

# Effect of Supporting Substrates on the Structure of DNA and DNA–Trivalent Complexes Studied by Atomic Force Microscopy

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**Abstract**—Linear DNA, circular DNA, and circular DNA complexes with trivalent (TV), a synthetic oligopeptide, were imaged by atomic force microscopy (AFM) using mica as a conventional supporting substrate and modified highly ordered pyrolytic graphite (HOPG) as an alternative substrate. A method of modifying the HOPG surface was developed that enabled the adsorption of DNA and DNA–TV complexes onto this surface. On mica, both purified DNA and DNA–TV complexes were shown to undergo significant structural distortions: DNA molecules decrease in height and DNA–TV displays substantial changes in the shape of its circular compact structures. Use of the HOPG support helps preserve the structural integrity of the complexes and increase the measured height of DNA molecules up to 2 nm. AFM with the HOPG support was shown to efficiently reveal the particular points of the complexes where, according to known models of their organization, a great number of bent DNA fibers meet. These results provide additional information on DNA organization in its complexes with TV and are also of methodological interest, since the use of the modified HOPG may widen the possibilities of AFM in studying DNA and its complexes with various ligands.

**Key words:** *atomic force microscopy, DNA compaction, highly ordered pyrolytic graphite, supporting substrates, trivalent*

## INTRODUCTION

Atomic force microscopy (AFM) is a relatively new technique that enables an analysis of the surface microrelief in the nanometer range.<sup>2</sup> The AFM approaches to DNA study are actively developed by some research groups, since they provide information additional to that obtained by such a traditional method as electron microscopy. The methodological efforts were mainly devoted to the development of techniques of preparation of DNA samples on various supporting substrates [1–4].

The supporting substrates used in AFM studies of biopolymers should meet the following general demands: their surface should be sufficiently flat and adsorb well the studied material. Mica is a commonly used substrate in AFM studies of biological samples, in particular DNA [4]. It has a hydrophilic surface with atomically flat areas of length larger than 100  $\mu\text{m}$ . However, already the early studies of DNA on the mica

support have revealed serious disadvantages of this substrate, such as the negative surface charge and strong deforming adhesive forces arising on adsorption and drying. To overcome these problems, the surface of mica used for ultrastructural studies of DNA was modified by bivalent cations [5, 6], silanes [4], or spreading agents [7].

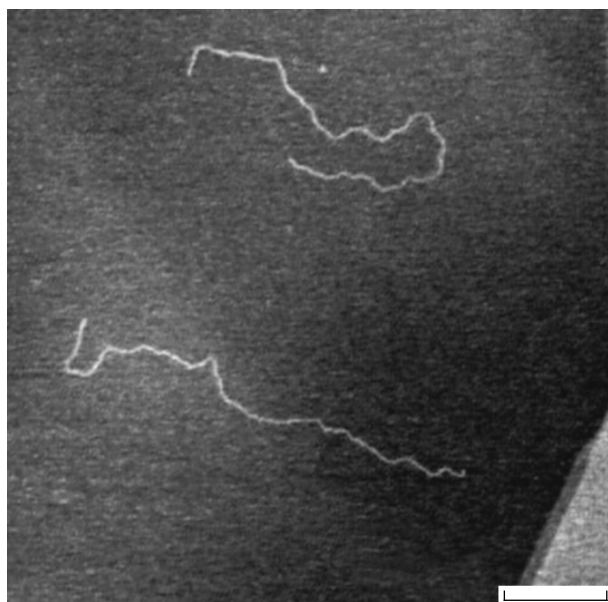
Like mica, HOPG has the surface with atomically flat areas, which can be easily renewed by plain cleavage. In contrast to mica, the HOPG surface is inert and hydrophobic. Therefore, the attachment of biological materials to its surface and obtaining reproducible AFM images on scanning of individual molecules or their complexes is not yet a trivial problem [8].

Single-stranded and double-stranded DNA were imaged by AFM using various techniques for the sample preparation [1]. AFM was proposed as a routine method for measuring the length of DNA fragments [3, 9]. It was successfully used for mapping DNA that forms R-loops [3].

