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Molecular Dynamics Studies of Axis Bending in $d(G_5\text{-}(GA_4T_4C)_2\text{-}C_5)$ and $d(G_5\text{-}(GT_4A_4C)_2\text{-}C_5)$: Effects of Sequence Polarity on DNA Curvature

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Gel retardation studies and other experiments indicate that DNA sequences containing the $d(GA_4T_4C)_n$ motif are curved, whereas those of identical composition but with a reverse sequence polarity, the $d(GT_4A_4C)_n$ motif, are straight. Hydroxyl radical cleavage experiments show that $d(GA_4T_4C)_n$ shows a unique signature, whereas $d(GT_4A_4C)_n$ behaves normally. To explain these results at a molecular level, molecular dynamics (MD) simulations were performed on the DNA duplexes $d(G_5\text{-}(GA_4T_4C)_2\text{-}C_5)$ and $d(G_5\text{-}(GT_4A_4C)_2\text{-}C_5)$ to 3.0 and 2.5 ns, respectively. The MD simulations are based on the Cornell force field implemented in the AMBER 4.1 modeling package and performed in a neutral solution of anionic DNA with K^+, Cl^- and Mg^{2+} at concentrations roughly comparable to a ligase buffer. Long range interactions were treated by the particle mesh Ewald method. Analysis of the results shows that the calculated dynamical structure of $d(G_5\text{-}(GA_4T_4C)_2\text{-}C_5)$ exhibits strong gross curvature, consistent with the observed behavior. The most significant locus of curvature in the MD structure is found at the central $C^{15}\text{-}G^{16}$ step, with an average roll angle of 12.8(±6.40) deg. The $d(G_5\text{-}(GT_4A_4C)_2\text{-}C_5)$ MD structure exhibited significantly less gross curvature. Analysis of results indicates that the reduction in gross curvature in the $d(G_5\text{-}(GT_4A_4C)_2\text{-}C_5)$ trajectory originates from the effect of the $T^{10}\text{-}A^{11}$ and $T^{20}\text{-}A^{21}$ steps, which showed average roll angles of 12.5(±5) deg. These three steps, $T^{10}\text{-}A^{11}$, $C^{15}\text{-}G^{16}$ and $T^{20}\text{-}A^{21}$, are half-helix turns away from one another, and their contributions to concerted bending cancel out. The A-tracts in the MD structure are essentially straight. The dynamical structure of $d(G_5\text{-}(GA_4T_4C)_2\text{-}C_5)$ exhibited minor groove deformation comprised of expansion at the 5′ end of A-tracts and progressive narrowing towards the 3′ end, consistent with and elaborating the interpretation of hydroxyl radical chemical probing results.

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Introduction

DNA sequences containing the motifs $d(A_4T_4)_n$ and $d(T_4A_4)_n$ may be identical in base composition, but gel retardation and DNA cyclization experiments indicate that they differ significantly in axis curvature and possibly related deformations in helix morphology (Berkhoff & Tullius, 1988; Hagerman, 1986). A full account of these phenomena, the effect of ''sequence polarity'' on structure and motions, has not been unequivocally established at the molecular level. With recent advances in DNA force field development and supercomputer power, dynamical models of DNA oligonucleotides have been obtained from molecular dynamics (MD) computer simulation. These models have been reasonably successful to date in describing the dynamical structure of DNA conformations (Cheatham *et al.*, 1995; Duan *et al.*, 1997; Young *et al.*, 1997a,b), conformational preferences and interconversion (Cheatham, 1997; Cheatham *et al.*, 1997; Jayaram *et al.*, 1998; Sprous *et al.*, 1998), and

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Abbreviations used: MD, molecular dynamics; PME, particle mesh Ewald; I, inosine.

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axis curvature (Young & Beveridge, 1998). The ability to reproduce sequence polarity effects in MD simulation is a further sensitive test of nucleic acid force fields and modeling protocols, and analysis of the MD results can yield explanations of observable properties at the molecular level.

Here, we describe MD simulations performed on two 30-mer DNA duplexes, $d(G_5\text{-}(GA_4T_4C)_2\text{-}C_5)$ and $d(G_5\text{-}(GT_4A_4C)_2\text{-}C_5)$, including counterions and water explicitly. The sequences under consideration are identical in base-pair composition, but are distinguishable by the sequential 5′ to 3′ order of the central A-tracts. These duplexes are models of the $d(XA_4T_4Y)_n$ and $d(XT_4AN_4Y)_n$ DNA sequence motifs, where X and Y are G or C bases. Both sequences have two A-tracts positioned in phase with a full helical turn of a *B*-form DNA double helix, a feature known to enhance curvature in intrinsically curved DNA segments (Marini *et al*., 1982, 1984). Both simulations were initiated from morphologically identical straight canonical *B*-DNA starting structures in explicit waterbaths with associated ions. No constraints based on assumptions of DNA curvature, A-tract structure or helix morphology were applied to either simulation, i.e. the structures progressed and developed in MD as a result intrinsic properties of the Cornell *et al.* (1995) force field. The MD results are consistent with solution structure and crystallography data for the A-tracts, and provide an interpretation of the nature of curvature in the $d(XA_4T_4X)_n$ and $d(XT_4A_4X)_n$ sequence motifs.

