Flexible Structure of DNA: Ion Dependence of Minor-Groove Structure and Dynamics

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Abstract: The structure and dynamics of the grooves of DNA are of immense importance for recognition of DNA by proteins and small molecules as well as for the packaging of DNA into nucleosomes and viral particles. Although there is general agreement that the minor groove of DNA varies in a sequence-dependent manner and is narrow in AT regions, alternative models have been presented to explain the molecular basis for the groove narrowing. In one model the groove narrowing results from direct, short-range interactions among DNA bases. In this model the minor groove width of a given sequence is fixed, and any localization of monovalent cations in the groove does not affect the groove structure. In an alternative model the narrow minor groove of A-tracts is proposed to originate from sequence-dependent localization of water and cations. Ion dynamics and exchange make experimental tests of these models difficult, but they can be directly tested by determining how DNA minor-groove structure responds to cation positions in the course of molecular dynamics (MD) simulations. To carry out such a test, we have conducted a long MD simulation on the sequence $d(CGCGAATTCGCG)_2$ in the presence of ions and water. We have analyzed the major structures that exist and the correlation between ion population and minor groove width. The results clearly show a time-dependent influence of ion positions on minor groove structure. When no ions interact with the groove, the groove is wide. Ion−water interactions narrow the groove through two distinct interactions: (i) ions interact directly with the DNA bases in the minor groove, such as cross-strand thymine oxygens (O2) in the sequence above, to give an internal ion-spine of hydration, or (ii) ions interact with phosphate groups in the AT sequence while water molecules in the minor groove interact directly with the bases. Some variations on these limiting models are possible in a dynamic DNA−water−ion structure, but it is clear that ion and water interactions at AT base pair sequence sites are required to yield the observed narrow minor groove in AT sequences.

Introduction

Crystallographic analysis of B-DNA, beginning with structures of $d(CGCGAATTCGCG)_2$ by Dickerson and co-workers,[1,2] demonstrated that the minor groove of DNA varies in a sequence-dependent fashion and is more narrow in A-tract sequences than in GC containing regions. Water molecules were observed in the minor groove of the crystal structure and these "structural" water molecules were termed the "spine of hydration". The concept of sequence-specific variations in local conformation and hydration has been supported by NMR,[3] chemical foot-printing,[4] and theoretical calculations.[5] The observation of these sequence-specific effects raises the obvious and important question of their molecular origin. Two different models are being used by those involved in the area to explain the groove narrowing.

One model for the conformational heterogeneity in the minor groove emphasizes direct, short-range interactions among DNA bases as the molecular interaction driving the sequence effects on DNA structure.[1,2,6] In this model the minor groove is narrow whether ions are locally present or not. A major modification of this base-dependent model incorporates ions and electrostatic interactions into the origins of DNA conformational heterogeneity.[7] The inclusion of ions in the model was suggested by recent crystallographic,[8−11] NMR,[12] and theoretical[13,14] results. In the electrostatic model, the narrow minor groove of A-tracts is proposed to originate from sequence-dependent localization of cations. In other words, an A-tract sequence should have a wide minor groove, due to repulsion of the negative phosphate groups in the absence of direct ion interactions. Crystallographic investigations of $d(CGCGAATTCGCG)_2$ at high resolution with different ion environments by the Williams group have dem-

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onstrated that monovalent cations are intrinsic components of minor-groove solvation.[8−10] In other words the spine of hydration can contain ions in addition to water. In this model specific sites in the minor groove can be partially populated with water and monovalent cations. Recent analysis of a crystal structure of d(CGCGAATTCGCG)$_2$, crystallized in the absence of monovalent cations,[6] as well as conclusions from a ^{23}Na NMR study of the same sequence,[15] however, have raised questions about the influence of ions on DNA structure. The X-ray and NMR results have been taken as evidence for the base-dependent model for DNA structure, although questions of interpretation remain.

The electrostatic and base-dependent models make specific predictions that have not been directly addressed by either experiment or theory at this point. In the base model the minor groove width depends only on sequence and interactions with cations do not influence the structure. The electrostatic model, on the other hand, predicts that the minor groove width of a given sequence will be strongly influenced in a time dependent manner by the positions of cations. As noted above, it is now clear from a number of experimental and theoretical analyses of the sequence d(CGCGAATTCGCG)$_2$ in the presence of different ionic environments that monovalent ions can replace water molecules in the minor groove in AT sequences. This still leaves open the question of whether these ions influence the DNA minor groove structure. Because of ion dynamics, multiple ion interactions with nucleic acids, and the averaging effects of ions bound at different sites, it is difficult experimentally to define specific site−ion interactions, particularly for monovalent ions, or their consequences for DNA structure. The question then is how does the DNA minor-groove width of d(CGCGAATTCGCG)$_2$ respond in a time-dependent fashion to monovalent cation positions during ion dynamics around the minor groove?

It is possible to directly address the question of the dependence of minor groove structure on ion interactions through theory and we have carried out a number of molecular dynamic (MD) simulations on the sequence d(CGCGAATTCGCG)$_2$ in the presence of ions and water to test the two models of minor groove structure. The test is powerful since the models predict quite different structural results during ion dynamics: (i) the base centered model predicts no significant correlated change in groove structure as ions move to and away from groove sites; (ii) the electrostatic model predicts that the groove will locally widen as ions move away from a minor groove site and will narrow as they move closer. A second question is how these ions interact with the minor groove in a dynamic fashion and the effect, if any, of "structured" water molecules on the minor groove structure. To address these questions, we have evaluated the correlations of ion and water positions with the minor groove width of the d(CGCGAATTCGCG)$_2$ duplex at the ApA, ApT, and TpT steps during a long MD simulation. MD simulations in the AMBER software package, when coupled with methods such as particle mesh Ewald (PME) for handling long-range electrostatic interactions,[16] yield stable trajectories in long MD simulations and have had excellent success in prediction of complex nucleic acid structures and interactions that agree with experimental results.[5,13,14,17−19] Therefore, we have used