AFM seems to be also promising for studying more complex biological subjects, such as DNA complexes with proteins and other ligands. Their study is additionally complicated, because they are less resistant than

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<sup>2</sup> Abbreviations: AFM, atomic force microscopy; HOPG, highly oriented pyrolytic graphite; EM, electron microscopy.



**Fig. 1.** The AFM image of DNA molecules (the pGem7zf+ plasmid, 3000 bp, linearized at the *Sma*I site) adsorbed onto a HOPG surface. The bar represents 200 nm.

DNA to many factors. AFM may provide additional information on packing of DNA strands within these complexes and eliminate artifacts that arise in electron microscopy due to the staining and contrasting of preparations.

The goal of this work was to develop a method for HOPG modification that could help study DNA and DNA–protein complexes on the AFM support. We wish to compare the morphology of linear and circular DNA, and complexes of circular DNA with the synthetic peptide trivaline, H-(L-Val)<sub>3</sub>–(NH)<sub>2</sub>–Dns, deposited on mica, a conventional support, and on a modified HOPG, an alternative support. We have chosen the complexes of the circular supercoiled DNA with trivaline as a test subject, since they have the easily recognizable circular structure that had well been studied morphologically and thoroughly characterized by EM [10–12].

## RESULTS AND DISCUSSION

The effect of supporting substrate on the structure of biological subject imaged by AFM is yet scarcely known. In many cases, structural changes in the subject interacting with mica are small because of its inherent stability. An assortment of AFM supports is rather limited.

HOPG is a support that is an alternative to mica. However, the HOPG surface should be modified for attachment of biomolecules. The treatment by pentylamine vapor in a glow discharge was previously used to activate supports in EM [13]. We recently proposed a method [14] that allows an even modification of the

HOPG surface in aqueous vapor plasma while keeping this surface flat enough. After this treatment, the HOPG surface is rugged (root-mean-square deviation of 0.3–0.5 nm), has level difference (peak-to-valley height modulations) of 0.4–1 nm, well binds DNA molecules and other biological subjects, and is suitable for AFM studies.

Figure 1 shows typical images of linear DNA molecules sorbed on the modified HOPG surface (DNA was imaged in air). The AFM images are rather stable and are not destroyed on repeated scanning despite the fact that DNA was imaged in contact mode that exerts a more destructive effect of cantilever to the biological subject during scanning than the tapping mode.

The heights of the DNA molecules adsorbed onto the modified HOPG surface are  $1.5 \pm 0.3$  nm in air and  $2.2 \pm 0.3$  nm when the specimen was blown through with warm nitrogen. The observed height is close to the diameter of DNA molecules in solution. On the other hand, the heights of DNA molecules measured by AFM on mica are 0.5–0.7 nm. This indicates significant changes in DNA conformation due to an interaction with mica [15]. It is also possible that the height of the DNA molecules may be reduced on mica due to the pressure exerted by cantilever on scanning. However, the available data permit the conclusion that the DNA molecules are strongly deformed even on their adsorption and subsequent drying on mica [15], and this is probably the main reason of the observed height reduction. Nevertheless, the general forms of DNA molecules (contour length and smoothness of winding) are nearly the same on the AFM images obtained on mica and HOPG.

As previously observed, larger biological subjects can also contract upon the adsorption onto a solid support. For example, AFM studies of bacteriophages have shown that the phage head is more compressed after the phage adsorption on mica (20%) than on HOPG (10%) [16]. The phage tails are more tightly packed and are incompressible upon the phage adsorption onto mica and HOPG.

When comparing these supports, it was of interest to ascertain how the supports will affect the subjects with a complicated but, at the same time, well-characterized organization. The complexes of the supercoiled DNA with trivaline can be assigned to the subjects of this sort [10].

