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Atomic Force and Electron Microscopy of High Molecular Weight Circular DNA Complexes with Synthetic Oligopeptide Trivaline

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Abstract

Intramolecular compact structures formed by high molecular weight circular superhelical DNA molecules due to interaction with synthetic oligopeptide trivaline (1) were studied by atomic force and electron microscopy. Three DNA preparations were used: plasmids pTbo1, pRX10 and cosmid 27877, with sizes 6120 bp, 10500 bp and 44890 bp respectively. Plasmid pTbo1 and pRX10 preparations along with monomers contained significant amount of dimers and trimers. Main structures in all preparations observed were compact particles, which coincide in their appearance and compaction coefficient (3,5-3,7) with triple rings described earlier. The size and structure characteristics of triple rings and other compact particles on atomic force images in general coincide with those obtained by EM (2). AFM (3) images allow to get additional information about the ultrastructural organization and arrangement of DNA fibers within the compact structures. Along with triple rings in pTbo1 and pRX10 – TVP complexes significant amount of compact structures were observed having the shape of two or three compact rings attached to each other by a region of compact fibre. Basing on the data of contour length measurements and the shape of the particles it was concluded that these structures were formed due to compaction of dimeric and trimeric circular DNA molecules.

Structures consisting of several attached to each other triple rings were not found for pTbo1, pRX10 monomers or cosmid preparations – TVP complexes where only single triple rings were observed. The conclusion is made that initiation of compact fibre formation within the circular molecules depends on the primary structure and for dimeric or trimeric circular molecules two or three compaction initiation points are present, located in each monomer unit within one circular DNA molecule. The nucleotide sequence dependent compaction mechanism providing independent compaction of portions of one circular molecule can be of interest for understanding of DNA compaction processes *in vivo.*

Introduction

Formation of closed circular DNA molecules can be regarded as a first step in nucleic acids packing. The circular organization of DNA is typical of most cell organelles. From this point of view the studies of compaction of circular DNA upon interaction with ligands modeling certain properties of the agents causing DNA compaction *in vivo* are of special interest.

Earlier the electron microscopic studies of DNA compact particles with several β-structure forming peptides were carried out (4-6). Among these peptides, which bind in the minor groove of DNA in a sequence specific manner (7) the tripeptide - trivaline peptide (1) has the simplest structure. It was demonstrated that TVP causes DNA compaction giving rise to different types of compact structures with circular and linear DNA (4-6, 8-10).

Very regular circular compact structures of the complexes of TVP with circular

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superhelical DNA molecules were described. It was shown that in these structures the segments of the same molecule are located in such a way that a fibre forming the compact ring contains three DNA duplexes arranged in a side by side manner. These structures were called “triple rings” (4). The ability of circular superhelical molecules to form triple rings upon interaction with TVP was demonstrated for the molecules of different molecular weights (9,10). It was demonstrated that starting from the size of 11 kb the structures having appearance of two or more triple rings attached to each other by a linear compact fibre could be formed and models for arrangement of DNA duplex fibres in these structures were described (9,10).

In this article the results of studies of compact particles formed by circular superhelical DNA of plasmids pTbo1 and pRX10 (6120 bp and 10500 bp) and cosmid 27877 (44890 bp) with the help of AFM and EM are described. In these studies special attention was concentrated on the compaction behaviour of high molecular weight molecules – dimers and higher oligomers of plasmids and cosmid. Also the appearance of compact structures on AFM preparations is described in details as the AFM images of DNA-trivaline structures were obtained for the first time and AFM is able to give information complimentary to that is obtained by EM.

AFM can be used for imaging of DNA and protein-DNA complexes without fixing or staining, the only constraint being that the molecule is bound to a surface (11-14). At present, the resolution of the AFM is comparable to conventional EM. However, AFM is a topographic technique, so additional information can be obtained about variations of the height of the sample.

The analysis of the compact structures corresponding to high molecular weight DNA shows that structures consisting of several attached to each other triple rings were not found for pTbo1, pRX10 monomers or cosmid 27877 preparations - TVP-complexes where only single triple rings were observed.