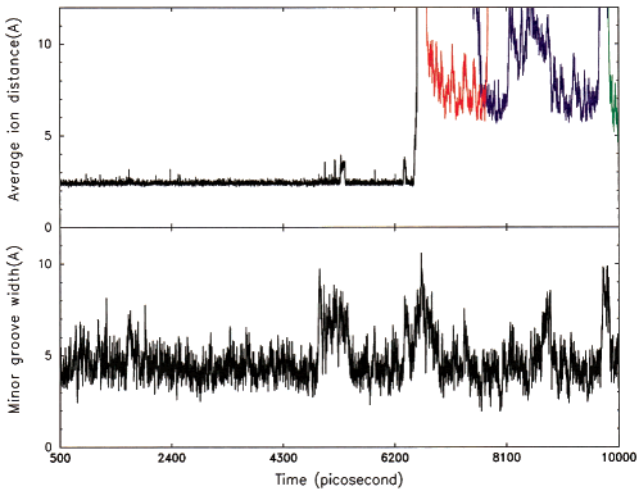

Figure 1. Minor groove width and Na^+ positions for the ApT sequence. The *x*-axis represents time in picosecond; the *y*-axis shows distances in Å. (Top) The black line represents the average of the distances of an ion in the minor groove from the cross-strand thymine oxygens (O2) at the ApT step. The red, blue, and green lines represent three different ions closest to the two phosphate−oxygen pairs (cross-strand O1P−O1P) around the ApT step at those time periods. Plotted are the average distances of the ions from the four central phosphate oxygen atoms on P7, P8, P18, and P19 that point into the minor groove around the ApT step. (Bottom) The time development of the minor groove width at the ApT step.

AMBER 5.0 and the force field of Cornell at al.[20] with the PME method[16,21,22] for our MD simulations. Many important questions have been addressed by taking average structural and energetic parameters from MD simulation results rather than thorough analysis of time-dependent structural fluctuations throughout the MD trajectory. In this work we are specifically interested, however, in the time-dependent influence of ion positions on minor groove structure.

The time-dependent MD results clearly show a dramatic influence of monovalent cation locations and interactions on DNA conformation, particularly on minor groove width, as well as on DNA dynamics. The results show some sequence-dependent effects of groove bound water molecules on the minor groove width, explain how different conclusions about ion influences on minor groove width arose from the different experiments described above, and provide a model to unify all results.

Results

DNA Minor Groove Width: Time Dependent Correlation with Na^+ Positions. We initially performed a qualitative evaluation of a 10-ns MD simulation to determine what major ion interactions with AT base pair sites in the minor groove were observed and what minor groove structures were associated with these interactions. Results from the simulation are illustrated with plots of the DNA minor groove width and ion interactions at the ApT step of the d(CGCGAATTCGCG) duplex (Figure 1) and the structures obtained at key times points are shown in Figure 2. During the equilibration steps of the

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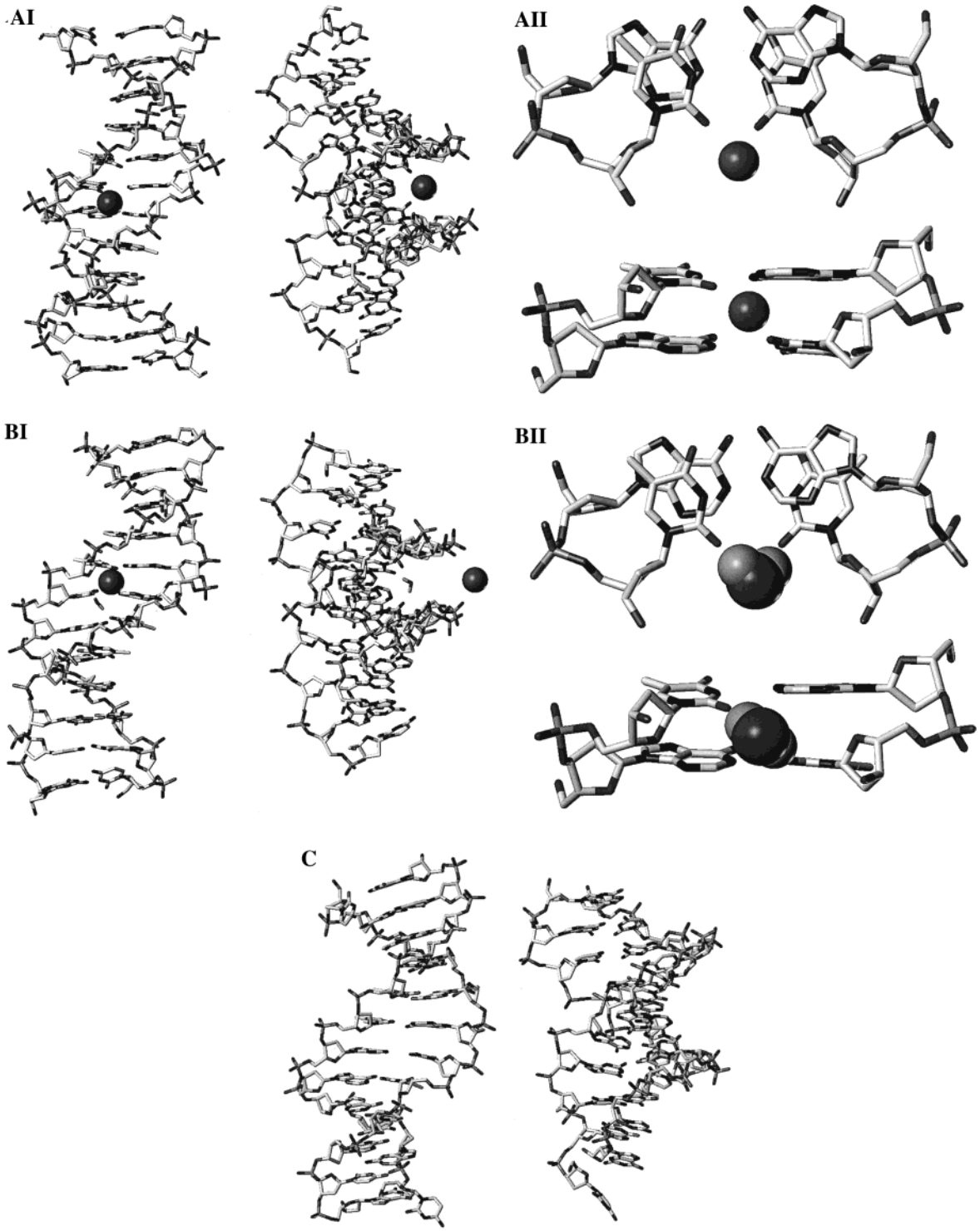

