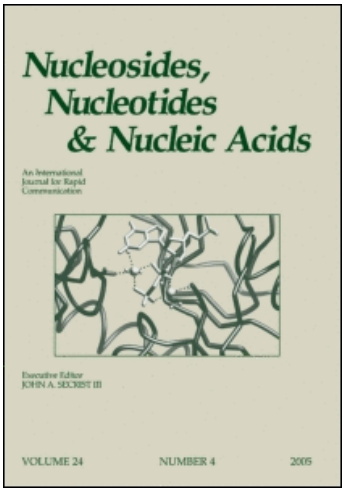

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STRUCTURAL PROPERTY OF DNA THAT MIGRATES FASTER IN GEL ELECTROPHORESIS, AS DEDUCED BY CD SPECTROSCOPY

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□ *Bent DNAs are known to migrate slower than ordinary DNA in non-denaturing polyacrylamide gel electrophoresis. In contrast, several satellite DNAs have been shown to migrate fast. The structural property that causes the fast migration, however, is not clarified so far on molecular basis. We have investigated the structural property of a satellite DNA, which contains consecutive purine sequences and migrates faster in gel, by CD spectroscopy. Partial formation of an A-form–like structure has been suggested. Reduction in DNA length due to the formation of the A-form–like structure may be responsible for the fast migration. The pronounced rigidity of DNA may also contribute to the behavior.*

Keywords Bent DNA; CD; Satellite DNA; A-Form

INTRODUCTION

Eukaryotic genomes contain tandemly repeated DNA sequences known as satellites.[1,2] The satellites cover a few percent to >50% of mammalian

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This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

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genomes.[2] Satellites are usually associated with regions of constitutive heterochromatin. Intrinsically bent structure has been found for many satellite DNAs and has been suggested to function in folding of DNA into chromosomes.[3–8] DNA curvature may aid tight winding of DNA in constitutive heterochromatin.

It is known that bent DNA migrates slower than ordinary DNA in nondenaturing polyacrylamide gel electrophoresis. In contrast, some satellite DNAs of box turtle, Komodo dragon, vulture, and cow migrate fast.[9] Fast migration is reported for DNA fragments from yeast centromeres[10] and those found in a study of illegitimate chromosomal recombination in mammalian cells.[11] Bovine satellite I DNA localizes in the centromeres of all autosomes.[12] Predominance (>80%) of purine–purine (or pyrimidine–pyrimidine) sequence over purine-pyrimidine (or pyrimidine–purine) sequence throughout the entire satellite I was noted.[13] Furthermore, it contains many runs of three or more consecutive purine (or pyrimidine) residues. It is found that one half of bovine satellite I DNA, an F fragment (704 bps), migrates faster, while the other half, an N fragment (710 bps), migrates normally.[13] The phenomenon of the fast migration of the F fragment is pronounced at lower temperature and the phenomenon disappears completely above 50°C.[13] Although it is well established that bent DNA structure causes slow migration, the structural property that causes fast migration is not clarified on molecular basis. The elucidation of the structural property will give us a clue to understand biological roles of DNA fragments that exhibit faster migration.

Here, we have studied structural properties of F and N fragments, together with another non-related fragment with a similar size (692 bps), by CD spectroscopy. It is suggested that the F fragment partially forms an A-form–like structure. The length of an A-form is known to be shorter than that of a B-form. Thus, the reduction in DNA length due to the partial formation of the A-form–like structure may account for the fast migration of the F fragment.

MATERIALS AND METHODS

Preparation of DNA Fragments

The F and N fragments of bovine satellite I DNA were prepared as described previously.[13] Briefly, the construct carrying the F fragment and that carrying the N fragment were digested with *Pst*I. Each digest was extracted with phenol, precipitated with ethanol, rinsed with 70% ethanol, and dried. A control fragment (692 bps), which is not related to the bovine satellite I DNA was obtained through digestion of a plasmid pUC19 with *Dra*I, and prepared in a similar way.

