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IMPROVED VISUALIZATION OF DNA IN AQUEOUS BUFFER WITH THE ATOMIC FORCE MICROSCOPE

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Abstract

An improved method has been developed for imaging deoxyribonucleic acid (DNA) in aqueous buffer with the atomic force microscope (AFM). DNA on untreated mica can be imaged in aqueous buffer with the AFM if the DNA is deposited onto the mica in a buffer with HEPES and $MgCl_2$, if the sample is rinsed thoroughly with high water pressure, and if the imaging is done with an electron beam-deposited (EBD) tip that has been deposited in the scanning electron microscope (SEM). The water rinse removes DNA that is otherwise easily scraped off the substrate. There is evidence that sharper tips may be more damaging to DNA when imaged in aqueous buffer especially when the DNA is bound tightly to the mica. The ability to image DNA in nearly biological conditions has potential applications for imaging biomolecular processes with the AFM.

Key Words: Atomic force microscopy (AFM), deoxyribonucleic acid (DNA), aqueous buffer, water, mica, imaging biological processes, imaging buffer, cantilever, electron beam-deposited (EBD) tip, scanning electron microscope (SEM).

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Introduction

There has been much interest in imaging biological samples [3, 5, 10] and processes [4] with the atomic force microscope (AFM) [2, 14]. Currently, two methods have been developed for imaging DNA in water: **1**) dehydrating the DNA onto the mica substrate using a pre-treatment in propanol [8], and **2**) imaging DNA adsorbed onto silylated mica [12, 13]. To see biological processes, one generally needs to image in aqueous buffer, which is more difficult with the AFM, because buffer salts loosen DNA from the substrate more readily than water does. Imaging in aqueous buffer has been done only with the propanol method. Thus, it would be desirable to develop a milder method of DNA deposition for imaging under physiological buffers. In this work, small DNA molecules were adsorbed onto mica in HEPES buffer and imaged stably in aqueous buffer without using an organic solvent to dehydrate the DNA onto the mica. These results will greatly facilitate the observation of molecular processes in physiological buffers with the AFM.

Materials and Methods

Materials

Ruby mica was obtained from New York Mica Co., New York, NY. Bluescript II SK(+) double-stranded plasmid DNA (2961 base pairs, 1 mg/ml) and lambda/Hind III DNA markers (250 mg/ml) were obtained from Stratagene, La Jolla, CA supplied in 10 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA). A commercial Water Pik® (Teledyne Corp., Fort Collins, Colorado) was used for rinsing the samples. A two liter glass desiccator and a mechanical pump were used for vacuum drying.

Sample preparation

Bluescript was diluted with buffer to a final concentration of 2.5 ng/μl. The buffer consisted of HEPES and $MgCl_2$ with concentrations ranging from 40 mM HEPES, 10 mM $MgCl_2$ to 0.4 mM HEPES, 0.1 mM $MgCl_2$, at pH 7.6. The ratio of HEPES to Mg did not change. One μl of this solution was then deposited onto

the center of a freshly split untreated mica disk. The drop was immediately rinsed with 200 ml of distilled water dispensed from the Water Pik®. The Water Pik® was held approximately 2 inches (5 cm) away from the sample and the stream of water was placed off-center on the disk. The sample was then blown dry with compressed air and subsequently dried in vacuum over P_2O_5 for 10 minutes or more before AFM-imaging.

Atomic force microscope imaging

Atomic force microscopy (AFM) was performed in aqueous buffer using a Nanoscope III (Digital Instruments, Santa Barbara, CA) as described previously [9]. Electron beam-deposited (EBD) tips [1, 7, 11] were grown on oxide-sharpened silicon nitride tips (supplied by Digital Instruments) in a scanning electron microscope (SEM). A new cantilever was used for each of these experiments, although it was also possible to image with an old cantilever as long as the grown tip was still intact. It was necessary to engage with as small a force as possible to avoid damaging the tip. Once engaged, it was useful to minimize the force by reducing the set-point voltage. Images were processed only by flattening to remove the background slope.

