Magnesium Increases the Curvature of Duplex DNA That Contains dA Tracts†

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ABSTRACT: Distinct structural features of DNA, such as the curvature of dA tracts, are important in the recognition, packaging, and regulation of DNA. Physiologically relevant concentrations of magnesium have been found to enhance the curvature of dA tract DNAs, as monitored by solution-state NMR, indicating that the structure of DNA depends on the cations present in solution. A model is presented which accounts for the sequence-dependent effects of magnesium on DNA curvature as well as for the previously known sequence-independent effect on DNA flexibility.

Variation in the local structure of DNA has been proposed to play a role in the recognition, packaging, and regulation of DNA (*1*−*10*). A structural feature that has been extensively studied is the curvature of dA tracts containing 4−6 consecutive dA residues. The effects of dA tracts have been examined by gel mobility, the rate of cyclization, and other properties of DNA (*3*, *4*, *9*, *11*−*16*). Our NMR structure (*17*) and the crystal structure (*18*, *19*) of a DNA containing the sequence element d(AAAAAT) are quite similar. When packaged into nucleosomes, DNA is curved about 1.75 turns per 150 base pairs. The curvature and flexibility of DNA appear to control the positioning of DNA in nucleosomes (*3*, *20*−*25*). DNA curvature can also arise from other sequence elements, such as d(GGGCCC), and some repeat sequences, including those in the centromere which make up about 5% of all human genomic DNA (*26*).

There is growing evidence that the local structure of DNA is a function not only of the sequence but also of the counterions and the temperature. If the local structure of DNA depends on the environmental conditions, then so may the recognition of the structural variations and the packaging of DNA.

The mechanism of the origin of the sequence dependence of DNA structure remains controversial (*4*, *27*). The "base-dependent" model proposes that steric clashes and other interactions between adjacent residues are the main forces that give rise to the sequence dependence of the structure of duplex DNA (*1*, *2*, *28*). Variations in the narrowing of the minor groove and other features of the DNA give rise to the curvature and other sequence-dependent structural features. The base-dependent model predicts that the minor groove will be narrow whether the DNA is or is not interacting with solvent and counterions.

A competing "electrostatic" model proposes to include the variation in neutralization of the backbone phosphates due to sequence-dependent interactions with cations in addition to the steric and other direct interactions between DNA bases (*3*, *4*, *29*). For example, the electrostatic model attributes the narrowing of the minor groove of dA tracts primarily, but not solely, to interactions of the bases with the counterions and water molecules in the minor groove. The electrostatic model predicts that curvature increases and the minor groove narrows as the residence time of counterions in the minor groove becomes longer while the base-dependent model does not. Crystallographic data have been ambiguous as to the effects of monovalent counterions on the local structure of DNA (*1*−*3*, *28*, *30*, *31*).

Methods have been presented that allow the prediction of the curvature and other structural properties of DNA from the sequence of the DNA (*10*, *32*). These methods have had limited success, which may be due, at least in part, to the results in the structural database having been collected on DNAs in the presence of different counterions and at different water activities and temperatures (*27*, *30*, *31*, *33*−*40*).

There is consensus that divalent metal ions can have significant effects on the structure of DNA (*33*, *37*, *38*, *41*−*44*). This consensus is in contrast to the controversy over the extent of the effects of monovalent cations on the structure of DNA. Structural studies have shown that magnesium binds as the hexahydrate in the major groove of DNA (*33*) or to the minor groove (*38*). The local charge neutralization and hydrogen bonding of magnesium can alter the structure of DNA (*33*, *38*, *44*). A magnesium that is not fully hexahydrated has been observed in one instance (*38*). Magnesium and other divalents can increase the thermal stability of DNA.

Our recent results indicate that the curvature of dA tracts is temperature dependent (*45*), and this is in accord with some earlier reports (*14*, *15*, *46*−*51*). The temperature dependence indicates that descriptions of the curvature and other structural properties of DNA have to take the full range of experimental conditions into account. The temperature dependence of the curvature of dA tracts, at temperatures below the melting temperature, may be due to changes in counterion residence times and hydration of the minor groove. Minor groove binding ligands have been proposed to straighten dA tracts partially by displacing counterions from the minor groove (*52*−*54*).