Background

DNA curvature can be determined using a number of experimental techniques, including anomalous slow polyacrylimide gel migration (Marini *et al*., 1982, 1984), cyclization assays (Ulanovsky *et al*., 1986), electron microscopy (Griffith *et al*., 1988) and electric birefringence (Hagerman, 1984). Hagerman also reported that sequences of the form $d(CA_4T_4G)_n$ or $d(GA_4T_4C)_n$ showed significant gel anomalies with respect to random sequence oligonucleotides, while sequences of the form $d(CT_4A_4G)_n$ or $d(GT_4A_4C)_n$ did not (Hagerman, 1986). In studies of the chemical probing patterns of these two sequence motifs, the hydroxyl radical cleavage pattern of $d(A_4T_4)$-based sequences exhibited a characteristic damped sinusoidal profile going in 5′ to 3′ within A-tracks (Berkhoff & Tullius, 1988). This is attributed to a progressive compression of the minor groove (Draganescu & Tullius, 1996). In contrast, the hydroxyl radical cleavage pattern of sequences containing the $d(T_4A_4)$ sequence motif exhibited a pattern characteristic of random sequence DNA. These results indicate that sequence polarity can be an important parameter in determining DNA structure and curvature. The origins of this phenomenon at the level of primary structure can be linked to a difference in the apposition of 5′ and 3′ ends of the A-tracts in the two sequence motifs (Hagerman, 1986; Sinden, 1994). How this translates into molecular modifications at the level of secondary structure has not yet been fully explained.

MD computer simulations at the all-atom level can, in principle, provide a complete view of all the DNA and solvent structure and motions (McCammon & Harvey, 1986; van Gunsteren & Berendsen, 1990). In practice, the approximations of the underlying force fields, sensitivity of simulated structures to artifacts from the treatment of long range interactions (Auffinger & Beveridge, 1995) and limitations of the computationally accessible time-range of simulation can place limits on the accuracy and applicability of the MD models (Beveridge, 1998; Beveridge *et al*., 1993). Recently, significant advances have been realized both in the force fields for nucleic acids in solution as well as in the associated simulation protocols. Improvements in supercomputer power and parallelization of dynamics code have increased the practical limit of simulated time from the range of picoseconds to nanoseconds. MD based on AMBER (Pearlman *et al*., 1995), the Cornell *et al*. (1995) force field, and the particle mesh Ewald (PME) treatment of long-range interactions (Cheatham *et al*., 1995; Darden *et al*., 1995; York *et al*., 1993, 1995) support a *B*-form DNA structure for greater than 14 ns in water (Young, 1998; Young *et al*., 1997b), exhibit an appropriate *A* to *B*-DNA transition in water on the order of 500-1250 ps (Cheatham & Kollman, 1996; Sprous *et al*., 1998), support an *A*-form DNA in low water activity conditions (Cheatham & Kollman, 1996; Sprous *et al*., 1998), and successfully describe axis curvature in sequences with A-tracts phased with respect to a full turn of a DNA double helix (Young & Beveridge, 1998). With this level of validity established for the current implementation of MD methodology, extension of MD studies to more challenging problems in the structural chemistry of DNA is appropriate and timely.

The essential problem addressed here is the nature of the structural differences in sequences of the form $d(XA_4T_4Y)_n$ and $d(XT_4A_4Y)_n$, where X and Y are C or G bases. Diverse experiments on A-tract DNA structure in solution indicate that net axis curvature is in the direction of the minor groove at the center of each A-tract (Brukner *et al*., 1993, 1994; Crothers & Drak, 1992; Crothers *et al*., 1992; Dlakic & Harington, 1995). There has been considerable discussion in the literature as to whether this is a consequence of negative roll angles for the ApA dinucleotide step (the ''wedge'' model; Bolshoy *et al*., 1991; Ulanovsky *et al*., 1986; Ulanovsky & Trifonov, 1987), curvature in the junction between relatively straight A-tracts and general sequence DNA (the ''junction'' model; Koo *et al*., 1986), or whether the A-tracts are essentially straight and the intervening general sequence DNA is curved (the ''curved general sequence'' model; Maroun & Olson, 1988). The wedge and junction models were both developed from data obtained from solution structure experiments, while the curved general sequence model emerged as a result of theoretical studies and analysis of

crystal structure data. All models to date have notable limitations (Goodsell & Dickerson, 1994; Haran *et al*., 1994). X-ray structures of oligonucleotides show A-tracts to be essentially straight in crystals (Haran *et al*., 1994; Young *et al*., 1995). However, interpretations of the models from crystallographic data suffer from still unresolved influences of crystal packing effects (Dickerson *et al*., 1994; DiGabriele *et al*., 1989; DiGabriele & Steitz, 1993) and reports that organic components of crystallographic liquor components influence curvature at concentrations less than those used to grow crystals (Dlakic *et al*., 1996; Ganunis *et al*., 1996; Harvey *et al*., 1995; Sprous *et al*., 1995). A molecular explanation of sequence-directed DNA curvature with respect to A-tract polarity requires a consideration of both computational modeling and experiments. Simulations from our laboratory have demonstrated a capability of MD to reproduce the A-tract curvature in solution (Young & Beveridge, 1998). Here we report studies which address the nature of A-tract polarity at the molecular level.

