# Minor Groove Hydration Is Critical to the Stability of DNA Duplexes

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*Received February 25, 2000*

Since the original observation of an ordered set of water molecules,[1,2] termed the "spine of hydration," within the primary hydration sphere of the minor groove of duplex B-form DNA, numerous studies have concentrated upon the function and role(s) of minor groove hydration. In the described spine of hydration, individual water molecules bridge the $N^3$-nitrogens of adenines and the $O^2$-carbonyls of thymines at adjacent base pairs. A second layer of water molecules bridges the oxygen atoms of the underlying first layer. Theoretical analyses,[3,4] NMR studies[5] and additional crystallography[6−11] have all largely confirmed the presence of this chain of ordered water molecules within the minor groove, but its importance to the structural integrity of B-form DNA remains to be clarified.

To probe the importance of minor groove hydration for duplex DNA, we designed an analogue dA-dT like base pair that lacks both the $N^3$-nitrogen of the adenine as well as the $O^2$-carbonyl of the thymine (Figure 1). When present in duplex DNA, this base pair would disrupt the minor groove spine of hydration, in fact it should eliminate the presence of any ordered water molecules or metal ions at these sequence positions since both sites used for hydration in the minor groove are absent. The analogue base pair used in this study contained as a purine element, 3-deaza-2′-deoxyadenosine ($dc^3A$) (Figure 1), a known[12] analogue in which the $N^3$-nitrogen is replaced by a C−H residue. Its pyrimidine-like partner was a 3-methyl-2-pyridone ($dm^3 2P$) (Figure 1). The latter derivative was prepared by a palladium-mediated coupling[13] between the iodo derivative of the 2-pyridone and the corresponding protected glycal. Further protection resulted in the phosphoramidite derivative suitable for DNA synthesis.

With the analogue phosphoramidites in hand, we prepared a series of double-stranded sequences similar to that first crystallized by Dickerson[14] but substituted the analogue base pair for one or more native base pairs within the central core $d(AATT)_2$ sequence (Table 1). Introduction of either a single $dc^3A$ or $dm^3 2P$ residue into the dodecamer sequence results in a relatively minor effect

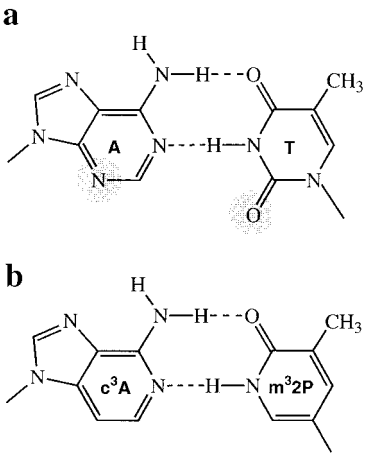


**Figure 1.** (a) Watson−Crick dA-dT base pair. (b) Watson−Crick-like base pair between $dc^3A$ and $dm^3 2P$.

in $T_M$ (<2 °C, entries 2 and 3, Table 1). With the introduction of a modified base pair (entry 4), roughly a 3 °C change in $T_M$ is present, and with two or four analogue base pairs (entries 5 and 6), the $T_M$ values decrease by about 10 and 26 °C, respectively. With four analogue residues distributed over the core sequence (entry 7) the $T_M$ value is about the same as that obtained with two analogue base pairs (entry 5). Thermodynamic analyses have assisted to characterize those complexes containing modified base pairs. The introduction of a single analogue base pair results in a 1.8 kcal/mol change in $\Delta G$ (entry 4), while the addition of a second modified base pair (entry 5) only results in a further 0.8 kcal/mol change. The presence of all four modified base pairs (entry 6) results in an additional loss of 1.4 kcal/mol in stabilization energy, an average of 0.7 kcal/mol per added base pair.

For comparison, we prepared the self-complementary Dickerson dodecamer containing a block of four $dc^3A{\cdot}dm^3 2P$ base pairs. At 4 $\mu$M total strand concentration this sequence exhibited a single transition at 70 °C. At increased concentration (16 $\mu$M) two transitions were present; one at 23 °C with moderate hyperchromicity and a second at 70 °C, moderately higher than that observed for the unmodified duplex. A biphasic transition, with a concentration dependence for the early transition, is a typical observation[15] for hairpin loops that can also adopt duplex structures with a central core devoid of Watson−Crick (W−C) base pairing. Using non-denaturing PAGE, we confirmed that the self-complementary dodecamer with four analogue base pairs preferentially adopts the hairpin conformation (data not shown). We additionally prepared a related self-complementary sequence in which the central A−T and T−A base pairs were replaced by G−C and C−G, respectively. This complex exhibited a single transition at all concentrations examined. When the remaining two T residues were replaced by the $dm^3 2P$ residues the $T_M$ was largely unchanged. Thermodynamic parameters for the two sequences were nearly identical (entries 8 and 9 in Table 1).

