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Adsorption of DNA to Mica, Silylated Mica, and Minerals: Characterization by Atomic Force Microscopy

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The nature of DNA adsorption to various substrates has been investigated by atomic force microscopy. The substrates used were mica, silylated mica, calcite, fluorite, and barite. The presence of a divalent cation, either in solution or accessible to the DNA on the substrate, greatly improves DNA adsorption. A comparison of DNA adsorption from different buffers onto mica and silylated mica shows that buffer composition is much less important for DNA adsorption to silylated mica and also suggests that for good adsorption DNA must be electrostatically adsorbed to the surface.

Introduction

Imaging DNA with an atomic force microscope (AFM) requires a substrate to which the DNA can be adsorbed from a variety of aqueous solutions. By choosing substrates with known surface chemistries, one can identify the important variables that affect DNA adsorption. DNA has been imaged with an AFM on a number of substrates including mica, silylated mica, carbon-coated mica, and thiols on gold.[1-8] We report here a comparison of DNA on mica and silylated mica in different buffers and a characterization of DNA on fluorite, calcite, and barite.

Early stable images of DNA were obtained on mica that was treated beforehand with a solution containing a divalent cation such as Mg^{2+}.[1-3] More recently it has been shown that DNA deposited onto bare mica from an aqueous buffer containing Mg^{2+} also yields stable images of DNA in air with contact[9] and tapping mode force microscopy.[10] DNA has also been imaged on mica treated with an amino silane (AP–mica).[5,6] Both substrates have limitations and advantages which differ according to the composition of the aqueous solution in which the DNA is deposited.

Fluorite, calcite, and barite are cleavable minerals, like mica, with well-known surface charges. Studying the adsorption of DNA on these surfaces can provide information about how DNA adsorbs in general. We have specifically looked at the density of DNA on the surface as well as the manner in which the DNA is spread on the surface.

Table 1. Adsorption of Plasmid DNA to Mica and AP–Mica[a] (Plasmids/μm^2)

plasmid DNA in	bare mica	AP–mica
H_2O[b]	0.4 ± 0.1	6.4 ± 3.0
HEPES–Mg		
40 mM HEPES–KOH	3.2 ± 2.2	3.9 ± 2.8
10 mM $MgCl_2$		
pH 7.6		
Tris–Mg		
40 mM Tris-HCl	1.9 ± 0.8	not measured
10 mM $MgCl_2$		
pH 7.6		
Tris–Mg–K		
50 mM Tris-HCl	0.4 ± 0.2	5.6 ± 2.5
10 mM $MgCl_2$		
50 mM KCl		
pH 7.6		
Tris–EDTA		
10 mM Tris	0	4.4 ± 2.4
1 mM EDTA		
pH 7.6		

[a] Data are means ± SD for three to nine independent DNA samples and are based on a DNA concentration of 1 ng/μL. Similar plasmid densities were obtained for sample volumes of 0.2 and 1 μL. Differences in density from place to place on a sample were typically less than 20%. [b] H_2O contained 0.1 mM Tris and 0.01 mM EDTA from the buffer in which the DNA was supplied. Data were taken from samples made with concentrations varying from 0.5 to 10 ng/μL. The data were normalized to a concentration of 1 ng/μL by dividing or multiplying the number of plasmids/μm^2 by the appropriate scaling factor.

Methods

DNAs. Bluescript II SK(+) double-stranded plasmid DNA (2961 bp, 1 mg/mL) was obtained from Stratagene (La Jolla, CA) supplied in 10 mM Tris, 1 mM EDTA. Bluescript was diluted either with water or with buffer as noted in Table 1. The extent of supercoiling was reduced by heating Bluescript DNA at 65 °C for 10–15 min or more.

ϕX174 RF1 double-stranded DNA (5386 bp, 950 μg/mL) was obtained from Pharmacia Biotech (Piscataway, NJ) supplied in 10 mM Tris, 1 mM EDTA. The ϕX174 double-stranded DNA was diluted to a final concentration of 100 ng/μL with Milli-Q water (filter system obtained from Millipore, Burlington, MA).