We demonstrated in this work that the complexes of DNA with trivaline give different AFM images on deposition on different supporting substrates (graphite or mica) and the type of support exerts an effect not only on the observed height of the complexes but also on their structure. The degree of DNA compaction in the complexes is defined as a ratio of the contour length of noncompacted DNA to the complex length. The shape of complexes adsorbed onto the surface of modified HOPG, the degree of DNA compaction within the complexes, and the general arrangement of complexes in

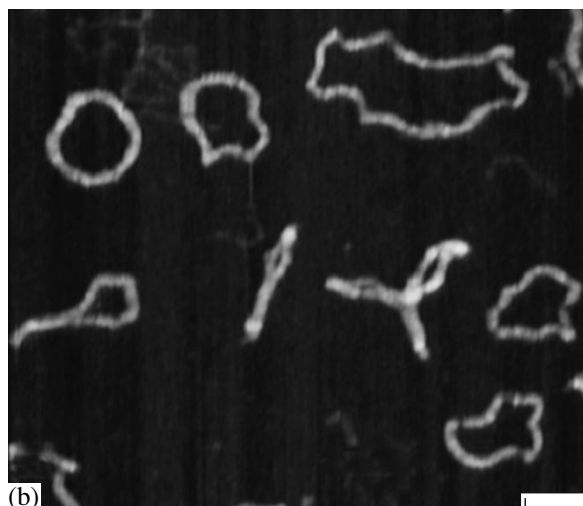
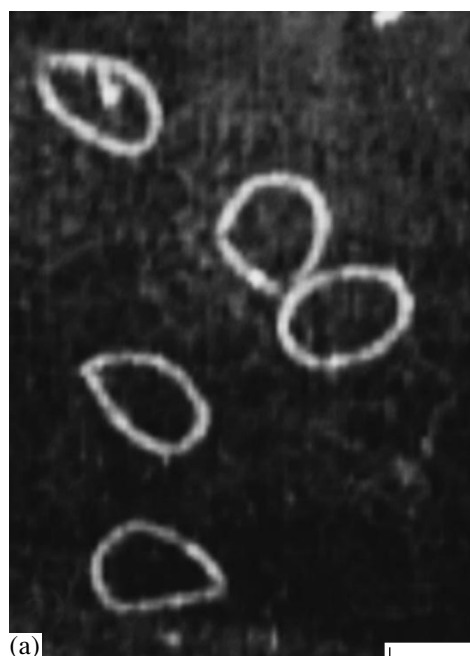
the AFM image (Fig. 2a) are similar to those observed by EM [17]. The AFM image of a circular supercoiled DNA preparation deposited on the HOPG support is given in Fig. 2c. It shows the morphology and thickness typical of such molecules. One can suppose that the procedure for preparation of specimen for AFM and the method of imaging cause no additional appreciable distortions in the structure of imaged biological subjects as compared with EM.

A different picture was observed when the complexes of DNA with trivaline were adsorbed on the mica surface (Fig. 2b): the majority of the circular structures lose their right shape but, nevertheless, retain their length and are quite recognizable. They are clearly strained and frequently contain the regions with uncharacteristic sharp bends and acute angles. All these structural distortions may probably be explained by a partial destruction of the complexes during their adsorption to mica, which releases the internal elastic energy accumulated in the complexes (much as in the supercoiled DNA molecules) and results in distortions of their backbone. It seems likely that the DNA–trivaline complexes deposited on mica are not flat anymore, and some regions of the fibers that form complexes are lifted above the support rather than adhere to it, which becomes apparent in the AFM images.

