Stable Binding of DNA to Zwitterionic Lipid Bilayers in Aqueous Solutions

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We found that DNA molecules bind strongly to zwitterionic dipalmitoylphosphatidylcholine lipid bilayers in solutions, facilitating in situ imaging with an atomic force microscope. The binding strength has a lower limit of about 2 kT per helical turn with kT the thermal energy at room temperature. The height of membrane-bound DNA is the same as the diameter of DNA, indicating no compression by the tip at a probe force of about 0.1 nN.

Introduction

Studies of how DNA interacts with lipid bilayers is important for understanding how foreign genetic materials can be efficiently delivered into cells. Cationic lipids have been favorable materials to mediate gene delivery,[1−3] in which the first stage is the formation of DNA−cationic lipid complexes that are granules of average diameter about one micron.[4] Recent scattering studies have revealed that complexed DNA−cationic lipid granules consist of stacked structures of ordered DNA molecules sandwiched between lipid bilayers.[5,6]

Structural studies with atomic force microscopy (AFM) have found that DNA molecules bind to millimeter-sized cationic lipid bilayers and condense into nematically ordered structures.[7−9] A promising development has been an experimental confirmation[7] of a theoretical prediction that the fluidity of a cationic bilayer facilitates the nematic ordering of bound DNA molecules, which induce hydrophobic mismatch of adjacent lipids to result in the needed attraction between neighboring DNA strands.[10] A mean field approach indicates possibility of nematic ordering of DNA bound to cationic lipid bilayers in 3-D through elastic coupling between the two entities.[11] Experiments also found that the structure of condensed DNA changes as the ionic strength increases and does not reverse several days after the ionic concentration is recovered to the original value.[7,12] This asymmetric behavior indicates that the 2-D condensation is intrinsically very complicated. However, that DNA binds to bare cationic lipid bilayers in solution suggests that DNA molecules encapsulate those complexed DNA−cationic lipid granules.

While investigating how DNA interacts with cationic lipids remains important, we need to study how DNA interacts with zwitterionic lipid bilayers, to have a better comprehesion of the gene delivery process. The reason is that successful gene delivery requires the proximity of DNA with cellular membranes that contain large amounts of zwitterionic lipids. In this article, we report our finding that DNA binds strongly to gel-phase dipalmitoylphosphatidylcholine (DPPC) bilayers in solutions, indicating that cellular membranes should not repel foreign genetic materials. Our results also demonstrate a new substrate for high-resolution imaging of DNA in solution and shed light on the advancement of the thriving field of biological applications of AFM.[13−16]

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Experimental Section

Materials. DPPC lipids in the powder form were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. The pTZ plasmid DNA molecules (2880 bp) were obtained from Sigma (St. Louis, MO) and used after appropriate dilution.

Specimen Preparation. Vesicle suspensions and supported DPPC bilayers on mica were prepared as described elsewhere.[17] For a bilayer with low coverage of defects and aggregates, plasmid DNA molecules at a concentration of 0.05 mg/mL were incubated with the bilayer in 20 mM NaCl at 4 °C overnight. Afterward, excess DNA in solution was removed by solution exchange of more than 20 times while avoiding exposure of the bilayer to air.

AFM Imaging. A NanoScope E AFM and oxide-sharpened Si_3N_4 tips with a nominal spring constant of 0.06 N/m from Digital Instruments (Santa Barbara, CA) were used in this work. In solution, images were obtained using a homemade fluid cell, at a probe force of about 0.1 nN in the contact mode, at a pixel number of 512 × 512, and with a scanning line speed of about 5 Hz.

Results

Typical supported DPPC bilayers are flat with several defects that are lower than the bilayer plane by a thickness of about 6 nm. Once assembled, the bilayer structure remained stable over several weeks, consistent with a previous report.[17] Figure 1 is a DPPC bilayer in the surface plot, showing a 3-D perspective of the planar structure. The bilayer has two defects, some debris, and several defects that are too small to allow the tip to touch the substrate.

Stable binding of DNA molecules to DPPC bilayer was obtained after incubating DNA molecules with the bilayer overnight. Figure 2 shows four typical examples, with roughly parallel DNA strands in many regions. Arrows indicate several segments of DNA with sharp turns. Since there is a large elastic energy associated with the radius of curvature of the turns, the binding and any possible DNA−DNA interaction combined must be even larger to allow these segments stably adsorbed to the bilayer. Very dark regions without any DNA strand and with some debris are bilayer defects. Some dark horizontal shades are scanning artifacts due to a slow feedback response of the instrument.

