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Atomic Force Microscopy of DNA Molecules Stretched by Spin-Coating Technique

Jing Yong Ye,*[,1] Kazuo Umemura,* Mitsuru Ishikawa,* and Reiko Kuroda†[,‡]

**Angstrom Technology Partnership and †National Institute for Advanced Interdisciplinary Research, Joint Research Center for Atom Technology, 1-1-4 Higashi, Tsukuba, Ibaraki 305-0046, Japan; and ‡Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, 3-8-1 Komaba, Meguro-Ku, Tokyo 153-8902, Japan*

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We have developed an effective approach to stretching DNA molecules with the flow of fluid generated by spin coating. Well-stretched λ DNA molecules were observed using atomic force microscopy. Substrate properties sensitively affected the stretching behavior of DNA. Our experimental findings revealed that a mica surface treated with crystal violet, a cationic dye molecule, is suitable to the spin-coating procedure for stretching DNA. Moreover, compared with relaxed DNA, we observed reduced height of the stretched DNA, which was attributed mainly to elongation force applied to the DNA molecules from the fluid flow and strong adhesion force between DNA and the substrate. This simple and effective method for preparing stretched DNA could be useful in physically mapping genomic DNA in a high throughput. **© 2000 Academic Press**

Key Words: **straightened DNA; spin coating; atomic force microscopy.**

Atomic force microscopy (AFM)[2] has a dramatic impact on biological sciences owing to its high spatial resolution. DNA is one of the most extensively studied molecules using AFM to understand its structure and interactions with protein molecules. However, the long DNA molecules are subject to entanglements or aggregations, which make measurements difficult or impossible to obtain detailed structural information. The sample preparation, therefore, becomes extremely important for AFM measurement of DNA. Several methods have already been developed to prepare straightened DNA molecules. Bensimon *et al.* established a method well known as molecular combing for alignment of DNA molecules (1, 2). Thundat *et al.* obtained stretched DNA in a sample rinsed with a jet of water and followed by air-drying or critical point drying (3). Wang *et al.* elongated DNA molecules using convective fluid flows generated within an evaporating droplet of DNA solution (4). Recently, a method based on a motor-controlled moving meniscus was developed to straighten DNA molecules and protein–DNA complexes as well (5). This technique allows large-scale physical mapping to identify protein-binding sites on DNA. We explored an approach to straightening DNA molecules using fluid flows generated by a spin-coating procedure. This approach is technically simple and effective.

The chemical and physical characteristics of substrates also play an important role in the preparation of DNA samples for AFM measurements. In addition to the requirement of surfaces with atomic flatness, anchoring sites are demanded on substrate surfaces to immobilize DNA molecules. Thus, substrate surfaces are often derivatized with positively charged silane compounds (1) or divalent metal ions (5, 6–8) to improve retention of DNA on the substrates.

In this paper we report the AFM imaging of the well-stretched DNA molecules prepared by the spin-coating technique. We tested mica substrates treated in different ways and found that a mica surface treated with crystal violet (CV), an organic cationic dye molecule, was a good substrate for the spin-coating procedure for stretching DNA, while other substrates including underivatized mica, silanized mica, and amine-terminated mica were not suitable for this study. We discuss the different binding forces between DNA and these substrates.

[1] To whom correspondence should be addressed. Fax: +81-298-54-2714. E-mail: jyye@jrcat.or.jp.

[2] Abbreviations used: AFM, atomic force microscopy; APTES, 2-aminopropyltriethoxysilane; MAPTMS, 3-methacryloxypropyltrimethoxysilane; TPM, triphenylmethane.

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MATERIALS AND METHODS

Preparation of a DNA Solution and Substrates

λ bacteriophage DNA (Takara Shuzo, Japan) was diluted to 0.2 nM in a 50 mM Mes buffer (pH 5.5). Substrates used in the experiments were freshly cleaved mica (Oken Shoji) and three types of derivatized mica. One derivatized mica was made by placing a freshly cleaved mica plate into a 0.35 mM CV (Nakalai-Tesque, Japan) water solution for 30 min and then the plate was rinsed in Milli-Q water and air-dried before use. The others were made in the same procedure as described above except for the solutions used to soak the mica plates: a 0.1% (v/v) 3-aminopropyltriethoxysilane (APTES) water solution and a 0.1% (v/v) 3-methacryloxypropyltrimethoxysilane (MAPTMS) water solution.

