VOLUME 123, NUMBER 32
AUGUST 15, 2001

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Influence of the Dynamic Positions of Cations on the Structure of the DNA Minor Groove: Sequence-Dependent Effects

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Received February 7, 2001

Abstract: Different models for minor groove structures predict that the conformation is essentially fixed by sequence and has an influence on local ion distribution or alternatively that temporal positions of ions around the minor groove can affect the structure if they neutralize cross-strand phosphate charges. Our previous studies show that the minor groove in an AATT dodecamer responds to local sodium ion positions and is narrow when ions neutralize cross-strand phosphate−phosphate charges [*J. Am. Chem. Soc.* **2000**, *122*, 10513−10520]. Previous results from a number of laboratories have shown that G-tracts often have a wider minor groove than A-tracts, but they do not indicate whether this is due to reduced flexibility or differences in ion interactions. We have undertaken a molecular dynamics study of a d(TATAGGCCTATA) duplex to answer this question. The results show that the G-tract has the same amplitude of minor groove fluctuations as the A-tract sequence but that it has fewer ion interactions that neutralize cross-strand phosphate charges. These results demonstrate that differences in time-average groove width between A- and G-tracts are due to differences in ion interactions at the minor groove. When ions neutralize the cross-strand phosphates, the minor groove is narrow. When there are no neutralizing ion interactions, the minor groove is wide. The population of structures with no ion interactions is larger with the GGCC than with the AATT duplex, and GGCC has a wider time-average minor groove in agreement with experiment.

Introduction

The influence of environment on the dynamics and average structures of nucleic acids is of fundamental importance to their interactions with proteins, drugs, and other cellular components as well as to DNA packaging in viral particles. Although it has been recognized that ions are required for the formation of stable nucleic acid structures, the direct influence of ion interactions on sequence-specific nucleic acid conformation and dynamics is still being debated.[1−4] It is important to consider how ion and water positions might affect well-established features of DNA conformation.

Two limiting models have been proposed to explain the origins of DNA conformational heterogeneity and the roles of cations. The traditional base-clash model[5−7] assumes that DNA duplexes have sequence-dependent conformations that are not

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10.1021/ja010341s CCC: $20.00
Published on Web 07/20/2001

significantly influenced by the local positions or fluctuations of ions. The alternative electrostatic model[1,8,9] proposes that the local positions and transient fluctuations of ions impact DNA conformation and dynamics, primarily through asymmetric neutralization of phosphate charges. In this model cations would have a strong influence on time-dependent conformation. These two limiting models are somewhat difficult to test by experiment because they both predict similar average structures that agree with experimental results. Their molecular explanations for a variety of conformational states are different.

From a number of experimental and theoretical studies, it is clear that on average the minor groove of A-tracts is significantly more narrow than the minor groove of G-tracts.[10−14] The study of the three-dimensional structure of the DNA fragment, d[(CGCGAATTCGCG)]$_2$, revealed that water molecules bind at specific sites within the minor groove of the A-tract.[13,15,16] This "spine of hydration" in the AATT minor groove was established by crystal structures of Dickerson and co-workers.[15] However, penetration of monocations into the spine now has been observed in molecular dynamic studies by Beveridge and co-workers,[17] in NMR studies by Hud et al.[18,19] and Denisov and Halle,[20] and in X-ray studies by Williams and co-workers[3,16,21] and Egli and co-workers.[22] Recently, using free solution electrophoresis, Stellwagen and co-workers[23] demonstrated that monocations can preferential bind in A-tract DNA.

As noted above, these observations do not resolve issues of cause and effect, and leave open questions of the influence of ions on DNA structure. In the base-clash model the narrow minor groove of A-tract DNA sequences arises from short-range base−base interactions. Monocations might bind there opportunistically, but with little or no conformational impact. Alternatively, in the electrostatic model, ions prefer the A-tract minor groove because of electrostatics. Cations localize there preferentially, causing the groove to narrow. It has been difficult to experimentally test whether there is a correlation between DNA structure and ion location and dynamics, and which is the dependent effect. The reasons for this difficulty can be attributed to ion dynamics, complexity in determining locations of monocations by X-ray diffraction and NMR spectroscopy, and multiple competing types of ion interactions with exchange among ions involved in the different interactions. The basic question is how does DNA conformation respond to cation positions around the double helix.

In an effort to directly address this question we carried out a number of molecular dynamics (MD) simulations on the sequence [d(CGCGAATTCGCG)]$_2$ in the presence of ions and water.[24] Our goal is to test the two limiting models described above. The models predict quite different conformational consequences of ion dynamics in MD simulations: (i) the base-clash model predicts no significant time-dependent correlated changes in DNA conformation and ion positions; (ii) the electrostatic model predicts that the conformation will change significantly with changes in ion positions. The dynamics of the AATT minor groove are particularly interesting since this region has a negative electrostatic potential[25,26] and is a favored cation interaction site. The MD results clearly show an influence of monovalent cation dynamics on transient DNA conformational states.[24] The minor groove is narrow when ions interact with bases deep in the minor groove or with cross-strand phosphate oxygens (Figure 1). It is wide when ions are transiently excluded. The MD simulations also define some sequence-dependent effects of minor groove-bound water molecules on the minor groove width. The conclusion from these results is that transient ion interactions that neutralize cross-strand phosphate repulsion have profound effects on the time-dependent minor groove conformation in AATT DNA sequences. The minor groove is not narrow with any significant probability without specific ion interactions. The results are consistent with the electrostatic model and provide a molecular basis for reduction of electrostatic repulsion of phosphate groups across the narrow minor groove. They bring experimental and theoretical results into agreement and demonstrate that the minor groove in AT sequences is narrow due to sequence dependent ion and water interactions. Sequence-specific base−base interactions are not sufficient to yield a narrow groove in the absence of ion-mediated base or cross-strand phosphate electrostatic interactions.

Interestingly, McConnell and Beveridge[27] have recently reported an MD analysis of ion effects on the minor groove width of the AATT duplex and concluded that ions near the floor of the minor groove do not impact the width of the minor groove. Here we demonstrate that the differences in their conclusions and ours arise from differences in reference states and methods of analysis, rather than from differences in the MD trajectories. Our reference state[24] is limited to structures with no direct base or cross-strand minor groove−ion interactions. The AATT groove is wide in this state but is narrow in cases where ions interact with bases, deep in the minor groove, or with cross-strand phosphate groups at the top of the minor groove (Figure 1). Our results with the AATT duplex clearly indicate that AT sequences have a significantly narrow average minor groove width due to preferential localization of cations in a manner to partially neutralize phosphate−phosphate repulsion and that GGCC sequences have a wider groove width due to fewer such cation interactions. It is apparent, however, that the electrostatic model makes an additional important prediction that could not be tested in our original analysis. This prediction is that the minor groove in GC sequences should also transiently

