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Circular Superhelical DNA Complexes with Synthetic Oligopeptide: Unusual Compact Structures and Influence of Bent Sequences on the Results of Compaction

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Abstract

The organization of synthetic oligopeptide trivaline (1) complexes with four types of circular superhelical DNA preparations was studied by electron microscopy. The DNA molecules in the preparations investigated had different sizes ranging from 2.9 kb to 21.0 kb. Two plasmids contained bent DNA sequences from minicircles of kinetoplast DNA of *Leishmania gymnodactili* and *Trypanosoma boissoni*.

The main structures in all preparations observed were circular compact particles which coincide in their appearance and compaction coefficient (3,5-3,7) with triple rings described earlier. But along with triple rings the new types of compact structures were observed having the shape of a ring with attached rod or the shape of two compact rings attached to each other by a region of compact fiber. The latter structures could be observed in significant quantities in case of DNA preparations longer than 10 kb. The conclusions can be made that due to TVP stimulated compaction of circular DNA molecules compact fibers containing both two or three DNA duplexes arranged side by side can be formed. It is shown that presence of bent DNA sequences stimulates the formation of structures containing more than one triple ring. It demonstrates the possibility of the primary DNA structure influence on the compaction process in case of the circular molecules. The new ways of circular DNA folding described can be of importance for understanding of DNA organization in different cell structures.

Introduction

Complexes of DNA with peptides folding into helix or β-stranded structure may be considered as model systems for studies of the interaction between DNA and various regulatory and structural proteins. Earlier we investigated DNA complexes with several β-structure forming peptides which as was demonstrated by Gursky *et al.* (2) bind to the minor DNA groove in a sequence specific manner. It was shown that some of these peptides can cause DNA compaction (3-6).

Among these peptides TVP (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 (3,4,7). Both for circular superhelical and linear DNA very regularly organized compact structurs were observed.

Especially interesting are the structural transitions of circular DNA due to interactions with TVP as information about circular DNA folding can be considered to be the first step in modeling of conformational events envolved in functioning of naturally circular nucleic acids (8) or topologically equivalent to circular DNA domains of eukaryotic chromosomes (9).

Earlier we described very regular circular compact structures in the studies of the

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complexes of TVP with circular superhelical DNA molecules. These structures were called "triple rings"(3). It was shown that in the triple rings the segments of the same molecule are located in such a way that a fiber forming the compact ring contains three DNA duplexes arranged in a side by side manner.

An important point of compaction process leading to the "triple ring" type particles is the formation of a "hairpin" structure, that means of a region where a segment of a DNA molecule makes a very sharp turn . It can be assumed that torsions caused by changes in topological parameters of the closed circular DNA due to peptide binding can contribute to formation of such a sharp turn ("kink") by DNA duplex. Similar conformational changes stimulated by binding of histone-like protein to small circular DNA were demonstrated by other authors who observed in electron microscope sharp kinks in small circular DNA-protein complexes (10).

Bending of DNA in the absence of ligands can be also caused by the presence of certain nucleotide sequences. Such sequences are found in minicircles of kinetoplast DNA of some tripanosomatids (11). These bent fragments of linear DNA were observed by electron microscopy as small circles (12).

As formation of triple rings involves rather complicated folding of DNA segments of one circular DNA molecule it was of interest to investigate whether the described compaction mechanism will be able to provide compaction of circular molecules of different molecular weights and whether the presence of bends influences the results of compaction process.

In this paper we present the results of electron microscopic investigations of complexes of TVP with four plasmids of different sizes ranging from 3 to 21 kb. Two of plasmids contained bent DNA sequences.

The main structures observed in all the plasmid preparations were triple rings which completely coincide in their organization and compaction coefficient with the triple rings described earlier for pBr 322 DNA (3). But along with triple rings the several new types of the structures were observed and are described in detail in the paper.

These structures probably arise when more than one primary "hairpin" is formed within single circular molecule at initial stages of compaction. In case of long circular DNA molecules it leads to formation of compact structures containing two or more rings connected to each other by linear portion of a compact fiber containing two duplex DNA segments. Presence of such structures demonstrate possibility of independent compaction of different parts of one circular molecule.

