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# Application of a Scanning Probe Microscopy to the Study of DNA Conformation

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Conformational properties of DNA have been studied by scanning probe microscopy and the advantages of this method have been shown for the study of biopolymer morphology. It has been shown that in aqueous-alcohol solutions the linear DNA has the toroid-like conformation without any compacting agents. The conformational transition of the supercoiled DNA into the unfolded circle occurs under the cyclic DNA–surfactant interaction. Morphology of linear DNA in the complex with a surfactant has been determined. It has been assumed that the toroid-like conformation of linear DNA is energetically favorable when the DNA charge is neutralized.

To study the morphology and conformational state of natural biopolymers which are in aqueous solutions or adsorbed on the surface of different substrates, many physical-chemical methods (circular dichroism, dispersion of optical rotation, double refraction in flux, X-ray diffraction studies etc.) have been used. However, to visualize and determine the morphology of such a complicated natural biopolymer as DNA, the application of atomic-force methods (AFM) [1] and scanning tunneling microscopy (STM) [2] is more prospective.

In the given work the complex STM and AFM investigations of the DNA morphology were carried out in different conditions and it has been shown that the obtained AFM and STM images of the objects studied are in good agreement with the results obtained earlier.

Mica (muscovite) was used as a substrate in AFM investigations, high-oriented pyrographite was applied in STM investigations. A drop of the solution (2 μl) containing the structures studied was deposited on the freshly sheared substrate surface, then the samples were held for 5 min, washed with distilled water, dried in air and investigated by probe microscopy methods. A scanning tunneling microscope SCAN-8 (Russia) [3] was used, tunnel parameters were varied in the range: $I_t$ from 0.2 to 1 nA, $U_t$ from 50 to 1000 mV. The AFM investigations were carried out in the contact and interrupted contact regimes [4] by Nanoscope-3 (USA).

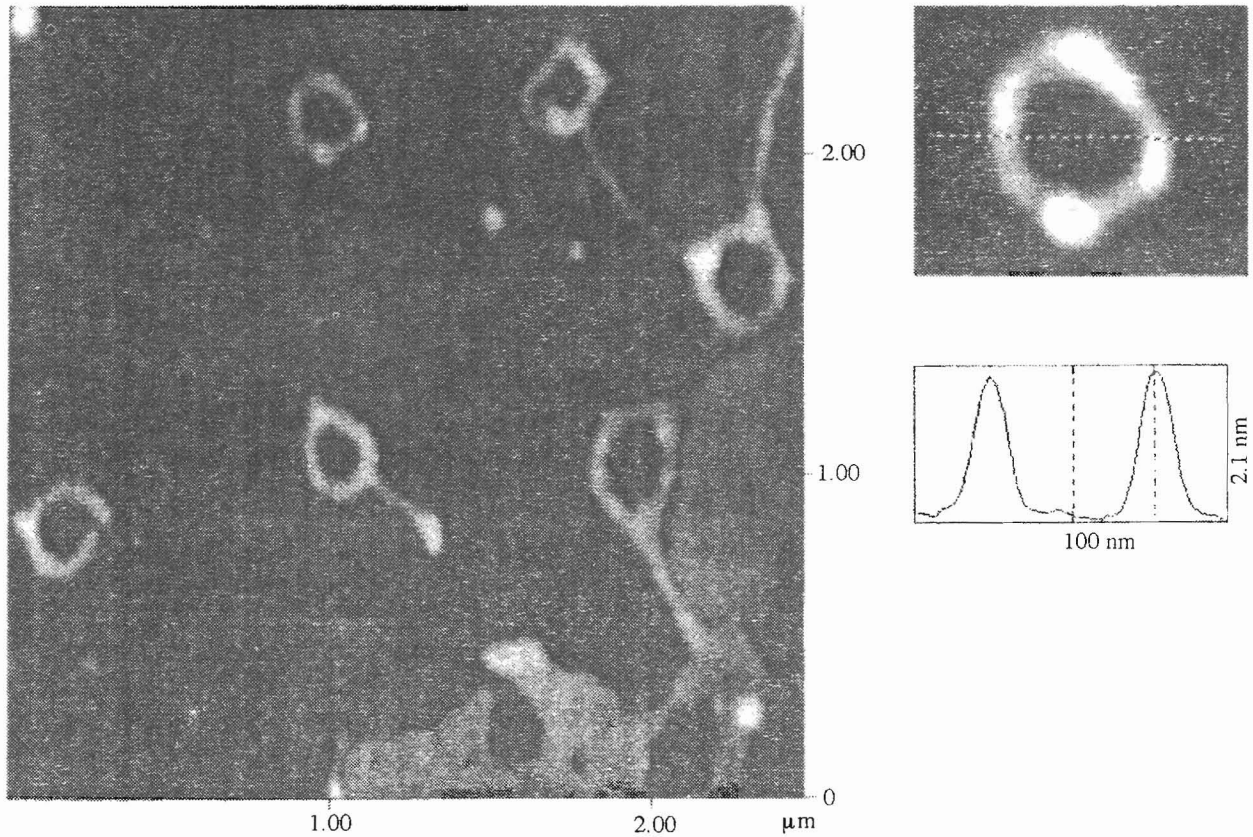


**Figure 1.** The AFM image of linear DNA from chick erythrocytes deposited from the 0.05 mole TBE buffer with 50% isopropanol solution on the mica surface. Contact regime. The frame size is 2.5 × 2.5 μm, the gray scale is 15 nm.

