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A Conformationally Constrained Nucleotide Analogue Controls the Folding Topology of a DNA G-Quadruplex

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Guanine-rich DNA and RNA sequences can fold into unique structures known as G-quadruplexes.[1] Interest in G-quadruplexes has increased since G-rich telomeric DNA sequences synthesized by telomerase were shown to form G-quadruplexes in vitro, and a number of biologically active RNA and DNA aptamers including anti-HIV, anti-proliferative, and anti-coagulation aptamers consist of G-quadruplex structures.[1] Furthermore, G-quadruplexes have recently been investigated as biosensors[2] and for use in nanotechnology.[3] The structures of G-quadruplexes can be divided into several classes, depending on the parallel or antiparallel nature of the strands and the number of G-rich tracts present in an oligonucleotide.[1] Oligonucleotides with single tracts of guanines form intermolecular parallel tetrameric G-quadruplexes. Oligonucleotides with two tracts of guanosines separated by two or more bases can form both intermolecular antiparallel fold-back dimeric and parallel tetrameric G-quadruplexes, and those with four tracts of guanosines can form both intramolecular parallel and antiparallel structures (Figure 1).[1] Intermolecular $[d(G_4T_4G_4)]_2$ (*Oxy*12) and intramolecular $(G_4T_4)_3G_4$ (*Oxy*28) G-quadruplexes formed by the *Oxytrichia trifalax* telomeric DNA sequence have been extensively studied by CD[4] and NMR spectroscopy[5] and by crystallography.[6] Despite several possible folding topologies, the G-quadruplex formed by *Oxy*28 has been observed only as an antiparallel crossover-basket structure (Figure 1, structure I). The ability to control the folding of G-quadruplexes would allow the physical, biochemical, and biological properties of these various folding topologies to be studied. Here we report a new method to control the folding of G-quadruplex DNA by specifically positioning the conformationally constrained nucleotide analogue 2′-*O*-4′-*C*-methylene-linked ribonucleotide (LNA) into a DNA G-quadruplex.[7]

The distribution of *syn* and *anti* glycosidic bond configurations differs in the various G-quadruplex-folding topologies. For example, the guanosine residues are alternatively *syn*−*anti*−*syn*−*anti* along the G_4 tracts in the crossover-basket form of *Oxy*28, and each sugar pucker is in the 2′-*endo* conformation,[5] whereas the guanosine residues are all *anti* in the intramolecular parallel, propeller G-quadruplex formed by the human telomeric DNA sequence[8] and other parallel G-quadruplexes (for example Figure 1, structure III).[1] Because sugars constrained in the 3′-*endo* conformation prefer the glycosidic bond to be in the *anti* conformation, we hypothesized that controlling the sugar pucker at selected positions would perturb G-quadruplex-folding topology. To test this possibility, we studied the ability of DNA oligonucleotides containing single LNA substitutions (Figure 2) to affect the conformation of the intramolecular G-quadruplex formed by *Oxy*28.

We evaluated the thermal stability of G-quadruplexes formed by *Oxy*28 with specifically positioned LNAs (see Table 1 for the oligonucleotides used in this study) by UV-monitored thermal denaturation experiments. Oligonucleotides were annealed by heating the samples in 10 mM phosphate buffer containing either 50 mM Na^+ or 50 mM K^+ at 95 °C for 5 min and then cooling the samples at 1 °C min^{-1} to room temperature. Samples were stored at 4 °C, with no special cooling, until they were analyzed. Prolonged storage at 4 °C did not influence the stability or the structure of the molecules; however, the cooling rate of the annealing reaction had a profound effect on stability with faster cooling rates giving rise to less stable G-quadruplex structures. The presence of compact

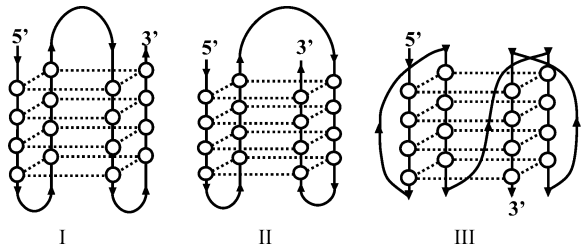

Figure 1. Three possible folding topologies of *Oxy*28. I, an antiparallel crossover basket; II, an antiparallel chair; and III, a parallel propeller. Open circles represent guanosine residues. Arrows indicate the directionality of the strands.

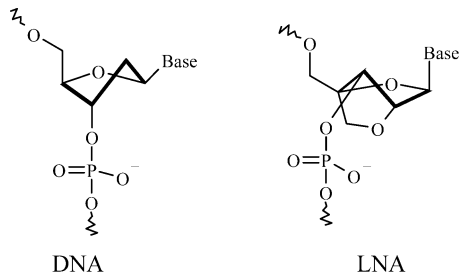

Figure 2. Structures of DNA and LNA. DNA is drawn in the C2′-endo conformation. LNA is locked in the C3′-endo conformation.

Table 1. Thermal Stability of LNA-Containing G-Quadruplexes

Oligonucleotide[a]	T_m[b] Na^+	T_m[b] K^+
1.5'd(GGGGTTTTGGGGTTTTGGGGTTTTGGGG)	64	85
2.5'd(-G-------------------------)	50	60
3.5'd(---G-----------------------)	60	72
4.5'd(----T----------------------)	59	80
5.5'd(-----T---------------------)	60	80
6.5'd(--------------T------------)	62	72
7.5'd(--------------T------------)	61	74
8.5'd(----------------G----------)	60	65
9.5'd(----------------G----------)	58	65

[a] Entry 1 is the *Oxy*28 DNA control. Underlined positions contain *syn*-glycosidic bonds in the *Oxy*28 DNA structure.[5] Entries 2−9 are LNA-containing oligonucleotides with the position of the LNA indicated. [b] T_m was determined from the first derivative of the melting curve at 295 nm with a heating rate of 1 °C min^{-1}. Values represent averages from experiments at DNA concentrations of 5 and 25 μM. Standard errors were <0.5 °C in duplicate determinations.