Spin-Coating Procedure

A spin coater (Model K-359SD-1, Kyowariken) was employed to coat the DNA solution onto various substrates in a procedure as follows. First, a DNA solution (80 μl) was deposited on a substrate 2 min before spin coating. This resulted in DNA molecules being bound to the anchoring sites of the substrate. Second, the substrate was accelerated up to its final, desired rotation speed ranging from 1000 to 7000 rpm. This step was accompanied by expulsion of the excess DNA solution from the surface owing to the rotational motion. Third, 1000 μl Milli-Q water was dropped from a pipet tip to the center of the rotating substrate to generate a water flow from the center to the edge of the substrate. The water flow aligned DNA molecules anchored on the substrate, while it washed out the remaining salts from the buffer solution. Fourth, after water was dropped, the remaining water formed a thin layer on the substrate, which had a gradually and uniformly thinning process with outward water flowing that further aligned and stretched the DNA molecules. Finally, when the thickness of the water layer reached a point where essentially no net water flow occurred due to the friction of the substrate, evaporation of the solvent became a dominant process (9, 10). The substrate continued to rotate at a constant rate for total 2 min. Thus, we obtained a dry and elongated DNA sample.

AFM Imaging

AFM images were collected in air at room temperature with a Nanoscope III (Digital Instruments) working in tapping mode with typical resonant frequencies of 300 kHz. The silicon cantilevers of 126-μm in length had a spring constant of ~60 N/m, and the tip had an estimated curvature of 10 nm. The scan rate was 1.97 Hz with a scan range of 1–5 μm. The images presented in this paper are free of modification except for flattening to remove the background curvature of substrate surfaces.

RESULTS AND DISCUSSION

The λ DNA molecule used is in B form with 48.5 kb corresponding to a contour length of ~16.5 μm by assuming the axial rise of 0.34 nm/bp. Such long molecules lead to aggregations and/or entanglements on substrates if no proper sample preparation is applied. We employed a spin-coating procedure to prepare straightened and immobilized DNA molecules for AFM imaging. The substrate used for spin coating was pretreated by soaking a freshly cleaved mica plate in a 0.35 mM CV solution to improve the retention of DNA molecules. In contrast to normal randomly distributed DNA with relaxed structures, Fig. 1A shows an AFM image of stretched DNA molecules spin-coated on the CV-soaked mica surface, although we also observed a slight variation in DNA straightness from one sample to another and from one region to another on the same substrate. The sectional image of the stretched DNA shown in Fig. 1B will be discussed in the latter part of this paper, together with the results of DNA spin-coated on other substrates.

The physical and chemical characteristics of the substrates used are critical factors of stretching DNA in the spin-coating procedure. We applied the spin-coating technique to different substrates. The finding that few DNA molecules were observed when a freshly cleaved mica was used (data not shown) indicated that an underivatized mica surface lacks anchoring sites for binding DNA molecules. A mica surface then was treated with MAPTMS, which reacted with SiOH groups on the mica surface, leaving the vinyl group exposed. However, we did not observe immobilized DNA molecules on the silanized mica surfaces after the spin-coating process (data not shown). This result implied that the binding force of the MAPTMS-treated mica is not strong enough to immobilize DNA molecules on the substrate. In contrast to the above observations, we found that DNA molecules were too tightly bound to an APTES-treated mica surface to be stretched in the spin-coating process with a rotation rate of 1000 rpm (Fig. 2). Therefore, we increased the rotation rate to 7000 rpm, and observed some partially stretched DNA molecules (Fig. 3A). This finding illustrated that the binding force between the amine-terminated surface and DNA is the strongest among the samples tested.

