried out. The single-chain surfactants in their solutions form semicylindrical (or full-cylindrical) aggregates on hydrophobic (or hydrophilic) surfaces. For the semicylindrical aggregates, the AFM images of these surface aggregates show parallel stripes, of which the width is twice the length of the surfactant molecules and the orientation is dependent on the surface lattice. In these cases, the aggregate structure on the interface is somewhat similar to the cylinder structure formed in the bulk solution,[9] both of which are dominated by hydrophobic interaction.

The aggregates of phospholipids and fatty acids containing aromatic chromophores of stilbene and azobenzene were also studied by spectroscopy.[10] It was proposed that these amphiphiles form stable aggregate units characterized by strong edge-to-face interaction among aromatic groups in the Langmuir−Blodgett (LB) films and aqueous solutions.[10,12,13] The aggregate units may be packed together to form an extended glide or herringbone

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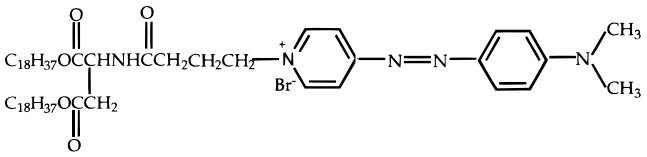
structure, and such a structure has been found by X-ray diffraction (XRD) in single crystal.[17] Recently, the LB film of a carboxyazobenzene compound has been studied by AFM. Fibrous supramolecular assemblies (80 nm wide and 100 μm long) composed of small 20−30 nm 2D clusters have been found.[18] Here, $\pi-\pi$ interaction is an important force to determine the superstructure.

It is well-known that phospholipids can self-organize into vesicles in aqueous solutions.[9] The method of vesicle fusion to prepare supported phospholipid bilayers was also well established,[19,20] but the self-organization from phospholipid LB films to a thermodynamically more stable structure in a different environment was hardly concerned.[21,22] However, this is closely related with the stability of LB films[23] and thus may be important for their application.[1]

To acquire more direct and detailed information on the DDPA membrane structure in air−solid and solid−liquid interfaces, we used AFM to detect the surface morphology of the membranes of DDPA and the mixed films of DDPA and α-L-dipalmitoylphosphatidylcholine (DPPC). In the AFM images, a line-shaped aggregate with a width roughly twice the length of the molecule was found in the LB monolayer deposited on mica. Maintained by strong aromatic interaction, the structure of the aggregate may be similar to the semicylindrical structure of the surfactant absorbed in the graphite−water interface but with the nonpolar tails pointing toward the air. We also found that the LB monolayer of DDPA underwent a self-organization process and turned into a bilayer and vesicles when it was hydrated. From the in situ AFM study of the mixed DDPA and DPPC LB film under aqueous solution, different stabilities of the DDPA and DPPC domains and their different self-organization properties were clearly revealed.

Experiment

The synthesis and identification of DDPA have been described elsewhere.[6] The structure of DDPA is shown as the following:



DPPC, purchased from ACROS (Geel, Belgium), was used without further purification. The chloroform solution of pure DDPA (1.0 mg·mL^{-1}) and an equimolar mixture of DDPA and DPPC (0.05 mmol·L^{-1}) was used in the LB film deposition. Other reagents were analytical grade and used without further purification. Deionized water was used except in the LB film deposition where the deionized water was further treated by an EASY pure RF system (Barnster Co.).

The deposition of LB monolayers was performed on a British NIMA LB 622 trough with the subphase of pure water (pH = 5.6). The subphase temperature was controlled by a HAAKE system (Mell-Technik GmbH a Co., Germany) at 20 ± 1 °C. The chloroform solution of DDPA or a DDPA/DPPC mixture was spread on the subphase by drops. After evaporation of chloroform, the surface was compressed at a speed of 40 cm^2·min^{-1}. The monolayer was transferred onto a freshly cleaved mica at a constant surface pressure of 3, 10, or 30 mN·m^{-1}. The mica substrate was immersed in the subphase and vertically pulled out with a lifting speed of 2 mm·min^{-1} so that the headgroup of the molecule faced the substrate. The transfer ratio was 1.0. For the absorption spectra study, the monolayer was transferred onto a fused silica surface.

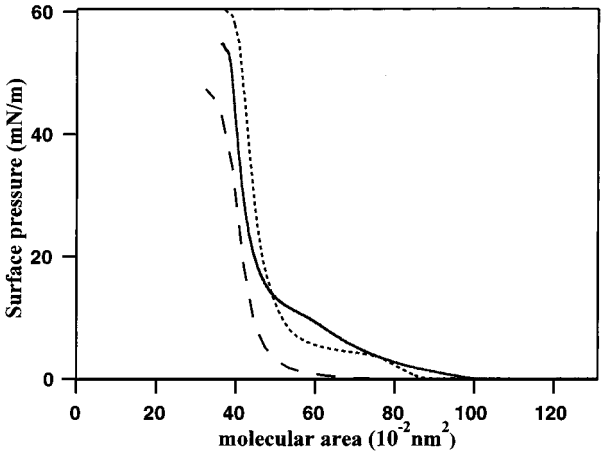


Figure 1. $\pi-A$ isotherm of DDPA (dashed line), DPPC (dotted line), and equimolar DDPA/DPPC (solid line) monolayers.

AFM measurements were performed on a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) equipped with a bioscope G scanner (90 μm). The scanner was calibrated by a 10 μm standard grid with 180 nm deep etch pits. The LB monolayer at the air−solid interface was imaged with the tapping mode, using silicon cantilevers with resonance frequencies of 260−340 kHz. The measurements in the aqueous solution were carried out with the contact mode or tapping mode in a homemade fluid cell using Si_3N_4 cantilevers with spring constants of 0.06, 0.12, and 0.32 N·m^{-1}. The water immersion process of DDPA LB films was performed in the AFM fluid cell directly. When the tapping mode is used in the solution, the driving frequency was around 20 kHz. The typical scan speed was 2 Hz.

The absorption spectra of the DDPA solution and DDPA LB film were taken with a Shimadzu UV-310 UV−vis−Nik recording spectrophotometer.