Mica. Disks of ruby mica (New York Mica Co., New York, NY) were glued with 2-Ton epoxy (Devcon Corp., Wood Dale, IL)

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to steel disks that had a transmission electron microscopy locator grid (Microscopy Sciences, Fort Washington, PA) glued to the center.

Adsorbing DNA to Mica. One microliter of Bluescript plasmid DNA, diluted in water, was placed on freshly cleaved mica. Some samples were immediately blown dry with compressed air (Figure 1a) and others sat on the mica for 30 s to 1 min (Figure 3a) before drying. A 0.2–1 μL portion of Bluescript plasmid DNA, diluted in buffer (see Table 1), was placed on freshly cleaved mica, immediately rinsed with 2 mL of Milli-Q water, and subsequently blown dry with compressed air.

Adsorbing DNA to AP–Mica. AP–mica was prepared by placing freshly cleaved mica into a 2 L glass dissector which contained 30–100 μL of (3-aminopropyl)triethoxysilane (APTES), 98%, obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) for 2 h. The AP–mica was then removed and stored under argon prior to making samples. The best AP–mica surfaces were prepared with 30 μL of APTES that had been redistilled in vacuum and stored under argon; the silylation was also done under argon.

A 1–5 μL portion of Bluescript plasmid DNA was placed on AP–mica for 3 min and then rinsed and dried as indicated above. When DNA was diluted in water, the samples were not rinsed.

Adsorbing DNA to Minerals. All minerals were cleaved within 5 min prior to deposition of DNA. A 0.5 μL portion of ϕX174 RF1 double-stranded DNA, at the dilution indicated above, was deposited on the freshly cleaved mineral surfaces. The samples were immediately blown dry with compressed air.

AFM Imaging. Prior to imaging, all samples were dried in a 2 L glass dissector in the presence of P_2O_5 (keep off skin). Tapping AFM was performed in dry helium using a Nanoscope III (Digital Instruments, Santa Barbara, CA) as described previously,[10] except for samples imaged at humidities above 40% relative humidity (RH). These samples were imaged by contact AFM with a Nanosocpe II. Adhesion forces cannot be calculated at present for tapping AFM, in which the forces vary continuously. The AFM head was leveled by using a feeler gauge in order to minimize tilt. The AFM was suspended from bungee cords for vibration isolation.

Heights and widths at half–height were measured with the Section–Cursor–Average command of the Nanoscope III version 3.12 software. This command allows one to measure the average height and width of DNA along a linear region.

Results

DNA Adsorbed to Mica. The amount of DNA adsorbed to mica depends strongly on the buffer composition. DNA in HEPES–Mg buffer adsorbs well to mica (Table 1, Figure 1c). Less DNA adsorbs from a Tris–Mg buffer (Table 1). Much less DNA adsorbs to mica from water or a Tris–Mg–K buffer (Table 1, Figure 1a), and DNA does not adsorb to mica from Tris–EDTA (Table 1) or 40 mM HEPES–20 mM KCl (unpublished results).

At high concentrations, circular DNA spreads on mica much better in HEPES–Mg than in water (Figure 2). In fact, DNA in water will aggregate on mica even at low concentrations. To prevent DNA aggregation in water, the sample must be blown dry immediately as in Figure 1a. The sample in Figure 2a was made with the same DNA concentration, but it was not blown dry immediately. Therefore, if the sample is allowed to sit for more than 30 s, DNA in water on bare mica will aggregate. Similar results have been reported previously.[11] The sample in Figure 2b was made with DNA at the same concentration but in HEPES–Mg buffer, and it did not aggregate even though it sat on the mica for 1 min before rinsing. In contrast to the aggregated DNA in Figure 2a, the DNA in Figure 2b forms a dense network of individual strands which are separate from each other over most of the surface.