CV used as a good anchor in this study contains three dimethyl amine phenyl rings joined by a central carbon atom. It is one kind of triphenylmethane (TPM) dyes, which were extensively studied to understand their fluorescence properties and were used as sensitive optical probes to reveal the microscopic dynamics of their local

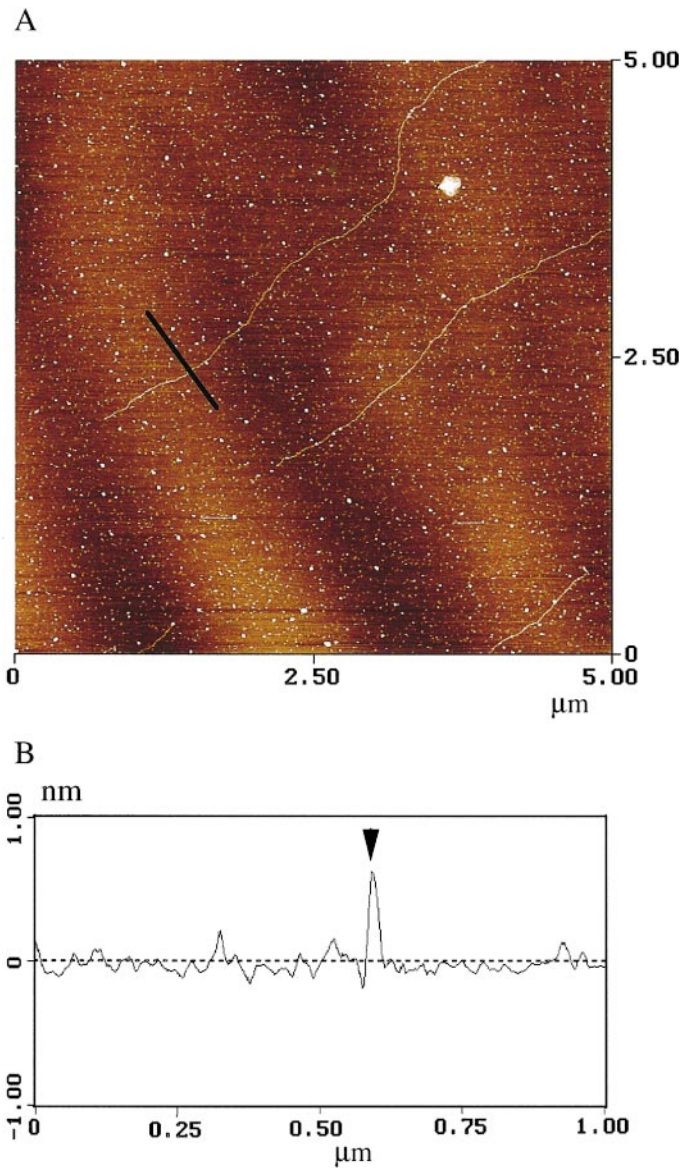

FIG. 1. (A) An AFM image of DNA molecules spin-coated on a CV-treated mica surface. The height is illustrated in gray code ranging from dark (0 nm) to light (1.5 nm). (B) The sectional image corresponding to the black line drawn in A. The peak marked with an arrowhead refers to the height of the stretched DNA.

environment (11–16). Strong interactions of TPM dyes with DNA were also found in the previous studies, although the exact binding mechanism, i.e., whether the TPM dyes bind to DNA by intercalation or by the interaction between the charged amino groups of TPM and the negative phosphate of DNA, remains unsolved (17). Our experimental finding that CV acted as an anchor between DNA and a mica surface is in favor of the latter possibility; i.e., the dye cation serves as a bridge between the negatively charged mica surface and the negatively charged DNA molecules. In previous experiments, strong binding of DNA molecules to mica surfaces was achieved by introduction of certain divalent transition metal cations (5, 6–8). However, the transition metal ions may cause significant perturbations to DNA structures (18, 19), and may not be compatible with biological processes (8). The organic ionic dye molecules, such as CV used in this study, might be good substitutes for the transition metal ions, although further studies should be carried out in the future.