The comparison of compact structures of trivaline complexes with long circular DNA molecules containing and not containing bent sequences shows that presence of bent sequences promotes formation of structures containing more than one triple ring. It can be assumed that DNA segments with bent sequences can influence DNA compaction either by stimulation of compaction at multiple sites or by separation of compaction events happening with different parts of the same molecule.

The tentative models of DNA fibers arrangement in new structures described are proposed. The possibilities of different types of DNA fibers aligning and spatial arrangement of parts of one circular superhelical molecule by complexing with simple oligopeptide can have important bearing for situations *in vivo* involving DNA duplex fibers alignment and bending.

Materials and Methods

TVP synthesis was carried out as described by Streltsov *et al.* (13). DNA-TVP complexes were prepared by direct mixing of TVP in trifluoroethanol and DNA in

0.001 M cacodylate buffer, pH 7.0 (2). The final solution contained 10 mkg/ml DNA and 25% trifluoroethanol. TVP concentration was $2x10^{-4}$ M.

Plasmids pLg19 and pTbo1, containing bent nucleotide sequences, were constructed on the base of vector BlueScript II KS+ (pBl). They contain insertions of minicircle kinetoplast DNA of *Leishmania gymnodactili* and *Trypanosoma boissoni* correspondingly. Minicircles of *Leishmania gymnodactili* contain one bend per the circle and *Trypanosoma boissoni* - four such ones. Plasmid pX10 does not contain any bends. The scheme of these constructs are represented on Figure 1. Electrophoresis was held in standard conditions as in (14). The purification of plasmid preparations was carried out with the help of "Wizard Minipreps DNA Purification System"("Promega"). Electron microscopic control of circular superhelical DNA was done by protein technique (15).

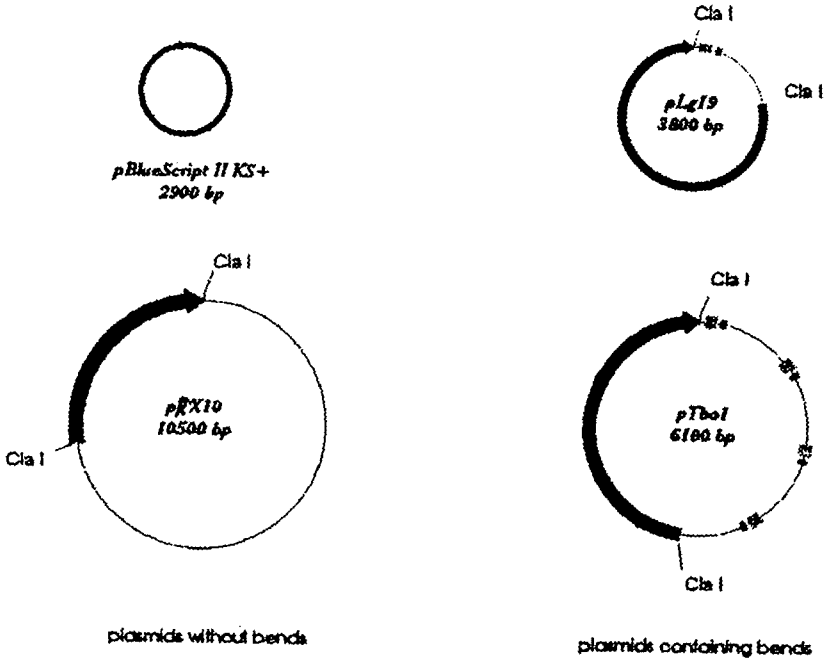

Figure 1: Schematic presentation of four plasmids used for DNA-TVP complexes preparation. The structural elements of plasmids and cloning sites are marked: black arrow is cloning vector; the grey mark is minicircle conservative region, including replication ori; the black mark is bend within minicircle DNA.

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 prepaprations was stained with ethanolic 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.

Results and discussion

Appearance of Uncompacted DNA in Electron Microscopic Preparations

Four circular superhelical DNA preparations were used in our study. Two plasmids pLg19 and pTbo1 were constructed on the base of pBl and contained insertions of minicircle sequences of *Leishmania gymnodactili* and *Trypanosoma boissoni*, respectively. pLg19 includes the insertion sequence of 900 b.p. containing one bend and pTbo1 contains 3200 b.p. insertion with four bends. Other two of plasmid preparations, namely pBl and pX10 do not contain any bent sequences and were used as a low and high molecular weight preparations of circular DNA without any particular sequences. The schematic presentations of all four preparations are shown in Figure 1.