Result and Analysis

***π*−*A* Isotherm of DDPA and DDPA/DPPC Monolayer at an Air−Water Interface.** The pressure−area isotherms of DDPA,[6] DPPC, and mixed equimolar DDPA/DPPC monolayers are presented in Figure 1. The isotherm of DDPA shows that DDPA is in the gel phase at ambient temperature. The limit molecular area, which is obtained by extrapolating the steep curve to $\pi = 0$, is 0.46 nm^2, which indicates a "stand up" arrangement of the headgroups. This result is consistent with the average headgroup orientational angle to the surface normal ($\theta = 37.8 \pm 0.4°$) measured by SHG.[6] The deviation of the area of the mixture from the average area of DDPA and DPPC at low surface pressure indicates some repulsive interaction[31] between DDPA and DPPC.

DDPA Monolayers in Air. Figure 2a shows the typical tapping mode image of the DDPA monolayer transferred at the surface pressure of 10 mN·m^{-1}. The LB monolayer consists of many long (several microns) line-shaped aggregates. The curved lines do not show clear directional preference to the lattice of mica or LB deposition direction.[14,15] This indicates that the aggregates were not produced during the transformation but formed at the air−water interface. On the basis of the images of different samples, the line structure covers about 75% of the surface. From the more magnified image which is presented in

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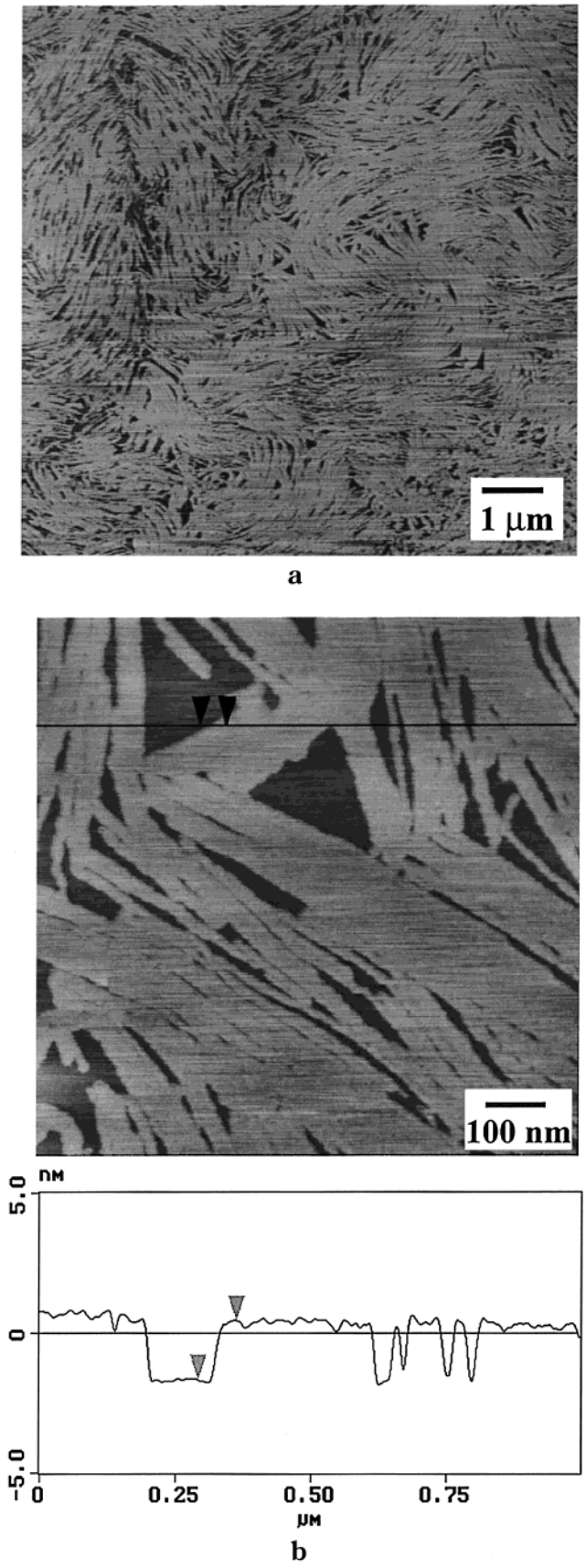

Figure 2. Typical tapping mode AFM images of the DDPA LB monolayer deposited on mica at a surface pressure of 10 mN·m^{-1}. The height scale of both images is 10 nm. (a) Larger scale image. (b) Magnified image and the cross section of the center part of part a. Along the line the cross section is shown.

