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On the tertiary structure of the *Citrus ichangensis* satellite DNA

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Abstract

A hexamer of the repeating unit of *Citrus ichangensis* satellite DNA was cloned. Polyacrylamide gel electrophoresis demonstrated that the shape of cloned hexamer is other than linear. As the computing of tertiary coordinates made by Eckdahl and Anderson's BEN program proved, the hexamer is a solenoid consisting of two turns termed coiled double helix (CDH)-form. An electron microscopic analysis revealed small diameter circles in the hexamer under investigation. Unlike the hexamer control molecules are s-shaped. It is concluded that the CDH-form is a characteristic of the satellite DNA of *Citrus ichangensis*.

Key words: Satellite DNA; Tertiary structure of DNA; Coiled double helix; Electron microscopy; *Citrus ichangensis*

1. Introduction

Satellite DNAs are tandemly arranged, highly reiterated sequences [1]. If a bend appears in a monomer due to the regular repeating organization, the long chains ought to reveal a tertiary structure. Computer modelling has proved that such molecules have to be of a solenoid form termed coiled double helix (CDH-form) [2,3]. The solenoid parameters, i.e. diameter and number of base pairs per turn, will vary with respect to the length and quality of A-tracts and their arrangement along the monomer chain. It is implied that bends are observed in DNA molecules wherever A-tracts are present, which is soundly confirmed in numerous published reports [4,5].

In our earlier study, oligomers of the *Citrus ichangensis* (a wild species of citric plants belonging to the subgroup Papeda of the genus *Citrus*) satellite DNA (dimer and trimer) were proved to migrate more slowly in a polyacrylamide than in an agarose gel, indicating the presence of a curvature in the monomer. This was confirmed by computer analysis of satellite DNA monomer molecules (181 bp) using Eckdahl and Anderson's BEN program to calculate three-dimensional coordinates of the molecule's axis. According to the computing, the satellite DNA octamer forms two solenoid turns [3]. Similar observations can be made for mouse and African green monkey satellite DNAs.

In this study a *Citrus ichangensis* DNA hexamer was cloned. Polyacrylamide gel electrophoresis of the cloned hexamer showed the shape of the molecule is other than linear at low temperatures. Electron micrographs of the hexamer show good agreement with the computer model. The resulting data indicate that a coiled double-helix form is characteristic of *Citrus ichangensis* satellite DNA.

2. Materials and methods

In earlier paper [3] we described the isolation of satellite DNA from *Citrus ichangensis*, sequencing of the DNA, and generation of oligomers by partial cleavage of the satellite DNA using restriction endonuclease *Bsp*I. The same report described computer modelling of satellite DNA tertiary coordinates using Eckdahl and Anderson's BEN program [6].

2.1. *Cloning of* Citrus ichangensis *satellite DNA hexamer*

The products of partial cleavage (with *Hae*III or *Bsp*I) of satellite DNA were electrophoresed in 1.5% low-melting agarose. The hexamer band was cut out and DNA was isolated by standard procedures [7]. The hexamer was cloned in plasmid pBS (Stratagene) in the *Sma*I site. The insert was excised by restriction endonucleases *Eco*RI/*Bam*HI in the poly-linker site.

Polyacrylamide gel electrophoresis at different temperatures was performed in an LKB apparatus (16 × 18 × 0.2 cm) in 0.05 M Tris–borate buffer, pH 8.3. After electrophoresis the gel was placed into ethidium bromide solution (1 μg/ml) for 10 min and then photographed with a Chroma 43 transilluminator (Helling).

