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Electron—Microscopic Study of Circular DNA Compaction in Model Systems. II. Complexes of Trivaline with High-Molecular-Weight Circular DNA

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Abstract—An electron-microscopic study of the morphology of complexes of a synthetic peptide trivaline with pRX10 supercoiled circular DNA was performed. Monomers of this plasmid are composed of 10,500 bp. The samples studied included also a considerable number of plasmid dimers and even oligomers. The compact structures including monomers of pRX10 most often had the characteristic triple ring morphology, although in some cases triple rings with an adjacent linear stretch of a compact fiber could be observed. Compact structures including plasmid dimers appeared either as triple rings of double length or as two triple rings connected by a stretch of linear fiber. Such complex structures have been described previously only in samples of circular DNAs containing bent sequences. The formation of intramolecular compact structures containing two or more rings in the process of pRX10 compaction demonstrates that the increase in size itself, regardless of secondary structure peculiarities, increases the topological freedom of fragments of a circular molecule so that they may undergo compaction independently. A detailed study of the morphology of unusual structures of trivaline–pRX10 complexes and determination of the DNA compaction coefficient shed light on the DNA packing in these structures. The data obtained suggest that DNA compaction under the influence of trivaline is based on the formation of multistranded compact fibers and is quite a flexible process. Compact fibers of similar morphology and compaction may include either three (as in triple rings) or two filaments of DNA duplexes.

Key words: DNA compaction, circular DNA, triple rings, trivaline, electron microscopy

INTRODUCTION

Earlier we have described compact circular structures formed by the interaction of a synthetic peptide trivaline with three types of circular DNA differing in molecular weight [1]. Trivaline dansyl hydraside (TVDH) is a simple oligopeptide capable of specific binding to some DNA sequences. The mechanism involved resembles the DNA complexing with regulatory β proteins [1, 2]. Specimens of trivaline complexes with DNA of one of the investigated plasmids, pTbo-1, alongside with triple rings, contained new, more complex compact structures composed of several rings interconnected with a compact linear fiber. Such structures were observed when dimers of pTbo-1 and its oligomers of up to 12 kbp participated in complex formation.

It was supposed that such structures may be formed oeing to the presence of certain nucleotide sequences in the pTbo-1 DNA causing its stable bending. In parallel, it was demonstrated that the increase of the circular DNA size increases the variability of the resulting compact structures. In this context, the study of compaction of high-molecular-weight circular DNA of approximately the size of pTbo-1 dimers or even higher under the action of trivaline seemed to be extremely promising.

In the present paper we describe some properties of the pRX10 DNA complexes with trivaline. Monomers of this plasmid are composed of 10,500 bp. Plasmid dimers and oligomers were also present in the plasmid samples studied.

The study of compaction of the pRX10 DNA under the action of trivaline revealed that such large circular DNAs which do not have any specific nucleotide sequences are nevertheless also able to give rise to compact structures composed of several interlinked triple rings differing from ordinary triple rings.

EXPERIMENTAL

Synthesis of TVDH was performed as described in [2].

DNA–TVDH complexes were prepared by mixing the peptide in water–trifluoroethanol with DNA in 1mM cacodylate buffer, pH 7.0 [3] to final concentra-