Results

Snapshots of structures taken in 500 ps intervals for the d(G_5-(GA_4T_4C)$_2$-C_5) simulation are presented in Figure 1A, with an expansion of the time region from 920 to 980 ps; the segment of the trajectory when maximum curvature is observed is shown in Figure 1B. Snapshots of structures taken in 500 ps intervals for the d(G_5-(GT_4A_4C)$_2$-C_5) simulation are presented in Figure 2. The simulations were performed using an all-atom model with explicit solvent treatment, but for clarity the snapshots presented are based on simplified representations of the DNA molecule with the calculated helix axis included.

A key feature of the d(G_5-(GA_4T_4C)$_2$-C_5) trajectory is a sampling of crescent shaped helix conformations resulting from a noticeable bend in the helix axis near the center of the sequence at the C^{15}-G^{16} step. Inspection of end-to-end distances for the MD structures revealed that the time region near 950 ps had the strongest signature of curvature (i.e. the lowest end-to-end distance). Several structures taken over this time region for the d(G_5-(GA_4T_4C)$_2$-C_5) trajectory are presented in Figure 1B. The helix axis in these structures shows three distinct bend locations, the C^{15}-G^{16} step and additional bends in the junctions adjacent to each A-tract, with the net effect that the entire DNA molecule exhibits bending in a concerted direction.

For d(G_5-(GT_4A_4C)$_2$-C_5), the MD also shows a significant bend located at the central C-G step, but there are also specific bends associated with each T-A step as well. Here the helix axis takes a ''zigzag'' space curve in the MD, with three bending loci. The net effect is that of less curvature, a consequence of the out-of-phase localized distortions canceling. The ''zigzag'' course does reduce end-to-end distance relative to contour length, making the ratio of the two a possibly questionable measure of curvature. With this caveat established, we note that the d(G_5-(GT_4A_4C)$_2$-C_5) molecule shows less curvature reduction by this ratio than the d(G_5-(GA_4T_4C)$_2$-C_5) molecule. Specifically, the ratio of end-to-end distance to contour length for d(G_5-(GT_4A_4C)$_2$-C_5) is 95.8(±1.5)% and for d(G_5-(GA_4T_4C)$_2$-C_5) 93.5(±2.0)%. The d(G_5-(GT_4A_4C)$_2$-C_5) MD structure shows less flexibility than that of d(G_5-(GA_4T_4C)$_2$-C_5) and other phased A-tract sequences studied (Young & Beveridge, 1998).

Ensemble average values of the parameters roll, tilt, twist and minor groove width, taken over 500-3000 ps and 500-2500 ps for the d(G_5-(GA_4T_4C)$_2$-C_5) and d(G_5-(GT_4A_4C)$_2$-C_5) simulations, respectively, are shown in Figure. 3. While the distribution of tilt is similar for both sequences, the distribution of roll values is distinctive. For the d(G_5-(GA_4T_4C)$_2$-C_5) simulation the central C^{15}-G^{16} step has an average roll angle of 12.8(±6.40) deg. In addition, the peripheral junctions between the central A-tracts and the flanking G·C base-pairs in

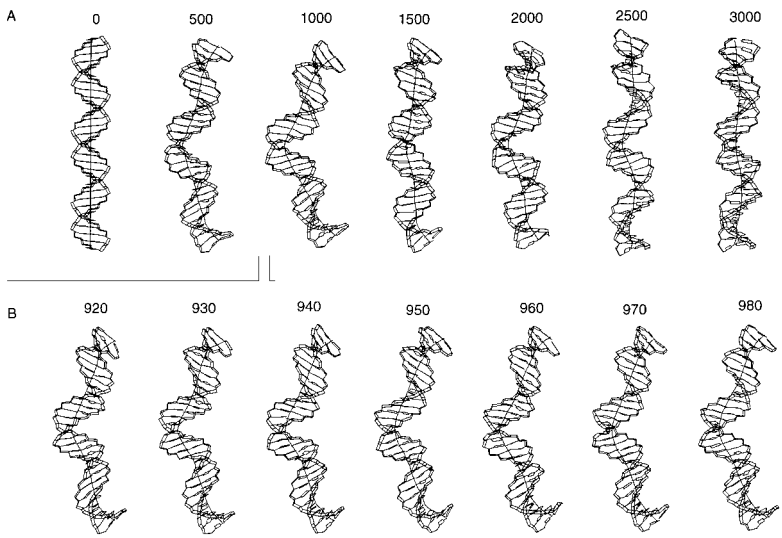

Figure 1. MD snapshots for A, d(G_5-(GA_4T_4C)$_2$-C_5) simulation in 500 ps intervals and B, for the d(G_5-(GA_4T_4C)$_2$-C_5) simulation over the time region of 920 to 980 ps. MD simulations are all-atom, explicit solvent simulations the cartoon representation of the DNA is used for clarity of display.