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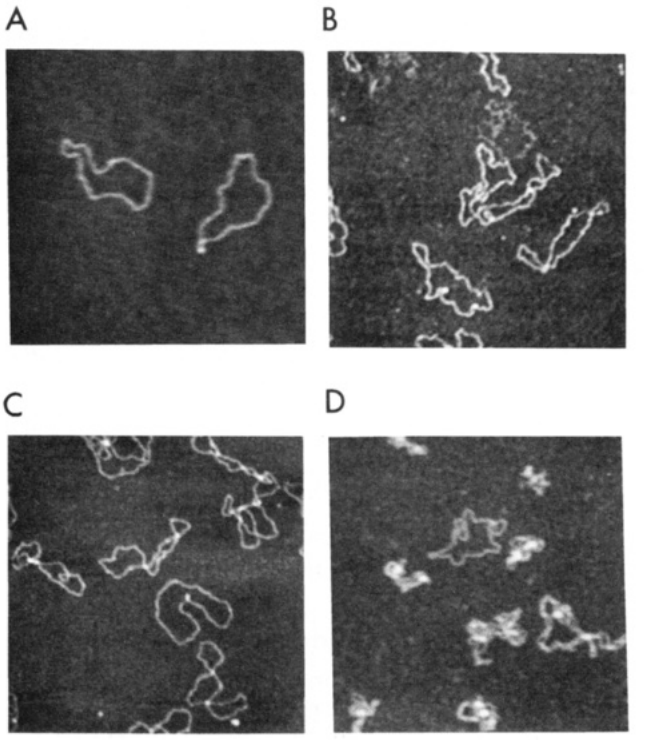

Figure 1. Comparison of plasmid DNA under different conditions on mica and AP–mica: (a) DNA in water on bare mica, (b) DNA in water on AP–mica, (c) DNA in HEPES–Mg (Table 1) on bare mica, and (d) DNA in HEPES–Mg (Table 1) on AP–mica. All images were taken in the tapping mode. All scan sizes are 1000 nm by 1000 nm.

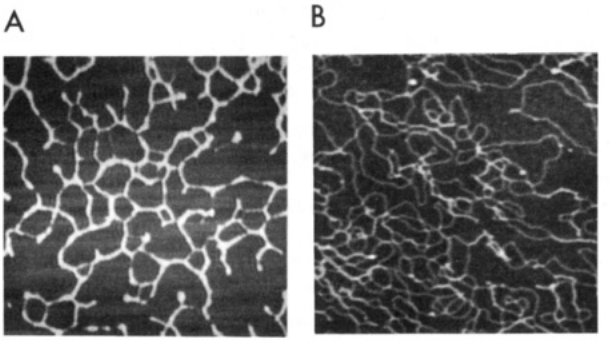

Figure 2. (a) Plasmid DNA in water at a concentration of 10 ng/μL on bare mica. This sample sat for 30 s on the mica before it was blown dry. (b) Plasmid DNA in HEPES–Mg (Table 1) at a concentration of 10 ng/μL on bare mica. This sample sat for 1 min on the mica before it was blown dry. Aggregation seems to appear only when DNA is in water; when DNA is in a buffer solution, it tends to spread well on the mica surface. Image a was taken in the contact mode. All scan sizes are 1000 nm by 1000 nm.

The measured dimensions of DNA on mica were 0.43 $\pm$ 0.08 nm height and 11.2 $\pm$ 1.8 nm width at half–height. There was no difference in the measured dimensions between DNA in water and DNA in HEPES–Mg buffer.

DNA Adsorbed to AP–Mica. DNA adsorbs better to AP–mica than to bare mica (Table 1)[5] in every solution except HEPES–Mg buffer. Even in Tris–EDTA (Table 1) and in solutions of high concentrations of salt such as 100 mM imidazole (unpublished result), DNA adsorbs well to AP–mica. Especially in HEPES–Mg (Figure 1d) and Tris–Mg–K, circular DNA molecules are more compact on AP–mica than on bare mica.

On AP–mica, DNA adsorbs well even in the absence of a divalent cation. This is a significant improvement over bare mica because it provides more flexibility with respect

to the kind of buffers present in the DNA solution at the time of adsorption. However, AP–mica can be more variable in quality than bare mica. When there is good silane coverage, the surface is flat and DNA is readily resolved, but when the silane coverage is uneven, it can be difficult to resolve small pieces of DNA. It is not clear what determines optimal silane coverage. The ambient humidity, the quality of the silane, and the quality of the mica surface are factors that may affect the silylation.