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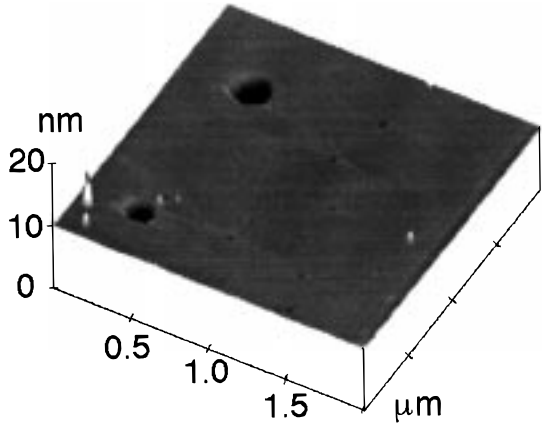

Figure 1. A DPPC bilayer in the surface plot.

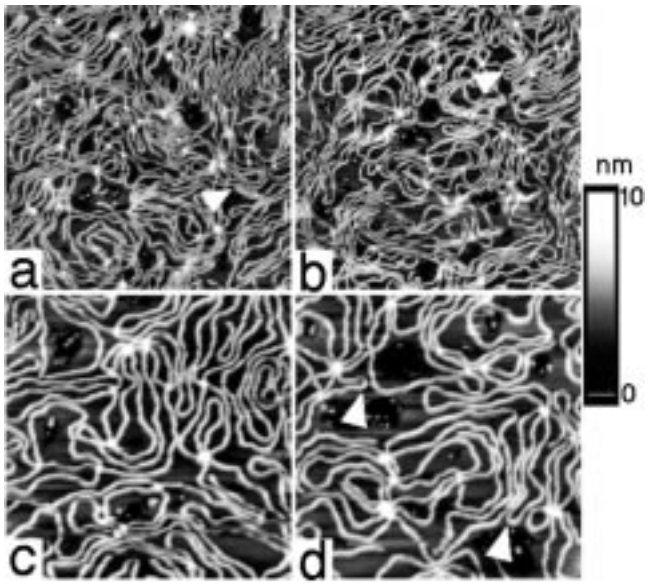

Figure 2. Four images of DPPC-bound DNA. Arrows indicate sharp turns. Defects on DPPC bilayers can be seen in these images. Image size for (a) and (b) is 1000 nm, and that for (c) and (d) is 500 nm. The vertical gray scale is shown at the right of the four images.

Good-quality images such as those in Figure 2 were not routinely obtained. In many cases, stable imaging was problematic. Figure 3 shows an example in which some obscured regions, due to unstable imaging, are indicated by arrows. However, the imaging force was always stable without any adhesion. Thus, the cause of unstable imaging is not a tip−sample attraction that has been a major factor for unstable in situ imaging of soluble proteins.[18]

The surface coverage of membrane-bound DNA could be reduced by incubating the sample with 1 M NaCl for 1 min, followed by washing the sample through exchanges with 20 mM NaCl. Figure 4 shows an example containing several plasmids widely distributed on the membrane. Figure 5 contains a group of six consecutive images at approximately the same area, with the images from (b) to (f) taken after image (a). Arrows in these images indicate three DNA segments that are seen intact in several images and disconnected in other images. Thus, those linear DNA segments in images other than those in Figure 5 are most likely also due to imaging artifacts.

One interesting result is that the height of DNA (2.0 ± 0.2 nm in 33 measurements) is the same as the diameter of DNA, indicating negligible vertical compression by the AFM tip. A similar phenomenon has also been observed for widely distributed DNA on gel-phase cationic lipid bilayers.[7] The width of DNA is about 7 nm, consistent with the tip-broadening effect.[19]

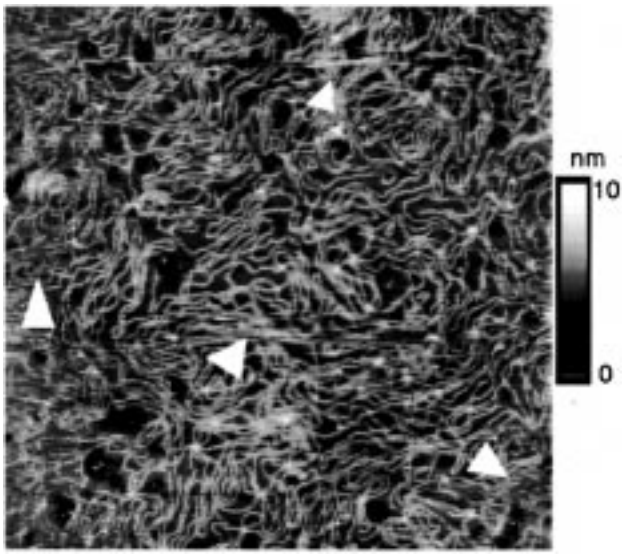

Figure 3. DPPC-bound DNA with an image size of 2000 nm. Arrows indicate regions of unstable imaging.

