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Evidence for opposite groove-directed curvature of GGGCCC and AAAAA sequence elements

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ABSTRACT

The repetitive sequence (AGGGCCCTAGAGGGGCCC-TAG)n was previously shown to be curved by gel mobility assays. Here we show, using hydroxy radical/ DNase I digestion and differential helical phasing experiments that the curvature is directed towards the major groove and is located in the GGGCCC, but not the CTAGAG segments. The effect of the GC step in the context of the GGGCCC motif is apparently about as large as that of AA/TT, i.e. enough to cancel the macroscopic curvature of helically phased A-tracts. These data are in an agreement with positive roll-like curvature of the GCC/GGC motif, predicted from nucleosome packing data and the 3D structure of the GGGGCCCC octamer, but they are not in agreement with the dinucleotide-based roll angle values predicted for AG/CT, TA, GG/CC and GC steps. Our results thus indicate the importance of interactions beyond the dinucleotide steps in predictive models of DNA curvature.

INTRODUCTION

Intrinsic DNA curvature was generally thought to originate from periodically repeating AnTm tracts (n+m>=3), with a period close to the helical repeat length. It was recently experimentally shown that curvature is possible without AnTm tracts, provided that a certain combination of dinucleotide steps occur (1–4). Several models were suggested (1,5,6,7) to quantitatively describe the DNA curvature as determined by gel-mobility experiments. There is increasing evidence that wedge-like features of a number of dinucleotides are not adequately described by these simple models (3,8,9,15,29). One of the main reasons for this disagreement could be the dynamic character of DNA curvature, caused by differential sequence-dependent DNA flexibility (8,9) and the existence of context-dependent interactions which could influence the wedge-like features of dinucleotide steps, as in the case of AnTm tracts (5,9,26).

In a previous paper we reported that the repeating sequence 5'AGGGCCCTAGAGGGGCCCTAG3' displays an anomalous gel mobility, characteristic for curved DNA, even in the absence of AnTm tracts (4). In view of the published data on roll angle values, it was suggested that the major groove-directed curvature is present in the CTAGAG, but not GGGCCC motifs (4). In this paper we determine the sequence elements underlying the curvature, as well as the groove orientation relative to the center of curvature. We used two independent approaches: i) analysis of digestion patterns obtained by DNase I and hydroxy radicals, and ii) varying the distance between centers of curvature and monitoring the effect on gel mobility data (22).

DNase I cleavage patterns mainly reflect the global changes in the geometry of the grooves, although there are some local structural features that also may influence the frequency of cutting to a lesser extent (2,13–20). DNase I and hydroxy radical cleavage rates are usually higher in 'convex' regions where the minor groove is oriented 180 degrees away from the local center of curvature, i.e. points outwards, then in 'concave' regions, where the minor groove is oriented toward the local center of curvature (13,15,17,20). In terms of the wedge model, groove-directed curvature is a result of roll angle contributions of dinucleotide steps (1,6,21).

In differential helical phasing experiments, synthetic oligonucleoties are prepared in which the distance of specific structural elements is systematically varied. Gel mobility anomaly is detected only in cases where the distance of the two elements is in an appropriate helical phase. Using an element of known direction of curvature one can thus conveniently determine the direction of curvature of another element.

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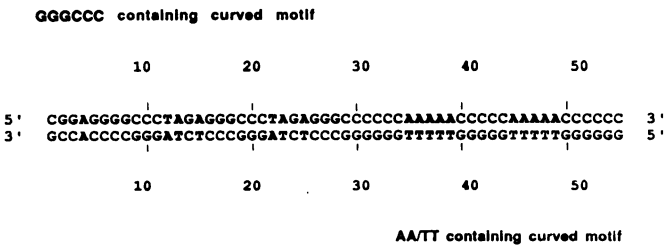

Figure 1. Sequence of 55-mer used for digestion experiments. The 5'-half of the upper (Watson) strand contains two copies of the motif 5'CTAGAG3' and three copies of the motif 5'GGGCCC3', but no AA/TT steps, while the 3'-half contains two A-tracts. The center of the 5'GGGCCC3' and 5'CTAGAG3' motifs, as well as the A-tracts, are separated by 10.5 base pairs, i.e. they are in phase with the helical repeat.

