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The SV40 termination region exhibits an altered helical DNA conformation

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ABSTRACT

The DNA structure of a fragment containing the SV40 termination sequences was examined using gel mobility assays. The region is shown to contain a DNA bend as evidenced by an abnormal mobility that is progressively accentuated as the temperature is lowered. This represents the strongest example of DNA bending among the collection of SV40 fragments studied. The same fragment was shown previously to uniquely support hyper-stable nucleosome formation in vitro, suggesting a possible relationship between DNA bending and nucleosome stability.

INTRODUCTION

Crystallographic studies of a nucleosome core particle have shown that the path of the DNA around the protein core is not smooth but is bent or even kinked at several locations (1). This has prompted the speculation that, within the nucleosome, certain short DNA sequences might preferentially conform to regions of sharp bends in the DNA while others might be excluded because of their lack of flexibility. It has been known for some time that DNA sequence can indeed influence nucleosome positioning (2). A detailed study of the relationship between DNA bending and nucleosome positioning has lent support to the idea that the exact positioning of DNA about the histone octamer depends largely on the bending preferences of the DNA (3). Thus, nucleosome-length DNA pieces that contain appropriately-placed bending sequences might support nucleosome formation more readily than DNA pieces lacking such sites.

Recently, we reported that, out of a mixture of SV40 restriction fragments, a 237 bp fragment containing the replication "termination" region of SV40 uniquely exhibited an enhanced ability to form nucleosomes in vitro (4). The nucleosomes formed by this fragment are also exceptionally stable to challenge by the destabilizing reagent heparin. When compared to the other SV40 fragments, the 237 bp fragment also exhibits anomalous behavior on

polyacrylamide gels, for example migrating more slowly than a 240 bp fragment. This behavior may imply that DNA sequences contained in the 237 bp fragment are capable of assuming an altered helical DNA conformation (5).

Of the mixture of SV40 fragments examined, the 237 bp fragment was the only one to display both of these unique properties, that is, forming hyperstable nucleosomes and exhibiting anomalous gel mobility. Since gel mobility can sometimes be related to DNA structure, we decided to investigate this phenomenon more thoroughly. Several reports have suggested that temperature may affect the extent of helical DNA bending exhibited by certain highly bent DNA sequences (6-8). Direct physical evidence linking the extent of helical bending to temperature has also been obtained recently (9,10). We have therefore analyzed the relative gel mobilities of the 237 bp fragment as a function of temperature. We find that the electrophoretic anomaly of the 237 bp fragment is exaggerated as the temperature is lowered, a feature which is characteristic of bent helical structures (6-8). Preliminary experiments are also consistent with the localization of the bending locus to sequences near a stretch of seven A residues.

MATERIALS AND METHODS

Preparation of DNA. Form I SV40 DNA was restricted with HindIII, KpnI, and MboI and end-labeled with -[^{32}P]-ATP as described previously (4). The end-labeled 237 bp MboI fragment was eluted from an 8% polyacrylamide gel. The purified 237 bp fragment was restricted with SfaNI or HpaI (New England Biolabs) in the presence of 50 µg/mL carrier DNA. After restriction digestion, the DNA samples were ethanol precipitated prior to gel electrophoresis.

Gel Electrophoresis. The DNA samples were electrophoresed on 0.6mm thick, 8% polyacrylamide gels using TBE buffer (11). Electrophoresis was continued for several hours at 2 watts, until the xylene cyanol tracking dye had migrated 20 cms into the gel. The polyacrylamide gels were dried onto Whatman 3MM paper and exposed to Cronex 4 x-ray film (DuPont). The temperature was varied during electrophoresis by placing the gels either in a cold-room or in a thermostat-controlled incubator.

In order to extrapolate the apparent length of each fragment from its mobility on polyacrylamide gels, semi-log plots of fragment length versus distance of migration were prepared from duplicate gels. Calibration curves were drawn using a least-squares fit, and the apparent lengths of fragments that fell within the linear portion of these curves (78-366 bps) were calculated. The accuracy of these calibration curves was confirmed by electrophoresis of Bst NI restricted SV40 fragments on 8% polyacrylamide gels.