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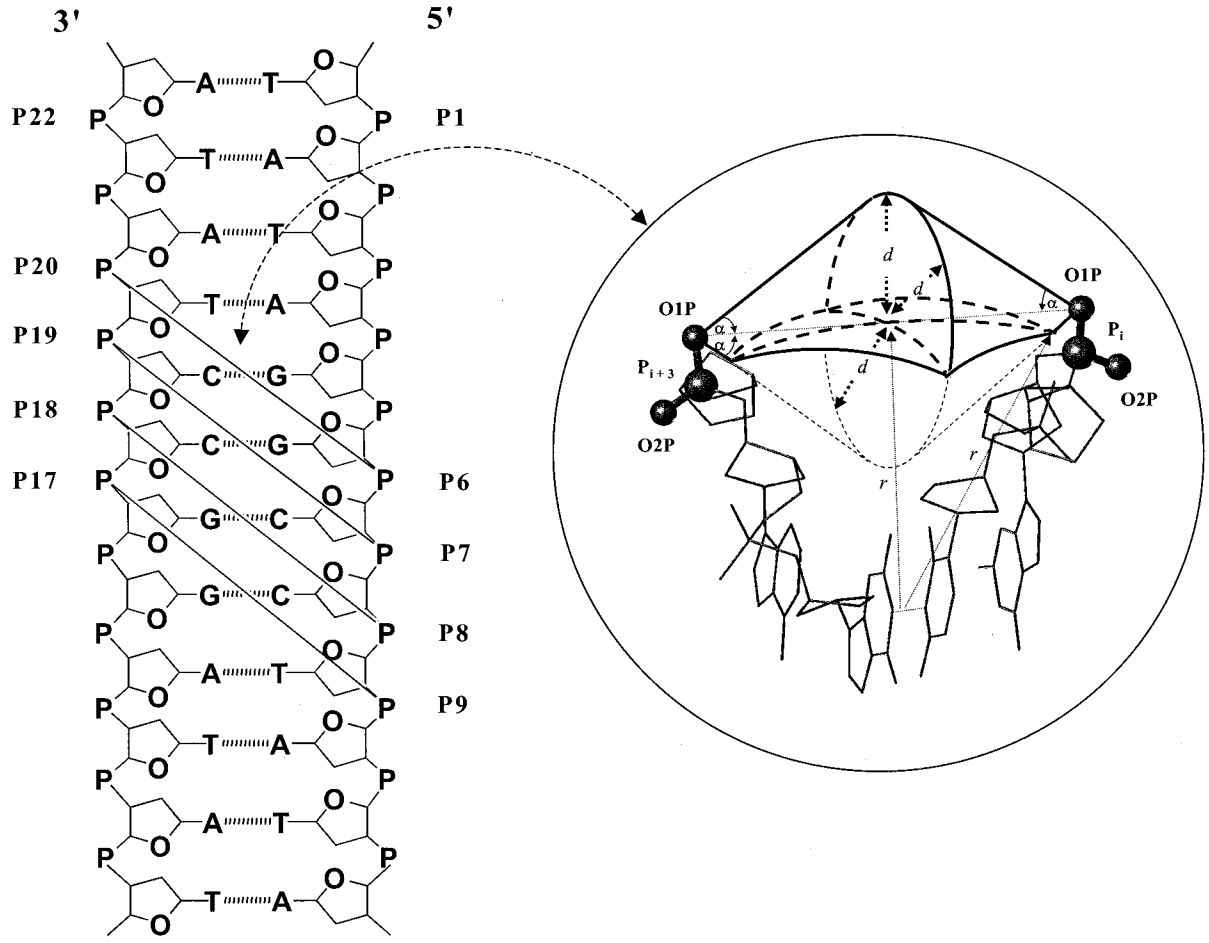

Figure 1. Method for estimating the importance of ion−phosphate interactions at the top of the minor groove. This figure illustrates the specific geometric definition of the region between two cross-strand phosphate oxygen atoms ($O1P_i$ on one strand and $O1P_{i+3}$ on the opposing strand) that is shown to be critical for groove narrowing by cations. An ion interacts simultaneously with both cross-strand phosphate oxygen atoms, and causes groove narrowing, if the ion center lies within the double cone region. The double cone region is defined in the following manner. (i) An O−O line passes between two cross-strand oxygen atoms. A perpendicular is drawn through the midpoint of the O−O line. (ii) A point on the perpendicular is defined by a distance, *d*, from the intersection with the O−O line. (iii) The double cone is defined by rotating the point around the O−O line. The parameter *d* controls the radii of the cones and was varied in a series of experiments designed to determine the effects of cation−phosphate proximity on groove width. The top half of the cone was distinguished from the bottom half. To isolate the top half of the double cone region, a locus of all points with distance equivalent to the length from the center of the base pair to the midpoint of the O−O line 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. Ion interactions with single phosphate or two phosphates on the same strand have a relatively small effect on the minor groove width and are not counted in this model.

narrow during fluctuations in cation positions that partially neutralize cross-groove phosphate repulsion.

This prediction can be tested, for example, by analysis of MD trajectories of an "inverted" duplex sequence such as $[d(TATAGGCCTATA)]_2$ (GGCC dodecamer). We report the results of such a simulation with the AMBER software suite, the force field of Cornell et al., and the particle Mesh Ewald (PME) method[28−31] for handling long-range electrostatic interactions. This study is carried out using sodium ions, which might behave slightly differently from other cations.[32,33] For example divalent cations are not as easily dehydrated as monovalent cations. It should be emphasized that in this work we are specifically interested in the time-dependent influence of ion positions on minor groove structure rather than the average groove structure. In addition, specific questions to be answered relate to the range of fluctuations of the minor groove in the GGCC dodecamer as compared to the AATT dodecamer, the structure at these limits, and the molecular basis for these structures. To minimize the influence of end effects we only analyze the central base pairs in both the AATT and GGCC duplexes.

Methods

Molecular Dynamic (MD) Simulations. The starting structure of our simulation was the canonical B-DNA[34] of $[d(TATAGGCCTATA)]_2$, generated using the SYBYL software package. The DNA duplex was solvated with approximately 4000 TIP3P water molecules[35]

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such that the solvents were placed up to 11 Å away from the duplex filling a periodic box size of approximately 45 Å × 45 Å × 60 Å. Thirty-two Na^+ and ten Cl^- counterions were placed around the DNA using LEAP module in AMBER to obtain electrostatic neutrality and a NaCl concentration of approximately 0.15 M. The simulation was carried out using the AMBER package[36] with the all-atom force field of Cornell et al..[37] We did not use the modified force field of Cornell et al. by Cheatham and Kollman[38] because we wanted to be consistent and be able to make comparisons with previous simulations that were carried out with the original force field.

The equilibrium protocol used in this simulation is similar to that described previously.[11,24,39,40] At the start of the equilibration, 500 kcal/mol restraints were placed on the DNA molecule. The water and ions 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. All simulations were carried out in the NPT ensemble with periodic boundary conditions, a constant temperature of 300 K and at a pressure of 1 bar. The SHAKE[41] algorithm was applied to all bonds involving hydrogen atoms and an integration time step of 2.0 fs was used. The Coulombic interactions were treated with the particle mesh Ewald (PME) methods, and Lennard-Jones interactions were subjected to a 9 Å cutoff. The nonbonded pair-lists were updated at every step. The total simulation length was 10 ns, and coordinates (trajectories) were written to output at 1 ps interval. The time-dependent structures at each picosecond were analyzed with the CURVES program.[42,43]

Correlation of Minor Groove Structure and Sodium Ion−Phosphate Pair Interaction. There are 9 possible types of O1P pair−cation interactions in an oligomer of this length: P4−P22, P5−P21, P6−P20, P7−P19, P8−P18, P9−P17, P10−P16, or P11−P15 (these will be referred to as site 1, 2, 3, 4, 5, 6, 7, 8, or 9). Since the goal is to isolate structures that have cation−phosphate interactions at any one site, structures wherein ions were interacting with multiple phosphate pairs 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 (see below) relative to the cross-strand phosphate oxygen pair (Figure 1), 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. The *d* value as shown in Figure 1 was varied from 1.5 to 6.5 Å. Evaluation of ion−minor groove interactions by this method is designed to test the effect of ion interactions on minor groove structure. This method is different from approaches that evaluate ion proximity to DNA atoms and that are designed to define specific ion (or water) binding sites.