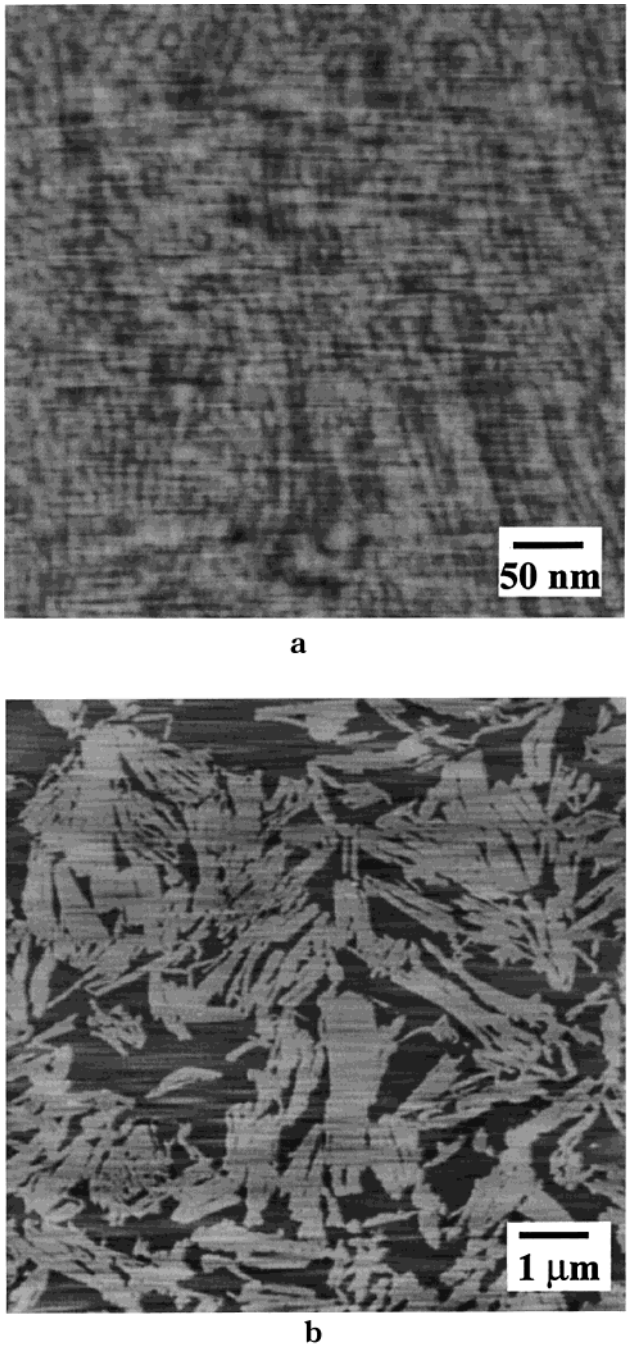

Figure 3. (a) Line-shaped aggregates of the LB monolayer of DDPA deposited on mica at a surface pressure of 30 mN·m^{-1} acquired by tapping mode AFM and 5 nm height scale. (b) Tapping mode AFM image of the LB monolayer of DDPA deposited on mica at a surface pressure of 3 mN·m^{-1} and 10 nm height scale.

Figure 2b, the wide line seen in Figure 2a was actually composed of many narrow lines. The measured width of the narrow lines is 13.2 ± 1.5 nm (average of 49 measurements). The actual width may be smaller, because the tip-broadening effect may exist.[24] The measured height of the aggregates is 2.1 ± 0.1 nm (average of 95 measurements).

At 30 mN·m^{-1} surface pressure, DDPA molecules were assembled into a flat monolayer, with only a few defects. The surface occupied by the solid-phase film exceeds 95% of the total surface area. The height difference between

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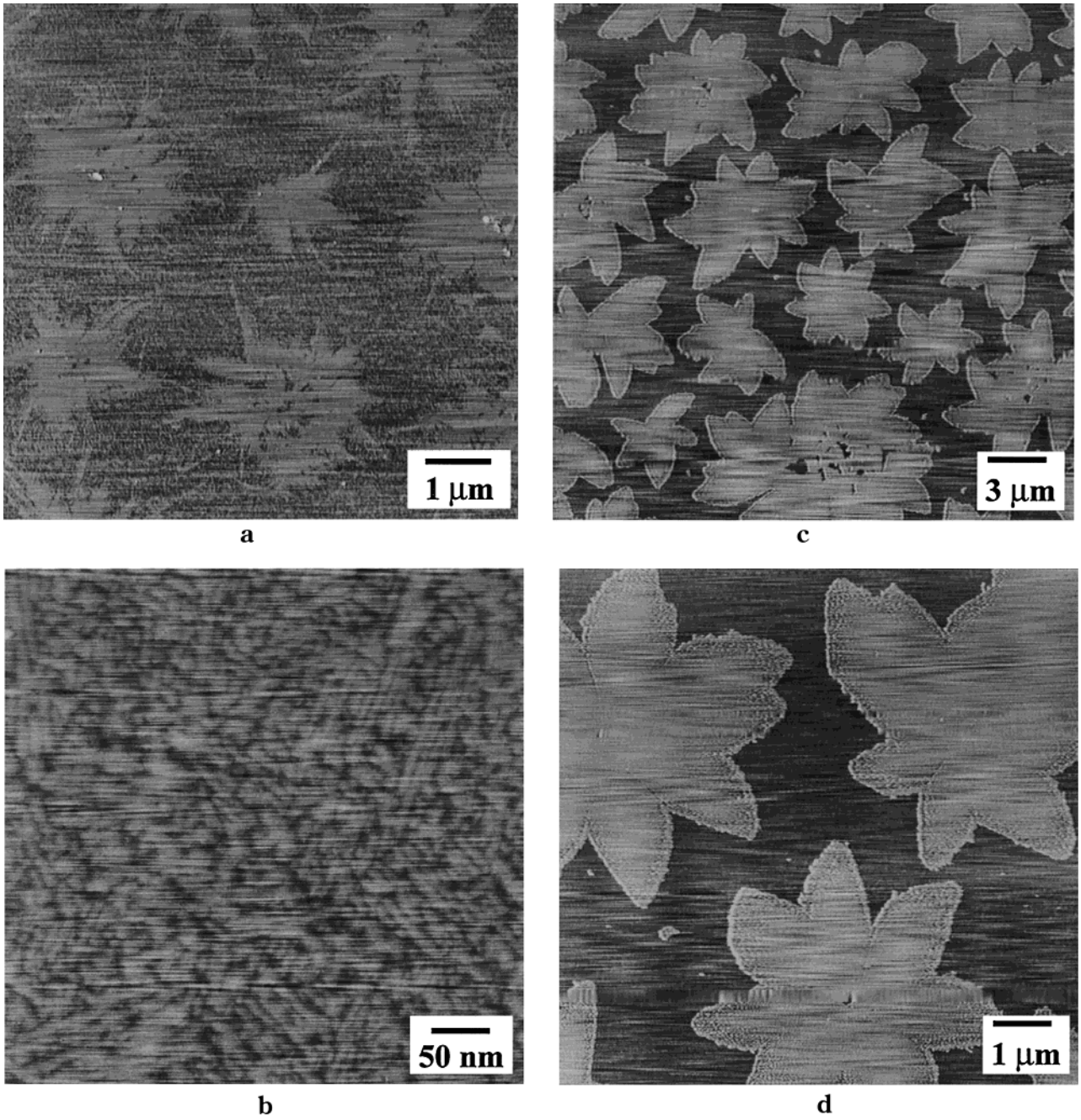


