Molecular Forces for the Binding and Condensation of DNA Molecules

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ABSTRACT Atomic force microscopy has been used to investigate the binding between a double-stranded DNA and bilayers of cationic lipids and zwitterionic lipids in low ionic-strength solutions. The binding of a DNA molecule to freshly cleaved mica surface in solution has also been measured. The binding of DNA molecules to cationic lipid bilayers has a minimal strength of ~45 pN. On zwitterionic lipid bilayers and mica surface, the minimal binding strength is approximately twice that value. The binding also has a dynamic nature, with only a certain percentage of recorded force curves containing the binding characteristics. Divalent Mg^{2+} ions enhance the binding by increasing that percentage without any effect on the binding strength. We have also observed a long-range attraction between DNA molecules and cationic lipid bilayers with a strength much larger than the minimum force and a range well over 50 nm, possibly related to the driving force responsible for the two-dimensional condensation of DNA.

INTRODUCTION

DNA molecules, important for their biological role of carrying genetic information (Saenger, 1984), are also charged polymers that display a number of distinct physical characters, such as a stretched state (Cluzel et al., 1996; Smith et al., 1996; Williams et al., 2001), dynamic polymeric behavior with individual DNA molecules (Quake et al., 1997; Perkins et al., 1997), and chain-chain repulsion at both short- and long-ranges in DNA liquid crystals (Strey et al., 1997). A great number of biological processes, including replication and recombination, involve complexes containing DNA molecules and macromolecular aggregates (Saenger, 1984; Cook, 1999). Complexes containing DNA molecules and lipid bilayers also form a class of biological systems that have potential applications in gene delivery trials (Felgner et al., 1987; Lasic, 1997; Lasic et al., 1997; Radler et al., 1997; Zhu et al., 1993). In all the above processes, there is a direct binding between DNA and other molecules. Some of these bindings have been extensively studied using chemical and biochemical methods where the association constants are measured on large ensembles with sufficient statistical significance (Saenger, 1984). At the molecular level, how DNA molecules interact with other entities may differ from the averaged results and may reveal molecular characteristics that are buried in an average of large ensembles. With atomic force microscopy (AFM), it is possible to investigate molecular interactions of a single bond (Moy et al., 1994; Hoh et al., 1992; Boland and Ratner, 1995; Grandbois et al., 1999) and, hence, to study how a double-stranded DNA interacts with other entities of biological or physical interest.

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In this article, we report our studies of the binding between DNA molecules and bilayers of cationic and zwitterionic lipids at the molecular level involving a single molecule or a few molecules, operating an atomic force microscope in solutions. We also measured the binding of DNA molecules to freshly cleaved mica surface in solution. The cationic lipid dipalmitoyl-dimethylammonium-propane (DPDAP) is similar to those that form complexes with DNA molecules, believed to be essential for efficient gene delivery (Felgner et al., 1987; Lasic, 1997; Zhu et al., 1993). The zwitterionic lipid dipalmitoylphosphatidylcholine (DPPC) mimic biomembranes (Cevc and Marsh, 1978). Mica surfaces are atomically flat and are widely used as substrate for biological applications of in situ AFM (Hansma and Hoh, 1994; Shao and Yang, 1995; Bustamante and Keller, 1995; Han et al., 1997).

To understand how to use AFM to investigate the binding between a single DNA molecule and a substrate, we describe briefly the basics of the instrument as a molecular force probe. The raw data of an AFM force curve without any tip-sample interaction have the typical feature shown in part (*A*) of Fig. 1. The vertical scale measures intensity difference from two photo-detectors (A-B signal), with the source being the laser beam reflected from the cantilever. The A-B signal is highly sensitive to the vertical movement of the cantilever tip. When the tip is apart from the sample surface, the cantilever is in the relaxed position and the A-B signal is a constant, corresponding to the flat region of the force curve and establishing a base line. After the tip is engaged to the sample surface, the cantilever follows the vertical movement of the piezo scanner, resulting in a linear slope of the A-B signal. Note that the A-B signal is proportional to the vertical movement of the cantilever, and that the restoring force is obtained on the basis that within the range of the movement the cantilever behaves like a linear spring. If there is a tip-sample attraction, we expect the approaching curve to be similar to the one in part (*A*). However, the retracting curve will be similar to the one shown in part (*B*), where the tip adheres to the sample

