საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, 175, №1, 2007
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, 175, №1, 2007

Biochemistry

Bends in Satellite DNA

Tengiz Beridze*, Ia Pipia**

* *Academy Member, S. Durmishidze Institute of Biochemistry and Biotechnology, Tbilisi*
** *S. Durmishidze Institute of Biochemistry and Biotechnology, Tbilisi*

ABSTRACT. The dependence of the mobility of three different stDNA oligomers (*Citrus limon, Poncirus trifoliata* and *Mus musculus*) on temperature in PAAG has been investigated. The dependence of mouse stDNA dimer's K-factor on temperature has chair-like form. First it decreases in 5-25ºC interval, reaches a plateau at the 25-35ºC interval, and finally decreases at 45ºC. The monomers of lemon and *P. trifoliata* are splitting into two components at 5ºC (181 and 186 bp). In the case of *P. trifoliata* they are presented in equal amount; in the case of lemon – the fast moving component is less than half. The same process occurs in the case of mouse stDNA monomer at 45-55ºC (234 and 240 bp). The amount of retarded component is about 10%. It was shown for the first time that in certain conditions stDNA molecules in solution may exist simultaneously in two - bent and straight forms. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *satellite DNA, DNA bending, electrophoresis, mouse, lemon, Poncirus trifoliata.*

Satellite DNAs (stDNA) represent tandemly arranged repeating sequences of nuclear DNA. This fraction often gives a separate peak in the CsCl density gradient [1,2]. It was shown by *in situ* hybridization that satellite sequences are located in the centromeric heterochromatin of chromosomes. StDNAs of different organisms differ by the length and GC-content of repeating sequences. They are not expressed by forming of RNA and protein.

Repeating units (monomers) of stDNA usually contain bends, which are provoked by the long adenine stretches in molecules [2]. The first experimental proof of anomalous gel migration of bent stDNA was obtained for 490 bp long DNA fragment of *Leishmania tarentolae* [3]. This DNA fragment migrated anomalously slowly in polyacrylamide (PAAG) gel at low temperature. In agarose gel this DNA-fragment migrated according to its actual length. The treatment of mammalian cells by AT-specific drugs (Hoechst 33258 or Distamycin A), which straightens the curvature, causes the decondensation of centromeric heterochromatin [4]. At the increasing of PAAG temperature up to 60ºC the bent molecules migrate according to their actual length [5]. Anomalous migration is characteristic of several animal stDNA [6]. It was demonstrated that 16 AT-rich centromeric DNA of yeast (*Saccharomyces cerevisiae*) also display retarded electrophoretic mobility [7]. The bends were observed also in GC-rich stDNA oligomers of *Citrus* species [8-10].

The presence of the bends in monomers of stDNA support the formation of solenoid-like tertiary structure in long stDNA molecules [11-13]. This structure was termed as coiled double helix - CDH-form [8]. It was assumed that CDH-form provides the basic function of stDNA - condensation of constitutive heterochromatin [1,8,9].

The aim of the present study was to determine the character of bends in mouse (*Mus musculus*) AT-rich and *Citrinae* plant (*Poncirus trifoliata, Citrus limon*) GC-rich stDNA. The comparison of the migration anomaly as a function of temperature was carried out.

Mouse stDNA is one of the most thoroughly studied molecule. It represents 9% of the nuclear genome. 234 bp long monomer consists of 12 A-rich tracts (n=5-6). The GC-content of these molecules is 36% [14].

Citrinae plants are also very suitable models for study of stDNA. In these plants stDNA represent approximately 20% of the genome. Most monomers are 181 bp long and contain five A-rich tracts. GC-content of these DNA molecules varies between 60-68%, which is significantly higher than the average GC-content of the *Citrinae* genome [10].

DNA isolation and restriction analysis. The isolation of nuclear DNA from mouse liver and young leaves of *Citrinae* plants was performed according to Beridze [15]. Liver and leaves were homogenized in 0.5 M sucrose, 0.005 M EDTA, 0.05 M Tris-HCl pH 8.0. Homogenate was centrifuged at 1000g for 20 min to yield nuclei. The nuclear pellet was dissolved in 0.15 M NaCl, 0.1 M EDTA, 0.2% SDS (Sodium dodecyl sulphate), pH 8.0, incubated at 60ºC 10 min. Further procedure of DNA isolation was described in detail earlier [15].