Correlation of the Minor Groove Width and Sodium Ion Occupancy. The effect of sodium ions around the cross-strand phosphate oxygen atoms on the DNA minor groove width was evaluated by determining the number of interacting sodium ions whose centers lie within the upper half of the space diagrammed in Figure 1, relative to the cross-strand oxygen pair, as determined for each time point. 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. The correlation of the sodium ions and minor groove width was done on the six central phosphate oxygen pairs on either end of the $[d(TATAGGCCTATA)]_2$ DNA duplex. For any sodium ion interaction that is within the defined space relative to the oxygen atoms for the cross-strand O1P pair of P_i-P_{i+3}, the ion population, P_j, is increased by 1, and the minor groove width across P_i-P_{i+3} is calculated. For example, if there were two sodium ions within the defined space between the phosphate oxygen pair at the *j*th time, P_j would be equal to 2. A moving average as previously described by Hamelberg et al.,[24] over *n* ps (where *n* can take any value, $n = 1$ to *N*), of the minor groove width was calculated and the total sodium ion occupancy, O_t, for the *n* ps window calculated. A plot of average minor groove width versus O_t (in percent) was made.

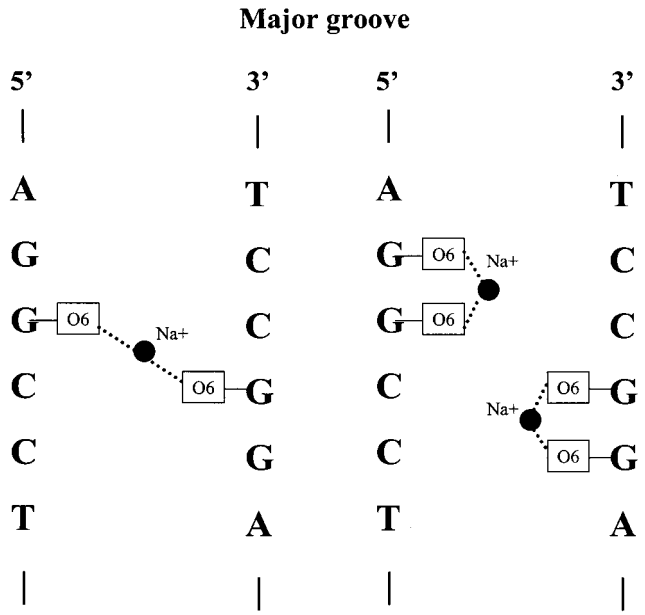

Figure 2. Schematic view of GGCC dodecamer base pair steps in the major groove and Na^+ coordinated to the two bases.

Effect of Major Groove Base-Coordinated Ions on the Structure of the DNA Duplex. To study the effect of major groove base coordinated ions on the structure of the $[d(TATAGGCCTATA)]_2$ duplex, the ion interactions at the GpC step in the major groove were defined. Both ions coordinated to the cross-strand O6s of G (Figure 2), and ions coordinated to the O6s of adjacent GpG (Figure 2) on both strands were determined and were correlated with the major groove width at the GpC step. As previously observed by Westhoff et al.,[32] ions were considered coordinated if their average distance from the two O6 oxygens was less than or equal to 3.5 Å.

Results

MD Simulation of $[d(TATAGGCCTATA)]_2$: Analysis of Structures. In this study as in the previous analysis of the minor groove in an AATT dodecamer,[24] only the central region of the duplex was considered to minimize contributions from end effects. By monitoring the trajectory for stable density and energies, the system of DNA, water and ions converged to a stable trajectory in less than 100 ps after the equilibration. As a conservative approach, however, the first 0.5 ns of the trajectory was discarded to ensure all structures analyzed were in a stable trajectory. During the course of the simulation, the DNA conformation, on average, stays close to the B-form average as can be seen from the plots of the deoxyribose phase angles of the GGCC element (Supporting Information, Figures S1 and S2) and the minor groove widths (Figure 3). However, there are occasional fluctuations of the deoxyribose phase angles

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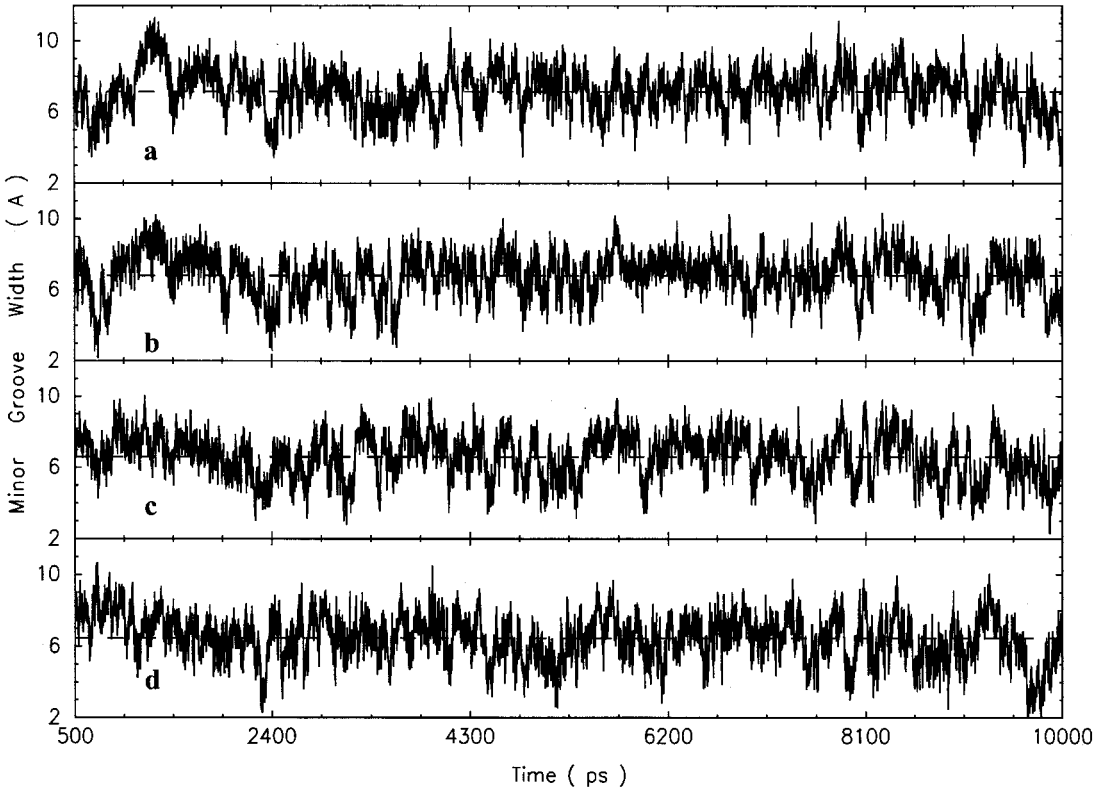