CD Analysis

Each fragment was dissolved in the solution containing 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA. The concentration of each fragment was 0.66 μM. CD spectra and thermal CD melting curves of each fragment were recorded with a Jasco J-720 spectropolarimeter. For melting, the temperature of the solution was raised from 5′ to 95°C at the rate of 1°C/min. The melting temperature was determined by the use of derivative of the melting curve.

RESULTS AND DISCUSSION

Characteristics of Nucleotide Sequences of F, N, and Control Fragments

Figure 1 shows nucleotide sequences of F, N, and control fragments. Predominance of purine–purine (or pyrimidine–pyrimidine) sequence over purine–pyrimidine (or pyrimidine–purine) sequence is seen for the F and N fragments.[13] Furthermore, these fragments contain many runs of three or more consecutive purine (or pyrimidine) residues, as revealed by shading in Figure 1. This characteristic is less pronounced for the control fragment.

Temperature-Dependent Spectral Change of the F Fragment Related to the Dissolution of a Certain Structure Responsible for the Fast Migration

It was reported that the F fragment migrates fast in non-denaturing polyacrylamide gel at low temperature.[13] Relative size, R_s, defined as (apparent size)/(actual size) was ca. 0.9 at 5°C. R_s gradually approached 1.0 when temperature was raised, and reached 1.0 at 50°C. Thus, the phenomenon of fast migration of the F fragment disappears at 50°C. In contrast, both N and control fragments migrate normally at any temperature between 5° and 50°C, an R_s value being ca. 1.0.[13] Therefore, it is expected that the difference in temperature dependency of mobility among three fragments may be reflected on their CD spectra.

Figures 2A–2C show CD spectra of each fragment at 5–80°C. For the F fragment, a gradual change of the CD spectrum was observed up to 50 (or 65)°C. When temperature was further raised to 80°C, the CD spectrum changed drastically, which implied melting of the F fragment duplex into single strands. In fact, the melting temperature of the F fragment was determined to be 73.4°C (Table 1). The spectral change up to 50 (or 65)°C, in turn, is supposed to reflect a structural change of the F fragment in the duplex state. A rate of the spectral change between 5 and 50°C $([\theta]_{5°C}-[\theta]_{50°C})/[\theta]_{50°C} * 100$ was 15% for the F fragment, as monitored at 265 nm. It is supposed that the gradual spectral change of the F fragment in the 5–50°C