On the contrary considerable amount of such structures corresponding to dimers and trimers of plasmid were found in AFM and EM preparations, despite the fact that molecular weight of pRX10 monomer is roughly the same as of pTbo1 dimers, and cosmid 27877 has higher molecular weight than pRX10 trimers. The conclusion is made that initiation of compact fiber formation within the circular molecules depends on the primary structure and for dimeric or trimeric circular molecules the compaction of monomer units, contained in one circular DNA molecule occurs independently.

Materials and Methods

Trivaline (H-Val-Val-Val-NH-NH-Dns, Dns is a residue of 5-dimethyl-aminonaphtyl-1-sulfonic acid) was synthesized as described in (16).

DNA-TVP complexes were prepared by direct mixing of TVP in trifluoroethanol and DNA in 0.001 M cacodylate buffer, pH 7.0 (7). The final solution contained 10 mkg/ml DNA and 25% trifluoroethanol. TVP concentration was 2×10^{-4} M.

Plasmid pTbo1, containing 4 bent nucleotide sequences was constructed on the base of vector BlueScript II KS+. It contains insertions of minicircle kinetoplast DNA of *Trypanosoma boissoni*. Plasmid pRX10 does not contain any bends. The purification of plasmid preparations was carried out with the help of “Wizard Minipreps DNA Purification System”(“Promega”) (17).

Cosmid clone 27877 was provided by Dr. L. Ashworth. Cosmid 27877 consists of the vector Lavrist16 and DNA fragment from human genome. Full length of cosmid DNA was defined by puls-electrophoresis as 44890 bp. Cosmid DNA was routinely isolated from *Escherichia coli* strain XL1-blue using QIAGEN plasmid midi kit (QIAGEN).

Electron microscopic control of circular superhelical DNA was done by protein technique (18).

For electron microscopic investigation of TVP-DNA complexes 5 mkl of solution was put on the electron microscopic grid, covered with freshly prepared collodion supporting film. In 5-10 sec the surplus of the solution was removed with a filter paper. After drying the part of the preparations was rotary shadowed with Pt/Pd alloy (4:1) at the angle 6°, the part of the preparations was stained with aqueous uranyl acetate. The preparations were studied in electron microscope JEM-100CX ("JEOL") under accelerating voltage of 80 kv and the working magnification of 5000-20000x on the screen. The measurements of contour lengths were made with the help of the computer, supplied with the digitizer.

For AFM study of TVP-DNA complexes 5 mkl of solution was placed on highly oriented pyrolytic graphite (HOPG). HOPG was kindly provided by Institute of Graphite (Moscow, Russia).

After drying the samples were imaged in Nanoscope II (Digital Instruments, Santa Barbara, CA). The AFM was operated in the constant mode using commercial silicon nitride tips with the apex curvature radius of 50 nm. The scan speed was 7 Hz.

Results and Discussion

Appearance of Circular DNA-Trivaline Complexes in Atomic Force Microscopic Preparations

The atomic force image of the plasmid pTbo1 DNA-TVP complex preparation is shown in Figure 1. It can be seen that the general appearance of the structures observed is similar to that was described earlier for EM preparations (9).

Most of the material is represented by the compact circular particles which in their appearance and contour length completely coincide with triple rings described earlier (4). The compact rings have relatively regular circular shape and according to their conformation on the support are rather rigid. Along with triple rings corresponding to compacted monomer plasmid molecules open compact rings are observed which represent compacted dimer plasmid molecules. Also the compact structures of more complicated organization are present in the preparation. They have appearance of compact rings with a short portion of linear compact fibre –RP (19) or of two or more compact rings connected by a short rod – SSP (20). Such structures were also observed by electron microscopy and their appearance on EM preparations is described in detail elsewhere (9,10).

Overall shape and contour length parameters of all the compact particles observed in AFM completely coincide with those obtained by electron microscopy. So in all