Figures 1B and 3B illustrate the section analyses of the spin-coated DNA molecules on the mica surfaces treated with CV and APTES, respectively. As shown in Fig. 3B the height of the stretched segment of DNA molecules (marked with two black arrows) is extremely small on the APTES-treated mica surface; thus, it is difficult to extract the accurate value of the height. However, because we observed both partially relaxed and stretched segments of DNA in the same image, it was possible for us to have a good comparison between DNA molecules with different structures. It is clear that the height of the stretched segment of the DNA molecule is notably smaller than that of the DNA molecules not being well stretched (average height of 0.38 nm). This implied the stretching caused an elongation of DNA molecules, although the length of the DNA molecules was not obtained owing to the limitation of the scanning range of our instrument. The height of the stretched DNA molecules on the CV-treated mica has an average value of 0.70 nm, which is

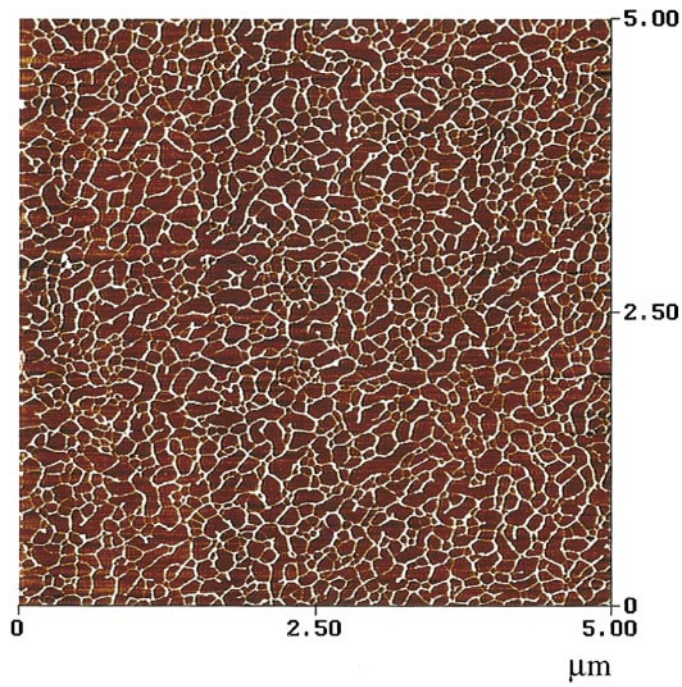

FIG. 2. An AFM image of DNA molecules on the APTES-treated mica surface prepared by a spin-coating procedure with a rotation rate of 1000 rpm. The height is illustrated in gray code ranging from dark (0 nm) to light (2.0 nm). DNA molecules densely adhered on the surface and formed an interconnecting network. There was essentially no stretching effect on the DNA molecules.