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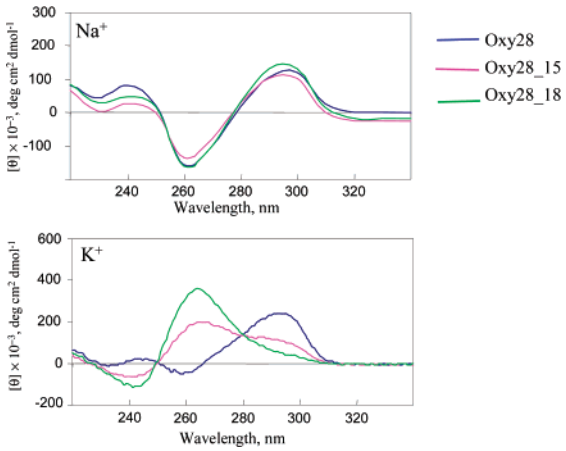

Figure 3. CD spectra of representative G-quadruplex structures. Spectra were obtained in 10 mM phosphate buffer (pH = 7.5) with either 50 mM Na^+ and 1 mM EDTA, or 50 mM K^+ and 1 mM EDTA.

structures indicative of a G-quadruplex was established for each oligonucleotide using nondenaturing gel electrophoresis (see Supporting Information). UV heating and cooling curves at 295 nm for each sequence were superimposable using a heating/cooling rate of 1 °C min^{-1}, suggesting folding kinetics faster than the cooling rate. Melting temperatures were determined from the first derivative of the melting curves (Table 1). Importantly, the melting temperature was not sensitive to the oligonucleotide concentration over a 5-fold concentration range, suggesting that each structure had a one-strand stoichiometry. In all cases, the presence of K^+ allowed a more stable structure than Na^+, a trend routinely observed with G-quadruplexes.[9] In general, the presence of an LNA was destabilizing to the G-quadruplex formed by *Oxy*28. This was particularly noticeable when an LNA was present at a guanosine residue. The G-quadruplex with an LNA in position 2 was severely destabilized in both K^+- and Na^+-containing solutions.

Next, we investigated the effects of LNA substitution on the structure of folded *Oxy*28. Oligonucleotides were annealed in either K^+ or Na^+, and CD spectra were obtained at 25 °C (Figure 3). In the presence of Na^+, unmodified *Oxy*28 gave rise to a strong maximum at 295 nm and a strong minimum at 260 nm (Figure 3, Na^+). Similarly, in the presence of K^+, the spectrum of unmodified *Oxy*28 displayed a strong maximum absorbance at 295 nm with a shallow minimum at 260 nm (Figure 3, K^+). These CD spectra are indicative of an antiparallel G-quadruplex (Figure 1, structure I).[1,4] The structures of LNA-containing oligonucleotides annealed in the presence of Na^+ were not affected by the presence or location of LNA substitution (Figure 3, Na^+). Each oligonucleotide produced a strong absorbance at 295 nm, consistent with an antiparallel structure. However, in K^+-containing buffer the presence and location of LNA substitution affected the structure (Figure 3, K^+). LNA substitution resulted in a complete change of the more stable G-quadruplex structure (LNA in positions 2, 17, or 18), a mixture of structures (LNA in positions 4, 15, or 16), or did not affect the structure (LNA in positions 5 and 6). The new structure displayed a CD spectrum with a maximum at 260 nm and a strong minimum at 240 nm. Intermolecular parallel G-quadruplexes are well-established structures, and intramolecular parallel G-quadruplexes have recently been observed by crystallography[8] and NMR spectroscopy.[10] In each of these reported cases, the CD spectra of a parallel G-quadruplex exhibited a diagnostic signal at 260 nm. Since we observed no concentration dependence on the CD spectra or the thermal stability, we conclude that LNAs at positions 2, 17, and 18 caused a shift in the thermodynamically preferred structure from an intramolecular antiparallel to an intramolecular parallel G-quadruplex (Figure 1, structure III).

Our studies represent the first to analyze the effect of conformationally constrained nucleotide analogues on DNA structures other than simple duplex and triplex.[11] LNAs increase thermostability of duplex and triplex structures and increase the A-form character of the nucleotides adjacent to the LNA. We found that LNA can induce a change in the thermodynamically preferred structure of the G-quadruplex formed by *Oxy*28 from an antiparallel to a parallel structure. Previously, the folding of a G-quadruplex was shown to be affected by the identity of the mono- and divalent cations used to stabilize the structure.[8,12] Here we show that internal modifications can be used to affect G-quadruplex-folding topology. By using LNAs, which favor *anti*-glycosidic bonds, we could produce parallel G-quadruplexes, which are known to contain *anti*-glycosidic bonds with the G tetrad. This method should find use in determining the biological significance of G-quadruplex-folding topology, controlling G-quadruplex folding for applications in nanotechnology, and in studying other nucleic acid-folding topologies.

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Supporting Information Available: CD Spectra and nondenaturing polyacrylamide gel analysis of all oligonucleotides tested. This material is available free of charge via the Internet at http://pubs.acs.org.

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