STRUCTURE AND FUNCTIONS OF BIOPOLYMERS

Compaction of supercoiled DNA on modified aminomica

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Stages of compaction of single molecules of supercoiled DNA pGEMEX, immobilized on modified aminomica, were visualized using atomic force microscopy. At the increase of the level of its compaction the length of molecule superhelix axis of the first order is decreased from ~ 580 nm down to ~370 nm with further formation of the superhelix axis of the second and third order with the length of ~260 nm and ~140 nm which makes ~20% and ~10% of outline length of the relaxed molecule respectively. Compaction of single molecules is completed with the formation of minitoroids, whose diameter is ~50 nm, and spheric conformation molecules. The model of possible conformational transitions of supercoiled DNA in vitro in the absence of proteins has been suggested. Compaction of supercoiled DNA molecules up to minitoroid level was shown to be caused by high surface charge density of aminomica on which DNA molecules were immobilized.

Key words: supercoiled DNA, atomic force microscopy, aminomica, DNA compaction, minitoroid, spheroid.

Introduction. Genomic DNA of giant length (from a millimetre to a meter) has been compacted using proteins in highly-organized structures in bacteria and eukaryotic nuclei, the volume of which makes up only from several units to hundreds of cubic micrometers. DNA volume can be easily calculated for microorganisms with sequenced genome by multiplication of DNA, diameter (2 nm), on the full length of molecule assuming DNA in B-form of (i.e. at the distance between nucleotides along the axis of the double spiral H=3.40 E). At the absence of proteins random compacted DNA molecule occupies considerably bigger volume than in the nucleus or in the bacterial cell. According to modern views, the mentioned eukaryote DNA compaction is due to its interaction with nuclear proteins, in the first place with histones, and further formation of nucleosomes, chromatin fibres and chromosomes [2-4]. Other proteins, connected to DNA in a sequence specific way, are the representatives of the HMG proteins group

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that modulate connection of histones with DNA. Besides, the structural maintenance of chromosome group (SMC group) proteins (condensins and cohesins) that are the members of ATPase family, playing the central role in chromosome condensation and causing direct DNA condensation have been identified. [5]

To investigate genome architecture in eukaryote nucleus different model systems are used. It was shown by atomic force microscopy (AFM) that *E.coli* nucleoid is a structure, which is changing throughout the cell growth – it is more compacted in the stationary phase, than in the log-phase. However, a fibre with the diameter of ~ 80 nm is a fundamental nucleoid structure for both phases [1]. In addition to 80 nm, a thinner fibre, with the diameter of 40 nm, and highly organized loop have also been visualized in the log-phase of nucleoid.

Another model system – nucleosome – is also often being used, as the basic chromatin unit, which is repeated. In this case, beads-on-a-string type of structures [6] have been visualized for linear DNA in complex with histone octamer, which is created by two molecules of each of the proteins H2A, H2B, H3 and H4. Compaction and condensation of DNA with the toroids and fibers (bundles) formation can also be achieved at different conditions, other than complex creation with proteins. For example, it has been shown that different polycationes (polylysine, spermine, spermidine, protamine) cause DNA condensation and toroids formation [7-9]. Moreover, it has been shown [10] that the presence of proteins or polycationes is not an obligatory condition for DNA condensation, and DNA condensation can be achieved in the way of DNA molecules immobilization on positively charged surface. It was stated in the works [10, 11] that condensation degree of DNA molecules can be controlled using the valency change of silanes which are used for mica modification, as well as in the way of NaCl concentration change. Nevertheless, highly-compacted DNA structures have not been visualized because of NaCl concentration changes in the narrow range (10 - 100mM). It is worth mentioning that at the conditions of linear DNA condensation [12, 13] the aggregates are formed by several DNA molecules, not single ones.

In the present work, we demonstrate for the first time the images of single molecules of supercoiled DNA, the compactizasion degree of which is considerably higher than earlier achieved. The usage of new substrate for DNA immobilization – modified aminomica with increased surface charge density and hydrophoby in comparison with standard aminomica – allowed both the visualization of different stages of single circular DNA compaction with the length decrease in superhelix molecule axis of by a factor of two and four with the formation of the superhelix axis of the second and the third order respectively, and the compaction of single supercoiled DNA molecules in minitoroids, spheroids, and bundles.

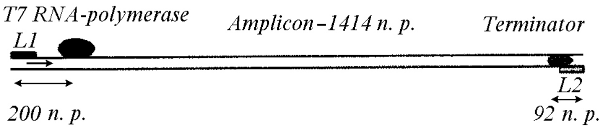

Figure 1. The schematic image of DNA matrix used for PCR. The primers, indicated with rectangulars flank the fragment of DNA pGEMEX with the length of 1414 pn, containing promoter and terminator for the T7 RNA polymerase transcription. The T7 RNA polymerase is indicated by big oval, the area of transcription termination for T7 RNA polymerase is indicated by a small oval.

Materials and Methods. *The preparation of DNA samples for PCR and AFM.* Linear and supercoiled DNA pGEMEX (Promega, USA) were used. To conduct the polymerase chain reaction (PCR), linear DNA has been obtained by treatment of supercoiled DNA pGEMEX of 3993 pairs of nucleotides (n.p.) with restrictase ScaI (New England Biolabs, England). L1 and L2 primers, constructed by us, has restricted DNA fragment, containing promoter and termination site for T7 RNA-polymerase transcription. The schematic image of the DNA fragment which has been amplificated with the length of 1414 pn is shown in Figure 1. L1 and L2 primers, the sequences of which with the corresponding positions of pGEMEX DNA are shown below, were obtained from Sigma (Japan):

5’-cgc tta caa ttt cca ttc gcc att c-3’ - a direct L1 primer (3748 - 3772)

5’-ctg att ctg tgg ata acc gta tta ccg-3’ - a reverse L2 primer (1168 - 1142)

Hot start PCR was conducted in the reaction volume of 50 мl on the amplificator GeneAmp 9700 (Perkin Elmer, USA) at the following temperature and time parameters: initial incubation - 95°C, 2 min; denaturation - 95°C, 1 min; annealing - 69°C - 73°C, 1 min; synthesis - 74°C, 1 min; cycles quantity – 35. The temperature of annealing has been defined theoretically using the Oligo programme. To minimize the amplification of non-specific fragments, several PCRs were conducted at different annealing temperatures - 69°C, 71°C, 73°C.

