

OBSERVATION OF DNA PARTIAL DENATURATION BY ATOMIC FORCE MICROSCOPY*

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Abstract Atomic force microscopy was used to investigate the DNA-cetyltrimethylammonium bromide (CTAB) complexes adsorbed on highly ordered pyrolytic graphite (HOPG). These complexes, at low concentrations, can automatically spread out on the surface of HOPG. The DNA-CTAB complexes display a typically extended structure rather than a globular structure. Partially denaturated DNA produced by binding CTAB to DNA is directly observed by AFM with high resolution. The three-dimensional resolution of partially denaturated DNA obtained by AFM is not available by any other technique at present.

Keywords DNA, Denaturation, Extended-structure, AFM

INTRODUCTION

The interaction between DNA and cationic surfactant holds special interest because of its significance in biomedical applications and because of the need for a better understanding of DNA behavior in living cells. Interaction between DNA polyion and oppositely charged surfactants results in “complex” formation, which has been studied extensively in recent years^[1–5]. This is due to the large and increasing interest in using cationic liposomes as a possible way for in vivo gene transfer^[6, 7].

DNA-surfactant complexes are generally insoluble in water, and the precipitation of various DNAs by cationic surfactants has been applied to DNA extraction, concentration, and counting. The DNA-cation complex is important not only for life science but also for material science, as the unique chemical structure of DNA can be applied in electron transfer, the formation of rodlike liquid crystals, and so on. As described in well-established protocols in molecular biology, DNA is recovered from the DNA-surfactant complexes in a pure state after being washed with mixed organic solvents, which suggests that such complexes are insoluble in hydrophobic solvents. These complexes are practically insoluble in aqueous solution because the phosphate groups of DNA molecules are screened by the positive charge of headgroups and also by hydrophobic moieties in the surfactants^[8].

Bathaie^[9] reported that the interaction of dodecyl trimethylammonium bromide (DTAB) to DNA did not cause any condensation in DNA, but led to DNA unfolding. The predominant forces in this conformational change are the electrostatic interaction between DNA and DTAB, and the hydrophobic interaction leading to partial DNA denaturation.

There are many techniques available to characterize the complexes of DNA-cationic surfactant, such as

* Dedicated to Professor Liang-he Shi on the occasion of his 80th birthday

This work was supported by the National Natural Science Foundation of China (No. 20174048).

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Received July 22, 2003; Revised August 21, 2003; Accepted August 22, 2003

fluorescence microscopy, UV spectroscopy, circular dichroism, capillary electrophoresis. However, few reports were found to use atomic force microscope (AFM) to directly verify DNA denaturation caused by cationic surfactant. AFM has become a helpful tool for obtaining ultrastructural information of biological samples^[10, 11]. AFM has also been used to obtain highly detailed topographical images with a typical resolution for biological samples of a few nanometers, and the three dimensional arrangement of the sample can be determined directly. AFM images are extremely accurate, particularly in the vertical dimension. Imaging is conducted under mild conditions and no vacuum is required. The samples need not be conducting and can be imaged in air or even in an aqueous environment. Imaging "in air" can be carried out under ambient air conditions with controlled humidity. In this work we use AFM to directly observe DNA denaturation caused by cationic surfactant. The extended-structure of single chains of DNA is presented.

EXPERIMENTAL

Materials

Salmon sperm DNA (Sigma) with $M = 1.2 \times 10^6$, ca. 2000 bp was purified using the standard phenol extraction procedure^[12]. Cetyltrimethylammonium bromide (CTAB) was obtained from Sigma. It was recrystallised twice from acetone and dried in a vacuum oven at 25 °C for 48 h prior to use.

Preparation of DNA-CTAB Complexes

The preparation of the DNA-CTAB complexes from purified DNA was carried out according to the methods described elsewhere^[13, 14]. DNA was dissolved in TBE buffer (45 mmol/L Tris-HCl, 45 mmol/L borate, 1 mmol/L EDTA (with pH 8.0) at 5.0×10^{-5} mol/L in nucleotide units. Under gentle stirring, an excess of aqueous solution of CTAB (1.0×10^{-4} mol/L) was added to the DNA solution. The resulting precipitate was centrifuged for 30 min at 1500 r/min. The precipitate was rinsed with ultrapure water ($> 18 \text{ M}\Omega \cdot \text{cm}^{-1}$, Millipore-UV, Millipore Co., Bedford, MA) and centrifuged. This process was repeated three times. Then the wet DNA-CTAB complexes were decanted and freeze-dried. The white powder complexes were dissolved in ethanol (AR, Aldrich) at 25 °C with concentrations of 1.0% and 0.1%.

