Modification of supported lipid membranes by atomic force microscopy

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ABSTRACT The atomic force microscope (AFM) was used to structurally modify supported lipid bilayers in a controlled quantitative manner. By increasing the force applied by the AFM tip, lipid was removed from the scanned area, leaving a cut through the lipid bilayer. Cuts were repaired with the AFM by scanning the region with a controlled force and driving lipid back into the cut. A slow self-annealing of cuts was also observed.

INTRODUCTION

The atomic force microscope (AFM) has proven to be an effective tool for the nondestructive imaging of biological specimens, often with sub-nanometer resolution. The most powerful attribute of the AFM, however, may be its ability to apply a known force to a material and then to observe the effect. The AFM can be used as a nano-indenter to observe the elastic and plastic response of a material to an applied normal force or to cut, tear, scratch, or study friction with an applied lateral force (1–7). These approaches are unprecedented in their ability to elucidate materials properties at the nanometer scale. Although there are many reports describing surface modification by the scanning tunneling microscope (STM), few similar modifications have been reported for the atomic force microscope (AFM), especially for biologically relevant materials (9–12). In this paper we present results showing the modification of supported membrane bilayers by the atomic force microscope. Specifically, the AFM was used to image, cut, or anneal the bilayer, based on the amount of force applied by the tip. Cuts through the bilayer could be repaired within minutes by scanning the region with a controlled force on the AFM tip. A slow partial self-annealing of unperturbed cuts was also observed. To our knowledge, this is the first demonstration in which an atomic force microscope has been used in a quantitatively controlled manner to write and erase a pattern in a substrate.

METHOD

Supported lipid membranes are of interest both as model substrates for membrane bound proteins and for their material properties as liquid crystals. Several groups have successfully imaged supported membrane systems, including membrane fragments (13, 14), Langmuir–Blodgett films (14–19), and collapsed vesicles (20). We have formed supported lipid membranes by the adsorption of self-assembled lipid tubules onto a graphite substrate. The formation of self-assembled lipid tubules from diacetylenic phospholipids was first discovered in 1984. The tubules, which are hollow cylinders made of lipid bilayer, are formed by slowly cooling a lipid–solvent dispersion through the main transition temperature of the lipid (21). The dimensions and number of bilayers in the tube wall vary, depending on the starting material and conditions. We chose to image a sample grown from 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycerol-3-phosphocholine ($DC_{8,9}PC$) in methanol/water. The majority of tubules prepared in this manner have single bilayer walls, average diameters of approximately 0.5 micron, and lengths of tens of microns (B. R. Ratna et al., submitted for publication). The diacetylenic groups of the acyl chains of $DC_{8,9}PC$ can be polymerized using ultraviolet radiation. Linear dichroism studies of these polymerized single bilayer tubules suggests that the polymerization direction is along the length of the tubule. Samples were prepared by depositing several microliters of solution containing either polymerized or unpolymerized tubules onto freshly cleaved highly oriented pyrolytic graphite, and allowing the solvent to evaporate. Images were taken with a NANOSCOPE III AFM (Digital Instruments, Inc., Santa Barbara, California) using microfabricated silicon nitride cantilevers.[1] All images were taken in air in the constant force mode. Typically, an imaging force of less than 10 nN was used.

RESULTS

The AFM image in Fig. 1 shows several unpolymerized tubules which have fully collapsed on the graphite substrate. Several tubes are seen overlapping in the lower right-hand corner of the image. The tubules conform to the substrate, such that features, like graphite steps, can be seen underneath the lipid. In the image each bilayer can be seen, as well as areas where the edges of the tube were "broken" during adsorption to produce ragged edges. In all figures, image processing consisted of a correction to account for sample tilt. A cross sectional profile through one of the tubes revealed that the tubules have completely collapsed to form a film two bilayers thick. The height of a single bilayer was measured by AFM to be approximately 61 Å and the thickness of the two stacked bilayers was measured to be approximately 122 Å. This is in good agreement (within 5.5%) with the 57.8 Å bilayer thickness measured by x-ray diffraction for dry $DC_{8,9}PC$ (22).[2]

The pressure at the contact area in these experiments is large (>10 atmospheres) and yet we observed no dam-

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[1] Microfabricated silicon nitride cantilevers with a calculated spring constant of 0.06 N/m were used.