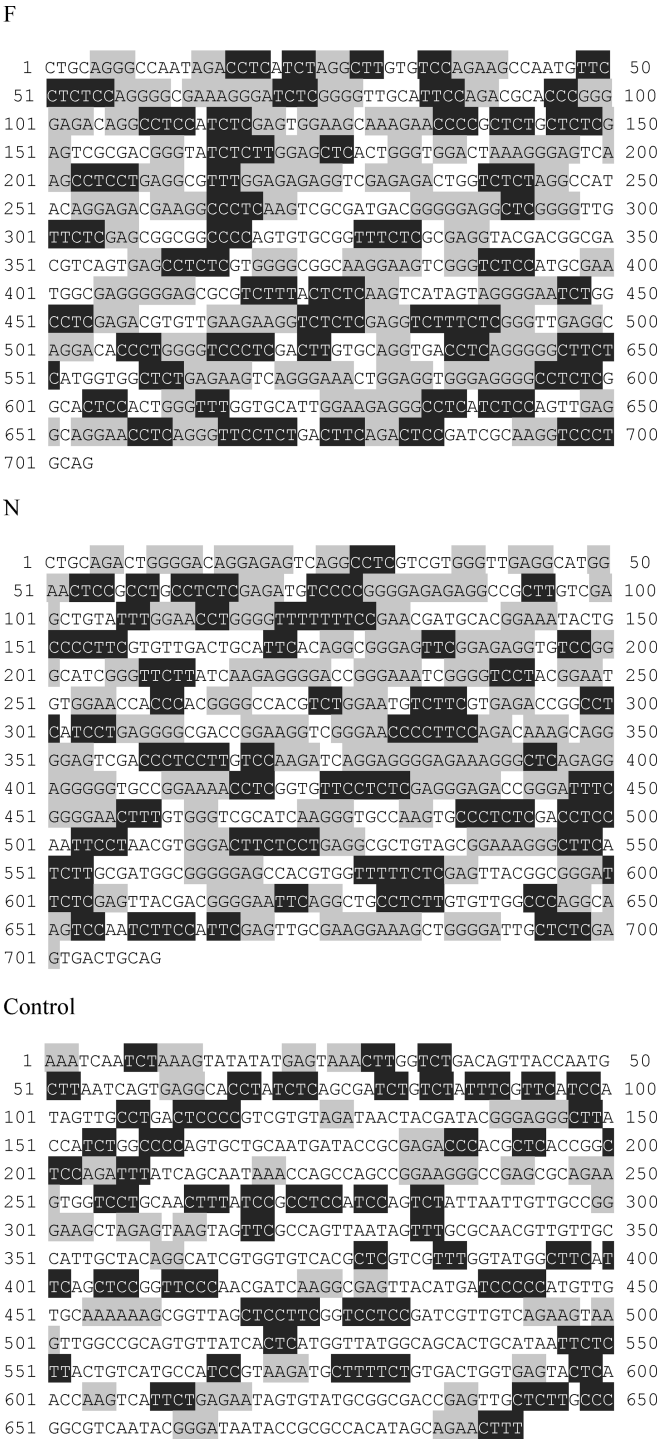

FIGURE 1 Nucleotide sequences of F, N, and control fragments. Runs of three or more consecutive purine or pyrimidine residues are shaded with gray and black, respectively.