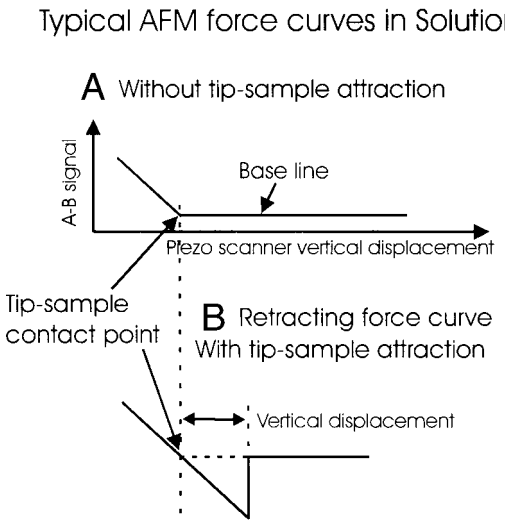

FIGURE 1 Illustration of the main basics of AFM force curves.

surface until the restoring force of the cantilever becomes large enough to snap it off the sample surface. With a known spring constant of the cantilever, the magnitude of the attraction can be obtained from the vertical displacement of the cantilever.

For a tip coated with a DNA molecule, any detected adhesion reflects the binding between DNA and the substrate. On this basis, we have developed a method to attach a DNA molecule onto an AFM tip. Force curves for such treated tips revealed typical binding forces of ~45 pN or a simple multiple of that, indicating the binding of a single DNA molecule or just a few molecules. Moreover, the binding showed a predominant dynamic nature. For repeated force curves on the same surface, only part of recorded curves, ranging from 5 to 16%, showed the binding. It was found that divalent Mg^{2+} ions caused an increase of the percentage of force curves with the binding character, with no effect on the binding strength. On cationic lipid bilayers, there were cases where an attraction occurred as the tip was clearly far away from the substrate. These attractions have a long-range character with a significant strength well over 50 nm, are much larger than 45 pN, and bear no resemblance to the attraction expected to an entropic elastic polymer. There were also force curves consistent with an attachment of a globular structure folded by an entangled DNA molecule.

MATERIALS AND METHODS

Materials

Zwitterionic and cationic lipids (DPPC and DPDAP) dissolved in chloroform were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. All DNA molecules were obtained from Sigma Aldrich (St. Louis, MO), and used after appropriate dilution. They include λ-DNA, Col E1 plasmid DNA of ~6600 bp, pZT plasmid DNA of 2880 bp, and λ-DNA EcoR I Digest. All divalent and multivalent salts ($MgCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, and spermine) were also obtained from Sigma Aldrich.

Supported bilayers

Supported unilamellar bilayers were prepared by the method of vesicle fusion as reported elsewhere (Fang and Yang, 1996, 1997b). Briefly, a glass culture tube was used to contain ~1.2 ml of a lipid suspension at a concentration of 0.2 mg/ml in 20 mM NaCl. The tube was then sonicated until clear under nitrogen gas. Afterward, a droplet (~0.3 ml) of the lipid suspension was applied to cover a piece of freshly cleaved mica. The substrate was then stored at 4°C overnight. Finally, the substrate was heated for 30 to 60 min at 50°C and 60°C for DPPC and DPDAP, respectively. The existence and the quality of the bilayer were examined by AFM. Bilayers of large surface coverage without excessive aggregates were used in our force studies.

AFM force curves

A NanoScope E AFM and oxide-sharpened Si_3N_4 tips with a nominal spring constant of 0.06 N/m, all from Digital Instruments (Santa Barbara, CA), were used in this work. Force curves were recorded with the commercial Instruments and data files were converted to ASCII text format to allow data analysis. All force curves were obtained in solution with a homemade fluid cell retrofit to the AFM head. The basis of a force curve is described in the introduction section. With an actual instrument, a force curve is recorded as the tip-sample distance is driven to oscillate at a giving frequency. Therefore, there is an approaching curve and a retracting curve in a complete driven cycle. The tip is in contact with the sample when the A-B signal is in the slope region. In our experiments, we chose to have the contact time long enough to get a good measurement of the slope so that it was ~10% of the time of a complete cycle. The time of the cycle is the inverse of the operating frequency.

Tip treatment

An oxide-sharpened Si_3N_4 tip was silanized by briefly immersing it in methacryloxypropyl trimethoxysilane (MAPS) liquid, followed by drying under ambient conditions. This procedure allows a coating of MAPS onto the oxidized tip without need of any additional tip oxidization procedure (Vansteenkiste et al., 2000). Then, the silanized tip was dipped into a solution containing DNA molecules and 0.2% each of ammonium persulfate (APS, by weight) and N,N,N′,N′-tetramethylethylenediamine (TEMED, by volume), respectively. The catalytic agents APS and TEMED together facilitate the reduction of the alkene double-bond of the MAPS and covalently link the opened bond to a reactive group, such as an OH group or an ether group (Hames, 1981; Rothe and Maurer, 1986). The time of the reaction (~1 h) was optimized to allow covalent binding of DNA strands to the tip without much aggregation.