To obtain stDNA oligomers DNA samples were partially hydrolyzed by restriction endonucleases. Mouse liver nuclear DNA was hydrolyzed by *Sau96 I* restriction endonuclease (BioLabs). 10 units of enzyme were added to 5ìg DNA and incubated at 37ºC for 30 min. *Citrinae* plant's nuclear DNA was partially hydrolyzed by *Sty I* restriction endonuclease (Promega). 1.6 units of enzyme were added to 5 ìg DNA and incubated at 37ºC for 30 min.

StDNA analysis on agarose and polyacrylamide (PAAG) gels. Agarose gel-electrophoresis was carried out on a horizontal plate (10 x 20 x 0.3 sm) of 2% agarose at room temperature in standard Tris-acetate buffer, pH 8.3 [16]. Gels were stained by 1 ì g/ml of ethidium bromide solution for 10 min and photographed with Chroma 43 transilluminator (Helling).

5% PAAG electrophoresis was performed in a LKB apparatus at different temperature in a Tris-borate buffer, pH 8.3 at 135 V. The duration of electrophoresis varied according to the gel temperature.

The molecular weight of DNA fragments was determined according to Southern [17]. The deviation from standard mobility - K-factor was determined as the ratio of apparent length to the actual length. The value of K-factor for normally migrating fragments is one; for retarded fragments - more than one. The experimental error of K-factor determination was ±0.01.

Computation of stDNA tertiary structure. Consensus sequences of lemon and *P. trifoliata* stDNA monomers were calculated by averaging the sequences of each of 11 monomer clones [10]. The computation of two-dimensional projection of mouse and citric plant stDNA tertiary structure was performed by computer program "DNACurve" [18].

Results and Discussion

For the identification of stDNA oligomers the digests of mouse and *Citrinae* plant DNA were subjected to electrophoresis in 2% agarose gel at room temperature.

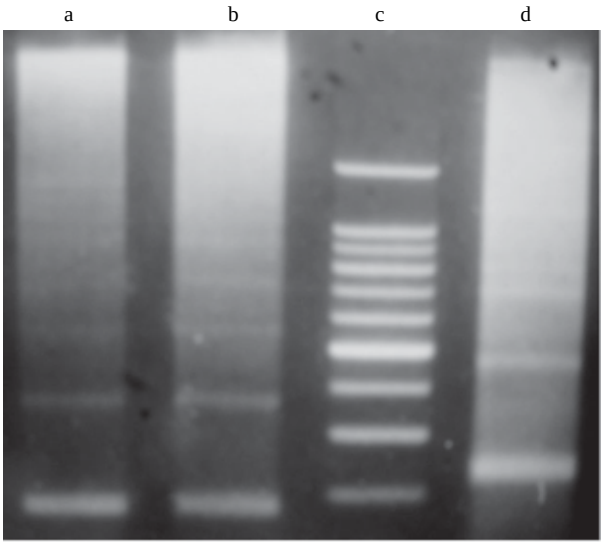

Fig.1. Electrophoresis of mouse, lemon and *P. trifoliata* stDNA oligomers in 2% agarose gel. a - *P. trifoliata***;** b - lemon; c - 100 bp DNA ladder (Promega); the 500 bp band is present at triple intensity; d - mouse.

Fig.1 represents the distribution patterns of satellite DNA oligomers in the agarose gel.

Determination of DNA fragments (oligomers) length and its K-factor value shows that each DNA fragment migrates in agarose gel in accordance to its actual length. In particular, the monomers of lemon and *P. trifoliata* migrate as 182 bp long molecule (K=1.0) and the dimers as 363 bp (K=1.0). The length of mouse monomer was detected as 237 bp long fragment (K=1.01) and the length of dimer - 470 bp (K=1.01).

The mobility of mouse stDNA monomer (234 bp) and dimer (468 bp) in a 5% PAAG at different temperature (5-55ºC) was investigated. Fig. 2 represents the migration pattern of mouse stDNA monomer at 5º and 45ºC in a 5% PAAG where the obvious retardation of the monomer at 5ºC in comparison to 45ºC is observed.

Dependence of K-factor on temperature has a chair-like form. Fig. 3 shows that K-factor's value decreases in the 5º-25ºC temperature interval, remains almost unchanged in the interval of 25º-35ºC and decreases to the final value of 1.02 at 45ºC.