Figure 3. Time-dependent fluctuations of the minor groove width of the GGCC duplex at the (a) P6−P20, (b) P7−P19, (c) P8−P18, and (d) P9−P17 cross-strand phosphate groups, as presented in Figure 1, for the last 9.5 ns of the simulation.

to low values. Typically, only one of the sugar residues of the GGCC element converts during any one time period, and this change has little effect on the minor groove width. Figure 3, a−d, presents the time dependence of the width across the GGCC minor groove at P6−P20, P7−P19, P8−P18, and P9−P17, respectively. The minor groove width ranges from approximately 3.0 to 10.0 Å during the course of the dynamics. This variation in amplitude is similar to the dynamic range of the minor groove of the AATT duplex (Supporting Information, Figure S3). As can be seen from Figure 3, the GGCC element fluctuates between narrow and wide minor groove structures as depicted in Figure 4, a and b, respectively. The minor groove of the GGCC duplex stays wide most of the time but transiently becomes narrow. In contrast, the minor groove of the AATT duplex stays narrow most of the time and occasionally becomes wide.[24] The average minor groove width of the GGCC duplex is approximately 6.0 Å, and that of the AATT duplex is approximately 4.0 Å.

Qualitative analysis of DNA structures throughout the 10 ns simulation revealed several types of ion interactions with the GGCC element. Unlike AATT, no significant ion base−cation interactions deep in the minor groove are observed in the GGCC duplex. The predominant type of ion−DNA interaction that affects the minor groove is between Na^+ and cross-strand phosphate oxygens. When ions interact closely with cross-strand phosphate oxygens in the central region, the groove becomes narrow as shown in Figure 4a. When there are no ion−phosphate interactions across the groove, the groove is wide as in Figure 4b. There are also ion interactions in the major groove as described recently by Auffinger and Westhof.[32] In our sequence these are specific interactions of a Na^+ and N7 nitrogen and O6 oxygen of G in the major groove of the GGCC duplex. Some of the Na^+ and O6 oxygen of G interactions involved interbase O6 oxygens of adjacent GpG on both strands and interstrand O6 oxygens at the GpC step as shown schematically in Figure 2.

Furthermore, a plot of atomic positional fluctuations, which are related to the B-factors calculated in X-ray crystallography, of the GGCC duplex (Supporting Information, Figure S4) shows larger values for the phosphate groups and DNA backbone as compared to other parts of the duplex. These larger values are seen in parts of the duplex most affected by the temporal positions of cations, the lip of the minor groove. The phosphate groups are more dynamic than other parts of the GGCC dodecamer and can, therefore, respond very quickly to cation positions. This observation is very similar to that observed for the AATT duplex in our previous study and is supported by experimental observations.[24]

Cation−Phosphate Interactions at Different Sites in the GGCC Duplex. Results for sites P6−P20, P7−P19, P8−P18, and P9−P17 illustrate the effect of cations at the central sites in this sequence. To focus on effects directly linked to the minor groove interactions, all structures (less than 2.5% of the total trajectory) with direct ion or water coordination to bases in the major groove at the GpC step were excluded. Shown in Figure 5, a−d, are the minor groove widths of $[d(TATAGGCCTATA)]_2$ with cation−phosphate interactions at P6−P20, P7−P19, P8−P18, and P9−P17, respectively. As schematically shown in Figure 1, the cation phosphate interactions are defined with *d* values ranging from 1.5 to 6.5 Å.

The top region of the minor groove (Figure 1) is designed to capture cross-strand ion−phosphate interactions. The *d* value can be varied to change the size of the space at the top of the groove that is included in the analysis. The cation−phosphate interactions at site P6−P20 caused the minor groove to become narrow at that site and get progressively wider to the other end of the groove. Similarly, there is narrowing of the minor groove at sites P7−P19, P8−P18, and P9−P17 due to cation−phos-