To visualize amplicons, 15 мl of PCR product have been separated using electrophoresis in 2% agarous gel with the further staining with bromide etidium.

To purify the amplificated DNA fragment, the following procedure was used. After electrophoresis, the line of gel, containing amplicon, was located by long wave UV

source of radiation of low intensity (BioRad, USA) and cut out. To purify amplicon from nucleotides, primers, DNA polymerase and bromide etidium, the QIAquick PCR purification kit (QIAgen, Japan) was used according to the producer's recommendations, and the phenol\chloroform extraction with further re-sedimentation by ethanol was made.

To conduct PCR, thermostable DNA polymerase of high accuracy of two types – Pyrobest DNA polymerase (TaKaRa Co., Japan) and Invirtogen Platinum DNA polymerase (Invirtogen, Japan) was used. Freshly cleaved mica, standard aminomica and modified aminomica with higher aminogroups density in comparison with standard aminomica were used as the substratum. To apply DNA on freshly cleaved mica, 10 mM HEPES buffer, containing 2.5 mM $MgCl_2$, was used. A drop of DNA solution of 10мl with the concentration of 0.1 мg/ml in TE-buffer (10mM tris·HCl pH7.9, 1mM EDTA) was applied on the 1cm2 spot of aminomica or modified aminomica, washed with deionized water after 2 min exposition, blasted with the flow of argon and then the sample was kept under the pressure of 100mm of mercury column for 20 minutes. The procedure of obtaining standard aminomica was conducted according to [14] using the modification of freshly cleaved mica with aminogroups in vapours of distilled 3-aminopropyltriethoxy silane (APTES) (Aldrich, USA). The distillation of APTES was conducted at the decreased pressure in the argon atmosphere. Freshly cleaved mica has been kept in a glass dessicator with the solutions of APTES and N,N-diisopropylethylamine for 1 hour for mica aminomodification. The modified aminomica was produced using slight changes in the technology of obtaining standard aminomica.

*Atomic force microscopy***.** The atomic force microscope (AFM) Nanoscope IV MultiMode System (Veeco Instruments Inc., USA) with E-scanner was used in the work. AFM images of DNA were recorded using oscillating AFM variant in the air in the mode "Height" using OMCL-AC160TS cantilevers (Olympus Optical Co., Japan) with resonance frequency of 340-360 kHz and hardness constant of 42 N/m. The images were captured in 512x512 pixels resolution, were flattened and analyzed using Nanoscope software (ver. 5.12r3) (Veeco Instruments Inc., USA).

The volume of individual DNA molecules was calculated on the basis of really measured parameters of molecules from AFM image. To calculate the molecules volume, the structure of lengthwise section of molecules was used by the means of the option of Nanoscope software.

Results and Discussions. It has previously been shown [15], that the length of supercoiled DNA (scDNA) axis remains constant at the increase of superhelix density and equals to ~35% of the contour length of a relaxed molecule. This condition is realized for DNA pGEMEX molecules, immobilized on freshly cleaved mica which is characterized by relatively not high surface charge density. The immobilization of scDNA on modified aminomica, which has increased surface charge density in comparison with both fresh cleaved and standard aminomica, results in considerable DNA compaction. Such single scDNA molecules, which constitute the axis of superhelix of the second order, are represented in Figure 2A. The length of the superhelix axis of these highly compacted molecules in comparison with plectonomic DNA has decreased to the value which equals to ~3 of the contour length of the molecule (l=260nm).

One more variant of highly compacted scDNA molecules which are generated on the surface of modified aminomica – spheroid – is indicated by an arrow in Figure 2A, and the section of spheroid, from which the parameters of the molecule have been defined, is shown in Figure 2B.

Having calculated the volume of the condensed structure one can define the quantity of compacted supercoiled DNA molecules, taking part in structure formation. The volume of the spheroid, shown in Figure 2A, which was calculated on the data of the section area and the diameter, equals to V=3140 nm^3. As theoretically calculated excluded volume of DNA pGEMEX in the assumption of B-form equals to V_{exclud}=3900 nm^3, the represented results show that the spheroid is created with a single scDNA molecule.

As a result of further compaction there is a formation of both the molecules with the length of the superhelix axis which is even two times less (the length of the axis of the third order equals to l=140 nm, position D2 in Table 1), and the molecules in spherical conformation (spheroids) (positions E2 and F2, Table 1).

The compaction on modified aminomica occurs not only for single DNA molecules. There is the image of a dimer (V=7080 cubic nm) in Fig. 2B – a compacted structure of two scDNA, the length of the superhelix axis of which is the same as the length of the superhelix axis of a single molecule, and equals to l=260 nm.

Another structure, visualized by us, which is similar outwardly to the spheroid in the AFM image with not high separating capacity, is a minitoroid. The minitoroid, formed by a single scDNA, its sections and three-dimensional molecule images are shown in Figure 3. The value of this molecule volume equals to V=3980 nm^3, which corresponds to the volume of the single molecule of DNA pGEMEX. It can be seen from the sections in Figure 3B and Figure 3C, that three out of four toroid segments have