The measured dimensions of DNA on AP–mica were 0.44 ± 0.07 nm height and 13.9 ± 2.1 nm width at half-height. There was no difference in the measured dimensions between DNA in water and DNA in HEPES–Mg buffer.

With contact AFM, DNA on bare mica is more easily damaged at humidities above 40% RH[1,2,12] AP–mica showed no advantage over bare mica for imaging DNA at 50% RH with contact mode AFM. DNA on both substrates was readily damaged at scan sizes on the order of 500 nm and below; imaging forces were ca. 5–10 nN.

AP–mica has a shelf life of approximately 1 month and has a more hydrophobic surface than bare mica, presumably due to the propyl chains that are attached to the surface amino groups.

DNA Adsorbed to Various Minerals. Circular double-stranded ϕX174 DNA was adsorbed to mica (Figure 3a), calcite (Figure 3b), fluorite (Figure 3c), and barite (Figure 3d). This DNA was more supercoiled than the Bluescript in Figure 1, but a significant percentage of the DNA is also relaxed. The supercoiling is evident in Figure 3a (arrow). Some relaxed DNA is also seen on fluorite but not on calcite. We believe that the DNA molecules on calcite are not supercoiled but that they have aggregated in order to avoid the calcite surface. Barite seemed to attract DNA the most of the four minerals, but the DNA aggregated into a dense network on the surface.

Discussion

Adsorption to Mica. Freshly cleaved mica has a negative surface charge due to the presence of hydroxyl groups recessed slightly below the surface, with a spacing of 0.5 nm. Potassium ions bridge between hydroxyl groups, or alkoxide ions, in adjacent layers. When exposed to an aqueous solution containing cations such as Mg^{2+} or Ca^{2+}, mica undergoes an ion exchange process in which the potassiums on the surface of the mica are replaced by the cations in solution. In this case, the Mg ions which have a valence of +2 can replace the potassiums which have a valence of +1.

If we consider that the adsorption of DNA to the surface is primarily motivated by an electrostatic interaction, then when the DNA is in solution with a divalent cation, the cation can form a charge bridge between the negatively charged mica surface and the negative phosphate groups of the DNA backbone. For a DNA concentration of 1 ng/μL there is a concentration of 2.8 μM phosphate groups. Typically our solutions contained 1–10 mM $MgCl_2$, which yields an excess of Mg ions by 3 orders of magnitude. The concentration of cations such as Mg^{2+} at the surface of a polyelectrolyte such as DNA can be as high as 1 M, even when the total cation concentration is low.[13] Calcium and other divalent and trivalent cations also promote DNA adsorption to mica.[11,14,15]

