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COMMUNICATIONS

Published on Web 08/22/2006

Removal of a Single Minor-Groove Functional Group Eliminates A-Tract Curvature

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When sequences of 3−7 dA residues are placed in phase with the DNA helical repeat (~10.4 bp), pronounced curvature of the DNA duplex is observed using gel migration anomalies[1] or cyclization kinetics.[2,3] NMR[4] and crystallography[5] have provided some quantitation of the effect. A consensus structural explanation for A-tract curvature has remained elusive for some 20 years.

Two models were originally employed to explain A-tract curvature. (i) Trifonov's "wedge model" suggested[6] each ApA step added an incremental contribution to the observed curvature. (ii) Crothers suggested[7] the "junction model" in which the A-tract sequence was structurally altered to a B′ helix, and curvature occurred as the result of optimizing base stacking interactions at the junction between the B′ and B helices. More recently, Williams and colleagues have suggested[8,9] that sequestering cations selectively within hydration structures in the minor groove accounts for A-tract curvature. Minor-groove-bound metal ions have been identified by both crystallographic[10,11] and NMR[12,13] techniques. Hud and colleagues have argued[14] that curvature is the result of a combination of cation and junction effects.

In addition to computational studies,[15,16] much of the experimental work to probe curvature effects has involved the use of A-tract sequences containing analogue nucleobases.[17−21] Many of these analogues have focused on the purine residues with relatively few pyrimidine residues examined. The latter have included U for T[17] and more recently the use of difluorotoluene[22,23] for T. These latter studies have indicated that pyrimidine methyl groups do not impact DNA curvature, and replacing the pyrimidine ring with fluorinated toluene opposite adenine or 3-deazaadenine results in moderate losses of curvature depending upon the location of the analogue.[21,22] In addition to analogue bases, external factors, most notably increased concentrations of Mg^{2+}, have been shown[24,25] to enhance the curvature effect.

To further probe the nature of the minor groove and possible electrostatic interactions in that region, we focused on the role of the thymine/uracil O2-carbonyl, in part because divalent metal ions, such as Mg^{2+}, prefer oxophilic ligands. We designed three dT/dU analogues in which the O2-carbonyl was replaced by −H, −F, or $-CH_3$ (Figure 1). To maintain the planar character of the heterocycles and "normal" interresidue bidentate hydrogen bonding with dA, these derivatives were prepared as C-nucleosides. Using this design, we have previously shown[26] that the dm^{3}2P analogue forms a normal bidentate base pair with dA in the context of a double-stranded dodecamer.

In this study, we used a 10-mer DNA sequence containing a $(dA\text{-}dT)_5$ A-tract and overlapping GC rich ends; the latter facilitate base pairing for the formation of ligation ladders. Analogue residues were inserted into the sequence using phosphoramidite chemistry. After purification and radioisotopic labeling ([γ-32]-ATP), the duplexes were treated with T4 DNA ligase and ATP. The resulting ligation ladders were resolved using 6% polyacrylamide nondenaturing gels. After autoradiography, the apparent length of each ligation band relative to a standard 25 bp ladder (*K*-factor) was plotted against its known sequence length (e.g., see Figure 2a).

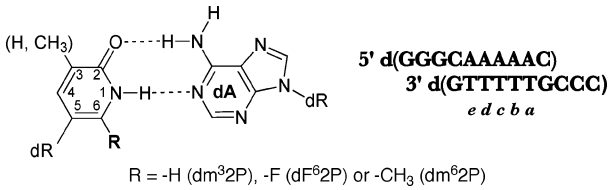

Figure 1. Structures of the modified dA−dT base pairs (left); dm^{3}2P has a methyl at C3, dF^{6}2P and dm^{6}2P have hydrogen (locants used for dF^{6}2P and dm^{6}2P are the same as those used for dm^{3}2P). Sequence used to generate ligation ladders to monitor A-tract curvature (right).

The native sequence $(dA\text{-}dT)_5$ migrates anomalously, and after graphic analysis results in a *K*-factor of 1.6 for 160 bp (0 mM Mg^{2+}). By comparison, replacing the central dA−dT base pair (position ***c*** in Figure 1) with dG−dC completely eliminates measurable curvature effects as has been previously reported.[27] We replaced two of the dT residues (positions ***b*** and ***c***, Figure 1) with the dm^3-2P analogue. This duplex is analogous to the native sequence but lacks two of the O2-carbonyls in the minor groove; no curvature was observed. We then performed each single substitution in which either the dT residue at position ***b*** or the dT residue at position ***c*** was replaced by the dm^{3}2P analogue. Loss of the O2-carbonyl from the dT in position ***b*** in the A-tract resulted in a significant loss of the observed curvature. Some DNA curvature remains, and this is likely to result from the three consecutive native dA−dT base pairs in positions ***c***−***e*** (Figure 2b). Sequences containing $(dA\text{−}dT)_3$ are known to result in moderate curvature effects. On the other hand, loss of the O2-carbonyl from the dT in the center of the A-tract (position ***c***) resulted in a complete loss of measurable curvature effects. In this case, the remaining native A-tracts are the dimers

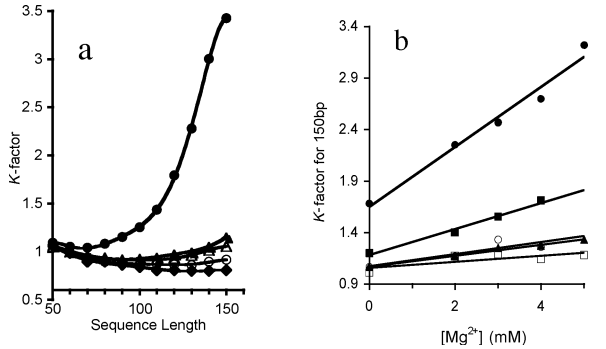

Figure 2. (a) Plot of *K*-factor (at 5 mM Mg^{2+}) from a single gel for ligation ladders containing the sequence d(AAXAA)·d(TTYTT), where X = dG and Y = dC (○), and X = dA and Y = dT (●), dm^{3}2P (▲), dF^{6}2P (△), or dm^{6}2P (♦). (b) Effects of [Mg^{2+}] on sequences (the 150 bp steps are plotted) d(AAGAA)·d(TTCTT) (□) or $d(A\text{−}T)_5$ (●) with analogue duplexes in which dm^{3}2P was placed at site ***b*** (■), ***b*** and ***c*** (○), and ***c*** (▲) of the A-tract.