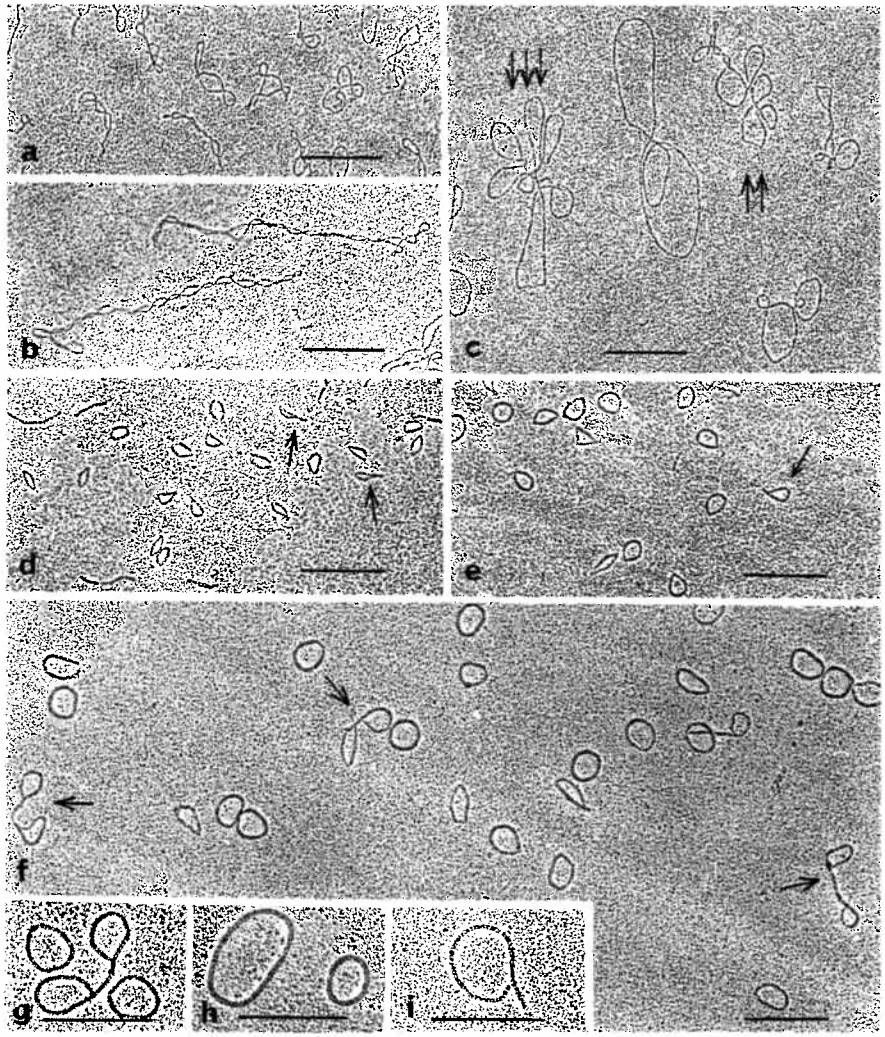

Figure 2: Appearance of circular DNA preparations (a-c) and DNA-TVP complexes (d-i) in electron microscope. Bar 0.5 mkm.
(a) pBl DNA preparation; (b) pX10 DNA preparation ;(c) pTbo1 DNA preparation.
DNA without peptide was prepared for EM by protein spreading technique according to Davis et al. (1971) and rotary shadowed (see Experimental procedures) . Most of the molecules have typical shape of superhelical circles. In pTbo1 preparation many DNA oligomers are seen. Dimers and trimer in (c) are indicated by two and three arrows, respectively.
(d-i) complexes of different DNA preparations with TVP. DNA-TVP complexes were prepared for EM by direct application of the complexes solution to the EM grids and rotary shadowed (see Experimental procedures):
(d) TVP-pBl DNA complexes; (i) TVP-pLg19 DNA complexes;
The ring structures with the attached short rod ("rackets") are indicated by arrows.
(f-i) TVP-pTbo1 DNA complexes. The structures containing more than one compact ring (related to as "spectacles shaped particles" - SSP) are present and are indicated by arrows in (f). The SSPs correspond to compact state of dimers of pTbo1 molecules.
(g,h) selected electron micrograph areas showing SSP and triple ring corresponding to pTbo1 dimers, respectively, along with pTbo1 monomeric triple rings; (i) "racket" structure, corresponding to pTbo1 dimer.

