G-Quadruplexes

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Inverting the G-Tetrad Polarity of a G-Quadruplex by Using Xanthine and 8-Oxoguanine

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Abstract: *G-quadruplexes are four-stranded nucleic acid structures that are built from consecutively stacked guanine tetrad (G-tetrad) assemblies. The simultaneous incorporation of two guanine base lesions, xanthine (X) and 8-oxoguanine (O), within a single G-tetrad of a G-quadruplex was recently shown to lead to the formation of a stable G·G·X·O tetrad. Herein, a judicious introduction of X and O into a human telomeric G-quadruplex-forming sequence is shown to reverse the hydrogen-bond polarity of the modified G-tetrad while preserving the original folding topology. The control exerted over G-tetrad polarity by joint X·O modification will be valuable for the design and programming of G-quadruplex structures and their properties.*

Guanine-rich (G-rich) oligonucleotides can assemble as four-stranded structures that are distinctly different from the well-known two-stranded DNA double helix.[1] These non-canonical structures, termed G-quadruplexes, are formed from consecutively stacked G·G·G·G tetrads (or G-tetrads).[2] Within a G-tetrad, four guanines adopt a square planar arrangement that is stabilized by Hoogsteen hydrogen-bonding interactions.[2]

G-quadruplexes exhibit high stability under physiological conditions[1] and their formation in cells can block enzyme progression in key biological processes.[3] This property of G-quadruplexes could be potentially exploited in therapeutics, with G-quadruplexes being anticancer targets[4] or applied as antiviral/anticancer agents.[5] In DNA nanotechnology, the ability of G-quadruplexes to transport charges between consecutively stacked G-tetrads has been exploited to construct conductive nanowires[6] and electronic switches.[7]

It has been observed that a single G-rich sequence can often form multiple structures. Moreover, slight modifications to a sequence that forms a well-defined predominant G-quadruplex structure can completely change the way it folds.[8] Studies have shown that structural diversity in G-quadruplexes arises from differences in stoichiometry, strand orientation, types of loop connecting the G-tracts, and the glycosidic angle of G-tetrad-forming guanine bases (*syn* or *anti*).[1] Such variability leads to a large pool of available G-quadruplex structures for applications in therapeutics[5] and nanotechnology.[6b] For example, computational and experimental data have shown that different stacking geometries in G-quadruplexes lead to differences in fluorescence and charge-transfer properties.[9]

Efforts have been made to control the G-quadruplex scaffold by adjusting the environment: the type and concentration of cation,[10] or the presence of co-solutes such as polyethylene glycol.[11] Another approach for manipulating the G-quadruplex scaffold involves the rational incorporation of guanine analogues within the G-quadruplex scaffold, such as sugar-modified nucleotides[12] [e.g., 2′-deoxy-2′-fluoro-guanosine (^{F}G), 2′-deoxy-2′-fluoro-arabinoguanosine (^{FANA}G), locked nucleic acid (LNA), peptide nucleic acid (PNA), unlocked nucleic acid (UNA)] and base-modified guanine analogues[13] [e.g., inosine, 6-thioguanine, 8-aminoguanine, 8-bromoguanine, 8-methylguanine, 8-oxoguanine].

By using a 5′-5′ inversion-of-polarity backbone modification, Martino et al.[14] showed that relative strand orientations in the G-tetrad core could be manipulated without altering the loop arrangements. In a new approach for manipulating