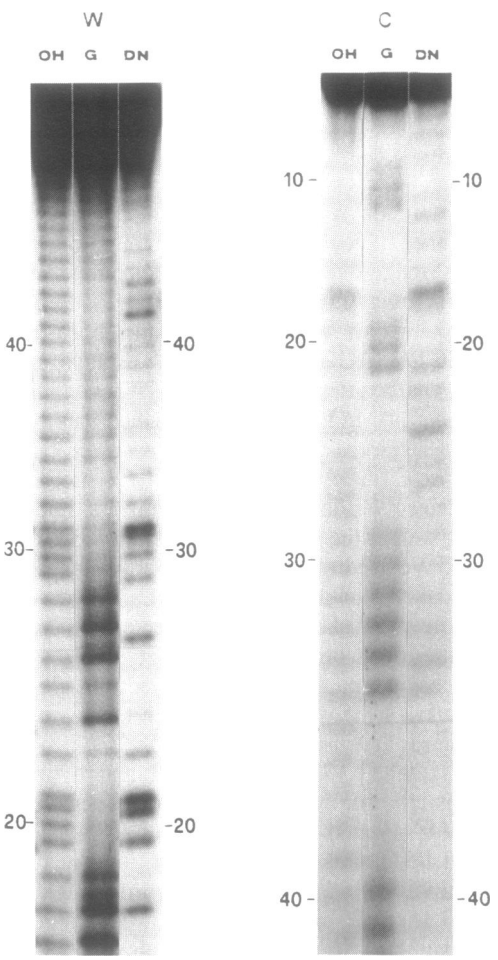

Figure 2. Cleavage patterns of DNase I (lane DN) and hydroxy radical (lane OH) of Watson (W) and Crick (C) strands of 55-mer . Lane G represents positions of guanines in the sequence, cut by dimethyl-sulfate-piperidine. Numbers refer to the numbering scheme of the sequence shown in Figure 1.

Our data suggest that the major groove-directed curvature of (AGGGCCCTAGAGGGGCCCTAG)n DNA is caused in fact by the GGGCCC, but not the CTAGAG motif. This result points to the influence of non AA/TT containing DNA motifs in DNA

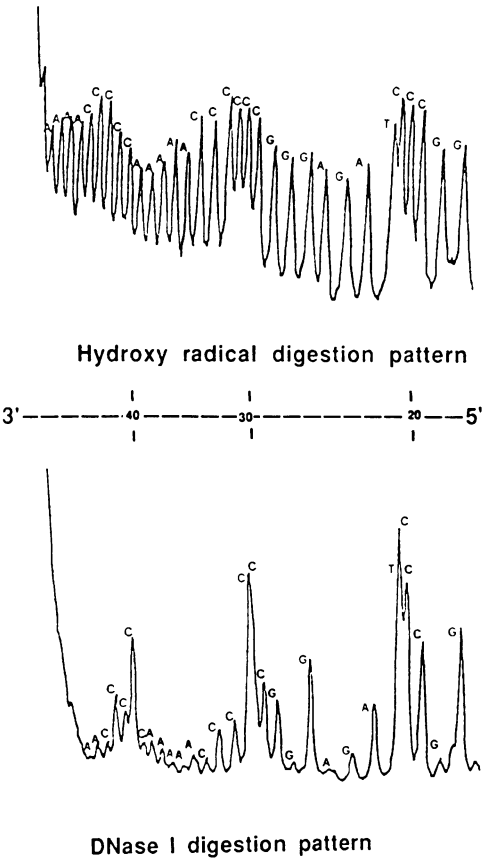

Figure 3. Phosphor imaging scan of DNase I and hydroxy radical cleavage patterns of the 55-mer (Watson strand).

curvature. The effect of the GC step in the context of GGGCCC motif is apparently about as large as that of AA/TT, i.e. it seems to be enough to cancel the macroscopic curvature of helically phased A-tracts. These results indicate that interactions beyond the dinucleotide level can be important for the prediction of wedge angles and DNA curvature.

MATERIALS AND METHODS

For DNase I and hydroxy radical digestion experiments the 55-mer 5'CGGAGGGGCCCTAGAGGGCCCTAGAGGGCC-CCCCAAAAACCCCCAAAAACCCCCC3' (Watson strand) and its complementary strand were synthesized using an Applied Biosystems 381A oligonucleotide synthesizer and purified by 8% denaturing polyacrylamide gel electrophoresis. The purified oligonucleotides (0.6 μg) were labelled separately in a total volume of 20 μl (70mM Tris−HCl (pH 7.6), 10 mM $MgCl_2$ and 5 mM dithiothreitol) with 10 μCi of (gamma ^{32} P) ATP (SA > 3000 Ci/mmol) and 6 units of T4 polynucleotide kinase, at 37°C for 30 minutes. The labelled Watson and unlabelled complementary strand were mixed in a concentration ratio of 1:2, respectively. The mixture was heated to 90°C and cooled down slowly to form the hybrids. DNA was cleaved by hydroxy radical (13,14) and DNase I (16,20) under conditions that leave more than 85% of uncleaved material. These conditions produce an average of 0.16 cuts per molecule (23), and the obtained cleavage