Figure 4. DDPA/DPPC (1:1) mixed monolayer. The height scale in all images is 10 nm. (a) Image of a 30 $mN \cdot m^{-1}$ monolayer. The DPPC-rich phase was leaf-shaped but with an irregular boundary. The surface of the matrix region was seen as more corrugated than that of a leaf-shaped domain. (b) Line-shaped aggregates found in the DDPA-rich area. (c) Image of the 10 $mN \cdot m^{-1}$ monolayer. The boundary of the domains was more regular. The sizes of the domains in both monolayers of 30 and 10 $mN \cdot m^{-1}$ varied, ranging from several microns to over 10 μm. (d) More magnified image of part of Figure 3c.

the monolayer and defect is 2.2 ± 0.2 nm (average of 25 measurements), consistent with the membrane thickness measured in the case of 10 $mN \cdot m^{-1}$. At a high magnification image, the line-shaped structure can also be seen as shown in Figure 3a, where because the scan size of the image is small, the lines are aligned in one direction. In larger scale, it can be seen that the aggregates orient themselves in all directions. The measured width of the aggregate is 10.1 ± 1.3 nm (average of 55 measurements). Because the line-shaped aggregates were more closely packed, the tip-broadening effect here was not as serious as that in the film deposited at 10 $mN \cdot m^{-1}$. We have also investigated the DDPA LB film deposited at low (3 $mN \cdot m^{-1}$) surface pressure, which is shown in Figure 3b. It can be seen that line-shaped aggregates were already formed at this surface pressure. The surface coverage of the aggregates is around 60%.

Because the nominal molecular areas at 10 (0.44 nm^2) and 3 $mN \cdot m^{-1}$ (0.50 nm^2) are only a little larger than that at 30 $mN \cdot m^{-1}$ (0.40 nm^2), some molecules might exist in the liquid expand (LE) phase occupying the darker region in the AFM image. It can be calculated from the surface coverage and molecular area in different surface pressures that the molecular area in the LE phase is about twice the molecular area in the solid phase.

DDPA/DPPC Mixed Monolayer in Air. From the tapping mode AFM image of the 1:1 DDPA/DPPC LB monolayer (30 $mN \cdot m^{-1}$) shown in Figure 4a, separated phases were obviously seen. We assign the leaflike domains to the DPPC-rich phase and the rest of the region

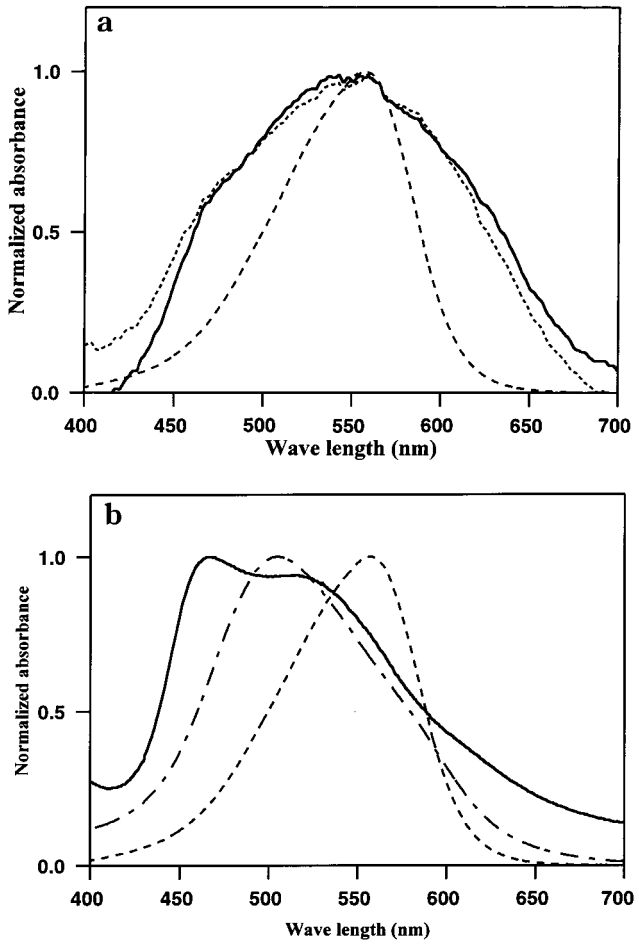


Figure 5. Absorption spectra of DDPA in solutions and on surfaces. (a) Normalized absorption spectra of a DDPA chloroform solution (0.02 mg·mL^{-1}, dash line) and a LB monolayer on hydrophilic quartz at 3 (dotted line) and 30 mN·m^{-1} (solid line). The absolute absorbances of LB monolayers with different surface pressures are very close. (b) Normalized absorption spectra of a DDPA chloroform solution (0.02 mg·mL^{-1}, dashed line) and an aqueous solution of pure DDPA vesicles (0.02 mg·mL^{-1}, solid line) and vesicles of a 1:10 molar DDPA/DPPC mixture (with 0.02 mg·mL^{-1} DDPA, dotted and dashed lines).

to the DDPA-rich phase, because of the following reasons. Previous AFM studies showed that the solid phase domain of DPPC has a similar shape.[32] The leaflike domain is nearly 1 nm higher than the matrix, and the thickness of the DPPC monolayer (2.8 nm)[33] is also a little higher than that of DDPA (2.2 nm). In the matrix region, characteristic line-shaped aggregates were found, as shown in a high-resolution image (Figure 4b), although the length of the aggregates was far shorter than that in the pure DDPA monolayer. The measured width of these line aggregates was 8.2 ± 1.2 nm (average of 60 measurements), close to twice the length of the DDPA molecule. Figure 4c shows the image of the mixed LB film under a surface pressure of 10 mN·m^{-1}. The leaflike domain is more clearly seen, higher than the matrix region by about 2 nm. Except for some indication of the aggregate formation on the edge of the leaflike domain, as shown in Figure 4d which is the expanded view of Figure 4c, no line-shaped aggregates were found. According to the pressure−area isotherm, solid and liquid phases coexisted under this pressure. The lower region must be in the liquid phase, for no line-shaped aggregates were found.

