Localization of Divalent Metal Ions in the Minor Groove of DNA A-Tracts

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Double-stranded DNA molecules which contain runs of four to eight consecutive adenine or thymine residues without a 5′-TA-3′ step (*i.e.*, A-tracts) exhibit a significant curvature of the helical axis. In spite of numerous investigations, the origin of A-tract bending remains unresolved.[1] A hallmark of curved DNA is an electrophoretic mobility in polyacrylamide gels that is anomalously large with respect to straight DNA (*i.e.*, DNA without static curvature).[2] The magnitude of this anomaly for A-tract DNA, and presumably its curvature, depends upon the concentration and species of cation(s) present in the running buffer of the gel.[3] Surprisingly, we find no reports of experimental investigations which address the possibility of site-specific interactions between cations and A-tract DNAs. We have initiated such a study by monitoring line broadening in ^{1}H NMR spectra of oligonucleotides containing A-tracts in the presence of the paramagnetic cation manganese(II). Here, we report what is, to our knowledge, the first experimental evidence of cations localized in the minor groove of DNA in the solution state.

One- and two-dimensional ^{1}H NMR spectra of the self-complementary dodecamers d(GCA$_4$T$_4$GC) [**A$_4$T$_4$**] and d(CGT$_4$A$_4$CG) [**T$_4$A$_4$**] have been collected as a function of $MnCl_2$ concentration. It has previously been shown that DNAs containing the sequence 5′-A$_4$T$_4$-3′ exhibit bending typical of A-tract DNA, while DNAs containing the sequence 5′-T$_4$A$_4$-3′ are either straight or perhaps only slightly curved.[4] 1D ^{1}H NMR spectra of the aromatic resonances of representative $MnCl_2$ titrations of the A-tract oligonucleotides are shown in Figure 1. While all aromatic resonances are affected to some extent by the presence of Mn^{2+}, which is expected considering the polyanionic nature of DNA, specific ^{1}H resonances in both sequences are preferentially broadened with respect to others. This indicates that both duplex oligonucleotides contain sites where Mn^{2+} association is relatively high and/or relatively close to the bases.[5]

In **A$_4$T$_4$**, the minor groove A3H2 and A4H2 resonances are the most broadened of the nonexchangeable aromatic resonances upon the addition of $MnCl_2$, indicating a favored site for Mn^{2+} association in the minor groove. Consistent with this observation is the selective line broadening of the minor groove H4′ resonances. Because of spectral overlap in the 1D ^{1}H spectra, broadening of the H4′ resonances can be appreciated more easily by a comparison of the H1′−H4′ cross peaks from 2D ^{1}H NOESY spectra in the absence and presence of $MnCl_2$. Reduction in the intensities of H1′−H4′ NOESY cross peaks accurately reflects the line broadening of the H4′ resonances because the H1′ resonances are far less affected by the presence of Mn^{2+}. From the spectra shown in Figure 2, it is clear that the resonances of A4H4′, A5H4′, A6H4′, and G11H4′ are preferentially broadened with respect to the G1H4′, C2H4′, A3H4′, and C12H4′ resonances. Broadening of the remaining H4′ resonances is difficult to assess due to spectral overlap.