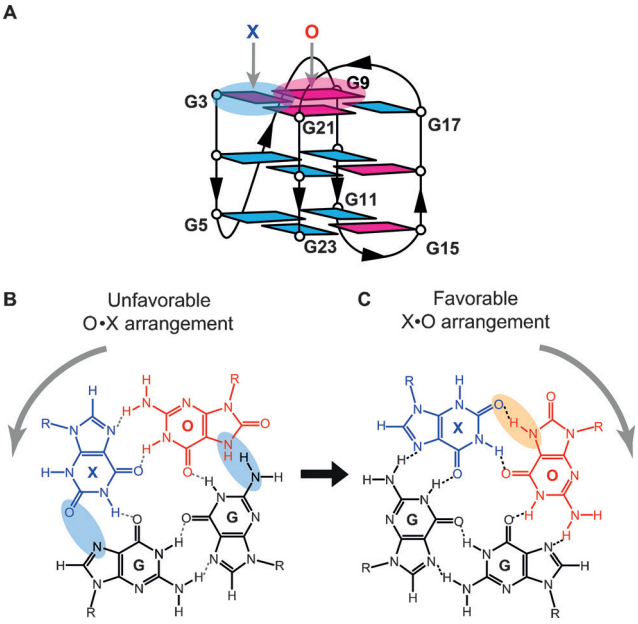

Figure 1. G-quadruplex DNA used in this study. A) Schematic structure of a human telomeric G-quadruplex with the proposed X3 and O9 modifications. B, C) The tetrad modified with xanthine and 8-oxoguanine (G·G·X·O tetrad) undergoes a conversion from the unfavorable O·X arrangement (B) to the favorable X·O arrangement (C). Hydrogen bonds affected by the modifications are shaded. Guanines in *syn* and *anti* glycosidic conformations are colored in magenta and cyan, respectively. The hydrogen-bond directionality in each tetrad is indicated by a gray arrow.

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the G-quadruplex scaffold, Dickerhoff and Weisz[15] successfully reversed the hydrogen-bond directionality of a G-tetrad without altering the global fold. Through the rational application of ^{F}G in *syn* positions within the modified tetrad, the preference for *anti* glycosidic orientations drove the inversion of G-tetrad H-bond polarity, while retaining the original folding topology of the G-quadruplex.[15]

Recent studies demonstrated that 8-oxoguanine (O) and xanthine (X), two major naturally occurring DNA base lesions formed via oxidation and nitrosative deamination of the guanine base respectively,[16] could be incorporated into a G-quadruplex in a complementary manner to form X·O·X·O[17] or G·G·X·O[18] tetrads.

In the current study, we show how the introduction of X and O in a non-complementary fashion can drive the reversal of G-tetrad hydrogen-bond polarity (Figure 1 B,C). By using this approach, the polarity of a G-tetrad can be inverted without affecting the overall fold of the molecule. We modified a human telomeric sequence (*HT*) that has previously been shown to form a well-defined (3+1) G-quadruplex[19] (Figure 1 A) consisting of three G-tetrad layers. The top G-tetrad of this molecule was modified with G3→X and G9→O substitutions (resulting in the *HT-3x9o* sequence, Table 1), which position X and O in an unfavorable O·X

Table 1: The DNA sequences used and their thermal stabilities.

Name	Sequence (5′-3′)	T_m [°C][a]
HT	TT GGG TTA GGG TTA GGG TTA GGG A	55.1 ± 0.3
HT-3x9o	TT **X**GG TTA **O**GG TTA GGG TTA GGG A	42.7 ± 0.6
HT-9x17o	TT GGG TTA **X**GG TTA GG**O** TTA GGG A	48.3 ± 0.5

X and O represent xanthine and 8-oxoguanine, respectively. [a] T_m was determined in buffer containing 10 mM KCl and 10 mM KPi (pH 9.0) from CD signals at 265 nm. The uncertainties (± values) indicate the hysteresis between heating and cooling curves. Compared with the T_m values measured at 265 nm, those measured at 290 nm were within the uncertainty limits for *HT* and *HT-3x9o*, but were significantly lower for *HT-9x17o* (Figure S8), which is consistent with the NMR data showing multiple conformations (Figure S1).

orientation based on the original G-tetrad arrangement (Figure 1 A, B). However, by inverting the glycosidic orientation of the bases, a G·G·X·O tetrad with the favorable X·O pair arrangement could be formed if the hydrogen-bond directionality of the tetrad is reversed (Figure 1 C).