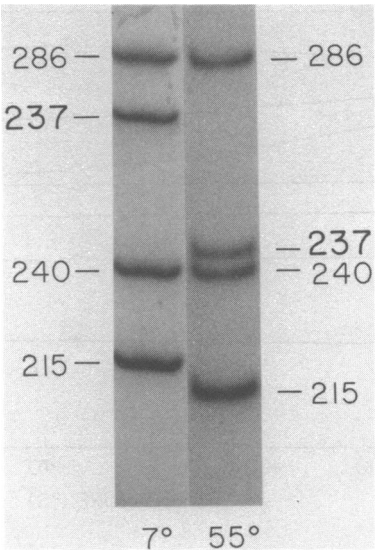

Figure 1. Relative gel mobilities of the 237 bp SV40 fragment at 7°C and 55°C. A mixture of end-labeled KpnI, HindIII, and MboI-restricted SV40 fragments was electrophoresed on 8% polyacrylamide gels at 7°C and 55°C. Electrophoresis at 55°C was allowed to continue until the separation between the 240 and 286 bp fragments was comparable to that obtained at 7°C.

RESULTS

In previous nucleosome reconstitution experiments, we separated restriction fragments from a combined KpnI, HindIII, and MboI digestion of SV40 DNA on 8% polyacrylamide gels (4). There were several anomalies in the relative migration patterns of the SV40 fragments. The most notable among them was the behavior of a 237 bp fragment and a 430 bp fragment, both of which migrated more slowly than would be expected based on their lengths. Figure 1 shows that, on an 8% polyacrylamide gel, the 237 bp fragment migrates much more slowly than a 240 bp fragment when the gel is run at 7°C. When the same gel is run at 55°C, the 237 bp fragment now migrates just behind the 240 bp fragment. Obviously, the anomalous migration of the 237 bp fragment is significantly influenced by temperature. The analysis of this fragment's relative mobility is complicated somewhat by the fact that another nearby fragment of 286 bp length also exhibits slightly anomalous behavior. Thus, at 7°C, the 237 bp fragment migrates with an apparent length of about 300 bps. However, it appears to run below the 286 bp fragment only because the 286 bp fragment migrates with an apparent length of about 310 at 7°C.

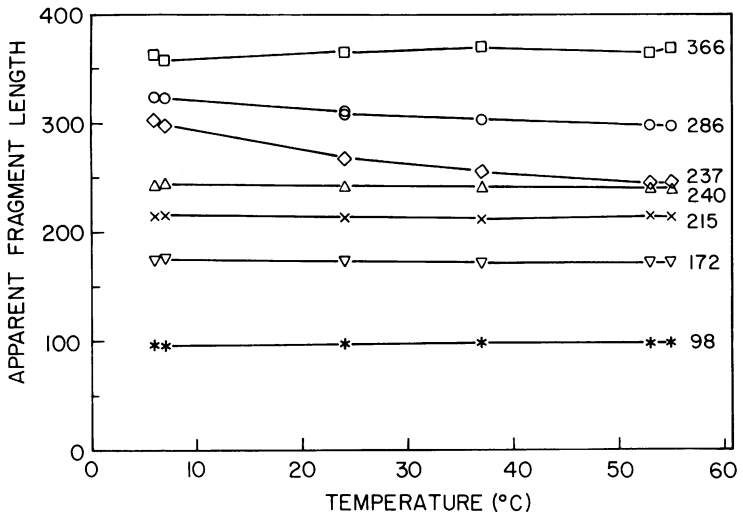

Figure 2. The effect of temperature on the apparent length of the 98 to 366 bp SV40 fragments. The end-labeled SV40 fragments were electrophoresed on 8% polyacrylamide gels at 6°C, 7°C, 24°C, 37°C, 53°C, and 55°C. The apparent length of each fragment was determined from least-squares calibration curves. The actual length of each fragment, in bps, is indicated to the right of each line.

In order to analyze quantitatively the migration of the collection of fragments, a series of gels were run at different temperatures. Semi-log plots of each fragment's relative gel mobilities were carefully calibrated using a least-squares fit. Since the longer fragments were not as well resolved on gels run at the lower temperatures, only those fragments in the size range of 78 to 366 bps were included in this analysis. The apparent length of each fragment was then extrapolated from the least-squares calibration plots. In order to confirm the calibrations of these gels, alternative sets of restriction digestions were also used. Data was included from gels run at six different temperatures ranging from 6°C to 55°C.

