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Introduction of Cationic Charge into DNA Near the Major Groove Edge of a Guanine•Cytosine Base Pair: Characterization of Oligodeoxynucleotides Substituted with 7-Aminomethyl-7-deaza-2′-deoxyguanosine

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The electrostatics of DNA is generally considered in terms of polyelectrolyte theory that treats DNA as a polyanionic cylinder.[1] Even at relatively low salt concentrations, the cation concentration in the vicinity of DNA is estimated to be 1 M, and diffusible cations are treated without regard to high vs low occupancy sites. Accordingly, the release of electrostricted ions, which is a powerful entropic driving force in the equilibrium binding of molecules to DNA,[1] is generally treated as nonsequence specific.

However, there are electrostatic factors associated with the DNA grooves, in addition to the phosphate backbone. NMR studies show that Mg^{2+} and related divalent cations preferentially bind in the major groove at G runs and appear to bend DNA.[2] A compilation of high-resolution structures of DNA shows a consensus for localization of monovalent cations in the major groove within 3.5 Å from the O^6- and/or N7-positions of dG.[3] The 1.2 Å resolution structure of 5′-d(CGCGAATTCGCG) in the presence of Tl^+ clearly indicates that there are high occupancy monovalent cation binding sites in the major groove near G/C regions as well as in the minor groove near A/T rich regions.[4]

To explore the role of groove associated cations on DNA stability, structure, and reactivity, we previously studied the effects of (i) removing a major groove cation binding site using 7-deazaguanine (**c^{7}G**)[5] and (ii) introducing a tethered cation into the major groove using a 5-(3-aminopropyl)uracil modified base.[6] The **c^{7}G** substitution results in lower thermodynamic stability that is mainly derived from a reduced enthalpy contribution.[5a] A crystal structure of DNA with **c^{7}G** shows the predicted loss of a cation from a conserved cation binding site, and the NMR indicates a destabilization at the flanking base pair.[5] The flexible cationic 3-aminopropyl side chain shows a complex sequence dependency in its impact on stability and structure that is related to its proximity to a major groove cation binding site.[6]

To create a structural model of groove associated cations that more accurately reflects cation distribution and location observed in DNA structures (Figure 1), we synthesized 7-aminomethyl-7-deaza-dG (**1**), which has a basic primary amine appended onto **c^{7}G** via a CH_2 linker.[7] **1** recapitulates the location of inorganic cations that are observed near the major groove edge of G: the restricted $-NH_3^+$ is ∼2.6 Å from the major groove face of the G (Figure 1), a distance that is also similar to that seen in crystal structures for cationic groups on basic amino acid residues of DNA binding proteins.[8] We also prepared 7-hydroxymethyl-7-deaza-dG (**2**) as a neutral isostere of **1**.[7] The spectroscopic and thermodynamic characterization of DNA containing **c^{7}G**, **1**, and **2** are reported.

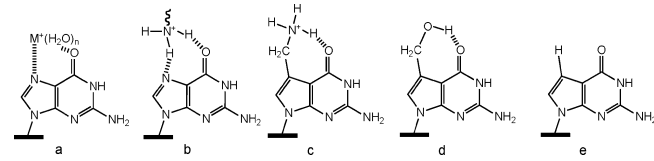

Figure 1. Models of (a) cation and (b) basic amino acid side chain associated with major groove edge of G, (c) tethered $-NH_3^+$ in 7-(aminomethyl)-7-deazaguanine (**1**), (d) 7-(hydroxymethyl)-7-deazaguanine (**2**), and (e) 7-deazaguanine (**c^{7}G**) lacking cation binding site.

The sequences of the oligomers studied along with their thermodynamic parameters from UV melting and differential scanning calorimetry (DSC) analyses for unmodified (G) and **c^{7}G**, **1**, and **2** substituted DNA are shown in Table 1. The sequence used (**OL-1**) was selected because it is self-complementary and forms an intermolecular duplex rather than an intramolecular hairpin. Similar to what was previously observed in another sequence,[5a] the **c^{7}G** substitution at G-5 (**OL-2**) decreases stability relative to **OL-1**: $\Delta\Delta G^\circ = 2.5$ and 4.1 kcal/mol at 10 and 100 mM NaCl, respectively. In contrast, the substitution of **1** (**OL-3**) makes the DNA thermodynamically more stable than **OL-1**: $\Delta\Delta G$ −2.2 and −1.4 kcal/mol at low and high salt, respectively. Relative to **OL-2**, the introduction of the tethered NH_3^+ increases stability by 4.7 and 5.5 kcal/mol at low and high salt, respectively. This translates to each $CH_2NH_3^+$ appendage increasing the stability by 2.3−2.7 kcal/mol. We attribute the stabilization predominantly to the tethered NH_3^+ ion, since DNA modified with **2** (**OL-4**) is thermodynamically similar to **OL-2** with **c^{7}G**. A similar trend is observed when **c^{7}G**, **1**, and **2** are introduced at G-3 (Supporting Information).