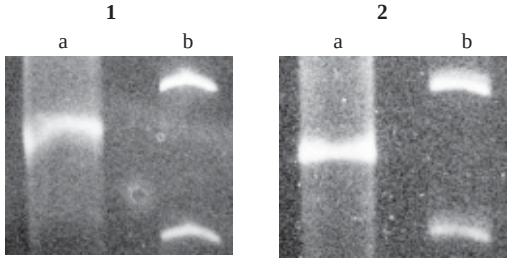

Fig. 2. Electrophoresis of mouse stDNA monomer in 5% PAAG. 1 - 5ºC; 2 - 45ºC. a – mouse stDNA monomer; b - 100 bp DNA ladder (Promega).

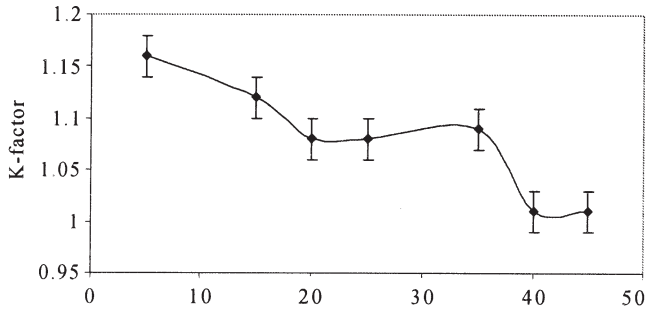

Fig. 3. The temperature dependence of K-factor's value of mouse stDNA dimer.

The temperature dependence of K-factor of mouse stDNA monomer practically coincides with dimer curves.

During electrophoresis of mouse stDNA oligomers intermediate thin lines (retarded) above each of the main DNA oligomer ladder were observed at elevated temperature (above 45°C). The amount of the retarded component is approximately 10% of the main component.

For a detailed examination of this phenomenon the study of mobility at 2°C temperature increment intervals from 45° to 55 °C was carried out. It was observed that at the temperature interval of 45° - 55°C mouse stDNA monomer splits into two components. At 45°C the distance between the monomer's thin (retarded) and thick lines are increased with elevation of temperature by each centigrade. The maximal distance between them was observed at 55°C. At 45°C the fast moving component (thick line) migrated as a 245 bp long molecule (K=1.05). The retarded component (thin line) migrated as a 256bp long molecule and its K=1.09. At 55°C the K-factors of fast moving and retarded fragments is 1.02 and 1.08 respectively - (Fig. 4).

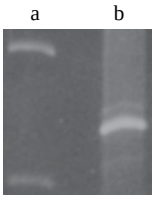

Fig. 4. The monomers of mouse stDNA in 5% PAAG at 55°C. a - 100 bp DNA ladder (Promega), b – mouse stDNA dimer.

It can be proposed that at elevated temperatures oligomers of mouse stDNA may exist simultaneously in two - straight and bent (retarded) - forms.

Like the mouse stDNA the anomalous gel migration was also detected for the lemon and *P. trifoliata* satellite sequences. But there are specific differences between them. In the case of both plants the monomers were splitted into two components at low temperature (5°C) (Table 1, Fig. 5). In the case of *P. trifoliata* they are represented in equal amount; in the case of lemon – the fast moving component is less than half. The length of retarded fragments of lemon and *P. trifoliata* are 184 and 186 bp respectively. The length of fast moving components in both plants is 181bp, which coincides with the monomers mobility in agarose gel. At an elevated temperature the retarded components disappear. The retarded component of lemon disappears at 25°C, and in the case of *P.trifoliata* - at 45°C. At this temperature the monomers are migrated as normal 181bp fragments.

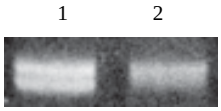

Fig. 5. The monomers of *P. trifoliata* (1) and lemon (2) stDNA in 5% PAAG at 5°C.

It can be proposed that satellite sequences of mouse and *Citrinae* plants have same properties and in some conditions in solution they simultaneously may exist in bent and straight forms. Principal differences between them is the following: the bent and straight forms of plant GC-rich monomers can be observed at low temperature (5°- 25°C for lemon; 5°- 45°C for *P.trifoliata*); the bent mouse AT-rich stDNA oligomers are more stable and their straightening begins only at elevated temperature (45°-55°C). At these temperatures the full straightening of mouse stDNA oligomers cannot be observed. This phenomenon can be explained by nucleotide sequence of the investigated molecules – the plant stDNA oligomers are less bent than mouse oligomers.