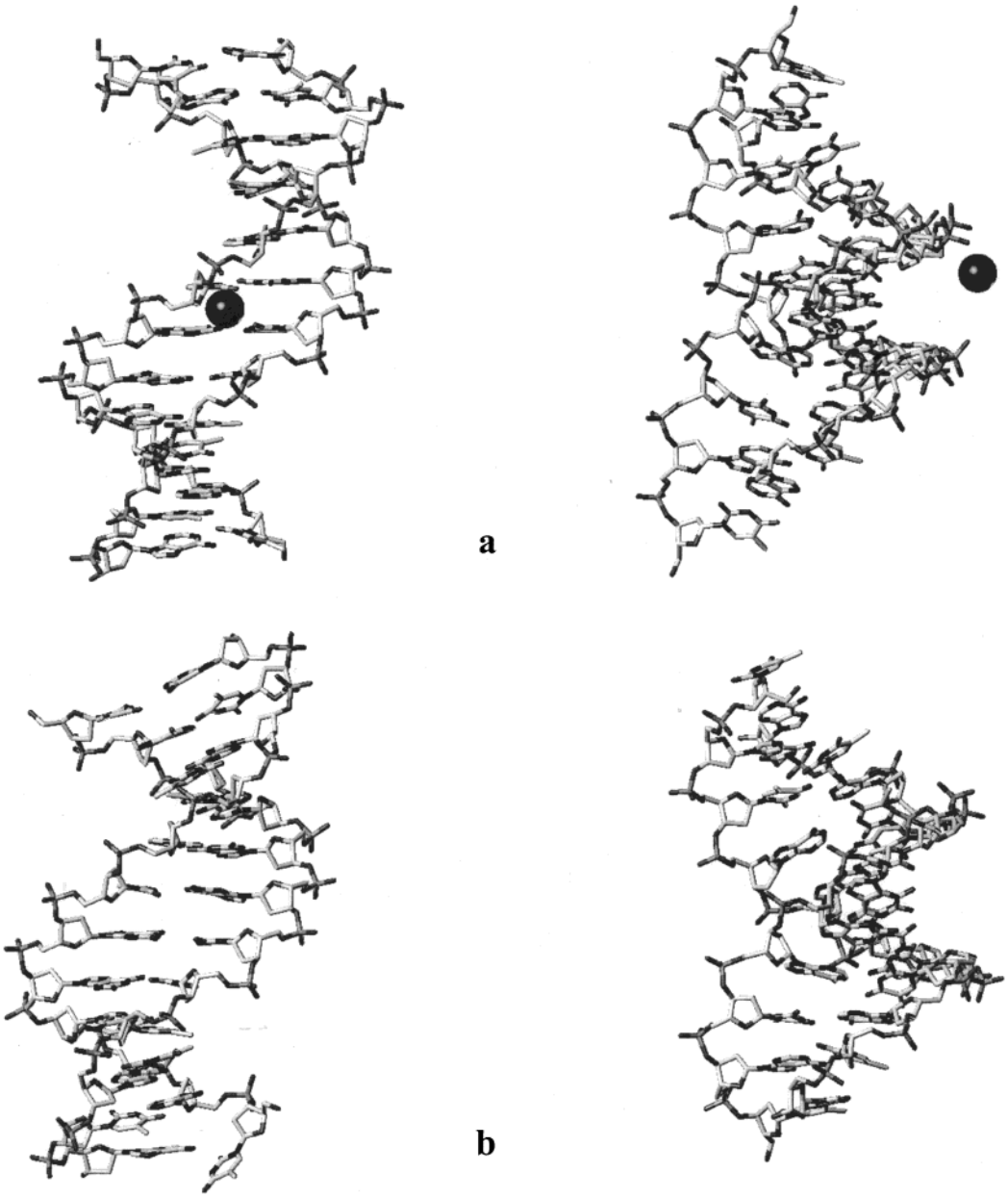

Figure 4. One structure of the d(TATAGGCCTATA) duplex during the simulation with a (a) narrow minor groove and a sodium ion interacting with the cross-strand phosphate oxygens of P8 and P18 within the region defined in Figure 1 and (b) wide minor groove. The DNA is shown in stick representation. The 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.

phate interactions at those specific sites (Figure 5). Also shown is the effect of changing the *d* value from 1.5 to 6.5 Å. As expected, cation effects on minor groove narrowing were lessened as the cutoff distance was increased. That is to say, as ions on average move closer to a particular site (Figure 1), the ion−cross strand phosphate interactions are strong and the groove narrows, while as ions move away from that site, the interactions weaken and the groove widens. The average minor groove width increases by ∼3.0 Å at all sites when the *d* value changes from 1.5 to 6.5 Å.

To compare Na^+ effects on the GGCC dodecamer with effects on the central AATT of $[d(CGCGAATTCGCG)]_2$, a constant *d* value of 5 Å was selected. The Na^+ ions interacting with these central regions throughout the simulations were determined and averaged. The results showed that throughout the simulation there were on average 19 Na^+ interactions within the 5 Å cutoff per 100 ps for the GGCC duplex as compared to 45 Na^+ per 100 ps for the AATT duplex at the top region of the minor groove as depicted in Figure 1. These numbers exclude all of the ions that interact outside of the defined region (Figure 1).

Correlation of Na^+−Phosphate Oxygen Interaction and Minor Groove Width. The average effects of cation−phosphate oxygen interactions on the minor groove of DNA at the central P−P pairs were analyzed over the entire time region of the simulation as described in the Methods. To correlate the effects Na^+−cross strand phosphate interactions and the minor groove width, a 10 ps sliding window was used with the groove−Na^+ interactions defined by Figure 1. The results, shown in Figure 6, were normalized to a 0−100% occupancy of the site by Na^+ ions. The average minor groove width decreases progressively from 7.0 to 4.0 Å as the occupancy of Na^+ ions in the region defined in Figure 1 ($d = 3.0$ Å) increases from 0 to 100%. The results are not strongly influenced by windows widths and *d* values, and results for other *d* values showed similar trends.

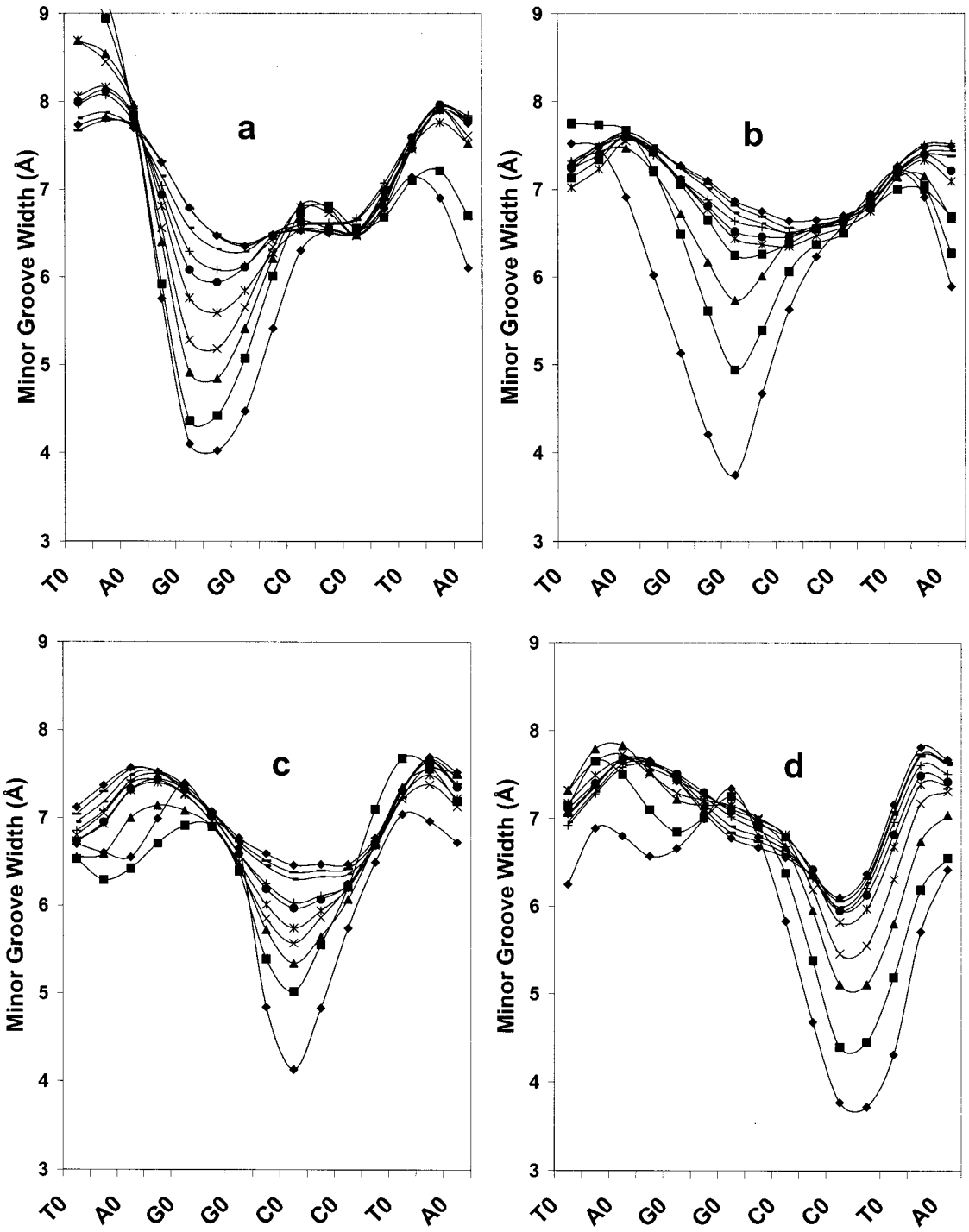

Figure 5. Plot of minor groove width along the d(TATAGGCCTATA) duplex for different ion−site interactions. The graph represents structures with exclusive phosphate oxygen−Na^+−phosphate oxygen interactions with oxygen atoms on (a) P6−P20, (b) P7−P19, (c) P8−P18, and (d) P9−P17 as schematically shown in Figure 1. The cation−phosphate interactions are defined with *d* values ranging from 1.5 to 6.5 Å in each plot. The interactions were determined as described in the Methods.