Figure 2. (A) The d(CGCGAATTCGCG) duplex with a narrow groove and a sodium ion coordinated at the ApT step. (I) The DNA is shown in stick representation and the ion in space-filling size. The view on the left is directly into the central minor groove. In the view on the right, the model on the left was rotated 90° counterclockwise and tilted 30° to show the ion in the minor groove. (II) The base pair views are of the central ApT step. The bottom view is directly into the minor groove while the top view is down the helix axis. (B) The DNA duplex with a phosphate−oxygen pair−sodium ion interaction and a water molecule coordinated at the ApT step.(II) Similar views to those in Figure 2A are shown for the phosphate−ion−water−base complex at the AT site. (C) The d(CGCGAATTCGCG) duplex with a wide groove, no coordinated water molecules at the AT base pairs, and no phosphate−sodium ion interactions. The duplex views are as described for Figure 2A.

simulation, a Na^+ enters the minor groove and interacts with cross-strand T bases for over 6000 ps. The ion is coordinated to the T carbonyl oxygens at the ApT step and is also loosely coordinated to the cross-strand sugar oxygens (O4′) as well as water molecules in the minor groove and the structure of this complex is illustrated in Figure 2A. The groove undergoes a transient thermally induced widening near the 1700−1800-ps time region (Figure 1). During that period the localized Na^+ at the ApT step remains in the groove but loses its optimum coordination position, from the cross-strand O2s of T (Figure 2A), and has increased dynamics.

From ∼1800−5000 ps the Na^+ remains localized at the ApT step and the groove remains narrow (average width of ∼4.3 Å versus 5.9 Å for B-form). At this point the groove again opens

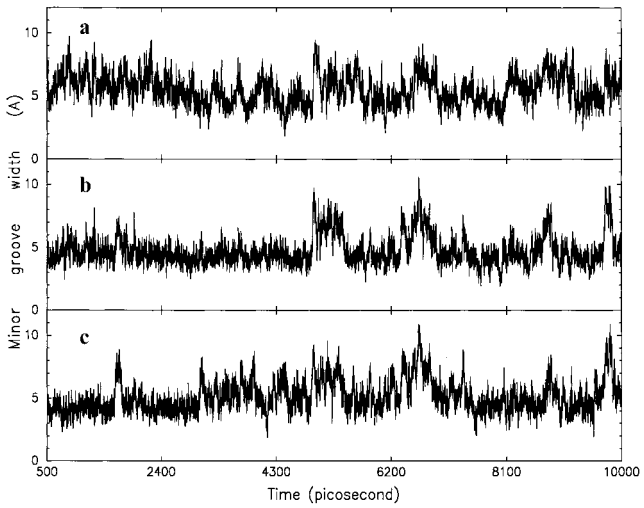

Figure 3. Time-dependent fluctuations of the minor groove width of the d(CGCGAATTCGCG) duplex at the (a) ApA, (b) ApT, and (c) TpT steps for the first 10 ns of the simulation.

due to thermal motions and remains wide (∼6.8 A) until approximately 5500 ps. In this time region, although the localized ion remains in the minor groove, it again loses its optimum ApT coordination. From 6400 to 6600 ps the groove again opens and the ion undergoes large dynamic motions. At ∼6600 ps, the ion moves farther away from the bases and forms an interaction with the cross-strand phosphate oxygens of the ApT sequence at ∼6700 ps. The ion interacts with phosphate groups until almost 7800 ps, and during this period, a water molecule in the groove enters the site to interact with the AT base pairs and the structure of this complex is shown in Figure 2B. During this period the minor groove is narrow but somewhat more dynamic than in the first 5000 ps of the simulation. Before the ion finally dissociates from the ApT site at ∼7900 ps, a second ion approaches the site. During the next 2000 ps, the second Na^+ remains associated with phosphate groups at the ApT step with water in the groove also as illustrated in Figure 2B. As thermal motion moves the ion farther from the phosphate oxygens, the groove opens in the 8700−8900 ps time range but closes again as the ion moves closer to the phosphate groups. When the groove is narrow, water molecules bridge the two strands by interacting with the cross-strand T carbonyl oxygens at the ApT step and cross-strand N3:O2 of the adjacent ApA and TpT sites. However, when the groove opens up, the strong interactions of the spine of hydration are lost and the water molecules interact more weakly with the individual strands. At approximately 9700 ps the second ion dissociates from the phosphate groups at the ApT step, and shortly thereafter, the groove opens with loss of water coordination. A view of the wide minor groove structure in the AT site, without tightly interacting ions, is shown in Figure 2C. Another ion approaches, however, and as it forms phosphate interactions, the groove again closes down with recoordination of water at the ApT step as shown in Figure 2B.