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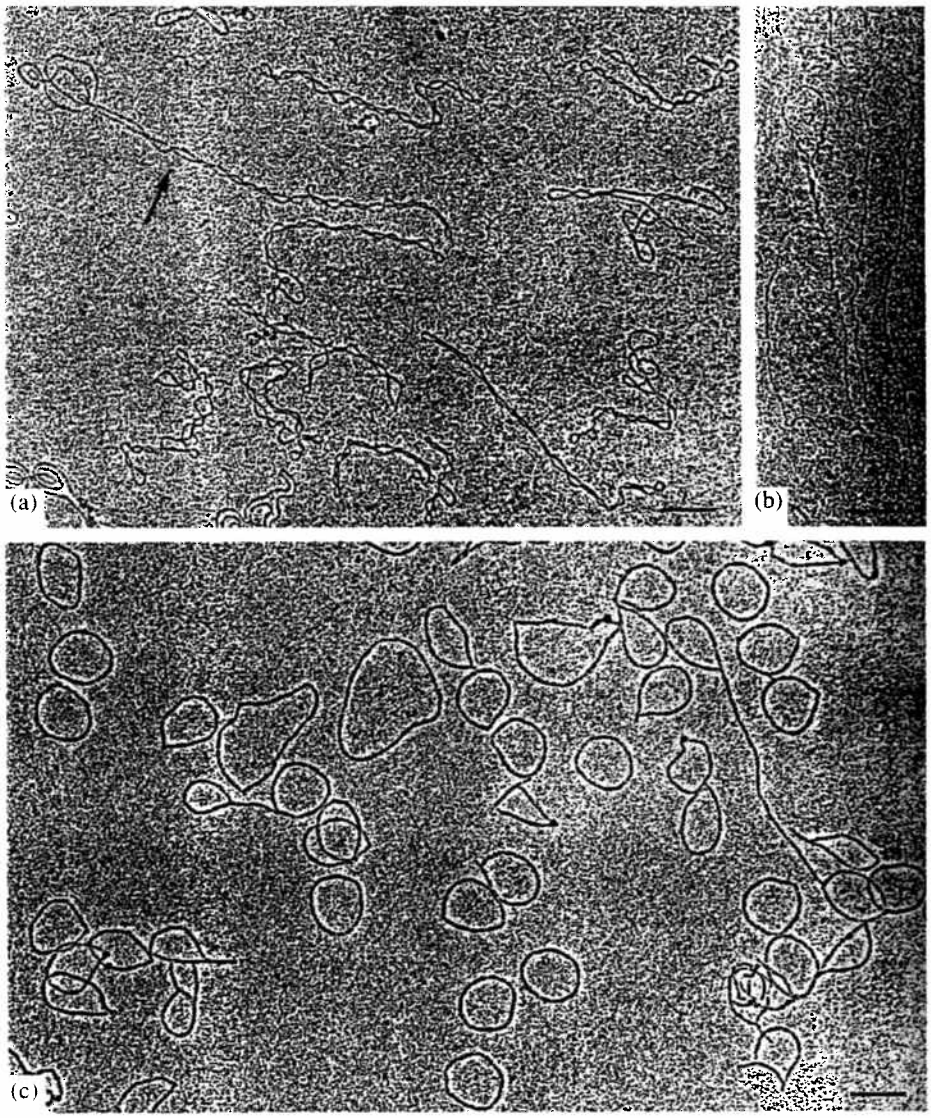

Fig. 1. (a) Micrograph of pRX10 DNA prepared by the protein technique [4]. A pRX10 dimer is indicated by arrow. (b) Micrograph of pRX10 complexes obtained at $4 \cdot 10^{-5}$ M TVDH. (C) Overview of compact structures of complexes obtained at $5 \cdot 10^{-4}$ M TVDH. Scale bar, 0.25 μm.

tions of 10 μg/ml DNA, 25% TFE, and up to 0.3 mM TVDH.

Samples of pRX10 plasmid were purified using Wizard DNA Purification System (Promega).

Electron-microscopic check of supercoiled circular plasmid DNA was performed as described in [4].

Electrophoresis of plasmid DNA was carried out under standard conditions in 0.8% agarose (Sigma) gel in 1× TAE buffer, at 5 V/cm. The bands of mono- and oligomers were stained with ethidium bromide [5].

Electron microscopic analysis of TVDH–DNA complexes was performed as follows: Specimens (5 μl) were placed onto fresh collodion-coated grids. In 5–10 s, excess liquid was drawn off with filter paper. After drying, some specimens were contrasted by rotary shadowing at 6°C with Pt/Pd (4:1), others with uranyl acetate in alcohol, and examined in a JEM-100CX (JEOL) electron microscope at an accelerating voltage of 80 kV and screen magnification of ×5000–20,000. Contour lengths were measured on ×20,000–80,000 microphotographs with a digitizing computer.