Correlation of Major Groove Coordinated Na^+ and Major Groove Width. In our previous study of the AATT dodecamer, no significant sodium ion−base coordination interactions in the major groove were observed. However, GC sequences are known to provide better major groove binding sites for cations than AT.[11,32,44−46] We, therefore, were also interested in ion−major groove interactions in our sequence as defined in Figure 2 and ion effects on the major groove width. Figure 7a is the correlation plot of Na^+ occupancy only at the GpC step defined by Figure 2, and major groove width at the GpC step. Figure 7b shows the correlation of the total ion occupancy at GpG on both strands (as shown in Figure 2) and at GpC with the major groove width at the GpC step. The results show that when the ion moves away from the bases and loses coordination at the site, that is zero total site occupancy during the 10 ps time period, the major groove has a width of approximately 15 Å.

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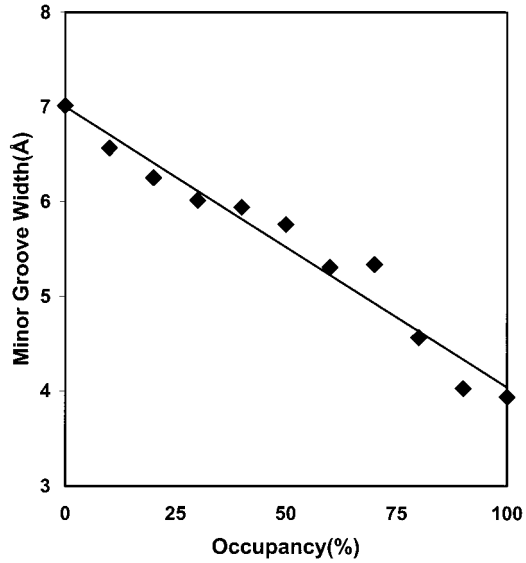

Figure 6. Correlation plot of phosphate oxygen−Na^+−phosphate oxygen interactions at the central cross-strand P−P groups (a model is shown in Figure 1) for the GGCC duplex. The total simulation time region was divided into overlapping 10 ps segments, each offset by 1 ps. Ion−phosphate interactions were determined as described in Figure 1 with a *d* value of 3.0 Å. The *x*-axis shows site occupancy in % and the *y*-axis represents minor groove width in Å.

When a Na^+ is tightly coordinated to the cross-strand O6s of G at each time point (100% total site interactions), the major groove is more narrow with a width of approximately 12 Å. When ion interactions at the adjacent GpG steps are included in the analysis (Figure 7b), the slope of the plot becomes steeper, and the lower bound is approximately 11 Å.

Discussion

General Overview. Here we attempt to observe and explain relationships between cation dynamics and local responses in DNA conformation. In our view, determining the structural origins of known relationships between sequence and conformation has broad application to questions of nucleic acid bending, folding, and global structure. It is known that on average the minor groove of A-tracts is relatively narrow, while that of G-tracts is relatively wide.[6,15] Three specific issues addressed here and in our previous work[24] relate to minor groove width and cation distribution. Our goals are to determine relationships between (i) counterion dynamics and time-dependent fluctuations in minor groove width, (ii) time-average counterion distributions and time-average groove widths, (iii) counterion distributions and DNA sequence.

We have previously conducted MD studies[24] on CGCGAATTCGCG (the AATT dodecamer), which was initially crystallized and studied in detail by Dickerson and co-workers[15]and has served as a model for many experimental and theoretical investigations. It should be emphasized that the focus of the work described here is minor groove width, not axial bending. Longer sequences and simulation times are needed to accurately examine bending of DNA. Using long DNA duplexes of 25 and 30 base pairs, Beveridge and McConnell, for example, have shown by MD that there is a correlation between DNA bending and ion distributions.[27]

Ion interactions with the minor groove have been extensively investigated by both experimental[3,16,18−20,23,47] and theoretical[17,24,32,44−46,48] methods. It is clear that monocations interact preferentially with the minor groove of A-tracts. However, previous work does not resolve whether changes in cation positions are correlated with changes in the groove width, or whether ions interact passively with preestablished conformational states that have nonelectrostatic origins. It has been proposed that the minor groove has a sequence-specific width profile that does not respond to positions of cations. In this model, groove width impacts cation distributions, but cation distributions do not impact groove width.[2]

Analysis of our previous MD simulations of the AATT dodecamer demonstrate a direct correlation of minor groove width fluctuations and transitient ion distributions.[24] The A-tract minor groove is narrow when ions are located either deep within

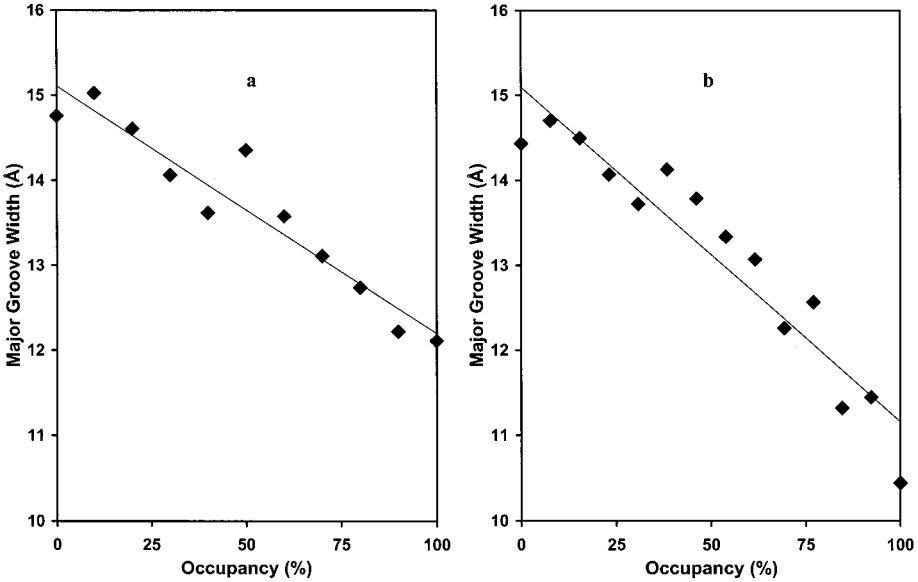

Figure 7. Correlation of base-coordinated Na^+ in the major groove (a) at the GpC step and major groove width at the GpC step (this model is shown in Figure 2). (b) Correlation of base-coordinated Na^+ in the major groove at GpG on one strand and GpG on the other strand or with the GpC step (Figure 2) and major groove width at the GpC step. The sodium ion is considered to be coordinated if the average distance from the ion to the two cross-strand oxygens (O6) of guanine or adjacent oxygens (O6) of guanine is within 3.5 Å. The simulation time region 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 major groove width of all 10 ps segment with the same occupancy.

the groove or higher up between cross-strand phosphate groups (in the region defined by Figure 1). The A-tract minor groove is wide when both of those regions are clear of cations. The width of the AATT dodecamer minor groove ranges from ~3.8 Å, when ions are proximal, for example in the region shown in Figure 1, to ~6.0 Å when ions are absent. These observations raise important questions about differences between G- and A-tracts that could not be addressed with analysis of the AATT dodecamer alone. Are differences in minor groove widths of G- and A-tracts sustained when the cation distributions are similar? If not, do time-average differences in widths correlate with time-average differences in cation distributions? Is the increased time-average width of the G-tract minor groove caused by decreased time-average cation localization?

