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DNA T4 Condensation in Water–Alcohol Media

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The compaction of high-molecular weight DNA T4 in a water–alcohol medium has been studied by AFM. The AFM images of compact globules formed by DNA molecules in water–alcohol media have been obtained. It has been found that at 40–50% alcohol concentrations, the DNA molecules form partially compacted formations in which particular coils of the macromolecule are twisted to form toroidal structures. Using the procedure of the recovery of the true geometric parameters of the object by the AFM profile, we have showed that the globule contains one DNA molecule. The model of DNA packing during the compaction has been proposed.

INTRODUCTION

It has been shown [1] that gigantic DNA T4 in a water–alcohol mixture at the alcohol concentration higher than 50% (for isopropanol) undergoes the conformational transition ball–globule. When the alcohol concentration decreases to 40%, the globules are partially untwisted. However, due to the restriction of the resolution ability of the used fluorescence microscopy method (the classic limit $\lambda/2$), it was difficult to determine the microstructure of the objects.

Scanning probe microscopy (SPM) can successfully be used for the study of the conformation and microstructure of DNA molecules [2, 3]. The traditional scheme of the preparation of specimens for probe microscopy in air includes the procedure of drying of a preparation droplet on the solid substrate surface. The drying process is nonequilibrium and accompanied by changes in the local values of the preparation concentrations, which are difficult to be monitored and can result in difficulties in the interpretation of the results. When the studies are performed in the liquid (using a liquid cell), the procedure of drying can be excluded from the scheme of the SPM experiment.

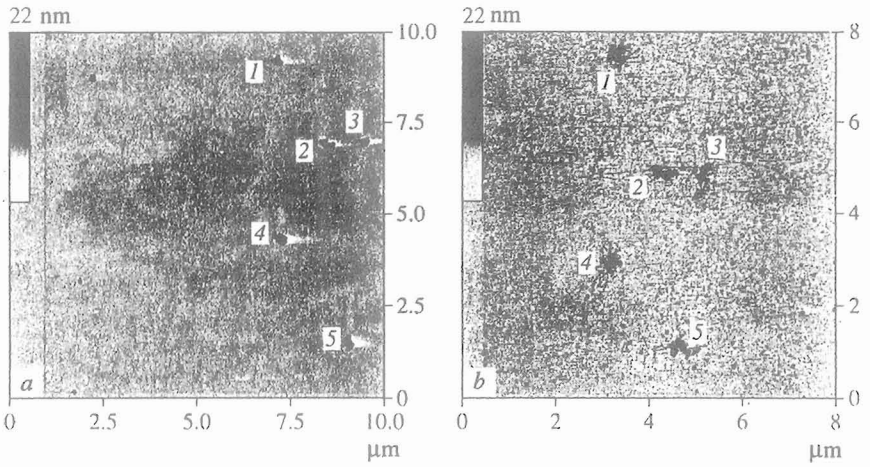

Figure 1. AFM image of compact globules formed by DNA molecules: *a*, in 80% isopropanol; *b*, after the decrease in the alcohol concentration to 40–50%.

EXPERIMENTAL

DNA of bacteriophage T4 (Nippone Gen) was used as the object of the study. Bidistilled deionized water was used in the experiments. Water–alcohol media were prepared using isopropanol (chemical purity grade).

The condensation of DNA molecule was studied by atomic force microscopy (AFM) directly in a water–alcohol mixture using mica substrates. The fresh cut of mica and an AFM probe were placed in a liquid cell, which was successively filled with the used solutions. The preparation containing DNA T4 molecules in a water–alcohol mixture with the 80% alcohol concentration was obtained by mixing one volume of a DNA solution in 0.5 TBE buffer with four volumes of isopropanol. The preparation of DNA molecules in 40% isopropanol was obtained by mixing two volumes of a DNA solution in the buffer with three volumes of isopropanol. In both cases, the final concentration of DNA in the mixture was 1×10^{-6} mol. The studied structures were immobilized on the substrate due to the adsorption from the solution.

The experiments were performed in a liquid cell of a Nanoscope-III AFM (Digital Instruments, USA) in the intermittent contact mode using sharpened cantilevers of silicon nitride (Nanoprobe) with a rigidity of 0.32 N/m.

RESULTS AND DISCUSSION

The preparation containing DNA T4 molecules in a water–alcohol mixture with the 80% concentration of isopropanol was introduced into the liquid cell pre-filled with a water–alcohol mixture with the same alcohol concentration. Under these conditions, DNA macromolecules were deposited on the mica surface as compact globules (Figs. 1*a*, 2*a*).