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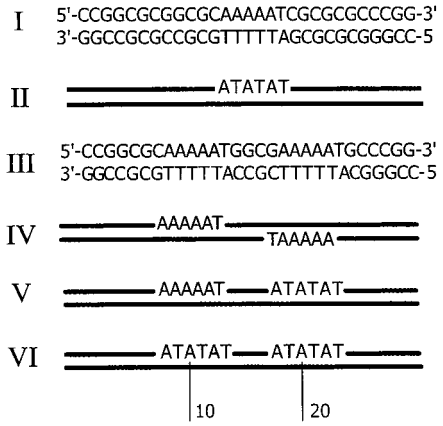

FIGURE 1: Sequences of the six DNA duplexes examined here.

Rouzina and Bloomfield summarized the effects of cations on the flexibility of DNA in a model in which multivalent cations give rise to sequence-independent, local, transient bending of DNA when the residence time of the cation is long enough for the DNA to bend (*12*). The multivalent cations induce bending by neutralizing the charge in one groove of the DNA. The transient bends increase the flexibility of DNA. Monovalent cations more uniformly neutralize the backbone of DNA and do not induce the transient bends according to this model (*12*).

To examine the effect of magnesium on the curvature of DNAs, a series of DNAs containing dA tracts have been examined. The shape functions of the DNAs, which are the molecule-specific parts of the diffusion coefficients (*45*, *55*), have been used to monitor the curvature as a function of magnesium concentration and temperature. In these experiments, the DNAs are examined in dilute solution and the method does not rely on the interaction of the DNAs with dyes, gels, enzymes, spin labels, or other components (*45*, *55*, *56*).

MATERIALS AND METHODS

Sample Preparation. The 29-mers were obtained from Integrated DNA Technologies Inc., Coralville, IA, and the sequences are given in Figure 1. The DNA samples were ethanol precipitated three times to remove salt, trace amounts of protecting groups, and other contaminants. NMR- and HPLC-based analysis of the samples showed no detectable impurities. The single-stranded DNAs were combined to produce duplex DNAs using calculated extinction coefficients. The proton NMR spectra of the resulting samples were examined and additional titrations carried out, as necessary, until there was no detectable presence of single-stranded material. Each of the duplex 29-mer NMR samples contained a total of 27 A_{260} of DNA in 500 μL of 100 mM NaCl, 10 mM phosphate buffer, and 50 μM EDTA at pH 7.0. One A_{260} is equivalent to an optical density of 1 at 260 nm in a 1 cm path length cell. The magnesium titrations were carried out by the addition of 1 M $MgCl_2$ to the NMR samples. The magnesium concentrations of the samples were 10, 15, 20, or 50 mM. The samples were diluted at most 4% during the titration.

NMR Experiments and Data Processing. The NMR experiments were carried out using a Varian 400 MHz UnityPlus spectrometer and a Nalorac double-resonance, pulsed field gradient, 5 mm probe. The diffusion experiments were carried out using the PFG-STE, pulsed field gradient stimulated echo, pulse sequence with $\delta = 8$ ms and $\Delta = 262$ ms as described previously (*45*, *55*, *56*). The strengths of the first and third gradient pulses were the same, and the experiment was carried out with 27 different values of the variable gradient strength from 13.7 to 87.9 G/cm. The strength of the gradient used to suppress transverse magnetization, applied at the middle of the Δ period, was 13.7 G/cm.

For each spectrum 5568 complex points were collected, with 600 transients, from 10 to 55 °C. A spectral width of 20 kHz and a delay time of 8 s were used. The free induction decays were Gaussian weighted, Fourier transformed, baseline corrected, and then integrated. VNMR version 6.1B software was used to acquire and process the data. A line broadening of 512 Hz was applied, and integration was over all of the proton resonances of the DNAs.

The diffusion coefficients, D, were calculated from the ratio of the intensity obtained with gradient strength G, I_G, to that obtained with no gradient, I_0, by use of $I_G/I_0 = \exp[-D\gamma_H^2\delta^2G^2(\Delta - \delta/3)]$. Plots of $\ln(I_G/I_0)$ versus G^2 were fit by nonlinear least squares, the correlation coefficients determined, and the results graphed using KaleidaGraph version 3.02, from Synergy Software, as previously described (*45*, *55*, *56*). The correlation coefficients for all of the fits of the diffusion coefficients were greater than 0.990, and the precision and accuracy of these methods have been described previously (*45*, *55*, *56*).