Figure 3 compares the time-dependent fluctuations of the minor groove width at the ApA, ApT, and TpT steps. The minor groove width varies from 3.0 to 10.0 Å and is strongly correlated with ion−phosphate interactions. Time-dependent fluctuations of the groove width at these sequences are weakly coupled. The average groove widths at the ApA, ApT, and TpT steps over the first 10 ns of the MD simulation are 5.4, 4.7, and 5.2 Å, respectively. To determine any dependence of the structural observations on the initial equilibration protocol, a 5-ns simulation was started from the 10-ns structure in the first simulation (Figure S1). No new structures were observed in this second simulation; however, the complex shown in Figure 2B was the dominant species in the simulation and was the major component in narrowing the minor groove. In summary, these results show that there are two major modes of interaction of Na^+ with the minor groove that cause a significant reduction of the groove width: (i) direct interaction of the ion with cross strand bases deep in the groove that is most favorable at the AT site (Figure 2A) and (ii) ion interactions with opposite strand phosphate groups that narrow the groove in association with water−base interactions (Figure 2B). Quantitative analyses of the effects of Na^+ on the minor groove are presented below in terms of these two distinct complexes. When there are no ions and no coordinated water molecules interacting with the minor groove, it is wide as shown in Figure 2C. During the entire simulation, the base coordinated structure, as shown in Figure 2A, is significantly populated only at the ApT step of the d(CGCGAATTCGCG) sequence. The phosphate−ion, water−base structure in Figure 2B is common to ApA, ApT, and TpT sites and appears to be the dominant species in narrowing the groove.

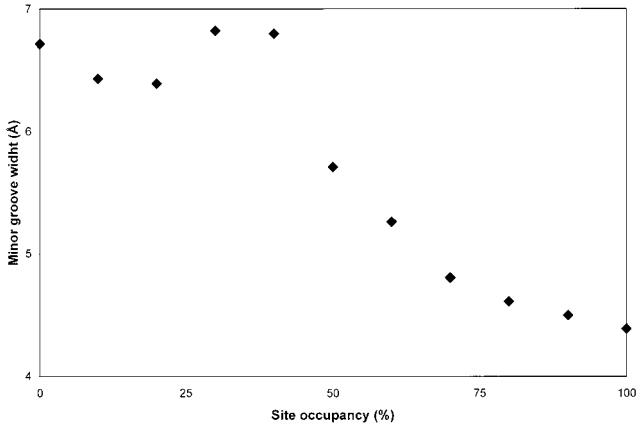

Figure 4. Correlation of base coordinated Na^+ at the ApT step and minor groove width (this model is shown in Figure 2A). The sodium ion is considered to be coordinated if the average distance from the ion to the two cross-strand oxygens (O2) of thymine is within 2.5 Å. The simulation time region (500−6600 ps) was divided into overlapping 10-ps segments, each offset by 1 ps. On the *x*-axis is the site occupancy over the 10-ps segment and the *y*-axis is the average minor groove width of all 10-ps segments with the same occupancy.

Correlation of Base−Na^+ Interactions and Minor Groove Width. As described in the Methods, the first 500 ps of the simulation were required to reach a stable trajectory and were discarded. In the next section of the trajectory, up to 6600 ps, a Na^+ was in the groove near the ApT step. To quantitatively evaluate the correlation of Na^+ interactions on minor groove width, the simulation time region from 500 to 6600 ps was divided into overlapping 10-ps segments, each offset by 1 ps. The average Na^+ ion occupancy of the ApT site and the minor groove width were determined for each segment as described in the Methods. The sodium ion was considered to be coordinated to the bases in the minor groove (Figure 2A) if the average distance from the ion to the cross-strand oxygen (O2s) of T was less than or equal to 2.5 Å (this 2.5-Å value is estimated based on distances from X-ray structural results[10]). The results for minor groove width and ion interactions in this section of the simulation are plotted in Figure 4 for the ApT step. The results show that when the ion moves away from the bases and loses coordination at the site, that is 0 total site occupancy during the 10-ps time period, the minor groove is

wide with a width of approximately 6.5 Å. When a Na^+ is tightly coordinated to the cross-strand O2s of T at each time point (100% total site interactions), the minor groove is narrow with a width of approximately 4.4 Å. The relatively flat region from 0 to 40% occupancy is caused by the fact that as thermal motions widen the groove, the Na^+ transiently can remain within the cutoff allowance to the T bases. This plateau region is reduced if a shorter cutoff distance is used as shown in Supporting Information for a 2.45 Å cutoff (Figure S2).

Correlation of Phosphate Oxygen−Na^+ Interactions and Minor Groove Width. The effects of cation-phosphate oxygen interactions on the minor groove of DNA at the ApA, ApT, and TpT steps were analyzed over the 6600−15 000-ps time region of the simulation as described in the Methods. The region evaluated is at the top of the minor groove (Figure 5) and is designed to capture ion−phosphate interactions of the type shown in Figure 2B but to eliminate the interactions shown in Figure 2A. The *d* value in Figure 5 can be varied to change the size of the space at the top of the groove that is included in the analysis. A 10-ps sliding window was used in the analysis as in Figure 4. The results were normalized to a 0−100% occupancy of the site by Na^+ ions. The minor groove width at the ApA site decreases progressively from near the B-form value of 5.9 Å down to 3.6 Å as the occupancy increases from 0 to 100% (Figure 6A). The TpT step behaves similarly to the ApA step as shown in Figure 6C. At the ApT step, Figure 6B, the minor groove width decreases progressively from approximately 5.1 Å at 0% total site occupancy to 3.6 Å at 100% site occupancy when only the effect of the sodium ions interacting with the phosphate oxygen pairs was considered. These results clearly demonstrate a direct minor groove width dependence on sodium ion interactions of the type shown in Figure 2B.

To determine the molecular basis for the groove width difference for the ApT versus ApA and TpT at the 0% occupancy point, we next looked at the effects of coordinated water on the groove widths. When the groove width is calculated for the array of structures at the ApT site that have no water coordinated directly to the bases (determined as described in the Methods) and no Na^+ at the phosphates (0% occupancy), the average groove width increases to 6.5 Å. A similar analysis for the ApA and TpT sites indicated that the average groove width also increases to approximately 6.5 Å when structures with no water or Na^+ interactions are present. Water interactions alone can thus narrow the groove at all these sites but the effect is larger at the ApT site than at the ApA and TpT sites (ApA and TpT sites at 5.7 Å and ApT at 5. 1 Å at 0% occupancy). The results are not strongly influenced for windows widths for 10−100 ps and *d* values for 3−5 Å. Results are shown in Figures S4 and S5 (Supporting Information) for cases where the sliding window was increased to 50 ps and the *d* value raised to 5 Å, respectively.