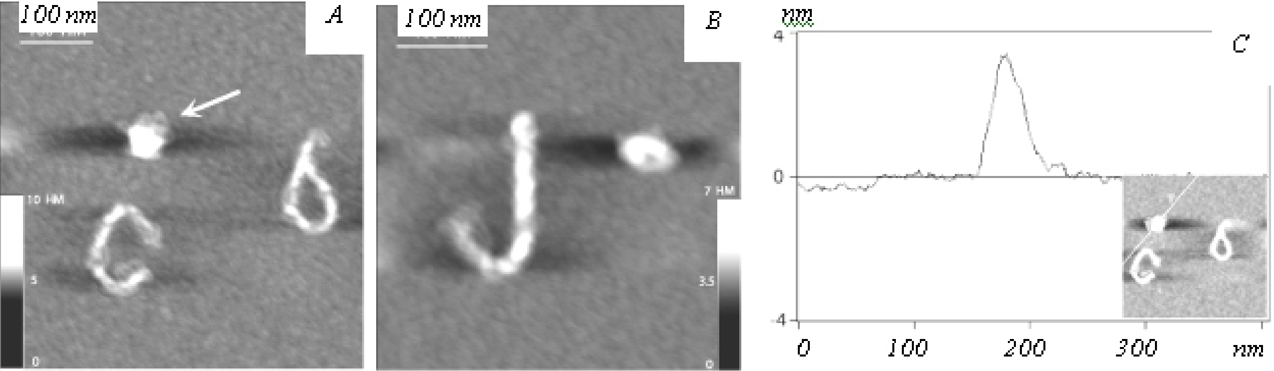

Fig. 2. (A) The AFM images of single over-supercoiled molecules of DNA pGEMEX on modified aminomica. The length of the superhelix axis of each DNA molecule equals to l=260-270 nm, i.e. approximately the quarter of the contour length of the relaxed molecule. The arrow indicates the DNA molecule, compacted to the level of spheroid. There is a scale of grey gradation, which corresponds to the range of Z coordinate from 0 to 10 nm, which allows estimating the height of the immobilized molecule. The size of the picture is 500 x 500 nm. (B) The DNA molecule cross cut in the spheric conformation, which allows defining the height of the molecule. The maximum height of the spheroid is h_{max}=3.65 n. The line, along which the section was done, is shown in the inset. (C) The AFM image of over-supercoiled DNA molecules, which constituted the dimer, the length of the superhelix axis of which equals to l=260 nm, and the volume equals to the double volume of the single supercoiled DNA molecule. The size of the picture is 500 nm x 500 nm. The scale grey gradation corresponds to the range of Z coordinate from 0 to 7 nm.

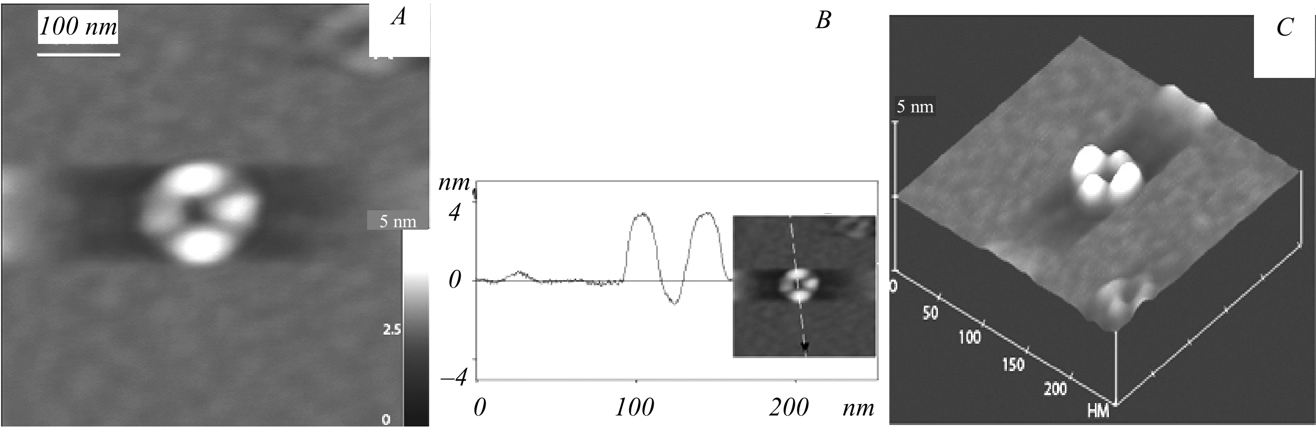

Fig. 3. The AFM image (A), cross cut (B) and (C) and the three-dimensional image (D) of the single over-supercoiled DNA pGEMEX which has constituted the minitoroid. (A) the size of the picture is 250 nm x 250 nm. (B) the height of two toroid fragments, through which the cross cut has been conducted, equals to 1.74 nm. (C) – the height of two other toroid fragments, defined from the sectionm equals to 1.74 nm and 0.84 nm respectively. The triangles indicate the corresponding peak on the cross cut and the level, according to which the measurement of the height of the highest peak was done. The height of this minitoroid fragment equals to 1.74 nm. (A, D) – the external diameter of the minitoroid is 50-60 nm, the internal one is 15-25 nm

the same height (h=1.74 nm), and the fourth segment has almost twice less height (h=0.84 nm). It means that the wind of scDNA braid is less than in three other segments, i.e. the minitoroid is a peculiar minicircle with cross cut in the upper part, the length of which equals to the quarter of the circle length.

It has previously been shown in the works [7, 10, 13, 16], that several linear or circular DNA molecules take part in the formation of toroids. We have also visualized toroids, formed by two over-supercoiled DNA (over-scDNA). The parameters of the majority of visualized compacted scDNA are shown in Table 1. The positions C1 and D1 correspond to this toroid of two DNA molecules, as the

The parameters of pGEMEX supercoiled DNA molecules (molecule contour length, distance between the nucleotides along the axis, molecule volume, etc.) determined from AFM images.