RESULTS AND DISCUSSION

The shape function, defined by $S_f = \eta D/T$, does not have the solvent viscosity, η, and temperature dependence, T, of the diffusion coefficients (*45*, *55*). The temperature dependence of a shape function reports on the changes in the curvature and flexibility of the DNA rather than on changes in the solvent viscosity or other nonspecific temperature effects (*45*, *55*). The DNAs with more compact structures will have larger shape functions than those with more extended structures. For example, a curved duplex DNA will have a larger shape function than a straight DNA of the same length. The sequences of the six 29-mer duplex DNAs examined in this study are shown in Figure 1.

Effect of Temperature on the Curvature of dA Tracts. The ratios of the shape functions of the dA tract DNAs are plotted in Figure 2 as a function of temperature. This mode of representation is reminiscent of optical melting curves (*45*, *56*). In the absence of magnesium the shape function of the DNA with a central dA tract, I, is larger than that of the DNA with a central scrambled dA region, II, at 10 °C, as shown in Figure 2. This difference in shape functions indicates that the DNA with the dA tract is curved at 10 °C (*45*). As the temperature is increased, the difference between the shape functions of the DNA with the central dA tract, I, and the DNA with a central scrambled sequence, II, decreases. By about 37 °C there is no appreciable difference between the shape functions of these two DNAs. This temperature dependence indicates that the curvature of the dA tract melts out by 37 °C. The same pattern can be seen for the DNAs with two dA tracts, III and IV, and for the DNA with a noncentral dA tract, V. The temperature dependence of the ratios of the shape functions is the same

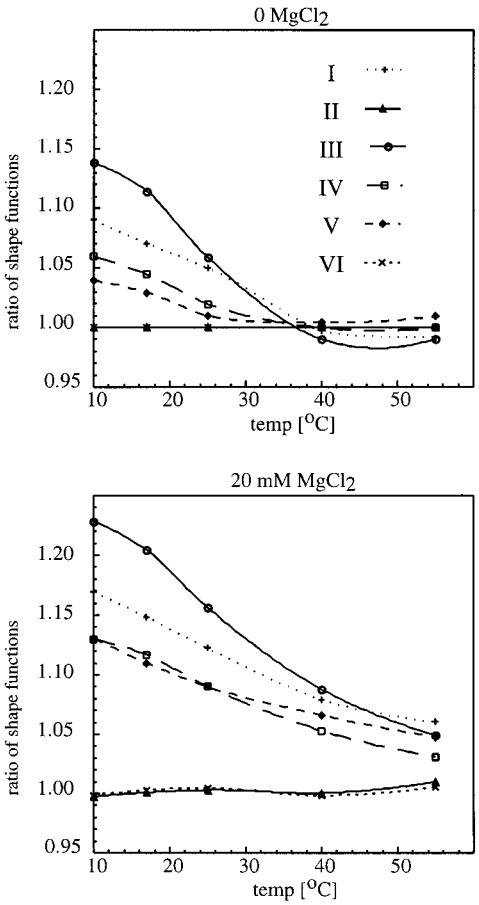

FIGURE 2: Ratios of the shape functions of the indicated DNAs to that of the DNA with a central scrambled sequence at 10 °C. The plot on the top is for the DNAs in the absence of magnesium and the plot on the bottom for the DNAs in the presence of 20 mM magnesium. The plots show that magnesium both increases the curvature of the DNAs containing dA tracts and increases the thermal stability of the dA tracts. The DNAs are numbered as given in Figure 1.

