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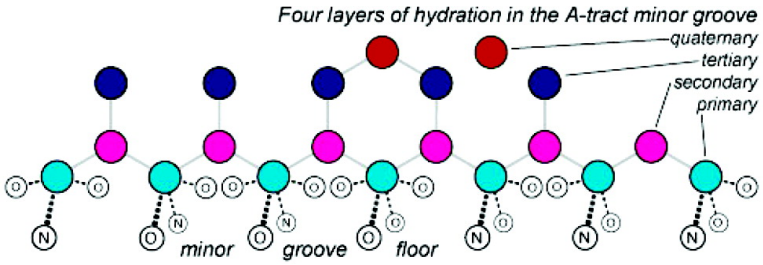
J. Am. Chem. Soc., **2004**, 126 (47), 15330-15331• DOI: 10.1021/ja045207x • Publication Date (Web): 09 November 2004



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Published on Web 11/09/2004

High-Resolution Structure of an Extended A-Tract: [d(CGCAAATTTGCG)]$_2$[†]

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DNA A-tracts are associated with narrow minor grooves,[1−3] axial bends,[4−6] high propeller twists,[7] and characteristic hydration motifs.[8−11] In addition, the minor grooves of A-tracts localize monovalent cations.[10−17] It was surprising that dodecamer duplexes with extended A-tracts, such as [d(CGCAAATTTGCG)]$_2$, here called A_3T_3, appeared in fundamental ways to differ from duplexes with shorter A-tracts, such as [d(CGCGAATTCGCG)]$_2$, called here A_2T_2. The hydration, minor groove width, and base−base hydrogen bonds of A_3T_3 differ from those of A_2T_2. A_3T_3 has been characterized in an unliganded form, by Rich[18] and Neidle,[19] and in complexes with minor groove-binding drugs, such as distamycin[18] or berenil.[20] The highest resolution unliganded A_3T_3 structure, solved to 2.2 Å resolution,[19] was interpreted to indicate disordered minor groove water molecules, which interact primarily with either one or the other DNA strands but not with both. The minor groove of A_3T_3 appeared to be relatively wide. Finally, A_3T_3 structures appeared to contain base−base hydrogen bonds, in addition to those proposed by Watson and Crick, with bifurcated hydrogen bonds within the A-tract major groove, between bases that are not base-paired.[18,21]

The archetypical A-tract, contained within the "Dickerson Dodecamer" (A_2T_2), has a narrow minor groove and an extended system of geometrically arranged water molecules,[8−11] some of which are long-lived in solution.[22,23] The primary hydration layer, on the floor of the A-tract minor groove of A_2T_2, forms bridges between DNA strands, linking N3 (A) and O2 (T) atoms from opposing strands. Additional layers of water molecules assemble atop the primary hydration layer. The hydrogen bonds between the bases of A_2T_2 conform to the scheme proposed by Watson and Crick.

To explore the differences and possibly reconcile the discrepancies between A_2T_2 and A_3T_3, a high-resolution A_3T_3 structure (1.5 Å resolution, >10 000 reflections) is described here, representing a significant increase in data quality in comparison with that in previous A_3T_3 structures (2000−3000 reflections). Electron density maps, along with information on data collection and refinement, minor groove hydration, spermine interactions, and a superimposition of A_3T_3 onto A_2T_2, are contained in the Supporting Information. Crystals were grown from a solution containing spermine, magnesium (Mg^{2+}), and lithium (Li^+).

The profile of the minor groove width of A_3T_3 at high resolution differs significantly from that of the previous low-resolution A_3T_3 structure (Figure 1). At high resolution, the A-tract minor groove is narrow, with the most acute narrowing at base pair 5−12 (where the width is 2.8 Å). By contrast, the groove width of the lower-resolution structure is wider and is relatively constant throughout the A-tract. The minor groove width profile of A_3T_3 at high resolution is seen to generally resemble those of high-resolution

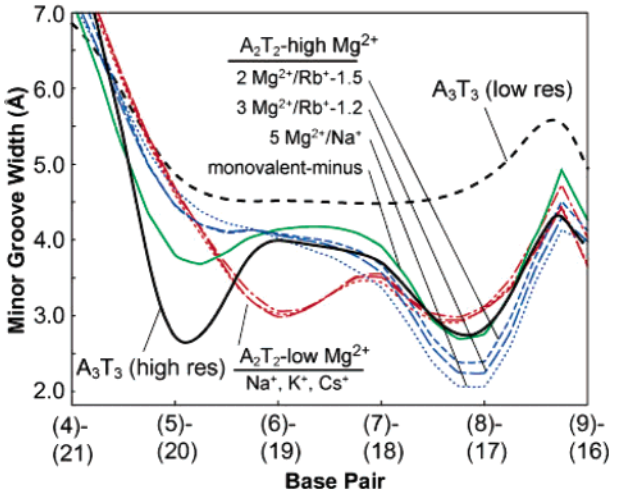

Figure 1. Minor groove widths of A_2T_2 and A_3T_3 structures. The groove width profile of the high-resolution A_3T_3 structure described here is given by a solid black line. The previous lower-resolution A_3T_3 (BDL038[19]) structure is in dashed black. Groove widths of three structures obtained from low [Mg^{2+}] are shown in red [dotted line; sodium (BDL084[24]), dashed line; potassium (BD0005[10]), dot-dashed line; cesium (BD0029[15]) forms]. Groove widths of three structures obtained from high [Mg^{2+}] are shown in blue [dotted line; magnesium/sodium (BD0007[25]), long-dashed line; magnesium/rubidium-1.2 (BD0012[11]), short-dashed line; magnesium/rubidium-1.5 (BD0013[11]) forms]. The monovalent-minus structure is solid green (BD0008[26]). Minor groove width profiles were calculated with the program CURVES (version 5.30),[27] which provides a continuous description of groove geometry. Coordinates were obtained from the Nucleic Acid Database.[28] In some structures, the DNA is covalently modified.

structures of A_2T_2 more closely than that of the previous low-resolution A_3T_3 structure.