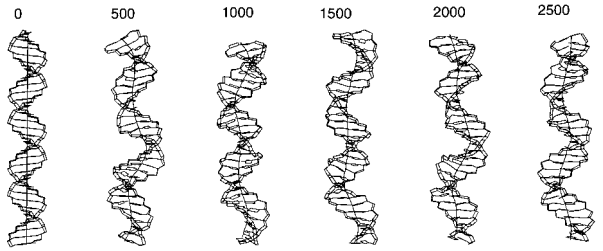

Figure 2. MD snapshots for the d(G_5-(GT_4A_4C)$_2$-C_5) simulation.

this sequence maintain significantly positive roll angles. The MD model of A-tracts is essentially straight. Since the central C^{15}-G^{16} step and the curved flanks are full helix turns apart, their in-phase bends lead to the gross curvature visible in Figure 1.

In the d(G_5-(GT_4A_4C)$_2$-C_5) sequence, the central C^{15}-G^{16} step has a roll of 10.4(±6) deg., slightly below the value found at the corresponding central step of the d(G_5-(GA_4T_4C)$_2$-C_5) sequence. However, whereas the A^{10}-T^{11} and A^{20}-T^{21} steps had slight negative roll angles in the d(G_5-(GA_4T_4C)$_2$-C_5) trajectory, the T^{10}-A^{11} and T^{20}-A^{21} steps of d(G_5-(GT_4A_4C)$_2$-C_5) show positive roll angles of 12.5(±5) deg. These three steps, T^{10}-A^{11}, C^{15}-G^{16} and T^{20}-A^{21}, are half-helix turns away from one another in the d(G_5-(GT_4A_4C)$_2$-C_5) sequence, causing their respective contributions to an overall bend to cancel each other out. As seen in the d (G_5-(GA_4T_4C)$_2$-C_5) trajectory, the peripheral junctions between the central A-tracts and the G·C base-pairs show significantly positive roll angles. Since these curvature loci are half-helix turn from the positive roll angles at the T^{10}-A^{11} and T^{20}-A^{21} steps, the net effect is again to cancel out the gross curvature. The bends at the T^{10}-A^{11}, C^{15}-G^{16} and T^{20}-A^{21} steps are visible in the instantaneous structures from the d(G_5-(GT_4A_4C)$_2$-C_5) trajectory in Figure 2.

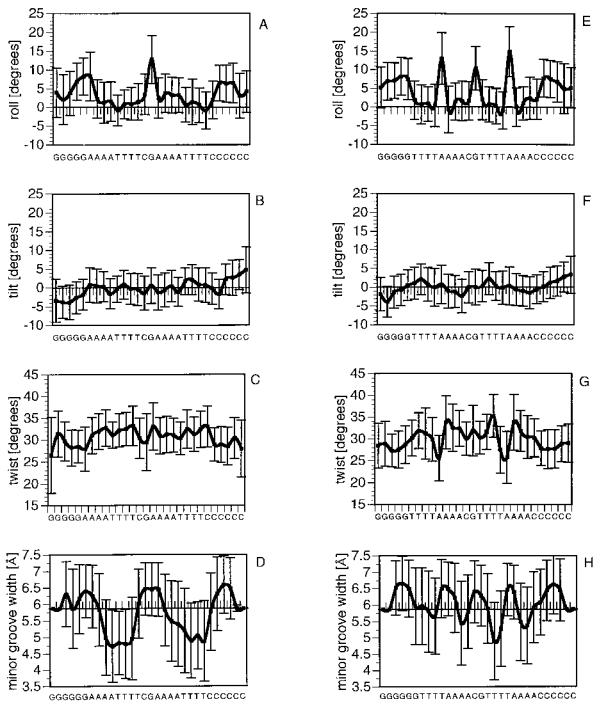

Figure 3. Average roll (A,E), tilt (B,F), twist (C,G) and minor groove (D,H) width as a function of sequence for the d(G_5-(GA_4T_4C)$_2$-C_5) (left, A-D) and d(G_5-(GT_4A_4C)$_2$-C_5) (right, E-H) simulations. Error bars are a single standard deviation about the mean. Averages were calculated for the 500-3000 ps and 500-2500 ps time ranges, respectively, for the two systems.

The value of twist for *B*-form DNA in solution is typically on the order of 34° per base-pair. Values obtained from MD for diverse sequences (Cheatham & Kollman, 1997; Young *et al.*, 1997b) tend to be slightly less than the value inferred from experimental (Kabsch *et al.*, 1982; Young *et al.*, 1995; Suzuki *et al.*, 1997). In the two sequences studied in this paper, the distribution of twist angles is below the MD average in the flanking sequences (see Figure 3C and G). The twist in the central A-tracts regions is closer to the normal value for the MD protocol, and exhibits larger standard deviations. Two additional points about twist deserve mention. First, the central C^{15}-G^{16} step in the d(G_5-(GA_4T_4C)$_2$-C_5) simulation is lower than the surrounding A-tracts and has larger standard deviation. Second, in the d(G_5-(GT_4A_4C)$_2$-C_5) simulation, the twist angles of the T^{10}-A^{11} and T^{20}-A^{21} steps are noticeably lower than adjacent base-pairs. Thus, helical twist is anti-correlated with roll angle at bending loci in both sequences.