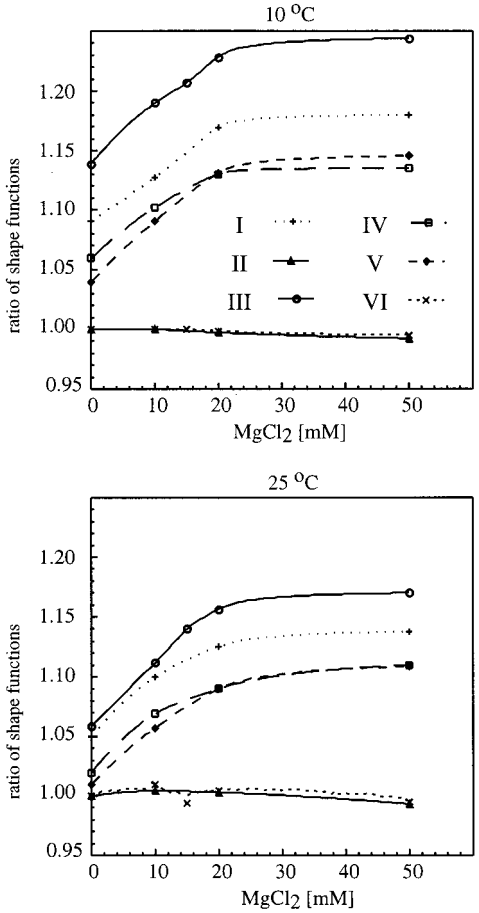

FIGURE 3: Ratios of the shape functions of the indicated DNAs to that of the DNA with a central scrambled sequence in the absence of magnesium. Data obtained at 10 °C are shown at the top, and those at 25 °C are shown at the bottom. An increase in curvature gives rise to an increase in the ratio of the shape functions. The DNAs are numbered as given in Figure 1.

for each DNA containing one or two dA tracts, indicating that the curvature of the dA tracts shows the same temperature dependence in all of the sequence contexts examined here (*45*).

Effect of Magnesium on the Curvature of dA Tracts. The ratios of the shape functions were used to monitor the effect of magnesium on the curvature of the dA tract DNAs. Plots of the ratios of the shape functions as a function of magnesium concentration are shown in Figure 3. At 10 °C in the absence of magnesium the DNA with a central dA tract, I, has a shape function that is about 9% greater than that of the DNA with a central scrambled sequence, II. At 10 mM magnesium the difference is about 13%, and at 20 mM magnesium the difference is about 17%. There is little additional change when the magnesium concentration is raised to 50 mM. These data indicate that the presence of magnesium approximately *doubles* the difference in shape functions between a DNA with a central dA tract, I, and a DNA with a central scrambled sequence, II. The same relative increases in the shape functions of the DNA with a noncentral dA tract, V, and the DNAs with two dA tracts, III and IV, are also observed.

Effect of Magnesium on the Thermal Stability of the Curvature of dA Tracts. When the titration with magnesium is carried out at 25 °C, there is an even larger effect on the percentage difference in the shape functions as the data in Figure 3 demonstrate. In the absence of magnesium the difference in the shape functions is about 5% between DNAs I and II, while in the presence of 20 mM magnesium the difference is about 13%.

The temperature dependence of the difference in the shape functions of the DNA with a central dA tract, I, and the DNA with a scrambled sequence, II, was also examined. The results obtained in the absence of magnesium and in the presence of 20 mM magnesium are shown in Figure 2. This difference between the shape functions is melted out by 37 °C when there is no magnesium in the samples, as discussed above. The shape function difference between DNAs I and II decreases by about half from 10 to 37 °C when 20 mM magnesium is present. In the presence of 20 mM magnesium the difference in the shape functions between DNAs I and II persists to at least 55 °C. At these higher temperatures there is localized melting of the DNAs, giving rise to increases in the shape functions of all of the DNAs (*45*).

The effects of magnesium on the DNAs with two dA tracts, III and IV, follow the same pattern as found for the DNA with a central dA tract. The effects of magnesium on the DNA with a noncentral dA tract are also quite analogous. In each case the addition of 20 mM magnesium approximately doubles the difference in shape functions and

increases the melting temperature of the curvature of the dA tracts by more than 20 °C. Magnesium has little or no effect on the shape functions of either of the DNAs with only scrambled sequences, II and VI, at concentrations up to 50 mM, which was the highest concentration examined.

Apparent Magnesium K_d for the Curvature of dA Tracts. The apparent K_d for the effect of magnesium on the shape functions of the DNAs with one or two dA tracts is approximately 12 mM. The increase in the melting temperature of the curvature and the apparent K_d are approximately the same for all of the DNAs with one or two dA tracts. The percentage increase in shape function depends on the number of dA tracts present. These results indicate that magnesium interacts with all of the dA tracts examined here in approximately the same manner regardless of their position in the DNA.

