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Stability of DNA Containing a Structural Water Mimic in an A-T Rich Sequence

Kerry J. Salandria,[†] Joseph W. Arico,[†] Amy K. Calhoun, and Larry W. McLaughlin*

Department of Chemistry, Merkert Chemistry Center, Boston College, 2609 Beacon Street, Chestnut Hill, Massachusetts 02467, United States

Supporting Information

ABSTRACT: We describe here the synthesis and properties of A-T rich DNA containing covalently bound water mimics located in the DNA minor groove.

Hydration in the minor groove of B-form DNA was initially observed in the early crystal structures described by Dickerson and co-workers.[1,2] More recent studies[3,4] have both confirmed the presence of the minor-groove spine of hydration and mapped the primary and secondary shells and hexagonal assemblies of water molecules. The primary water layer is hydrogen-bonded to the adenine N3 nitrogens (and/or thymine O2 oxygens) and then reaches across the minor groove to a second N3 nitrogen or O2 carbonyl in the opposite strand, one base pair displaced. The second water layer bridges the oxygens of the primary hydration layer.

Perhaps more challenging to understand is the contribution of such hydration patterns to duplex conformation and stability. Simple deletions of the adenine N3 nitrogens[5] and/or thymine O2 carbonyls[6] selectively eliminate hydrogen-bonding sites in the minor groove. Studies with such analogue nucleosides present in DNA have reported destabilization of the duplex in spite of the presence of the Watson−Crick functional groups that should permit essentially normal complementary base pairing. CD studies[7] have indicated that in the absence of selected N3 nitrogens and O2 carbonyls, duplex DNA readily undergoes a conformational change to a structure that is very A-like in nature. Also notable are studies showing that the presence of an uncompensated amino[8] or carbonyl[9] group in the minor groove is dramatically destabilizing.

We have designed an analogue nucleoside (Figure 1b) that attempts to mimic the N3-hydrated state of adenine (Figure 1a). Here we report on the synthesis of the analogue and related compounds, their incorporation into DNA, and the stability of duplexes containing one or more of the "covalently hydrated" adenine residues.

Our synthetic strategy was designed to prepare the 3-deaza and 3-deaza-3-methyl derivatives of dA as controls that would help us better understand the effects of the loss of the N3 nitrogen as well as the effects resulting from the introduction of steric bulk into the minor groove by using the methyl-substituted analogue. The final synthetic target was the covalently hydrated analogue, the 3-deaza-3-hydroxymethyl derivative. The syntheses of the 3-deaza and 3-deaza-3-methyl heterocycles are known, and that of the 3-deaza-3-hydroxymethyl derivative was straightforward. The challenge in all of the syntheses involved the glycosylation reactions. As the steric bulk increases (even from hydrogen to methyl) at the 3-position of the corresponding 6-chloropurine (the most common glycosylation target), the ratio of N7- to N9-glycosylated product becomes extremely unfavorable (N7 > N9). We note that the glycosylation of 6-chloro-3-deaza-3-methylpurine was reported[10] to yield the desired N9-glycosylated product in only 20% yield. We have largely solved[11] the difficulties related to effective glycosylation of 3-substituted purines with the introduction of the tetramethylsuccinimide (M_4SI) protecting group at the 6-position of the purine (Scheme 1). This group effectively blocks access to the N7 nitrogen, causing glycosylation to occur predominantly at the desired N9 nitrogen. Notably, with this protecting group the 3-deaza-3-(*O*-tributylsilyl)hydroxymethylpurine derivative could be glycosylated at the N9 nitrogen in 74% isolated yield (Scheme 1). The succinimide is easily hydrolyzed after the glycosylation (or after DNA synthesis) to unmask the 6-amino group, thus avoiding the harsh conditions necessary to convert the 6-chloro derivative to the corresponding amine. After elaboration of the analogue nucleosides into the fully protected phosphoramidites, they could be incorporated into DNA with yields that were sufficient to prepare the desired sequences.