The dependence of stDNA oligomer's electrophoretic mobility on temperature can be explained by analysis of the tertiary structure of these molecules. Wheeler's computer program - "DNACurve", which is based on Ulanowski-Trifonov's wedge model [18,19], was used to construct two-dimensional projections of tertiary structure of mouse and *Citrinae* stDNA oligomers (Fig.7 and 8). The consensus sequence of stDNA monomer of lemon and *P. trifoliata* was received by averaging each of 11 clones sequenced by Fann et al [10] (Fig.6). The length of monomers is 181 bp. The stDNA monomer of lemon contains four adenine rich stretches. There are

Table 1

The length of stDNA monomers in 5% PAAG at different temperatures

Temperature	Mouse	*P. trifoliata*	Lemon
5°C	282	186/181	184/181
45°C	256/245	181	181
55°C	253/239	181	181

a

cttggtgggtggcgtgggcgaagttcgtccgcgggactcggaatggccccagacttggcgagcggcctcc

gtgtgcc78aaaaataggccacgggcacagccgcgccc107aaaaatcagcccccgaaggtcggggtcccaagac

cccccagcgg151aaaaatggcc161aaaaaacgggtgggctatagc

b

cttggtgggggggtggggcgaagttggtccgccggactcggaatggggcgcaactttgcgagcggcctcc

gtgtgcccgagggatgccccgggcacagccgcgccc107aaaaacgagcgcccgaaggcccccgtccc136aaaa

cccccccagcgg151aaaaagggcc160aaaacaggggggtgggctatagc

c

ggacctggaatatggcgag20aaaactg27aaaatcacgg37aaaatgag45aaatacacactttaggacgtg66aaata

tggcgag78aaaactg85aaaaaggtgg95aaaattag103aaatgtccactgtaggacgtggaatatggcaag136aaaac

tg143aaaatcatgg153aaaatgag161aaacatccacttgacgacttg182aaaaatgacg192aaatcact200aaaaaacgtg

aaaaatgag210aaa219atgcacactgaa

Fig. 6. Consensus sequences of lemon (a), *P. trifoliata* (b) and mouse (c) stDNA monomers.

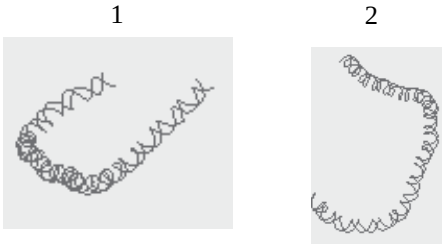

Fig. 7. Two-dimensional projection of *Citrinae* plant stDNA tertiary structure. 1 - Monomer of lemon stDNA. 2 - Monomer of *P. trifoliata* stDNA.

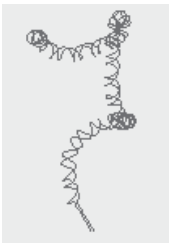

Fig. 8. Two-dimensional projection of mouse stDNA monomer tertiary structure.

five adenine residues at the position 78, 107 and 151 positions and six adenines at the position 161. The stDNA monomer of *P. trifoliata* contains four adenine-rich sites. There are four adenine residues at the position 136,151 and 160. The position 107 contains five adenines.

In Fig. 6 the 234 bp long sequences of mouse stDNA monomer are also presented [14]. This sequence contains six sites with three adenine residues (45, 66, 103, 161, 192, 219), 8 sites with four adenines (20, 27, 37, 78, 95, 136, 143, 153), three sites with five adenines (85, 182, 210) and one site with six adenines (position 200).

The chair-like curves of the temperature dependence of electrophoretic mobility of oligomers can be explained by analyzing the two-dimensional projections of their tertiary structure. In the case of all three organisms two essential bends in the monomer can be observed. It can be proposed that the increase of temperature up to 20°C partially straightens bent molecules (possibly one of the bend is straightening) and retardation in PAAG decreases. Further elevation of the temperature to 35°C does not change the molecular structure At 45°C the full straightening of molecules occurs and the molecules migrate in accordance with the actual length.

This is the first report of a simultaneous existence of stDNA oligomers in two forms - straight and bent. Conformational isomers of curved pBR222 restriction fragments by PAAG were described earlier [20].