All DNA preparations before obtaining complexes and electron microscopic studies were analysed by electrophoresis (data not shown). The electrophoretic data demonstrated that most of DNA in all four preparations was in superhelical state. We also checked whether DNA changes its topological state after complexing with TVP. For this purpose the DNA-TVP complexes were obtained, checked for formation of compact particles by electron microscopy, and afterwards analysed by electrophoresis in conditions which disrupt the complexes. It was found that process of complex formation is completely reversible and DNA conserves its superhelicity after formation and disruption of DNA-TVP complexes.

Electrophoretic data also demonstrates that in all the preparations the monomer plasmids were present along with certain amount of dimers and higher oligomers. Especially significant share of DNA molecules in the oligomeric form which could be roughly estimated as 20-30% of total amount of DNA was found in pTbo1 preparations. Such significant share of dimers and higher oligomers is characteristic of plasmids with big insertions having complicated structure (Kolesnikov and Yurchenko, unpublished results).

All DNA preparations were analysed by electron microscopy using cyt *C* spreading method (15). Electron micrographs of pBlueScript, pX10 DNA and pTbo1 preparations are shown in Figures 2 a-c respectively. In the micrograph of Tbo1 preparation the dimers are indicated by two arrows and trimer by three arrows. Data for pLg19 is not shown.

The conformation of the molecules on electron micrographs for all four preparations is

typical of circular superhelical DNA. The size distribution obtained by DNA molecules length measurements on the electron micrographs coincides with the electrophoresis results for all preparations and shows the presence of oligomeric molecules.

Morphology of Compact Particles in DNA-TVP Complexes Preparations

Figures 2 d- i and 3 a-d show electron microscopic photographs of complexes of TVP with different DNA preparations. Most of the material in all preparations is represented by the compact circular particles which in their appearance completely correspond to triple rings described earlier (3). These structures are formed by fibers with the thickness of 180-220 A° on the rotary shadowed preparations. The compact rings have relatively regular circular shape and according to their conformation on the micrographs are rather rigid. Also it is evident that the fibers forming these compact rings have regular substructure with apparent cross striations. All these features are characteristic of triple rings and are described in details elsewhere (6).

The compaction coefficient of DNA in all the triple rings (determined as ratio of contour length of unfolded DNA to contour length of compact ring) was found to be 3,5 - 3,7 (Figure 4) and coincides with the value obtained earlier for pBr322.

Along with the perfect triple rings in DNA-TVP complexes preparations certain amount of the structures are observed where compact rings are connected with a short rod (Figures 2d, 2i, 3a, 3d). These structures in their shape resemble tennis rackets. The attached rod (racket handle) is also formed by the compact fiber which is in its thickness and organization similar to that of the fiber forming the triple ring.

The "racket"structures are indicated by arrows (Figure 2d, 3a). Such structures are observed in certain amount in all preparations investigated.

If in preparations of pBl and pLg19 -TVP complexes most of the compact DNA particles have the appearance of triple rings and "racket"-shaped particles in higher molecular weight circular DNA preparations significant amount of structures with more complicated organization is observed.

The representative large field micrograph of pTbo1-TVP complexes preparation in Figure 2 f demonstrates that along with triple rings the structures containing more than one triple ring attached to each other are observed (indicated by arrows). The rings in such structures are connected by a fiber which have the same thickness and structure as fiber forming the rings themselves.

The contour length measurements of the compact structures and known compact coefficient for triple rings allows to estimate easily to which uncompacted DNA length this or another structure corresponds. It is evident that the structures containing two or more triple rings in preparations of Tbo1 -TVP complexes refer to compact form of dimers or higher oligomers of pTbo1 molecules. As the principles of organization of such structures are the same for dimers and higher oligomers, and dimers are met much often than higher oligomers, the illustration material will refer to compact structures containing dimers. As their shape resembles spectacles rim, such structures will be further refered to as "spectacle shaped particles" (SSP).

