Scanning Probe Microscopy of DNA on Mica and Graphite

D. V. Klinov[a], E.V. Dubrovin[b], I.V. Yaminsky[b]

[a]*Institute of Bioorganic Chemistry, 119871 Moscow, Russia*
[b]*Dept. of Chemistry, Dept. of Physics Moscow State University, 119922 Moscow, Russia*

Abstract. Method of modification of highly oriented pyrolytic graphite (HOPG) is proposed for deposition of biological objects especially DNA for scanning probe microscopy. Atomic force microscopy (AFM) images of DNA on HOPG are compared with those on conventional support - mica. The advantages of HOPG as a substrate for DNA for using in STM imaging and DNA mapping are discussed.

INTRODUCTION

In the early 90-th probe microscopy imaging of DNA on graphite has met well-described puzzles and problems. First papers reported that the images with atomic resolution were acquired with scanning tunneling microscopy (STM), providing the data about helix type, its periodicity [1-2] and even purine bases [3]. In further investigations it was demonstrated that the surface of highly-oriented pyrolytic graphite (HOPG), that was a support to the DNA molecules in the most cases, posses DNA-like defects [4]. The difficulties with immobilization of DNA molecules on graphite [5] as well as low reproducibility of STM images of DNA also questioned their validity. Has not been solved, the problem was replaced by the task of imaging of DNA on mica by means of atomic-force microscopy (AFM) technique [6], that was progressively developed in the last decade. Still mica as a substrate for nucleic acids has some evident disadvantages: negative charge in water, high polarity, extremely low conductivity. DNA on mica was imaged in STM only at ultra low currents [7]. Typically double helical pitch of DNA remains unresolvable.

As it was mentioned above it is very difficult to fix DNA on electrically conductive and rather inert HOPG. However, the graphite surface has layered structure with anisotropic oxidation rate. It substantially complicates the surface modification. In this paper we propose a new method of modification of HOPG to suit this support for DNA deposition. The surface of the substrate is kept flat enough for the AFM and STM investigations. We acquired reproducible AFM and STM images of DNA molecules adsorbed on HOPG.

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MATERIALS AND METHODS

We used modified HOPG as a substrate for the DNA deposition. HOPG was glow-discharged in the presence of pentylamine vapor.

The DNA used in this study was pUC19 plasmid DNA digested by PvuII. The DNA adsorption on the HOPG was carried out by the usual droplet procedure.

In the droplet method [8] HOPG surface was carefully put on a 10-15 μl drop of DNA solution at a concentration of 1-2 μg/ml in a buffer containing 10-30 mM of ammonium-acetate and 7-9 mM of magnesium chloride. The samples were incubated for 5 - 10 minutes. After completing the adsorption, the HOPG was washed with water, blotted with filter paper and dried with argon.

STM images were acquired with the scanning tunneling microscope FemtoScan (Advanced Technologies Center, Moscow, Russia) in the constant current mode (I_t=49 pA, U_t= 1V).

The samples were imaged in AFM tapping mode using NanoScope (Digital Instruments, Santa Barbara, USA) and commercial silicon nitride cantilevers. The images were processed using FemtoScan Online software.

RESULTS AND DISCUSSIONS

We have found the procedure (see materials and methods), which allows modification of the HOPG surface homogeneously keeping it sufficiently flat. After the modification the surface has the roughness (a mean square deviation) 0.3-0.5 nm. The corrugation height lies in the range 0.4-1.0 nm. When HOPG surface is modified chemically in a liquid medium, the oxidation occurs only at the lattice defects, whereas the rest of the surface remains unmodified. As the number of defects is limited, the degree of modification is quite low. The increase of the number of defects leads to the surface degradation making it irregular and unsuitable for AFM and STM investigations of biological objects. In addition, this multi-stage modification complicates the routine using of this method that is essential for DNA-mapping by AFM.

