Topographical structure of pBR322 DNA studied by scanning tunneling microscope and atomic force microscope

P.-C. Zhang, C. Bai, Y.-J. Cheng, Y. Fang, and Z.-H. Wang
STM Laboratory, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

X.-T. Huang
Department of Biology, Nankai University, Tianjin 300071, China

(Received 9 August 1993; accepted 14 December 1993)

Plasmid pBR322 DNA (0.5 mg/ml) isolated from *Eschericha coli* HB101 was suspended in Tris-HCl/EDTA (1/0.1 mol/L, *p*H 8.5), deposited on a highly oriented pyrolytic graphite surface, then imaged with scanning tunneling microscope (STM) in air and under paraffin liquid in constant current mode. The methods for preparing DNA samples used in the STM study are discussed. Dynamic changes of apparent width of supercoiled pBR322 DNA were captured during an STM imaging course. The pBR322 DNA is also visualized with an atomic force microscope (AFM) built by this laboratory after the sample was absorbed on the freshly cleaved mica surface. Characteristically relaxed circular structures of pBR322 DNA have been observed in the AFM experiment.

I. INTRODUCTION

The potential capability of the atomic force microscope (AFM) to generate atomic resolution images of biological surfaces has motivated immense interest in imaging DNA molecules. Currently, AFM images of DNA in air and under solutions routinely display a resolution comparable to that obtained by electron microscopy. AFM can also be used to observe some dynamic phenomena in cell biology and organic chemistry. Häberle *et al.* have observed a dynamic process that could be associated with the penetration of a virus into the cell.[1] Henderson *et al.* have imaged actin filament dynamics in living glial cells with AFM.[2] Rabe *et al.* has used the scanning tunneling microscope (STM) to investigate structures and dynamics of long chain alkanes and alkyl derivatives *in situ* at the solid–fluid interface.[3,4] Recently, several successful reports about DNA molecules imaged with AFM have been published.[5–9] AFM seems to be a more efficient tool for the study of DNA structure than STM. In this paper we present results of the same sample of plasmid pBR322 DNA studied by STM and AFM.

II. EXPERIMENT

A. Materials and methods

1. Preparation methods for STM specimens

The highly oriented pyrolytic graphite (HOPG) surface was freshly cleaved before use, then was dc glow discharged for 1–2 min under vacuum between 100 and 200 mTorr, similar to the method reported by Vensenka *et al.*[5] Plasmid pBR322 DNA (0.5 mg/ml) isolated from *Eschericha coli* HB101 was suspended in Tris-HCl/EDTA (1/0.1 mol/L, *p*H 8.5). A small metal ring was put into the sample solution, then taken out. A thin film of the sample solution containing DNA was formed in the metal ring. After that, the film kept touch with the above freshly cleaved HOPG surface for about 30 s. When the sample solution was close to dry but not completely dry, a thin film of liquid paraffin was covered over the sample. Immediately afterwards, the sample was imaged with STM in a constant current mode.

2. Preparation method for AFM specimens

3–5 μl of the same solution containing plasmid pBR 322 DNA mentioned above was dropped on a freshly cleaved mica surface. About 1 min later, the residual solution on the mica was removed by attachment of a slice of a filter paper to the edge of the liquid drop. Immediately afterwards 5 μl of 0.5% buffered phosphotungstic acid solution (adjusted to *p*H 7 with NaOH solution before use) was added over the DNA molecules for about 1 min, the residual phosphotungstic acid solution was removed by a slice of filter paper as mentioned in the above method. Afterwards, the DNA samples treated by the above procedures were imaged with an AFM.

B. Instrumentation

The STM instrument used in this work was a STM-3000 that was built by this laboratory. Tip bias and tunneling current were about +21 mV and 0.31 nA, respectively. The images were collected in a constant current mode.