№ at Fig. 4	Molecule (s)	Height $\mathbf{h}_{max}$ (nm)	Height $\mathbf{h}_{min}$ (nm)	Contour length of super-coiled molecule **L** (nm)	Super-helix axis length **l** (nm)	Contour length of relaxed molecule $\mathbf{L}_{rel}$ (nm)	The distance between nucleotides along the superhelix axis H, **(?)**	Volume V, **nm^3**
A3[c]		0.80	0.35[x]	1243	466	1243	3.11	3510
A2[d]		0.99	0.35[x]	1216	-	1216	3.05	3530
E1		2.00	0.87	269	269[b]	1076	2.69	7080
B2		1.35	0.28[x]	260	260[b]	1040	2.60	3470
C2		1.36	0.30[x]	270	270[b]	1080	2.70	3520
D3		1.74	0.84	260	260	1040	2.60	3980
B1		1.40	0.35[x]	548	548	1096	2.74	6300
C1		2.00	0.45[x]	-	401	-	-	6840
E2		2.60	1.85	-	-	-	-	3620

№ at Fig. 4	Molecule (s)	Height h_{max} (nm)	Height h_{min} (nm)	Contour length of super-coiled molecule **L** (nm)	Super-helix axis length **l** (nm)	Contour length of relaxed molecule L_{rel} (nm)	The distance between nucleotides along the superhelix axis H, **(?)**	Volume V, **nm³**
F2		3.45	0.30[x]	-	-	-	-	3140
C3		1.69	0.78	285	285[b]	-	-	3280
D2		3.00	1.25	140	140	-	-	5180
B3		0.95	0.35[x]	580	580	-	-	4440
D1		2.10	0.30[x]	267	267	-	-	6570

x – double-stranded DNA; [a] – the length of the first order supercoiled axis; [b] – the length of the second order supercoiled axis; [c] – picture was captured on mica, in the buffer containing $MgCl_2$; [d] – DNA picture was captured on standard aminomica.

volume of structure equals to the double volume of the single scDNA pGEMEX. The characteristics of AFM images of single DNA molecules are shown in all the other positions of Table 1, except the dimer braid in position E1.

We have suggested the scheme of step-by-step compaction for single DNA molecules, as well as for dimers, on the basis of the analysis of the obtained AFM images (Figure 4). The positions B1, C1, D1, E1, marked with the rectangular, correspond to scDNA dimers, which was defined on the basis of the volumes calculation of the above-mentioned molecules, all the other positions correspond to single molecules. The least compacted molecules are shown in positions A1-A3, the most compacted ones are in positions E1-E3. The image of scDNA pGEMEX, immobilized on freshly cleaved mica with Mg^{2+} ions, is shown in position A3. This molecule has 7-8 supercoiled winds (knots or self-intersections), and the value of superhelix density equals to y=-0.024. The scDNA pGEMEX molecules, immobilized on standard aminomica (positions A1, A2) with the high value of surface charge density in comparison with freshly cleaved mica, have a different view. They are similar to plectonomic DNA molecules but are more compacted, i.e. localized on the smaller area of aminomica.

Several variants of scDNA compaction are observed at the transition to modified aminomica which is characterized by a considerably higher surface charge density in comparison with standard aminomica. The number of knots increases at the first stage and over-supercoiled DNA molecules are formed (B3), the length of the superhelix axis of which equals to approximately a half of the contour length of the relaxed molecule, i.e. specific braids are

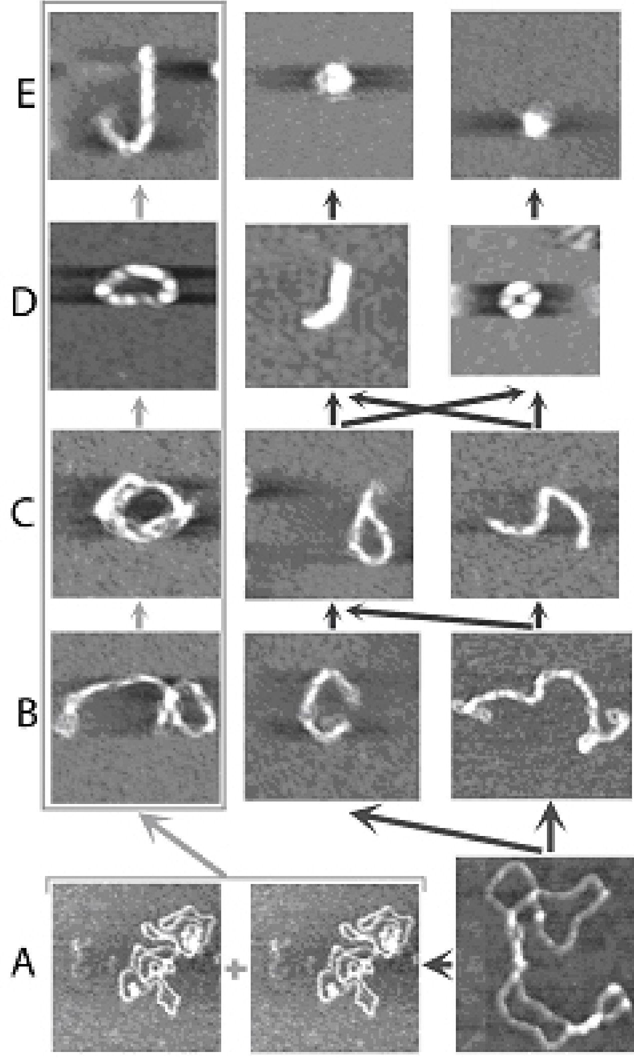

Fig. 4. The model of compaction of the supercoiled DNA molecules, suggested on the basis of obtained AFM images of the over-supercoiled DNA pGEMEX. A1, A2 – a standard aminomica, A3 - a freshly cleaved mica, other AFM images were captured on modified aminomica. The arrows indicate the direction of DNA compaction. The rectangular indicates four dimers. Different variants of compacted DNA molecules are formed at the increase of the surface charge density or the quantity of protonated aminogroups (i.e. at the transition from fresh cleaved and standard aminomica to modified aminomica). B3 – over-scDNA which constitutes the superhelix axis of the first order, and the contour length of the compacted molecule equals to a half of the contour length of the relaxed molecule; B2, C2, C3 – over-scDNA, which constitute the superhelix axis of the second order, and the contour length of the compacted molecule equals to the quarter of the contour length of the relaxed molecule; D2 – over-scDNA, which constitutes the superhelix axis of the third order (the contour length equals to the eighth part of the contour length of the relaxed molecule); E2, E3 – spheroids; D3 – minitoroid, constituted with a single DNA molecule. B1, C1, D1 – condensed structures, of two DNA molecules.

formed. This braid-like molecule folds in half at the second stage – the length of the superhelix axis becomes two times less and equals to approximately the quarter of the contour length of the relaxed molecule (C3, B2, C2). The further formation of braids (C3) is as possible at this stage as the formation of toroids (C2). The shorter braids with even twice less length of the superhelix axis 1~140 nm (D2) are formed at the third stage. Besides, the formation of the minitoroid (D3) is possible from the toroid (C2) as well as from the braided molecule (C3). The further compaction of toroids and braids leads to the appearance of half-spheroids (E2) and spheroids at the fourth stage.