Visually, the area of the leaflike domain in Figure 4c is larger than that in Figure 4a. Our explanation is that, on the edge of the leaflike domain of Figure 4c, the aggregates are actually composed of the DDPA-rich solid phase instead of the DPPC-rich solid phase, as is indicated by Figure 4d.

Spectroscopic Evidence of the DDPA Aggregate. Figure 5a shows the absorption spectra of the DDPA chloroform solution and the DDPA LB films deposited on a fused silica surface at surface pressures of 30 and 3 mN·m^{-1}. In the chloroform solution, the absorption maximum was at 557 nm. The spectra of the LB films transferred at different pressures were nearly the same. Both have a broader absorption band whose absorption maximum was at 550 nm. In both broadened absorption bands, a blue-shifted absorption band at 470 nm and a red-shifted absorption band at about 590 nm exist as shoulders of the absorption. It is unlikely that the broadening was caused by the heterogeneous environment of the molecules in the LB films, because their spectra at different surface pressures were nearly the same. However, it can be explained by the interaction between the

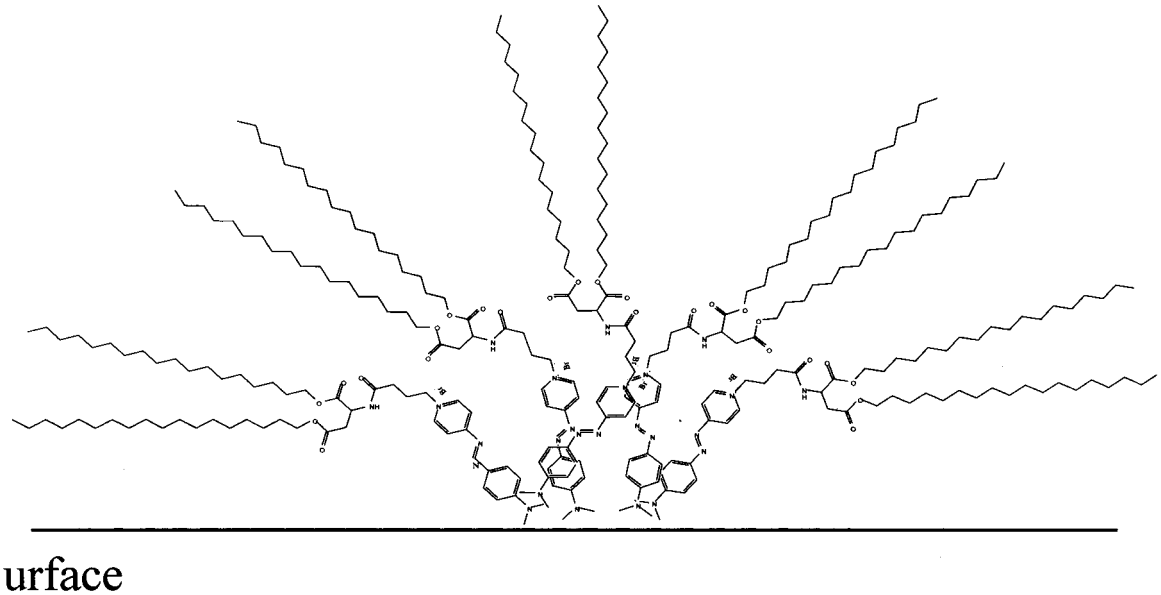


Figure 6. Schematic cross-sectional structure of a DDPA semicylindrical aggregate. The headgroups were bound to the substrate. The force to associate the amphiphiles in the axial direction was mainly aromatic interaction.

