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Atomic force microscopy of DNA molecules

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DNA–cytochrome *c* complexes adsorbed on carbon-coated mica surfaces were directly imaged by atomic force microscopy in air using commercially available cantilevers, with a routine resolution of 6 nm. Images of M13 phage DNA and M13–DNA polymerase complex are also shown.

AFM imaging; DNA; DNA–protein complex

1. INTRODUCTION

Although scanning tunneling microscopy (STM) has been applied to various biological specimens with considerable success [1–3], the lack of understanding of the contrast formation and the difficulty of preparing stable and super-thin specimens along with the profound artifacts from substrates have been the major obstacles for STM application in routine biological experiments. The subsequently developed atomic force microscope (AFM) [4] has long been recognized as the preferred imaging tool in the scanning probe family (SPM) for the study of biological structures [1,5–10], for its relatively simple imaging principle and its suitability for non conductors. It has been reported recently that DNA molecules were directly imaged by AFM in air on treated mica surface [11,12]. It has been reported [12] that 10 nm resolution was obtained with DNA bound to the magnesium acetate-treated mica surface, using an electron beam fabricated stylus. It was claimed that the resolution was limited by the stylus geometry and humidity control was crucial to the reduction of the probe force for reproducible imaging [12]. In the present study, we report that stable and reproducible AFM images of DNA–protein complexes in air using commercially available AFM cantilevers were obtained with a resolution of 6 nm on cytochrome *c*–DNA copolymers. When specimen surface charge was removed, probe force could be minimized without the need of humidity control. Images of M13 phage DNA and M13–DNA polymerase complex, prepared by this method, will also be presented.

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2. METHODS AND MATERIALS

2.1. *Sources of DNA*

Double-stranded plasmid DNA (10 kbp), consisting of a cDNA encoding the $(Na^+ + K^+)$-ATPase α-subunit and a mammalian expression vector, was kindly provided by Dr. H. Yu. M13 DNA (7.1 kb) supplied in a Sequenase kit was used as the single-stranded DNA sample. DNA (2 μg/ml) was dissolved in cytochrome *c* (horse heart, type III; Sigma) (100 μg/ml) solution with 10 mM Tris, 1 mM EDTA, pH 8, and 50% formamide. NH_4-acetate can also be used instead of formamide, but the resolution is much worse than the present method.

2.2. *Substrate*

Freshly cleaved mica was carbon-coated at low current (~45 A, 30–40 min) in an evaporator (DV502A, Denton Vacuum) for smaller grain size. When imaged by AFM, well prepared substrates showed very small grain structures with very low height variations on the surface. Other substrates, such as freshly cleaved mica or graphite did not provide stable adhesion and are unsuitable for AFM imaging. Microscope cover slips showed too many large scale features which could interfere with the recognition of DNA molecules, thus, are not useful in this resolution range.

2.3. *Monolayer adsorption*

The procedure outlined by Inman and Schnos [13] was used to adsorb the specimen to the substrate. A 0.5 ml deionized distilled water drop was applied to a 0.5" drilled dent on a teflon block, serving as the hypophase. A 5 μl DNA solution was directly drained to the water drop surface via a glass rod [13]. Too much reduction in the water drop surface area could result in too thick a film on the substrate. After the specimen was picked up by the carbon-coated mica, excessive fluid on the surface was blotted-off by optical tissue papers and it was dried in a desiccator.

2.4. *Instruments*

A home-made AFM head with a motion stage retrofitted to a NanoScope II SPM base was used to acquire most AFM images. Cantilevers were purchased from Digital Instruments, Inc. and used directly without further manipulation. Among the types of stylus available, the one with a spring constant of 0.06 N/m was used.