AFM images of these highly compacted DNA molecules with high separating capacity are shown in Figure 5. It is worth mentioning that such toroids appear in groups (Figure 5A), as well as in single structures (Figure 2A). It indicates that (i) protonated aminogroups are immobilized not uniformly on the surface of the modified aminomica, (ii) spheroids and half-spheroids are formed on the sites of the modified aminomica with the maximum density of active aminogroups.

Another variant of scDNA compaction is the compaction of dimers, rather than single molecules. The molecules, the AFM images of which are shown in positions B1, C1, D1, E1, constitute dimers, which was defined by us on the basis of their volumes calculation. At first two plactonomic molecules (A1, A2) form braid-like structures (B1).There is a possibility of further formation of the toroid (C1, D1) or the braid (E1), the length of the superhelix axis of which equals to the quarter of the contour length of the relaxed molecule. Position D2 also corresponds to the toroid, formed by two molecules of over-scDNA.

The parameters of pGEMEX supercoiled DNA molecules (molecule contour length, distance between the nucleotides along the axis, molecule volume, etc.) determined from AFM images.

Let us consider the mechanism of over-supercoiled DNA compaction. Plactonomic DNA molecules with the low value of superhelix density are seen in the AFM image at the immobilization of DNA pGEMEX molecules on the surface of freshly cleaved mica from the buffer, containing Mg^{2+} ions (position A1 in Table 1). Over-scDNA and highly compacted DNA molecules have not been visualized in numerous works on model systems, including nucleosomes, devoted to the investigation of DNA compaction with different proteins (histones, condensine) [2, 4, 5]. In our opinion, the main reason for it is the fact that mica with the low value of the surface charge density, mainly with the usage of Mg^{2+} ions, was used in the majority of works as AFM substratum. Mg^{2+} ions, similar to Ca^{2+} ions, can hinder scDNA compaction. Thus, it was shown

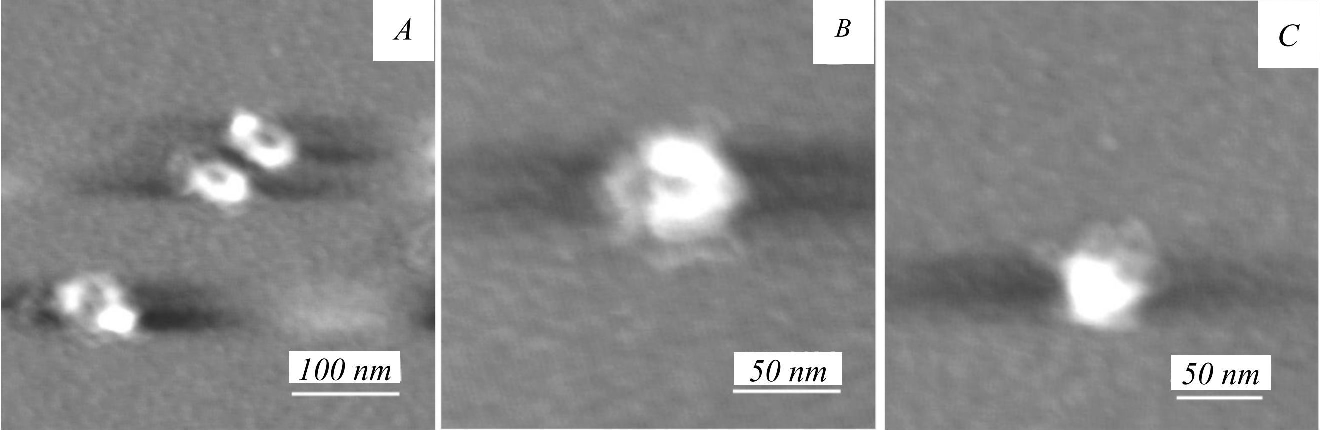

Fig. 5. The AFM image of compacted molecules of supercoiled DNA pGEMEX. (A) Three minitoroids, formed by single DNA molecules. The size of the picture is 400 x 400 nm. (B) Half-spheroid, the height of which at the maximum is h_{max}=2.6 nm. The height of the non-compacted DNA strand equals to h_{min}=0.3 nm, which corresponds to double-chain DNA. The size of the shot is 250 nm x 250nm. (C) The spheroid, the height of which equals to h_{max}=3.45 nm. The size of the picture is 250 nm x 250 nm

in the work [17], that Ca^{2+} ions hinder DNA compaction in the complex with histone protein HMGB1.

While modeling the processes of DNA over-supercoilization and compaction using a circular polypropylene tube, one can face one more apparent contradiction. At first sight, the general tension and curve rigidity of such a compacted molecule should increase at the increase in the quantity of supercoiled winds as well as at the formation of the superhelix axis of the second and the third order (i.e. at the specific folding of the braid two and four times). Still the braid comes to toroid conformation at a slight decrease in mechanic rigidity of the braid while keeping the maximum quantity of the coils (knots).On the other hand, it was shown in the work [18] that conformational rigidity of the supercoiled DNA increases considerably at the interaction with histone proteins while forming a tetrasome, a nucleosome, and a chromatosome. The authors explain the increase of DNA conformational flexibility with the increase in the radius of the DNA supercoiled, which, in its turn, can be necessary for the functioning of nucleosome DNA included in chromatine.