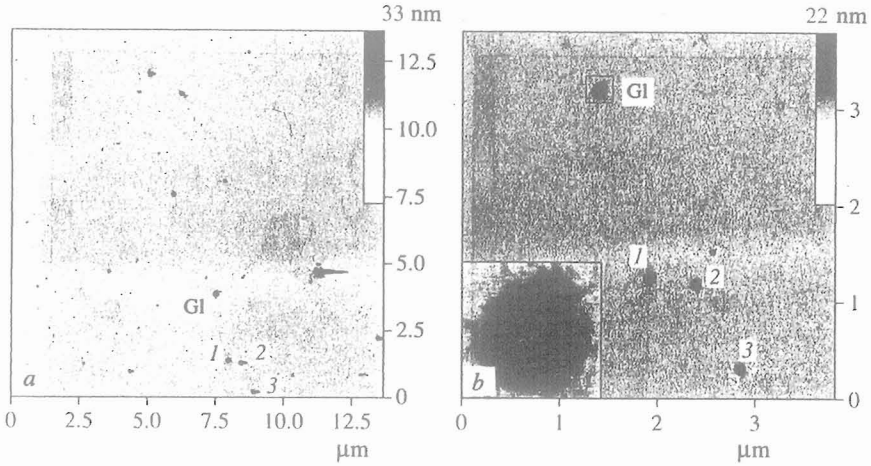

Figure 2. AFM image of compact globules formed by DNA molecules: *a*, in 80% isopropanol; *b*, after the decrease in the alcohol concentration to 40–50%. One of the globules with a greater resolution is shown in insert.

The use of the procedure of the recovery of the true sizes of the studied objects (taking into account the broadening effect) [4] makes it possible to determine the volume and geometry of the globular structures by three known parameters: the height of the AFM image above the substrate, the width at the half-width of the AFM profile (two values of the width corresponding to the directions of the main axes should be used for an ellipsoid), and the curvature radius of the tip. We performed the calculations for two values of the curvature radius of the tip: 5 and 10 nm. According to our estimates using the test-objects, the radius of the used tip lies in the indicated interval (Table 1).

Table 1. Geometric parameters of globules formed by DNA T4 molecules due to the compaction by alcohol.

R, nm	a, nm	b, nm	c, nm	V, 10^5 nm^3
5	41±12	55±17	23±9	2.5±1.7
10	37±12	52±17	23±9	2.1±1.5

Designations: a, b, c, and V are the parameters of an ellipsoid; c is the half-height of the AFM image of the globule.

The values of the globule parameters determined by two boundary values for the curvature radius of the tip are close (taking into account the considerable standard deviation, which is due to the statistical scatter of the analyzed parameters for different globules). The recovered values of the parameters of the semiaxes a and b suggest that a flattened and slightly extended ellipsoid is the geometric shape of the globule.

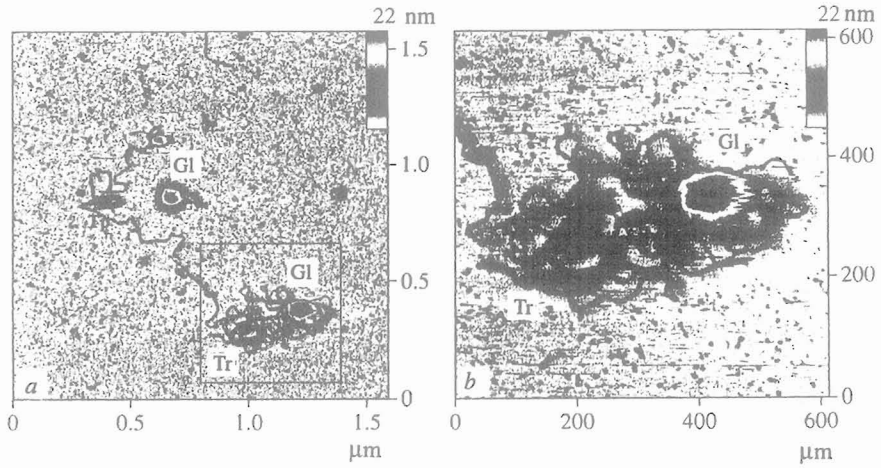

Figure 3. Partially compacted DNA molecules formed and visualized in the AFM liquid cell with an increase in the isopropanol concentration (from 40%). Globular (Gl) and toroidal (Tr) formations are marked. Figure *b* has a higher resolution than Fig. *a*.

It follows from Table 1 that the globule volume is $(2.5\pm1.7)\times10^5$ nm^3, which exceeds the volume of the DNA T4 molecule of 1.7×10^5 nm^3 ($L = 55\times10^3$ nm). Thus, we may assume that each globule is formed by one DNA molecule, which is in a close-packed state.

To study the partially decompacted structures of the DNA T4 macromolecules, a mixture of isopropanol with water (40% isopropanol + 60% water) was added to the liquid cell containing DNA T4 as globules in 80% isopropanol (20% water).