[2] Since the AFM measurements were made while working at ~50% relative humidity, there is expected to be some swelling of the bilayer with respect to the dry x-ray sample.

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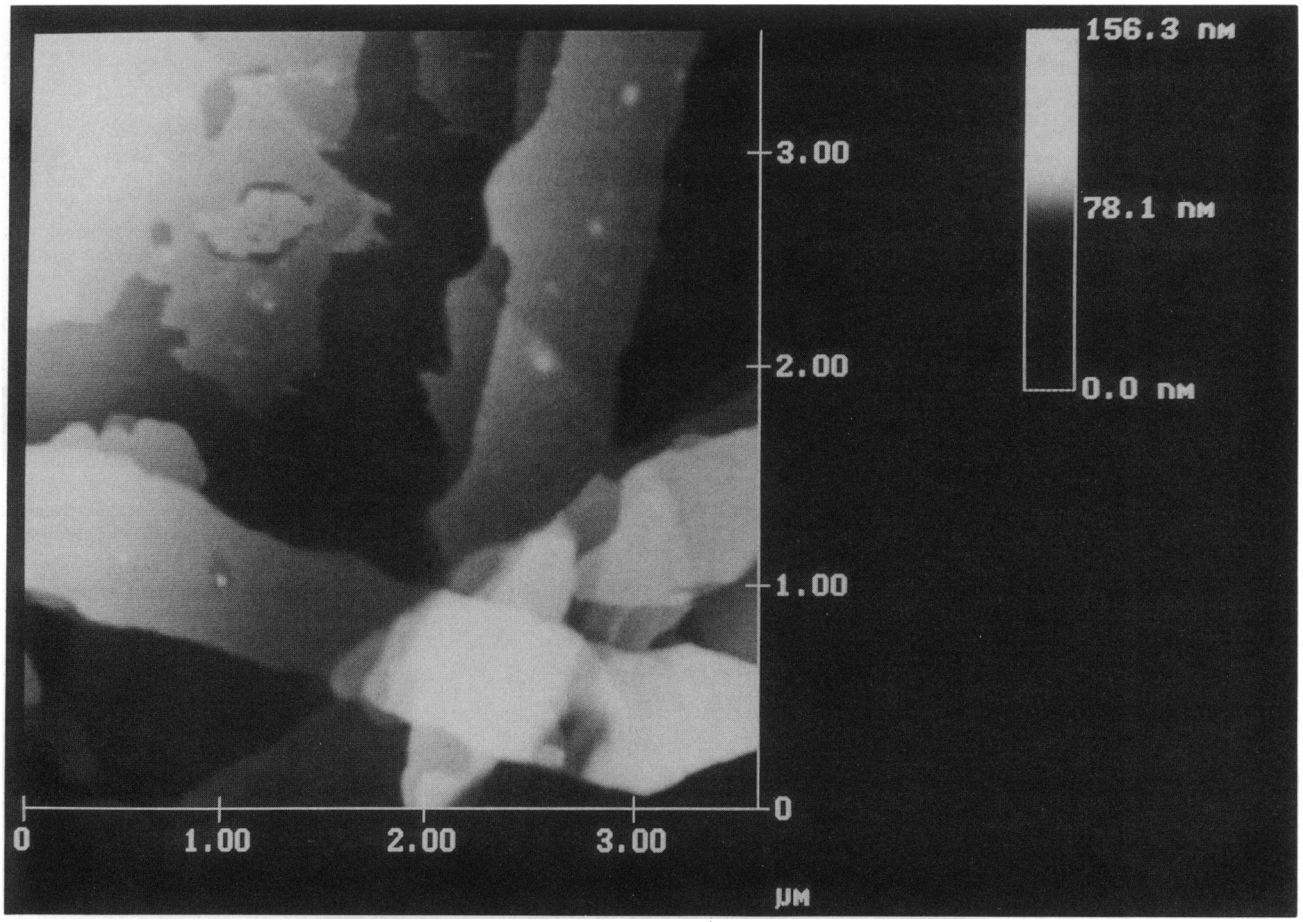

FIGURE 1 The image is a 3.5 × 3.5 μm AFM scan showing several unpolymerized lipid tubules, which have collapsed on the substrate to form flat lipid layers.