Figure 2 shows a plot of apparent fragment length as a function of temperature for a series of SV40 fragments. Most of the fragments migrate in the manner expected of fragments of these lengths. The apparent lengths of these fragments are also unaltered at temperatures between 6°C and 55°C. The 237 bp fragment clearly deviates from this behavior. At 6°C, it migrates with an apparent length of 300 bps. As the temperature is increased, its apparent length gradually decreases, reaching a value of 245 bps at 55°C.

TABLE I
Ratios of apparent lengths at 7°C to actual lengths for a series of SV40 restriction fragments

Fragment Length (bps)	$R_L^{7°}$
366	0.98
286	1.13
240	1.02
237	1.27
215	1.00
172	1.02
98	0.98

The 286 bp fragment experiences a much more modest decline in apparent length with increasing temperature, reaching a value of 297 bps at 55°C.

These results demonstrate that the 237 bp fragment possesses by far the largest structural anomaly among these seven SV40 DNA fragments. This effect is temperature-dependent, since the anomalous behavior of this fragment largely disappears as the temperature is increased. The 286 bp fragment also exhibits slightly anomalous behavior, however, this behavior is not as severely influenced by temperature as that of the 237 bp fragment. Finally, the majority of the SV40 fragments in this series show no anomaly in their electrophoretic mobility.

Another way to compare the electrophoretic anomalies of these fragments is to calculate the ratio of their apparent lengths (as deduced from their relative gel mobilities) to their actual lengths (8). These "R_L" ratios are listed in Table I for the series of fragments run at 7°C. Of all the fragments, the 237 bp fragment exhibits the greatest deviation from expected behavior. At 7°C, it has a calculated R_L value of 1.27, signifying that it migrates as if it were about 25% larger than its true size. The 286 bp fragment is the only other fragment which deviates significantly from the norm, with an R_L value of 1.13.

Taken together, these results indicate that the 237 bp fragment may contain a region of sequence-directed bending. In general, maximal retardation of gel mobility is observed when a bend is positioned near the center of the fragment; as the bend is moved closer to a fragment end, the fragment's

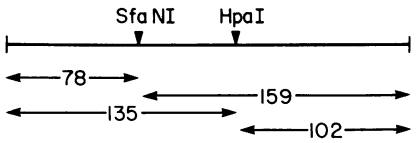

Figure 3. Sub-digestion of the 237 bp fragment. The 237 bp DNA fragment was digested with the restriction endonucleases Sfa NI or HpaI to generate two distinct pairs of sub-fragments. The lengths of these sub-fragments are indicated below by double-headed arrows.

electrophoretic anomaly is diminished (12,13). In order to locate the region of maximal bending within the 237 bp fragment, we cleaved it with restriction enzymes, separating it into two separate pairs of sub-fragments (Figure 3). The electrophoretic mobilities of these sub-fragments were then examined in parallel with the fragments generated by KpnI, HindIII, and MboI digestion of SV40. The ratios of apparent to real fragment lengths at 7°C were calculated for the sub-fragments of the 237 bp parent fragment. These values are listed in Table II. The R_L ratios of the 78, 102, and 159 bp sub-fragments are clustered around 1.0, indicating that they migrate in the manner expected of fragments of these lengths. Only the 135 bp sub-fragment, which covers the left-most portion of the parent fragment, exhibits a higher R_L ratio of 1.13.

Of this set of sub-fragments, the 135 bp fragment exhibits anomalous gel mobility, while the 78 and 159 bp fragments, which overlap its left-most and right-most halves, do not (see Fig. 3). This implies that the bending locus of the 237 bp fragment may be situated near the center of the 135 bp sub-fragment. Since this would place the presumptive bending locus very near the ends of either the 78 or 159 bp sub-fragments, they would not be expected to exhibit much deviation in their relative gel mobilities. Unfortunately, this preliminary mapping of the bending locus is only approximate and a more definitive localization would probably require the very extensive circular permutation experiments of Wu and Crothers (13). However, the data are most consistent with the localization of the DNA bending locus in the 237 bp fragment to sequences within its left-most half, probably near the center.