A Model for Increased Curvature Due to Magnesium Binding. The extent of binding of magnesium to DNA has been examined by both theoretical and experimental methods (*44*). The experimental binding isotherms of magnesium to nucleic acids are well described by the Poisson−Boltzmann theory (*44*). This theory, and the supporting experimental results, indicates that at 1 mM Mg there is approximately 1 magnesium bound per 10 nucleotides when the sodium concentration is about 100 mM. The predictions are for 2.5 magnesiums bound per 10 nucleotides at 10 mM and 3 magnesiums bound per 10 nucleotides at 20 mM. The saturation level is approximately 0.4 magnesium per nucleotide, and this occurs near 50 mM Mg. The maximum effect of magnesium on the curvature of dA tracts is observed by 20 mM. The Poisson−Boltzmann model predicts approximately one magnesium bound per four nucleotides at this concentration of magnesium (*44*).

The model of Rouzina and Bloomfield (*12*), for the sequence-independent effect of magnesium on the flexibility of DNA, can be extended to include the sequence-dependent effect of magnesium on the curvature of DNA. At low magnesium concentrations, below about 1 mM, the magnesium ions exhibit rapid diffusion between the binding sites. At higher concentrations, in the range of 5−20 mM magnesium, the residence time of the magnesium at the dA tract sites becomes long enough, on the order of 0.1 μs, for the magnesium-induced charge neutralization to enhance and stabilize bending. A residence time on the order of 0.1 μs is needed to allow the DNA to bend (*57*). At higher concentrations, above 50 mM, the DNA is saturated with magnesium and the residual highly mobile magnesium ions can localize for a long enough residence time, in a sequence-independent manner, to induce transient bends. The transient bends give rise to an increase in the flexibility of the DNA.

This model and our results do not discriminate between two possible origins of the magnesium effect on the curvature. The magnesium could preferentially bind to the dA tracts, or the curvature of the dA tracts could be enhanced by uniform binding of the magnesium. The addition of magnesium increases the curvature of the dA tract DNAs at all concentrations. This indicates that magnesium adds to, or increases, the curvature that is already present in the dA tracts. Thus, it appears that magnesium induces curvature in generally the same orientation as that found in the absence of magnesium. The results also indicate that magnesium has no appreciable effect on the shape functions of the DNAs containing scrambled dA regions.

The presence of magnesium will reduce the average charge on the phosphates. A uniform reduction in the charges could lead to an increase in bending by reducing the charge−charge repulsion that can occur during bending. If uniform charge reduction were a significant contributor to the increase in bending observed upon addition of magnesium, then the addition of other counterions, including monovalents, that can reduce the average charge should also increase the bending. This possibility can be experimentally tested.

Structural Interpretation of the Magnesium Effect on Curvature. The shape functions give information on the ensemble average of the structure of the DNA but do not offer information about the details of any of the structures that make up the ensemble. There have been extensive studies to determine the structural basis of the curvature of dA tracts and how this curvature changes due to the presence of magnesium and other multivalent ions. These prior theoretical, structural, and other studies have been discussed elsewhere (*3*, *4*, *10*, *31*, *33*, *45*). The longest simulations of DNA carried out to date approach the 0.1 μs time scale that experiment has indicated is required for DNA to bend (*57*).

Arguments have been presented that have the dA tract induced bending occurring into the major groove and other arguments for the bending to occur into the minor groove. Arguments have been presented for the bending to occur at the dA tract and other arguments for the dA tracts being straight and the curvature occurring either at the junctions or in the rest of the DNA. However, there is consensus that magnesium generally binds to the major groove of DNA as the hexahydrate and there can be contacts between the hydration waters and the DNA. There is one example of magnesium binding to the minor groove, and this magnesium is partially dehydrated (*38*). It is of interest to examine how our results relate to these models for the curvature induced by the presence of dA tracts.

It is important to note that the literature results on dA tracts are actually on a wide range of DNAs. The results on the d(AA) sequence element, found in the "Dickerson dodecamer", have been extrapolated to dA_n in some cases. This may not be entirely justified since repeats of d(AA) do not show the gel retardation and other effects observed with dA_n. Similarly, the results on dA_4, dA_5, and dA_6 tracts may have considerable overlap while each of these DNAs may have some distinct features not found in the other sequences.