Comparison of the GGCC and AATT Dodecamers: Here we address the structural origin of the wide minor groove of G-tracts by MD simulations of the GGCC dodecamer (the DNA duplex $[d(TATAGGCCTATA)]_2$.) A comparison of the AATT and GGCC dodecamers allowed us to distinguish differences between A-tract DNA and G-tract DNA, and to eliminate contributions from end effects. Our MD simulations are consistent with those of Beveridge and co-workers,[17] who demonstrated previously that monocations can penetrate to the floor of the groove of A-tracts, substantially dehydrate, and interact directly with base functional groups. These cations deep within the minor groove would have long lifetimes, enabling detection by NMR experiments such as those of Halle and Denisov.[20] These cations might also account for the preferential interactions observed by Stellwagen and co-workers by free-solution electrophoresis[23] Surprisingly we find that the ions deep in the groove, with long lifetimes and defined positions, are a small fraction of the total ions within the A-tract minor groove. Substantially more cations are localized higher up within the lip of the minor groove (in the region defined in Figure 1).

Our MD simulations suggest substantial differences in the cation distributions of A- and G-tracts. In comparison with the A-tract, cations show little propensity to penentrate to the floor of the groove and to interact with the base functional groups of the G-tract. In addition, cation localization is less probable higher up in the lip of the G-tract than in that region of the A-tract minor groove. The frequency of cross-strand ion−phosphate interactions (Figure 1) in the AATT dodecamer is double that of the GGCC dodecamer. In sum, cation localization is less probable at both the floor and in the lip of the G-tract minor groove than in those regions of the A-tract minor groove.

Cation Interactions with the Minor Groove of GGCC and AATT Dodecamers. A critical issue to be addressed is why, on time-average, the minor groove is narrow in A-tracts and wide in G-tracts. If one controls for the effects of cations, A-tracts and G-tracts have similar minor groove widths. The effects of a given ion distribution on groove width are similar for A- and G-tracts. Therefore, the difference in time-average groove widths arises from differences in time-average cation distributions.

For a particular ion distribution the structural effects on A- and G-tracts are very similar. When ions are located between cross-strand phosphate groups, the minor groove is narrow for both A-tracts and G-tracts as illustrated in Figures 4−6. These combined results are some of the most important of our studies. The results indicate that for both A- and G-tracts, the dynamics of the DNA and of the counterions are coupled. The amplitudes of the conformational fluctuations are similar for both types of tracts. From the time-dependent fluctuation of the minor groove of AATT and GGCC, Figures 3 and S1 show that the minor groove narrows to approximately 3.0 Å when ions fully attenuate cross-strand phosphate repulsion, and open up to approximately 10.0 Å when no ions are proximal.

It should be emphasized that there do not appear to be discrepancies between the MD trajectories published by various laboratories. Differences in conclusions appear to arise from different methods of analysis, in particular from different reference states. McConnell and Beveridge (2000) for example, recently concluded that ions near the floor of the minor groove do not have significant effects on minor groove width. We concur with that conclusion; localization of cations near the floor of the minor groove is not a good predictor of groove width. The groove width will not vary substantially as an ion transits from the floor of the minor groove to localize in the lip region. In our analysis, we divided the ion distributions into three states. In state I an ion is located deep within the minor groove in close proximity to base functional groups that line the floor of the groove. In state II an ion is located high up near the lip of the groove in a region where it is directly attenuating cross-strand phosphate repulsion (Figure 1). In state III ions are absent from the floor of the minor groove and from lip region. We have chosen state III as the reference state. With the system defined in this way the locations of ions correlate with groove width for both A- and G-tracts. For both types of tracts the minor groove is wide in state III and is narrow in states I and II. Sequence-specific differences in time-average groove width arise from differences in the time-average populations of the three states. State I, with direct ion−base interactions, can occur in A-tracts but is essentially unpopulated for G-tracts. State III is much more probable for G-tracts than for A-tracts.

In their analysis of ion−minor groove interactions, McConnell and Beveridge (2000) compared structures with direct ion−base interaction (our state I) to structures with no ion-base interactions (our combined states II and III). They noted that the average minor groove width is narrow in both cases. We have reanalyzed our AATT simulation with the same two-state method (Supporting Information, Figure S5), observed the same trend, and conclude that the trajectories for the two simulations are in good agreement. McConnell and Beveridge (2000) infer that ions near the floor of the minor groove cannot be used as a predictor of groove width. They are correct in this conclusion. However our three-state method of analysis suggests a greater importance of electrostatic interactions and ion distribution on groove width than does the two-state method of McConnell and Beveridge. It should be emphasized that the goal of our studies is to determine whether ion interactions affect the local DNA conformation, and in this case it is essential to separate ion-free and ion-bound states.

Origin of Differences in Ion Distributions. Differences in cation localization within the minor groove would be favored by differences in coordination geometry[16,17] and electronegative potential[25,26,49] of the A-tract DNA in comparison with G-tract DNA. However, these factors alone do not explain differences in cation localization higher up in the lip of the minor groove. It is probably significant that water molecules near the floor of the A-tract minor groove are restrained in position and orientation by A-tract-specific functional groups. The floor of the minor groove is lined with hydrogen-bond acceptors but lacks hydrogen-bond donors, and thus water molecules that form hydrogen