The origin of the differences in ΔG for the duplexes is derived from the ΔH term for the G-5 substitutions; **c^{7}G** in **OL-2** causes a marked reduction (21.9 and 24.8 kcal/mol at low and high salt, respectively) in enthalpic stabilization, while **1** in **OL-3** is enthalpy stabilizing by −14.7 and −7.7 kcal/mol at low and high salt, respectively. DNA with **2** (**OL-4**) behaves similar to the **c^{7}G**-modified DNA. At the 3-position in **OL-1**, the ΔH term for DNA modified with **1** is similar to that of unmodified DNA and remains higher than that for duplexes with **c^{7}G** and **2**, particularly in 100 mM NaCl (Table 1 and Supporting Information).

The differences in the ΔH term can reflect changes in base stacking and/or hydration. Therefore, the effects of the different modifications on cation and water release upon unfolding were examined by T_M dependencies on salt concentration and osmolyte molality, respectively.[9] There is a correlation between the ΔH term and DNA hydration. **OL-1** and **OL-3** have similar Δn_W values, which are significantly higher than those observed for **OL-2** and

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Table 1. Thermodynamic Parameters for DNA Formation at 20 °C[a]

OL	sequence	NaCl[b]	T_M[c]	$\Delta G°$[d,e]	$\Delta H°$[e]	$T\Delta S°$[e]	Δn_{Na+}[f]	Δn_W[f]	$\Delta\Delta G$ vs OL-1 or -5
1	5'-GAGAGCGCTCTC	10	48.7	-6.9	-78.2	-71.3	-3.35 ± 0.17	-41 ± 3	-
		100	66.1	-12.5	-92.0	-79.5	-3.61 ± 0.18	-43 ± 4	
2	5'-GAGA-**c^{7}G**-CGCTCTC	10	44.7	-4.4	-56.3	-51.9	-2.14 ± 0.11	-25 ± 2	2.5
		100	62.0	-8.4	-67.2	-58.8	-2.31 ± 0.12	-27 ± 2	4.1
3	5'-GAGA-**1**-CGCTCTC	10	52.0	-9.1	-92.9	-83.8	-2.86 ± 0.14	-38 ± 4	-2.2
		100	67.3	-13.9	-99.7	-85.9	-2.90 ± 0.15	-36 ± 3	-1.4
4	5'-GAGA-**2**-CGCTCTC	10	47.2	-4.6	-54.5	-49.9	-1.63 ± 0.10	-18 ± 2	2.3
		100	63.9	-7.6	-58.2	-50.6	-1.52 ± 0.10	-17 ± 2	4.9
5	5'-CGCGTTTTCGCG	10	68.4	-4.4	-31.0	-26.6	-0.26 ± 0.02	-18 ± 2	-
6	5'-CGCGTTTTC-**c^{7}G**-CG	10	63.7	-3.5	-27.0	-23.5	-0.21 ± 0.02	-15 ± 2	0.9
7	5'-CGCGTTTTC-**1**-CG	10	63.0	-4.0	-31.3	-27.3	0	-14 ± 2	0.4
8	5'-CGC-**1**-TTTTCGCG	10	71.3	-4.3	-28.9	-24.6	0	-16 ± 2	0.1
9	5'-CGC-**1**-TTTTC-**1**-CG	10	65.6	-3.6	-27.0	-23.4	0	-13 ± 1	0.8

[a] Parameters are measured from UV (T_M) and DSC melting curves in 10 mM sodium phosphate buffer (pH 7.0). The observed standard deviations are T_M (±0.7), ΔH_{cal} (±3%), $\Delta G°_{20}$ (±5%), $T\Delta S_{cal}$ (±3%). [b] Salt concentration in mM. [c] °C. [d] Determined at 20 °C. [e] kcal/mol. [f] per mol DNA.

OL-4. Interestingly, the release of cations upon unfolding varied little between **OL-1** and **OL-3** but was significantly lower for **OL-2** and **OL-4** (Table 1).

CD spectra provide global DNA conformational information of the different duplexes and some indication of changes in base stacking by analysis of the relative intensities of the negative band at ~250 nm.[10] The CD spectra of **OLs 1**−**12** are consistent with a B-conformation, and there is a correlation between the intensity of the negative bands and the ΔH term at 10 mM NaCl that reflects base stacking for the oligomers (Supporting Information).