Results and Discussion

DNA can be imaged directly in aqueous buffer if the DNA is deposited in the HEPES-Mg buffer, if it is rinsed with high water pressure (in this case we used a Water Pik®), and if the imaging is done with an EBD tip [8]. The Water Pik® rinse washes all loosely bound DNA off, only leaving the tightly bound DNA on the mica. About 80% of the DNA is removed by the Water Pik® rinse. A HEPES-Mg buffer is used for depositing the DNA onto the mica because using a dilute Tris-EDTA buffer for deposition results in less DNA bound to the mica [6].

All successful images in aqueous buffer were obtained by first leaving the sample in Milli Q® purified water in the AFM for 10 to 20 minutes then imaging in buffers. Stable images have been obtained in several different buffers. The imaging buffer that gives the best results is 10 mM HEPES, 1 mM $MgCl_2$ at pH 7.6. Stable images could also be obtained in 10 mM HEPES, 50 mM NaCl, 10 mM $MgCl_2$, with 1 mM mercaptoethanol or 1 mM DTT, pH 7.6 as well as in 25 mM Tris, 4 mM MgAc, at pH 7.5. Lower concentrations of the latter buffer also produced stable images. As the salt concentration in the imaging buffer is increased, imaging becomes less stable, presumably because the DNA is more likely to go into solution when there are more salts present. Also it is often easier to get stable images after a new buffer has been in the AFM for a few minutes because the force on the sample stabilizes after 20 minutes. If the imaging is done immediately after changing solutions in the fluid cell, there is a considerable amount of drift in the force exerted on the sample which could be due to thermal fluctuations near the cantilever (J.P. Cleveland, M. Radmacher, personal communication).

Once the DNA has been imaged in water for at least 10 minutes, imaging in low salt buffer is stable. Figure 1 is an image of Bluescript in 10 mM HEPES, 1 mM $MgCl_2$, pH 7.6; this particular area has been imaged for 25 minutes. Figure 2a is a plasmid that has been imaged for 14 minutes in the buffer mentioned above. Figure 2b is the same plasmid 5 minutes later at the same scan size of 500 nm. DNA can also be imaged stably for at least an hour in water. The image with the smallest scan size was obtained under water (Figure 3).

The contour length of the plasmid DNA is comparable to what has been observed previously [8]. As in our previous work [8], the DNA in aqueous buffer is wider and taller than what has been observed in propanol and in air [3, 8]. There is evidence that sharper tips damage the DNA more easily. Figure 3 shows DNA in water that has been damaged by the tip. It is clear though that the DNA is not being pushed around, which indicates that it is bound well to the mica. The most stable images of DNA in aqueous buffer always show very wide DNA (widths of about 19 $\pm$ 4 nm [8]). Figure 3 has widths of 7.2 $\pm$ 1.4 nm, which indicates that the tip used for imaging this plasmid was very sharp.

Imaging DNA in aqueous buffer for an extended period of time without damaging the molecules has the potential for observing processes involving DNA in the AFM. The imaging conditions are now more nearly physiological, since DNA can be imaged in aqueous buffer without a propanol pre-treatment.

Acknowledgments

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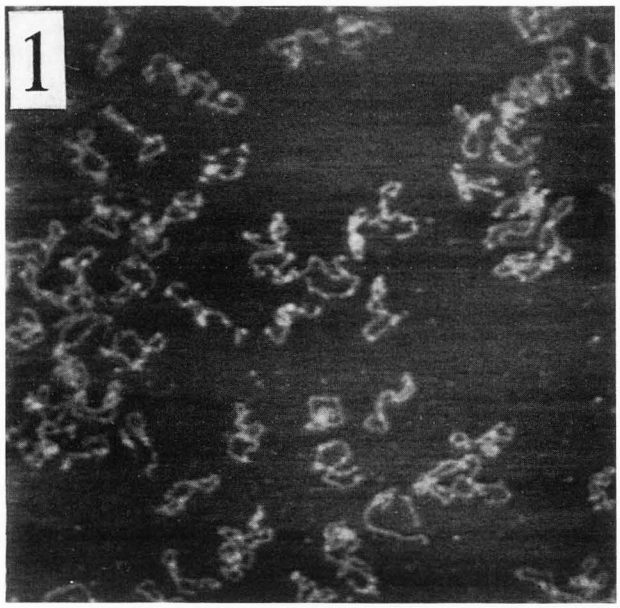