age to the adsorbed lipid bilayers, even after repeated scanning. To determine if the lipid bilayer could be damaged or "cut" by the tip, the AFM was continuously scanned in a single line across the width of an unpolymerized tube. The force applied by the tip was slowly increased until the first signs of lipid removal were observed at the edge of the bilayer. The scanning force was maintained at this "damage threshold" until all material was removed from the scan line. This required approximately 2,500 scans across the tubule's width to complete the cut. Fig. 2 *A* shows an unmodified section of an adsorbed tubule before a cut. Fig. 2 *B* shows the same tubule after a 75 nm wide cut was made through the lipid bilayer. The width of the cut is dependent upon several factors including the tip shape, the thickness of the lipid layer, and thermal drift during the cutting process. We found there was a distinct damage threshold of 12.9 ± 1.0 nN required to cut the tubules.[3] Any applied force below this threshold did not result in lipid removal, while any increase in the force above the threshold rapidly accelerated the cutting procedure. Additional cuts were made at different angles to the tube's long axis but no dependence of the threshold cutting force on the cutting direction was observed.

Samples prepared with the polymerized tubules also collapsed to form flat bilayer structures. However, unlike the unpolymerized tubules which collapsed with ragged edges, the collapsed polymerized tubules exhibit clean, straight edges as would be expected if polymerization occurs along the length of the tubule. If the polymerization does in fact occur along the long axis of the tubule, rather than a random polymerization, we should observe some dependence of the cutting force on the cutting direction. The polymerized tubules, however, could not be cut by scanning across their width with the cantilevers used in this experiment, even when the applied force reached almost four times (49 nN) the cutting force used to modify the unpolymerized tubules. As expected, a much greater applied force will be required to sever the chemical bonds formed during polymerization. Experiments are currently being undertaken to see if the polymerized tubules can be cut with larger applied force and if the modification is directionally dependent.

[3] This value is an average of 10 cuts made with several different cantilevers and samples.