To understand the mechanism of scDNA compaction on the surface of modified aminomica, it is important to consider the question concerning DNA conformation on the surface of charged mica. To answer this question, we have visualized the fragments of DNA pGEMEX with of 1414 pn length (see Figure 1), which were obtained linearizing supercoiled DNA pGEMEX and amplifying with PCR. AFM images of amplicons, immobilized on the standard and the modified aminomica, are shown in Figure 6. The contour length of amplicons was measured directly from the AFM image in two different ways – using the option of Nanoscope software, as well as using curvometer at measuring the contour length of amplicons in the image with the increased scale. Both methods have given results which coincide with the accuracy of ±2.5 %.

The diagram of the distribution of the contour length of the amplicon, immobilized on the standard aminomica, is shown in Figure 7. The contour length of this amplicon equals to L=435 ±15 nm, which corresponds to the distance between nucleotides along the axis of the helix H=3.10 . This value is in the range of internucleotide distance, characteristic of both B-form of DNA (3.03 < H < 3.37) and A-form (2.56 < H < 3.29) [19]. At the same time the contour length of the same amplicon, immobilized on modified aminomica equals to L=296 ± 14 nm, which corresponds to the distance between nucleotides along the axis of the duplex H=2.09 . DNA molecules with considerably decreased value of the H ~ 2 have been referred by us to the new form of DNA – S-DNA ("S" from the English word 'spring').

Thus, intramolecular rebuilding of the molecules themselves due to the screening of negatively charged DNA phosphate groups with positively charged aminogroups of modified aminomica [20], which leads to the decrease in the distance between nucleotides along the axis of the duplex, can be a crucial factor of the compaction of over-supercoiled DNA molecules. The results, obtained

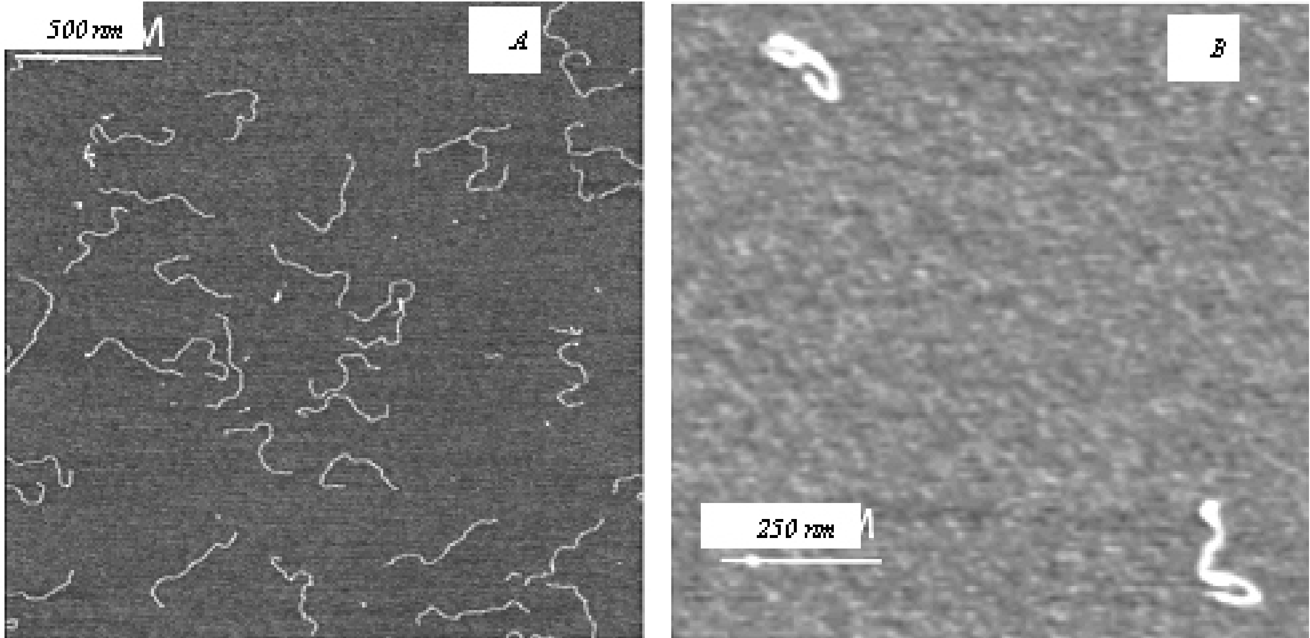

Fig. 6. The image of the amplified DNA pGEMEX fragment, obtained using atomic force microscopy. (A) **The freshly cleaved mica.** The size of the picture is 2.2 micrometer x 2.2 micrometer. The expected contour length of the amplicon is L=485 nm in the assumption of B-form of DNA. The contour length of the amplicon, calculated from the AFM image, equals to L=435 ± 15 nm, which corresponds to the distance between nucleotides along the axis of the double helix H=3.07 ?. (B) **The modified aminomica** with the increased surface charge density (of the protonated aminogroups). The size of the picture is 1 micrometer x 1 micrometer. The contour length of the amplicon, calculated from the AFM image equals to L=296 ± 14nm, which corresponds to the distance between nucleotides along the duplex axis H=2.09 ?

by us, show that the compaction of single DNA molecules is possible *in vitro* at the absence of proteins, and the necessary condition for it is high surface density of the positive charge of substratum, on which supercoiled DNA is immobilized. Apropos, it has been shown in the previous investigations using X-ray photoelectronic spectroscopy [21], that only 50 % of aminogroups are active (protonated) on the surface of standard aminomica.

It is worth mentioning that freshly cleaved mica, which has small total negative charge, influences considerably on the properties of aminosilane aminogroups (APTES), used for its modification. If the ionization constant of APTES in the water solution is pC ~ 10 [22], the ionization constant of APTES on the surface of mica decreases in ~ 3 units of pH and equals to approximately pC ~ 7 [23, 24].