RESULTS

The attachment of DNA molecules to the end of an AFM tip allows an investigation of the binding of DNA molecules to the substrate materials. We have found a striking feature in our force curves that reveal the molecular detail of the binding of DNA to a substrate surface. Fig. 2 *A* shows a typical example: the raw data of the A-B signal as the tip were retracted from the sample surface. The flat region slightly below the base line signals a constant pulling force.

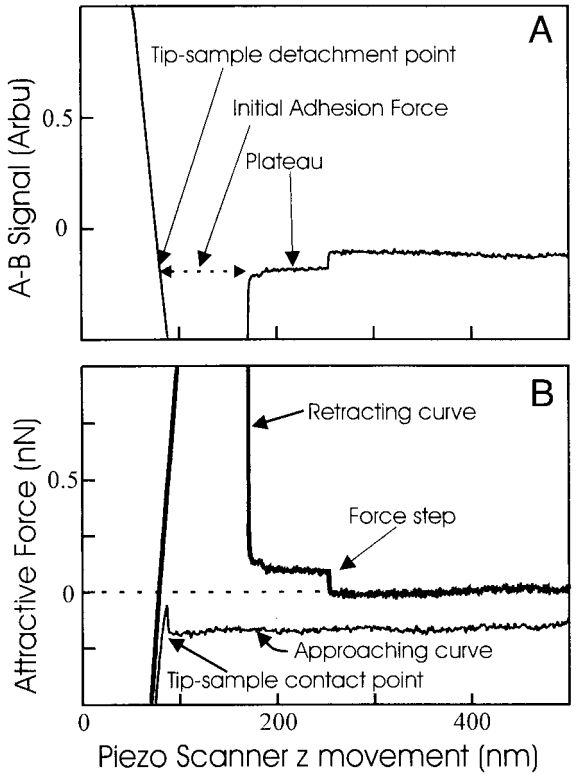

FIGURE 2 Part (*A*) are original raw data of the A-B signal vs the piezo scanner *z* movement. Part (*B*) are plots of retracting and approaching force curves. The force curves were obtained by a tip with attached λ-DNA on a DPDAP bilayer in 4-mM $MgCl_2$ solution.

According to the spring constant of our tips, we converted the raw data into a relative measurement of a tip-sample attractive force by first calculating an average slope, followed by mapping the A-B signal to the cantilever vertical displacement and setting the base line as zero. Fig. 2 *B* shows both the retracting force curve with a plateau above the base line and the approaching force curve with an artificial offset to bring out the entire feature of the curve. A negative attractive force means a repulsive force. Several key positions in the force curves are marked and briefly explained on the graph. The small attractive force spike in the approaching force curve indicates the existence of a tip-sample attraction that gives rise to the large initial adhesion force as seen in the retracting force curve. Note the same slope for the retracting and approaching force curves. This is because the slope is caused by the vertical movement of the piezo scanner while the tip is in contact with the sample.

The plateau of the retracting force curve represents a new feature attributable to the binding of a double-stranded DNA to the surface. Fig. 3 illustrates the scenario. As a uniformly bound DNA chain is pulled from the surface, a constant attraction plateau occurs until the contact is being pulled to snap off. The final detachment of bound-DNA

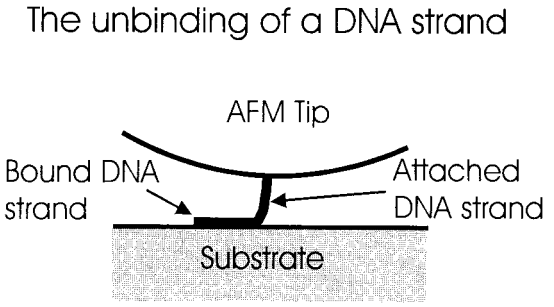

FIGURE 3 A model depicting a tip with one attached DNA molecule that has a part uniformly bound to a substrate.

molecule leads to a force step, providing a quantitative measurement of the binding strength between the DNA molecule and the sample surface. In this scenario, the same phenomenon occurs if only part of a DNA molecule is uniformly bound to the surface. Fig. 4 shows an example with two force plateaus, corresponding to a situation of binding to two DNA strands.

In our force measurements, it was found that for a tip with attached DNA molecules, not all of its force curves contained the binding-characteristic plateau. Moreover, there was always a large initial attractive force that tends to bury any short plateau. To maximize the effect, we have used