Contour length of plasmids and of DNA-TVDH complexes. Plasmid compaction coefficients in complexes with TVDH

Plasmid	Size (bp)	Contour length (μm)	Number of measurements	Type of compact structure	Contour length (μm)	Number of measurements	Compaction coefficient
pRX10 Monomers	10500	3.82 ± 0.09	30	Triple rings	1.05 ± 0.06	30	3.64
				Rackets	1.1 ± 0.07	20	3.47
pRX10 Dimers	21000	7.64 ± 0.17	17	Triple rings	2.20 ± 0.12	16	3.47
				Dumbbells	2.15 ± 0.11	20	3.5
pRX10 Trimers	31500	–	–	Dumbbells	3.0 ± 0.17	5	3.57

RESULTS AND DISCUSSION

Electron microscopic photograph of the pRX10 DNA prepared by the protein technique of Davis *et al.* [4] is shown in Fig. 1a. Most molecules have a typical supercoiled DNA conformation. Among the monomeric molecules, several dimers may be observed (indicated by arrow in Fig. 1a) and even oligomers. Electrophoresis demonstrated that most of the molecules in the pRX10 sample are in supercoiled conformation, including higher plasmid oligomers (data not shown).

Properties of DNA–trivaline complexes obtained at were investigated different DNA/peptide ratios. At low peptide concentrations, unfolded circular plasmid molecules with a substantially lower number of self-crossings were the most abundant ones (Fig. 1b). Their appearance on the grids was close to that of relaxed molecules. Probably, the extent of supercoiling decreased upon peptide binding. This phenomenon was described for pBR322 in our previous work [6].

An overview of the pRX10–TVDH complexes obtained at higher peptide concentration is presented in Fig. 1c. Most of the molecules are triple rings with the morphology typical of compact structures. Comparison of DNA contour length in the absence of trivaline and its length in DNA–TVDH compact complexes indicates that the degree of compaction is the same as in the case of other plasmids, 3.5–3.7 [1, 6].

Among the "perfect" triple rings which constitute the bulk of the material, there are triple rings with attached stretches of linear compact fibers with organization and thickness identical to the fibers forming the triple rings. Such molecules look like lawn tennis rackets. Some of them can be seen in Fig.2. Compact rings with two such stretches were also observed (Fig. 2a).

Racket-like structures have been observed in DNA samples of lower molecular weight (unpublished). The peculiar feature of such structures is that the linear compact fiber connected to the ring may be composed of only an even number (probably, two) of laterally organized DNA filaments. In other words, racket structures may comprise fibers of similar morphology but composed of a different number of DNA duplexes.

Moreover, two compact rings connected by a compact fiber could be seldom observed in pRX10 monomer samples. Such structures look like dumbbells or spectacles. They were originally described for pTbo-1 dimers in our previous publication [1]. A micrograph of such a dumbbell is given in Fig. 2c.

Alongside with the typical triple rings of pRX10 complexes and other compact structures of monomeric molecules, larger compact rings corresponding to dimers with a compaction coefficient of 3.47 may be observed (Fig. 3a,b). Although in the initial pRX10 samples a certain amount of trimers and oligomers of higher order is present (data not shown), we failed to detect unfolded triple rings corresponding to molecules bigger than pRX10 dimers.

Noteworthily, in experiments with pTbo-1 the unfolded rings were also detected, but only for dimers, whereas trimers in trivaline–DNA complexes always gave a dumbbell structure, i.e., they were composed of several interconnected compact rings. However, if circular pRX10 DNA dimers (21,000 bp, approximately equal in length to pTbo-1 tetramers) were analyzed, we registered the formation of unfolded triple rings, probably due to the presence of several sequences inducing bends. The presence of such sequences may underlie the more complex mode of circular molecule compaction and induce several centers of compact fiber formation within a single molecule [1].

One may hypothesize that the presence of the bent regions in pTbo-1 may explain why the ideally unfolded triple rings corresponding to dimers are much less abundant than the more complex dumbbells, whereas the pRX10 monomers of the same molecular weight but lacking bent structures give rise to predominantly triple rings.

It is evident that the increase of molecular weight itself, even if the molecules do not possess such specific regions, should extend the relative conformational independence of different regions of a large circular molecule.