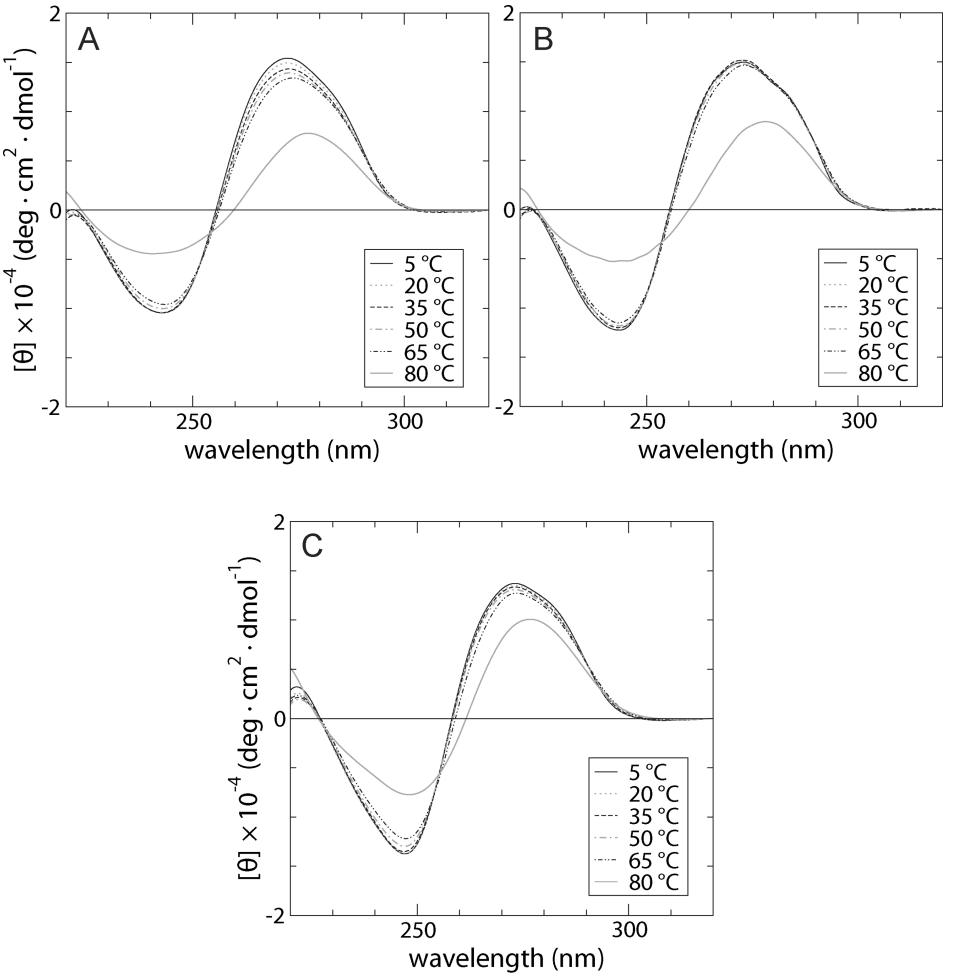

FIGURE 2 CD spectra of the F fragment (A), the N fragment (B), and the control fragment (C).

range correlates with the gradual disappearance of the phenomenon of the fast migration in the same temperature range. Temperature-dependent spectral change may reflect the disappearance of a certain structure that is responsible for the fast migration.

For the N fragment, in contrast, a spectral change is quite limited up to 50°C. The rate of the spectral change between 5 and 50°C is only 2%. At 80°C, a drastic change of the spectrum corresponding to melting of the N fragment duplex was observed, as observed for the F fragment. The melting temperature of the N fragment was determined to be 70.6°C. A very limited change of the spectrum up to 50°C suggests that the N fragment undergoes almost no structural change up to this temperature. A spectral change was comparatively modest also for the control fragment up to 50°C, the rate of the spectral change between 5 and 50°C being 6%. The melting

TABLE 1 Summary of Properties of the F, N, and Control Fragments

	F fragment	N fragment	Control fragment
Origin	One half of bovine satellite I DNA	The other half of bovine satellite I DNA	pUC19 plasmid
Size (bps)	704	710	692
Mobility in gel			
at 5°C	Fast	Normal	Normal
at 50°C	Normal	Normal	Normal
Melting temperature (°C)	73.4	70.6	65.7
Rate of CD spectral change between 5 and 50°C (%)	15	2	6

temperature of the control fragment was determined to be 65.7°C. The modest spectral changes suggesting modest structural changes, if any, of N and control fragments in the 5–50°C range are consistent with the observation that these fragments exhibited an almost constant R_s value of 1.0 in this temperature range.

Partial Formation of an A-Form–Like Structure for the F Fragment at Low Temperature

For the temperature range of 5 to 50°C, F, N, and control fragments all gave CD spectra basically characteristic of B-form, a positive peak around at 275 nm and a negative peak at around 245 nm with the comparable intensity.[14–16] This indicates that not only the N and control fragments, but also the F fragment mainly takes on B-form, even at 5°C. It is supposed that the population of a certain structure that is responsible for fast migration is small even at 5°C, although the phenomenon of the fast migration of the F fragment is pronounced at this temperature. In order to extract the CD spectrum corresponding to the structure responsible for the fast migration, a difference spectrum was obtained by subtracting the CD spectrum at 50°C from that at 5°C (Figure 3). It should be remembered that the phenomenon of the fast migration of the F fragment becomes less pronounced at higher temperature and disappears at 50°C. The difference spectrum is different from the spectrum of a canonical B-form. The difference spectrum gives a positive peak around at 265 nm and a comparatively rather weak negative peak around at 240 nm. These features are characteristic of the spectrum of an A-form.[14–16] Thus, it is suggested that the F fragment may partially take on an A-form–like structure at low temperature and that this structure is responsible for the fast migration in gel. Partial formation of the A-form–like structure for the F fragment which contains many runs of three or more consecutive purine (or pyrimidine) residues is consistent with the claim that runs of consecutive purine sequence have a tendency to set up an A-form–like structure.[17,18]