On the other hand, not only pC of APTES, but also pC of nucleotides can decrease under the influence of mica surface properties. It means that some basic nitrogens can be protonated at certain conditions (i.e. in the conditions of DNA immobilization on the modified aminomica). AFM images of a single supercoiled DNA pGEMEX, immobilized on modified aminomica, are shown in Figure 8. We have visualized several of such unusual molecules, the fragments of which constitute a specific net. One of the reasons of its appearance can be the formation of intramolecular triplexes or H-DNA. It is known that H-DNA is formed for fragments purine\pyrimidine DNA sequences at pH~4 in water solution, i.e. the structural transition from B-DNA to H-DNA occurs at pH decreasing in 3 units from the physiological value [25]. The values of molecule ionization constants, immobilized on mica, move in 3 to acid pH. The analogous net of molecules of plasmid DNA, immobilized on freshly cleaved mica, was visualized in the work [26] only for the certain value of Mg^{2+} ions concentration. These results indicate that supercoiled DNAs constitute the net only in the narrow interval of both surface density of mica charge and DNA concentration.

Thus, the usage of modified aminomica with the high value of surface charge density results in extreme compaction of single supercoiled DNA molecules. The following processes can occur as a result of screening negative

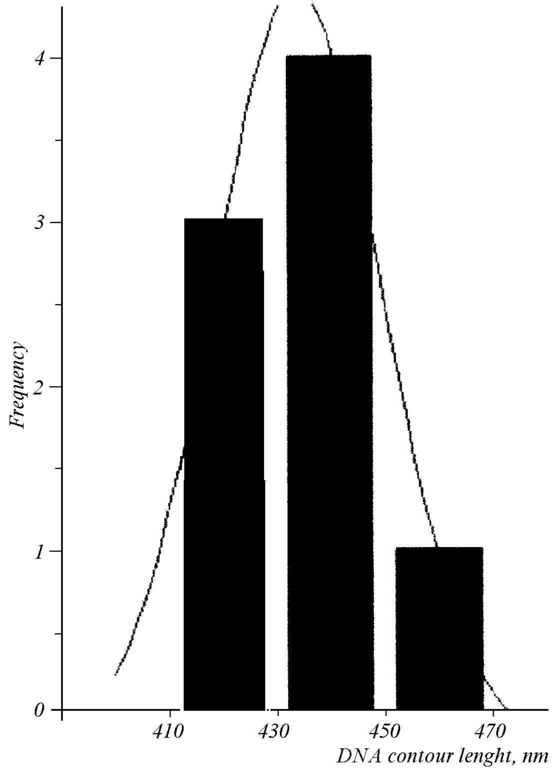

Fig. 7. The contour length of amplicons after conducting PCR and the further cleaning, measured from the AFM image of the DNA on freshly

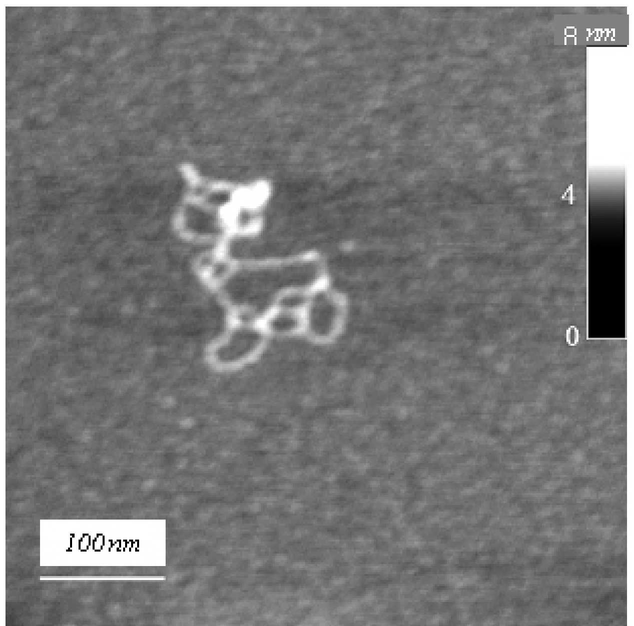

Fig. 8. The AFM image of a single molecule of supercoiled DNA pGEMEX on modified aminomica. The segments of the molecule constitute a peculiar net. The size of the picture is 502 nm x 502 nm. There is a scale of grey gradation, which corresponds to the range of Z coordinate from 0 to 8 nm

charges of DNA phosphate groups with positive charges of protonated aminogroups of modified aminomica in DNA molecules: (i) the decrease in the distance between nucleotides along the duplex axis and the formation of S-DNA; (ii) the formation of intramolecular triplexes, or H-DNA. We assume on the basis of the analysis of the above-mentioned results, that it is possible to model processes, occurring with natural DNA molecules *in vivo* using supercoiled DNA, immobilized on modified aminomica, as DNA in the nucleus is in the environment with the high density of charged residues of different molecules, proteins in the first place [27, 30].

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Компактизация суперсприральной ДНК на модифицированной аминослюде

Резюме

С помощью атомно-силовой микроскопии визуализированы этапы компактизации единичных молекул суперспиральной ДНК pGEMEX, иммобилизованных на модифицированной аминослюде. При повышении уровня компактизации ДНК длина суперспиральной оси первого порядка молекул уменшается от ~580 до ~370 нм с последующим образованием оси суперспирали второго и третьего порядков длиной ~260 и ~140 нм (~10 % от контурной длины релаксированной молекулы) соответственно. Компактизация единичных молекул завершается образованием минотороидов диаметром ~50 нм и молекул сферической конформации. Предложена модель возможных конформационных переходов суперспиральной ДНК in vitro в отсутствие белков. Показано, что компактизация суперспиральных молекул ДНК до уровня минитороидов и сфероидов обусловлена высокой поверхностной плотностью заряда аминослюды, на которой иммобилизованы молекулы ДНК.

Ключевые слова: суперспиральная ДНК, атомно-силовая микроскопия, аминослюда, компактизация ДНК, тороид.

