Atomic force microscopic study on topological structures of pBR322 DNA*

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Abstract Plasmid pBR322 DNA (0.5 mg/mL) isolated from *Escherichia coli* HB101 was suspended in Tris-HCl-EDTA (1 mol/L — 0.1 mol/L, pH 8.5); then a drop of the above solution was deposited on freshly cleaved mica substrate. After adsorption for about 1 min, the sample was stained with phosphotungstic acid. The residual solution was removed with a piece of filter paper. Afterwards the sample was imaged with a home-made atomic force microscope (AFM) in air. The AFM images of pBR322 DNA with a molecular resolution have been obtained. These images show that pBR322 DNA exists in several different topological structures: (i) relaxed circular DNA with a different diameter; (ii) supercondensed DNA with different particle sizes; (iii) dimeric catenane connected by one relaxed circular molecule and another close-compacted molecule which might be either supercoiled or intramolecular knotted form; (iv) oligomeric catenane with multiple irregular molecules in which DNA is interlocked into a complex oligomer; (v) possibly-existing intermediates formed from monomer to oligomer.

Keywords: pBR322 DNA, atomic force microscopy, topological structure, image.

Microscopes are always considered to be important tools in the developmental history of biology. The successful development of scanning tunneling microscope (STM) is a revolution in microscopy. Their invention leads to the birth of a series of scanning probe microscopes (SPM) such as atomic force microscope (AFM), magnetic force microscope (MFM), ballistic electron emission microscope (BEEM), and photo scanning tunneling microscope (PSTM)[1]. These instruments use a probe to approach the surface of a determined object and obtain its surface local properties in spatial distribution, such as tunneling current, interacting force, ion conductance, magnetic domain. AFM is a most promising one among the above microscopes that may be used in biological research.

AFM has been used to determine several materials with very poor conductivity, e.g. mica, rutile, and an atomic resolution has been achieved. Also, AFM has been employed

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to study DNA, proteins and biological membranes. The work concerning the structures of nucleic acids determined with AFM has just begun in recent years. The previous studies were concentrated on exploring conditions of sample preparations and imaging quality[3—6]. Several kinds of DNA structures have been examined by STM at this laboratory[7, 8], but STM usually requires of a determined sample a good conductivity. Therefore, the stable STM image of a DNA sample cannot be obtained in the usual case and one tends to use AFM to investigate the structures of biological macromolecules. In this paper AFM will be used to image pBR322 DNA and to further investigate its topological structures.

1 Materials and methods

1.1 Preparation of pBR322 DNA

The pBR322 DNA obtained from *E. Coli* HB101 was separated and purified by gel electrophoresis. The detailed procedures were carried out according to the procedure described in Huang and Chen's paper[9]. Tris (hydroxymethyl) aminomethane (Tris), Ethylenediaminetetraacetic acid (EDTA), etc. were local products with analytical grade.

1.2 Procedures of sample preparation for the AFM experiments

About 5 μL plasmid pBR322 DNA (2 μg/mL, 0.1 mmol/L TE, pH 8.5) was dropped on a freshly cleaved mica surface. About 1 min later, the residual solution on the mica was removed by attachment of a piece of filter paper to the edge of the liquid drop; then 5 μL 0.5% buffered phosphotungstic acid solution (adjusted to pH 7 with NaOH solution before use) was immediately added over the DNA molecules for about 1 min, and the residual phosphotungstic acid solution was removed by a piece of filter paper in the same way. After the treatment with this procedure, the DNA samples were visualized in air.

1.3 Experimental instrument

The AFM instrument used for our investigations was home-made and their detailed description can be seen in Wu *et al.*'s report[10]. All images were obtained from AFM observation in air 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. 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 adsorption in this study.

2 Results and discussion

2.1 Measurement of DNA length

The molecular weight of plasmid pBR322 DNA is 2.8×10^6 u. The DNA molecule

contains 4 363 base pairs and is 1.49 μm in length. There are linear, relaxed and supercoiled forms in tertiary structures of plasmid pBR322 DNA. Recently, Huang *et al.* have successfully demonstrated via electrophoresis etc. that pRB322 DNA is capable of existing in a novel topological form that is called supercondensed form. The plasmid pBR322 DNA obtained from the above methods was absorbed on the freshly cleaved mica surface and imaged with AFM in air. The AFM findings obtained from a scan size of 12 μm × 12 μm is shown in fig. 1. The topological structure of most pBR322 DNA molecules shown in fig. 1 exhibits a relaxed circular form, but their diameters are different. Because the linear length of a plasmid pBR322 DNA monomer is known to be 1.49 μm, it can be figured out that the theoretical diameter of the relaxed ring for a monomer is 475 nm. For their dimer or trimer, the corresponding diameters should be the integral times of the above value. Namely, the diameter of the relaxed pBR322 DNA dimer or trimer is 949 or 1 424 nm, respectively. The diameters of pBR322 DNA observed in fig. 1 were measured and the statistic results are listed in table 1. As can be seen in table 1, the measured values of the diameter for the monomer, dimer and trimer are basically in agreement with the calculated data. It has been found in fig. 1 that about 56% of relaxed circular pBR322 DNA belong to monomers, about 36% of them exist in dimer form, and the remaining 8%