2.2. *Electron microscopy*

The cloned hexamer of the satellite DNA as well as control DNA were obtained by electrophoresis in low-melting agarose [7]. DNA preparations were subjected to overnight dialysis at 4°C against 10 mM Tris, 1 mM EDTA, pH 8.0, followed by gel-filtration through sepharose CL-2B. Elution was performed by 10 mM Tris, 1 mM EDTA, pH 8.0. The samples were diluted with a 1:30 buffer containing 5 mM triethanolamine, 0.2 mM EDTA. A drop of the solution was applied to

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carbon-coated copper nets with a Formvar substrate. Using uranyl acetate as a contrasting agent, the samples were further shadowed by the Pt–Pd alloy at 8°C with rotation. The preparations were analysed under an electron microscope JEM 100 C (Jeol, Japan).

3. Results

3.1. *Cloning the hexamer of* Citrus ichangensis *satellite DNA*

Hexamer of the repeating unit of *Citrus ichangensis* satellite DNA was cloned in plasmid pBS in the *Sma*I-site. The insert was 1.1 kb long (1,086 bp of the actual insert plus 20 bp of polylinker). The cleavage products of plasmid pBS, pBS with the hexamer insert and the satellite DNA of *Citrus ichangensis* using restriction endonuclease *Hae*III were compared with the intention of proving that the cloned sequence is a fragment of *Citrus ichangensis* satellite DNA. Plasmid with a hexamer insert produces bands characteristic of both pBS and satellite DNA. This is confirmed by the observation of low-molecular weight fragments of identical mobility. No fragment of this kind was produced by plasmid pBS.

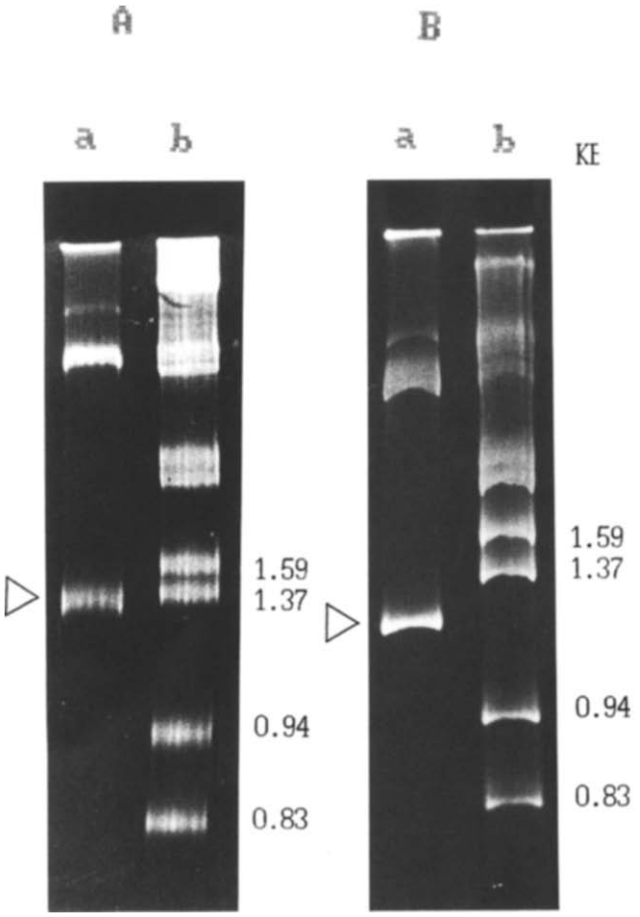

Fig. 1. Electrophoresis in 5% polyacrylamide gel of hexamer of *Citrus ichangensis* satellite DNA. Hexamer is marked by a triangle. (A) 4°C; (B) 60°C.
(a) Plasmid pBS with a hexamer insert cleaved by *Eco*RI/*Bam*HI.
(b) DNA size marker (*Eco*RI/*Hin*dIII digest of bacteriophage λ).