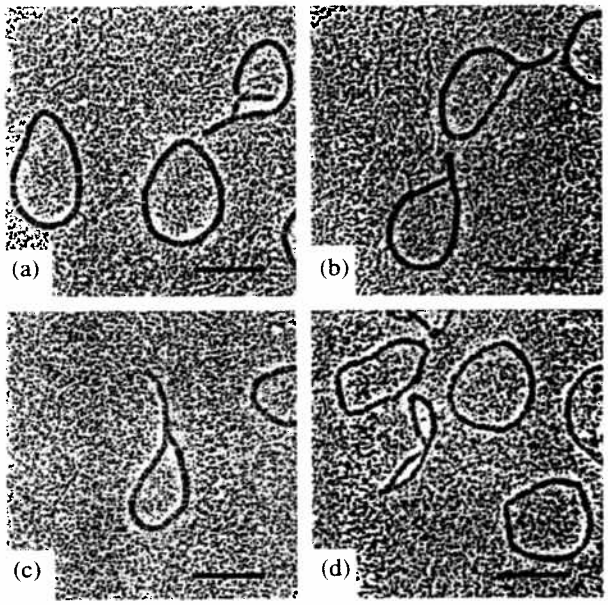

Fig. 2. Micrograph of compact structures corresponding to the pRX10 monomer–TVDH complexes. Scale bar, 0.25 μm.

The study of pRX10–TVDH complexes revealed that compact structures formed by dimers are mostly dumbbells and are much more variable than the compact structures formed by monomers. Micrographs of selected dumbbell compact structures formed by pRX10 dimers are presented in Fig. 3d–i.

All these entitites have a characteristic organization: their constituent rings are formed of morphologically typical fibers identical to the fibers of ordinary triple rings. The fibers connecting individual rings in such structures are practically identical in morphology and thickness to the fibers present in ordinary triple rings. This is not a trivial fact, because the connecting fibers in dumbbells may include only an even number of DNA duplex filaments. If one analyzes this phenomenon basing on the mode of DNA packing in compact fibers, then it appears that the links between the rings of the dumbbells are analogous to the fiber stretch in the rackets.

A certain part of structures look like two interconnected rings without any spacer between them (Fig. 3c). Sometimes an electron-dense spot could be observed in the junction of the two rings. In such structures the rings may be oriented in a specific manner. Similar structures were detected in experiments with pTbo-1 DNA: they seem to represent final stages of DNA packing, which will be described in our future publications.

Contour lengths of uncompacted monomeric and dimeric DNA of pRX10, of compact triple rings, and

