Reconstitution of Phospholipid Bilayer by an Atomic Force Microscope Tip

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In a recent paper, Carlson et al. (1997) reported the use of the atomic force microscope (AFM) to initiate bilayer fusion in a controlled manner. In their study of reconstituted high-density lipoproteins (HDL) they obtained discoidal bilayers of dipalmitoyl phosphatidylcholine (DPPC) and apolipoprotein A-I (apoA-I), the primary protein component of HDL. Under certain experimental conditions the fusion of the discoidal bilayers into a planar bilayer was induced by the scanning process in the AFM. In other cases, scanning of the HDL particles resulted in digging a monolayer-thick hole. The difference between fusion and digging did not appear to depend on force or scan rate. It was not clear whether protein played a role in the fusion process.

Independently, we observed a similar effect on a phospholipid bilayer in the absence of protein. The results were first obtained on a control sample used in our study of the interaction of the polyene antibiotic filipin with planar bilayers (Santos et al., 1998). The bilayer of 7:3 (w:w) dipalmitoyl phosphatidylethanolamine (DPPE):cholesterol was prepared by Langmuir-Blodgett deposition on freshly cleaved mica at a surface pressure of 25 mN/m. The sample was then imaged by contact-mode AFM under water. Silicon nitride tips with a nominal spring constant of 0.12 N/m (Digital Instruments, Santa Barbara, CA) were used. The bilayer was unintentionally exposed to air for a moment during transfer to the fluid cell of the AFM. The short exposure to air probably led to the disruption of the bilayer into monolayer and trilayer domains; subsequent respreading led to a highly defective bilayer.

Fig. 1 *a* shows the AFM image of the respread bilayer. The bilayer contains numerous holes with a depth of about 2.5 nm, which is consistent with the thickness of a monolayer. A 5×5 μm^2 square region in the central area of the image was generated by scanning at a force of 20 nN for 2 minutes, then imaged by increasing the scan area and immediately recording the image at a force of 5 nN. The scanning process heals the defective bilayer and produces a flat hole-free area. Fig. 1 *b* displays the AFM image of another DPPE/cholesterol sample prepared under the same conditions as the previous sample with an air exposure of 2 s. Monolayer patches are clearly seen within holes, which are also one layer thick. We could not produce a hole-free area like in Fig. 1 *a* by applying a higher force, although fusion of patches could be induced. Fig. 1 *c* shows the same area as in Fig. 1 *b* after 10 min of scanning with a force of 25 nN and a scan rate of 10 Hz. Some patches fused with their nearest neighbors. Applying a higher force did not accelerate fusion and eventually led to the rupture and removal of the bilayer.

Exposure to air was not necessary to induce the healing of holes. Fig. 2 *a* shows a pure DPPE bilayer deposited at a surface pressure of 25 mN/m. The sample was kept under water during transfer to the AFM. A flat hole-free square region in the center of the image was generated by scanning for 2 min with a force of 100 nN, then imaged by increasing the scan size and recording the image at a force of 5 nN. Note that such healing could be induced only for samples with monolayer-thick holes. It is not clear why Langmuir-Blodgett deposition sometimes produces monolayer-thick holes in DPPE bilayers. It is much more common to have bilayer-thick holes in bilayers (Mou et al., 1995, Shao et al., 1996) although monolayer-thick holes have also been observed (Hui et al., 1995). In the samples with bilayer-thick holes we did not observe any healing. Applying a higher force led to the coalescence of nearby holes and then to removal of the bilayer.

The mechanism of the tip-induced reconstitution is not clear although it is logical that energy brought by the AFM tip helps to convert unstable holes or disks into a lower-energy bilayer. One possible explanation is that the AFM tip drags lipid molecules that are loosely attached or extracted from bilayer until they fill in holes in bilayer. These molecules might also adsorb on the AFM tip during this process, forming a layer on it. Fig. 2 *b* shows an AFM image of a hole in a DPPE/cholesterol bilayer. The hole appears to be one layer thick and to contain another monolayer-thick hole inside. However, it is clearly seen that the lower edges of both holes have exactly the same shape, which is a very unlikely event. Such an image might be generated if the hole were two layers thick and the AFM tip had an adsorbed monolayer on it. As the substrate step is deeper than the step on the AFM tip, the tip cannot reach the bottom of the hole and the hole edge is imaged twice, once with the tip itself and once with monolayer on it (double tip effect). Thus, the image in Fig. 2 *b* suggests that lipids can adsorb to the AFM tip during the scanning of bilayers in water.