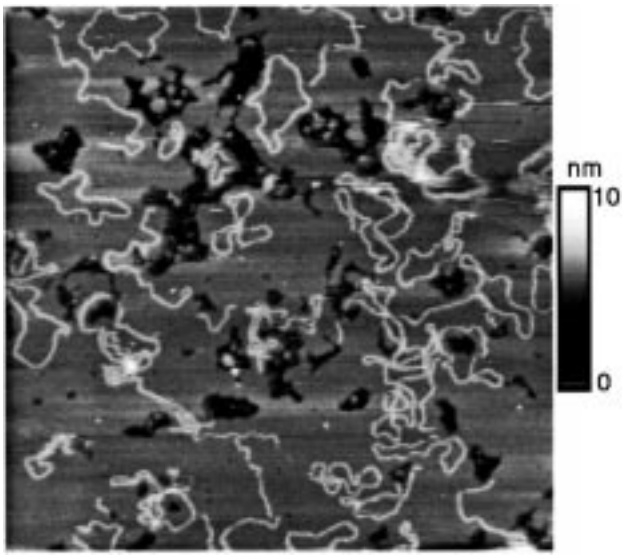

Figure 4. An image of widely distributed DPPC-bound DNA with an image size of 2000 nm. Bilayer defects and some relaxed plasmids are seen.

For fifteen mostly relaxed plasmids, their average contour length is 1027 ± 68 nm, measured directly from AFM images. Thus, membrane-bound DNA is B-type, similar to the conformation of DNA adsorbed to a mica surface.[20] We also imaged these plasmid DNA molecules in air with samples prepared as described elsewhere.[9] Figure 6 shows an example, in which most plasmids are intact, indirectly supporting our conclusion that most linear DNA segments observed with in situ imaging are due to imaging artifacts.

Discussion

Our results indicate that the binding of DNA to DPPC bilayers must be fairly strong so that membrane-bound DNA molecules can withstand the probe force to allow high-resolution in situ imaging. That the surface coverage of membrane-bound DNA can be reduced by brief incubation with 1 M NaCl solution indicates that the electrostatic interaction between DNA and the

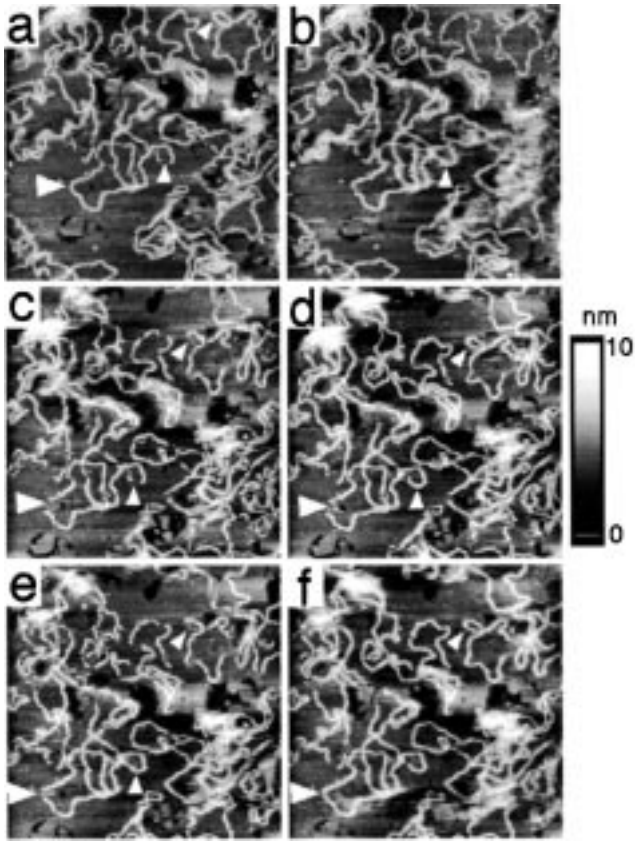

Figure 5. Six images, each with a of size 1135 nm. Arrows point to those segments that are seen in some images but not in others. Other similar segments are not labeled with arrows.