The AFM instrument used for our investigations has been described elsewhere.[10] The results presented here were obtained by optical detection of a cantilever deflection, which was sensed by measuring the deflection of a reflected laser beam at a photodiode, as has become common practice in the AFM field. All results were imaged in air at room temperature and the images were recorded in a constant-force mode. Commercial cantilevers microfabricated from Si_3N_4 were used in this study (Nanoprobes, Digital Instruments Inc.), having a spring constant of $k=0.12$ N/m (200 μm long) and a pyramid-shaped tip. The piezoelectric scanner was calibrated by taking an image of mica. AFM images were stored as 180×180 point arrays. Raw (unfiltered) data are presented unless otherwise stated. Freshly cleaved mica was used as a substrate for DNA absorption in this study.

 0734-211X/94/12(3)/1461/4/$1.00

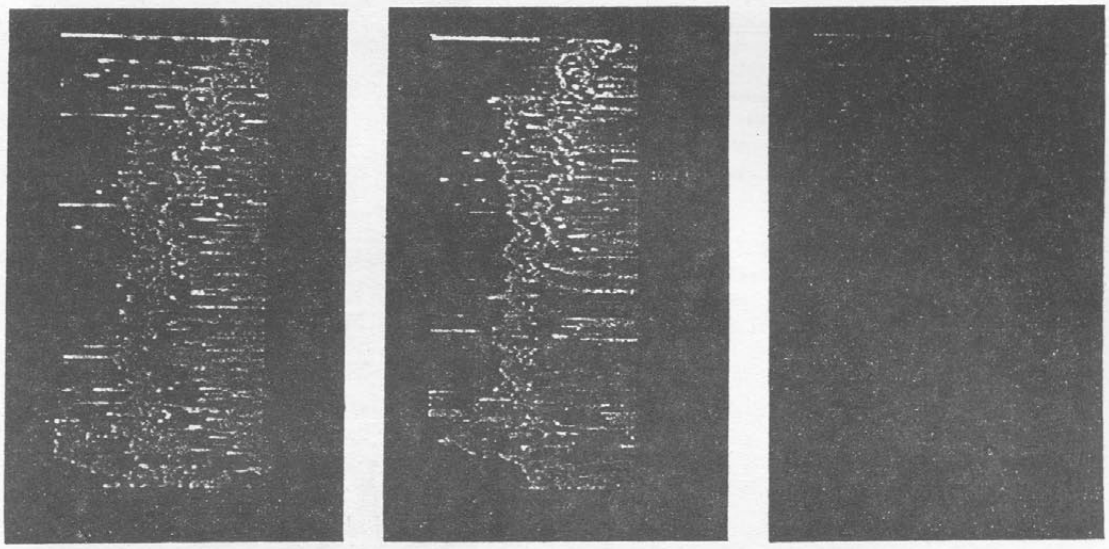

FIG. 1. STM images of plasmid pBR322 DNA under liquid paraffin. (a) At the beginning of scan; (b) a few minutes after (a); (c) a few minutes after (b). Scan size is 426 nm×1404 nm.

III. RESULTS AND DISCUSSION

A. STM images of pBR322 DNA and change of DNA apparent width during the scan course

The plasmid pBR322 DNA prepared by the above method was imaged with STM in air at room temperature, keeping under a liquid paraffin. An image of pBR322 DNA on a HOPG surface with a range of 426 nm×1404 nm is shown in Fig. 1(a). The plasmid pBR322 DNA in Fig. 1(a) is a supercoiled molecule and from its length it can be expected that the image of DNA observed here may be a dimer of pBR322 DNA. In this figure, the apparent width of DNA was found to be about 19.5 nm but the STM image of pBR322 is not stable. Following Fig. 1(a) an obvious change in apparent width along the DNA strand *in situ* can be observed, as shown in Fig. 1(b). The apparent width of the DNA is reduced to 11.4 nm.

A few minutes later, after obtaining the image of Fig. 1(b), an image of this DNA molecule with a narrower apparent width was obtained, as given in Fig. 1(c). The apparent width of the DNA was reduced to 4.0 nm which is close to the helical diameter of A-form DNA in a crystalline state.

The dynamic change in DNA apparent width might be due to the two following reasons. One reason might be that change in the conformation of DNA took place, owing to the interaction between a tip and a sample molecule or drying of solvent during the scan course. Another reason might be that small molecules (e.g., H_2O, salt ions) adsorbed on the DNA molecules are moved away, so that the real DNA chain with a few or without small molecules around the DNA strand was exposed.