3. RESULTS AND DISCUSSION

Figure 1 shows a high-resolution image of double-stranded circular plasmid DNA (10 kbp)–cytochrome *c* copolymer adsorbed onto the surface of carbon-

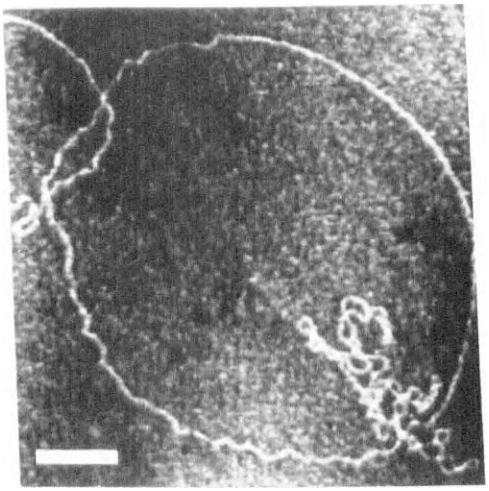

Fig. 1. AFM image of 10 kbp double-stranded circular plasmid DNA, copolymerized with cytochrome *c*, adsorbed onto a carbon-coated mica surface. Only plane fit was used to flatten-out the background. The measured molecular length of the fully relaxed molecule is 3.4 μm. Molecular width measured is 9–10 nm. A part of another molecule (upper left corner) and a molecule in a supercoiled form (lower right corner) are also shown. The scanning speed was 8.68 Hz. Scale bar = 200 nm.

coated mica with a resolution of 9–10 nm. When NH_4-acetate was used, lateral resolution was much worse, up to 80 nm on many occasions. With the present method, the best resolution consistently achieved was about 6 nm (Fig. 2). These images were extremely stable with repeated scans if the set-point voltage was kept below zero. Optimization in regard to reducing the probe force by lowering the set-point voltage was not critical on image quality. The stable adhesion between the cytochrome *c*–DNA copolymer and the carbon-coated mica may be due to the increased surface roughness which not only increases the contact area between the polymer and the substrate, but also increases the friction to enable the polymer to better withstand the disturbance of the AFM probe. Cytochrome *c* may also play a role in specimen stabilization. To fully understand its function, some details of cytochrome *c*–DNA interaction in air are needed. In this study, we have used more than 10 cantilevers without pre-selection, and almost all of them achieved excellent resolution, indicating that the commercially fabricated stylus is sufficiently sharp for this type of application. This fact also seems to indicate that, at least down to 6 nm, the resolution is probably limited by the cytochrome *c* bound to DNA, rather than stylus geometry. For the plasmid DNA, the molecular length measured from 8 individual molecules is 3.4 ± 0.2 μm. The expected molecular length of 10 kbp DNA in the B form is 3.2 μm [14]. Since all images were taken in air without humidity control, it appeared that humidity setting is not important for AFM imaging in this case. The soft nature of the protein–DNA complex and the sub-10 nm resolution obtained in air seem to disagree with the accepted value for surface adhesion force of the order of 100 nN [15]. We found that residual surface

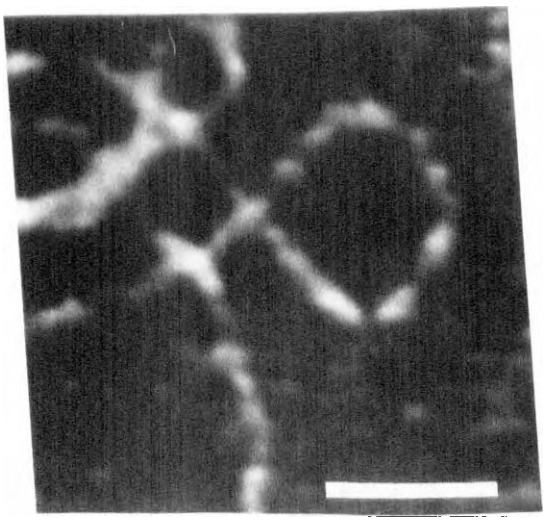

Fig. 2. Part of a 10 kbp plasmid DNA viewed at a smaller scale. The smallest molecular width measured here is about 6 nm, demonstrating the exellent resolution of AFM. Scale bar = 50 nm.