Characterization

The IR measurements were carried out with a BRUKER Equinox 55 spectrometer equipped with a wire-grid polarizer and a MCT detector. Spectrum resolution is on an average of 32 scans.

A droplet of each solution (1% or 0.1%) was deposited onto a freshly cleaved surface of HOPG (quality ZYB, Digital Instruments). Microscopic analysis was carried out by using a commercial atomic force microscope (Digital Instruments). All AFM images were obtained in air at room temperature by using contact mode with a spring constant of 0.06 nN/nm. The cantilever was 200 μm in length. The set point was adjusted to minimize the force between the tip and the sample in each measurement.

The lateral dimensions of biological structures in AFM are overestimated because of the finite dimensions of the tips. In order to minimize the broadening effect of AFM, we measured the diameters of DNA with full width at half-maximum height.

RESULTS AND DISCUSSION

FTIR spectra of the original DNA, CTAB, and DNA-CTAB complex samples are shown in Fig. 1. For CTAB (Fig. 1a), the absorption bands at 2918 and 2848 cm^{-1} are assigned to the characteristic peaks of $-\text{CH}_2$ and $-\text{CH}_3$, while DNA Fig. 1(b) exhibits the absorption bands of base pairs at 1603 cm^{-1} (adenine), 1658 cm^{-1} (thymine), 1690 cm^{-1} (guanine), and 1484 cm^{-1} (cytosine)^[15]. The absorption of phosphate ester is located at 1236 cm^{-1} . From Fig. 1(c), it can be seen that there exist strong absorption bands at 2920 and 2850 cm^{-1} which are the characteristic bands of $\nu_{\text{as}} \text{CH}_2$ and $\nu_{\text{s}} \text{CH}_2$, respectively. Both of these bands are the characteristic peaks of CTAB. In addition, there exist the absorption bands of base pairs. As a result, Fig. 1(c) overlaps Fig. 1(a) and Fig. 1(b), suggesting the formation of a DNA-CTAB complex^[15].