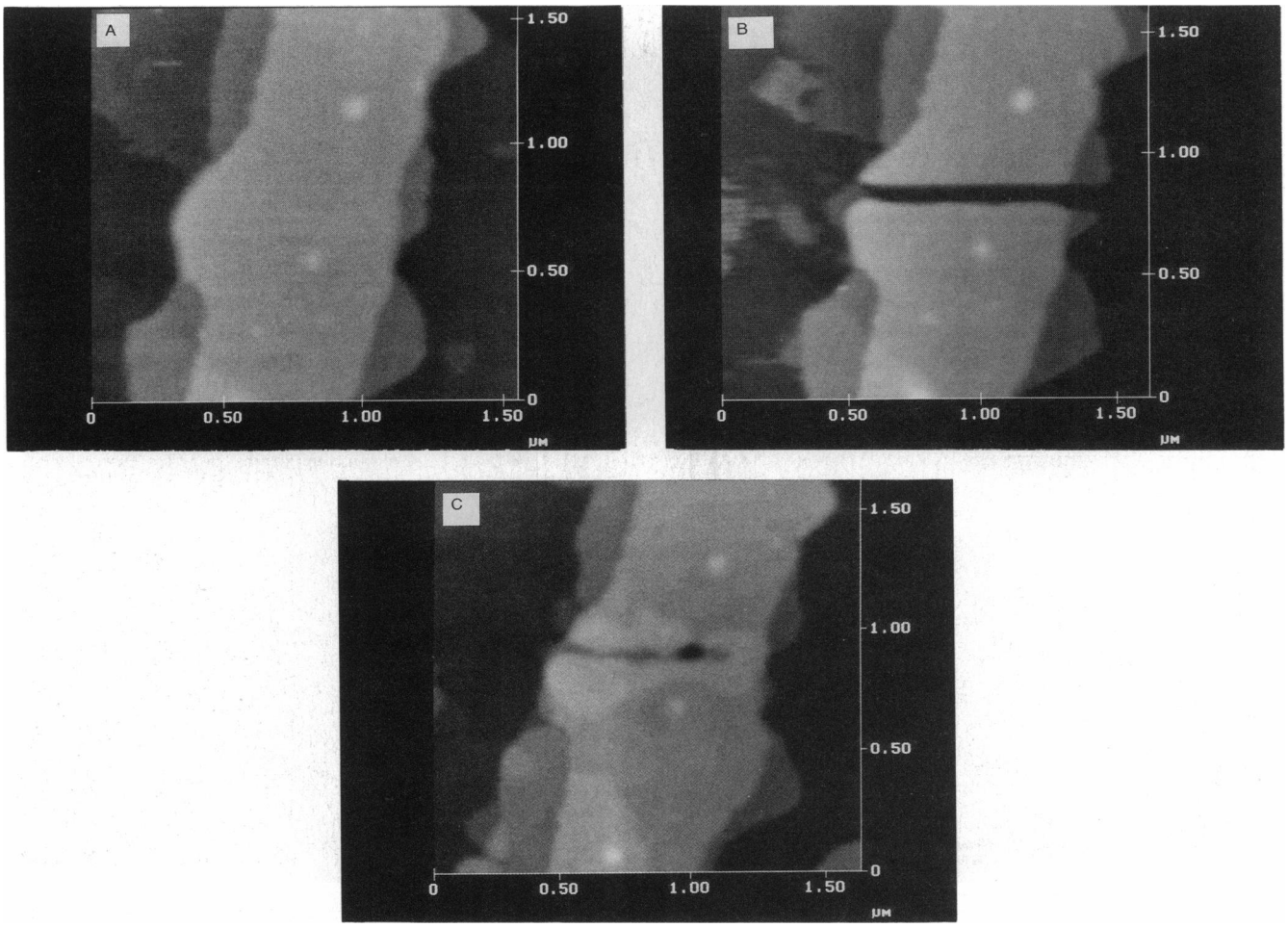

FIGURE 2 (*A–C*) The images show 1.9 μm × 1.9 μm AFM scans of a collapsed unpolymerized lipid tubule (*A*) imaged at a low force (<8 nN), (*B*) after lipid has been removed by scanning across the tube's width with increased force (~13 nN), and (*C*) after the cut has partially self-annealed over a 24-h time period.

Cuts through the unpolymerized lipid bilayer were found to undergo a slow self-annealing process. Fig. 2 *C* shows the partial self-annealing of a 75 nm wide cut (Fig. 2 *B*) after 24 h. By scanning with reduced force, the cut could be imaged without obviously affecting the annealing process. The annealed cut in Fig. 2 *C*, however, was imaged only three times over the 24-h period, to avoid the possibility of unintentionally clearing lipid from the cut during the annealing process. The annealing mechanism in this case is believed to result from viscous flow of lipid at the boundary of the cut, possibly coupled with diffusion driven by the necessity to reduce the energy of the exposed hydrocarbon at the edge of the cut. We found the self-annealing mechanism could be prevented by increasing the width of the cut. Wider cuts were observed over a two-day monitoring period and no annealing was observed. This may indicate that self-annealing is dependent on the presence of residual lipid within the cut, which facilitates flow across the graphite substrate. In smaller cuts, the AFM tip may simply move residual lipid back and forth within the cut during each scan. This movement of material would make it difficult to image any lipid left in the cut. By widening the cut, and thus the cutting time, the cut may be swept clean leaving a bare graphite surface.

A second annealing mechanism was also observed. We found that the AFM could be used to repair or heal a cut by using the AFM tip to force lipid into the empty cut. By scanning perpendicular to the cut with a force approximately 14% smaller than the threshold cutting force, the cut could be forced to anneal in minutes rather than hours. This "induced annealing" was due to the shear stress applied by the AFM tip driving viscous flow of lipid into the cut. Fig. 3 shows a cut 80 nm in width which has been shear annealed in 15 min. Induced annealing was observed to occur on even the widest cuts that we studied.

DISCUSSION

To our knowledge, this is the first example of an AFM being used to modify a lipid bilayer in a quantitatively