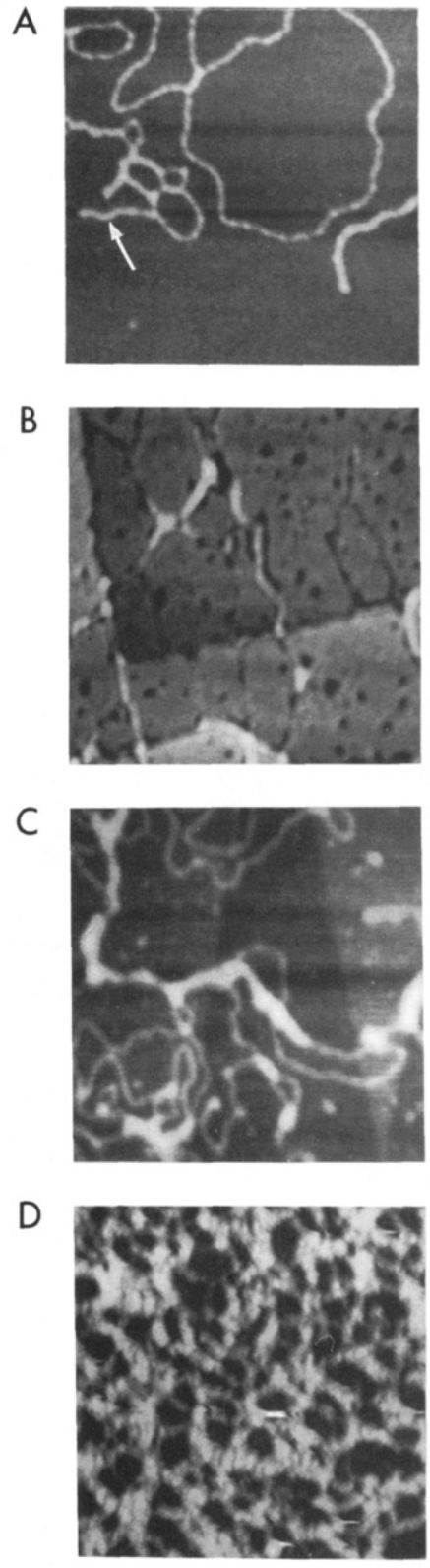

Figure 3. Comparison of circular double-stranded ϕX174 RF1 DNA deposited onto (a) mica, (b) calcite, (c) fluorite, and (d) barite. All samples were made with DNA at a concentration of 50 ng/μL. All images were taken in the tapping mode. All scan sizes are 400 nm by 400 nm.

The preceding argument refers mainly to divalent and trivalent cations in solution, not taking into account the other ions that might be present in solution. It is evident from Table 1 that a HEPES buffer allows more adsorption

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to bare mica than a Tris buffer. This might be due to the fact that Tris is positively charged in solution at neutral pH. Thus Tris may interfere with the binding of Mg^{2+} to the mica and to the DNA. HEPES has heterocyclic rings and both positively and negatively charged groups at neutral pH, which give it the capability for a variety of different interactions with DNA and mica.

DNA plasmids spread well on bare mica, especially in the presence of Mg ions. The minimum energy configuration for each DNA molecule is for it to have as many of the phosphate groups electrically neutralized as possible. In Mg-buffer, the phosphate groups and the mica surface can both be neutralized by the large excess of Mg ions. The good spreading of DNA seen in Figures 1c and 2b suggests that Mg^{2+}-induced cross-bridges are formed primarily between mica and DNA phosphates. In contrast, DNA in water tends to aggregate on mica (Figure 2a). This might be due to the fact that in water there are few counterions. Those that are present will be near the DNA in order to electrically neutralize the phosphate backbone. Therefore the DNA will tend to stick to itself around these counterions, instead of to the mica surface since the surface is negatively charged. If the samples are blown dry within 30 s of placing the drop on the mica, the DNA in water on mica does not aggregate because there is not a high enough concentration of DNA. In such samples, the individual DNA molecules are generally well spread on the mica, as in Figure 1a. A similar result has been seen with supercoiled DNA, imaged in solution with an electron microscope, which is relatively uncoiled in a dilute salt-free buffer.[16] But if DNA in water sits on the mica for more than 30 s, the DNA aggregates on the surface to reduce its contact area with the negatively charged mica surface. When the DNA is dissolved in Mg-containing buffer, it does not aggregate when adsorbed to mica (Figure 2b). Even at high concentrations, the DNA in Mg-buffer on mica tends to avoid itself as much as possible. Thundat et al. have also observed this with longer, linear DNA molecules.[17]

The AFM is imaging bare, uncoated DNA molecules in these experiments. The evidence for this is that the Bluescript DNA as supplied in Tris–EDTA has only 1.6 ng of Tris + EDTA per ng of DNA. When this DNA is diluted 100-fold in water, applied to mica, and blown dry immediately, as in Figure 1a, it is statistically most probable that the Tris and EDTA are bound primarily to the mica, not to the DNA. Measured heights and widths of DNA are similar for DNA in water and in the buffers used here and are not changed significantly by rinsing the sample with high-pressure water. Therefore it appears that the AFM is also imaging DNA without much adsorbed solute in samples prepared from DNA in buffer.

Adsorption to AP–Mica. AP–mica has amino groups exposed on the surface. Aliphatic amino groups have a pK of approximately 10.6. Although the close packing of the aliphatic amino groups on the AP–mica surface decreases the pK, the surface will still be positively charged in solution at neutral pH. Therefore, the negatively charged DNA adheres well to this surface. The buffer composition has little effect on DNA binding to AP–mica.