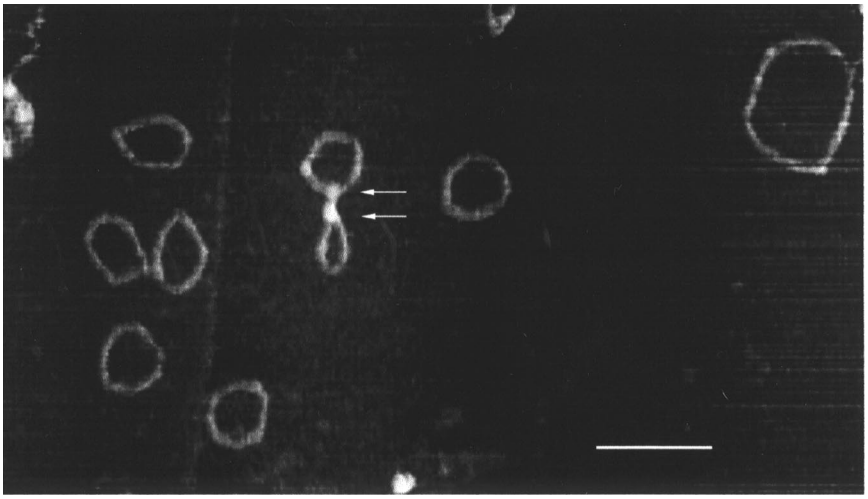

Figure 1: AFM image of the plasmid pTbo1 DNA - TVP complexes adsorbed onto the HOPG surface. Numerous single triple rings are observed as well as compact structures corresponding to dimers and having appearance of open longer ring and of a spectacle shaped particle (SSP). The highest intensity regions in the SSP corresponding to points of junction of the rings with the portion of linear fibre connecting them are indicated by arrows. Scale bar = 250 nm.

tables only the results of contour length measurements carried out on electron microscopic preparations are given. The fact of such structural identity of compact particles observed by AFM and EM shows that the conditions of complexes attachment to the chosen supports for AFM (HOPG) and EM films and influence of further manipulations in the course of sample preparations for analysis are comparable in both methods and allow to conserve main structural features of the complexes. It should be noted that DNA-TVP complexes on AFM preparations obtained on traditional mica supports had different appearance and their structure was changed and partially disrupted (to be described in detail elsewhere).

Despite the similarity of results AFM images can give some important additional information about ultrastructural organization of DNA-TVP compact particles.

Figures 2 and 3 show 3D high magnification AFM images of triple ring and racket particle, respectively. It can be seen that the fibre forming these structures is not uniform in thickness and has some kind of not very regular knobby substructure. This type of ultrastructure can be explained by the fact that the thick fibre is formed by 3 interwound DNA duplex fibres. In the case of rotary shadowed EM preparations such organization is revealed as “cross-striations” of the fibres observed. Apparent width of compact rings on AFM preparations is 23±3nm. It is well known the AFM is very sensitive in the measurements of the height of the objects. In our case it was possible to follow variations of height within compact structures. The average height of the compact fibres forming triple rings measured on AFM preparations is equal to 6.1±1 nm, what is close to the thickness of triple ring according to the measurements on stained by uranyl acetate EM preparations (4). The differences in triple ring width measured by AFM and EM can be explained by the influence of cantelever thickening in the course of scanning what leads to artificial overestimation of the fibre width.

It is of interest that the points of the maximal height (or compact fibre thickness) revealed by AFM microscopy as the highest intensity points on the AFM images in

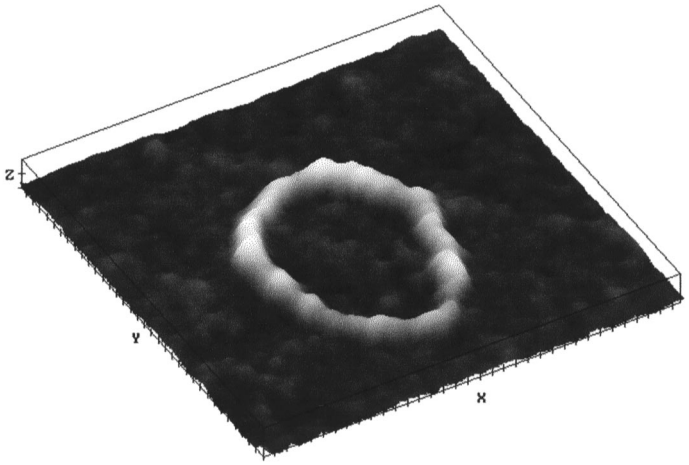

Figure 2: 3D magnified image of the triple ring formed by plasmid pTbo1 DNA in the presence of trivaline. Irregular knobby structure is clearly seen. Scale X, Y, Z = 10 nm.

