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Optimizing DNA Visualization with a Solver P47H Atomic-Force Microscope

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Abstract—The conditions for visualizing DNA molecules with a Solver P47H atomic-force microscope (NT-MTD, Moscow, Russia) were optimized. The DNA samples had different sizes, types, and conformations (pBR-322 plasmid DNA and chicken erythrocyte DNA) and were immobilized on mica. The microscope was equipped with a Smena-B detecting head and was operated in a tapping mode. The dependence of the amplitude of tip oscillations on the spacing between the tip and the test sample's surface was used to determine the optimum parameters of scanning. The highest quality and reproducibility of the DNA images were attained by scanning with a small initial amplitude (9–23 nm) of cantilever oscillations and an optimum gain (0.08–0.3). Images with the highest contrast were obtained in the amplitude curve's region corresponding to a repulsive interaction regime. The operating amplitude was set at one-half (or slightly less than) the initial amplitude of tip oscillations.

INTRODUCTION

Atomic-force microscopy (AFM) based on measuring the force interaction between a tip and the surface of a test sample is widely used to analyze biomolecules. The quality of AFM images, their contrast, and the reproducibility of experimental results are all governed by the selected conditions and regimes of scanning, which is particularly important for biological samples (proteins, nucleic acids, their biological analogues, etc.).

A DNA molecule (a genetic information carrier) is a double-stranded negatively charged polymer. Modern biochemistry places special emphasis on investigations both of the DNA, which changes its conformation in the course of functioning through interactions with the other components of the cell and its nucleus, and of damages to the DNA that rive rise to pathologic processes in the organism and other detrimental effects. Bacterial extrachromosomal circular supercoiled DNA molecules (plasmid DNA) are often used in biochemistry as a model DNA. Formation of an isolated single-stranded breakage in a plasmid DNA molecule causes its conformation to change and its supercoiled shape to be replaced by a circular one (Fig. 1).

The objective of this work is to optimize the methods for visualizing DNA molecules by means of a Solver P47H atomic-force microscope with a Smena-B detecting head, which is used to investigate changes in the DNA structure when solving the fundamental and applied biochemical problems.

MATERIALS AND METHODS

DNA samples were visualized with a Solver P47H atomic-force microscope (NT-MTD, Moscow, Russia) operating in the tapping mode on air at room temperature. The Smena-B detecting head, equipped with a scanning piezoelectric element (piezoscanner) capable of scanning a region of $50 \times 50 \times 2.5$ μm, allows a sample to be analyzed both in air and in liquid medium.

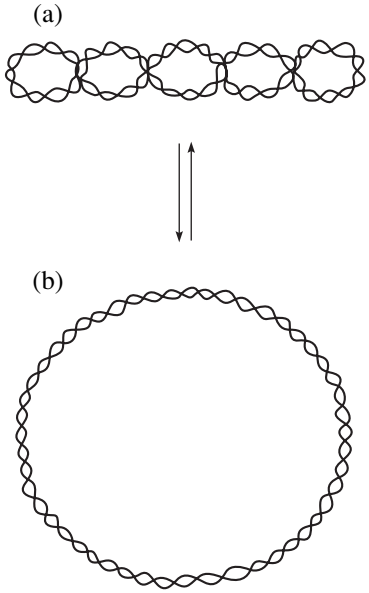

Fig. 1. Transconformations of plasmid DNA: (a) supercoiled and (b) circular DNA shapes.

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Standard NSG11 silicon cantilevers (NT-MTD) with a length of 100 μm, a spring constant of 11.5 N/m, and resonance frequencies of 190–275 kHz were used. The typical curvature radius of the tip cantilever did not exceed 10 nm. The scan resolution was 512 × 512 pixels. The Nova RC 1.0.26.578 software package for the NT-MTD probe microscopes was used for processing the AFM images and determining the sizes of scanned samples.

The DNA samples were prepared based on a buffer solution of 25 mM tris-HCl (pH = 7.5) and 5 mM $MgCl_2$. The plasmid DNA concentration was brought to values of 2.5–5.0 μg/ml. The DNA was presented by circular (30–35%) and supercoiled (65–70%) forms: pBR-322 plasmid DNA (4362 nucleotide pairs). The chicken erythrocyte DNA concentration was brought to values of 0.125–1.0 μg/ml. A test sample (2 μl) was applied to a freshly cleaved mica (1 × 1 cm); held at room temperature for 3–5 min; then rinsed with bidistilled deionized sterile water (1 ml); and dried on air and, subsequently, on silica gel.