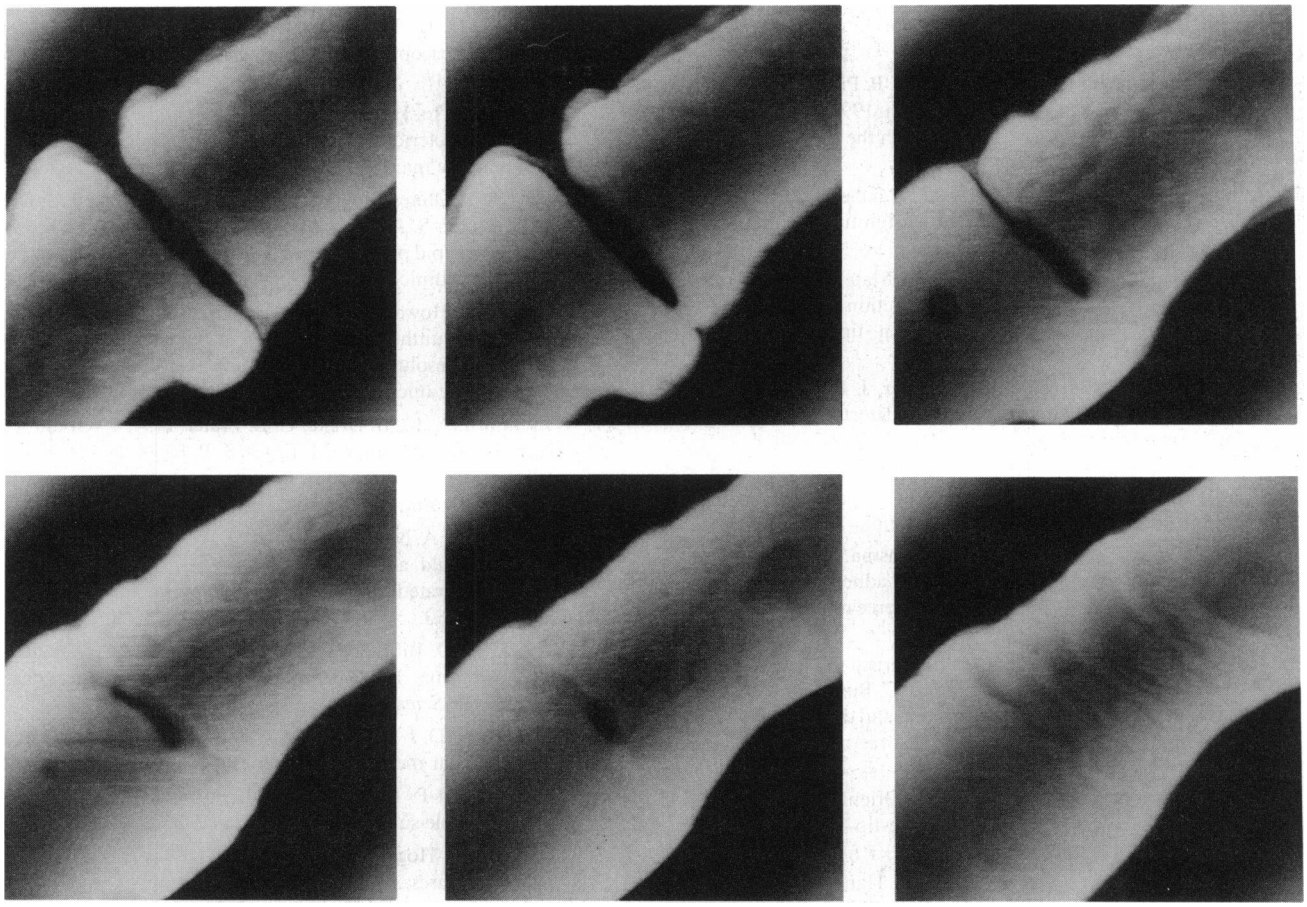

FIGURE 3 Time evolution of a cut through a collapsed unpolymerized lipid tubule as the cut is forced to anneal by scanning the AFM tip perpendicular to the cut. Total time elapsed is approximately 15 min.

controlled manner. The AFM can cut, anneal, or image the bilayer, based on the amount of force applied. Both a rapid shear stress induced annealing, resulting from pressure applied by the AFM tip, and a slower self-annealing of the supported bilayers was observed. The threshold force required for modification can yield information about the relative strength of adhesion of the adsorbed layer and the strength of interactions within the layer, whether they be weak van der Waals forces or strong covalent bonds. By cutting through the bilayer and watching the annealing processes, it may be possible to measure some of the fundamental physical parameters of this supported bilayer system. Although these annealing processes are more complicated to interpret than simple diffusion, they may lead to a determination of the viscosity of this lipid system. From a technical standpoint, it is worth noting that the lipid bilayer can act as a lithographic resist, and when patterned with the AFM, could be processed to build up a metallized replica of the pattern. Thus, it appears that the AFM can be used to generate patterns with a resolution of at least 75 nm with this system. Furthermore, the line width might be reduced by using higher aspect AFM tips and moving to monolayer systems. These experiments are a clear demonstration of how the AFM may be used both to modify biological surfaces and also to study materials properties of samples on the nanometer scale.

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