Table 1 Diameters of relaxed rings of plasmid pBR322 DNA measured by AFM

Molecule	Diameter/nm	Type
a	1 056	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	1 276	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	1 420	trimer

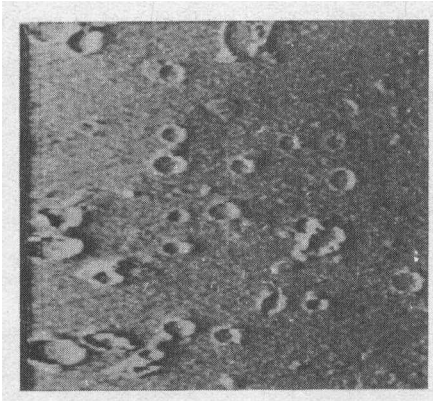

Fig. 1. AFM image of pBR322 DNA. The imaged was determined in air and in contact mode. The force constant of the cantilever is 0.12 N/m and its length is 200 μm. The scan size of the image is 11 890 nm × 13 020 nm.

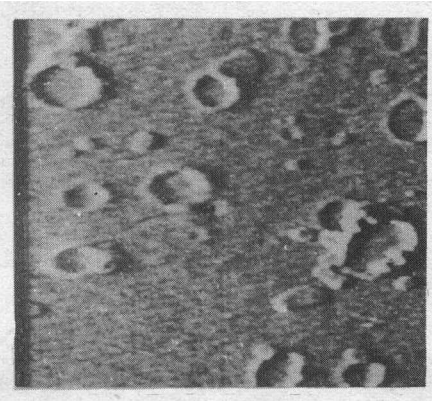

Fig. 2. AFM image of pBR322 DNA. The experimental conditions are as the same as in fig. 1 and the scan size of the image is 5 960 nm × 6 510 nm.

are connected to trimers. Obviously, most of the pBR322 DNA molecules exist in a monomer form and part of them in a dimer or trimer forms.

2.2 Topological structures of pBR322 DNA

Although topological structures of DNA have been observed by conventional transmission electron microscope (TEM), it usually requires complicate procedures for sample preparation. The determined sample is necessary to be shadowed or stainned with heavy metal, and has to be observed under vacuum condition, whereas the procedures for sample preparation used for AFM experiments are relatively simple and the observation of samples can be performed in air. The samples were imaged with AFM after dropping its solution on mica surface, removing the residue solution, and slightly drying. The results of pBR322 DNA visualized by AFM are illustrated in figs. 1 — 3. Although the

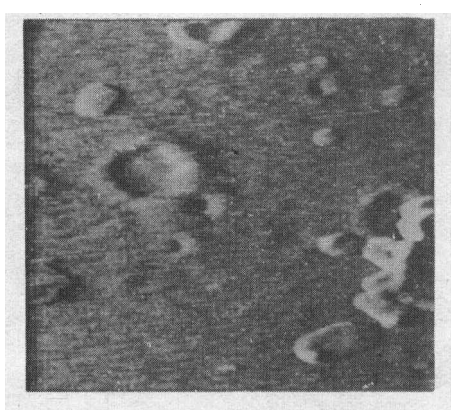

Fig. 3. AFM image of pBR322 DNA. The experimental conditions are as the same as in fig. 1 and the scan size of the image is 5 960 nm × 6 510 nm.

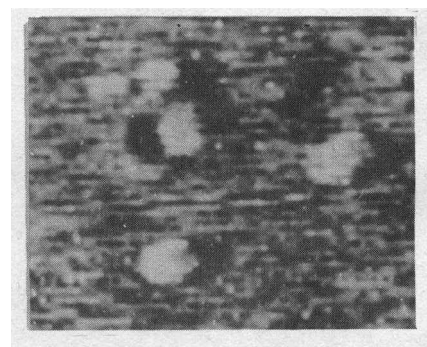

Fig. 4. AFM image of supercondensed pBR322 DNA.

sample was stained with phosphotungstic acid, it should be pointed out that the procedure is not necessary for sample preparation. By using attractive or tapping mode, or minimizing the interacting force between an AFM tip and a sample, the staining procedure can be omitted.