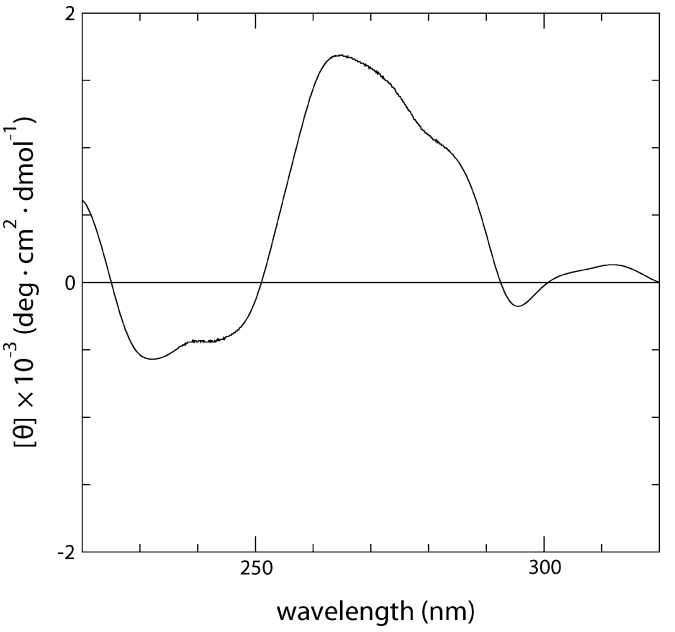

FIGURE 3 A CD difference spectrum for the F fragment between 5 and 50°C.

On the addition of 10 mM of Mg^{2+}, R_s value of the F fragment changed from 0.9 to ca. 1.0.[13] We have tried to examine the effect of Mg^{2+} on the CD spectrum of the F fragment. Under the CD conditions, however, the addition of Mg^{2+} caused some kind of precipitation and qualitative CD analysis was not possible. Higher DNA concentration applied for the CD experiment, compared to that applied for the gel electrophoresis experiment, may be responsible for the precipitation, although the exact reason of the precipitation is not clear.

Possible Explanation on the Fast Migration of the F Fragment from the Structural Viewpoint

The length of A-form DNA is ca. 30% shorter than that of B-form DNA with the same base pairs.[19] Thus, reduced length of the F fragment DNA due to the partial formation of the A-form–like structure may account for the fast migration in gel. It should be noted that a complete switch to the A-form–like structure is not required to explain the observed R_s value of 0.9. Partial formation of the A-form–like structure is enough to achieve the reduction of the length to 90% of ordinary B-form DNA. This is qualitatively consistent with the result that the spectral change of the F fragment between 5 and 50°C is not so drastic.

The F fragment contains many runs of three or more consecutive purine (or pyrimidine) residues, as described above. It was proposed that

purine–purine stacking interactions provide most of the mechanical rigidity of the DNA helix.[20] A stiff and rod-like molecule may exhibit minimal frictional drag as it passes through tight pores of polyacrylamide gel. The rigidity or the narrowness of allowed conformational space of the F fragment might also contribute to the fast migration.

Interpretation of the Mobility of the N Fragment

It was found that a part of the N fragment forms bent structure that causes slow migration.[13] The center of the curvature locates approximately between positions 600 and 610 of the N fragment. It was suggested that periodically spaced $(dT)_{2-5}$ tracts between positions 570 and 640 are responsible for the formation of the bent structure. In spite of the existence of the bent structure at a part of the N fragment, however, the N fragment migrates normally, R_s being 1.0 at any temperature. In order to interpret this phenomenon, it was proposed that like the F fragment, the N fragment containing many runs of three or more consecutive purine (or pyrimidine) residues also possesses the A-form–like structural property which causes fast migration and that the tendency of this fast migration cancels the tendency of the slow migration caused by the bent structure.[13] The CD spectral change for the N fragment between 5 and 50°C was very limited compared to those for the F and control fragments. The result may imply that both of the bent structure and the A-form–like structure remain in this range of temperature, which would result in the continuation of the cancellation. This may account for the observation that R_s of the N fragment is kept at 1.0 in this range of temperature, although further research is needed to clarify the behavior of the N fragment.

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