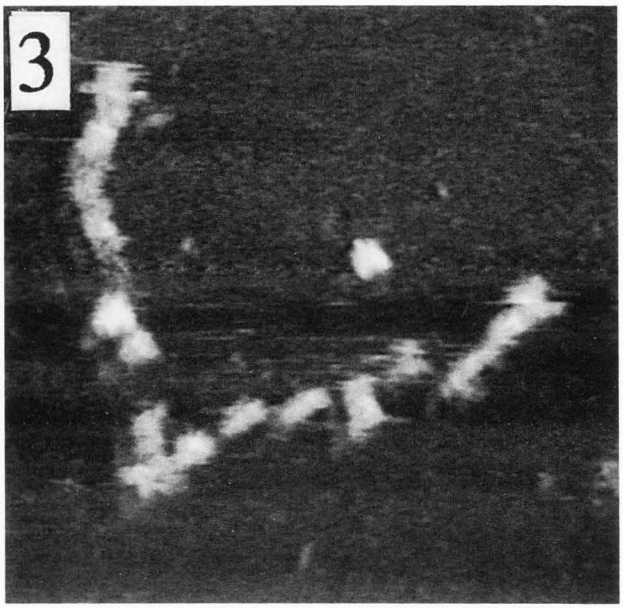

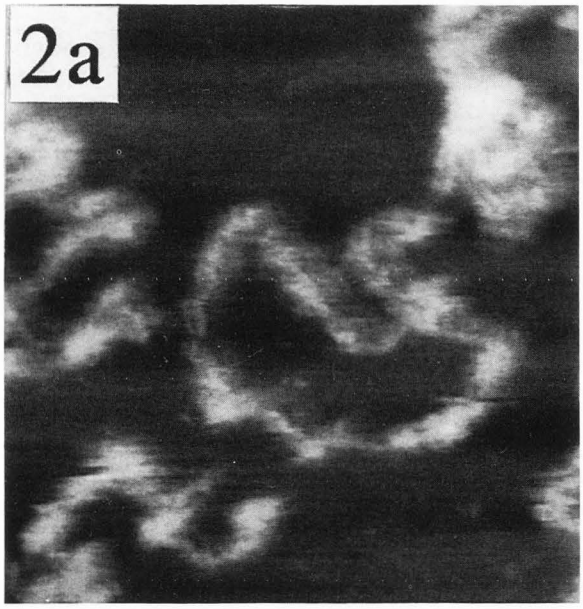

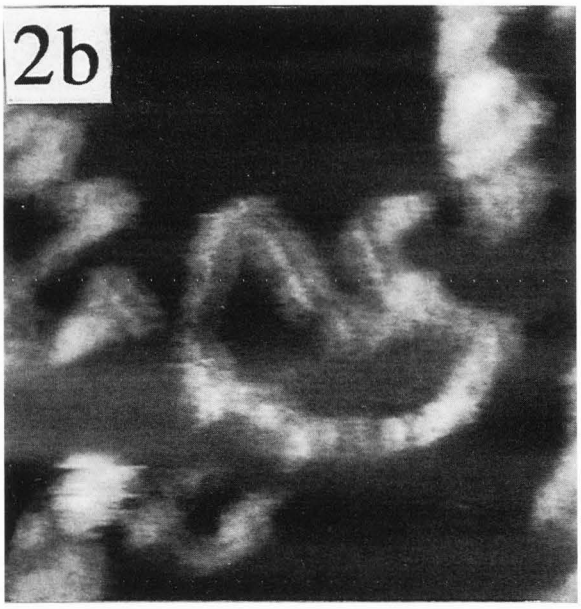

Figure 1. Bluescript plasmid DNA in 10 mM HEPES, 1 mM $MgCl_2$, at pH 7.6. The scan size is 3 μm by 3 μm.