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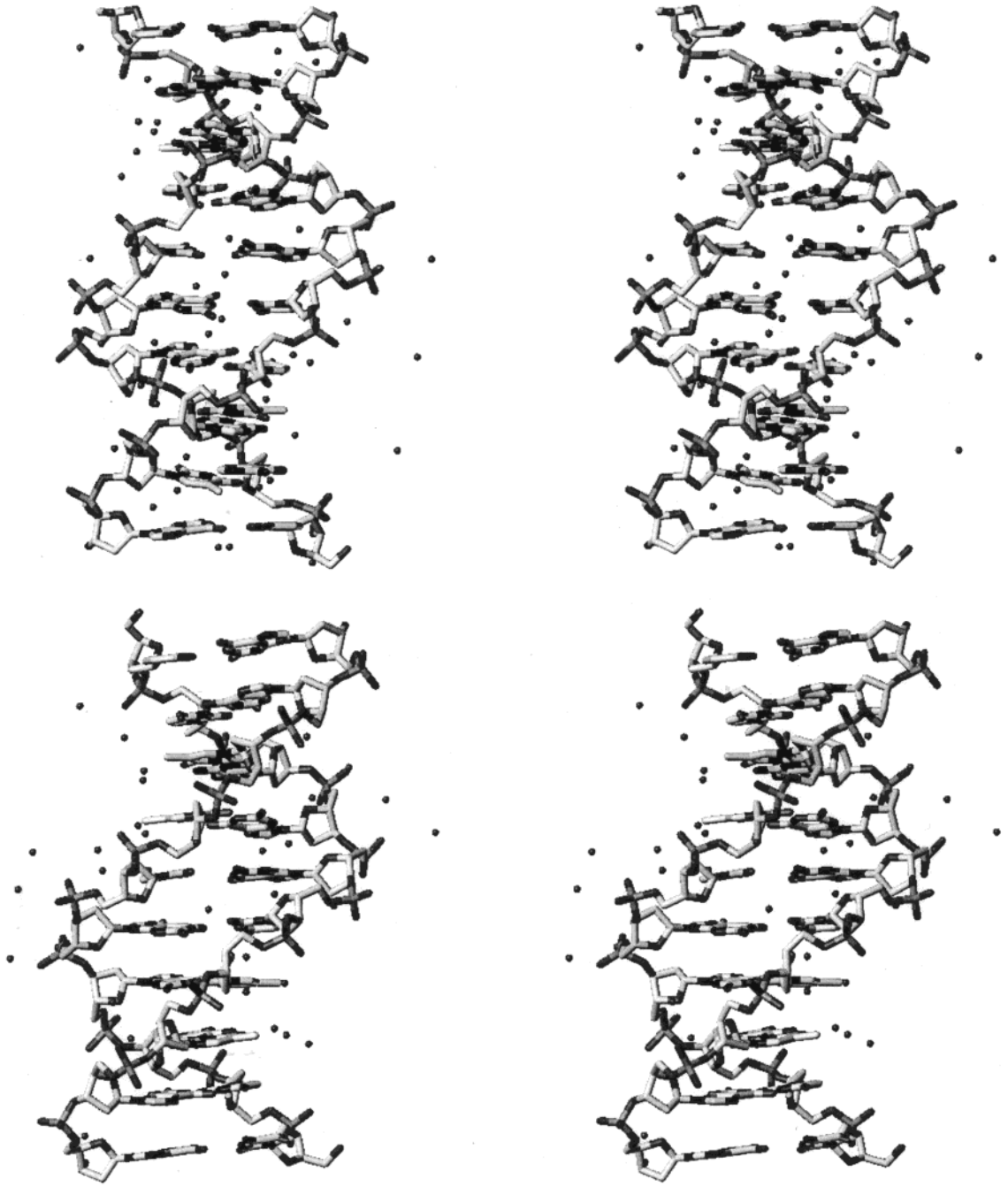

Figure 8. Two X-ray structures of GGCC duplex shown in stereoview. Top: NDB ID bdj051 with a wide minor groove structure.[52] Bottom: NDB ID bdjb49 with a narrow minor groove structure.[54] The dots were assigned as water molecules in the PDB files.

bonds with the floor of the A-tract minor groove are not free to rotate. When the groove is narrow, the restrictions on water molecule position and orientation propagate up, via a fused hexagon motif[16] to the lip region. The restraints on water orientation and position increase with the extent of groove narrowing. Therefore, it appears that base functional groups, water molecule position and orientation, groove narrowing, and cation localization act in concert to stabilize conformation states with short interstrand phosphate distances. We believe that these factors provide the structural origin for observed differences in G- and A-tract minor groove width. In other words the narrow average minor groove width of the A-tract can result from an optimization of solvent interactions, both water and ions, with specific sequences of DNA. In fact, it has been shown experimentally that a classic DNA minor groove compound, netropsin, can convert the A-form DNA to B-form DNA upon addition of the minor groove binder.[50,51] However, the GGCC dodecamer would not interact with water molecules or classical minor groove binding molecules in the same way because of the amino group of guanine in the minor groove. Thus, an appropriate DNA sequence, coordinated water, and cations appear to be required to give an average narrow DNA minor groove. Although the minor groove width fluctuations are similar for AATT and GGCC dodecamers, the AATT groove has a number of cations interacting across the minor groove higher than that of GGCC and a significantly higher population of

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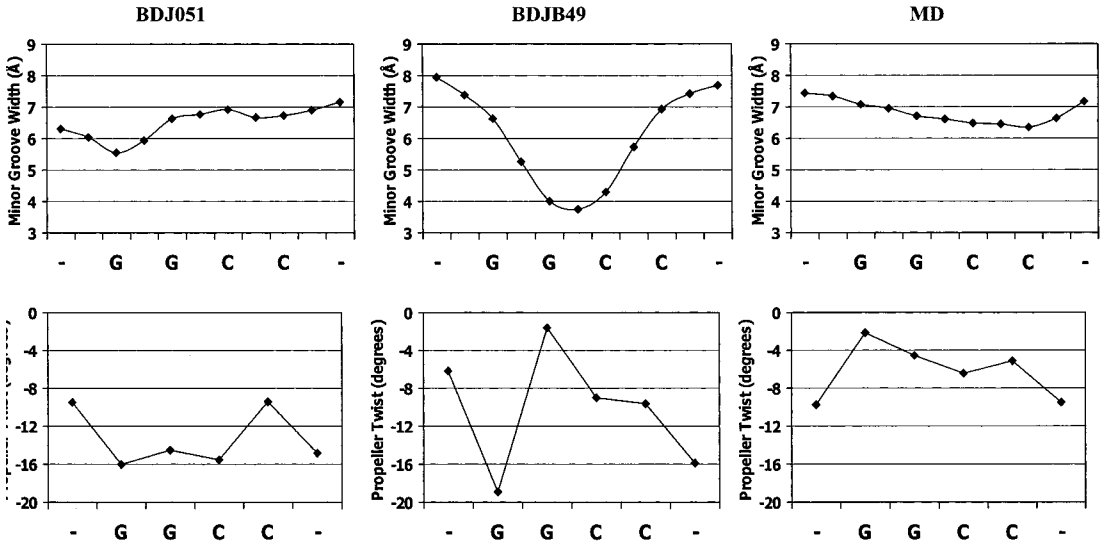

Figure 9. Plots of minor groove width and propeller twist of the two X-ray structure of GGCC duplex (NDB ID: bdj051 and bdjb49) and the average structure of the MD simulation of the GGCC duplex.

narrow minor groove structures, hence, a more narrow average minor groove width.

The range of minor groove widths observed experimentally for G-tracts agrees well with the range in minor groove widths observed in our MD simulations. It is informative to specifically compare the variations observed in experimental studies of minor groove width with variations observed in MD simulations. Although the AT minor groove is generally assumed to be narrow while GC sequences have wide minor groove, X-ray structures of AT and GC sequences with both narrow and wide minor grooves have been observed[52−55] (Figure 8). Shown in Figure 9 are plots of the minor groove width and propeller twist of two crystal structures with sequence element, GGCC,[52,54] one with a narrow and one with a wide minor groove. Also plotted is the average minor groove width and propeller twist of the GGCC dodecamer of this MD simulation. It appears that the crystal structures are the same conformational species, with wide and narrow groove, observed in the MD simulation.[56] Calladine rules and other base-clash models[5−7] are not consistent with observed narrow minor groove of GC sequence or wide minor groove observed in AT sequence.[57] Our results and previous work clearly show how sequence, cations, and water molecules act in concert to affect the minor groove structure of DNA in a dynamic fashion.

Acknowledgment. This work was supported by an NIH Grant to W.D.W. and an NSF Grant to L.D.W.. Computers were purchased with support from the Georgia Research Alliance.

Supporting Information Available: Additional figures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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