The minor groove width of the MD structures is shown as a function of sequence in Figure 3D and H. The MD results for d(G_5-(GA_4T_4C)$_2$-C_5) show minor groove narrowing in both A-tracts. In contrast, the width of the minor groove of the d(G_5-(GT_4A_4C)$_2$-C_5) structure shows no distinguishable compression within the A-tract regions. Comparison of the roll profiles in Figure 3A and E with the minor groove widths in Figure 3D and H, indicates that perturbations of the two properties are correlated: the base-pair steps with high positive roll have wide minor grooves while the A-tracts with relatively low roll angles have compressed minor grooves. In addition to the relative narrowing of the minor groove width in the A-tract stretches, the regions at the 5′ end of the A-tract have a wider minor groove width than the 5.87 Å expected for canonical *B*-form structures. As the strand progresses 5′ to 3′ along A bases (or 3′ to 5′ along the T bases), the minor groove narrows and ultimately adopts values below the expected *B*-DNA average. Experimental evidence of progressive narrowing in A-tracts has been reported for A-tracts in diverse sequences (Draganescu & Tullius, 1996), and the MD results are consistent with the specific results of hydroxyl radical cleavage experiments on d(XA_4T_4Y)$_n$ and d(XT_4A_4Y)$_n$ motifs (Berkhoff & Tullius, 1988).

Ensemble average values for the helicoidal parameters which differentiate *B*-form from *A*-form

DNA, i.e. sugar pucker, x-displacement, base-pair inclination, and helical twist, define the structures for both trajectories to be clearly within the *B* family. Although the A-tracts have distinct compressed minor grooves, we see no evidence that the structures resemble previously described unusual DNA forms such as *H*-DNA or *B*′-DNA (Alexeex *et al*., 1987; Arnott *et al*., 1983).

Discussion

The MD on d(G_5-(GA_4T_4C)$_2$-C_5) showed significant sampling of structures which are curved in a gross, macroscopic sense. The bending in the MD structures exhibited a wide dynamic range, sampling both curved and straight conformations within the 3.0 ns. In contrast, the d(G_5-(GT_4A_4C)$_2$-C_5) MD did not sample macroscopically curved conformations, and showed relatively less flexibility than d(G_5-(GA_4T_4C)$_2$-C_5). These computational results are consistent with the experimental results reported by Hagerman (1986). The d(G_5-(GA_4T_4C)$_2$-C_5) MD structure exhibited a progressive narrowing of the minor groove in the A-tract region, supporting the inferences drawn from hydroxyl radical cleavage experiments by Berkhoff & Tullius (1988). One interesting feature of the MD model is that the 5′ end of the minor groove is wider than normal (see the text below). Together with results described for d(CGCGAATTCGCG) (Sprous *et al*., 1998; Young *et al*., 1997b), a set of DNA decamers (Cheatham & Kollman, 1996; Cheatham, 1997; Cheatham *et al*., 1997) and a series of sequences with and without phased A-tracts (Young & Beveridge, 1998), this body of evidence provides support of the quality of the MD models for DNA. The absence or presence of gross curvature, the underlying roll, tilt and twist angles, and the minor groove structure are sequence dependent features intrinsic to the MD model and the force field, since no disposable parameters are chosen with respect to any of these properties.

The MD modeling indicates that the molecular origin of sequence-dependent curvature lies largely in the roll angles. This result agrees with conclusions from the recent energy minimization studies of Sanghani *et al*. (1996) on d($A_4T_4CGA_4T_4$), d($A_4T_4GCA_4T_4$), d($T_4A_4CGT_4A_4$) and d($T_4A_4GCT_4A_4$) duplexes using JUMNA (Lavery, 1988) and a continuum electrostatic treatment of the solvent. Sanghani *et al*. (1996) reported large, positive roll angles in the central G-C or C-G steps and also T-A steps in both d($A_4T_4NNA_4T_4$) and d($T_4A_4NNT_4A_4$) sequence. The roll angles at the center of the A_4T_4 elements were on the order of -6° in JUMNA, considerably lower than what we see from the MD average (Figure 4), and closer to the inferences from solution studies for A-tract structures (Bolshoy *et al*., 1991; Haran *et al*., 1994; Ulanovsky *et al*., 1986; Ulanovsky & Trifonov, 1987).