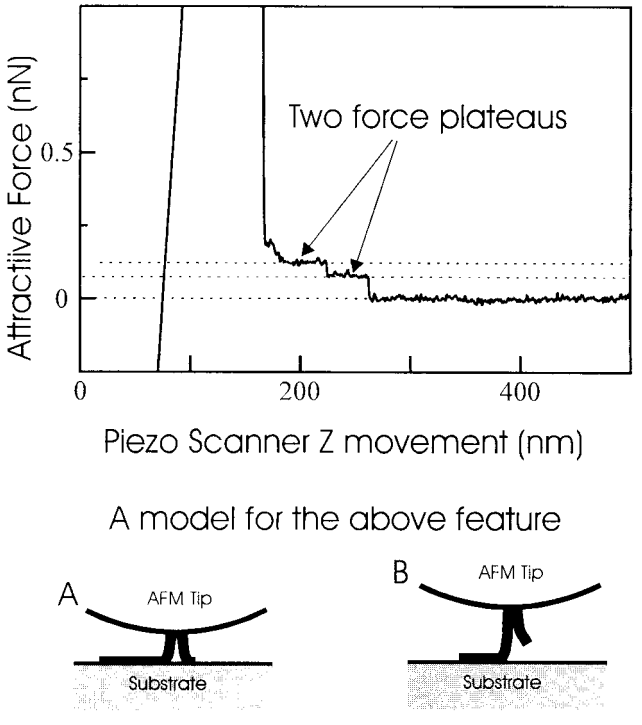

FIGURE 4 The top part shows a force curve with two attraction plateaus and the bottom part models a scenario corresponding to the two plateaus. The force curve was obtained in 4 mM $MgCl_2$ with a tip attached with λ-DNA on a DPDAP bilayer.

λ-DNA molecules extensively for the binding studies, because of their relatively long length that would result in a longer plateau. For tips with attached λ-DNA molecules, it was found that on cationic lipid bilayers, ~9% of the force curves contained the DNA-binding character in 20 mM NaCl (26 of 296 at 1 Hz and 19 of 210 at 10 Hz). This percentage increased to 16% in 4 mM $MgCl_2$ (46 of 281 at 1 Hz and 49 of 314 at 10 Hz). Similar features were observed when ionic strength was changed to 1 M NaCl or 100 mM $MgCl_2$, consistent with our earlier study (Fang and Yang, 1997a). For ~10% of the time the tip was in contact with the sample, the timescale involved in our experiments was <0.1 s.

On mica surface, the overall percentage of force curves with the binding plateau in 4-mM $MgCl_2$ solution remained to be high (27 of 186 at 1 Hz and 12 of 96 at 10 Hz). However, in 20-mM NaCl solution, no binding was detected within the instrumental limit (for a total of >100 curves at each operating frequency). On zwitterionic DPPC bilayers, the binding occurred only at a very low percentage in 4 mM $MgCl_2$ (9 of 196 at 1 Hz and none at 10 Hz for a total of 192 force curves). Again, we did not observe any binding character in 20 mM NaCl (>100 curves for each operating frequency). Therefore, within the timescales of our experiments, the binding of double-stranded DNA to the three kinds of surfaces is strongly favored in the presence of divalent Mg^{2+} ions. In the presence of monovalent Na^+ ions, we only detected a significant binding of DNA molecules to DPDAP lipid bilayers.

Fig. 5 summarizes our results. It shows the number of occurrence of force curves with the binding plateau for different force steps at two vertical raster frequencies on the three surfaces containing either divalent Mg^{2+} ions (4 mM) or monovalent Na^+ ions (20 mM). On cationic DPDAP bilayers, the binding of DNA was notable in the presence of either divalent Mg^{2+} ions or monovalent Na^+ ions. At the lower frequency, a fine structure of binding character is more distinct, showing that the strengths of the binding force are multiples of a minimal value of ~45 pN. At the higher frequency, the overall distribution function of the binding strength shifts slightly to the right, although still retaining some characters of the fine structure. A similar fine structure was also found in NaCl solution at 1 Hz. On zwitterionic DPPC bilayers in 4-mM $MgCl_2$ solution, a dependence of the binding of DNA on the operating frequency is notable because of the absence of detected biding at 10 Hz, indicating that the timescale of establishing a stable binding is rather long. On both mica surface and DPPC bilayer, any fine structure of the binding character is lost, with only a force unit of ~90 pN for the strength. These results provide a molecular level strength for the binding of DNA to these surfaces in low ionic-concentration solutions.

We have also investigated the binding of DNA molecules to these three surfaces in solutions containing two high-