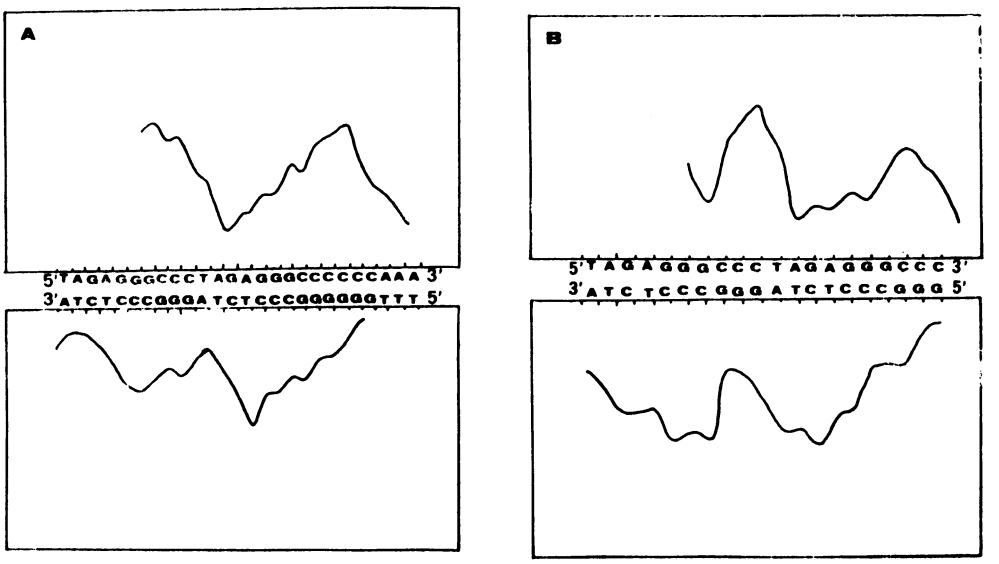

Figure 4. Plots of 3-bond averaged relative frequencies of cleavage obtained by **A)** hydroxy radical and **B)** DNase I digestion, for both strands in the non AA/TT containing part of the molecule.

intensities therefore reflect the affinity of the cleavage agent for different parts of the DNA. The fragments resulting from DNA cleavage and Maxam–Gilbert G-reaction (24) were separated on 8% polyacrylamide denaturing gels containing 8M urea. The cleavage patterns were recorded using autoradiography, quantitative evaluation was carried out by scanning image plates of a Molecular Dynamics Phosphor Imager system. The relative frequencies of cutting for each bond were calculated, including the correction for the cuts that have occured between a cut and the radioactive label (16).

Five double stranded DNA fragments with four base long 5'-protruding ends were designed in which the GGGCCC and the AAAAA motifs were separated by varying number of nucleotides (Fig. 5). The ten resulting 42-mers were synthesized, purified, kinased and the hybrids prepared as described above. Two μl of the mixture (corresponding to 0.6 μg of oligo) were used in ligation reaction with 1 unit of T4 DNA ligase in a total volume of 10 μl (70 mM Tris–HCl (pH 7.6), 10 mM $MgCl_2$ and 5 mM dithiotreitol), plus 2.3 nM cold ATP. The ligation reaction was allowed to proceed about 4 hours at room temperature. Ligated products were run on 8% polyacrylamide gels (mono:bis acrylamide ratio = 29:1; 90 mM Tris-borate, 10 mM EDTA, pH 8.3). The applied voltage was 5 V/cm and electrophoresis was carried at 22°C. Gels were dried and autoradiographed with an intensifying screen. Mobility measurements were made relatively to the migration of pBR322 fragments cut by Hae III and Hinf I.