From the AFM images of pBR322 DNA, we can see several typical DNA topological structures: (i) characteristic relaxed circular DNA homogeouneously distributed on mica surface (these molecules with different sizes are the oligomers of pBR322 DNA); (ii) in

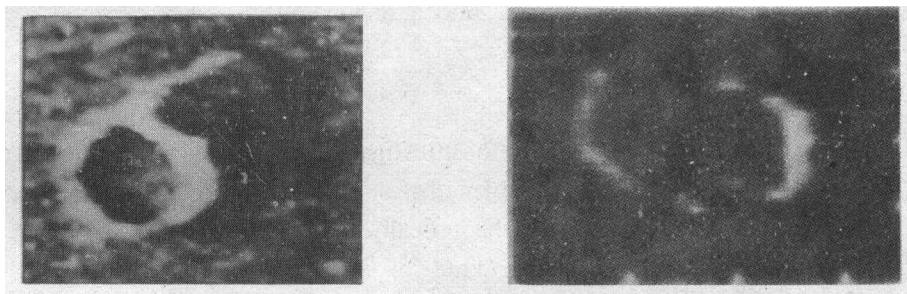

Fig. 5. Topological structure catenated by two pBR322 DNA molecules. One of them may be a relaxed circular molecule and another is in a condensed, supercoiled, and intramolecular-knotted state.

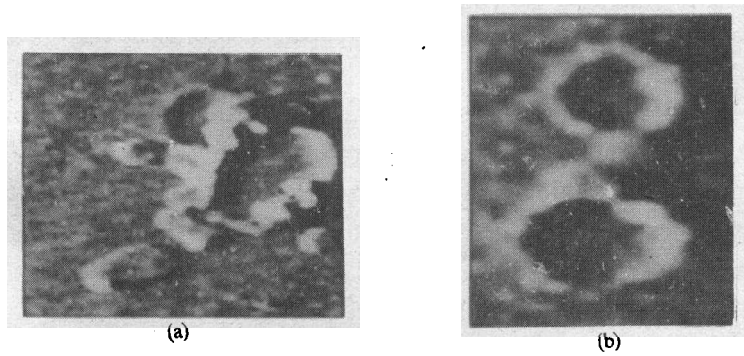

Fig. 6. Topological structure catenated by multiple pBR322 DNA molecules. There are multiple-molecular ring-like structures connected by pBR322 DNA and condensed into cantenes. (a) Parallel connection by multiple DNA molecules; (b) tandem connection by multiple DNA molecules.

fig. 1, a few globoid grains can be distinguished, and in figs. 2 and 3 the morphology with the grain-like shape is clearer. Fig. 4 was obtained from zooming into the top-right area of fig. 3 containing two grain-like species. We designate the morphology of this kind of DNA molecules as supercondensed DNA, which is in agreement with Huang's results observed with TEM[9]; (iii) the image

Fig. 7. Intermediate of pBR322 DNA from a monomer to a oligomer.

of fig. 5 was obtained by a zoomed-in scan from an area in fig. 1. Topological structure is catenated by two pBR322 DNA molecules. One of them may be a relaxed circular molecule and the other is in a condensed, supercoiled, and intramolecular-knotted state; (iv) there are probably ring-like structures connected by multiple molecules in a few areas in fig. 1 and they are aggregated into oligomeric catenanes, as shown in fig. 6(a) and (b); (v) more interestingly, a kind of structure formed from connection of three ring-like molecules can be found in the bottom-left area of fig. 1 and its magnified image is shown in fig. 7. The first and third rings in the oligomeric catenane contain a nick. This kind of structure might be an intermediate from a monomer into a oligomer.

3 Conclusions

AFM may be convenient to be used to investigate topological structures of pBR322 DNA. The above results indicate that the lengths of pBR322 DNA oligomers observed by AFM are basically in agreement with the theoretically calculated results. The AFM images of pBR322 DNA demonstrate that this kind of DNA is able to exist in multiple topological structures such as relaxed, supercoiled and catenated forms and the intermediate from a monomer into oligomer may also be in existence.

As can be seen above, AFM is an important tool for observing topological structures of pBR322 DNA. The sample determination can be carried out in solution by a reequipped instrument, so DNA structures are possible to be measured in nearly native-existing forms. Because AFM requires neither sample fixation nor shadowing nor staining, it may image a sample in physiological buffer and gain the related dynamic data, and therefore it is promising in biological research. Besides imaging, AFM can realize the fabrication of biological materials at micrometer scale even so at nanometer scale, and measure local physical and biophysical properties, so AFM has a potentially important significance in the investigation of biological problems.

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