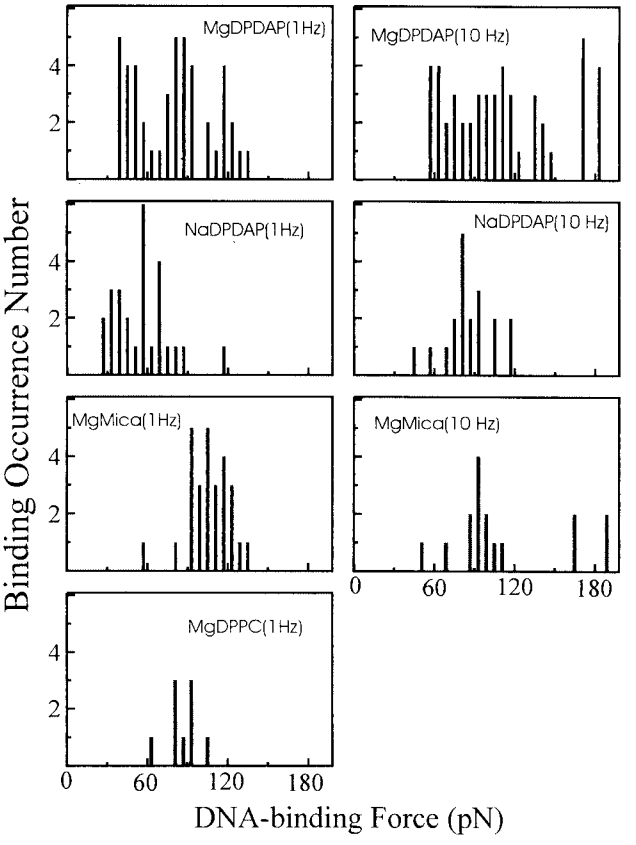

FIGURE 5 Here are seven distribution plots showing the occurrence of binding events at two frequencies in two ion species on three kinds of surfaces. Mg stands for 4-mM $MgCl_2$ solution and Na for 20-mM NaCl solution. Tips with attached λ-DNA were used for all datapoints here. Only force curves containing the binding character are included in these plots.

valence ions. No significant binding was detected in solutions containing tetravalent salt spermine at a concentration of just 0.005 mM. In the presence of divalent Mn^{2+} ions, binding events was observed only at concentrations <20 mM. These results are complementary to our earlier study (Fang and Yang, 1997a).

For shorter DNA fragments, the length of force steps was much shorter and the percentage of the force curves with a plateau was much lower, as was the case with circular plasmid DNA molecules. However, the initial adhesion force was still notable and similar for all kinds of DNA molecules used.

Increasing the concentration of catalytic agents, such as at a higher concentration of APS and TEMED in the process of attaching DNA molecules to a silanized tip or increasing the reaction time, we obtained force curves mostly with much too large initial adhesion forces. Such an effect prevented the detection of any force plateau with the step length buried in the large adhesion force, although some plateaus of a longer step length were detected. This phenomenon indicates that increasing catalytic agents caused a

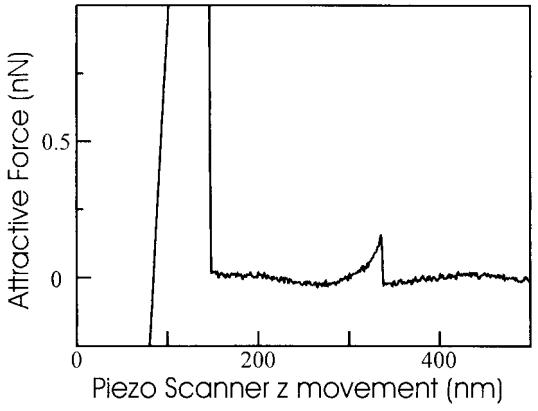

FIGURE 6 A force curve obtained using a tip with attached λ-DNA in 4 mM $MgCl_2$ on a DPDAP bilayer.

formation of an aggregate at the tip so that the magnitude of the initial adhesion force became larger. The aggregate could contain a number of DNA molecules and their interactions with the sample were superimposed, losing the information pertinent to individual double-stranded DNA. Therefore, it is not desirable to involve too many catalytic agents during the process of tip treatment. We realize that it is also difficult to guarantee the attachment of only a single DNA molecule to a tip during the treatment. The condition finally adopted for our investigation was a result of a great deal of trials to attain an optimization.

Besides the unique force curves that reveal the value of the binding strength, we have also noticed another interesting feature in our force curves. An example force curve is shown in Fig. 6. Here an apparent increase of the tip-sample attraction arises after the tip is well away from the tip-sample contact point as the tip retracts, indicating a different phenomenon from a uniform binding of the DNA molecule to the surface. This phenomenon was most prominent on cationic lipid bilayers and was occasionally observed on mica surface in 4 mM $MgCl_2$. Fig. 7 shows four more examples, with the horizontal axis representing the tip-sample distance. For each force curve, an arbitrary constant was added to separate the four curves to demonstrate the feature of this far-away attraction.

On DPPC bilayers in the presence of Mg^{2+} ions, we observed yet another kind of phenomenon. Fig. 8 shows an example force curve. As the tip was pulling away from the surface, the surface behavior changed. The tip-sample interaction showed first the feature of a hard, incompressible surface with a very steep slope, then the character of a very soft and compressible surface with a much less inclined slope (*arrow*), and finally a stretched character because the tip is above the relaxed position. At last, the tip snapped back to the relaxed position as it completely detached from the surface.