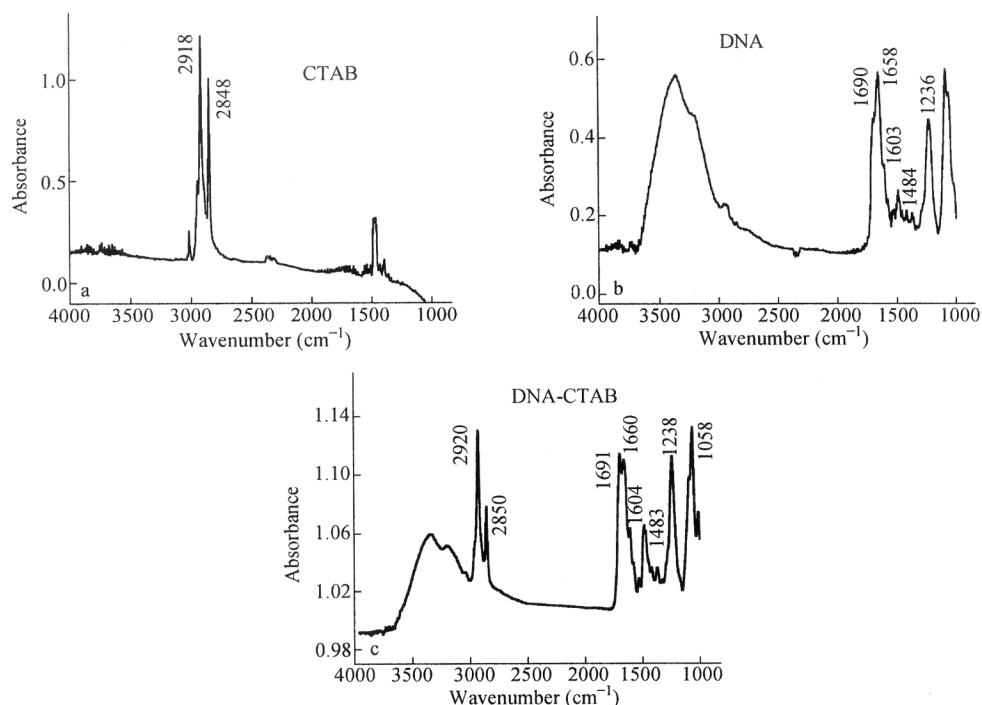


Fig. 1 FTIR spectra of (a) CTAB, (b) original DNA and (c) DNA-CTAB complex samples

Figure 2 shows the morphology of DNA-CTAB complexes adsorbed on HOPG at high concentration (1%). Most of the DNA-CTAB complexes are in the form of compacted coil (area a of Fig. 2), and some are extended coil (area b of Fig. 2) on the surface of HOPG. Binding of cationic surfactant to DNA results in the formation of hydrophobic complexes. These complexes are formed through the interaction of negatively charged DNA phosphate groups with the surfactant counterions and stabilized by hydrophobic interaction of the hydrocarbon tails of surfactant molecules^[13]. This image demonstrates that these complexes are different from those formed as a result of DNA condensation by cations such as spermidine^[16, 17, 18], polyamines^[19], protamine^[20], or polylysine^[21, 22]. The result is compatible with the concern that the DNA within the complex does not exist as a globular structure^[23].

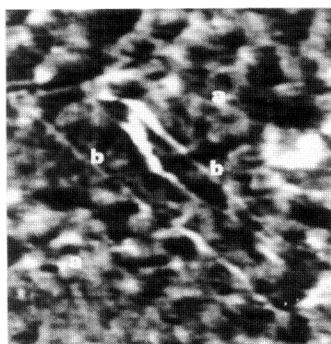


Fig. 2 Contact mode AFM image showing the morphology of DNA-CTAB complexes (1%) adsorbed on HOPG
Scan area: $2.090 \times 2.090 \mu\text{m}$, Height scale: 0–30 nm

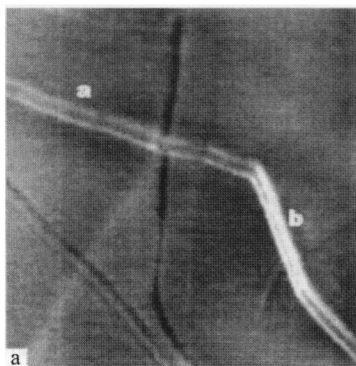


Fig. 3a AFM image showing the morphology of extended DNA-CTAB complexes (0.1%) on the surface of HOPG

Scan area: 537.6×537.6 nm, Height scale: 0–15 nm

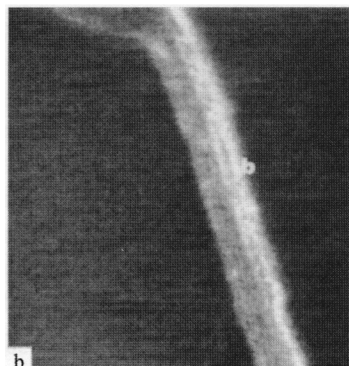


Fig. 3b High-resolution AFM image of DNA-complexes (0.1%) adsorbed on HOPG

Scan area: 175.3×175.3 nm, Height scale: 0–15 nm

Figure 3(a) shows the morphology of extended DNA-CTAB complexes on the surface of HOPG at extremely low concentration ($< 0.1\%$). Two linear extended structures (area a of Fig. 3a) are almost parallel on the surface. From Figs. 2 and 3, it is evident that at low concentration (0.1%), DNA-CTAB complex exists as an extended coil structure and then assumes a compacted coil and extended coil structure when the complex concentration is increased to 1.0%. These phenomena are similar to the result reported in the literature^[8]. At high concentration, the elongated coil DNA-CTAB gradually shrinks. This trend is attributable mainly to the occurrence of interchain segregation within individual single DNA molecules and partly to the enhancement of counterion condensation in low-polarity medium.