Along with SSP long triple rings are sometimes observed apparently corresponding to compacted dimers of pTbo1 (Figure 2h). It should be noted that in pTbo1-TVP preparations we never observed triple rings of the size corresponding to trimers or higher oligomers of pTbo1, despite the fact that such molecules were not rare in cyt C preprations of pTbo1 DNA. The compact particles relating to trimers or higher oligomers of pTbo1 found in pTbo1-TVP preparations always have the shape of several triple rings connected to each other.

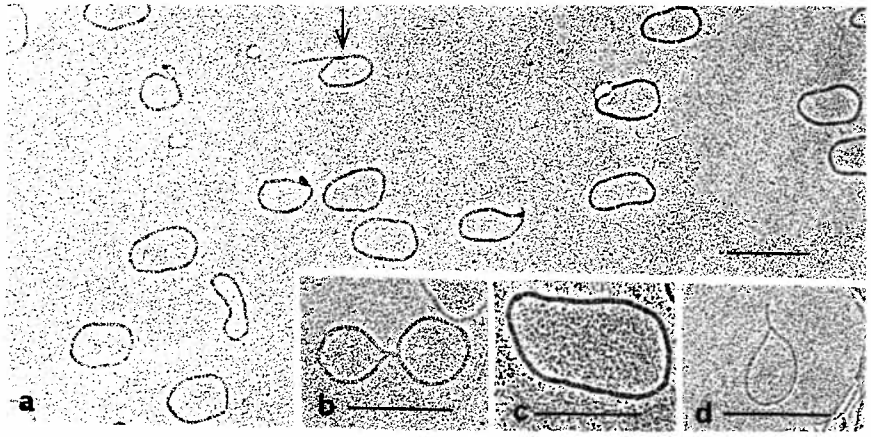

Figure 3: Appearance of complexes of pX10 DNA with TVP. Bar 0.5 mkm.
The complexes were prepared for EM as described in Experimental procedures.
(a) The large field micrograph demonstrates that most of the material is represented by large triple rings. In their contour length these rings are very close to triple rings corresponding to pTbo1 dimers. (b,c) selected SSP and triple ring corresponding to dimer of pX10. (d) "racket" structure corresponding to pX10 monomer.

The analysis of pBl, pLg19 and pTbo1-TVP complexes preparations shows that nearly all the SSPs correspond to molecules of pTbo1 dimer size or longer. So it can be assumed that only starting from certain length of circular DNA alternative ways of DNA compact particle formation become possible leading to SSP formation.

If only dimeric pTbo1 moleclues are considered in pTbo1-TVP complexes preparations the SSPs are met much more often than long dimeric triple rings. It can be assumed that the formation of long triple rings for dimers or higher oligomers of pTbo1 is hindered by the presence of bent sequences.

To check this possibility we investigated trivaline complexes with plasmid pX10 with the length of 10500 b.p. which is very close to that of dimer of pTbo-1. The micrographs of this preparation are shown in Figures 3 a-d. The large field micrograph in Figure 3a demonstrates that in this case most of the material is represented by perfect triple rings corresponding to monomers of pX10. These triple rings are nearly of the same contour length as triple rings containing dimeric pTbo1 DNA molecules (Figure 4). In this preparation the very long triple rings (Figure 3c) as well as SSP particles (Figure 3b) relating to dimeric pX10 molecules are also observed.

The comparison of results relating to dimers of pTbo1 and monomers of pX10 allows to make a conclusion that presence of bent sequences shifts the compaction process towards more complicated pathways leading to formation of SSP. These assumptions