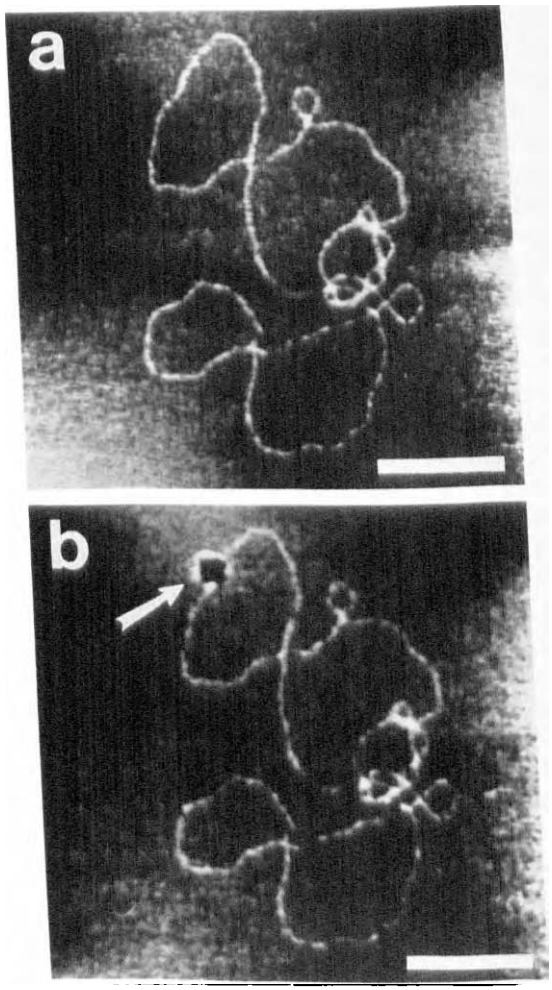

Fig. 3. AFM images of a 10 kbp plasmid DNA before (a) and after (b) cutting. The arrow in (b) indicates the small area scanned at 78.13 Hz and a higher force, in which the DNA is completely destroyed. Scale bars = 200 nm.

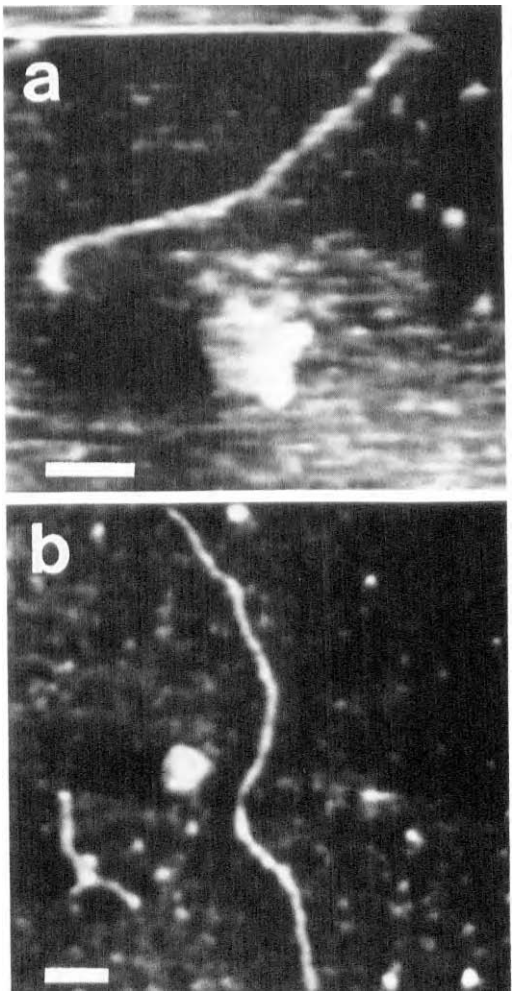

Fig. 4. Typical AFM images of M13 single stranded DNA: (a) a single phage DNA (7.1 kb) measured 888 nm long (scale bar = 200 nm); (b) a filament appeared to contain 2 phage DNA's with a measured length of 1650 nm (scale bar = 200 nm).

charge on the specimen appeared to facilitate a large adhesion force as indicated by the decrease of the detector differential signal at the initial approach. This large adhesion force prevented any stable image being obtained. But proper grounding of the specimen removed this problem entirely. The same specimen, unstable when floating, yielded excellent images after grounding. We also experimented with cutting part of a DNA molecule directly via a higher scanning speed and higher probe force. Figure 3 shows the same molecule before (a) and after (b) cutting. As shown, the part of the molecule within the small area scanned at higher speed and force was completely destroyed, but the rest of the molecule remained intact which was not dragged around by the probe. Since the entire molecule can be imaged, this method would be of some use if we could put markers on the DNA at selected sites.