References

1. *Kim J., Yoshimura S., Hizume K., Ohniwa R., Ishihama A., Takeyasu K.* Fundamental structural units of the Escherichia coli nucleoid revealed by atomic force microscopy // *Nucleic Acids Res.* – 2004. – V. 32, № 6. – P. 1982-1992.
2. *Hizume K., Yoshimura S., Maruyama H., Kim J., Wada H., Takeyasu K.* Chromatin reconstitution: development of a salt-dialysis method monitored by nanotechnology // *Arch. Histol. Cytol.* – 2002. – V. 65, № 5. – P. 405-413.
3. *Gonzalez-Huici V., Salas M., Hermoso J.* Genome wide, supercoiling-dependent in vivo binding of a viral protein involved in DNA replication and transcriptional control // *Nucleic Acids Res.* – 2004. – V. 32, № 8. – P. 2306-2314.
4. *Yoshimura S., Hizume K., Murakami A., Sutani T., Takeyasu K., Yanagida M.* Condensin architecture

and interaction with DNA: regulatory non-SMC subunits bind to the head of SMC heterodimer // *Curr. Biol.* – 2002. – V. 12, № 6. – P. 508-513.

5. Kemura K., Rybenkov V., Crison N., Hirano T., Cozzarelli N. 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation // *Cell.* – 1999. – V. 98. – P. 239-248.
6. Sato M., Ura K., Hohmura K., Tokumasu F., Yoshimura S., Hanaoka F., Takeyasu K. Atomic force microscopy sees nucleosome positioning and histone H1-induced compaction in reconstituted chromatin // *FEBS Letters.* – 1999. – V. 452. – P. 267-271.
7. *Golan R., Pietrasanta L., Hsieh W., Hansma H.* DNA toroids: stages in condensation // *Biochemistry.* – 1999. – V. 38, № 42. – P. 14069-14076.
8. *Allen M., Bradbary E., Balhorn R.* AFM analysis of DNA-protamine complexes bound to mica // *Nucleic Acids Res.* – 1997. – V. 25, № 11. – P. 2221-2226.
9. *Dunlap D., Maggi A., Soria M., Monaco L.* Nanoscopic structure of DNA condensed for gene delivery // *Nucleic Acids Res.* – 1997. – V. 25, № 15. – P. 3095-3101.
10. *Fang Y., Hoh J.* Surface-directed DNA condensation in the absence of soluble multivalent cations // *Nucleic Acids Res.* – 1998. – V. 26, № 2. – P. 588-593.
11. *Cherny D., Jovin T.* Electron and scanning force microscopy studies of alterations in supercoiled DNA tertiary structure // *J. Mol. Biol.* – 2001. – V. 313. – P. 295-307.
12. *Hud N., Downing K.* Cryoelectronmicroscopy of phage DNA condensates in vitreous ice: the fine structure of DNA toroids // *Proc. Natl. Acad. Sci. USA.* – 2001. – V. 98, № 26. – P. 14925–14930.
13. *Lin C., Wang C., Feng X., Liu M., Bai C.* The observation of the local ordering characteristics of spermidine-condensed DNA: atomic force microscopy and polarizing microscopy studies // *Nucleic Acids Res.* – 1998. – V. 26, № 13. – P. 3228-3234.
14. *Limansky A., Shlyakhtenko L., Schaus S., Henderson E., Lyubchenko Y.* Aminomodified probes for atomic force microscopy // *Probe microscopy.* – 2002. – V. 2, № 3-4. – P. 227-234.
15. *Boles T., White J., Cozzarelli N.* Structure of plectonomically supercoiled DNA // *J. Mol. Biol.* – 1990. – V. 213. – P. 931-951.
16. *Hansma H., Golan R., Hsieh W., Lollo C., Mullen-Ley P., Kwoh D.* DNA condensation for gene therapy as monitored by atomic force microscopy // *Nucleic Acids Res.* – 1998. – V. 26, № 10. – P. 2481-2487.
17. *Поляничко А., Чихиржина Е., Андрущенко В., Костылева Е., Wieser H., Воробьев В.* Влияние ионов Ca^{2+} на компактизацию ДНК в комплексе с негистоновым хромосомным белком HMGB1 // *Молекулярная биология.* – 2004. – Т. 38, № 4. – С. 701-712.
18. *Sivolob A., Prunell A.* Nucleosome conformational flexibility and implications for chromatin dynamics // *Phil. Trans. R. Soc. Lond. A.* – 2004. –V. 362. – P. 1519-1547.
19. *Зенгер В.* Принципы структурной организации нуклеиновых кислот. – М.: Мир, 1987. – 584 с.
20. *Лиманский А.* Атомно-силовая микроскопия: от визуализации молекул ДНК и белков до измерения силы межмолекулярных взаимодействий // *Успехи современной биологии.* – 2003. – Т. 123, № 6. – С. 531-542.
21. *Shlyakhtenko L., Gall A., Weimer J., Hawn D., Lyubchenko Y.* Atomic force microscopy imaging of DNA covalently immobilized on a functionalized mica substrate // *Biophys. J.* – 1999. – V. 77. – P. 568-576.
22. *Лиманский А.* Исследование аминомодифицированной слюды как субстрата для атомно-силовой микроскопии нуклеиновых кислот // *Біополімери і клітина.* – 2001. – Т. 17, № 4 – С. 292-297.
23. *Vezenov D., Noy A., Rozsnyai L., Lieber C.* Force titrations and ionization state sensitive imaging of functional groups in aqueous solutions by chemical force microscopy // *J. Am. Chem. Soc.* – 1997. – V. 119. – P. 2006-2015.
24. *Zhang H., He H., Wang J., Mu T., Liu Z.* Force titration of amino group - terminated self –assembled monolayers using chemical force microscopy // *Appl. Phys.* – 1998. – V. A66. – P. S269-S271.
25. *Lyamichev V., Mirkin S., Frank-Kameneskii M.* Structure of $(dG)_n$ C $(dC)_n$ under superhelical stress and acid pH // *J. Biomol. Struct. & Dyn.* – 1987. – V. 5, № 2. – P. 275-282.
26. *Wu A., Li Z., Yu L., Wang H., Wang E.* Plasmid DNA network on a mica substrate investigated by atomic force microscopy // *Analytical Sciences.* – 2001. – V. 17. – P. 583-584.

УДК 577.2:577.32
Найшла до редакції 29.03.05