B. AFM images of pBR322 DNA

Plasmid pBR 322 DNA treated by the above method was also imaged with AFM in air. About 25 individual molecules of pBR322 DNA can be found on the mica in an area of about 12 μm×12 μm. Molecular weight of plasmid pBR322 DNA is 2.8×10^6 dalton, containing 4363 bps. The linear length of the monomer molecule is 1.49 μm. Plasmid pBR322 DNA usually exists in different topographical structures with linear, relaxed, and supercoiled forms. Assumed that one pBR322 DNA molecule exists in a circular form, the theoretical diameter of the monomer molecule is about 475 nm. pBR322 DNA can associate to dimer or trimer. Their diameters, corresponding to dimer or trimer, will increase to lengths that are double or three times the monomer, respectively. Because the linear length of pBR322 DNA is 1.49 μm, the diameters of the relaxed ring of a pBR322 DNA molecule consisting of its monomer, dimer, and trimer can be figured out to be 475, 949, and 1424 nm in turn. The diameters of each relaxed ring corresponding to different individual pBR322 DNA can be measured from the AFM image in Fig. 1(a). The results are shown in Table I. The measured values of the diameters of monomer, dimer, and trimer are basically in agreement with the calculated data. In Fig. 1(a), of pBR322 DNA belongs to monomer, 36% of which exists in dimer form, and the rest of them, the remaining 8%, are connected to trimer with a longer length.

C. Comparison of STM results with AFM

The effects of STM and AFM methods on both the appearance and imaging stability of circular DNA molecules

TABLE I. Diameters of relaxed rings of plasmid pBR322 DNA measured by AFM.

Molecule	Diameter (nm)	Type
a	1056	dimer
b	528	monomer
c	528	monomer
d	528	monomer
e	426	monomer
f	528	monomer
g	594	monomer
h	990	dimer
i	594	monomer
j	798	dimer
k	924	dimer
l	528	monomer
m	963	dimer
n	1276	trimer
o	594	monomer
p	528	monomer
q	829	dimer
r	528	monomer
s	813	dimer
t	570	monomer
u	858	dimer
v	528	monomer
w	438	monomer
x	825	dimer
y	1420	trimer

have been observed. Because the HOPG surface is hydrophobic, the hydrophilic treatment of the HOPG surface before use is in favor of adsorption of the DNA molecules, but the glow discharged treatment of the HOPG surface requires a longer time than that of mica surface for DNA adsorption. This hydrophilic treatment may be important for the STM image. Figures 1(a)–1(c) show the STM images of pBR322 DNA absorbed on the HOPG surface by a hydrophilic treatment. When the DNA sample is adsorbed on the freshly cleaved mica surface and stained with phosphotungstic acid, a steady AFM image can usually be observed in air, but the AFM image resolution is not so high as that of the STM image. The DNA molecules in the AFM image show a wider apparent width than in STM image. The images of pBR322 DNA observed in the STM experiment tend to exist in supercoiled form on the HOPG surface. For the hydrophilic mica surface, the images of pBR322 DNA obtained from AFM seem to more easily form a topographic structure with a relaxed ring. Although DNA molecules absorbed on the HOPG surface can be occasionally captured in the STM experiment, the adsorption of DNA on the HOPG surface is usually unstable. In contrast, DNA easily adsorbed on the hydrophilic mica surface, and a stable AFM image can be obtained. An AFM image similar to that of Fig. 2(a) can be obtained both easily and reliably.