Tapping Method of Scanning

The tapping mode has gained the widest acceptance among the vibration-based methods, particularly in investigations of biological samples. Its specific feature is that the oscillating tip of the cantilever is located so close to the sample surface that, in the course of scanning, it regularly comes in contact with the surface in the lower part of its double-amplitude region. In this case, the cantilever does not touch the surface for almost the whole period of its oscillations and its interaction with the sample is relatively weak. As the tip approaches the surface, the interaction becomes stronger. The character of interaction may affect both the amplitude of cantilever oscillations at a resonance frequency and the phase shift of the fundamental harmonic of oscillations with respect to the actuating signal. At each point of the scan space, a feedback system maintains the amplitude of tip oscillations at a user-set level (the operating amplitude) by moving the tip at a right angle to the sample surface, thereby mapping the surface topography.

In comparison to the amplitude, the phase of oscillations is more sensitive to variations in the intensity of the tip–sample interaction. Obtaining an image of the phase signal simultaneously with the topographical mapping of the sample surface provides additional information on the details of the surface structure. This mode of measurements was called the phase contrast method.

The interaction between a cantilever operating in the tapping mode and the surface represents the Van der Waals interaction, i.e., a combination of long-range attractive and short-range repulsive forces. In the general case, these forces depend on the spacing between the tip and the sample surface and exhibit characteristic nonlinear effects [1–4].

The behavior of the oscillating tip near the sample surface is described, according to [1], by the following expression:

$$z(z_c t) = z_0(z_c) + A(z_c)\cos[wt - \phi(z_c)], \qquad (1)$$

where z_0 is the tip displacement; A, w, and ϕ are the amplitude, angular frequency, and phase shift of the tip oscillations, respectively; and z_c is the spacing between the sample and the tip at rest in the absence of any interaction between them. The tip's displacement, the amplitude, and the phase shift depend on the tip–sample separation. As z_c is reduced, the influence of the forces acting between the tip and the surface increases, which manifests itself as a deformation of the cantilever resonance curve [1]. As a result, two main regions of high and low amplitudes are discernible in the resonance curve. The first of these is attributable to the attractive forces, and the other corresponds to the repulsive forces. A change between two states during a force approach cycle can be observed by measuring an amplitude curve [5, 6].

RESULTS AND THEIR DISCUSSION

A Solver P47H atomic-force microscope is designed primarily for measuring with a nanometer resolution the surface characteristics and the near-surface physical fields of extended samples [7]. A Smena-B detecting head contains a piezoscanner with a scan space of 50 × 50 × 2.5 μm. A cantilever is installed on the piezoscanner. Since this design of an atomic force microscope is versatile, it is not optimized for visualizing small samples such as DNA molecules [5–8].

Scanners with a low sensitivity and a small scan space (on the order of 3 × 3 × 1.3 μm) are conventionally used to achieve atomic resolutions (the DNA diameter is ~2 nm), since these scanners have a more compact design, a higher resonance frequency, and a lower sensitivity to control voltage noise. The scanning range of our instrument is 50 × 50 × 2.5 μm.

Our task was to optimize the procedure for visualizing DNA molecules, from the sample preparation phase to the acquisition of high-quality images of nanostructures with sizes comparable to that of the DNA (diameter, 2 nm), using a Solver P47H atomic-force microscope with a Smena-B detecting head.

In a number of papers, e.g., in [5, 6], scanning of a sample was performed with the control of a change between the attraction and repulsion interaction regimes. The initial amplitude of tip oscillations substantially affected the resolution and the image contrast. Sharp repeatable images of immunoglobulin (IgG) molecules were recorded when scanning was carried out with a low initial amplitudes of oscillations that could not deform the molecule [6]. The results