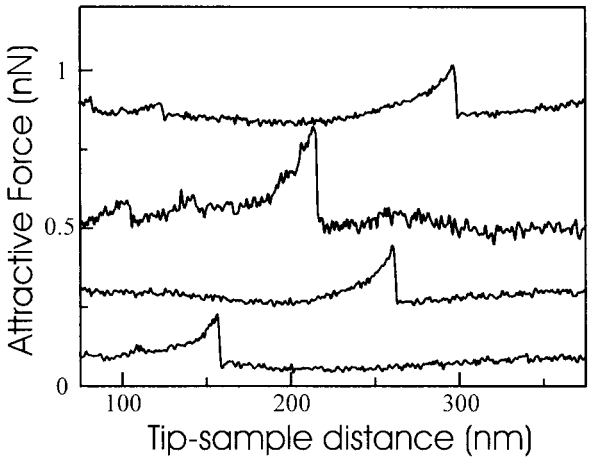

FIGURE 7 Four force curves with arbitrary offsets to demonstrate their individual features. Left end of the horizontal axis is far from the origin of these force curves to enhance the feature of the long-range attraction far away from the tip-sample contact point.

DISCUSSION

Minimum binding strength

Our tip-treatment resulted in a nonspecific attachment of a DNA molecule to an AFM tip. This method is not desirable for simultaneously obtaining molecular information correlating to the length of DNA molecules, such as whether a DNA molecule is stretched (Cluzel et al., 1996; Smith et al., 1996). However, it still marks a considerable advancement in the study of interaction between DNA molecules and other entities and provides, for the first time, the binding strength between individual double-stranded DNA and the three kinds of surfaces. It is conceivable to extend the method to investigate the interaction between DNA and other biomacromolecules or macromolecular complexes.

The intermolecular force, instead of the binding energy, is directly measured with AFM. With a reasonable physical

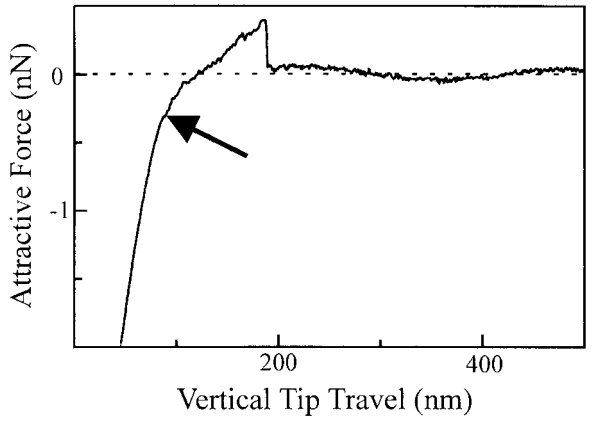

FIGURE 8 A force curve obtained by a tip with attached λ-DNA in 20 mM NaCl on a DPPC bilayer.

assumption, it is possible to estimate the binding energy at the molecular level. The detected force measures the binding energy per unit length. According to the assumption that all typical noncovalent bonds break at a stretched length of ~1 Å (Zaccai, 2001), the minimal binding energy corresponding to the minimum strength of 45 pN is then just slightly above the thermal energy at room temperature (k_BT). If the length of interest is an average distance between neighboring charges without taking into account of the helical nature of DNA molecule, we obtain a minimum binding energy twice the room temperature thermal energy. Comparing to an earlier estimation of the binding energy based on structural studies of the two-dimensional (2-D) condensation of DNA with a lower binding limit of ~8 k_BT per helical turn (Yang et al., 1996); the present direct force measurements yield a stronger binding energy per unit length.

The minimum attractive force as revealed in our experiments indicates that the binding between a DNA molecule and the three kinds of surfaces has a quantized character. Because this binding is intrinsically nonspecific and is likely of electrostatic nature, our finding actually indicates that any binding with a dominant electrostatic interaction in solution may require a minimal force of ~45 pN. We note that on DPPC bilayer and on mica surface, the minimal binding force was actually ~90 pN, twice the minimum force on cationic DPDAP bilayers. At present, we do not have an explanation for this phenomenon. One attempt to explain the result is to relate the binding to the number of bases involved. However, more experiments are needed to elucidate the physical origin of the phenomenon.

The minimum binding force measured here leads to an estimated binding energy just slightly above the thermal energy at room temperature, implying that for a stable binding of DNA molecules, a cooperative involvement of several to several tens bases must occur. Then, the overall binding strength can be sufficient to overcome any thermal fluctuation to result in a stable association.