Minor Groove Structure Variation and Na^+ Interactions. To better visualize the positional influence of Na^+ interactions on the minor groove width of d(CGCGAATTCGCG)$_2$, the groove width was determined as a function of cross-strand cation−phosphate oxygen pair interactions as described in the Methods section. The minor groove width of d(CGCGAATTCGCG)$_2$ is compared in Figure 7 for cation−phosphate interactions at P5−P21, P7−P19, and P8−P18. The ion−phosphate interactions at P5−P21 cause the minor groove to become narrow at that site and get progressively wider to the other end of the groove. Similarly we see narrowing of the minor groove when there are ion−phosphate interactions at P7−P19 and P8−P18. Clearly the minor groove is most narrow at the site of ion interactions and in each case the minor groove progressively widens away from the site of interaction. Similar specific effects of ions on minor groove width were observed by Feig and Pettitt[14] for a different AT base pair DNA sequence.

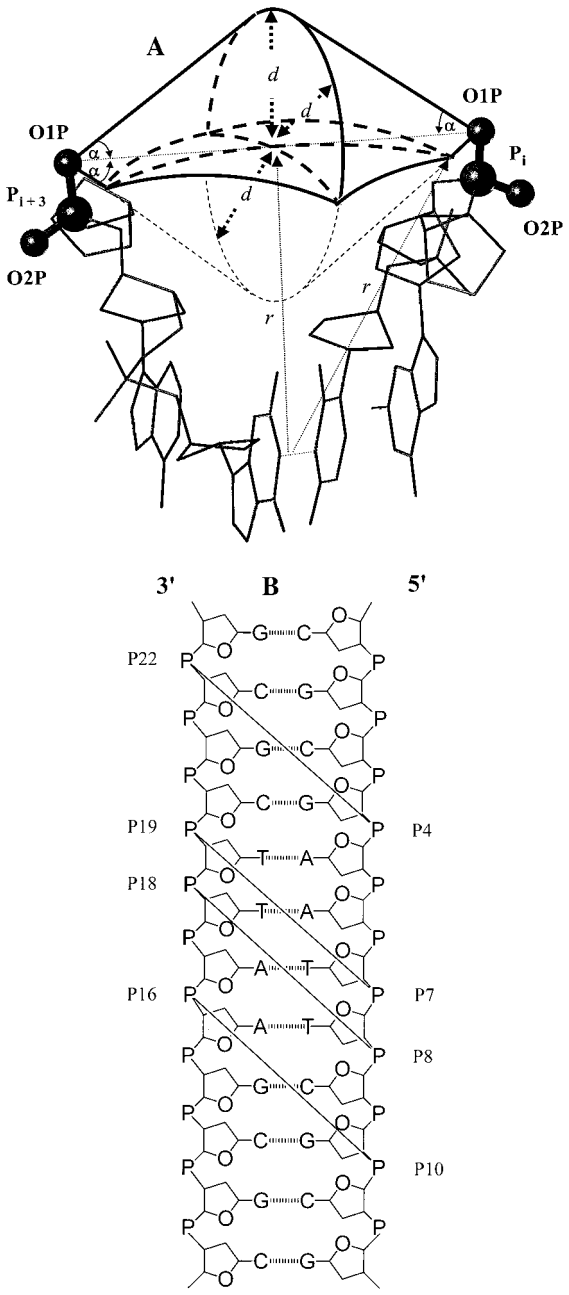

Figure 5. Model for calculation of Na^+−site interactions for the structure shown in Figure 2B. Schematic representation of a defined space relative to two cross-strand phosphate oxygen atoms (O1P), P_i on one strand and P_{i+3} on the other strand. (A) An ion is considered to interact with the cross-strand phosphate oxygen atoms if its center lies within the space at the top of the groove that is defined by the solid lines. The space is defined by taking the midpoint of the two cross-strand oxygen atoms and defining a variable distance, *d*, from the midpoint. To isolate the top half region of the double cone, a locus of all points with distance equivalent to the length from the center of the base pair to the midpoint of the oxygen atoms was defined. This distance can be used to define a sphere of exclusion with its center at the base pair center and a radius, *r*. If the center of an ion lies out of this sphere and is within the top half of the double cone, a cross-strand phosphate oxygen−Na^+−phosphate oxygen interaction is counted. (B) Some examples of the cross-strand phosphate atoms that represent the shortest distances across the minor groove are shown for illustration.