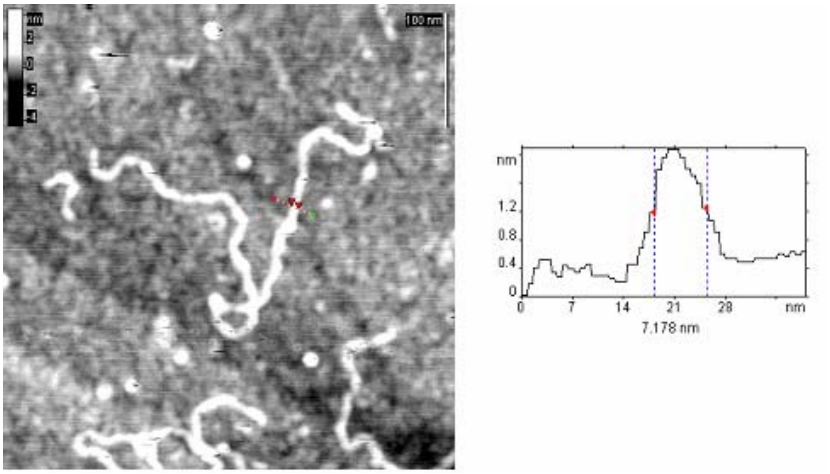

FIGURE 1. Tapping mode AFM image of DNA on HOPG with cross section of the molecule.

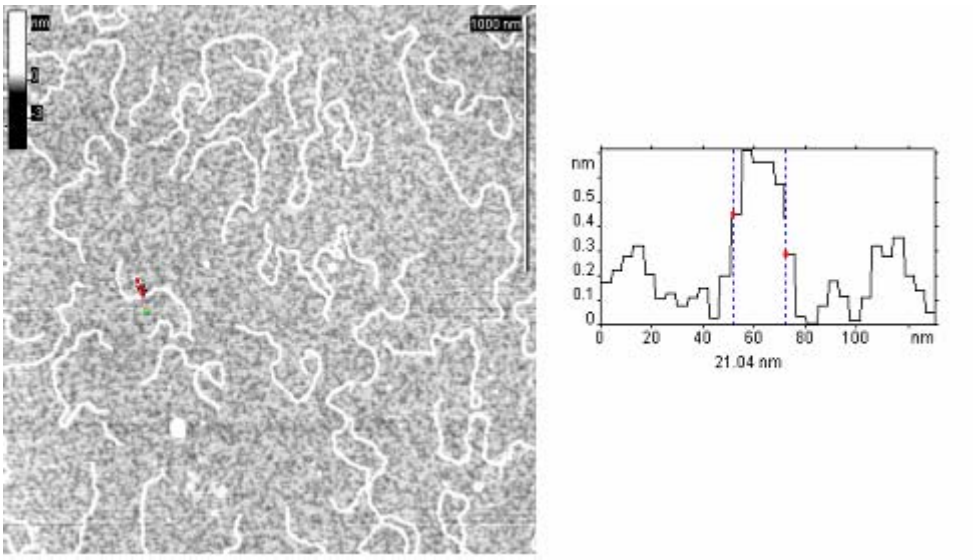

FIGURE 2. Tapping mode AFM image of DNA on mica with cross section of the molecule.

The typical images of DNA molecules absorbed on the modified HOPG surface in the air are shown in fig.1. The value of the length dispersion is about 3-6%. This indicates that molecules are not "outstretched" and are proper for DNA-mapping.

AFM images of DNA molecules absorbed on the modified HOPG surface are similar to those on mica (fig. 2), but typical molecule widths and heights for both supports are different (table 1). For the case of HOPG, the molecule width is smaller than for the case of mica (measurements have been carried out on the half height). The heights of DNA molecules on HOPG (1.6 ± 0.3 nm) are closer to the diameter of double helix of DNA *in vivo* (2 nm) than on mica (0.7 ± 0.1 nm). Because the same cantilever as well as operation mode has been used for both supports we attribute this difference to the influence of the support on DNA: mica makes the molecules more flattened out than HOPG (fig.3). The measured lengths of the molecules absorbed on HOPG and mica under identical saline conditions are close for both cases for different fragments (table 2).

TABLE 1. The measured heights and widths of DNA molecules deposited on mica and HOPG.

Substrate	Height, nm	Width, nm
HOPG	1.6 ± 0.3	8.5 ± 1.1
Mica	0.7 ± 0.1	19 ± 4

In our case, the tangential adhesion force increases due to the modified HOPG surface asperity preventing the molecules to displace. At the same time, the interaction force between the cantilever and biological object decreases. The interaction force between the cantilever and modified HOPG surface (pull out force) lies in the range 0.5-10 nN and 0.3-5 nN when measured in the air and in the hot nitrogen stream, respectively. AFM images of DNA molecules absorbed on the modified HOPG surface are sufficiently stable. The images remain unchanged after the repeated scans.