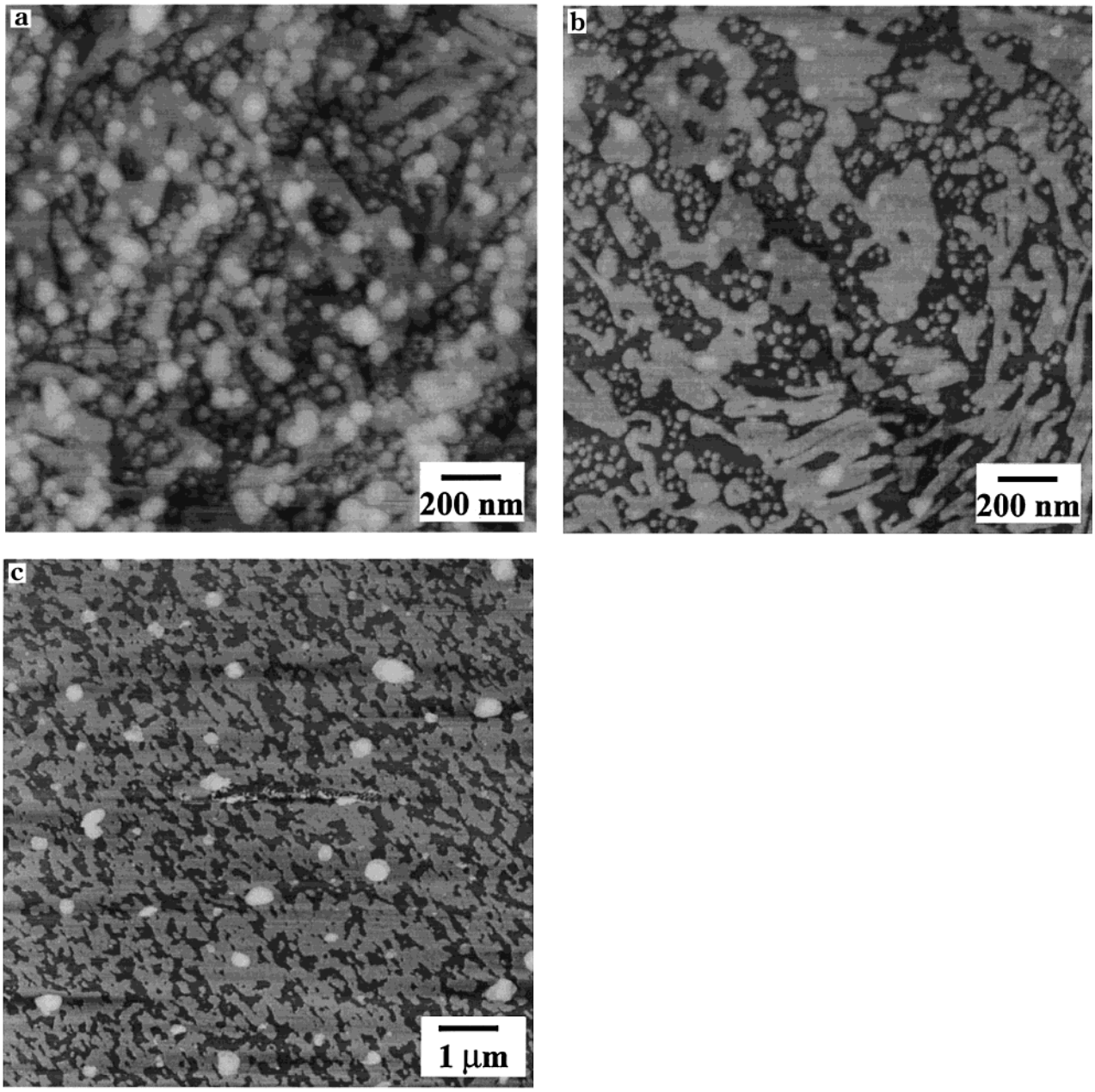
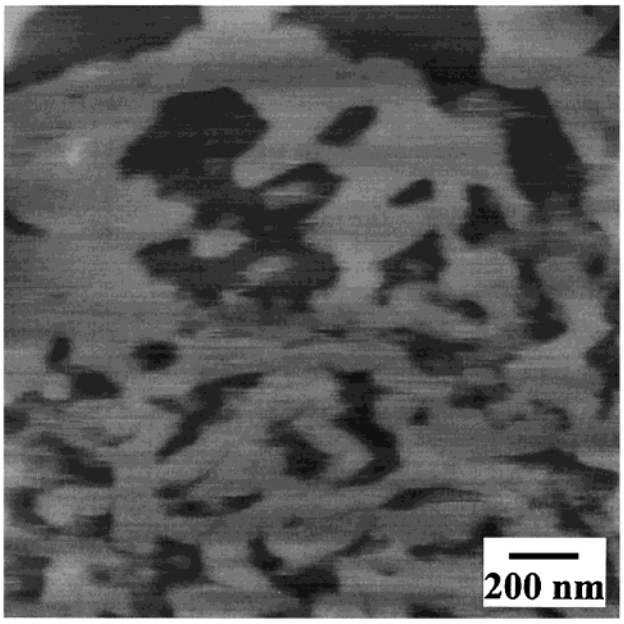


Figure 7. Tapping mode image of a DDPA bilayer under water. The height scale in all images equals 50 nm. (a) Image captured immediately after engagement. Vesicles can be clearly seen. (b) Image obtained after 30 min of scanning. Vesicles had been turned into bilayers. (c) Bilayer after heating; there were larger bilayer patches and the surface coverage changed little.

oblique packed chromophores in the aggregates formed in the LB films, which will cause splitting of the absorption band, according to the exciton theory.[25,26] The aggregates can be classified as H-character aggregates because the blue shifts were more obvious.

The absorption spectra of DDPA in an aqueous solution were also taken. The solubility of DDPA in water is very low; we prepared the vesicle solution of DDPA and a 1:10 (molar) DDPA/DPPC mixture by sonication of the suspension of the corresponding lipids with water.[20] Figure 5b presents the spectra in an aqueous solution, along with the spectra in $CHCl_3$. The mixture of DDPA with excess DPPC solution had one absorption band at 505 nm in the visible region. The DDPA/water solution had absorption bands at 513 and 465 nm. The blue-shifted band indicated that H-type aggregates were also formed in the aqueous solution.[12,13,25−27]

Proposed Structure of the Line-Shaped Aggregate. From the AFM images of the pure DDPA and DDPA/DPPC monolayers at the air−mica interface, we found a line-shaped aggregate with the least measured width of only 8.2 nm, nearly twice the length of the DDPA molecule. Because of the tip-broadening effect of AFM, the actually width may be smaller. In previous AFM studies of surfactant hemimicelle absorbed on the hydrophobic surface, a semicylindrical structure was found.[14,15] Amphiphiles bearing aromatics similar to DDPA were also studied,[12,13,17] and an extended glide structure was found.[17] On the basis of the similar structure found by AFM investigation and the spectroscopic evidence, we propose a similar structure shown in Figure 6: The headgroups were assembled by aromatic interaction to form a one-dimensional ordered array with the alkyl tails pointing outward from the polar surface (water or mica surface); thus, the amphiphile molecules were organized into



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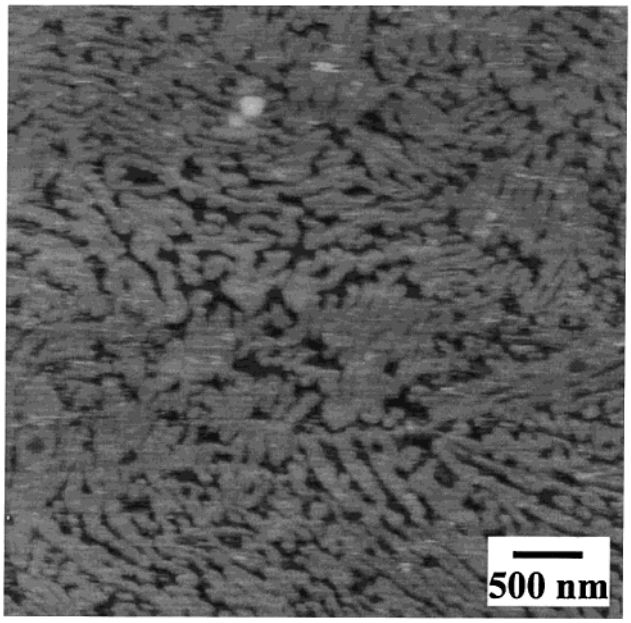

b

Figure 8. Contact mode images of a DDPA bilayer reorganized by a LB monolayer under water. (a) Bilayer image acquired with an applied force of about 1 nN and 50 nm height scale. (b) Line-shaped bilayer of DDPA image acquired with an applied force of about 1 nN and 50 nm height scale.

semicylindrical aggregates with structure comparable to that formed by surfactant in the graphite−water interface. The alkyl tails of DDPA in the semicylindrical structure do not necessarily stick up because they were not packed closely. Therefore, the measured height of the aggregates was less than the length of the DDPA molecule (3.4 nm), as estimated by the space-filling model.