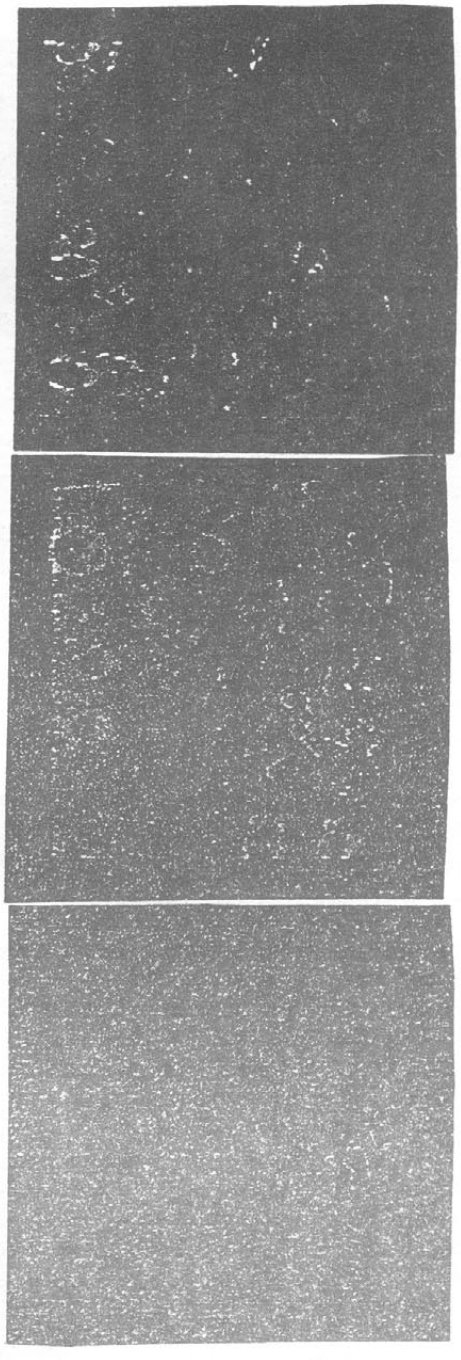

FIG. 2. AFM image of plasmid pBR322 DNA in air. (a) Scan size is 11890 nm×13020 nm; (b) scan size is 5950 nm×6510 nm; (c) scan size is 3960 nm×4340 nm.

IV. CONCLUSION

As seen above, STM can be used to investigate the structure of the DNA molecules absorbed on a hydrophilically treated HOPG surface, but the obtained STM image of DNA samples seems to be unstable. The DNA image observed by AFM is stable, but the resolution of the AFM image is not as high as that of the STM image. AFM can provide a convenient method for measurement of DNA length and as a direct evidence for observing topographic structures of the DNA molecules, but the AFM image resolution needs to be improved by development of new methods.

ACKNOWLEDGMENTS

The authors express their special appreciation to G.-Z. Huang for maintaining the instrument in order during the experimental course, R.-L. Li for helping with data acquisition and image process. We also would like to thank T. Wu and J.-S. Zhang for checking the whole paper. This work was supported by the National Natural Science Foundation of China.

[1]W. Häberle, J. K. H. Höber, F. Ohnesorge, D. P. E. Smith, and G. Binnig, Ultramicroscopy **42**, 1161 (1992).
[2]E. Henderson, P. G. Haydon, and D. S. Sakaguchi, Science **257**, 1944 (1992).
[3]J. P. Rabe and S. Buchholz, Science **253**, 424 (1991).
[4]J. P. Rabe and S. Buchholz, Phys. Rev. Lett. **66**, 2096 (1991).
[5]J. Vesenka, M. Guthold, C. L. Tang, D. Keller, and E. Delaine, Ultramicroscopy **42–44**, 1243 (1992).
[6]T. Thundat, D. P. Allison, R. J. Warmack, and T. L. Ferrell, Ultramicroscopy **42–44**, 1101 (1992).
[7]H. G. Hansma, J. Vesenka, C. Siegerist, G. Kelerman, H. Morrett, R. L. Sinsheimer, V. Elings, and C. Bustamante, Science **256**, 1180 (1992).
[8]Y. L. Lyubchenko, B. L. Jacobs, and S. M. Lindsay, Nucleic Acids Res. **20**, 3983 (1992).
[9]H. G. Hansma, R. L. Sinsheimer, M.-Q. Li, and P. K. Hansma, Nucleic Acids Res. **20**, 3585 (1992).
[10]J.-H. Wu, Y.-J. Cheng, C.-C. Dai, G.-Z. Huang, Y.-C. Xie, L.-X. Gong, and C.-L.Bai, Chin. Sci. Bull. **38**, 790 (1993).