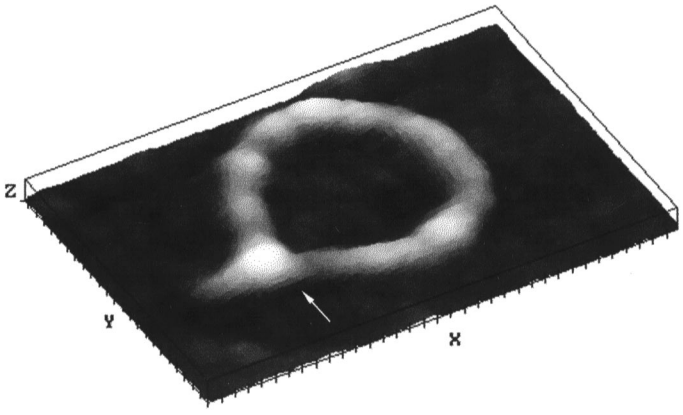

Figure 3: 3D magnified image of the racket structure formed by plasmid pTbo1 DNA in the presence of trivaline. The highest intensity region corresponds to the point of junction of the ring with the attached portion of the linear compact fibre (indicated by arrow). According to earlier proposed model in these junction points many DNA fibres are located in the vicinity of each other. Scale X, Y, Z = 10 nm.

many cases are located in the meeting points of many DNA fibres in the RP or SSP (Figure 4) according to the earlier proposed models of DNA fibres arrangement in circular DNA-TVP complexes (9). These models show that in the points of junction of the rings and pieces of linear compact fibre in the RPs (Figure 4B) and SSPs (Figure 4C) such DNA fibres arrangement can be seen most probably.

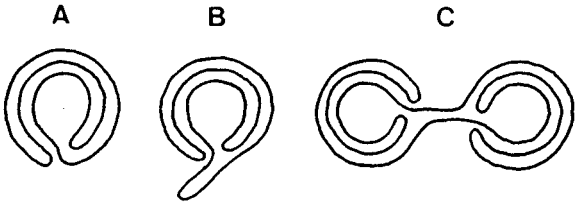

Figure 4: Models of DNA fibres arrangement in triple rings (**A**), racket particles (**B**) and spectacle shaped particles (**C**).

Organization of Circular High Molecular Weight DNA-Trivaline Complexes

Due to the fact that plasmid pTbo1 and pRX10 preparations contained significant amounts of dimers and higher order oligomers and that compact structures corresponding to plasmid oligomers are easily recognized in AFM and EM preparations it was possible to obtain significant amount of information on organization of such compact structures. The micrographs of compact particles corresponding to dimers of pTbo1 and pRX10 plasmids are shown in Figures 5 and 6. Compact particles

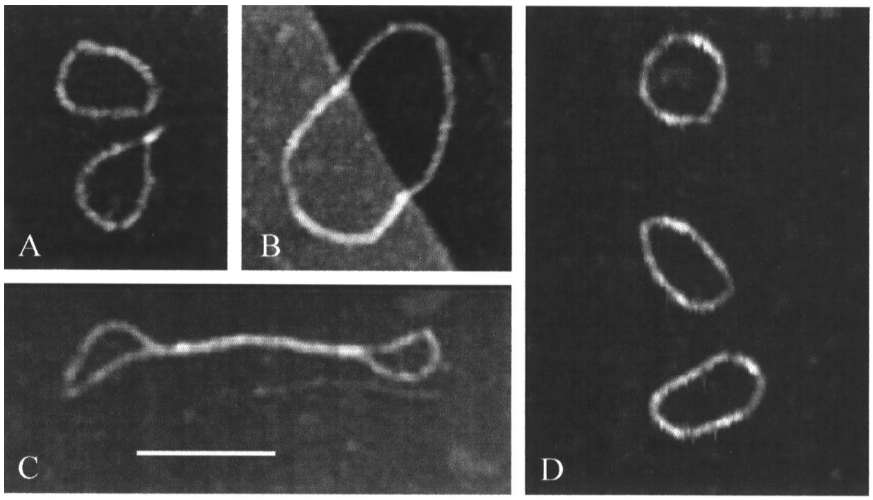

Figure 5: Selected AFM images of the plasmid pTbo1 DNA - TVP complexes. (**A**) A triple ring and a racket particle. (**B**) Open dimeric triple ring. (**C**) Spectacle shaped particle corresponding to pTbo1 dimer. (**D**) Triple rings corresponding to pTbo1 monomers. Scale bar = 250 nm.