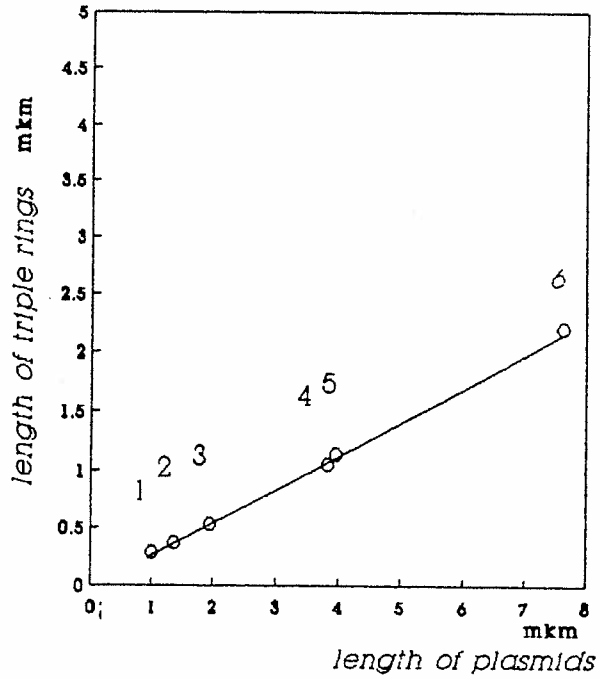

Figure 4: The contour length of compact structures of TVP-DNA complexes plotted against the length of corresponding uncompacted DNA measured in protein spread preparations. The open circles correspond to triple rings. They are marked in the following way: (1) - pBl; (2) - pLg19; (3) - pTbo1 -monomer; (4) - pX10-monomer; (5) - pTbo-dimer; (6) - pX10-dimer. The corresponding mean values of uncompacted molecules and triple rings are given in the Table I.

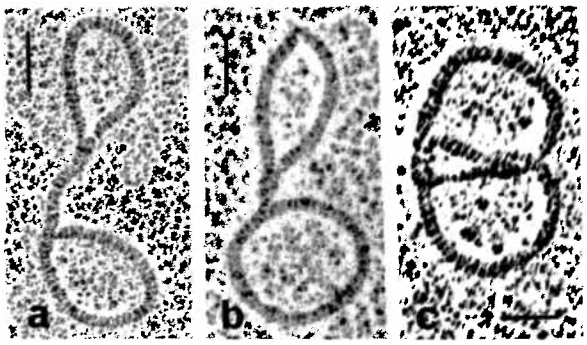

Figure 5: Selected SSPs, corresponding to pTbo1-dimers in higher magnification. Bar 0.1 mkm. (a,b) SSPs with typical structure. The fiber forming "neck" connecting two rings in SSP has the same thickness and structure as the fiber forming the rings. (c) different type of SSP particle more rarely present in EM preparations.

are also supported by the fact that for dimers of pX10 which are significantly longer than even trimers of pTbo1 perfect open triple rings could be observed.

So it can be assumed that the presence of bent sequences in pTbo1 favours independent compaction of different parts of the same circular molecule. Probably these bent sequences can either serve as initiation sites of primary "hairpin" formation or can cause such interwinding of long DNA molecules that parts of the same molecule can be compacted into triple ring structures independently.

Table I

Contour length of uncompacted circular DNA and triple rings of DNA-TVP complexes for different plasmids DNA preparations.

Plasmid	Length of plasmid in mkm	Number of measurements	Length of triple rings in mkm	Number of measurement
pB1	0.99±0.05	80	0.28±0.03	80
pLg19	1.36±0.08	42	0.37±0.02	48
pTbo1 monomers	1.94±0.08	174	0.53±0.03	84
pX10 momomers	3.82±0.09	15	1.05±0.06	15
pTbo1 dimers	3.89±0.24	45	1.06±0.08	17
pX10 dimers	7.64±0.17	17	2.20±0.12	16

Morphology of Unusual Compact Particles and Possible Ways of DNA Folding in their Structure

According to their unusual organization and complicated DNA fibers arrangement in their structure SSPs deserve special detailed description. Several selected SSPs are shown in higher magnification in Figures 5 a-b. In higher magnification it is evi-