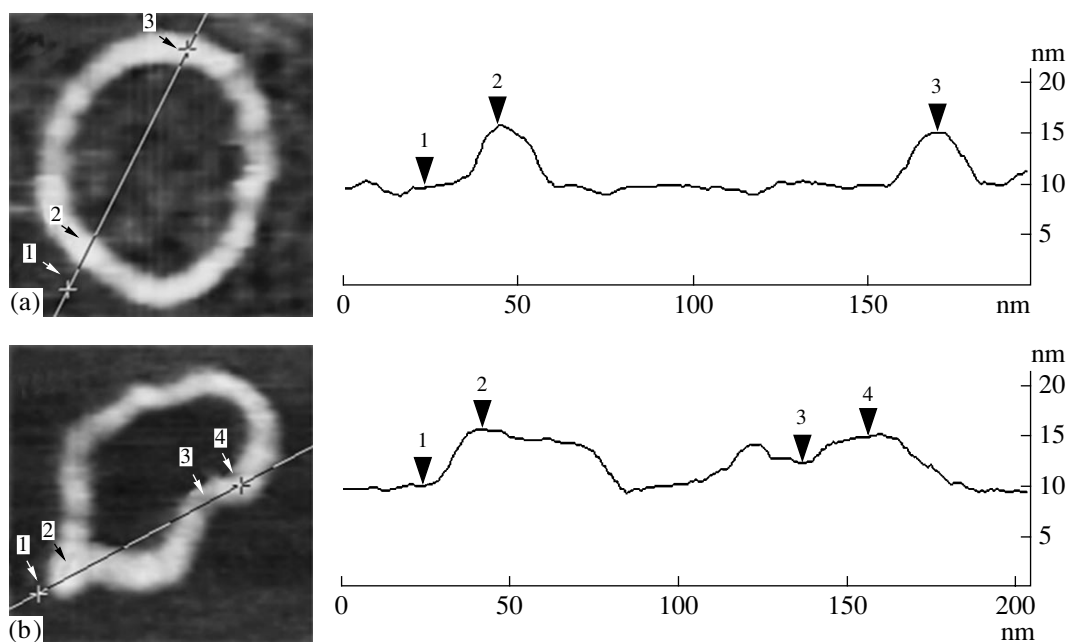
The AFM images with cross-sectional surface profiles of two typical DNA–trivaline complexes deposited on HOPG and mica, respectively, are shown in Figs. 3a and 3b. The mean value of height measured for the complexes deposited on HOPG is  $6.1 \pm 0.2$  nm (Fig. 3a). This value is in good agreement with data on the thickness of complexes measured on the EM preparations stained by uranyl acetate [10]. The mean value of height of the ring structures in AFM images on mica is  $4.9 \pm 0.7$  nm (Fig. 3b), which indicates the fiber contract on this support. Note also that there is a significant spread in heights measured for the complexes adsorbed to mica: the deviation from the mean value is 0.7 nm compared with 0.2 nm in the case of HOPG. Most likely, the destruction of complexes is partial and some regions of the ring structures are left intact.

The AFM image on mica (Fig. 3b), which is typical of this support, shows segments with the height in the range of 5.5–5.8 nm, which are joined to the regions with the height of 3.9–4.8 nm. The highest points of these ring structures frequently coincide with sharp bends in fibers, which indicates that the fibers are either thicker at these points or lifted above the support surface. The AFM images of the DNA–trivaline complexes on HOPG demonstrate much larger height uniformity of the fibers that form these complexes.

Thus, we showed that the triple rings are subjected to appreciable deformations upon the preparation of specimens for AFM on mica, whereas their regular structure remains intact when modified HOPG is used as a support according to the procedures we developed in this work.



**Fig. 2.** The AFM images of complexes of the circular supercoiled pBR232 plasmid (4360 bp) with trivaline adsorbed on the surface of (a) modified HOPG and (b) mica; (c) AFM image of the circular supercoiled DNA of the supercoiled pBR232 plasmid (4360 bp) deposited on the HOPG support. The bar represents 100 nm.



**Fig. 3.** The AFM image of the complex of the circular supercoiled pBR232 plasmid (4360 bp) with trivaline adsorbed on the surface of (a) modified HOPG and (b) mica. The size of image is 180 nm on a side. Cross-sectional surface profile along the line drawn is shown to the right. The measured height corresponds: (a) 6.1 nm between points 1 and 2, and 5.5 nm between points 1 and 3; (b) 5.5 nm between points 1 and 2, 2.5 nm between points 1 and 3, and 4.8 nm between points 1 and 4.

The AFM data on the height of various segments in the subjects under investigation may be of considerable interest, especially when the shape of the subject is more complicated than an ordinary ring. For example, the unusual structures formed by monomeric and dimeric circular molecules of the pTbo-1 plasmid with trivaline [17, 18] have bulges well recognizable in AFM images (Figs. 4a and 4b) at the points where two fibers merge into one (marked by arrows).

According to the previously proposed model for the packing of DNA fibers in such compact structures [17], a bulk of bent DNA fibers merges exactly at these points and, therefore, imperfect packing of fibers is possible, which can be visualized as bulges. In any case, these points become clearly visible as peculiar in the AFM images of the structures described here. The AFM technique with the use of modified HOPG developed in this study may be useful for studying DNA and DNA-protein complexes.