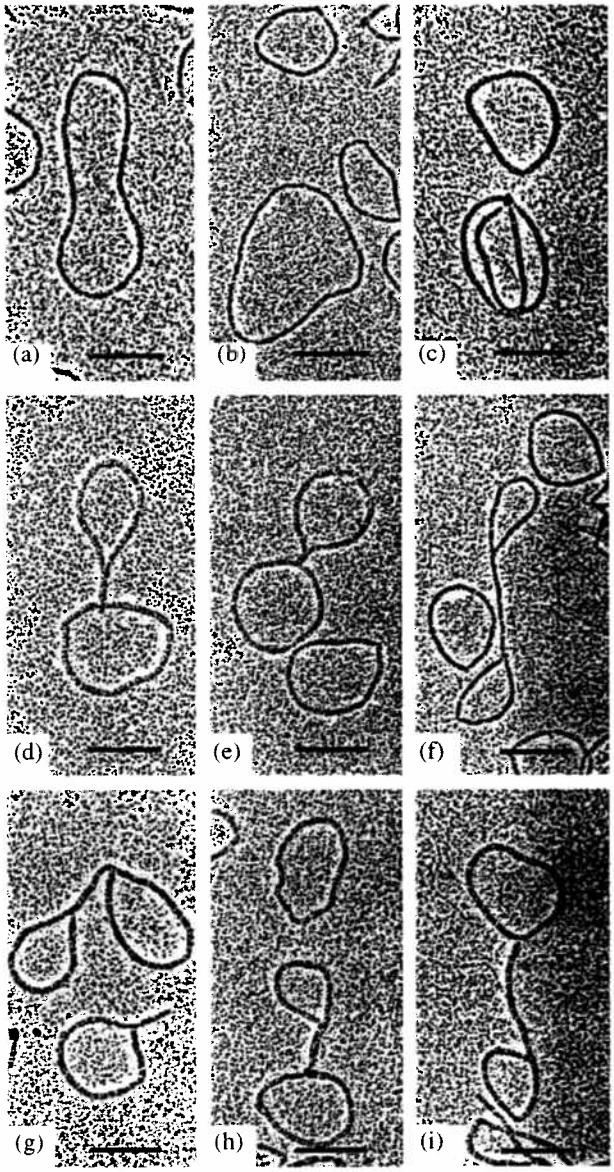

Fig. 3. Micrograph of compact structures corresponding to the pRX10 dimer–TVDH complexes. Scale bar, 0.25 μm.

of compact structures of unusual morphology corresponding to DNA monomer and dimer complexes with trivaline are presented in Fig. 4. For unusual structures, the lengths of all the elements were summed up. It follows from Fig. 4 that neither dumbbells nor rackets differ from triple rings in their contour length. Hence, the DNA compaction coefficient for the connectors in dumbbells and for the stretches of compact fibers in rackets does not differ from that in triple rings.

It follows from these data that the mechanism of DNA compaction under the influence of trivaline is

