Substrate for Scanning Probe Microscopy of DNA: HOPG versus Mica

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The procedure of highly-oriented pyrolytic graphite (HOPG) modification for DNA imaging is found. Advantages of the HOPG in comparison with mica as a substrate for scanning probe microscopy of biomolecules are discussed. SPM images of DNA on HOPG were acquired and analyzed for various conditions of adsorption.

1. Introduction

At present time available AFM substrates for DNA imaging are limited in types. The conventional support for the biological objects is mica [1] which has hydrophilic surface with atomically flat regions of several microns and more. Its surface can be easily updated by a simple cleavage. However, mica has several important disadvantages as AFM support, namely the negative surface charge in solution [2] and a large adhesion. Besides, an immobilization of DNA on mica requires the modification of its surface by two-valence cations or silans, or by using the spreading-agents. Moreover, mica as a support is appropriate only for ultralow current STM imaging.

It is difficult to fix DNA and many other biological objects to unmodified HOPG surface [3–5]. However, the graphite surface has a layered structure with anisotropic oxidation rate which substantially complicates the surface modification. In this paper we realized the method of the HOPG surface modification that allows to immobilize DNA molecules and other biological objects. 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.

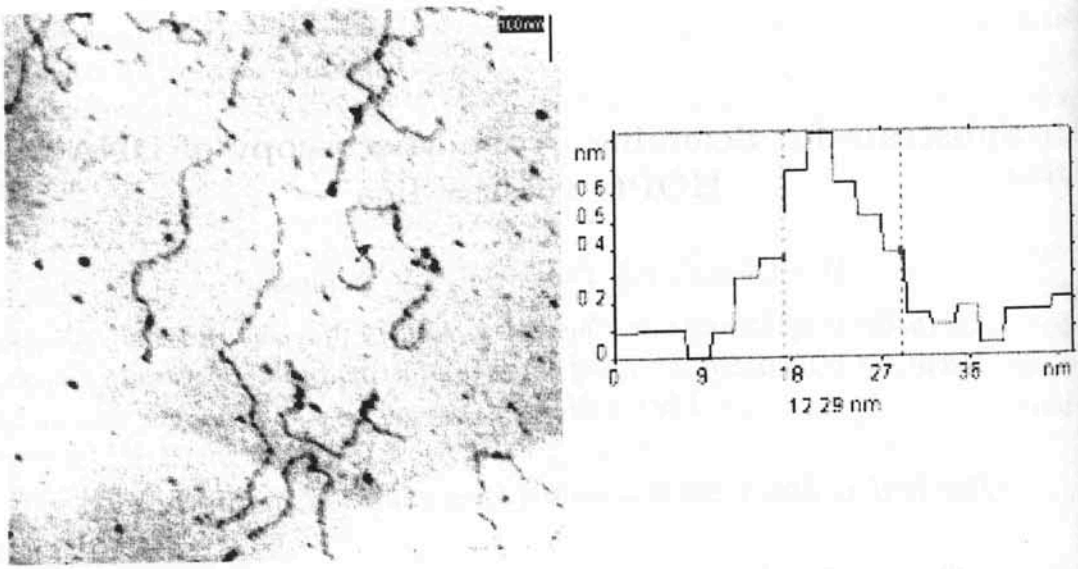

Figure 1. The typical images of DNA molecules absorbed on the modified HOPG surface in the air. Tapping mode; negative contrast; maximum height difference 3.5 nm.

Solvent	small fragment (332 b. p.)	large 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

Table 1. The measured lengths of the molecules absorbed on HOPG and mica under identical saline conditions for different DNA fragments.

2. 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 [6] 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

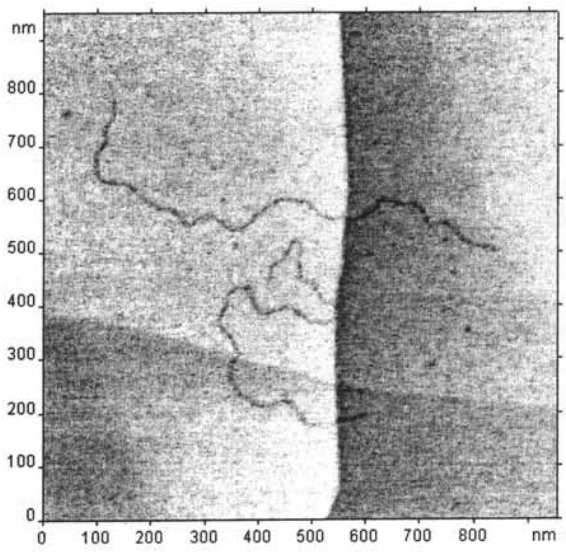

Figure 2. AFM image of DNA on mica. Contact mode; negative contrast; maximum height difference 3.9 nm.