Self-Organization of DDPA under Water. Biological samples can be easily deformed by the AFM tip. If there are vesicles absorbed on the membrane or mica surface, the pressure exerted by the tip with a low loading force (about 200 pN) can press them into bilayers.[28] To minimize the force exerted on the sample, we used the tapping mode to image the DDPA membrane under water. The effective loading force can be lower than 200 pN in this imaging mode.[29] Adding some electrolyte can reduce the repulsive electrostatic force between the tip and the surface.[30] Thus, an alternative method to reduce the imaging force is applying contact mode imaging in the presence of some electrolyte.

After the DDPA LB monolayer deposited at 30 $mN \cdot m^{-1}$ was immersed into water for about 30 min to reach thermal equilibrium, a surprising structural change was observed. Figure 7a shows the tapping mode image obtained immediately after engagement. Much thicker (about 8 nm) membranes along with spherical structures were clearly discerned, suggesting that the DDPA monolayer on mica self-organized into bilayer and vesicles. Such a kind of image can only be obtained under optimal conditions at an early stage of scanning because the vesicles are easily fused into bilayers by the AFM tip even using tapping mode imaging in fluid. Figure 7b shows an image obtained after scanning for 30 min. The measured thickness of the bilayer is 8.9 ± 0.4 nm (average of 100 measurements). After the sample was heated in water to 70 °C, which is above the phase transition temperature of DDPA (67.4 °C),[6] for about an hour, a flat bilayer was seen as shown in Figure 7c, with some large vesicles perhaps formed during heating. The measured thickness is 7.2 ± 0.3 nm (average of 110 measurements), significantly lower than the thickness before heating. This thickness reduction might arise from the removal in the heating process of the water molecules which are trapped between two layers during the self-organization. Thus, the thickness of the DDPA bilayer is close to that of phospholipid with the same length of the acyl chain α-L-distearoylphosphatidylcholine (DSPC, 6.9 ± 0.5 nm).[20] In all of the image acquired under water, the surface coverage of the bilayer was about 70%.

We have also imaged the DDPA membrane under water with the contact mode. A typical image obtained with an applied force of about 1 nN is presented in Figure 8a, where no vesicle can be seen. The quality of this contact mode image is not quite good. A few membranes were found to be swept away by the tip, found from larger scale scanning. It was also found that the measured height of the bilayer changed substantially with the applied force. Figure 9 shows the relationship of the measured height with the applied force, in which each datum was obtained by averaging about 100 measurements. If low force was applied, the measured height was close to that obtained by tapping mode imaging. We noted that when the applied force exceeded 20 nN, the membrane was swept away, and the quality of the acquired image was not acceptable. This compressibility was far larger than that of common phospholipid monolayers.[27]

In some other region, different aggregates were found. Figure 8b shows these structures. The measured height

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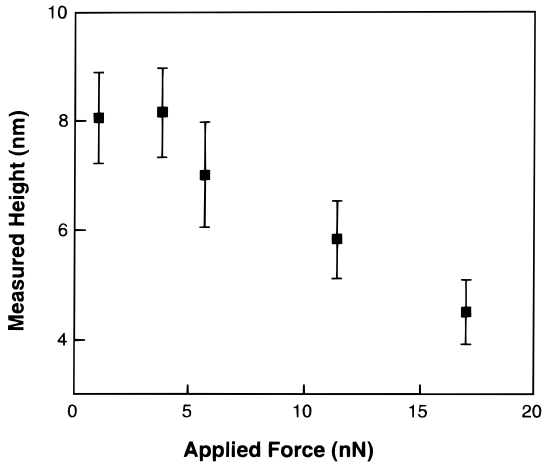


Figure 9. Relationship of the measured height of the DDPA bilayer with applied forces. The bilayer was compressed from 8 to about 5 nm by increasing the imaging force.

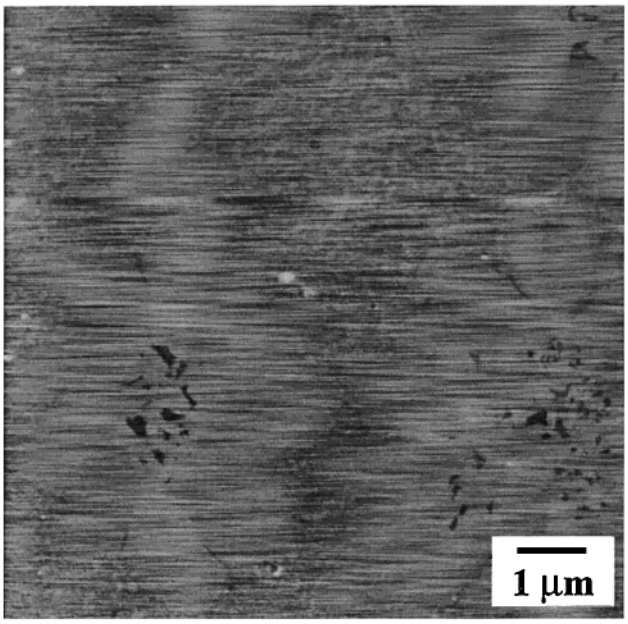
was about 8 nm. The aggregates extended over 1 μm, with the widths ranging from 50 to 100 nm. These structures were probably formed by the hydration of the previous line-shaped aggregates at the air−mica interface.