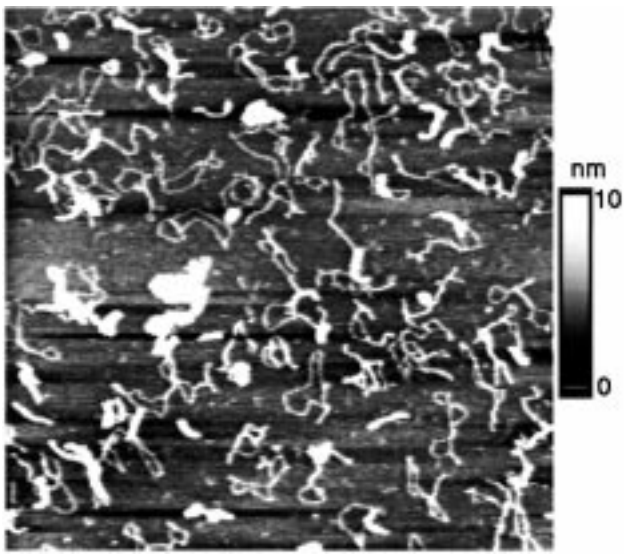

Figure 6. An image of widely distributed DNA on mica obtained in air with an image size of 2000 nm. Some large debris, possibly condensed salt as the sample was dried, is seen on the surface.

large dipole moment of DPPC headgroup[21] plays a major role to tether DNA.

According to a recent study, DNA molecules are in the fluctuation-enhanced repulsive regime at low densities, with an exponential decay length of 1.76 nm.[22] For DPPC-bound DNA, the smallest inter strand distance is about 11 nm, more than 6 times larger than the decay length of the fluctuation-enhanced regime. Thus, any inter strand interaction between DPPC-bound DNA molecules must be negligibly small and they can be considered as noninteracting molecular threads confined in 2-D.

For noninteracting DPPC-bound DNA molecules, an estimation of the lower limit of the binding strength can be obtained. There are segments with sharp turns that have a radius of curvature about 6 nm. It has been shown that at length scales about the persistent length of DNA, the elastic energy of DNA is approximately proportional to the square of curvature (reciprocal of the radius of curvature).[23,24] For the sharp turns with a radius of curvature about 6 nm, the minimum elastic energy is about 7 kcal/mol, distributed over the length scale of about five helical turns, corresponding to an elastic energy of about 2 kT per helical turn, with kT the thermal energy at room temperature. Thus, the binding of DNA to DPPC should be stronger than 2 kT per helical turn.

A recent theoretical calculation indicates that the binding strength of DNA sandwiched between a pair of cationic lipid bilayers is about kT/b, with b the average distance between neighboring charges.[25] For double-stranded DNA, the number of charged groups is about 20 per helical turn. The binding of DNA to a single bilayer should be about one-half kT/b, corresponding to 10 kT per helical turn. Our results indicate that the binding strength between DNA and DPPC is about one-quarter that between DNA and a cationic lipid headgroup.[9]

The occurrences of unstable imaging despite stable imaging force without any adhesion suggests the existence of dangling DNA segments that interfere with the imaging. These dangling segments would not attract the tip but would result in tip fluctuation as it scanned across their top and, thus, causing unstable imaging without any adhesion force. They may also give image artifacts since the tip does not detect them so that some plasmids are seen as linear DNA.

Any rearrangement of membrane-bound DNA should be severely restricted because DPPC is well into the gel phase at room temperature. Thus, if the rearrangement is one of the major factors to promote long-range order of membrane-bound DNA, as predicted for the 2-D condensation of DNA on cationic bilayers,[10] there should be no long-range ordering for DPPC-bound DNA. The lack of any nematic ordering in DPPC-bound DNA molecules is clearly shown in our AFM images. For DNA molecules bound to cationic lipids, the ordering of DNA remains in the presence of 1 M NaCl for more than 1.5 h except that the inter helical distance increases.[7] The coverage of DNA was drastically reduced by only briefly exposing the system to 1 M NaCl for DPPC-bound DNA. This phenomenon is consistent with the much weaker binding strength of DNA to DPPC so that the attraction between the two entities decreases by the screening of monovalent ions at high concentration. The possibility of any nematic ordering is drastically reduced since the binding strength may be too weak to overcome the fluctuation-enhanced repulsive interaction[22] to allow the high-density packing required for any nematic ordering to occur.