Figure 2. Bluescript plasmid DNA from center of Figure 1 in 10 mM HEPES, 1 mM $MgCl_2$, at pH 7.6. **(a)** Plasmid after 14 minutes of imaging in aqueous buffer. Scan size is 500 nm by 500 nm. (Scan Rate: 7.6 Hz). **(b)** Same plasmid after 5 more minutes of scanning at 500 nm by 500 nm.

Figure 3. Lamda/Hind III marker imaged in water. The scan size is 200 nm by 200 nm.

Note: Scan Rate: 7.6 Hz for all figures.

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Discussion with Reviewers

R. Balhorn: If some of the DNA is not stuck down tightly enough, will the tip simply move those molecules around on the surface without imaging them? Or does this "loose" material contaminate the tip?
Authors: Molecules, that are not tightly bound, are pushed around on the surface by the tip, and as a result, they contaminate the tip.

R. Balhorn: Magnesium is known to aggregate DNA and affect its structure. Might this thicker fiber represent a DNA fiber condensed or coiled to some extent by the magnesium ions in the buffer?
Authors: As advised by Dr. B. Samori (personal communication), "The very drastic rinsing by the Water Pik® certainly prevents the sample to ever reach Mg^{++} concentrations required to induce this kind of effect... (Chaires and Sturtevant, Biopolymers 1988, **27**, 1375)".

Z. Shao: What are the physiological differences for drying in vacuum and drying in alcohol?
Authors: We are not aware of any physiological difference for the DNA but if a DNA-protein complex is imaged, drying in alcohol will readily denature the complex, while drying in air will be less destructive.

Z. Shao: Figure 2b showed a clear double-line structure. Is this a double tip effect? Figure 3 also showed a weak line in parallel to the DNA. Could the authors offer some explanation?
Authors: We interpret the double lines as double-tip images, which can arise during scanning.

E. Henderson: It is not mentioned that baking DNA onto mica has also been used as a preparation for imaging in aqueous solution.
Authors: In this paper we mention two deposition methods used previously for imaging DNA in aqueous buffer. Reference 8 does mention the method that involves baking DNA onto mica but since this has not proven to be as reliable, we did not include it here.

E. Henderson: A brief explanation of why DNA attaches poorly to mica in high salt conditions would be useful.
Authors: We think that the salts compete with the DNA in binding to the mica. Also, DNA may be more soluble in an environment of high ionic strength.

E. Henderson: Is there an hypothesis as to why HEPES works better than Tris?
Authors: We have no good hypothesis at this time.

E. Henderson: Is this work somewhat redundant with regard to previous descriptions from the same laboratory about aqueous imaging of DNA by AFM?
Authors: This paper presents a method that eliminates the pre-treatment in propanol which will hopefully facilitate the imaging of processes involving DNA and proteins.

B. Samori: This drastic rinsing is likely to be able to induce mechanical modifications of the structures of the DNA molecules not firmly attached. Can we rule out that possibility for the most firmly attached ones, i.e., for those imaged afterwards?
Authors: Images of DNA in air have not revealed any structural differences between samples that are rinsed with a Water Pik® and those that are not.

S.M. Lindsay: The use of HEPES is a real improvement. A strong drying effect is needed (as described here) and this seems to be common to many of these preparation techniques (including ours). This raises the question of whether the DNA is held by electrostatic forces alone or whether 'embedding' in a layer of salt plays a role.
Authors: Since the sample is rinsed extensively, it is unlikely that embedding in a salt layer plays a role. Also our previous [7, 8] imaging was done with DNA in a very dilute buffer containing only 1.4 ng buffer solids per ng DNA, which is not enough salt to embed DNA.