The experimental results strongly suggest that in the presence of the central core of analogue residues the dodecamer sequence results in decreased helix stability, and when present in a self-complementary sequence results in a strong preference for the hairpin conformation rather than the duplex. At least three parameters could be responsible for this preference, (i) changes in interstrand hydrogen bonding, (ii) changes in base stacking, or (iii) changes in hydration/metal ion binding.

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10.1021/ja000686v CCC: $19.00 
Published on Web 06/23/2000

**Table 1.** Effects of $dc^3A$ and $dm^32P$ Substitutions on the Stability of a Duplex Dodecamer[1]

5' d(C C G G **W X Y Z** C G C C)
3' d(G G C C **W'X'Y'Z'**G C G G)

| Entry | Sequence[2] W-W' | X-X' | Y-Y' | Z-Z' | $T_M$ | Thermodynamic Parameters[3] $\Delta G_{25}$ | $\Delta H$ | $\Delta S$ |
|---|---|---|---|---|---|---|---|---|
| 1 | A-T | A-T | T-A | T-A | 63.4 | -18.9 | -90.7 | -241 |
| 2 | A-T | **a**-T | T-A | T-A | 62.8 | | | |
| 3 | A-T | A-**t** | T-A | T-A | 61.5 | | | |
| 4 | A-T | **a**-**t** | T-A | T-A | 60.1 | -17.1 | -94.6 | -260 |
| 5 | A-T | **a**-**t** | **t**-**a** | T-A | 52.7 | -16.3 | -95.3 | -265 |
| 6 | **a**-**t** | **a**-**t** | **t**-**a** | **t**-**a** | 37.0 | -14.9 | -101 | -289 |
| 7 | A-**t** | A-**t** | T-**a** | T-**a** | 51.7 | | | |
| 8 | 5'C G C G A G C T C G C G | | | | 72.0 | -21.1 | -101 | -269 |
| 9 | 5'C G C G A G C **t** C G C G | | | | 70.6 | -21.1 | -102 | -271 |

[1] $T_m$ values were obtained at a concentration of 3 $\mu$M in 20 mM $NaH_2PO_4$, 1 M NaCl, pH 7.0 buffer using an AVIV 14DS spectrophotometer. Samples were heated at 0.5 °C/min. [2] **a** = $dc^3A$ and **t** = $dm^32P$. [3] Calculated with $T$ = 25 °C and reported in kcal/mol for $\Delta G$ and $\Delta H$ and in cal/mol °K for $\Delta S$.

**(i) Interstrand Hydrogen Bonding.** We designed the analogue base pair such that bidendate W−C-like hydrogen bonding would not only be possible but probable (Figure 1). The $dc^3A$ residue is essentially a ring-fused derivative of 2-aminopyridine, and UV studies of 2-aminopyridines have clearly established that these derivatives prefer the amino rather than the imino form.[16] Studies of acid dissociation constants have led to the same conclusions.[17] For the pyrimidine analogue we chose to prepare the C-nucleoside of the requisite 2-pyridone. Also as the result of ultraviolet studies, 2-pyridones have been shown to prefer the keto rather than the enol form.[18] In the absence of the $O^2$-carbonyl, less hindered rotation about the glycosidic bond will occur, but it seems likely that as the hydrogen bonding face of the $dc^3A$ residue approaches the $dm^32P$ analogue, the complementary hydrogen bonding face will be selected. If free rotation about the glycosidic bond represented a significant entropic disadvantage to base pairing, then the thermodynamic parameters measured for entries 8 and 9 in Table 1 would differ. Had either analogue undergone an unfavorable tautomeric shift, the base pair containing the analogue residue would have a lone pair−lone pair clash or an N−H) (H−N steric interaction—both lead to severe helix destabilization when present at even a single site.[19,20] Other effects could be present and related to the change in the length of the glycosidic bond for $dm^32P$ resulting from the replacement of the $N^1$-nitrogen by carbon, but when present in a G−C rich sequence (entry 9 in Table 1) no loss of stabilization was observed. Additionally, pseudouridine is a related derivative in which the uracil base has been rotated and attached to the carbohydrate through the C5 position. It exhibits essentially normal base pairing with adenosine in simple duplexes.[21]