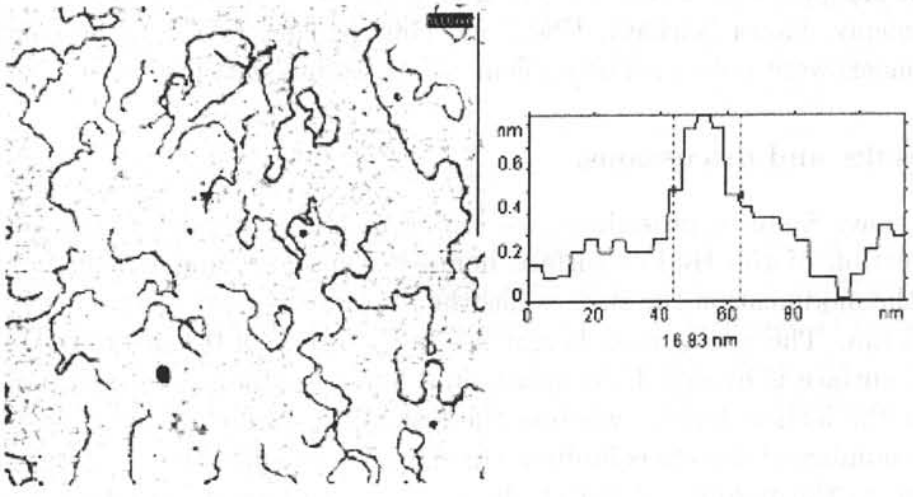

Figure 3. AFM image of DNA on mica. Tapping mode; negative contrast; maximum height difference 6.6 nm.

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 Femto-

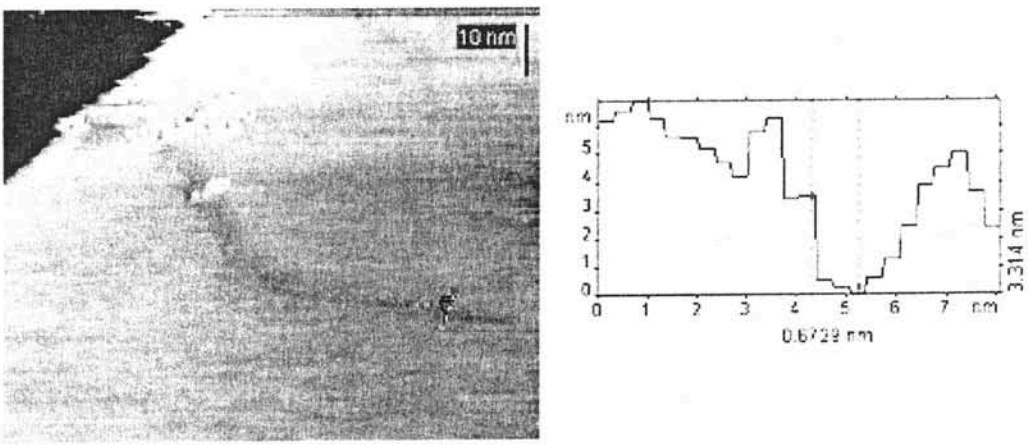

Figure 4. STM image of DNA molecule absorbed on HOPG. I_t = 49 pA, U_t = 1 V.

Scan (Advanced Technologies Center, Moscow, Russia) in the constant current mode (I_t = 49 pA, U_t = 1 V).

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.

3. Results and discussions.

We have found a procedure (see materials and methods) which allows the modification of the HOPG surface homogeneously keeping it sufficiently flat. After the modification the surface has the roughness (mean square deviation) of 0.3–0.5 nm. The corrugation height lies in the range of 0.4–1.0 nm. When the 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 in 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 usage of this method that is essential for DNA-mapping by AFM.

The typical images of DNA molecules absorbed on the modified HOPG surface in the air are shown in Fig. 1 (tapping mode) and Fig. 2 (contact mode). The value of the length dispersion is about 3–6%. This indicates that molecules are not "outstretched" and are suitable for DNA-mapping.

AFM images of DNA molecules absorbed on the modified HOPG surface

are similar to DNA molecules images on mica (Fig. 2), but typical molecule widths for both supports are different. For the case of HOPG, the molecule width is smaller than for the case of mica (measurements have been carried out at the half height and the same cantilever has been used for both supports). The measured lengths of the molecules absorbed on HOPG and mica under identical saline conditions are close for both cases for different fragments (Table I).

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 of 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 $MgCl_2$.

The samples of DNA adsorbed onto modified HOPG were investigated by STM. DNA molecules in the STM images were found in inverse contrast (Fig.3). The very important result is that the observed in STM image DNA width is smaller than that obtained from AFM data. So it may prove that the STM imaging of DNA on HOPG may become a more accurate way for mapping DNA than the AFM method.

References

[1] H.G.Hansma, R.L.Sinscheimer, M.Q. Li, P.K.Hansma, Nucleic. Acids Res., 20 (1992) 3585.

[2] D.J. Muller, M. Amrein, A. Engel, Journal of Structural Biology, 119 (1997) 172.

[3] P.G. Arscott, G. Lee, V.A. Bloomfield, D. F. Evans, Nature, 339 (1989) 484.

[4] D. Keller, C. Bustamante, R. W. Keller, Proc. Natl. Acad. Sci. USA, 86 (1989) 5356.

[5] R.J. Driscoll, M.G. Youngquist, J. D. Baldeschwieler, Nature, 346 (1990) 294.

[6] 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 (1998) 4603.