## EXPERIMENTAL

**Trivaline** [H-(L-Val)<sub>3</sub>-(NH)<sub>2</sub>-Dns] was synthesized as described by Makarov *et al.* [15].

DNA plasmid pGem7zf+ (3000 bp) was linearized at the *Sma*I site (Promega). Preparations of the circular supercoiled plasmids pBR322 (4360 bp) and pTbo-1 (6120 bp) were purified with the use of Wizard Mini-preps DNA Purification System (Promega) according to the standard procedure.

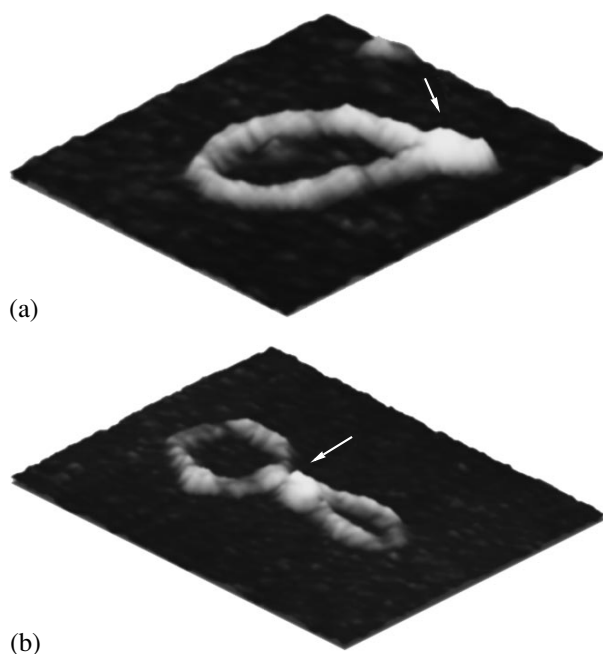
**Complexes of trivaline with DNA** of the circular supercoiled plasmids pBR322 and pTbo-1 were prepared by direct mixing of the peptide in aqueous trifluoroethanol and DNA in 1 mM cacodylate buffer, pH 7.0 [11]. The final solution contained 10 µg/ml DNA and 25% trifluoroethanol. Trivaline concentration varied from 0 to 0.3 mM.

**Modification of the HOPG surface.** HOPG was kindly donated by the Institute of Graphite (Moscow, Russia). The freshly cleaved HOPG was placed into a water vapor atmosphere at pressure about 10<sup>-2</sup> Torr, the voltage from 500 to 1000 V was applied to electrodes, which resulted in a slight violet glow. Treatment in the glow discharge was continued for 15–30 s [16, 17]. The supports modified in this way were used no later than after 30 min.

**Preparation of specimens for AFM.** A solution of the pGem7zf+ plasmid DNA (5 µl, 1–2 µg/ml of DNA) in 10 mM MgCl<sub>2</sub>, 20 mM NH<sub>4</sub>OAc, pH 7.0 was deposited onto the modified HOPG surface and left for 1–5 min, the surface was then washed with water and dried in argon.

A solution of the DNA-trivaline complex (5 µl) was deposited onto the surface of freshly cleaved mica and left to adsorb for 1 min. The support was then blot with filter paper and dried in argon without preliminary washing.

**AFM imaging** was performed on a Solver P47 BIO instrument (NT-MDT, Zelenograd, Russia) and a Nanoscope II instrument (Digital Instruments, United States)



**Fig. 4.** The AFM image of the complex of the circular supercoiled pTbo-1 plasmid (6120 bp) with trivalent adsorbed on the surface of modified HOPG: the structure formed by (a) a monomeric circular molecule and (b) a dimeric circular molecule. Arrows indicate bulges localized at the points of merger of two fibers into one. Image size is (a)  $333 \times 293$  nm and (b)  $546 \times 606$  nm.

using D and J scanners. The instruments were calibrated using colloidal gold particles of 10 nm (Sigma, United States) and diffraction gratings. Scanning was carried out in the contact mode using the feedback system. The scanning rate corresponded to 7–14 Hz. Cantilevers (Digital Instruments, United States) were used with a spring constants of 0.06, 0.12, 0.38, and 0.53 N/m. Scanning angles were  $90^\circ$  and  $180^\circ$ . The images were processed with the Nanoscope II software.

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