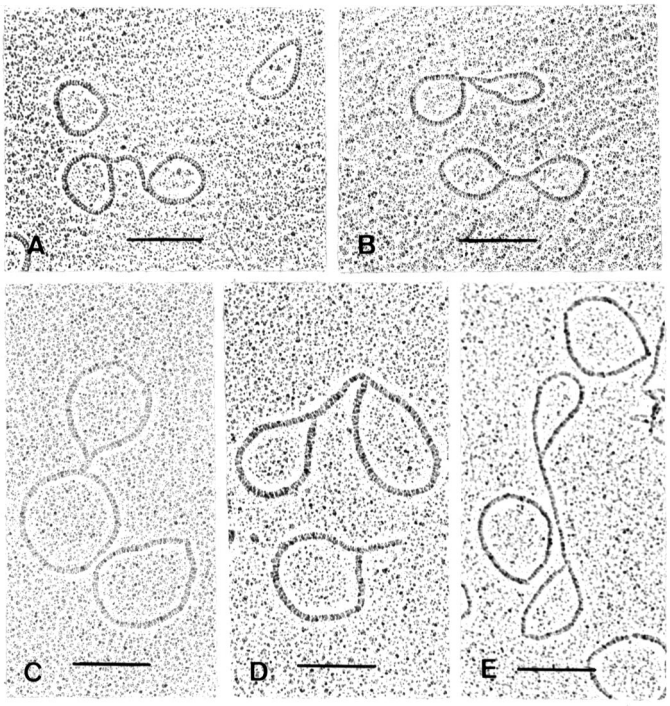

Figure 6: Selected EM micrographs of dimeric pTbo1 DNA - TVP complexes (**A, B**) and dimeric pRX10 DNA - trivaline complexes (**C-E**). Scale bar = 250 nm.

having shape of open rings are observed along with the SSPs. These two types of structures are observed in comparable quantities. Evidently separate rings forming SSP do not look as if they are produced due to selfinterwinding of a big open ring and they are connected by a well-defined fragments of a fibre with the structure similar to that of a fibre forming triple rings. Earlier it was shown that SSPs have the same contour length as the open triple rings (10). The results of the contour length measurements for all these compact particles and corresponding free DNA are shown in Table I and Figure 7.

Another interesting feature of SSPs is that the size of both rings for most of the structures is nearly the same and if the "neck" connecting two rings is small the size of the rings in SSP is naturally very close to the size of compacted plasmid monomers. It is also of interest that despite the fact that pRX10 monomers have molecular weight very close to that of pTbo1 dimers we have not observed any SSPs corresponding to pRX10 monomers. This indicates that formation of separated rings within compact particles formed by long circular molecules occurs not because of the high molecular weight but is stimulated by the presence of two identical DNA fragments corresponding to plasmid monomers in this long molecule. The similar size of rings in SSPs shows that they manage to maintain a kind of compaction dynamical equilibrium in the course of their formation.

The same conclusions are supported by the analysis of organization of compact par-

Table I

Results of contour length measurements of uncompacted DNA molecules and compact particles of DNA-TVP complexes and calculated linear compaction coefficients. (*) Compaction coefficient calculations were carried out basing on theoretically calculated length of uncompacted DNA.

DNA	Size,bp	Contour length,μm	Number of measurements	Triple ring length, μm	Number of measurements	Compaction coefficients
PTbo-1 monomers	6120	1.94 ±0.08	174	0.53 ±0.03	84	3.66
PTbo-1 dimers	12240	3.89 ±0.24	45	1.06 ±0.08	17	3.67
PTbo-1 trimers	18360	5.9±0.12	16	1.62±0.05	8	3.65
PRX10 monomers	10500	3.82 ±0.09	30	1.10 ±0.07	50	3.5
PRX10 dimers	21000	7.64 ±0.17	17	2.20 ±0.12	36	3.49
PRX10 trimers	31500	-	-	3.0 ±0.17	5	3.57 *
Cosmid 27877	44980	-	-	4.2 ±0.23	16	3.6 *