RESULTS

The 55-mer was designed to contain an 'atypically' curved 5'GAGGGGCCCTAGAGGGCCCTAGAGGGCCC3' region at the 5'-end and a 'typically' curved A-tract containing segment 5'$C_6A_5C_5A_5C_6$3' at the 3'-end of the Watson strand (Figure 1). This design allows the comparison of the behaviour of the two regions under identical experimental conditions. Cleavage of this molecule either with DNase I, or with hydroxy radicals, gave

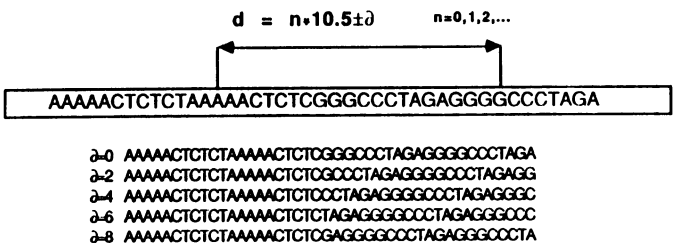

Figure 5. Sequences of the 5 42-mers with differential deviation (∂) from the full helical turn distance between GGGCCC and AAAAA motifs. It is important to note that the 10.5 bp periodicity between the same type of motifs is preserved, while the phasing between AA/TT and non AA/TT centers of curvature is varied.

a sinusoidal pattern in both regions of the molecule, as shown by autoradiography (Figure 2) and densitometry (Figure 3). The curved, A-tracts containing segment shows the typical phasing of minima with the A-tracts (13–16) reflecting sequence-dependent curvature towards the minor groove (1,7–9,13–15, 21,25,26). In the segment not containing A-tracts, (Figure 3 and Figure 4) the relative cutting activity was maximal in the C-rich part of the 5'GGGCCC3' motif, while the minimal cutting rate was detected in the 3' region of the 5'CTAGAG3' motif. The stagger in the 3' direction between cutting patterns of the complementary strands (Figure 4) is characteristic for minor groove recognition (13,16) . The above results illustrate that the segments with and without A-tracts behave essentially in a similar way in hydroxy radical and DNase I digestion experiments, suggesting that a similar, groove-directed curvature may be present in both types of curved DNA. The regions of the wider minor groove are located in the GGGCCC motifs of the non AA/TT containing DNA fragment.

The five oligonucleotides shown in Fig. 5 were designed in such a way that the two motifs GGGCCC and AAAAA are differentially separated. After annealing, inter-motif distances deviate from a multiplication of the full helical turn by 0, 2, 4,

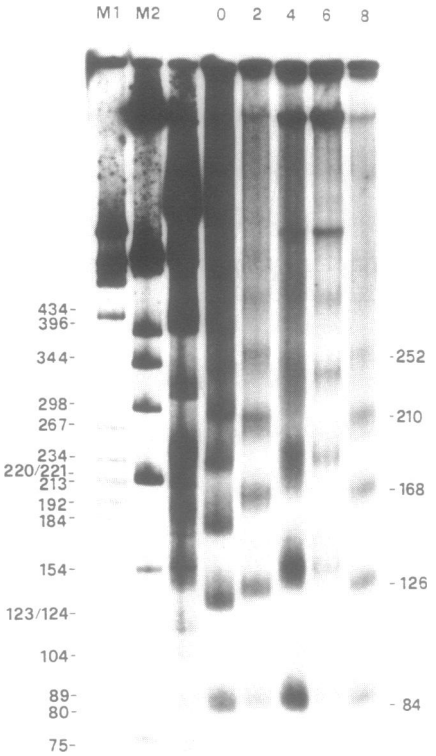

Figure 6. Autoradiogram of mobility of 5 ligated 42-mers with differential deviation ∂ from the full helical turn distance between the GGGCCC and AAAAA motifs ($\partial=0$, 2, 4, 6 and 8 bp) on a 8% nondenaturing polyacrylamide gel. Lane M1: pBR 322 cut by Hae III; M2: pBR 322 cut by Hinf I. The size (in bp) of various DNA fragments is indicated.

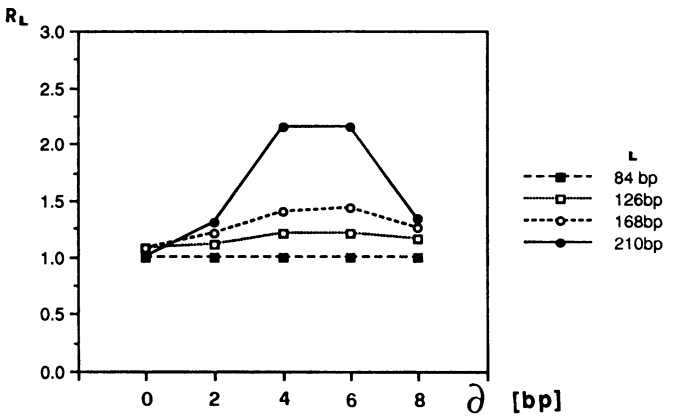

Figure 7. Plot of relative mobility RL of 5 ligated 42-mers, versus deviation ∂ from the helical turn distance between the GGGCCC and AAAAA motifs. The multimers with the same actual size (L) are connected into a curve. The data point for multimers with the same actual size L are connected. The mobility anomaly is the largest for $\partial=5$, corresponding to a separation of motifs by odd multiples of half a helical turn. In the case $\partial=0$, i.e. with the motifs separated by multiples of a full helical turn, the curvatures are canceling each others and no mobility anomaly is found.