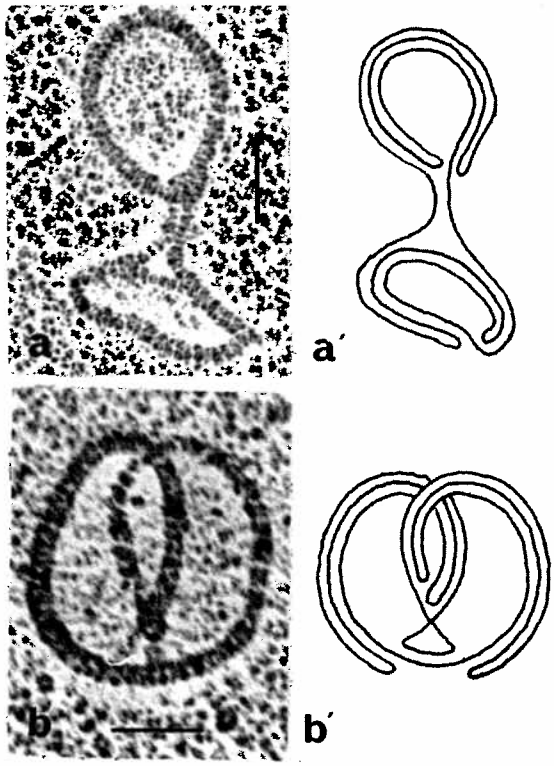

Figure 6: Compact particles with distorted structure in high magnification (a,b) with the simplified graphical interpretation (a', b')of the possible DNA fibers arrangement. The drawings on a) and b) do not reflect the DNA fibers interwinding. Bar 0.1 mkm.

dent that fiber forming the "neck" of SSP have completely the same organization as the fiber forming the rings themselves.

Also in small amounts the SSPs having different appearance are observed (Figure 5c) as well as structures where two (or more) rings are attached to each other without any "neck" (not shown). These structures will be discussed in more detail further.

As each SSP most probably contains one long circular compacted molecule it is evident that the "neck" connecting two rings can contain only even number of DNA duplex fibers. The contour length measurements show that compaction coefficient of DNA in SSPs is very close to that of the triple rings and their overall contour length is roughly equal to that of the dimeric triple rings. It means that compaction coefficients of DNA in the fibers forming the "neck" and the rings are very close to each other.

Most probably the compact fiber forming the "neck" of SSP is formed by two aligned DNA duplex fibers. According to its structural features the "neck" is equivalent to the "handle" fiber of the racket structures which also can contain only even number of DNA fibers. So the data demonstrates that mechanism involved in DNA folding in compact DNA-peptide structures can provide compaction of not only three but of two DNA duplex segments arranged in a side by side way as well.

In our earlier publications a model of DNA-peptide arragement in β-oligopeptide-DNA complexes was proposed where DNA is wound around the the rod-like core formed by assembled peptide molecules (5). It can be assumed that the same type of organization can be observed both for two and three DNA duplex fibers segments arranged around such peptide core and that structural features of such compact fiber are determined more by geometry of the peptide component of the complex. It should be noted that proposal about side by side arrangement of two DNA duplexes in compact rod-like structures of linear DNA-TVP complexes was offered by us earlier (7).

The proposed conclusions about arrangement of DNA fibers in SSPs are supported by analysis of compact particles with distorted structure or where the compaction process was not completed. One of such SSP particles is shown in Figure 6 a-a′.with the proposed model of DNA folding in its structure. In this structure the composition of the "neck" of two DNA fibers arranged in a side by side way is evident. The graphic interpretation of DNA folding in Figure 6 a′ shows two possible types of junctions of the"neck" fiber with the fibers forming triple rings within SSP. The proposed way of arrangement of DNA fibers in usual (type 1) SSPs (shown in Figures 2f, 2g, 3b, 5a,5b, 6a) allows to make the assumption that such particles can be formed when two primary "hairpins" emerge in the long circular DNA molecule and are separated by a region where two opposite segments of the molecule are joint to each other due to interaction with peptide. The same model gives the explanation of formation of the SSPs of the more rarely met type 2 (shown in Figures 5c,6b). The difference with respect to the previous case is the relative direction of the DNA fibers forming the "neck" separating two primary "hairpins".

The described formation of different regular compact structures by circular superhelical DNA molecules due to interactions with simple oligopeptide can be considered as a model of DNA compaction *in vivo*. The formation of SSPs demonstrates the possibility of independent condensation of different parts of single circular molecule. The influence of the presence of bent sequences on the final morphology of the compact structures demonstrates the possibility of modulation of DNA compaction processes in cell by changes in the circular DNA primary structure. The variety of the structures observed demonstrates the compaction flexibility of circular superhelical molecules in very simple model system.

The described ways of DNA compaction and DNA fibers alignment and folding

may be of importance in different processes of fuctioning of DNA *in vivo*.

Acknowledgements

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