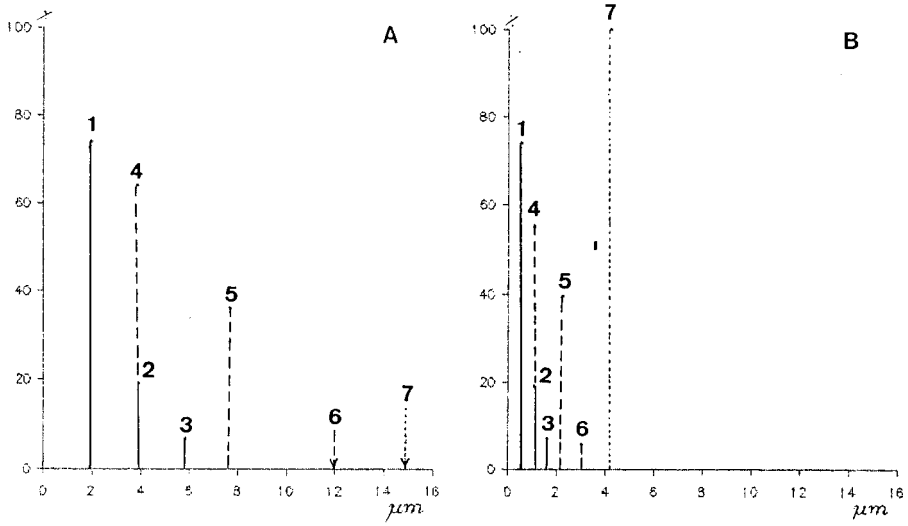

Figure 7: Histogram of the contour length of uncompacted DNA molecules (**A**) and compact particles of DNA-trivaline complexes (**B**). Percent content of corresponding monomers or oligomers is plotted for uncompacted plasmid DNA and plasmid DNA-TVP complexes. (**1-3**) - pTbo1 monomers, dimers, trimers, respectively. (**4-6**) - pRX10 monomers, dimers, trimers respectively. (**7**) - cosmid 27877. For uncompacted pRX10 and for cosmid 27877 arrows show the calculated lengths.

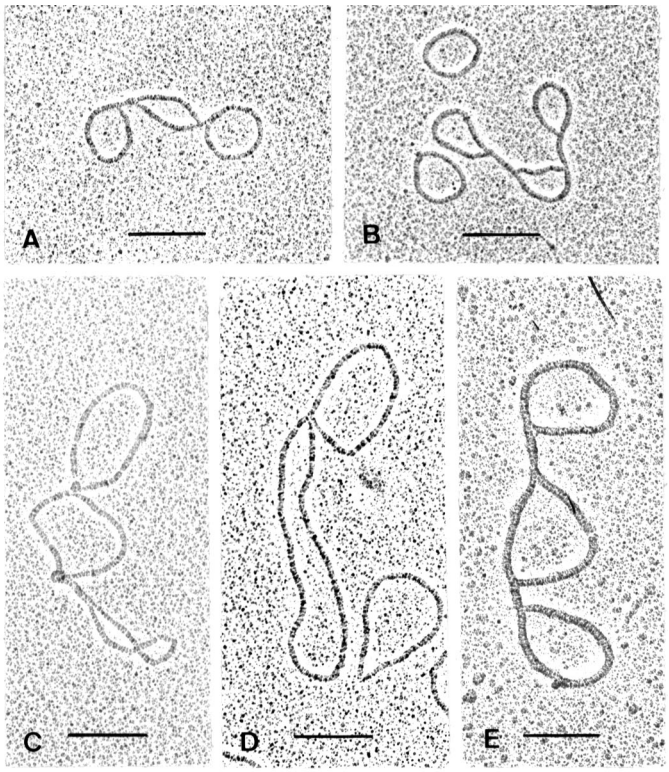

Figure 8: Selected EM micrographs of trimeric pTbo1 DNA - TVP complexes (**A, B**) and trimeric pRX10 DNA - TVP complexes (**C-E**). Scale bar = 250 nm.