After the method was established, we imaged M13 single-stranded circular DNA. It is known that M13

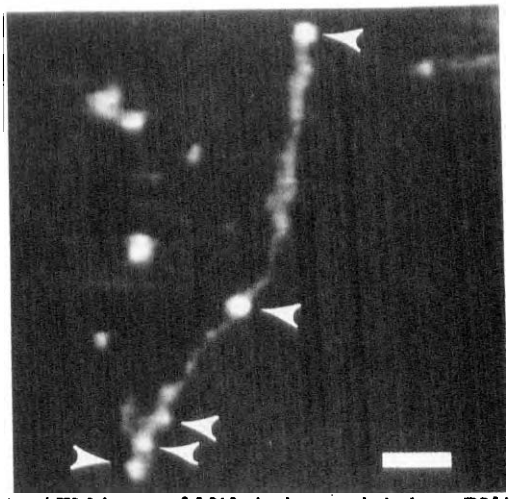

Fig. 5. An AFM image of M13 single-stranded phage DNA with 5 bound Klenow fragments of DNA polymerase I (see arrows). The molecule measures 860 nm long. The slightly shorter length may be due to less compact molecular packing. A random priming reaction was conducted at room temperature for 10 min using a Prime-a-Gene labeling system. The system (10 μl) consisted of M13 DNA (0.2 μg/μl), dNTPs (200 μM each), random primers (50 μM), Klenow fragments (5 μM), $MgCl_2$ (10 mM) and Tris-HCl (50 mM, pH 7.6). Scale bar = 100 nm.

DNA is packed into an 895 nm filamentous phage particle [16]. Among the specimens imaged without the native coat proteins, 890 nm long filaments were found everywhere. But we did not find any relaxed circular form. The measured length from 17 such filaments is 894 ± 62 nm, similar to the phage particle in length. Figure 4a shows a typical AFM image of a single phage DNA. This result indicates that M13 DNA is not relaxed by cytochrome *c* treatment under these conditions, probably due to some internal complementary sequences [17] and bond-angle constraints. We also found some longer filaments as well, as shown in Fig. 4b. Most long filaments so far measured have a length that is an integral multiplication of about 890 nm, and the longest one has a length of 4.4 μm. The reason for this polymerization is not clear. To make sure these observations were not artifacts of cytochrome *c* polymerization, we also imaged specimens with cytochrome *c* only without M13 DNA added, prepared by the same method. Similar structures were never found. In addition, these observations were also confirmed by transmission electron microscopy with uranyl acetate staining, where carbon film on a copper grid was used to pick up the specimen (50 mM uranyl acetate, 50 mM HCl [18]). Despite the broadening effect of cytochrome *c*, when random primer and Klenow fragment of DNA polymerase I were added, enzyme–DNA complex were also observed by AFM. Figure 5 shows one such image at high resolution, with 5 DNA polymerase molecules on the DNA. On control specimens, without added Klenow fragment, similar structures were not found. This

success in imaging DNA–enzyme complexes suggests the possibility that the present approach with a specific primer could be used to identify a specific position of a DNA molecule. It should be also possible to study protein–DNA interactions.

In conclusion, we have demonstrated that DNA–protein complexes can be routinely imaged by AFM in air, with a resolution of 6 nm. Based on AFM images, it is found that, with the present preparation method, single stranded M13 phage DNA is in a filamentous form with the length similar to the natural phage, and, in addition, longer filaments are also observed. We have found that the force exerted on the specimen by the AFM probe seems much smaller than previously believed. If this conclusion can be confirmed by future experiments, our current thinking on probe-surface interaction must be re-examined.

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