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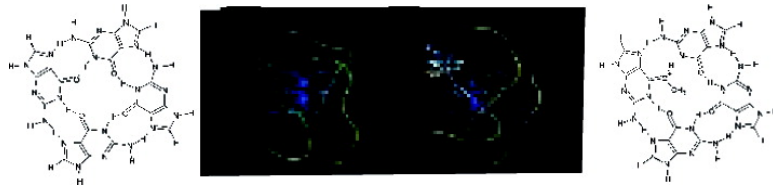

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Effect of O^6-Methylguanine on the Stability of G-Quadruplex DNA

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The incorporation of O^6-methylguanine (6mG) into DNA is linked to the induction of mutations and cancers.[1] Methylation at the O^6-position of guanine can result in a point mutation from the transition of a G:C to A:T base pair, preventing accurate replication.[2] 6mG is the major mutagenic lesion introduced by methylating environmental carcinogens.[3,4]

DNA methylation rates are significantly higher in repetitive DNA sequences than in nonrepetitive genomic DNA regions.[5,6] Telomeres are unique structures found at the ends of chromosomes that function to protect chromosome integrity. The DNA sequence of human telomeres consists of a large number of guanine-rich repeats.[7] It is well established that the guanine-rich sequences from the human telomere can fold into unimolecular G-quadruplex structures with several distinct conformations that form depending on the solution environment.[8,9] In Na^+ solution, an antiparallel "basket" structure forms featuring two lateral loops and one diagonal loop.[10] In K^+ solution, an antiparallel "hybrid" structure forms, featuring two lateral loops and one side loop.[11−14] The propeller X-ray crystal structure was shown by biophysical methods to not be the predominant structure in solution.[15]

G-Quadruplex formation and stabilization are influenced by many factors, including sequence, loop size and composition, and strand concentration. Perhaps the most important element in G-quadruplex formation is the monovalent cations needed to stabilize the negative electrostatic potential created by the guanine O^6 oxygen atoms within the quadruplex core. Monovalent as well as divalent cations are an integral part of G-quadruplex structure, and without cations, G-quadruplexes are unstable.[8]

The effects of 6mG on quadruplex formation and stability have not been previously investigated. We hypothesized that O^6-methylation within a G-quartet would either inhibit quadruplex DNA formation or destabilize the quadruplex structure because of at least two factors. First, O^6-methylation could weaken interactions with Na^+ or K^+ ions within the quadruplex core by alteration of the metal coordination site. Second, methylation might weaken the Hoogsteen hydrogen bonding that stabilizes G-quartets because of the decrease in electronegativity or steric hindrance.[16] To test this hypothesis, we studied the structure and stability of G-quadruplexes formed by the human telomere sequence with single modifications in which 6mG was substituted for guanine. All experimental materials and methods used can be found in Supporting Information. The oligonucleotides used in this study are shown in Table 1.

The effects of O^6-methylation substitution on the structure of the G-quadruplexes were first studied by circular dichroism (CD). Oligonucleotides were annealed in either Na^+ or K^+ solution, and CD spectra were obtained at 20 °C (Figure 1). In the presence of Na^+, unmodified human telomere sequence I (Table 1) gave the characteristic CD signature expected for an antiparallel structure, with a maximum at 295 nm and a minimum at 260 nm (Figure 1, Na^+).[8] The modified sequences (II, III, IV, Table 1) containing a single 6mG substitution displayed CD spectra similar to the unmodified control, but with a slight reduction in the magnitude of the CD (especially near 260 nm) and a slight shift in the wavelength of the minima (Figure 1, Na^+). In contrast, greater perturbations of CD spectra were seen in presence of K^+ (Figure 1, K^+). The unmodified sequence I showed the characteristic spectrum for the hybrid form. The sequences (II, IV) containing 6mG residues at the ends of the G-quartet stack yield CD spectra distinct from the unmodified control, with decreased CD near 260 nm. 6mG substitution in the central G-quartet perturbs the CD spectrum most and yields a radically different spectrum with positive maxima at 260 and 295 nm. Sedimentation velocity experiments (Figure S1) and gel electrophoresis experiments (Figures S2 and S3) show that, under the solution conditions of the CD experiments, the oligonucleotides appeared to behave as either monomeric or