10.1021/ja0632310 CCC: $33.50

ApA on either side of the analogue site; native ApA is not sufficient for measurable curvature. These results suggest an important role for the thymine O2-carbonyls as well as the presence of cooperative effects such that removal of a single but central O2-carbonyl eliminates the curvature effect. To confirm that these effects were not due to the electronegative nature of the carbonyl (relative to hydrogen) or the steric bulk of the carbonyl (relative to hydrogen), we prepared two additional sequences. In one case, we replaced the O2-carbonyl of dT at position ***c*** with the more electronegative fluorine (using $dF^6$2P; see Figure 1). In the second case, we replaced the same O2-carbonyl with the sterically bulkier methyl (using dm^6-2P; see Figure 1). Neither of the ligation ladders formed from these analogue sequences exhibited any measurable DNA curvature (e.g., see Figure 2a).

Metal ions have been implicated in the curvature phenomenon.[8,9,14,28] Increasing concentrations of Mg^{2+} are reported[24,25] to enhance curvature. To probe this phenomenon, we analyzed the ligation ladders containing various $dm^3$2P substitutions in the A-tract using PAGE under varying concentrations of Mg^{2+} (0−5 mM). At 5 mM Mg^{2+} (Figure 2a), the *K*-factor for the native A-tract is dramatically increased (3.5 for 160 bp), with minimal curvature effects for sequences with $dm^3$2P in position ***c*** or ***b*** and ***c*** (Figure 2b), or for the $dF^6$2P or $dm^6$2P analogues at position ***c*** at any tested [Mg^{2+}].

The present results suggest that the thymine O2-carbonyls are critical to the observation of A-tract curvature and that Mg^{2+} is an important modulator. Hud et al. have suggested[28] that divalent metal ions can only enter the A-tract minor groove at either end, so the narrowing of the minor groove in the center of the A-tract likely prevents hydrated Mg^{2+} from penetrating the groove. However, monovalent ions, such as Na^+, appear to locate within the minor groove[8] and might coordinate to the thymine carbonyls to narrow the minor groove. Mg^{2+} binding to charged phosphates across the minor groove then results in charge neutralization and enhances curvature.[10] Loss of an O2-carbonyl should release Na^+ from the floor of the minor groove, widening the groove and cooperatively releasing Mg^{2+} to straighten the A-tract conformation. Such effects are likely to be cooperative and more dramatic at the center of an A-tract. On the other hand, in the absence of divalent metal ion, A-tract curvature is still present. So penetration of the minor groove by Na^{2+} and coordination to O2-carbonyls narrows the minor groove and seems to be sufficient for the observed curvature effect.

The analogues used in this study create other potential effects for consideration. The C-nucleosides replace an sp^2 nitrogen with the corresponding sp^2 carbon. While the bond geometry remains the same, the C−C glycosidic bond is slightly longer than the C−N bond, but such effects do not appear to alter base pairing geometries.[26] ΔG_{25} values for self-complementary dodecamers, each containing two analogue residues, decrease in the order dT > $dF^6$2P > $dm^3$2P > and $dm^6$2P. While some duplex destabilization is observed with these analogues, it seems unlikely to be the primary contributing effect. The presence of a single dG−dC in an A-tract eliminates curvature effects; its replacement by the bidentate dI−dC destabilizes the helix, yet DNA curvature is enhanced;[18] a $dm^3$2P near the end of an A-tract is destabilizing, but curvature is partially recovered (Figure 2b). Single substitutions do not alter conformation.[26,29]

Can cooperative Na^+/Mg^{2+} binding explain the results obtained for various minor groove analogues? With a dG−dC base pair in the center of an A-tract, the N2-amino group binds one lone pair of the O2-carbonyl and likely makes it a less effective ligand for metal coordination and could explain the lack of DNA curvature when dG−dC interrupts an A-tract. Removal of the N2-amino group (use of dI−dC) results again in a curved DNA sequence.[18] Introduction of 3-deazaadenine impacts the curvature effect moderately only when present at the ends of the A-tract.[20] Other mechanisms, such as hydrated Mg^{2+} penetrating the ends of the minor groove,[28,29] might also explain this observation. More difficult to explain are the analogue substitutions using difluorotoluene. These hydrophobic isosteres reside opposite dA in a duplex, but the positional dynamics of such residues in the absence of hydrogen bonding is less clear. Positional flexibility for a difluorotoluene residue may permit the remaining four dA−dT base pairs to adopt a conformation that is still essentially curved in nature.

The absence of the central O2-carbonyl in the $d(A-T)_5$ sequence eliminates the intrinsic curvature of the sequence. Our results suggest this site as a key ligand for cooperative metal-ion binding and that A-tract curvature is strongly mediated by a divalent metal ion(s) associated with the minor groove.

Acknowledgment. Support for this work was provided by the NSF (MCB-0451488).

Supporting Information Available: Synthetic procedures as well as procedures for assays and selected gels. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0632310