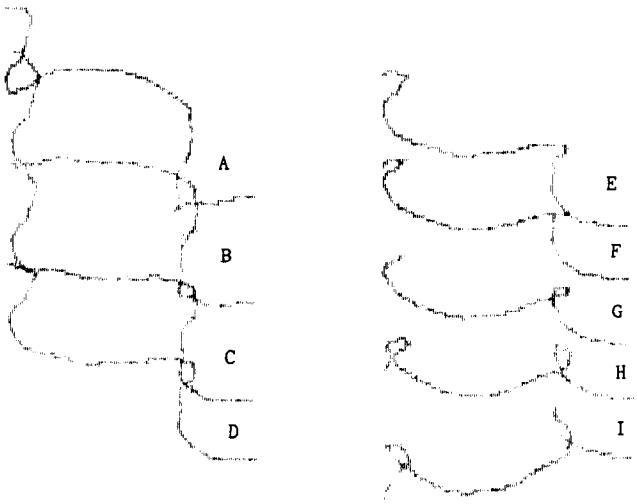

Fig. 2. A two-dimensional projection of *Citrus ichangensis* satellite DNA hexamer axis calculated by the BEN program. The projections were obtained by rotating the molecule axis by 18° (A–I).

3.2. *Mobility of hexamer of* Citrus ichangensis *satellite DNA in polyacrylamide gel*

As a curved DNA migrates normally in polyacrylamide gel at high temperatures [8], the difference in the mobility of the DNA fragment at a low temperature (4–10°C) (i.e. retarded migration) and at high temperature (60°C) can be considered as an evidence of a curvature in the DNA [8,9]. A comparative analysis of satellite DNA hexamer revealed that it migrates in a 5% polyacrylamide gel at 60°C as a 1.1 kb long molecule, which corresponds to its actual length and agrees with its mobility in the agarose gel. However, the hexamer migrates as a 1.3 kb molecule in the same gel at a lower temperature (4°C) (Fig. 1). Therefore, the K-factor, i.e. the apparent length divided by the sequence length, for the hexamer is 1.18 at 4°C.

3.3. *Computer modelling and electron microscopic analysis of hexamer of the* Citrus ichangensis *satellite DNA*

We computed the tertiary coordinates of hexamer of the *Citrus ichangensis* satellite DNA and made two-dimensional projections by Eckdahl and Anderson's BEN program, based on Ulanovsky–Trifonov's wedge model. The results obtained by this program confirmed best the experimental data [10]. The hexamer appears to be a solenoid made up from two turns (Fig. 2).

Hexamer of the satellite DNA was analyzed under an electron microscope (Fig. 3A). A 1.1 kb fragment of *Allium porrum* chloroplast DNA, containing genes of two tRNAs and an ATPase gene fragment, was examined as control (Fig. 3B). (The sequence of this fragment is included in the EMBL Data Library, Accession Number X66734). The primary structure of the fragment could be regarded as a random sequence. They were