Table 1. Thermal Stability and Sedimentation Coefficient

		T_m[a] [°C]		$S^*_{(20,w)}$[b] [Svedbergs]	
	oligonucleotide	Na^+	K^+	Na^+	K^+
I	$AGGG(TTAGGG)_3$	62	70	1.76	1.95
II	$A\text{-}6mG\text{-}GG(TTAGGG)_3$	53	56	1.85[c]	2.01
III	$AG\text{-}6mG\text{-}G(TTAGGG)_3$	37	36	1.86	1.94[c]
IV	$AGG\text{-}6mG\text{-}(TTAGGG)_3$	48	52	1.89	2.03

[a] T_m was determined from nonlinear curve fitting of the melting curve at 295 nm with a heating rate of 1 °C min^{-1}. [b] Distributions of sedimentation coefficient from velocity ultracentrifuge experiments at 20 °C in water for A_{260} 0.8 samples. [c] Sedimentation coefficient for the major peak only, other species present are shown in Table S1.

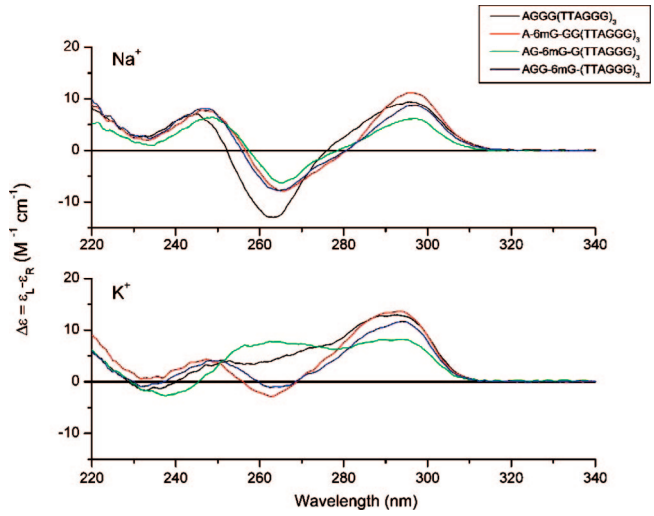

Figure 1. CD spectra of G-quadruplex structures. Spectra were obtained in 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl or KCl at pH 7.

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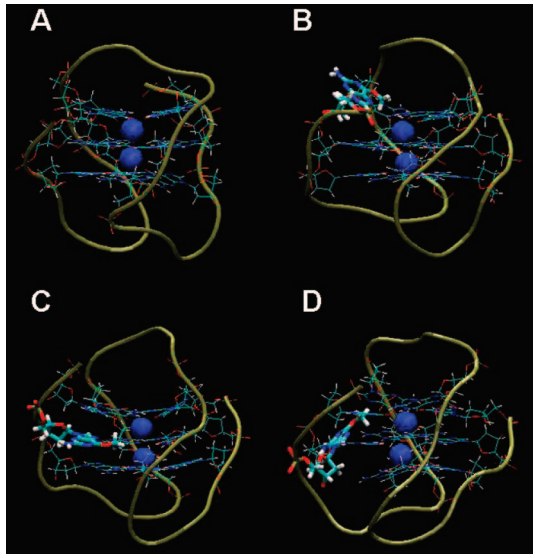

Figure 2. Average structures from MD trajectories in Na^+ buffer. (A) $AGGG(TTAGGG)_3$, (B) $AG\text{-}6mG\text{-}G(TTAGGG)_3$, (C) $AGG\text{-}6mG(TTAGGG)_3$, and (D) $AGGG\text{-}6m(TTAGGG)_3$. The methylated residue is drawn in stick form. The loop residues are not shown.

dimeric species, but no G-wires formed (Figure S1).[17,18] Electrophoretic mobilities differed for each sequence, but O^6-methylation did not promote aggregation to higher molecular weight forms.