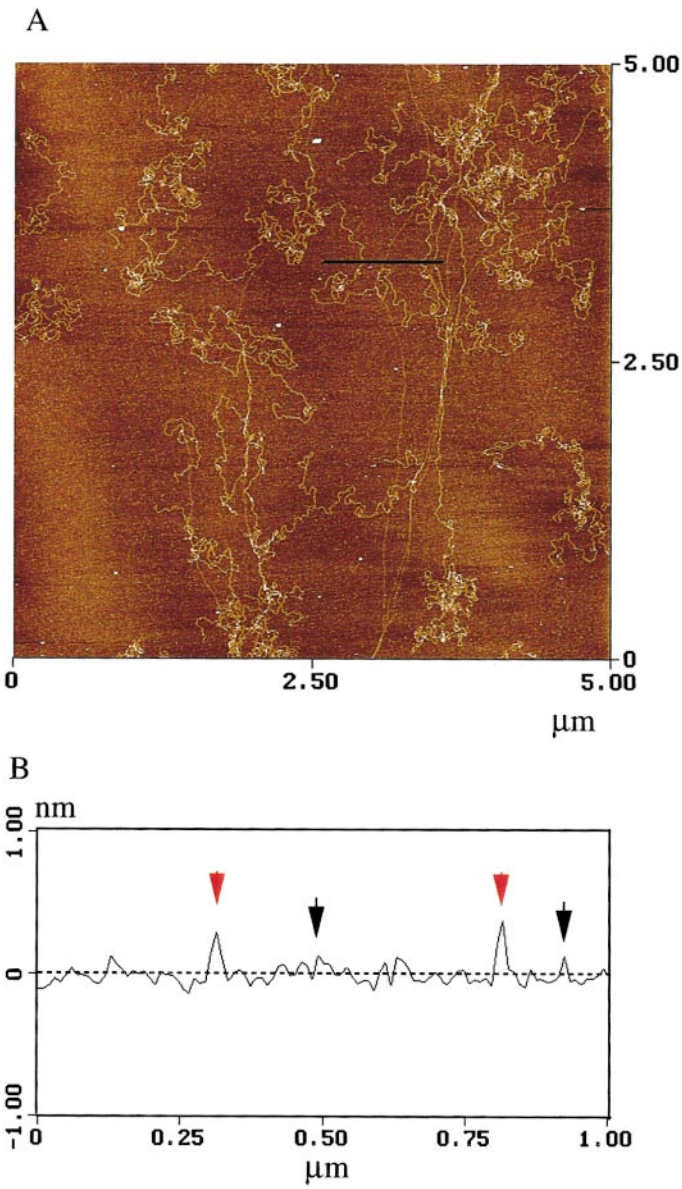

FIG. 3. (A) An AFM image of DNA molecules on the APTES-treated mica surface prepared by a spin-coating procedure with a rotation rate of 7000 rpm. The height is illustrated in gray code ranging from dark (0 nm) to light (1.5 nm). Both partially relaxed and stretched DNA molecules appeared in the image. (B) The sectional image corresponding to the black line drawn in A. The peaks corresponding to the partially relaxed and stretched segments of DNA molecules are marked with red and black arrows, respectively. Differences in height exist between them.

even larger than that of the partially relaxed DNA on the APTES-treated mica. There are two possible explanations for this experimental finding. One is that the force between DNA and the CV anchoring sites was moderate compared with the strong adhesion of DNA to the APTES-treated mica surface, and the other is due to the milder stretching force given by the fluid flow generated with slow rotation rate (1000 rpm) used for spin coating on the CV-treated mica in contrast to the high rotation rate (7000 rpm) for the APTES-treated mica. To clarify this point, we further measured the sample prepared with a 7000 rpm spin-coating procedure on a CV-treated mica. The height of DNA on the CV-treated mica was essentially independent of the rotation rate in the range of 1000 and 7000 rpm, and was larger than that on the APTES-treated mica even when the spin coating was in the same rate for both substrates. This result indicated the extent of elongation of DNA on the CV-treated mica was limited by the strength of the binding force; the binding between DNA and the anchoring sites on CV was not strong enough to keep the large tension of DNA given by the fast fluid flow generated with 7000 rpm rotation. On the other hand, for DNA on APTES-treated mica, the adhesion force from the substrate was extremely strong; thus, the change of shape of DNA caused by stretching force was retained as it happened, and the strong adhesion force itself also caused reducing the height of DNA. This explains the observed small height of DNA on the APTES-treated mica compared with that on the CV-treated mica and as well as the difference in height between DNA with stretched and partially relaxed structures on the APTES-treated mica.

The heights of DNA observed here were all smaller than the calculated value of 2 nm for B-form DNA. Reduced heights of DNA molecules were normally observed in other studies as well (3, 4, 20, 21). In addition to the possible reasons for the reduction of height, such as deformation of DNA under the AFM tip (20) and presence of buffer salts (21), the main reason for the reduced height observed in this experiment may be due to the adhesion force between DNA and CV- or APTES-treated mica surfaces and the elongation force applied to DNA from the fluid flow generated by the spin coating.

CONCLUSIONS

We developed an effective method of preparing stretched DNA samples for AFM imaging. Stretched DNA molecules were obtained using a spin-coating procedure. The stretching behavior of DNA was sensitively affected by the interactions between the DNA molecules and the substrates used for spin coating. We discussed several different treatments of surfaces for stretching DNA. We found that CV, an cationic dye molecule, was a good anchor, which applied moderate binding force to DNA in the spin-coating process. This technically simple approach for preparing stretched DNA molecules on CV-treated mica surfaces using the spin-coating procedure has potential applications for high-throughput physical mapping of large DNA molecules.

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