**(ii) Base−Base Stacking.** The base stacking interactions between neighboring heterocycles are more difficult to quantify. In general it seems that heterocycles exhibit better stacking interactions than simple aromatic compounds but the reasons for these observations are less clear. Both dipole−dipole interactions[22] as well as the hydrophobic effect[23] have been suggested by recent studies as being origin for base stacking. We have calculated the dipole moment for the two native and analogue nucleosides used in this study in an aqueous environment both by AM1[24] and PM3-SM3[25] methods. By AM1 analysis, the dipole magnitude increases from 2.35 D (dA) to 3.46 D ($dc^3A$) with the analogue, but in the second case the magnitude decreases from 4.49 D (dT) to 3.73 D ($dm^32P$). The average for the base pair remains largely unchanged, and a similar phenomenon is observed using PM3-SM3 methodology. Hydrophobicity, as least as measured by mobility during reversed-phase HPLC is only moderately affected. With more significant detrimental base stacking effects for the analogue, the $T_M$ values for entries 8 and 9 in Table 1 would differ. These observations suggest that while some alteration in the nature of the base stacking interactions due to changes in dipolar or hydrophobicity effects may be present, the magnitude of these differences are not responsible for signifcant losses in duplex stabilization.

**(iii) Hydration/Metal Ion Binding.** Sequences containing adjacent $dc^3A$ residues (opposite dT's) have been characterized has having moderately reduced $T_M$ values,[12] but appear to still exist primarily as duplex structures. In those sequences the $O^2$-carbonyls of the partner dT residues are still available as sites for hydration/metal ion binding in the minor groove. Hydration in those sequences may not be as effective as in native dA-dT containing sequences and consequently the helix is destabilized. In the present study, the complete absence of both the $N^3$-nitrogens and the $O^2$-carbonyls at as many as four sites within the center of a dodecamer eliminates all such possible interactions, and dramatically alters helix stability. Thermodynamic analyses indicate that the presence of one analogue base pair results in a 1.8 kcal/mol loss in stabilization energy, while the presence of each additional analogue results in an additional 0.7−0.8 kcal/mol loss. This observation is consistent with a cooperative effect relating to the spine of hydration. The initial disruption of the ordered water molecules arising from the introduction of a single analogue base pair may propagate along the minor groove and disrupt additional ordered water molecules at adjacent sites. Adjacent analogue base pairs then have a correspondingly moderate effect. These observations are consistent with theoretical calculations[3] suggesting that in dA-dT sequences the spine of hydration should be considered an integral part of the helix and enhances the stability of the helix against base-pair opening events. The corollary to this suggestion is that in the complete absence of minor groove hydration, base pair opening at dA-dT base pairs can be expected to be enhanced and to contribute to the destabilization of the B-form duplex.

In addition to the absence of hydration, the inability to localize metal ions[6−8,10,11] to either the $N^3$-nitrogens or $O^2$-carbonyls in the minor groove, or to water molecules hydrating these sites (there is an alternative to this electrostatic view of the minor groove[9]), may increase phosphate−phosphate repulsion across the minor groove to destabilize the duplex, or in the case of the self-complementary sequence, facilitate the noted conformational change. Although bidentate hydrogen bonding base pairs with minimally altered base stacking properties are present in the core sequence, these effects alone do not appear to be sufficient for duplex stabilization. The spine of hydration associated with possible metal ion binding in the minor groove is critical for the structural integrity of duplex DNA.

**Acknowledgment.** We thank Tim Searls and Dr. Narendra Vaish, Boston College, for experimental assistance. We thank Professor Loren Williams, Georgia Institute of Technology, for helpful discussions. This work was supported by a grant from the NSF (MCB-9723844).

**Supporting Information Available:** Synthesis procedures for the $dm^32P$ nucleoside analogue, sample HPLC analyses of hydrolyzed oligonucleotides, sample absorbance vs temperature plots, and gel electrophoresis experiments (PDF). This material is available free of charge via the Internet at http//:pubs.acs.org.

JA000686V

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