6 and 8 base pairs, respectively. The value of deviation was termed ∂. Two motifs of opposite direction but equal magnitude of curvature will show mobility anomaly if spaced by odd multiples of half a helical turn ($\delta=5$). In the case $\delta=0$, i.e. with the motifs separated by multiples of a full helical turn, the curvatures will cancel each others and no mobility anomaly will be found. As generally accepted, AAAAA is a structural element causing minor groove-directed curvature (1–3, 6, 7–9, 13, 21, 22); so it was chosen as a reference in our experiments. Figure 7 shows the relative mobility of the ligated 42-mers plotted as a function of ∂. As can be seen, the maximal gel mobility anomaly was detected around $\partial=5$ and no anomaly was obtained at $\partial=0$. In light of the above discussion this indicates that GGGCCC is curved in a direction opposite to the curvature of AAAAA.

DISCUSSION

The curvature of (AGGGCCCTAGAGGGGCCCTAG)n DNA was previously reported (4), but the sequence elements responsible for the curvature and the direction of their curvature were not experimentally established. Considering the published data of AG/CT, TA, GG/CC and GC wedge-roll values (1,6), the preferential curvature of PyPu (TA), compared to PuPy (GC) steps (12) and crystallographic data (10,11), we expected that the region with the major groove-directed curvature is located in the CTAGAG, and not in the GGGCCC part of the curved (AGGGCCCTAGAGGGGCCCTAG)n DNA (4). However, the results of DNase I/hydroxy radical digestion experiments and gel mobility assays show, on the contrary, that the GGGCCC, and not the CTAGAG element has the inherent major groove directed curvature. Furthermore the experiments show that the curvature of the GGGCCC and AAAAA motifs is of opposite sign. The effect of the GC step in the context of GGGCCC motif seems to be about as large as that of AA/TT, i.e. it is apparently enough to cancel the macroscopic curvature of helically phased A-tracts.The origin of the macroscopic curvature for the (AGGGCCCTAGAGGGGCCCTAG)n DNA can be explained by an overall roll angle difference between the GGGCCC and CTAGAG sequence elements, which will add up if the two motifs are separated by a distance close to the helical repeat length. This explanation does not include the wedge-tilt component which can also contribute, although to a lesser extent, to DNA curvature (1,6, 7, 12,21).

It was reported that the dinucleotide-based roll angle value of GC step is one of the smallest found in all dinucleotides (1,6). Our results, on the other hand, suggest that in the context of our oligonucleotide the GC step has in fact a high positive roll angle value. This bimodal, context-dependent behaviour of GC step was originally suggested by Calladine and Drew (21). They reported that the nearest neighbour interactions within the GGGCCC motif could produce a widening of the minor groove and an increase of the overall roll. Nucleosome packing data (27), X-ray analysis of an 5'GGGGCCCC3' octamer (28) and the effect of sequence context on base pair stacking geometries (29) also support this view, indicating that the context-dependent influence on DNA curvature may be difficult to explain in the terms of dinucleotide-based models.

The fact that the oligonucleotides with $\partial=0$ show no mobility anomaly is noteworthy since they contain an A-tract repeated in phase with the helical turn. The A_nT_m tracts are known to strongly contribute to gel mobility anomaly and so to DNA

curvature (1,3,4,9,26). Also, since A_nT_m tracts are by far the most frequent examples of curved structures, the quantitative models describing sequence-dependent DNA curvature assign a relatively stronger influence to the AA/TT step (1,6,7). Our data for the first demonstrate that A-tracts repeated at helical turn periods can display no mobility anomaly if they are appropriately phased with GGGCCC motifs. On the other hand, GGGCCC motifs without AnTm tracts do not drastically effect gel mobility (2,3,4). This phenomenon could be explained simply by the fact that the stiffness of A-tracts and not just curvature influences the electrophoretic behaviour as was previously suggested (8,9).

Summarizing, we can conclude that the GGGCCC motif seems to adopt a strongly curved conformation with a direction of curvature opposite to and the magnitude similar to the A-tracts. The presently available data point towards the general conclusion that conformational interactions beyond the dinucleotide steps have an impact on DNA curvature.

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