It appears that Li^+ recapitulates a monovalent-minus conformation of DNA.[26,29] The minor groove profile of the high-resolution A_3T_3 structure most closely resembles that of a modified A_2T_2 structure crystallized in the presence of Mg^{2+} and the absence of monovalent cations. For example, the A-tract minor groove of A_3T_3 is widest at base pair 6 (4.0 Å), where it is 1 Å wider than our high-resolution structures of A_2T_2. These differences may arise from differences in the cationic environment. The A-tract minor groove has previously been shown to localize monovalent cations.[10−16,30] In the structure presented here, Li^+ was the only monovalent cation in the crystallization solution. Li^+, with relatively high charge density, exhibits coordination chemistry that is significantly different from that of the group I ions (Na^+, K^+, and Rb^+) that are commonly present in DNA crystallization solutions. These ions dehydrate more readily than Li^+ or Mg^{2+}, allowing more facile interaction with ligands on the floors of the grooves. It would not appear likely that Li^+ would dehydrate and localize within the narrow minor groove. No Li^+ ions were observed in the electron density maps. However, failure to observe Li^+ ions should not be interpreted as

[†] Atomic coordinates and structure factors have been deposited in the NDB (entry code BD0067 and PDB (entry code 1S2R).

10.1021/ja045207x CCC: $27.50

evidence for a lack of Li^+ localization. Crystallographic determination of Li^+ is problematic due to the very weak scattering of X-rays.

The minor groove hydration of high-resolution A_3T_3 is consistent with observations from high-resolution structures of A_2T_2 but not with those of the lower-resolution A_3T_3 structure. The high-resolution A_3T_3 minor groove contains ordered solvent sites that are readily fit to a monolayer of water molecules. Rising from the floor of the groove are primary, secondary, tertiary, and quaternary layers.[10,11] The primary layer forms bridges between the strands. Each internal primary site is coordinated by at least one, but commonly two, O4′ atom(s) and one O2 (T) atom and one N3 (A) atom. The single exception is the central site at the ApT step, which is coordinated by two O2 (T) atoms but not an N3 (A) atom. Two intact hexagon hydration motifs[10,11] are observed. The primary and secondary layers correspond to the "spine of hydration". The hydration pattern of A_3T_3 is extended relative to that of A_2T_2 to include seven, rather than five, sites in the primary layer.

Two partial spermine molecules are observed in the G-tract major groove of A_3T_3, one at each end of the duplex. Previous high-resolution structures of A_2T_2, crystallized in the presence of magnesium and spermine, have uniformly exhibited a fully hydrated Mg^{2+} at one end in the major groove, and in some cases, a spermine molecule at the opposite end.[24,29,31] In the current structure, two spermine molecules, but no Mg^{2+} ions, are observed. The second spermine molecule replaces the Mg^{2+}. With 1.5 Å data, the octahedral geometry of a well-ordered hexahydrated Mg^{2+} ion would be obvious in the electron density maps. No such octahedral geometry was seen in our maps of A_3T_3. This replacement of magnesium by spermine could be caused by the sequence or the presence of Li^+ in the crystallization solution. Both spermine molecules are partially disordered, with eight atoms of each visible.

Previous crystal structures of A_3T_3 and other DNA fragments with extended A-tracts exhibited bifurcated hydrogen bonding in the A-tract major groove.[18,21] Adenine amino groups appeared to form hydrogen bonds with both a Watson−Crick partner and with the O4 of an adjacent thymine. These extra Watson−Crick hydrogen bonds were thought to be important, for example, in stabilizing high propeller twist in A-tracts. However, the high-resolution A_3T_3 structure described here does not show evidence of this extra Watson−Crick hydrogen bonding. Of six potential extra Watson−Crick hydrogen bonds in the high-resolution A_3T_3 structure, the shortest is 3.5 Å, which is at the far limit for interaction.

In summary, the 1.5 Å structure of A_3T_3 described here differs significantly from a previous A_3T_3 structure,[19] but it is consistent with high-resolution A_2T_2 structures. The high-resolution A_3T_3 structure exhibits a monolayer of ordered solvent sites in the minor groove and a narrow minor groove but lacks extra Watson−Crick hydrogen bonding. There is at least one plausible explanation for some of the differences between the previous and current A_3T_3 structures. It is conceivable that the minor groove binder present in the previous crystallization solution, although not observed in the maps, was actually bound in the minor groove as a side-by-side dimer. It may have been positionally disordered to the extent that it could not be observed in the electron density maps. This hypothesis may explain the unusual hydration and the width of the minor groove.

Acknowledgment. This work was funded by the National Science Foundation (MCB-9976498). The authors thank Drs. Nick Hud and Martin Egli for helpful discussions.

Supporting Information Available: Electron density maps, along with information on data collection and refinement, minor groove hydration, spermine interactions, and a superimposition of A_3T_3 on A_2T_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA045207X