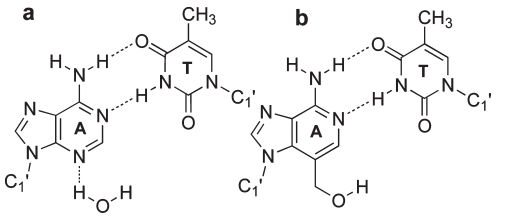
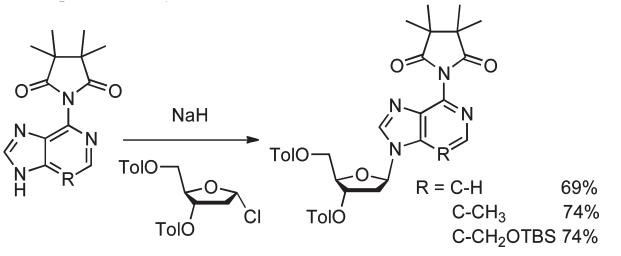


Figure 1. (a) Water molecule hydrogen-bonded to the N3 of dA as part of the minor-groove spine of hydration. (b) Covalently bound water mimic.

Scheme 1. Glycosylation of 3-Substituted Purines Using the Tetramethylsuccinimide (M_4SI) Protecting Group



After ammonia deprotection and purification of the oligonucleotides, the TBS group was removed by dissolving the 12-mers

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Table 1. Thermodynamic Stabilities of Selected DNA Duplexes

duplex	sequence at 5 μM duplex	T_M (°C)	ΔG (kcal/mol)
1	d(CCGG AAAA CGCC)	63.4	-19.1 ± 0.6
2	d(CCGG AAA^{H}A CGCC)	61.0	-16.4 ± 0.4
3	d(CCGG AAA^{CH_3}A CGCC)	58.3	-14.8 ± 0.3
4	d(CCGG AAA^{CH_2OH}A CGCC)	60.9	-16.4 ± 2.3
5	d(CCGG $(A^{H})_4$ CGCC)	51.1	-13.9 ± 0.2
6	d(CCGG $(A^{CH_3})_4$ CGCC)	44.4	-10.8 ± 0.4
7	d(CCGG $(A^{CH_2OH})_4$ CGCC)	54.7	-15.2 ± 0.7

in dimethyl sulfoxide followed by treatment with fluoride ion. Confirmation of the complete deprotection of the oligonucleotides was obtained by mass spectral analysis.

We prepared three types of oligonucleotides: (i) those containing 3-deazaadenine, (ii) those containing 3-deaza-3-methyladenine, and (iii) those containing 3-deaza-3-hydroxymethyladenine. The design parameters were as follows: The first modified oligos did not contain the N3 nitrogen, a site that is used in the formation of the spine of hydration but otherwise introduces no unfavorable steric effects. In the methyl-containing sequences, the N3 nitrogen was also removed, and additional unfavorable steric effects were introduced into the minor groove through the presence of one or more methyl groups. In the final sequences, hydroxyls were introduced onto the unfunctionalized methyls to generate a covalently bound water mimic capable of interacting by hydrogen bonding in the minor groove.

Thermodynamic parameters were obtained for the formation of all duplexes (Table 1). Even the simple elimination of a single N3 nitrogen from a centrally located dA residue (duplex **2**) resulted in a 2.4 °C decrease in the melting temperature (T_M), consistent with previous reports.[11] In that same study, the introduction of three dA residues lacking the N3 nitrogen resulted in a 12 °C change in T_M, which also compares favorably with the 12.3 °C reduction in T_M observed here with four deazaadenines present (duplex **5**). We then examined the methyl-substituted sequences (duplexes **3** and **6**). The 3-deaza-3-methyl analogues both lack the N3 nitrogen but also add steric bulk within the minor groove. Introduction of a single methyl group (duplex **3**) reduced the T_M value by 2.7 °C relative to a sequence containing a single 3-deaza analogue (duplex **2**) and by 5.1 °C relative to the unmodified standard. The presence of four analogues containing methyls (duplex **6**) reduced the T_M by a dramatic 19.0 °C, attesting to the duplex instability resulting from increased steric effects in the minor groove. The hydroxymethyl group is sterically even larger than a methyl group and could have an even more dramatic destabilizing effect should the hydroxy groups be unable to take part in advantageous interactions in the minor groove. Introduction of one $-CH_2OH$ group did result in helix destabilization relative to the control sequence (compare the T_M for duplex **4** with that for duplex **1**). However, relative to the single-methyl sequence (duplex **3**), the T_M for the hydroxymethyl sequence was some 2.6 °C higher. This observation is further supported by the sequence with four $-CH_2OH$ groups (duplex **7**), which exhibited a T_M 10.3 °C higher than that for the sequence containing four methyl groups. No significant additional cooperativity appeared to be present for any analogue duplex.