Our results show that patterning similar to that shown by Carlson et al. (1997) can be obtained on samples without

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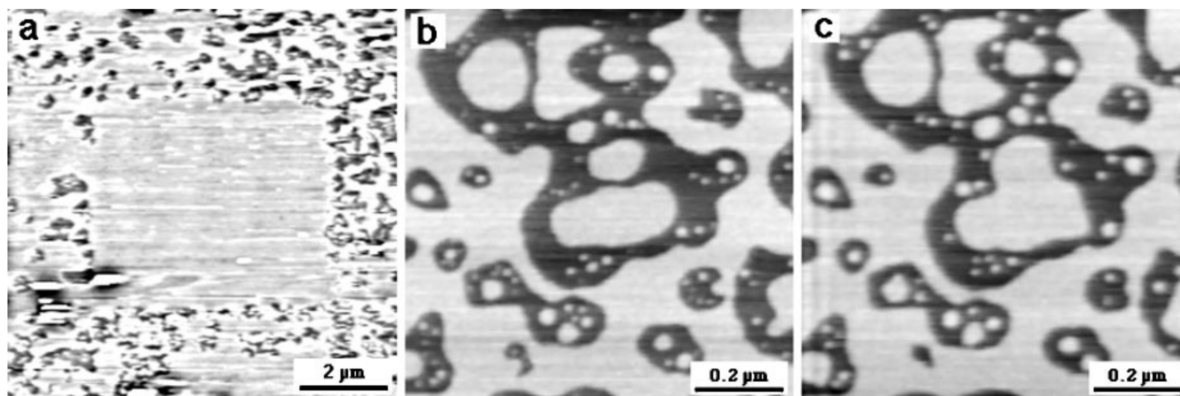

FIGURE 1 AFM height images of a DPPE/cholesterol bilayer which was exposed to air for a short time. All images are taken in a contact mode under water. (*a*) Area: 9 × 9 μm^2. A square defect-free region in the central part of the image was reconstituted by the AFM tip during several scannings at a force of 20 nN. (*b*) Area: 900 × 900 nm^2. Monolayer-thick patches within holes are clearly seen. (*c*) Area: 900 × 900 nm^2, the same region as in (*b*) after scanning at 25 nN for 10 minutes. Some patches fused with their nearest neighbors.

protein. We could not establish the precise conditions necessary for patterning but have shown that it is crucial to have a bilayer with monolayer-thick holes. While additional work will be necessary before the mechanism of patterning is fully understood, the effect opens new possibilities for manipulation of phospholipid bilayers and formation of nanoscopic defect-free regions with a desired size and shape.

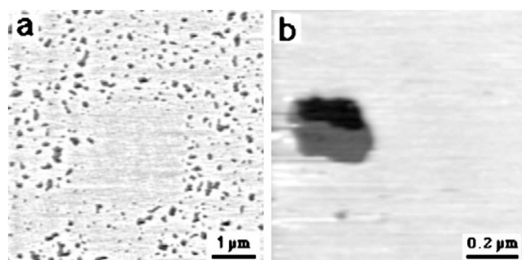

FIGURE 2 (*a*) AFM height image of a DPPE bilayer under water. A square defect-free region in the central part of the image was reconstituted by the AFM tip after scanning for 2 minutes at a force of 200 nN. Area: 6 × 6 μm^2. (*b*) AFM image of a hole in a DPPE/cholesterol bilayer. Note that the lower edges of the hole have the same shape (double tip effect). Area: 1 × 1 μm^2.

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