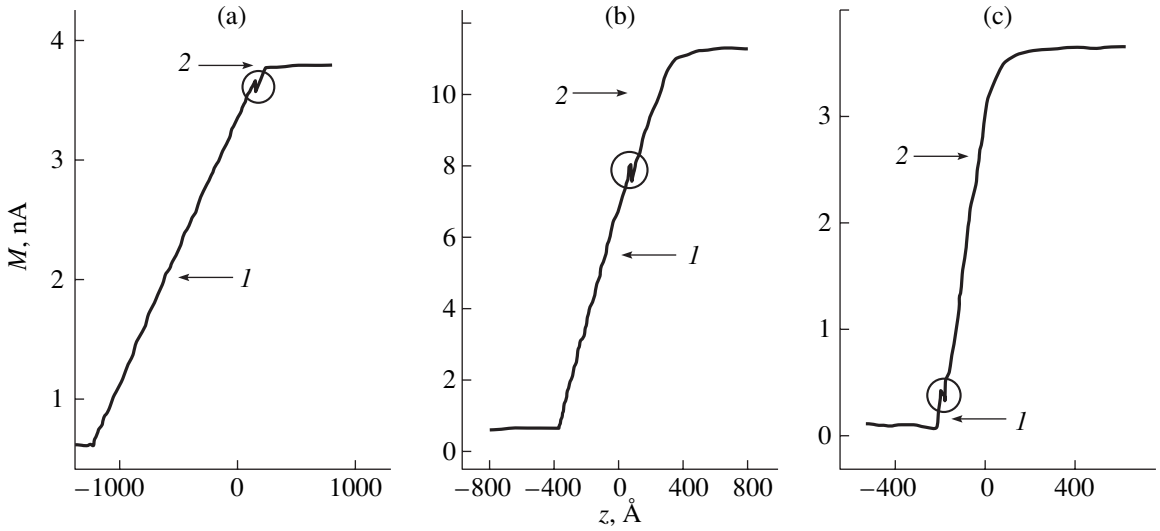

Fig. 2. Experimental dependence of signal M (nA), which is proportional to the amplitude of cantilever oscillations, on distance z (Å) between the tip cantilever and the surface, measured on a portion of the mica sample that was free from DNA molecules. After determining the proportionality factor that relates the amplitude of oscillations A (nm) to signal M (nA), the initial amplitude of cantilever oscillations was (a) 137 nm, (b) 78 nm, and (c) 40 nm [7]; (*1*) repulsion interaction zone and (*2*) attraction interaction zone.

presented in [6] were obtained with the same microscope as in our work, with the only difference being that it was equipped with a Smena-A detecting head and an AA10 (NT-MDT) adapter, which allowed resolutions as high as the atomic resolution to be achieved.

To optimize the scanning parameters (e.g., the amplitude), we used the dependence of the amplitude of tip oscillations on the tip–sample separation. Typical experimental dependences, measured on a portion of the mica that is free from DNA molecules, are shown in Fig. 2. The amplitude of oscillations is constant as long as the tip–sample separation is less than the initial amplitude. As distance z_c diminishes, the amplitude decreases according to a linear law until the regime of the attraction interaction is replaced by that of the repulsion, which manifests itself by an amplitude jump in Fig. 2.

The highest quality and the best reproducibility of DNA images were achieved when the scanning was performed with a small (9–23 nm) initial amplitude of cantilever oscillations, which was determined by the cantilever exciting voltage applied to the piezoscanner and was dependent both on the cantilever type and on the physical properties of the test sample surface. The sharpest images were obtained in the repulsive interaction region of the amplitude curve. The operating amplitude was set at one-half (or slightly less than) the initial amplitude of tip oscillations. The optimum operating gain of the feedback circuit in our instrument, with which the maximum scan velocity was attained without deteriorating the quality, ranged between 0.08 and 0.3.

The quality of images also depends on the conditions under which the sample is prepared (i.e., on whether a water film is present on the mica surface after drying).

The samples were scanned in three regimes: at a constant amplitude, at a constant height, and in the phase-contrast mode. As a result of the optimization described, we obtained high-quality images of DNA molecules of different sizes, types, and conformations (Figs. 3 and 4). The figures show that higher-contrast images can be obtained in the latter two regimes, which provides additional information on the details of the surface structure. The vertical and lateral DNA dimensions (see Fig. 3c) lie within the following limits: height, 0.22–0.97 nm (0.5 nm on the average), and width, 8.3–19.8 nm (12.6 nm on the average). These results comply with the numerous data presented in the literature [8–13]. In all these studies, the measured DNA height is <1 nm, whereas the molecular diameter, calculated on the basis of the Watson–Crick model of DNA [14], is 2 nm. This discrepancy may result from the deformation of the sample by the cantilever [8–10], the molecular dehydration [11], the salt deposition [10, 12, 13], and the electrostatic interaction between the molecule and the substrate [13]. These explanations are in general applicable to our study as well, since our samples were scanned in air (dehydration) and preparation of the substrate involved application of Mg^{2+} bivalent cations ($MgCl_2$ salt was used) on it in order to adsorb negatively charged DNA molecules on a mica