The mica surface in solution is charged negatively. DNA molecules also have a negative charge. Therefore, their immobilization requires an additional surface

modification or the presence of two-valence cations when applying DNA. The proposed method allows us to apply DNA without two-valence cations. This essentially expands its applications for the studies of DNA and DNA-proteins complexes. AFM images of DNA molecules absorbed on the modified HOPG surface from the three times distilled water (with low ionic force) are visually similar to fig. 1, but in this case the contour length of DNA molecules is 8-20% less than for the adsorption on mica or modified HOPG under 40 mM NH_4Ac+10 mM $MgCl_2$.

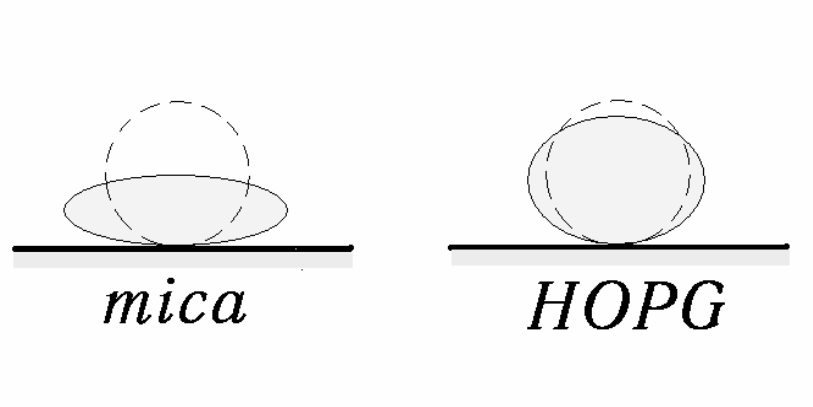

FIGURE 3. The proposed scheme of DNA deposition on mica and HOPG. Cross-section of the molecules are shown. Dashed circles demonstrate natural size of the molecule in solution.

The samples of DNA adsorbed onto modified HOPG were investigated by STM. DNA molecules in the STM images were found in inverse contrast (fig.4). The very important result is that the observed in STM image DNA width is smaller than that obtained from AFM data. So it may be turned out that STM imaging of DNA on HOPG may become a more accurate way for mapping DNA than AFM method.

TABLE 2. The measured lengths of different DNA fragments deposited on mica and HOPG under identical saline conditions.

Solvent	small fragment (332 b. p.)	big fragment (2364 b. p.)
Modified HOPG, 40 mM NH_4Ac 10 mM $MgCl_2$	113 ±7 nm	798 ±28 nm
Mica, 40 mM NH_4Ac 10 mM $MgCl_2$	114 ±6 nm	810 ±16 nm
Three times distilled H_2O	95 ±10 nm	748 ±26 nm

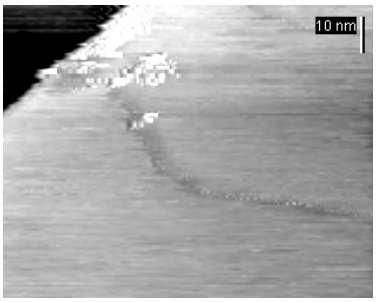

FIGURE 4. STM image of DNA on graphite.

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REFERENCES

1. Patricia G. Arscott, Gil Lee, Victor A. Bloomfield & D. Fennel Evans, *Nature* **339**, 484-486 (1989).
2. David Keller, Carlos Bustamante, and Rebecca W. Keller, *Proc. Natl. Acad. Sci.* USA **86**, 5356-5360 (1989).
3. David D. Dunlap & Carlos Bustamante, *Nature* **342**, 204-206 (1989).
4. C.R. Clemmer, T.P. Beebe, *Science* **251**, 640-642 (1991).
5. G. Travalgini, H. Rohrer, M. Amrein and H.Gross, *Surf. Sci.* **181**, 380-390 (1987).
6. H.G.Hansma, R.L.Sinscheimer, M.Q.Li, P.K.Hansma, *Nucleic. Acids Res.* **20**, 3585-3590 (1992).
7. Guckenberger R., Heim M., Cevc ., Knapp H.F., Wiegrabe W., Hillebrand A., *Science* **266**, 1538-1540 (1994).
8. D.V. Klinov, I.V. Lagutina, V.V. Prokhorov, T. Neretina, P.P. Khil, Y.B. Lebedev, D.I. Cherny, V.V. Demin, E.D. Sverdlov, Nucleic Acids Res. **26** No. 20, 4603-4610 (1998).