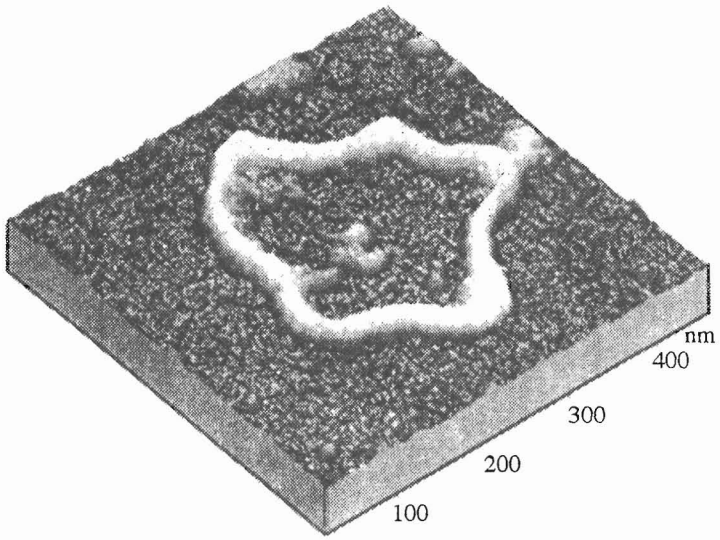


**Figure 2.** The AFM image of the cyclic DNA complex (pUC 19) with a surfactant (cetyltrimethylammonium bromide) adsorbed on the mica surface, contact regime. The frame size is 0.45 × 0.45 μm, the gray scale is 15 nm.

Figure 1 illustrates the AFM image of linear DNA from chick erythrocytes deposited from the TBE buffer with 50% isopropanol. It has been shown that in these conditions DNA has the toroid-like morphology even if the compacting agents are absent. We assume that if alcohol is present (analogous result were obtained for methanol, ethanol and isobutanol), the approach of $Na^+$ counterions with negative charged DNA phosphate groups occurs up to the complete charge screening on a macromolecule. As a result the internal stresses release the DNA double spiral, which takes the most energetically advantageous space conformation, occurs.

However, the type of the most advantageous DNA conformation appeared to depend on the initial macromolecule conformation. Figure 2 illustrates the AFM image of the cyclic DNA complex (pUC 19) with the opposite-charged surface-active substance CTAB (cetyltrimethylammonium bromide). Taking into account that the initial cyclic DNA was in the supertwisted conformation, one can conclude that, if the negative charge is neutralized, DNA with CTAB takes another most advantageous conformation – an unfolded circle. It should be noted that the application of AFM allowed us to answer the question about the morphology of cyclic DNA in the complex with a surfactant.

As was shown earlier [5] the complexes of linear DNA with a surfactant also remain a compact conformation in low-polarity organic media. However, it is not possible to determine the morphology of DNA included in the complex by the usual physical-chemical methods, such as viscosimetry, double refraction, UV spectroscopy etc. As is seen from Figs. 3 and 4 the analogous problem was solved by STM. Figure 3 illustrates the STM image of the complex of linear DNA (from chick erythrocyte) with an opposite charged surfactant (DSDMAC – distearyldimethylammonium chloride) prepared from an aqueous