It is seen in Fig. 2*a* (marked by Gl) that at 80% isopropanol the DNA species are compact globules. When a 40% solution of isopropanol is pumped through the cell, the dynamic process of decompaction begins: the globules decrease in the height, and the central cavity appears in some globules (Fig. 2*b*, insert). However, the globules are not untwisted further. This can be explained by the presence of the interaction forces between the molecule and substrate, which prevent the untwisting when the alcohol concentration decreases. Therefore, DNA macromolecules in the intermediate state (between the globule and ball) were obtained as follows.

The DNA molecules in 40% isopropanol were introduced into the liquid cell, and then the alcohol concentration in the cell was increased by pumping 80% isopropanol. The molecules precipitated on the substrate surface in the partially compacted state. The further compaction of DNA adsorbed on the substrate was not observed, which is most likely explained by the interaction of the DNA molecules with mica.

The AFM images of the partially compacted structures are presented in Figs. 3 and 4. It was found that the initial compaction process is twisting of individual regions of the DNA macromolecules to form toroidal structures, which are likely the centers of the further compaction.

Based on the observations, we can assume that the compact globules (Figs. 1*a*, 2*a*) are

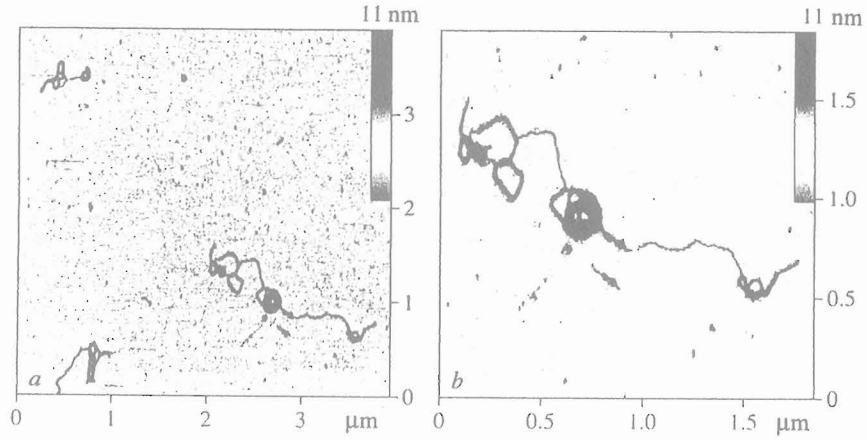

Figure 4. Partially compacted DNA molecules formed and visualized in the AFM liquid cell with an increase in the isopropanol concentration (from 40%). Figure *b* has *a* higher resolution than Fig. *a*.

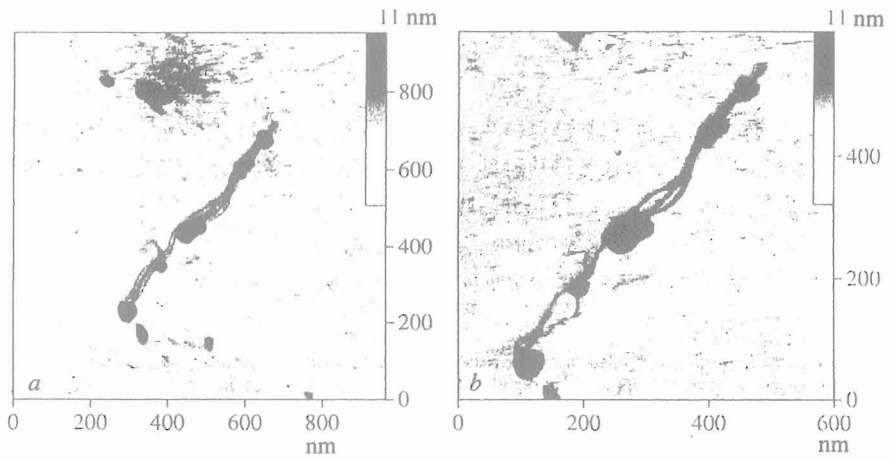

Figure 5. Rod-like structure: *a*, formed due to the compaction of the DNA molecule; *b*, partially "untwisted" under the action of the microscope probe after several scans.

the product of the compaction of DNA macromolecules and represent high-density particles in which particular regions of the molecule are twisted into toroidal structures. Perhaps, the compact particle has a cavity in the center: the globule volume somewhat exceeds the volume of one DNA T4 molecule, and the partially decompacted globules allow one to visualize the central cavity (Fig. 2*b*, insert).

It should be mentioned that, when the alcohol concentration in the cell increases (from 40%), DNA macromolecules form not only toroidal, but also (more rarely) rod-like structures (Fig. 5). The scanning process results in the situation when the rod-like structure is partially "untwisted" under the probe action. The compacted structures with the morphology different from the toroidal morphology observed in several cases can be explained by the fact that the intermediate stages of the compaction process, in which some molecules can exist in the nonstationary (nonequilibrium) morphological states, were studied in the experiments.

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