DISCUSSION

We have characterized the anomalous gel migration of a 237 bp MboI restriction fragment of SV40 which was previously shown to form unique, hyperstable nucleosomes in *in vitro* reconstitution experiments (4). At 7°C, this

fragment migrates with an apparent length of 300 bps on 8% polyacrylamide gels, but at 55°C it migrates to a position much closer to that expected. Such reductions in electrophoretic mobility may be due either to a lower than expected end-to-end distance of the DNA or a higher than expected contour length (14,15). The latter possibility is extremely unlikely since the 27% reduction in mobility (Table 1) would require that the fragment assume a structure with an average 8 base pairs per turn, a seeming impossibility. Either bent or melted DNA can be accomodated in the first possibility. However, melted or pseudo-cruciform (16) DNA would have the opposite temperature dependence to that observed here and we have seen no evidence for unpairing using enzymatic probes (not shown). Therefore, we conclude that the 237 base pair fragment is very likely to be bent, in accord with analogous conclusions in other systems (see ref. 8 for a discussion). The magnitude of the bend is comparable to that observed in several other cases, including the origins of replication of plasmid pT181, SV40, and phage λ (17-19) and the hisR promoter of Salmonella (20). It is less, however, than the extensive bending exhibited by kinetoplast minicircle DNA (6,12,21).

DNA bending has been postulated to arise either from helical periodicities in the occurrence of dinucleotides (22), or from structural polymorphisms associated with poly(dA) tracts (8,23). SV40 sequences have been analyzed for the presence of purine-purine and pyrimidine-pyrimidine dinucleotides which occur with a period of 10.5 bases (24). The sequences corresponding to the 237 bp fragment do not appear to have a particularly striking harmonic component, especially when compared to other SV40 sequences, such as those spanning nucleotide number 427. The lack of helical periodicity among dinucleotides in this region indicates that it is more likely that the DNA bend arises from poly(dA) tracts.

Three such (dA•dT) tracts are located within a 46 base pair region which coincides with the locus of bending mapped near the center of a 135 b.p. fragment (Table II). These are underlined in the following DNA sequence between nucleotides 2591 and 2641: 5'-TGAAAAAAAT GCTTTATTTG TGAAATTTGT GATGCTATTG CTTTATTTGT. The first (dA•dT) tract is clearly expected to be a strong locus of bending (8), but is not likely to be the sole cause of DNA bending since several other SV40 fragments contain internal A_7 tracts. The latter two tracts are interrupted by an A•T transversion; although this is the least disruptive substitution, the extent of associated bending is expected to be substantially lessened (8). However, the three tracts are phased in such a way that the overall bending will be reinforced. That is,

TABLE II
Ratios of apparent lengths at 7°C to actual lengths for the subfragments of the 237 bp fragment

Fragment Length (bps)	$R_L 7^\circ$
159	1.03
135	1.13
102	1.02
78	0.98

Koo et al. (8) found that tracts occurring at approximately 10 base pair intervals were reinforcing even when A tracts and T tracts appeared on the same strand. Therefore, it seems likely that the 237 b.p. SV40 termination fragment has an altered gel mobility because three in phase bending elements cause it to assume a curved shape.

This same SV40 termination fragment was singled out as uniquely able to form hyper-stable nucleosomes in vitro (4) and the above analysis leads to a plausible explanation for that phenomenon. Nucleosomal DNA is strongly bent at four unique sites per nucleosome (3). Beginning from the first of these, the separation in base pairs is successively 30, 20 and 30 (ref. 1). The SV40 termination fragment has three bending determinants that are separated by distances (see above) that could allow occupancy of three of the four nucleosome bend sites with intrinsically bent DNA. Thus, the usual energy loss associated with wrapping stiff DNA around a curved histone core would be minimized and a nucleosome of unusual stability could form.

Several recent studies of the effects of DNA sequence on nucleosome positioning have suggested that relatively short regions of DNA may contribute, in an additive fashion, to the establishment of the final nucleosome "frame", or position (25-27). These studies are also consistent with the idea that short regions of bent DNA might conform more readily to those positions on the nucleosome core that require the DNA helix to bend or kink sharply (1). From a series of SV40 fragments, we singled out the 237 bp fragment for its ability to form hyper-stable nucleosomes (4). Independently, we have now singled out this same DNA fragment based on its ability to assume a bent DNA structure. The above analysis suggests how the two phenomenae may be related. Explicit testing of this model is possible and would

require extensive mutagenesis and nucleosome positioning experiments. Independent of the precise model, the 237 b.p. fragment encompasses sequences where DNA replication and possibly early mRNA transcription terminate, allowing the possibility that nucleosomes of unusual stability could potentially play a role *in vivo*.

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