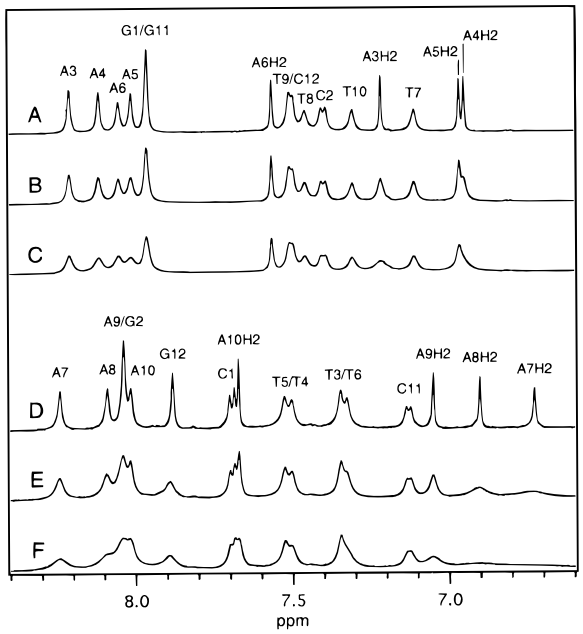

Figure 1. 500 MHz ^{1}H NMR spectra of the aromatic region of d(GCA$_4$T$_4$GC) in the presence of (A) 0.1 mM EDTA, (B) 1.0 μM $MnCl_2$, and (C) 3.0 μM $MnCl_2$; and d(CGT$_4$A$_4$CG) in the presence of (D) 0.1 mM EDTA, (E) 1.0 μM $MnCl_2$, and (F) 3.0 μM $MnCl_2$. All samples in this study were 2 mM in oligonucleotide strand in D_2O, 50 mM NaCl, pH 6.0; 128 fids were collected at 283 K with 16K data points and a 5000 Hz sweep width at 283 K.

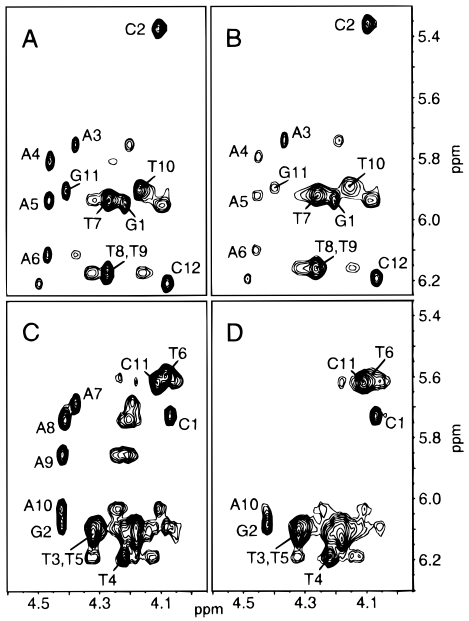

Figure 2. Contour plots of the H1′−H4′/H5′/H5″ region of 2D NOESY spectra with intra-residue H1′−H4′ cross peaks labeled for d(GCA$_4$T$_4$-GC) in the presence of (A) 0.1 mM EDTA and (B) 1.5 μM $MnCl_2$, and for d(GCT$_4$A$_4$GC) in the presence of (C) 0.1 mM EDTA and (D) 1.5 μM $MnCl_2$. Spectra were collected at 283 K with τ_m = 150 ms; 2048 points were collected in t_2 and 512 blocks in t_1 using States−TPPI phase cycling. Spectra were apodized with squared-sine-bell window functions phase-shifted 72° and 64° in t_2 and t_1, respectively.

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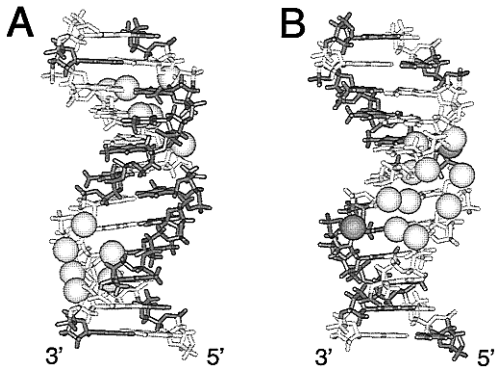

Figure 3. Model B-form helices of (A) $[d(GCA_4T_4GC)]_2$ and (B) $[d(CGT_4A_4CG)]_2$. Spheres designate the AH2 and H4′ protons which qualitatively exhibit the largest resonance line broadening in the presence of $MnCl_2$. Purine and pyrimidine residues are shown in light and dark gray, respectively.

Since H2 protons are not expected to participate in cation binding, the most likely source of A3H2 and A4H2 resonance broadening in $\mathbf{A_4T_4}$ is a close approach of Mn^{2+} to the electronegative O2 oxygens of T10 and T9 (~3 Å center-to-center distance from the cross strand A3H2 and A4H2, respectively). The equal broadening of these two resonances indicates that the site of deepest Mn^{2+} penetration into the minor groove is located near the center of 5′-A3pA4·T9pT10-3′. In contrast, A6H2 is the least broadened of the aromatic ^1H resonances, indicating that Mn^{2+} penetration into the minor groove varies significantly along the A-tract in $\mathbf{A_4T_4}$. The locations of the preferential Mn^{2+} association sites of $\mathbf{A_4T_4}$ are illustrated in Figure 3.