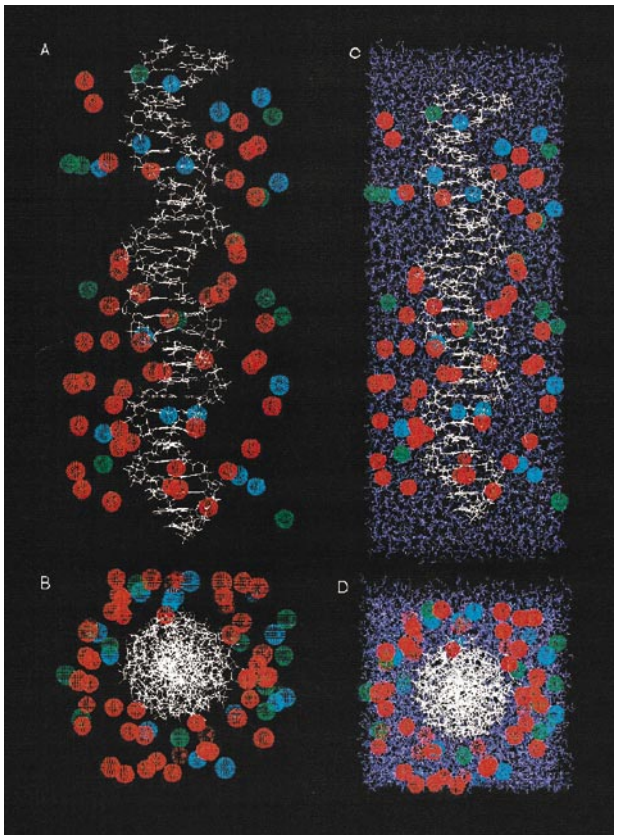

Figure 4. A, The starting structure for the d(G_5-(GA_4T_4C)$_2$-C_5) simulation showing the initial random placement of ions; B, the same structure, looking down the helix axis. C, the same structure after solvation with ~6500 TIP3P water molecules; D, the same structure looking down the helix axis. K^+ are represented as red dotted spheres, Mg^{2+} as green, and Cl^- as cyan.

Average values of helicoidal parameters for each step found in the MD structures differ with key values of the model developed by Bolshoy *et al*. (1991), which was particularly successful at predicting the macroscopic curvature of a number of known sequences. Their model generated an account of observed DNA curvature in terms of average roll, tilt and twist parameters for each of the unique ten dinucleotide steps, assigned on the basis of a linear regression analysis of gel and cyclization data for XY sequences. The key trait of the Bolshoy *et al*. (1991) model is strong negative roll for A-A steps, whereas the A-A steps in the MD simulations reported here are, on average, relatively straight. With regard to some other highly bent steps in the MD, the Bolshoy *et al*. (1991) model attributes the T-A step a roll of only 0.8°, which is clearly not in accord with the TA step seen in the d(G_5-(GT_4A_4C)$_2$-C_5) simulation, nor does the model suggest a strong roll for the C-G step. The data set used by Bolshoy *et al*. (1991) can be fit equally well by a number of other helicoidal values, including one with straight A-tracts

(Y. Lui & D. L. Beveridge, unpublished results). Another dinucleotide step model for DNA bending proposed by DeSantis (1990, 1992) is based on conformational energy maps. Like the Bolshoy *et al.* (1991) model and the observations of Sanghani *et al.* (1996), a strong negative roll for A-A steps is presumed. Unlike the Bolshoy model, the DeSantis *et al.* (1990, 1992) helicoidal set incorporates a strong positive roll for the T-A step, much like we see in our current simulations.

The MD simulations described here support the view that A-tracts in DNA sequences in solution are essentially straight, consistent with the results from crystal structures (see Young *et al.*, 1995, and references therein). In the two other structures we have studied, there are five or six adenine bases in a row (Young & Beveridge, 1998), and there is a significant locus of curvature at the 5′ end of the A-tract. While it has been suggested that this location of bending is not consistent with an A-tract centered minor groove bending direction (Dickerson *et al.*, 1994; DiGabriele & Steitz, 1993; Goodsell *et al.*, 1993, 1994; Grzeskowiak *et al.*, 1993; Haran *et al.*, 1994), our results illustrate how this is possible through the additive effects of bending at numerous other positions in the sequence. In the structure of d(G_5-(GA_4T_4C)$_2$-C_5), there are five base-pairs between the high roll angle at the C^{14}-G^{15} step and each of the A^{10}-T^{11} or A^{20}-T^{21} steps at the centers of the two A-tracts. Hence, to measure the overall curvature from the reference frame of the A^{10}-T^{11} or A^{20}-T^{21} steps at the centers of the *A*-tracts we rotate through five twist angles or roughly 170°. Viewed from this reference frame at the center of either A-tract, the additive effect of the two loci of bending positions the net direction of curvature directly into the minor groove. Thus, whereas the type of curvature loci seen in our d(G_5-(GA_4T_4C)$_2$-C_5) simulation and previous *A*-tract simulations is similar to that seen for single isolated A-tract junctions in crystal structures, the net direction of curvature is actually in accord with inferences from solution measures (Bolshoy *et al.*, 1991; Brukner *et al.*, 1993, 1994; Crothers & Drak, 1992; Crothers *et al.*, 1992; Dlakic & Harington, 1995) which assign the net direction to be towards the minor groove at the center of the *A*-tracts.