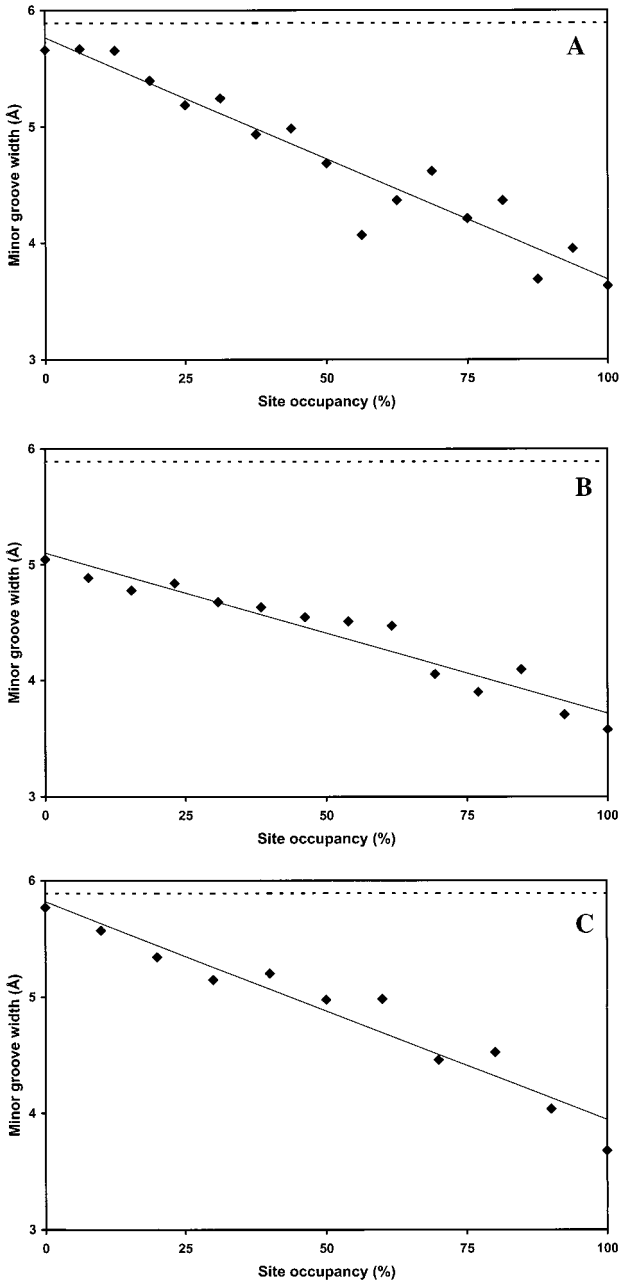

Figure 6. Correlation plot of phosphate oxygen−Na^+−phosphate oxygen interactions at the (A) ApA, (B) ApT, and (C) TpT steps. (A model is shown in Figure 2B.) The simulation time region (6600−15000 ps) was divided into overlapping 10-ps segments, each offset by 1 ps. Ion−phosphate interactions were determined as described in Figure 5 with a *d* value of 3.0 Å. The *x*-axis shows site occupancy in percent and the *y*-axis represents minor groove width in angstroms.

Discussion

We initiated the MD studies reported here to test the two alternative models, described in the Introduction, for the narrow minor groove structure in AT base pair sequences of DNA. The base interaction model predicts that there will be no significant influence of ion position on minor groove width, while a model that includes ion interactions predicts that the minor groove will be narrow when ions interact and wide when they do not. While it is difficult to directly test these two models experimentally, modern molecular dynamics methods provide a powerful test for the alternative predictions of the two models.

The first questions that we wish to address in this work are

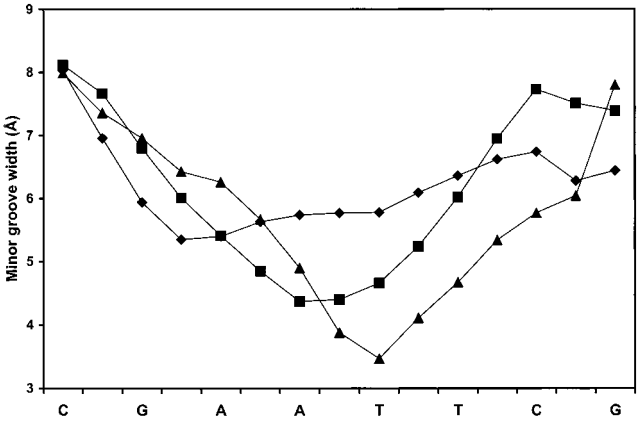

Figure 7. Plot of minor groove width along the d(CGCGAATTCGCG) duplex for different ion−site interactions. The graph represents structures with exclusive phosphate oxygen−Na^+−phosphate oxygen interactions with oxygen atoms on P5−P21 (◆), P7−P19 (■), and P8−P18 (▲). The interactions were determined as described in the Methods.

what ion interactions with the minor groove in AT sequence dominate, how do these interactions affect the structure of the groove, if at all, and what is the structure of the ion complexes with the minor groove in AT base pair sequences? A 15 ns MD trajectory was obtained for the sequence d(CGCGAATTCGCG)$_2$ that has been structurally analyzed in detail starting with the pioneering crystallographic work of Dickerson and coworkers.[1,2] In all trajectories and as illustrated in Figures 1 and 2, two major types of Na^+ interactions with the minor groove at the central AATT sequence are observed: (1) interaction of Na^+ with cross-strand phosphate oxygens and a spine of hydration that interacts with A and T bases (Figure 2B) and (2) direct interaction of Na^+ with cross-strand thymine oxygen (O2) groups at the ApT step (Figure 2A). Both of these interactions result in a narrow minor groove. A relatively rare species had no Na^+ interactions, no structured water molecules at the AATT site, and a wide minor groove (Figure 2C). The DNA structure at 10 ns in Figure 1 was used to initiate a 5-ns additional MD analysis of the d(CGCGAATTCGCG) sequence and the time-dependent results of the minor groove width are shown in the Supporting Information (Figure S1). As in Figure 1, the minor groove is predominately narrow throughout the 5-ns time period and the major ion interaction is with phosphate oxygens as in Figure 2B. These results and experimental observations discussed below strongly suggest that the structure shown in Figure 2B is the dominant ion interaction for producing a narrow minor groove. Water is an intrinsic component of the narrow minor groove in Figure 2B and it is the cooperative interaction of water and ions with a particular sequence that results in a narrow groove in AT base pair sequences in this structure. It should be emphasized, however, that when both ion complexes are dissociated, the minor groove is wide as illustrated in Figure 2C.

The next questions that we wished to address were whether a quantitative correlation exists between ion interactions and minor groove width and what correlation exists between ion location at a particular site and groove width at that site. The total 15-ns trajectory shown in Figures 1 and S1 was divided into two regions: one dominated by base−Na^+ interactions from 500 to 6600 ps and one dominated by Na^+−phosphate interactions from 6600 to 15000 ps. Correlations between ion interactions and minor groove width are shown in Figures 4 and 6. It is clear for both of these regions that when there are ion interactions with specific sites, the minor groove is narrow at

those sites and when there are no ion interactions, the groove is significantly wider and approaches a "B-form" type minor groove structure.