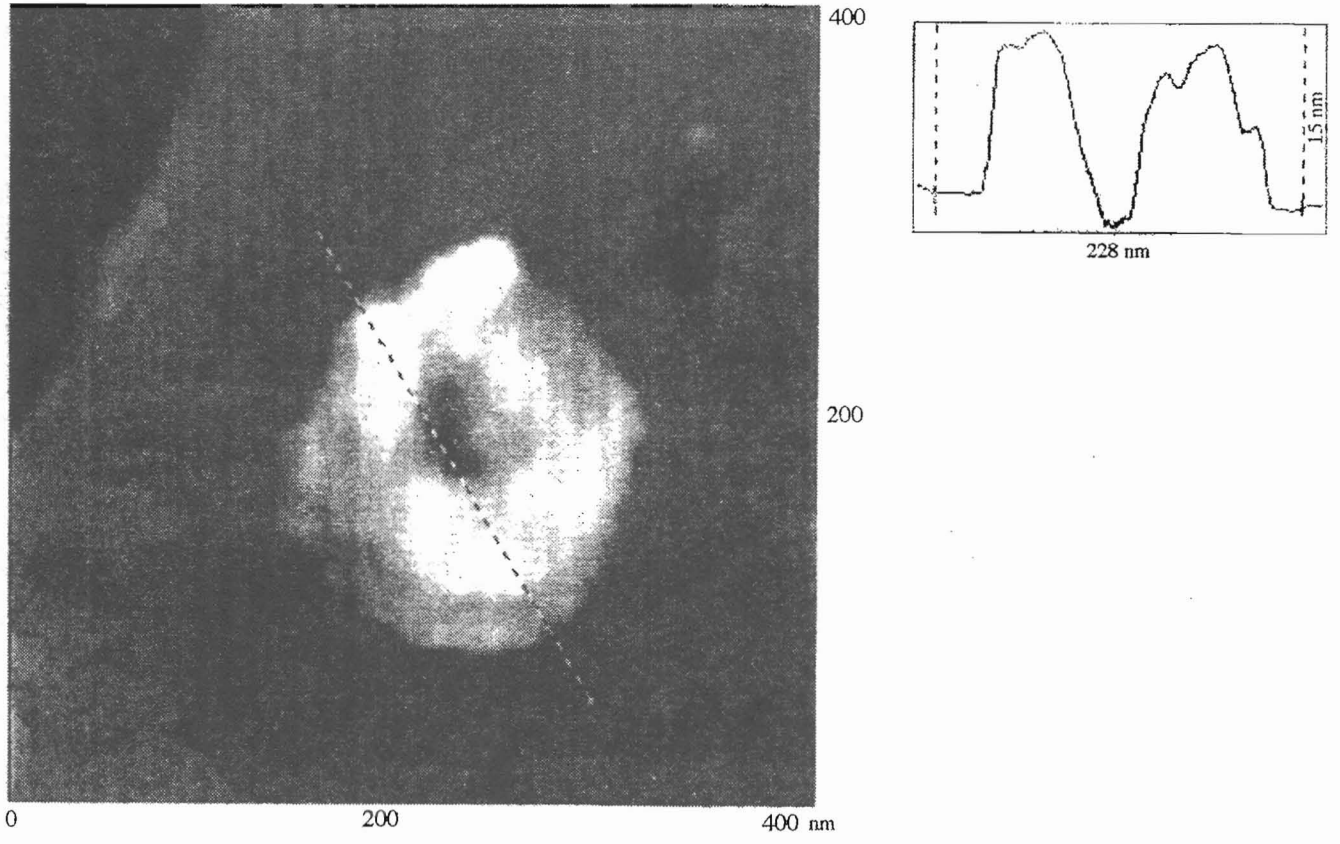


**Figure 3.** The STM image of the complex of linear DNA (from chick erythrocyte) with a surfactant (distearyldimethylammonium chloride) prepared in aqueous solution and adsorbed on the pyrographite surface. The constant current regime. The frame size is 0.4 × 0.4 μm, the gray scale is 15 nm.

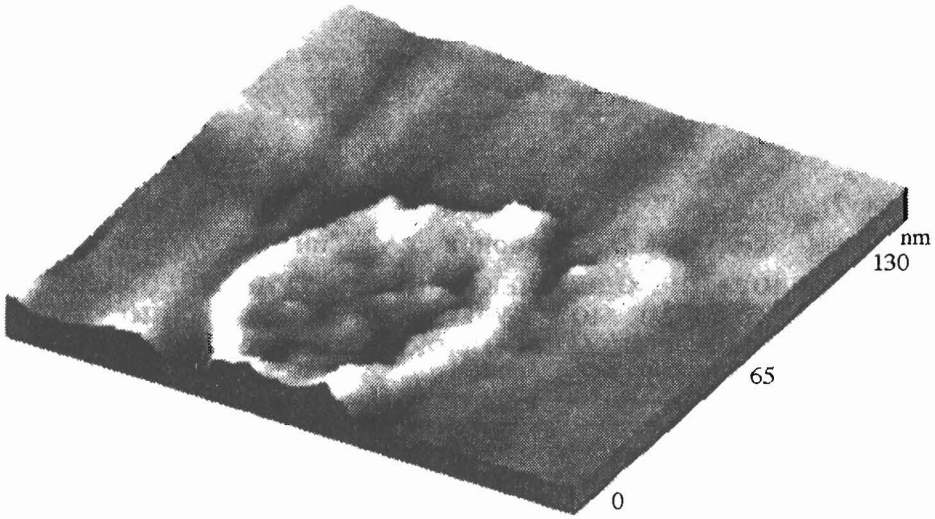


**Figure 4.** The STM image of the complex of linear DNA (from chick erythrocyte) with a surfactant (distearyldimethylammonium chloride) prepared in chloroform and adsorbed on the pyrographite surface. A constant current regime. The frame size is 0.13 × 0.13 μm, the gray scale is 6 nm.

solution. Figure 4 illustrates the STM image of the complex prepared from chloroform. The obtained results confirm the conclusion about the preservation of the compact DNA conformation in chloroform and allow us to propose the toroid-like conformation as the most energetically advantageous one for linear DNA if its charge is neutralized.

It should be noted in conclusion that the obtained results testify to the advantages of the use of probe microscopy methods to investigate the complicated complexes of biopolymers and synthetic lipid analogs, in particular the morphology of bioobjects. The above-mentioned physical-chemical methods of the investigation of biopolymers in a solution allow us to obtain only an indirect representation of their morphology, whereas STM and AFM reflect the real space structure of the object.

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