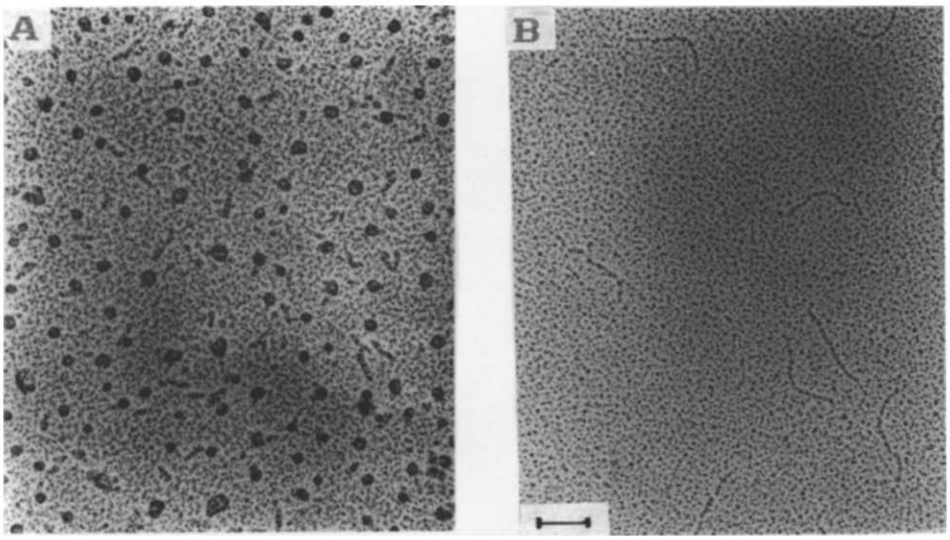

Fig. 3. Electron microscope visualization of *Citrus ichangensis* satellite DNA hexamer.
(A) The hexamer.
(B) 1.1 kb control DNA. The bar represents 0.2 μm.

S-shaped as they could be in the case of a DNA molecule with a random sequence. Unlike the control, the hexamer was discovered to be mainly in the form of small circles of differing diameters. Assuming the CDH-form of the hexamer, one could expect circular structures to be observed for the molecules adsorbed onto the surface, with their butt ends.

Fig. 3A demonstrates that the length of the hexamer molecules considerably exceeds (3 times) the perimeter of the observed rings. This can be achieved with only about two helix coils formed by the hexamer molecules, which was suggested by the respective computer data.

4. Discussion

Satellite DNAs are located in the constitutive heterochromatic regions of the eucaryotic chromosomes participating in the compaction of heterochromatin in a way still unknown to us. There is evidence indicating the presence of proteins specific for heterochromatin; nevertheless, it is clear that it is satellite DNA that causes the compaction of heterochromatin, since these proteins are connected with satellite DNA [11].

It was suggested that the compact state of the heterochromatin can be determined by a specific tertiary structure of satellite DNA [1–3]. A coiled double helix form presented in this paper can possibly be such a structure. It is actually typical of all the satellite DNAs whose monomers possess A-rich sites [3]. Ample data confirming this hypothesis can be found in the latest literature. Thus, Reinert et al. observed the temperature-dependent helix elongation component only for the eucaryotic DNA in solution and postulated a tertiary structure composed of solenoidal super-helix components [12,13]. It is very likely that these components are highly reiterated sequences. Benfante et al. found a sequence-directed curvature of oligomers of repetitive *Alu*I DNA of *Artemia franciscana* [14]. An electron microscopy study of the hexamer revealed small rings. The authors proposed that there were two overlapping circles suggesting a solenoid struture for *Artemia* repetitive DNA. There is also evidence indicative of bends in monomers of mouse, rat and green monkey satellite DNA [15].

Satellite DNA of *Citrus ichangensis* is a very suitable model for the study of tertiary structure. The primary structure of a monomer has four sites consisting of five adenine residues [3]. A curvature in the hexamer of satellite DNA can be observed as abnormally slow mobility at 4°C while at 60°C the molecule migrates in accordance with its actual length.

It was discussed earlier that the bend angle in DNA molecules due to termal fluctuation is close in size to a static bend [3]. This ought to give rise to an actual ensemble of structures in solution for satellite DNA oligomers. A strictly identical CDH-form for all of the analysed molecules is valid only at the temperature of absolute zero. It is therefore likely that the molecules analyzed by electron microscopy will be of the solenoidal form differing only in the diameter and, consequently, the helix length. We consider that the circular structures observed under the electron microscope confirm computer data and can be considered as sound proof of elements of a coiled double helix in the satellite DNA of *Citrus ichangensis*.

The existence of a tertiary structure being recognized, a new approach to the organization of satellite DNA-containing chromatin can be used. Whenever a hexamer

of the *Citrus ichangensis* satellite DNA is concerned, about two solenoid turns per six nucleosomes can be found. In what way is the static form of the coiled double helix related to DNA winding histone octamers? Does a solenoid determine nucleosome phasing? What is the role of a coiled double helix in heterochromatin compaction? All of these essential questions call for further studies.

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