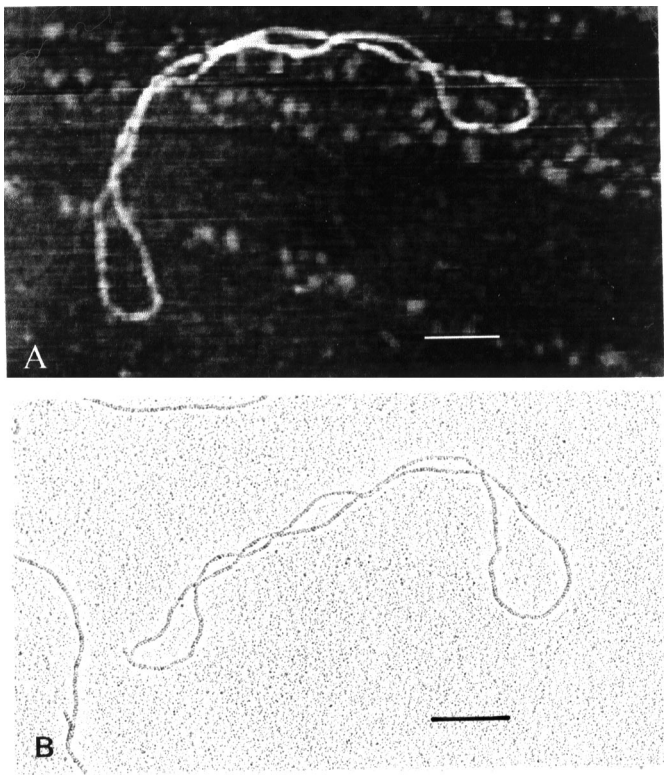

Figure 9: AFM image (**A**) and EM micrograph (**B**) of the cosmid 27877 DNA - TVP complexes. Scale bar = 250 nm.

ticles corresponding to trimers of plasmid DNA. The micrographs of compact particles formed by trimers of pTbo1 and pRX10 plasmids are shown in Figure 8. In this case most of the particles have the shape of three rings with the size close to that of plasmid DNA monomer. Attached to each other by a fragment of a compact fibre. Sometimes only two rings are observed and in this case one of them according to the contour length correspond to monomer and the other to dimer of plasmid DNA (Figure 8D). The compaction coefficients calculated as a ratio of measured contour length of compact particles to the theoretically evaluated length of non-compacted molecules are completely the same for trimers as for dimers (Table I and Figure 7).

These results allow to suppose that initiation of compaction stimulated by trivaline can be sequence dependent and can occur at specific regions of the monomers contained in one long oligomeric circular DNA molecule. This effect is independent on presence of stationary bent DNA sequences in pTbo1 plasmid as the same effect was observed for dimers and trimers of pRX10 which is a high molecular weight plasmid not having any peculiarities of primary structure (9).

In order to follow compaction behaviour of circular high molecular weight molecule containing a single not repeating DNA sequence we studied trivaline stimulated compaction of cosmid 27877 with the size of 44980 bp. The AFM and EM micrographs of corresponding compact particles are shown in Figures 9A and B respectively.

They have appearance of long interwinding closed ring structures characteristic of superhelical structures. Both AFM and EM images show that in this case we do not observe independent rings within one compact ring particle. On AFM preparations where it is possible to follow the relative positions of overlaying compact fibres it is especially evident that there are no “necks” typical of SSPs formed by oligomers of pTBo1 and pRX10 DNA.

The compaction coefficient for cosmid 27877 compact rings obtained by comparison of measured contour length of the compact rings and known cosmid molecular weight is typical of triple rings (Table I, Figure 7).

So, it can be concluded that in case of cosmid 27877 despite its very high molecular weight exceeding molecular weight of pRX10 tetramer or pTbo1 octamer only single triple rings are formed.

The results show that mechanisms of circular superhelical DNA compaction in the presence of trivaline are able to provide intramolecular compaction of very long molecules. The compaction is probably sequences dependent and leads to formation of separate compact rings within one long circular DNA molecule if it contains identical DNA fragments. Stimulated by trivaline DNA compaction deserves further studies and can be a useful model for understanding of mechanisms of DNA compaction processes *in vivo*.

Acknowledgements

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References and Footnotes

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3. AFM – atomic force microscopy.
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19. RP – racket particle.
20. SSP – spectacle shaped particle.

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