The simplest interpretation of these results is that the absence of hydrogen-bonding acceptors in the minor groove destabilizes the duplex as a result of disruption of the ordered spine of hydration. The presence of four methyl groups also disrupts the spine of hydration but additionally introduces further steric effects and additional destabilization of the duplex. The sequence containing four hydroxymethyl substituents in principle has the greatest steric effects in the minor groove, but the hydroxyl groups appear to provide complementary stabilizing interactions through a modified water structure guided by favorable interactions from the $-CH_2OH$ groups. A possible interaction mimicking the corresponding water molecule is illustrated in Figure 2.

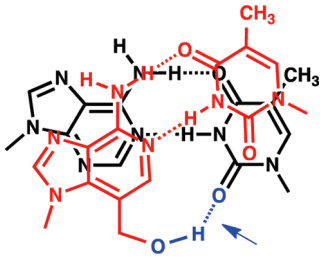

Figure 2. Structural water mimic interacting with the carbonyl of the cross-strand dT one base pair removed.

CD spectra of the various modified helices (Figure 3) also offer some insight into the effects of various analogues. All of the spectra suggest the presence of B-form helices. The CD spectra for the native sequence **1** and sequence **5** containing four dc^{3}A residues are the most alike of the four spectra, suggesting similar chromophoric stacking (dA vs dc^{3}A) within the helix. The interactions of the purine heterocycles are dramatically different for duplex **6**, in which four methyl groups have been added to the central core of base pairs. This spectrum suggests a quite different stacking of the dmc^{3}A chromophores, leading to dramatic shifts in the positive and negative displacements in the observed CD spectrum. The final spectrum, for duplex **7**, is intermediate in character relative to those of duplexes **5** and **6**. It seems likely that the minor-groove interactions present with the structural water mimic drive the conformation back toward a more nativelike B-form, but much like the temperature data, the final native B-like structure is not fully attained.

The data presented here suggest that simple methyl groups located at the C3 position of dA residues are sufficient for dramatic structural perturbation but that the introduction of a $-OH$ group as a structural water mimic assists in partial stabilization of the analogue structure, presumably through interactions within the minor groove.

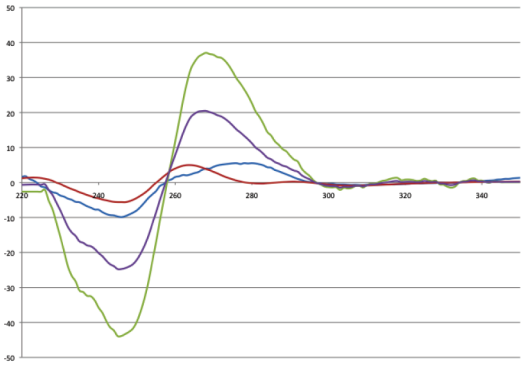

Figure 3. CD spectra (ellipticity vs wavelength at 3 μM duplex concentration) of duplexes **1** (blue), **5** (red), **6** (green), and **7** (purple) obtained in 20 mM sodium phosphate (pH 7) and 1 M NaCl.

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■ ASSOCIATED CONTENT

Supporting Information. Synthetic schemes, procedures, and thermal analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
mclaughl@bc.edu

Author Contributions
[†]These authors contributed equally.

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