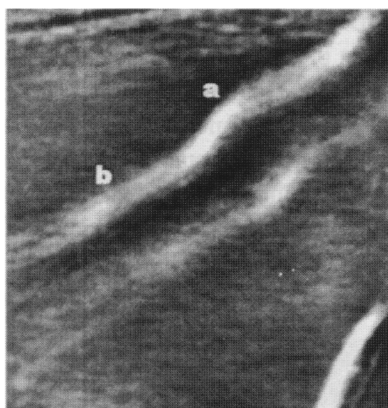


Fig. 4 High-resolution AFM image showing the DNA-CTAB complexes (0.1%) on HOPG

Scan area: 289.5×289.5 nm, Height scale: 0–10 nm

It has been proposed that organic molecules with covalently attached long alkyl chains can be immobilized on the basal face of HOPG. The Van der Waals interaction between the alkyl chains and the graphite substrate is strong^[24, 25]. Therefore, it is possible that the alkyl chains of CTAB molecules can be adsorbed on the hydrophobic surface and act as the anchoring points for the DNA molecules. Figure 3(b) is magnified from Fig. 3(a) (area b of Fig. 3a). From the image, partial denaturation of DNA (area b of Fig. 3a) is visible. The separation of the two single-stranded DNAs is distinguishable. The hydrophobic interaction between the nitrogenous bases in DNA and the hydrocarbon chains in CTAB molecules may even cause separation of the two strands that constitute the double-stranded DNA. This agrees with the results reported in the literature^[4, 9]. The

hydrophobic association of the surfactant chains may stabilize the two single-stranded molecules due to the hydrophobic interactions in solution. Figure 4 shows the DNA-CTAB complexes spreading on HOPG and DNA partial denaturation (area b of Fig. 4) can also be seen. The single-stranded parts show the elongated coil structure. The other part of the linear structure kept a double-stranded structure (area a of Fig. 4). The structure of pure DNA may be found in Refs. [26, 27].

The average distance between the two single-stranded DNA fragments is 4.0 ± 0.8 nm ($n = 50$, n is the density of links per unit volume). The single-stranded DNAs have an average width of 5.0 ± 1.1 nm ($n = 100$) and a mean height of 1.85 ± 0.4 nm ($n = 50$). The double-stranded DNAs have an average width of 14 ± 3.0 nm ($n = 100$) and a mean height of 2.2 ± 0.4 nm ($n = 100$). The limited structural detail observed from DNA is partly due to a number of factors involving the AFM tip. For instance, the DNA width observed in these AFM images is broader than the accepted DNA width due to constraints imposed by the tip geometry. The factors that significantly influence the resolution are the relative humidity and tip contamination. Tip contamination resulting from particles such as salt crystallites adhering to the tip is frequently observed. Generally, the primary effect of relative humidity in AFM imaging is the variation of capillary force. The capillary force, which is derived from the formation of liquid bridges between the tip and the substrate, increases with increasing humidity. The measured width of DNA was found to increase significantly with increasing capillary force. This increased capillary force results in poor resolution probably due to increased contact area^[28]. However, in the present work, HOPG is a hydrophobic substrate, which rules out the possibility of forming bridges between the tip and HOPG substrate. In addition, the alkyl chains of the surfactant molecules interact with the hydrophobic surface and function as the anchoring points for the DNA molecules. All of these factors are helpful for us to obtain highly resolved DNA images for distinguishing the partial separation of the two strands that constitute the double-stranded DNA.

We would also like to mention that it is well known some surface features of HOPG resemble DNA and other biomolecules in scanning tunneling microscopy studies^[29]. The AFM figures presented in this work are very different from the features of HOPG, and therefore could be reliably attributed to the DNA molecules. It should be emphasized that the three-dimensional resolution of partially denaturated DNA obtained by AFM is not obtainable by any other technique at present.

CONCLUSION

The DNA-CTAB complexes display an extended structure rather than a condensed structure. These complexes at low concentrations can automatically spread out on the surface of HOPG. Partially denaturated DNA formed by binding CTAB to DNA can be directly observed by AFM with high resolution.

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