To test whether a fluid phase phosphatidylcholine (PC) bilayer would promote the 2-D condensation of DNA, we have tried similar experiments without any success with dimyristoylphosphatidylcholine (DMPC) that has a main phase transition temperature at 23 °C, although DMPC bilayers were readily formed on mica. Stable imaging on DPPC-bound DNA is already very hard and the failure of successful imaging of any DNA on DMPC may be the result of the fluidity of DMPC bilayers. Our results indicate that the binding of DNA to DPPC is electrostatic so that the key player for the binding to DNA must be the PC headgroup. Thus, DNA should also bind to DMPC. It is possible that PC bilayers behave so differently from cationic lipid bilayers that no nematic ordering of PC-bound DNA molecules could occur. Thus, the fluidity of the

bilayer makes the substrate and, hence, the bound DNA, much less susceptible to the AFM tip to allow any successful imaging.

Last, it is worth noting that our results may provide structural information at the molecular level toward an understanding of the pathways in gene delivery trials. We show here that DNA binds to zwitterionic lipids, although not so strongly as it binds to cationic lipids. Thus, cellular membranes that contain large amount of zwitterionic lipids would not repel, but rather, attract foreign genetic materials and facilitate their intracellular delivery. Experimental investigation has found that using mixtures of PC and cationic lipids to complex with DNA resulted in higher delivery efficacy (D. D. Lasic, unpublished results), suggesting the involvement of membrane fusion in the gene delivery process because zwitterionic lipids are more suitable to fuse with cellular membranes.[26] The requirement of cationic lipids indicates the need of stronger binding of DNA and, most likely, the involvement of DNA condensation in the complexes to catalyze the gene delivery. More experiments are needed to better understand details.

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References and Notes

(1) Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7413.
(2) Lasic, D. D.; Templeton, N. *Adv. Drug Del. Rev.* **1996**, *20*, 221.
(3) Zhu, N.; Liggitt, D.; Liu, Y.; Debs, R. *Science* **1993**, *261*, 209.
(4) Lasic, D. D. *Liposomes in Gene Delivery*; CRC Press: Boca Raton, 1997.
(5) Lasic, D. D.; Strey, H.; Stuart, M. C. A.; Podgornik, R.; Frederik, P. M. *J. Am. Chem. Soc.* **1997**, *119*, 832.
(6) Radler, J.; Koltover, I.; Salditt, T.; Safinya, C. R. *Science* **1997**, *275*, 810.
(7) Fang, Y.; Yang, J. *J. Phys. Chem.* **1997**, *101*, 441.
(8) Mou, J.; Czajkowsky, D. M.; Zhang, Y.; Shao, Z. *FEBS Lett.* **1995**, *371*, 279.
(9) Yang, J.; Wang, L.; Camerini-Otero, R. D. *Nanobiology* **1996**, *4*, 93.
(10) Dan, N. *Biophys. J.* **1996**, *71*, 1267.
(11) Podgornik, R. *Langmuir* **1997**, *13*, 4791.
(12) Fang, Y.; Yang, J. *J. Phys. Chem.* **1997**, *101*, 3453.
(13) Bustamante, C.; Keller, D. *Physics Today* **1995**, *48* (12), 32.
(14) Han, W.; Lindsay, S. M.; Dlakic, M.; Harrington, R. E. *Nature* **1997**, *386*, 583.
(15) Hansma, H. G.; Hoh, J. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 115.
(16) Shao, Z.; Yang, J. *Quart. Rev. Biophys.* **1995**, *28*, 195.
(17) Fang, Y.; Yang, J. *Biochim. Biophys. Acta* **1997**, *1324*, 309.
(18) Yang, J.; Mou, J.; Shao, Z. *Biochim. Biophys. Acta* **1994**, *1199*, 105.
(19) Yang, J.; Mou, J.; Yuan, J.-Y.; Shao, Z. *J. Microscopy* **1996**, *182*, 106.
(20) Rhodes, D.; Klug, A. *Nature* **1980**, *286*, 573.
(21) Shepherd, J. C. W.; Buldt, G. *Biochim. Biophys. Acta* **1978**, *514*, 83.
(22) Strey, H. H.; Parsegian, V. A.; Podgornik, R. *Phys. Rev. Lett.* **1997**, *78*, 895.
(23) Camerini-Otero, R. D.; Felsenfeld, G. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1708.
(24) Schellman, J. A. *Biopolymers* **1974**, *13*, 217.
(25) Bruinsma, R. *EuroPhys. J.* **1997**, in press.
(26) Siegel, D. P. *Biophys. J.* **1993**, *65*, 2124.