Plasmid DNA molecules on AP–mica are much more extended if they are deposited in water (Figure 1b) than if they are deposited in buffer (Figure 1d). Plasmids in buffer have fewer exposed negative sites on the DNA due to the excess of counterions in solution. In contrast, the negative charges on plasmids in water have a strong electrostatic attraction to the positive surface of AP–mica. Thus the minimum energy configuration in water is for the plasmid to be well spread. The condensed form of plasmids in Mg-buffer on AP–mica (Figure 1d) may be due to intrastrand Mg^{2+} cross-bridges. A similar Mg^{2+}-induced condensation of circular DNA has been observed by electron microscopy of DNA in solution.[16]

A silylated surface has also been used as a substrate for covalent attachment of proteins and biological membranes for AFM,[18,19] but more recently, biological membranes have been adsorbed to bare mica,[20] which produces a flatter membrane surface for AFM imaging.

Adsorption to Minerals. Since divalent cations are implicated here and elsewhere[1,2,11,14,15] in the binding of DNA to mica, a relevant comparison is the binding of DNA to the cleavage planes of other crystals containing divalent cations. DNA shows varied adsorption behavior on the four minerals investigated (Figure 3). We believe that the explanation involves both the solubilization of the minerals and the surface charges at the solution interface.

When the drop of DNA solution is placed on the mineral surface, the latter dissolves until saturation is reached. In the case of calcite, $CaCO_3$, the saturation density of Ca^{2+} ions is 140 μM at neutral pH. This is enough to neutralize much of the DNA in solution before the drop is blown dry, since the PO_4^- density is approximately 280 μM for the DNA concentration used. Unlike mica, there is no net charge on the calcite surface to facilitate binding to the surface Ca^{2+} ions. Thus the DNA does not spread on the calcite surface and tends to be trapped in small etch pits and at cleavage steps (Figure 3b).

Cleavage of barite, $BaSO_4$, produces a surface containing both Ba^{2+} and SO_4^{2-}. The solubility of barite is much lower than that of calcite; the saturation concentration of Ba^{2+} is 9 μM. Since this is not nearly enough to neutralize the DNA, the phosphate groups bind to the fixed Ba^{2+} ions on the cleavage plane when the solution is dried. Although the surface is overall neutral, the presence of divalent positive charges is apparently enough to cause strong binding to the DNA backbone, resulting in dense adsorption (Figure 3d).

The case of fluorite, CaF_2, is more complicated. The cleavage plane of fluorite, unlike calcite and barite, consists of a surface layer of only F^- ions,[21] with a layer of Ca^{2+} slightly below the F^- layer. The fluorite surface reconstructs to form needles and small triangular etch pits, with the release of Ca^{2+}. Fluorite saturates water with 200 μM Ca^{2+}, which is approximately enough to bind to all of the DNA in solution. Thus the DNA-bound Ca^{2+} ions sense a negative surface layer, which probably facilitates some surface binding, leading to stretched filaments (Figure 3c), even though the crystal is electrically neutral overall.

AFM of DNA in Air and Aqueous Solutions. DNA on mica can be imaged in air or dry gas, as described here, and also in water or dilute aqueous buffer.[14,15,22] DNA on AP–mica has been imaged in water[6] and aqueous buffer (D. Laney, unpublished results). Imaging in air or dry gas is a convenient way to routinely assay DNA samples for characteristics such as DNA bending.[23,24] The ad-

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vantage of imaging DNA in aqueous buffers is that the DNA is in a more nearly physiological environment, where enzyme activities on DNA have been observed by AFM.[15,25] Under some conditions, even DNA in motion can be imaged in the AFM.[15]

Conclusion

Studying the adsorption of DNA to different surfaces under varying conditions has provided a greater understanding of how DNA adsorbs to surfaces of known chemistry. Bare mica is a good substrate for obtaining clear, crisp images of well-spread DNA, especially in low-salt Mg-buffers. DNA in other buffers, especially those lacking a divalent or trivalent cation, adsorbs better to AP–mica. Electrostatic models can explain the results obtained for binding of DNA to silylated mica and minerals.

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