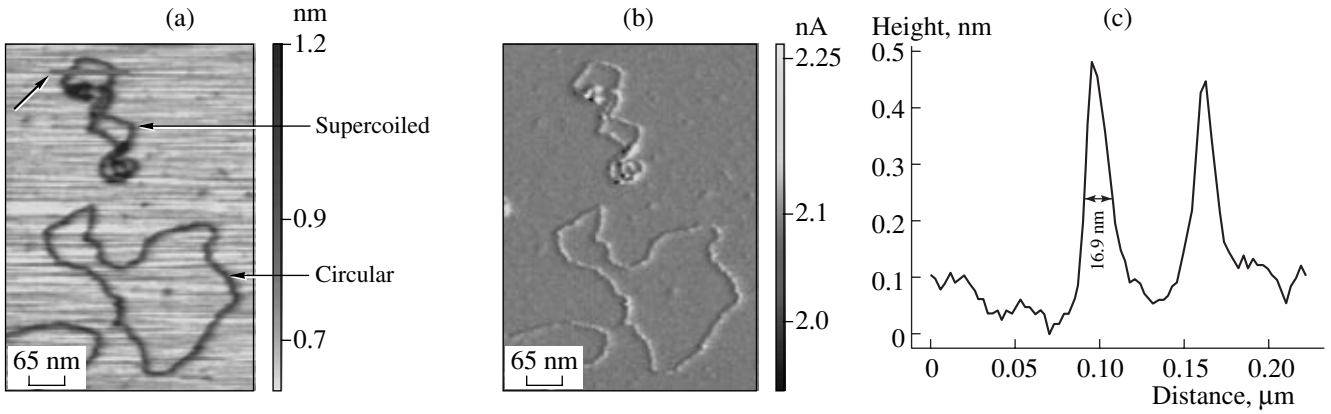

Fig. 3. AFM visualization of pBR-322 plasmid DNA on a mica substrate, performed in the tapping mode: (a) surface geometry, (b) constant-height regime, and (c) profile of the DNA cross section in the zone marked with an arrow in Fig. 3a.

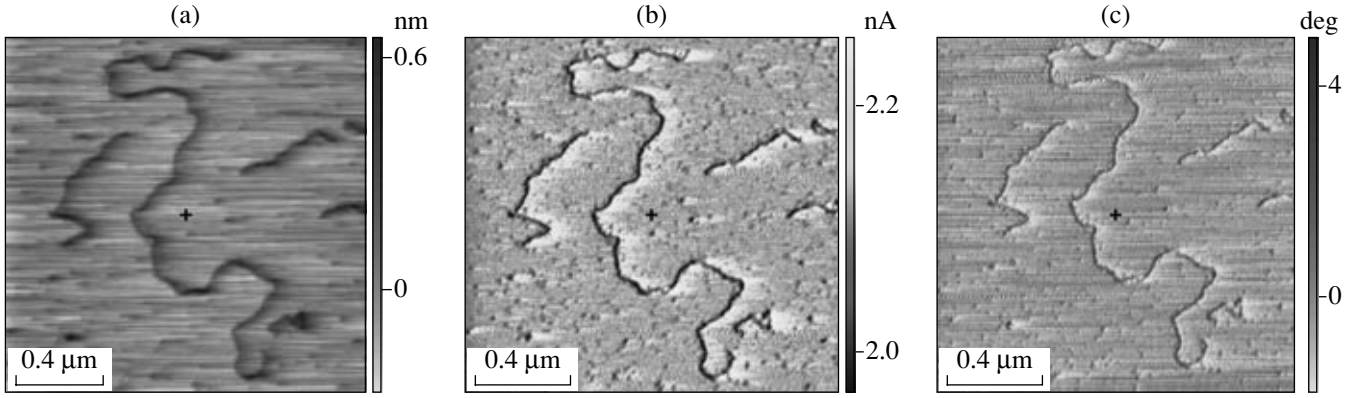

Fig. 4. AFM visualization of chicken erythrocyte DNA on mica substrate, performed in the tapping mode: (a) surface topography, (b) regime of a constant height (detection of varying amplitude), and (c) phase contrast mode (detection of varying phase).

substrate being negatively charged during its cleavage. The lateral dimensions of the recorded images were overestimated due to the finite radius of the tip's curvature, and the broadening of the test sample profile results from the superposition of the tip shape on the recorded image.

CONCLUSIONS

In this paper, we presented the optimum conditions for the AFM visualization of DNA molecules having different sizes, types, and conformations and immobilized on mica. The Solver P47H atomic-force microscope had a Smena-B detecting head with an extended range of scanning. The investigations of DNA molecules were carried out in the tapping mode. The dependence of the amplitude of tip oscillations on the spacing between the tip and the test sample surface was used to determine the optimum parameters of scanning. The best quality and reproducibility of DNA images were achieved by scanning with a low initial amplitude of cantilever oscillations (9–23 nm) and an optimum operating gain of the feedback circuit (0.08–0.3). The sharpest images were obtained in the amplitude curve's region corresponding to the repulsive interaction regime.

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