In addition to the intermolecular duplex DNA, an intramolecular hairpin with a higher T_M (**OL-5**) was modified with a **c^{7}G** (**OL-6**) or a single residue of **1**. The introduction of **c^{7}G** results in a minor destabilization (Table 1), while **OL-7** and **OL-8** are very similar to the unmodified hairpin. The addition of a second **1** (**OL-9**) places the two cations as close as 4.0 Å of each other assuming a normal B-conformation. This causes a modest reduction in the thermodynamic parameters. The minimum distance between the tethered NH_3^+ ions in **OL-3** is 8.3 Å. Of note is that substitution of **1** (**OL-7** and **OL-8**) or two residues (**OL-9**) into the hairpin removed any T_M salt dependency; Δn_{Na+} is 0.

In summary, the tethering of cationic and neutral polar functionalities at a distance from the floor of the major groove that mimics the distance observed for diffusible cations and basic amino acid residues is reported. In DNA with a **c^{7}G** substitution, a conserved cation binding is eliminated and the DNA is thermodynamically destabilized.[5] In the current study, it is demonstrated that the insertion of **1** into DNA, which permanently refurbishes a cation that is lost with **c^{7}G**, increases the stability of DNA relative to that of the natural sequence. Locating a polar hydroxyl group on the major groove edge of **c^{7}G** does not restore stability. The effect of the tethered cationic modification provides an insight into the stabilizing role that "diffusible" cations associated with high occupancy sites in the major groove play in maintaining DNA structure.

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Supporting Information Available: Thermodynamic and CD data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Manning, G. S. *Q. Rev. Biophys.* **1978**, *2*, 179–246. (b) Record, M. T., Jr.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *2*, 103–179. (c) Honig, B.; Nicholls, A. *Science* **1995**, *268*, 1144–1149.
(2) (a) Braunlin, W. H.; Nordenskiöld, L.; Drakenberg, T. *Biopolymers* **1991**, *31*, 1343–1346. (b) Buckin, V. A.; Kankiya, B.; Renteperis, D.; Marky, L. A. *J. Am. Chem. Soc.* **1994**, *116*, 9423–9429.
(3) Howerton, S. B.; Nagpal, A.; Williams, L. D. *Biopolymers* **2003**, *69*, 87–99.
(4) Howerton, S. B.; Sines, C. C.; VanDerveer, D.; Williams, L. D. *Biochemistry* **2001**, *40*, 10023–10031.
(5) (a) Ganguly, M.; Wang, F.; Kaushik, M.; Stone, M. P.; Marky, L. A.; Gold, B. *Nucleic Acids Res.* **2007**, *35*, 6181–6195. (b) Wang, F.; Li, F.; Ganguly, M.; Marky, L. A.; Gold, B.; Egli, M.; Stone, M. P. *Biochemistry* **2008**, *47*, 7147–7157.
(6) (a) Soto, A. M.; Kankia, B. I.; Dande, P.; Gold, B.; Marky, L. A. *Nucleic Acids Res.* **2002**, *30*, 3171–3180. (b) Li, Z.; Huang, L.; Dande, P.; Gold, B.; Stone, M. P. *J. Am. Chem. Soc.* **2002**, *124*, 8553–8560. (c) Moulaei, T.; Maehigashi, T.; Lountos, G.; Komeda, S.; Watkins, D.; Stone, M.; Marky, L.; Li, J.-S.; Gold, B.; Williams, L. D. *Biochemistry* **2004**, *43*, 7458–74648.
(7) Wang, R.-W.; Gold, B. *Org. Lett.* **2009**, *11*, 2465–2468.
(8) See Protein Data Bank (http://www.rcsb.org/pdb, e.g., PDB id: 1GU5, 1CF7, 1DP7, 1GCC, 3BPY, 1C7U).
(9) (a) Spink, C. H.; Chaires, J. B. *Biochemistry* **1999**, *38*, 496–508. (b) Kaushik, M. N.; Suehl, N.; Marky, L. A. *Biophys. Chem.* **2007**, *126*, 154–164.
(10) (a) Tinoco, I.; Sauer, K.; Wang, J. C.; Puglisi, J. *Physical Chemistry: Principles and Applications in Biological Sciences*, 4th ed.; Prentice Hall, 2001. (b) Basse, W. A.; Johnson, W. C., Jr. *Nucleic Acids Res.* **1979**, *6*, 797–814. (c) Kypr, J.; Kejnovská, I.; Renčiuk, D.; Vorlíčková, M. *Nucleic Acids Res.* **2009**, *37*, 1713–1725.

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