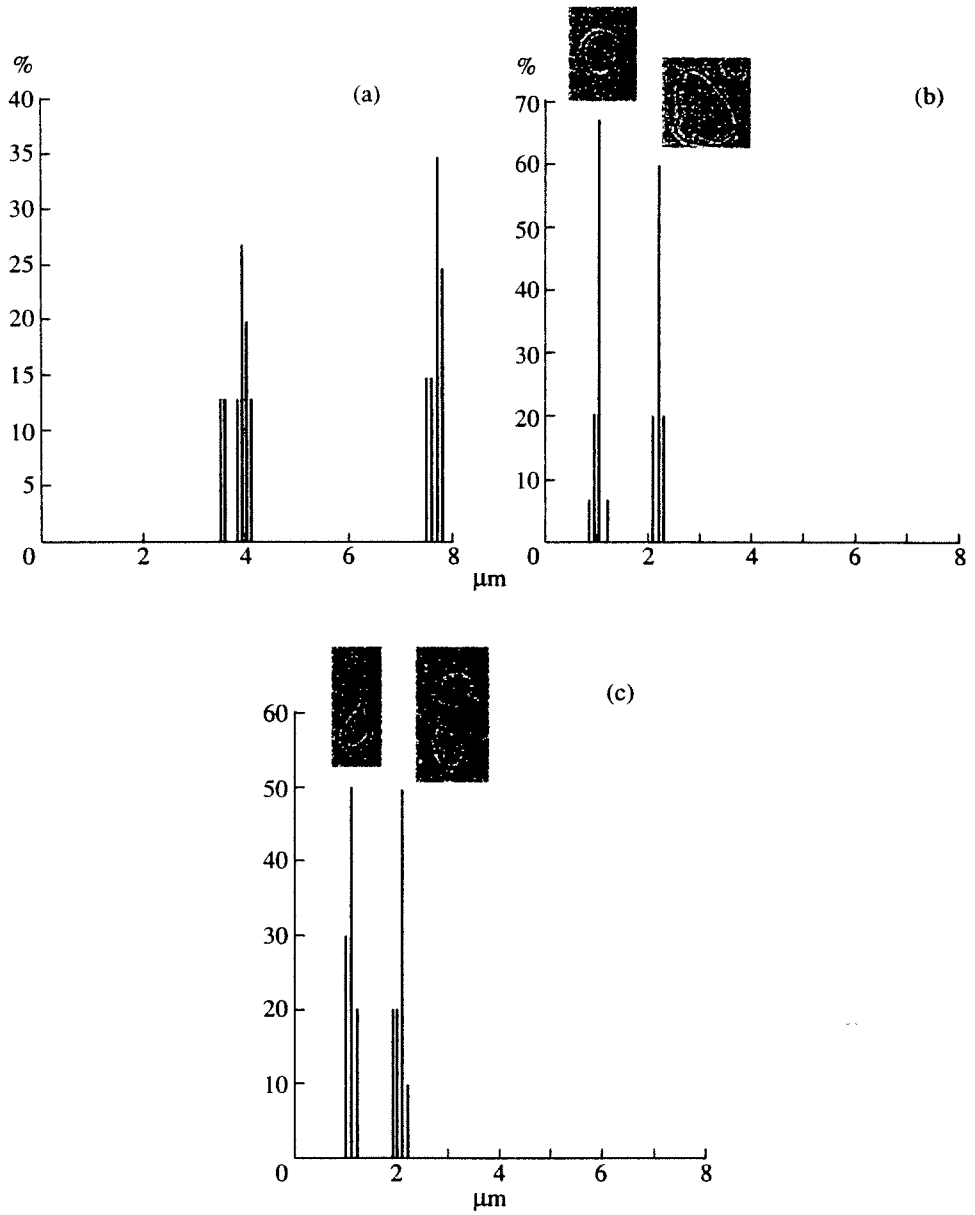

Fig. 4. Histograms of contour lengths for (a) uncompacted monomers and dimers of pRX10, (b) triple rings corresponding to DNA monomer and dimer complexes with TVDH, (c) compact structures with unusual (racket- or dumbbell-like) organization corresponding to monomer and dimer DNA complexes with TVDH.

quite flexible, and may produce morphologically similar compact fibers composed of three or two filaments of duplex DNA lying side by side.

A model of DNA packing in compact fibers formed by DNA complexes with synthetic oligonucleotide has been suggested in [7, 8], according to which peptides associate into a scaffold for DNA filaments to wind on. Obviously, if these filaments are wound closely enough, the thickness and general morphology of such a compact fiber will not be directly determined by the number of DNA filaments wound around the protein scaffold, i.e., the architecture of such compact fiber will be determined only by the protein component.

Moreover, one may conclude that long fragments of a circular DNA molecule, even if they do not have

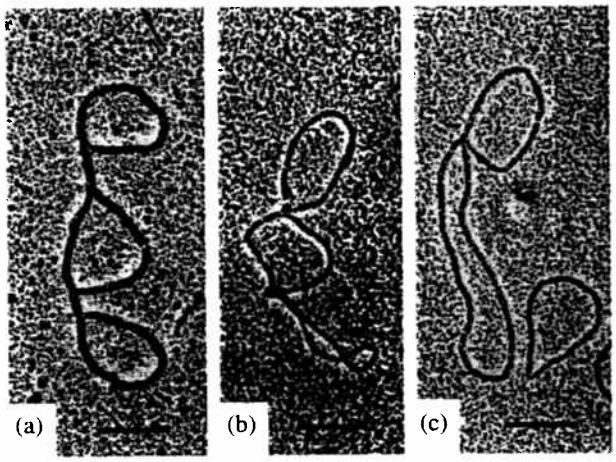

Fig. 5. Micrograph of compact structures of pRX10 trimer–TVDH complexes. Scale bar, 0.25 μm.

a peculiar primary and secondary structure, possess sufficient topological flexibility and may undergo compaction independently under the action of trivaline.

It appears that the "critical" size of such molecules is close to that of the pRX10 monomer. This is supported by the morphology of compact structures corresponding to pRX10 trimers, the micrographs of which are presented in Fig. 5. They never look like unfolded triple rings, but are made of three monomer rings, or of monomer and dimer rings connected by a stretch of compact fiber.

Examination of long circular pRX10–TVDH complexes demonstrates that when a certain DNA length is attained, even DNA molecules lacking specific nucleotide sequences possess a sufficient conformational flexibility allowing some parts of a circular molecule to behave independently in the process of compaction.

The data obtained in this study, taken together with the data of some previous publications, suggest a model of DNA packing in all the types of structures observed in these experiments. Such data may add to the general understanding of DNA–protein compaction *in vivo*, e.g., in the case of DNA–histone interactions. They may help in analyzing the possibilities of compaction/decompaction processes within a DNA domain (loop) of eukaryotic chromosome or analogous structures of prokaryotic genomes [9, 10].

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