The crystal structure of the molecule d(CGCAAATTTGCG) is the closest sequence to be solved crystallographically to the d(G_5-(GA_4T_4C)$_2$-C_5) sequence we have simulated (Edwards *et al.*, 1992). The values for the helicoidal parameters for this structure were retrieved from the NDB (Berman *et al.*, 1992). Unlike the structure of the A-tract sequence in the simulated molecule, the crystal structure of d(CGCAAATTTGCG) exhibits strong positive roll angles within the central A-tract, (7.6° for the A^5-A^6 step) and negative roll-angles in the flanking sequences (−6.2° for G^2-C^3 and −9.3° for G^{10}-C^{11}). The structure of d(G_5-(GA_4T_4C)$_2$-C_5) from simulation is thus quite different from the structure of the related d(CGCAAATTTGCG) sequence solved *via* X-ray crystallography.

Young *et al.* (1995) determined the average roll, tilt and twist angles for the 16 possible (ten unique) dinucleotide steps from crystallography. Comparing these results for A-tracts with those from the MD simulations described here, the MD A-A steps are relatively straight and agree with observed A-A steps in crystal structures, where roll and tilt are seen to be 0.7(±4.8) deg. and −1.1(±3.6) deg., respectively. However, MD results on other steps diverge from those of crystal structures. For C-G steps, a roll angle of 3.7(±6.6) deg. and tilt angle of 0.0(±4.4) deg. is observed for oligonucleotides in crystals (based on 99 observations), whereas the MD results for C^{15}-G^{16} step show an average roll angle of 12.8(±6.4) deg. for the d(G_5-(GA_4T_4C)$_2$-C_5). The T-A step shows roll value of 12.5(±5) deg. in MD, compared with 1.9(±5) deg. (12 observations) in crystals. However, high roll values for the G-G steps are seen in both our MD models (Figure 3) and the crystal structures, in which the roll value is 6.6(±5) deg. (34 observations). In MD, the G-G steps closest to the *A*-tracts show the strongest positive rolls. Specifically, G^4-G^5 in the d(G_5-(GA_4T_4C)$_2$-C_5) shows an average roll of 6.9° and $C^{25}C^{26}$ ($G^{35}G^{36}$) shows a roll of 6.4° For the G^2-C^3 step roll drops to 4.0° and roll for $C^{29}C^{30}$ ($G^{31}G^{32}$) is 4.2°. Though the standard deviation of the angles (4-5°) places all of these G-G steps seen in simulation within agreement with one another, this trend is leading evidence that there is a higher order context effect beyond the dinucleotide step level.

If a dinucleotide model is deemed insufficient to predict DNA curvature, an emerging question is how the A-tracts might alter the conformations of nearby base-pairs. A possible explanation is that the distinct minor groove structure of A-tracts sets up a structural disturbance which is propagated into the neighboring base-pairs and influences their structures. An example of this emerging from the MD is the way in which the minor groove at the 5′ terminus of an A-tract is abnormally wide compared to normal *B*-DNA, in addition to the previously reported narrowing of the A-tract minor groove. This widening of the minor groove at the 5′ terminus must be propagated out into surrounding base-pairs, which allows sampling of higher roll values. This induced minor groove widening mechanism could explain how a sequence of base-pairs is curved only when adjacent to A-tracts themselves. This mechanism is also consistent with the experimental observation that phased A-tract dependent DNA curvature is only marginally influenced by the content of flanking sequences (Haran *et al.*, 1994). This model shares similarities to the junction (Koo *et al.*, 1986) or tilt (Diekmann, 1986) models, but does not invoke an *H*-DNA or a *B*′-DNA form for the A-tract. This view is consistent with previous simulations which find G-rich segments to be conformationally flexible (Young & Beveridge, 1998) when contrasted

with the more rigid and structurally distinct A-tracts. We extend this view to suggest that the G-rich sequence flanking A-tracts are malleable, and the structural distortion of being adjacent to the abnormally wide 5′ A-tract minor groove allows them to sample high roll angles. This is seen here and has been reported by Sanghani *et al.* (1996).

The proposed mechanism for A-tract dependent curvature (Young & Beveridge, 1998) can explain several other experimental observations. Ligands such as distamycin and berenil widen the minor groove and reduce curvature, as seen in gel anomaly experiments (Barcelo *et al.*, 1991; McCarthy *et al.*, 1993). The base analog 2,6-diamino-adenosine differs from adenine only in that it has a bulky amino group in the minor groove, which potentially could prevent minor groove compression. This base analog will reduce phased A-tract curvature when substituted for an adenine (Diekmann *et al.*, 1987; Koo & Crothers, 1987). In contrast, inosine (I), which is identical with guanine other than the absence of a minor groove amino group, does not disrupt phased A-tract curvature nearly as much. A possible explanation is that I can accommodate the compressed minor groove more than G or 2,6-diamino-adenosine (Diekmann *et al.*, 1987; Koo & Crothers, 1987).