The low probability of binding events in recorded force curves indicates that an adsorption of DNA molecule to these surfaces is of a dynamic nature. It is feasible that an adsorption actually involves two processes, a probability for the binding to take place, and the strength of the binding being large enough to overcome thermal and entropic fluctuations. Taking this probability into account, which is essentially the percentage of the occurrence of binding events, our results give an overall binding strength of ~7 pJ/m, or ~6 k_BT over the length of a helical pitch.

At longer timescales, it is likely to have a larger binding probability and, hence, a stronger overall binding strength. This was actually notable in our experiments of the binding of DNA to DPPC bilayers in 4 mM $MgCl_2$, where no binding was observed at 10 Hz, indicating that a longer time did enhance the chance of adsorption of DNA molecules to the surfaces. On this basis, without detecting any binding of DNA molecules to DPPC bilayers in 20-mM NaCl solutions in our current study only reveals that a stable binding has not been achieved at the timescale of our experiments (<0.1 s). This result is not in conflict with our earlier study in which DNA molecules did show binding to DPPC bilayer after 1 day of incubation (Malghani and Yang, 1998), or a recent study that indicated the binding of DNA to gel-phase phosphatidylcholine bilayers in a binding essay study using short nucleotides and phosphatidylcholine vesicles (Lu and Rhodes, 2001). We note that the timescale involved here is much longer than the inverse of the molecular bond resonance frequency, which is responsible for the dynamics of the molecular adhesion bonds (Evans and Ritchie, 1997).

A strong dependence of the binding of DNA on the species of ions in the solution suggests that ions are not just simply agents to screen any long-range electrostatic interaction. On negatively charged mica surface, it is understandable that divalent Mg^{2+} ions mediate the binding of DNA molecules via salt bridges. On zwitterionic DPPC bilayers and on cationic DPDAP bilayers, Mg^{2+} ions no longer just play the simple role of establishing salt bridges. We note that overall effect of Mg^{2+} ions is to increase the probability of the binding of DNA to the three kinds of surfaces. Their presence did not change the binding strength. According to a recent work and theoretical analysis, the persistent length of DNA in 20-mM NaCl and 4-mM $MgCl_2$ is ~50 nm (Baumann et al., 1997; Podgornik et al., 2000). Therefore, it is likely that Mg^{2+} ions also mediate intra-strand interactions to stabilize the conformation of DNA to render it more likely to be bound to an attractive surface.

To understand molecular details of the binding of DNA molecules on these surfaces, it may require appropriate molecular modeling for any future theoretical analysis. A recent theory on the attractive interaction between DNA molecule and cationic lipid bilayers showed a binding force of the order k_BT/b, with b the average distance between bases (Bruinsma, 1998). This value is within one order of magnitude of our experimental results. Other related theories on the attraction between DNA molecules in the 3-D condensation of DNA resulted in a binding strength approximately one order of magnitude smaller (Gronbech-Jensen et al., 1997). Therefore, our results indicate that new approaches may be required to understand the phenomenon of the molecular level binding processes involving DNA molecules. It is also possible to modify or refine current theories to account for the molecular level details of how DNA interacts with other entities, especially in light of recent development of a more comprehensive theory on DNA and cationic lipid complexes (May et al., 2000).

Long-range attraction

The observation of an attraction between DNA and cationic lipid bilayers well away from the tip-sample contact point is

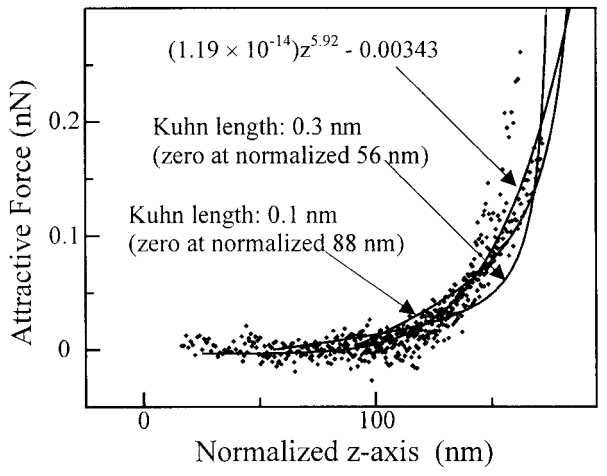

FIGURE 9 A normalized force curve and three fits. Dots are normalized datapoints from five force curves. The best fit is a power law with parameters shown. Two other fits are those of elastic polymers with Kuhn lengths and initiation points specified on the graph.

a new phenomenon. We note that the magnitude of the attraction just before the tip snapping back to the relaxed position is several times the minimum binding force as mentioned in the above, indicating that there is a different mechanism to hold DNA tightly bound to the bilayers. The simple binding does not play a parallel role to hold the DNA to the surface because the attraction shows a smooth and long-range character without any force steps. Therefore, our results reveal a different aspect of the DNA-lipid association.