The shapes of the curves for these two regions that represent complexes 2A and 2B (Figure 2) are different, probably due to the significantly different dynamics for DNA bases and phosphate groups. The difference in base and phosphate group dynamics is illustrated by the plot of atomic positional fluctuation in Figure S3. Similar results have been observed by Feig and Pettitt and are supported by experimental observations.[14] The phosphate interaction model follows a linear dependence of average width on ion interactions (Figure 6). The base interaction model has a more cooperative appearance (Figures 4 and S2). As an ion dissociates from the base interactions, there is a transient period when the groove remains narrow, even though the ion has moved outside of the cutoff distance. In the same manner, the groove transiently remains wide as an ion enters the cutoff distance due to the response time of DNA bases. The DNA phosphate groups have significantly lower response time and greater motional dynamics than the DNA bases (Figure S3) and the more linear plot is obtained for phosphate ion interactions. It is clear in both cases, however, that the minor groove width is strongly correlated with ion position. In both cases the groove is wide when there are no ion interactions and narrow when there are interactions. The plots of groove width versus ion interaction site (Figure 7) strongly re-enforce this view. The groove width is always most narrow at the ion interaction site and widens as you move away from that site. Again, similar results have been observed by Feig et al. for a different DNA sequence.[14]

The inescapable conclusion for the above results is that ions have a profound effect on minor groove structure and that a narrow minor groove structure cannot exist for any significant length of time without ion interactions. This model is in agreement with a broad array of other experimental and theoretical studies and provides a molecular basis for reduction of electrostatic repulsion of phosphate groups across the narrow minor groove. The presence of monocations in the minor groove in the AT sequence was observed theoretically by Beverigde and co-workers, by NMR studies of Hud et al. and Halle et al., and in X-ray studies by Williams et al. and Egli et al. The importance of electrostatic effects on DNA structure is also supported by experiments in which selected phosphate groups were replaced by neutral methyl phosphonates.[23] Two experimental observations, a narrow groove in a crystal structure without monovalent cations and observation of a low percentage of monovalent cations bound tightly with the minor groove by NMR have raised questions about the importance of ion interactions on DNA structure. Our model, however, predicts that the complex shown in Figure 2B with Na^+−phosphates and base−water interactions is the dominant species for producing a narrow minor groove in the AT sequence. The monovalent cations in this structure could also be replaced by divalent cations to narrow the minor groove. Such ion−DNA interactions are more dynamic than ion−base interactions (compare regions in Figure 1) and specific phosphate−ion interactions would not be detected in either X-ray or NMR experiments. The models presented in Figure 2A,B are, however, required to produce a narrow minor groove, and without these interactions, the groove is wide as in Figure 2C. These models bring all experimental and theoretical results into agreement and demonstrate that the minor groove in the AT sequence is narrow due to sequence-dependent ion and water interactions. Sequence alone, however, is not sufficient to yield a narrow groove in the absence of these interactions.

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Methods

Molecular Dynamics (MD) Simulations. MD simulations were carried out on the d(CGCGAATTCGCG)$_2$ DNA duplex in water with more than enough salt to neutralize the phosphate charges (32 Na^+ and 10 Cl^-), NaCl concentration of approximately 0.15 M. In a typical simulation a DNA duplex was surrounded by counterions and approximately 4000 water molecules, using the Leap module within AMBER,[24] to fill a periodic box size of approximately 45 Å × 45 Å × 60 Å. All MD simulations were performed using the SANDER module of AMBER 5.0 on several Silicon Graphics Origin servers. The Cornell et al. all-atom force field for DNA and counterions[20] and the TIP3P model for water[25] were used for all the simulations. In order not to generate any bias in the results, the starting structures were canonical B-DNA, generated using the SYBYL software package rather than specific PDB files. All simulations were carried out in the NPT ensemble with periodic boundary conditions, at constant temperature of 300 K and a pressure of 1 bar. The SHAKE[26] algorithm was applied to all bonds involving hydrogen atoms with a tight tolerance of 10^{-6}, and an integration time step of 2.0 fs was used. The coulomb interactions were treated with particle mesh Ewald (PME) methods[21,22,27,28] and van der Waals interactions were subjected to a 9-Å cutoff.

The following equilibration protocol was applied to the system before the actual MD runs. 500 kcal/mol restraints were placed on the DNA. The water and counterions were minimized for 1000 steps, followed by 25 ps of 300 K MD, which allowed the solvent to relax. This was then followed by five rounds of 600 step minimization on the entire system, starting with a 25 kcal/mol restraint on the solute and reducing it by 5 kcal/mol during each round. During the final step, with no restraints, the entire system was slowly heated from 100 to 300 K over 10 ps and then equilibrated for another 10 ps at 300 K. The coordinates (trajectories) were written to output at 1-ps intervals. The temperature of the solute and solvent, the potential energy, and the kinetic energy were monitored throughout the entire simulations to make sure that they remained constant. To avoid "the flying ice cube" phenomena,[29,30] a tight SHAKE tolerance was used and nonbonded pairlists were updated at every step.

Data Analysis. The simulation converged within 0.5 ns and the first 0.5 ns of results were discarded. The coordinates of the entire system at each time point (1 ps) of the output trajectories were saved, automatically extracted, and saved in PDB format using a combination of programs in C++ and Unix shell scripts that were written for this purpose. The time-dependent structures at each picosecond were analyzed with the CURVES program.[31]

Correlation of the Minor Groove Width and Sodium Ion Occupancy. For any time period, *t*, in the simulation the local average minor groove width was determined by eq 1.