Summary

MD simulations based on all-atom, explicit solvent, nanosecond time scale were carried out to obtain dynamic models of the sequences d(G_5-(GA_4T_4C)$_2$-C_5) and d(G_5-(GT_4A_4C)$_2$-C_5) in solution. The structure of the d(G_5-(GA_4T_4C)$_2$-C_5) sequence exhibited a strong gross curvature, consistent with the experimental studies of Hagerman (1986). The most significant locus of curvature in the MD structure was found at the central C^{15}-G^{16} step, with an average roll angle of 12.8(±6.40) deg. In comparison with the d(G_5-(GA_4T_4C)$_2$-C_5) sequence, the structure of the d(G_5-(GT_4A_4C)$_2$-C_5) sequence exhibited significantly reduced gross curvature. The reduction in gross curvature in the d(G_5-(GT_4A_4C)$_2$-C_5) structure originates from bending centered at the T^{10}-A^{11} and T^{20}-A^{21} steps, each of which showed average rolls values of 12.5(±5) deg. Because these three steps, T^{10}-A^{11}, C^{15}-G^{16} and T^{20}-A^{21}, are half-helix turns away from one another, their contributions to the overall bend cancel out. The A-tracts are themselves on average relatively straight, in accordance with crystal structure data. The MD structure of d(G_5-(GA_4T_4C)$_2$-C_5) exhibited minor groove deformation in the A-tract region consisting of an expansion at the 5′ end which rapidly progresses to a relative narrowing while moving towards 3′ end. This result supports the minor groove narrowing interpretation of hydroxyl radical footprinting data advanced by Berkhoff & Tullius (1988).

These simulations compliment recent MD simulations on other A-tract sequences from this laboratory (Young & Beveridge, 1998). In the MD model, A-tracts are essentially straight, but non-A-tracts are not, as a rule, curved either. Rather, it appears that A-tracts set up local distortions which are propagated into the surrounding flanking sequences, inducing them into adopting high positive roll angles and other structural perturbations. The roll angle effects are most pronounced at the junctions, but propagate for several steps into the flanking sequences (Young & Beveridge, 1998). This model is consistent with experimental data, but does not exclude the possibility of other interpretations.

Computations

Molecular dynamics simulations were performed on duplexes of d(G_5-(GA_4T_4C)$_2$-C_5) and d(G_5-(GT_4A_4C)$_2$-C_5) using the AMBER suite of programs (Pearlman *et al.*, 1995) and the Cornell *et al.* (1995) force field. These duplexes are representative examples of the d(XA_4T_4Y)$_n$ and d(XT_4A_4Y)$_n$ motifs, where $n = 2$. Six base-pairs flanking the A-tracts on either end isolate the motifs of interest from end effects. The AMBER utility NUCGEN was used to create canonical, uniformly straight *B*-DNA starting structures for the two sequences. Inorganic counterions and coions, 12 Mg^{2+}, 48 K^+ and 14 Cl^-, were randomly added about the starting structures subject to the requirement that no added ion be closer than 5 Å to any other atom in the system (see Figure 4). The Mg^{2+} parameters were obtained from the Weiner *et al.* (1984) force field. The concentration of added salt in the MD corresponds to 10 mM Mg^{2+}, 70 mM Cl^-, and 50 mM K^+, in addition to that required for neutralization of the DNA sugar-phosphate backbone. The salt concentrations correspond closely to the conditions of a ligase buffer, and match those of a simulation performed in this laboratory (Young & Beveridge, 1998). The DNA-ion complex was solvated with ~6500 water molecules described by the TIP3P model (Jorgensen, 1981a,b,c) using the AMBER edit utility. Final box dimensions were 120 Å × 45 Å × 45 Å. The minimization, heating, equilibration and production dynamics protocols have been described (Young *et al.*, 1997a,b). The PME method was used to treat long range electrostatics (Cheatham *et al.*, 1995; Darden *et al.*, 1995; York *et al.*, 1993). Production dynamics were performed under constant pressure and temperature conditions with target values of 1.0 atmosphere and 300 K, respectively. The total simulation lengths for d(G_5-(GA_4T_4C)$_2$-C_5) and d(G_5-(GT_4A_4C)$_2$-C_5) were 3.0 ns and 2.5 ns, respectively.

Analysis of the results involved visual inspection of MD structures using computer graphics, plots of the helicoidal parameters associated with structure and curvature, tabulating overall curvature as the ratio of helix end-to-end distance over helical axis path length, evaluating the minor groove width, and inspecting the helicoidal parameters which define *B*-form DNA. Curvature models of DNA are commonly based on the helicoidal parameters roll (positive bending into towards the major groove), tilt (bending towards the phosphodeoxyribose backbone) and twist (Bolshoy *et al.*, 1991; Calladine & Drew, 1986; Ulanovsky & Trifonov, 1987; Zhurkin *et al.*, 1979). Analysis was performed using the MD Tool Chest suite of programs (Ravishanker & Beveridge, 1998) with Curves (Lavery & Sklenar, 1996) used to calculate all helicoidal parameters for the MD structures and helix

end-to-end distances. Groove width calculations are based the algorithm proposed by Boutonnet *et al*. (1993).

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