To analyze this phenomenon, we normalized five force curves, by aligning them at 0.05 nN above the base line, and the result is shown in Fig. 9. The normalized force curve lacks the information about how far away from the tip-sample contact the attraction occurs. However, it allows us to examine the nature of the attraction after an average. Note the three fitting curves on the graph. The best fitting line is a power law with the zero as that of the normalized z axis in the figure, with fitting parameters shown. Although a polynomial function would fit the curve just as well, we chose the power law function so that the fitting has less variable parameters. At present, we do not have a theoretical model for this attraction. These fitting parameters will be useful for future theoretical modeling to compare with our experimental results.

The other two fitting curves in Fig. 9 correspond to modeling the DNA as freely jointed chains, because it has been shown that both DNA molecules and polysaccharides do behave like elastic polymers (Austin et al., 1997; Smith et al., 1992; Rief et al., 1997; Marszalek et al., 1998). However, both curves do not fit well to our experimental data. Even worse, the two fits result in Kuhn lengths of 0.1 nm and 0.3 nm, respectively, much shorter than the commonly accepted Kuhn length for DNA molecules (Schellman, 1974). These fits were also at different zero points, assuming only part of the molecule was under elastic strain, instead of the entire molecule between the tip and the sample. From these fits we see that if we set zero to be at the tip-sample contact point, an elastic polymer model would have no resemblance at all to our experimental force curves. Therefore, simple entropic elastic response by no means contributes to the observed attraction spikes.

The long-range character of the attraction indicates that the binding of DNA to cationic bilayers may involve some kind of attractive configuration potential and the measured force reflects the derivative of such a potential energy. It has been shown that on DPDAP cationic lipid bilayers, bound DNA molecules can condense into 2-D nematically ordered arrays (Fang and Yang, 1997b). Some initial successes in theoretical modelings have counted for several aspects of the 2-D condensation of DNA (Dan, 1996; Podgornik, 1997). Because we rule out the possibility that the observed phenomenon is attributable to a simple binding of a DNA molecule to the bilayer, it is likely that the detect long-range attraction is attributable to the attractive potential that drives the 2-D condensation of membrane-bound DNA.

The condensation of DNA on 2-D bilayers has been shown in several DNA-cationic lipid systems (Fang and Yang, 1997b; Mou et al., 1995; Clausen-Schaumann and Gaub, 1999). The actual potential energy responsible for the 2-D condensation of DNA must involve more than one base in the condensed DNA molecules. Because an exact length of the molecule involved in the condensation is not known, the energy per base for the condensation can not be determined from our force curves. According to the force curves, notable attraction was detected over a range of ~70 nm. That the breaking-away force is larger than the minimum binding strength shows that the interaction between condensed DNA molecules is stronger than a simple adsorption so that the energetic basis for the condensation exists. The long-range force also reflects the cooperative nature of the phenomenon so that a consideration of individual bases may not apply, further supporting the conjecture that the long-range force is more likely attributable to the condensation of DNA because that process is intrinsically cooperative. We hope our results provide a useful basis for future theoretical investigation at the molecular details.

We also note that our data are somewhat similar to an earlier report of a long-range attraction between DNA bases (Pincet et al., 1996). However, the origin of the attraction must be different. In that case, the interaction is between DNA bases in solution, possibly because of a correlation of orientation ordering (Pincet et al., 1996). In our experiments, the long-range is a restoring force from pulling on a single DNA chain, with one end of the chain bound to a cationic lipid bilayer. The recent theoretical work on complexes of DNA and cationic lipids may be extended to account for the observed phenomenon in real space (May et al., 2000).

Attachment of a globular structure

The change of the surface behavior from hard to soft, in addition to an attraction between the tip and the sample, is consistent with the picture that part of the DNA molecule below the tip was folded into some kind of global structure. Then, instead of a polymeric string, a soft globular structure was attached to the tip so that the overall response would be similar to the ones we observed (Fig. 8). The attraction still is the result of the binding of DNA to the surface. The breaking-away force is also larger than the minimum binding strength, which is consistent with the globular structure of folded DNA molecules, as several segments of DNA are likely to be involved in the binding of the soft globe to the surface.

CONCLUSION

Our results show, once again, the potential of AFM in biological applications. At the molecular level involving a single DNA molecule, the binding of DNA to the three kinds of surfaces contains several distinct physical characteristics. We show that the binding of a DNA molecule to a nonspecific target is associated with a minimum strength of ~45 pN. The divalent ion Mg^{2+} is likely to bridge intrastrand interaction to render a long DNA molecule more likely to adopt a conformation facilitating a stable bond to an attractive surface, rather than directly bridging the DNA to a substrate. The observed long-range attraction provide an experimental base for any future theoretical analysis or refinement of current theories on DNA-cationic lipid interactions.

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