ბიოქიმია

გადაღუნვები სატელიტურ დნმ-ში

თ. ბერიძე*, ი. ფიფია**

** აკადემიის წევრი, ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, თბილისი*
*** ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, თბილისი*

შესწავლილია სამი განსხვავებული სატელიტური დნმ-ის (*Citrus limon, Poncirus trifoliata* და *Mus musculus*) ოლიგომერების ძვრადობის დამოკიდებულება ტემპერატურაზე პოლიაკრილამიდის გელში. თაგვის სტ-დნმ-ის ოლიგომერების K-ფაქტორის ტემპერატურაზე დამოკიდებულების მრუდს აქვს სავარძლისებრი ფორმა. K-ფაქტორის სიდიდე მცირდება 5-25°C-ის ინტერვალში, შემდეგ აღინიშნება პლატო 25-35°C-ის ინტერვალში და შემდგომი შემცირება 45°C-ზე. ლიმონისა და *P. trifoliata*-ს სტ-დნმ-ის მონომერი 5°C-ზე იყოფა ორ კომპონენტად (181 და 186 ფწ). *P. trifoliata*-ს შემთხვევაში მათი რაოდენობა დაახლოებით თანაბარია, ხოლო ლიმონის შემთხვევაში სწრაფად მოძრავი კომპონენტის რაოდენობა ნახევარზე ნაკლებია. თაგვის სტ-დნმ-ის მონომერის ორ კომპონენტად დაყოფა (234 და 240 ფწ) ხდება 45°C-ზე. შეკავებული ძვრადობის მქონე კომპონენტის რაოდენობა ორივე კომპონენტის ჯამური რაოდენობის დაახლოებით 10%-ს შეადგენს. პირველად იქნა ნაჩვენები, რომ სტ-დნმ-ის მოლეკულები გარკვეულ პირობებში, ხსნარში ერთდროულად შეიძლება არსებობდნენ ორი — გადაღუნული და წრფივი ფორმით.

REFERENCES

1. *T. Beridze.* Satellite DNA, Springer Verlag. 1986.
2. *V. Hemleben, T.G. Beridze, L. Bachmann, A. Kovarik, R. Torres.* Russian Advances in Biochemistry, 43, 267-306, 2003.
3. *J.C. Marini, S.D. Levene, D.M. Crothers, P.T. Englund.* Proc. Nat. Acad. Sci. USA, 79, 7664-7668, 1982.
4. *M.Z. Radic, K. Lundgren, B. Hamkalo.* Cell, 50, 1101-1108, 1987.
5. *S. Dieckmann*. In: Nucleic Acids and Mol.Biology, (F.Eckstein, D.J.M.Lilley eds) Springer, 1, 138-156, 1987.
6. *D.J. Fitzgerald, G.L. Dryden, E.C. Bronson, J.S. Williams, J.N. Anderson.* J. Biol. Chem., 269, 21303-21314, 1994.
7. *T. Bechert, S. Heck, U. Fleig, S. Dieckmann, J.H. Hagemann.* Nucleic Acids Res., 27, 1444-1449, 1999.
8. *T. Beridze, N. Tsirekidze, M.A.Roytberg.* Biochemie, 74, 167-194, 1992.
9. *T. Beridze, N.I. Tsirekidze, M.S. Turishcheva*. FEBS Letters, 338, 179-182, 1994.
10. *J-Y. Fann, A. Kovarik, V. Hemleben, N.I. Tsirekidze, T.G. Beridze.* Theor. Appl. Genet., 103, 1068-1073, 2001.
11. *T.G. Beridze, M.S. Turishcheva, G.N. Kokonashvili, M.V. Masyukov.* Abstracts of 14th International Congress of Biochemistry, FR, 136, 1988.
12. *R. Benfante, N. Landsberger, G. Tubiello, G. Badaracco.* Nucleic Acids Res., 17, 8273-8282, 1979.
13. *M. Plohl, B. Borstnik, D. Ugarkovic, V. Gamulin.* Biochemie, 72, 665-670, 1990.
14. *W. Hörz, W. Altenburger.* Nucl. Acids Res., 9, 683-696, 1981.
15. *T. Beridze.* Plant Sci.Lett., 19, 325-338, 1980.
16. *T. Maniatis, E.F. Fritsch, J. Sambrook.* Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory. 160-162, 1982.
17. *E. Southern.* Anal. Biochem., 100, 319-323, 1979.
18. http://courses.mbl.edu/Medical_Informatics/2000/wheeler.htm
19. *L.E. Ulanovsky, E.N. Trifonov*. Nature, 326, 720-722, 1987.
20. *N.C. Stellwagen.* Electrophoresis, 21, 2327-2334, 2000.

Received February, 2007