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$$GW_t = \frac{1}{n}\sum_{i=1}^{n} gw_{t-i+1} \quad (1)$$

where n is the number of periods (picosecond) in the moving average, gw_j is the actual groove width at time j, GW_t is the tth point of the moving average of the groove width, t goes from n to N where N is the final picosecond. The total number of GW_t points is therefore ($N - n + 1$).

The total number of water molecules or sodium ions that occupy a particular site within the t time period is given by eq 2

$$O_t = \sum_{i=1}^{n} P_{t-i+1} \quad (2)$$

where site occupancy, P_j, is 0 if none of the sodium atoms or water molecules satisfies a particular condition, 1 if only one sodium atom or only one water molecule satisfies a particular condition for being bound, 2 if only two sodium atoms or only two water molecules or one sodium atom and one water molecule satisfy a particular condition for being bound, etc. at j picosecond. O_t is the total site occupancy of sodium atoms and/or water molecules over n picoseconds.

A plot of AGW versus O_t (in percent) is made, where AGW is the average groove widths of all GW_t with the same occupancy (O_t) over n picoseconds. O_ts are considered insignificant and not plotted if the number of all GW_t with the same occupancy (O_t) is less that 0.25% of ($N - n + 1$).

Base Coordinated Sodium Ion in the Minor Groove at Specific Sites. For each picosecond, the minor groove width at a particular site was calculated and if the average distance of the sodium cation was within m Å from the two cross-strand interacting atoms in the minor groove, the sodium ion was consider to be coordinated and given a site occupancy, P_j, of 1; otherwise it is 0 (Figure 2A). Throughout this period, a moving average, over n ps (wherein n can take any value, e.g., 10 or 20 ps), of the minor groove width was calculated and the total sodium ion site occupancy, O_t, for the n-ps window calculated. A plot of AGW versus O_t (in percent) was made.

To determine if Na^+ was coordinated at the ApT step, the average distance of sodium ions to the cross-strand thymine oxygen atoms (O2) was measure and m was given a value of 2.5 Å. This could also be done for the ApA/TpT step by determining the average distance from the N3 of adenine on one strand and O2 of thymine on the other strand.

Cross-Strand Phosphate Oxygen−Na^+ Interactions. To evaluate the effect of sodium ions around the cross-strand phosphate oxygen atoms on the DNA minor groove width, the number of interacting sodium ions whose center lies within the upper half of the space diagrammed in Figure 5, relative to the cross-strand oxygen pair, was determined for each time point and correlated with the minor groove width. The interstrand oxygen atom, O1P, on the phosphate atom of base pair i is closest to the oxygen atom on the phosphate atom of base pair $i + 3$ on the opposite strand. In terms of the two phosphates of the DNA molecule studied, the pairing can be represented a follows; P4−P22, P5−P21, P6−P20, P7−P19, P8−P18, P9−P17, P10−P16, and P11−P15. The correlation of the sodium ions and minor groove width was done on the two phosphate oxygen pairs on either end of ApA, ApT, and TpT sites of the $d(CGCGAATTCGCG)_2$ DNA duplex. P6−P20 and P7−P19 are on either end of ApA, P7−P19 and P8−P18 are on the either end of ApT, and P8−P18 and P9−P17 are on either end of TpT. The correlation method is illustrated using the ApT site. For any sodium ion interaction, that is, within the defined a space relative to the oxygen atoms for the cross-strand O1P pair of P7−P19 or that of P8−P18, P_j is increased by 1 and the average minor groove width over the ApT step is calculated. For example, if there were two sodium ions within the defined space the phosphate oxygen pair at the jth time, P_j would be equal to 2. A moving average, over n ps (where n can take any value, $n = 1-N$), of the minor groove width was calculated, and the total sodium ion occupancy, O_t, for the n-ps window was calculated. A plot of AGW versus O_t (in percent) was made.

Also, water molecules coordinated at the ApT, ApA, and TpT steps in the minor groove combined with the sodium ions interacting with the phosphate oxygen pair and the minor groove width were correlated by a similar procedure. For each jth ps, if there was a water molecule coordinated to the O2s of thymine at the ApT step (N3 of adenine and O2 of thymine for the ApA and TpT steps), 1 was further added to P_j. If there was no water molecule coordinated with the bases or no sodium ion interacting with either of the cross-strand oxygen (O1P) pairs, P_j was equal to 0. A moving average, over n ps, of the minor groove width was calculated and the total sodium ion occupancy, O_t, for the n-ps window was calculated.

Correlation of Minor Groove Structure and Interacting Sodium Ion Positions. For each simulation the goal was to isolate coordinates (structures) for analogies that had cation interactions only with a particular phosphate oxygen pair. Therefore, there could be nine possible types of O1P pair−cation interactions. The different possibilities are as follows: ions interacting exclusively with either the O1P phosphate oxygen pair of P4−P22, P5−P21, P6−P20, P7−P19, P8−P18, P9−P17, P10−P16, or P11−P15 or not interacting with any of them at all. Consequently, structures wherein ions were interacting with multiple phosphate pair were excluded, so that the effect of the cations on a particular site could be evaluated. The total number of structures with multiple interactions was generally small (less than 5%). If a sodium ion is within the defined space relative to the cross-strand phosphate oxygen pair (Figure 5), it is considered to interact with that site and that coordinate set was grouped with structures of similar ion interactions exclusively to that site. The minor groove width for each structure along the duplex in each set was then calculated and averaged over all structures in that set.

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Supporting Information Available: Figures showing the time-dependent fluctuations of the minor groove width of the d(CGCGAATTCGCG) duplex from 10 to 15 ns of the simulation, correlation of base coordinated Na^+ at the ApT step and minor groove width as in Figure 4, atomic positional fluctuations of $d(CGCGAATTCGCG)_2$ for the first 10 ns of the simulation, and a correlation plot of phosphate oxygen−Na^+−phosphate oxygen interaction as in Figure 6 for different values of d and overlapping time segments. This material is available free of charge via the Internet at http://pubs.acs.org.

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