In a model, referred to here as "major groove", recently proposed by the Dickerson group (*33*), magnesium binds to the major groove, has a preference of d(GG) > d(AG), d(GT), and induces bending into the major groove. This model has the bending occurring at the magnesium binding sites. The results on the effect of magnesium on the shape functions of the DNAs with scrambled dA regions indicate that there is no significant net bending consistent with this model. The shape function results on the DNAs with dA tracts can be included if the model is extended. The extended model has magnesium binding to the major groove of the dA tracts that induces bending in the same direction as was present in the absence of magnesium. The difference in the bending between dA_n and d(AA) may arise from dA_n sites being able to simultaneously bind two magnesiums. Thus,

in the extended major groove model, magnesium binds to dA tracts, in the manner the Dickerson group has proposed for other sites, and the bending induced by magnesium binding is into the major groove.

There have been a number of biochemical studies whose results are consistent with dA tract bending into the minor groove. A high-resolution NMR structure of a DNA containing a dA_6 sequence element and a comparable sample with a d(AAGAAA) sequence element have been recently completed by the Lu group (*58*). The DNAs used in that study were in low ionic strength, 20 mM potassium, relative to most other studies (*58*). The structural results have led to a model, referred to here as "minor groove", to account for the curvature of dA tracts as observed by a wide range of experimental methods. This model incorporates many of the features present in prior proposals for bending into the minor groove, especially those from the Crothers group (*10*, *16*, *29*, *59*, *60*). The minor groove model attributes the major groove bending by dA tracts observed in structural studies, most of which are crystallographic, to crystal packing forces and other factors.

The shape function results indicate that magnesium increases the curvature of dA tracts in the same orientation as the bending found in the absence of magnesium. To make the shape function results consistent with the minor groove model seems to require magnesium binding inducing an increase in bending toward the minor groove. For magnesium binding to cause an increase in bending toward the minor groove, the magnesium most likely has to bind to the minor groove as suggested elsewhere (*38*). The minor groove model can be extended to account for the shape function results. The extension is that magnesium binds to the minor groove of dA tracts, inducing bending into the minor groove, and that dA tracts are preferentially curved by magnesium.

Thus, the results on the effects of magnesium on the shape functions can be made consistent with either the minor or major groove model for the curvature of dA tracts if the models are suitably modified. The shape function results indicate that dA tracts have specific interactions with magnesium that are not well accounted for in either model in their current form.

Phasing of the dA Tracts. Crothers and Drak pointed out that if the bending of a dA tract, or other sequence element, is centered at the middle of the sequence element and toward either the minor or major groove, then the direction of bending would be the same for both polarities of the sequence (*16*). This appears to be approximately the case for some, but not all, dA tracts. Thus, in general, curved sequence elements cannot be switched from cis to trans by changing the polarity of one of the sequence elements. However, the structural data, for both major and minor groove bending, tend to have bends that are not symmetrical with respect to the center dA tract. The shape function data presented here indicate that changing the polarity of d(AAAAAT) tracts separated by the helical repeat, samples III and IV, does change the overall shape of the DNA. This indicates that either the curvature is not entirely symmetrical with respect to the center of the dA tract or the bending is not directly into the major or minor groove. Both of these factors can contribute to the observed differences in the shape functions of DNAs III and IV.

Summary. The results presented here are in general agreement with attributing a significant portion of the sequence dependence of DNA structure to electrostatic effects (*3*, *4*, *29*). However, the main points of this study are that the curvature of dA tracts in DNA is a function of the magnesium concentration and that at a physiological concentration magnesium increases the thermal stability of the curvature of dA tracts to physiological temperatures. The apparent K_d for the magnesium effect is about equal to the total concentration of magnesium in cells, which is about 15−20 mM (*61*−*63*). The curvature of dA tracts is only one sequence-dependent structural feature of DNA. It is likely that magnesium and other cations found in nuclei, such as polyamines, may have pronounced effects on the structure of other sequence elements of DNA. Thus, the sequence dependence of the structure of DNA is dependent on the environmental conditions. The results also indicate that methods for determining the ensemble average of the properties of DNA from structural information are not yet fully developed.

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