Sites of preferential cation association are also observed for $\mathbf{T_4A_4}$. For this sequence, A7H2 and A8H2 are the aromatic ^1H resonances most broadened by the presence of $MnCl_2$ (Figure 1).[6] With respect to the H4′ resonances, the H1′−H4′ cross peaks in 2D NOESY titration spectra which involve T6H4′, A7H4′, A8H4′, and A9H4′ are broadened virtually to base line by a concentration of $MnCl_2$ at which other H1′−H4′ cross peaks (*e.g.*, those involving C1H4′, G2H4′, T4H4′, and C11H4′) exhibit comparatively little line broadening (Figure 2c,d). Thus, for $\mathbf{T_4A_4}$, Mn^{2+} association is again favored in the minor groove, but for this sequence the most favored sites are near the center of the duplex (Figure 3). The relative lack of line broadening of the A10H2 resonance indicates that Mn^{2+} does not enter into the minor groove at the outer ends of the A-tracts.

The minor groove of A-tract DNA is narrow with respect to B-form DNA (9.0 Å versus 11.5 Å for the shortest phosphate−phosphate distance across the minor groove),[7] and there is substantial evidence that this narrowing progresses in the 5′ → 3′ direction.[8] The distance from an AH2 to the nearest cross strand H1′ (≅ 5 Å in B-form DNA) has been used as an indicator of minor groove width.[8b−e] For $\mathbf{A_4T_4}$, we measure distances of 4.5, 3.9, 3.7, and 3.8 Å for A3H2−G11H1′, A4H2−T10H1′, A5H2−T9H1′, and A6H2−T8H1′, respectively.[9] Similarly, for $\mathbf{T_4A_4}$, we measure ~4.5, 4.1, 3.9, and 3.9 Å for A7H2−T7H1′, A8H2−T6H1′, A9H2−T5H1′, and A10H2−T4H1′, respectively. These measurements reveal a definite correlation between A-tract minor groove width and Mn^{2+} localization. This suggests that the absence of Mn^{2+} penetration at the 3′ ends of the DNA A-tracts may result from the minor groove becoming too narrow for the entry of a fully hydrated Mn^{2+}. The closer approach of phosphate groups in the narrowing minor groove is, however, necessarily accompanied by an increased negative electrostatic charge density which should *increase* cation association.[10] Thus, if cation association is enhanced over the entire length of an A-tract minor groove, any Mn^{2+} associated with the 3′ end of an A-tract is restricted to interaction with phosphate groups without entrance into the minor groove.

Although both $[d(GCA_4T_4GC)]_2$ and $[d(CGT_4A_4CG)]_2$ have A-tracts, it is known that the polymer $[d(A_4T_4GC)]_n$ is curved, while $[d(T_4A_4CG)]_n$ is essentially straight.[4] Thus, the localization of cations in the minor groove alone cannot fully explain A-tract bending. However, the helical position of the preferred cation association sites are different for the two sequences (Figure 3). Taken together with the preference of sequences with G·C base pairs to bind cations in the major groove,[11] the cation distribution along DNA helices containing the two different A-tract repeats would be quite different. It has been shown that changes in the relative screening of phosphates by tethered cations can result in considerable bending of the DNA helical axis.[12] Thus, we propose that any complete explanation of sequence directed DNA bending will have to take into account the localization of cations in the minor groove of A-tracts. Consistent with the results presented here is a theoretical study published while this manuscript was in preparation which suggests that Na^+ enters the minor groove of AT-rich sequences.[13] Thus far, all Mn^{2+} binding sites identified experimentally have been located in the major groove and involve at least one guanine residue.[11] This suggests that minor groove cation binding sites may be unique to A-tract sequences. Determining the actual range of sequences which generate such sites and the extent to which cations localized in the minor groove contribute to A-tract bending will require further investigations. These studies are presently underway.

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(6) The H8 resonances of G2 and G12 in $\mathbf{T_4A_4}$ also exhibit appreciable broadening. The broadening of G2H8 is understandable since the 5′-GT-3′ step has been identified as a major groove ion binding site.[11] Broadening of the G12H8 resonance, on the other hand, appears to be the result of an end effect.

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(9) Distances were calculated from cross peak integrals of 150 ms mixing time NOESY spectra using the r^{-6} relationship and a cytosine H5−H6 distance of 2.5 Å as a reference.

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