UV-monitored thermal denaturation experiments were performed to evaluate the thermal stability of the methylated G-quadruplexes containing 6mG (Table 1 and Figure S4). Table 1 shows that the substitution of 6mG significantly destabilizes the quadruplex structure regardless of its position. Substitution within the central G-quartet is the most destabilizing, with the T_m decreased by 25 °C in Na^+ and 34 °C in K^+.

Analytical ultracentrifugation sedimentation velocity experiments were performed at 60 000 rpm at 20 °C to determine the distribution of sedimentation coefficient(s) (Table 1). G-Quadruplexes in Na^+ have *s* values around 1.8; whereas in K^+ solution, the *s* values for the G-quadruplexes are closer to 2.0 (Table 1). Sedimentation velocity experiments show multiple species for sequences II and III in Na^+ and K^+, respectively (Figure S1 and Table S1). In both ionic solutions, the sedimentation velocity experiments were not sensitive to G-quadruplex concentration, suggesting that they do not aggregate to form higher order G-wire structures (Table S1 and Figure S1).[17]

To further investigate the structural effect of methylation on the structure of the G-quadruplexes, molecular dynamics (MD) simulations were performed as described in Supporting Information. Starting structures were generated using deposited coordinates for NMR structures for the Na^+ form or the hybrid form in K^+.[10] After an equilibration period, each starting structure had an unrestrained production trajectory of 5 ns at 300 K. The simulations were performed with both Na^+ and K^+ ions. Figure 2 shows the average minimized structures over the last 50 ps of the MD trajectories for the Na^+ forms of the control sequence I and the substituted sequences II−IV.

The results of the simulations indicate that, for all the substituted sequences in Na^+, the methylated guanine does not participate in the G-tetrad formation and may flip out from the stacked quartets. However, they do not cause major distortion to the overall antiparallel structure but weaken the stabilization as the quadruplex melting temperature significantly decreased (Table 1). MD simulations of the K^+ hybrid forms show that the structures of substituted sequences II and IV remain stable, but the substitution within the central quartet results in a complete destruction of the whole G-quartet stack. Notably, the measured sedimentation coefficient of sequence III in K^+ is radically different from all others (Table S1), suggesting that 6mG substitution favors an alternate conformation. Additional MD simulations are in progress to explore this possibility.

A study of the effects of 6-thioguanine substitution on duplex and quadruplex stability was reported.[19] In that study, 6-thioguanine was reported to destabilize duplex DNA and to completely inhibit formation of quadruplex DNA as represented by the 15 nt thrombin binding aptatmer sequence. The aptatmer sequence quadruplex structure contains only two stacked quartets, instead of the three present in all forms of the human telomere quadruplex structure. In contrast to this earlier report, we find the substitution at the O^6 position of guanine can be accommodated but is destabilizing.

This study shows that G-quadruplex stabilization is directly related to the cooperative nature of the four guanines and its ions. Each quartet needs the interactions of all four members for stability. Disruption of the interactions in the middle G-quartet can destabilize the entire quadruplex structure. In both ionic conditions examined here, disruption of the top and bottom G-quartets reduces the overall G-quadruplex stability but not as great as a disruption in the middle G-quartet.

Our studies represent the first to analyze the effect of methylation on G-quadruplex DNA. The stability of G-quadruplex is decreased by half if a guanine involved in the middle G-quartet is methylated at its O^6 position. We found that, in Na^+ solution, methylation at any site insignificantly affects the overall antiparallel G-quadruplex structure. In K^+ solution, methylation in the top and bottom G-quartets results in a conformational change similar to those seen in Na^+ solution. In both solutions, the effect of methylation is most dramatic in terms of conformation and stabilization of the overall G-quadruplex when a guanine in the middle G-quartet is methylated. Here we show that the folding of a G-quadruplex can be affected by the methylation of guanines involved in G-quartets.

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Supporting Information Available: Supplemental figures, materials, and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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