The DDPA bilayer is stable at ambient temperature; no significant structural change was observed after storing it in water for 72 h. The stability was also checked by replacing deionized water by phosphate buffer (40 mmol·L^{-1}, pH = 7.4). The phenomenon observed here is different from that of a phospholipid monolayer. We have prepared the LB monolayer of DPPC at a surface pressure of 20 mN·m^{-1} and found that the monolayer remained intact when we imaged it under water.

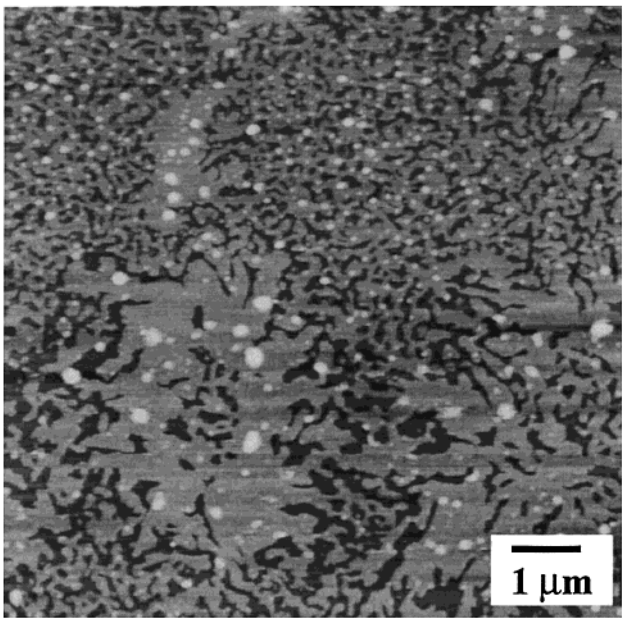
Self-Organization of a DDPA/DPPC Mixed Film. The self-organization property of the mixed LB film in an aqueous solution was also studied by in situ AFM. Figure 10a shows the contact mode image of the film transferred at 30 mN·m^{-1}. Figure 10b shows the contact mode image of the same place under a 100 mmol·L^{-1} NaCl solution, taken at 30 min after the addition of the NaCl solution. It can be seen from Figure 10b that the border and the shape of the two domains in Figure 10a was roughly maintained even after the self-organization. An interconnecting line-shape structure between the leaf domains can be seen, like that in a pure DDPA bilayer. Vesicles were also seen on both leaf domains and the line-shape region. The measured height of the bilayer was 7.6 ± 0.5 nm (average of 93 measurements), lying in the median of the thickness of supported DDPA and DPPC bilayers.[20] The result above again suggests different self-organization properties of DPPC and DDPA LB monolayers.

Discussion

In our experiments, a line-shaped aggregate of DDPA was revealed. It is not likely a substrate-induced structure,[14,15,35] because the aggregates did not show a clear orientational preference to the substrate lattice. We believe that the formation of the line structure was mainly driven by $\pi-\pi$ interaction, because amphiphiles bearing a similar chromophore can form a one-dimensional ordered structure,[17] yet common phospholipids do not form this kind of aggregate. Absorption spectra of both the LB film and aqueous solution of DDPA showed evidence of aromatic interaction. The much higher (about 15 °C higher) phase transition temperature of DDPA[6] compared with those of



a



b

Figure 10. In situ image of a 30 mN·m^{-1} DDPA/DPPC monolayer in the air−mica and water−mica interface. (a) Contact mode image in air and 10 nm height scale. (b) Contact mode image in solution and 50 nm height scale. The stable imaging force was about 1 nN.

common phospholipids with the same alkyl chain[34] is also an indication of a strong $\pi-\pi$ interaction. The packing mode of chromophores may be similar to that in the literature,[10,17] though the exact structure remains to be confirmed by further experiments. The observation that the line-shaped aggregates were formed at low surface pressure (3 mN·m^{-1}) by AFM imaging and by the absorption spectra indicates that these aggregates may be formed at the air−water interface even before compression, as previously found.[10,18]

The basic superstructure of amphiphile aggregates depends to a great extent on the relative sizes of the headgroups and nonpolar tails.[9] In the aqueous solution, phospholipids have nearly equal sizes of headgroups and

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nonpolar tails,[9] so that they tend to form a lamellar structure. At first sight, it is hard for DDPA to form a semicylindrical structure. However, such a structure is possible for two reasons. First, the strong aromatic interaction forces the headgroups to arrange in a specific linear way.[10,17] Second, the headgroup of DDPA in the LB monolayer may not be fully hydrated as in the aqueous solution (see explanation below), and therefore the headgroup size is less than that in the aqueous solution.

The surface coverage of the DDPA bilayer should be 48%, if the nominal molecular area remains unchanged after the self-organization. The increase of nominal molecular area (as the surface coverage was actually 70%) indicates the increase of the headgroup size. The increase of the headgroup size may come from the intimate hydration layer and the weakening of the close aromatic packing because of the competition between the hydration and $\pi-\pi$ interaction. Calculated from the surface coverage and $\pi-$area isotherm, the molecular area of DDPA in the bilayer is about 0.6 nm^2, which is much larger than the area occupied by two closely packing alkyl chains.[36] Therefore, the alkyl tails have more fluidity than expected, so that the DDPA bilayer shows better compressibility than the common phospholipid monolayer.

Different self-organization properties of the DPPC and DDPA LB monolayers were clearly seen from the hydration experiments of pure and mixed films. The packing of the nonpolar tails may account for the difference. In the DPPC LB monolayer, the alkyl chains are so closely packed that water molecules cannot easily get access to the hydratable headgroups underneath. Consequently, the DPPC LB monolayer is relatively stable under water, while it would be readily destroyed in the presence of organic solvents. If the DDPA LB monolayer adopts the semicylindrical arrangement proposed above, it is impossible for the alkyl tails to pack themselves very closely. Water molecules can easily penetrate the hydrophobic surface and complete the hydration of the headgroups, so that the reorganization can occur. Also considering the extremely low solubility of DDPA in water, the self-organization may take place in the local area. During the organization process, it is possible that some water molecules are trapped between the two layers, and the bilayer formed in this way is thicker than expected.

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