Characterization of *HT-3x9o* by NMR spectroscopy identified 13 sharp signals at 10.0–12.5 ppm corresponding to imino protons (Figure 2 A), in contrast to the 12 signals seen for the unmodified *HT* G-quadruplex.[19] As previously reported,[18] the additional signal arises from the H7 proton of the 8-oxoguanine in the G·G·X·O tetrad. Upon pH titration, specific imino proton signals undergo major changes in chemical shift and line broadening (Figure S1 in the Supporting Information). Our previous work on the G·G·X·O tetrad has identified these changes to be associated with a protonation–deprotonation equilibrium (pKa of 6.7) of the xanthine base.[18] Our NMR data indicate successful formation of the G·G·X·O tetrad *HT-3x9o* at pH 7.0 and 9.0.

To establish whether the G-tetrad polarity had been successfully reversed by the O·X modification, we determined

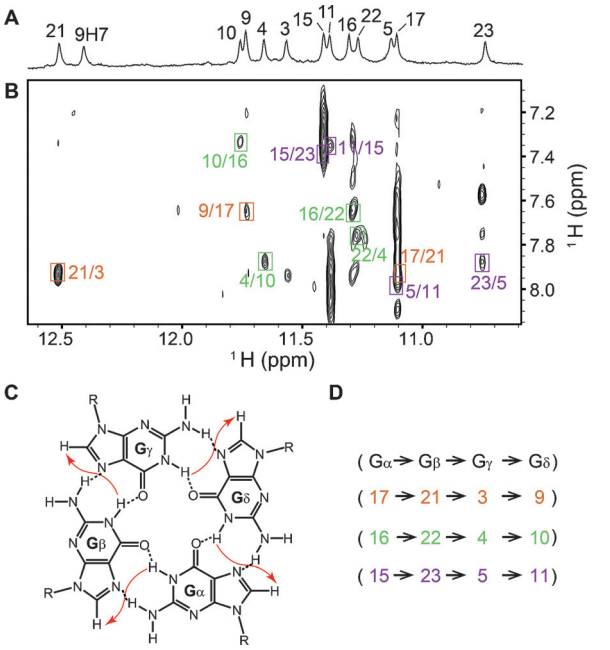

Figure 2. Characterization of *HT-3x9o* in buffer containing 70 mM KCl and 20 mM KPi (pH 9.0). A) Imino proton spectrum of *HT-3x9o*. Except for 9H7, signals are labeled with their respective residue numbers. B) NOESY spectrum of *HT-3x9o* (mixing time, 250 ms). H1–H8 cross-peaks used for the folding topology determination are framed and labeled with the imino proton number, followed by that of H8. C) Characteristic H1–H8 NOE connectivity patterns around a Gα·Gβ·Gγ·Gδ tetrad, as indicated with red arrows. D) Characteristic H1–H8 NOE connectivities determined for the G17·G21·X3·O9 tetrad (orange), G16·G22·G4·G10 tetrad (green), and G15·G23·G5·G11 tetrad (purple).

the folding topology of *HT-3x9o* by using two-dimensional NMR spectroscopy (Figure 2 B). This can be achieved through tracking the characteristic cyclic H1–H8 NOE patterns observed for *HT-3x9o* (Figure 2 B, C), with unambiguous resonance assignments (Figure S2–S4) obtained by using site-specific ^{15}N-labeled samples.[20] The established hydrogen-bond pattern identifies X and O to be situated within the top G17·G21·X3·O9 tetrad (Figure 2 D), in which X and O adopt a favorable arrangement, as illustrated in Figure 1 C. Although the overall fold adopted by *HT-3x9o* is unchanged from the (3+1) G-quadruplex fold of the original sequence *HT*,[19] the modified tetrad has undergone a polarity inversion of hydrogen-bond directionality (Figure 3).

A consequence of this G-tetrad inversion is a change in the circular dichroism (CD) profile. The CD spectrum of *HT-3x9o* is notably different from the typical pattern originally observed for the (3+1) G-quadruplex of *HT* (Figure 4 and Figure S5), with the CD spectrum of *HT-3x9o* exhibiting a negative peak at 240 nm and a positive peak at 265 nm, similar to the CD signature of a parallel G-quadruplex. The dramatic shift in the CD profile of *HT-3x9o* is associated with its non-alternating G-tetrad polarity, wherein the G-tetrads are aligned and stacked in a manner similar to that observed in parallel G-quadruplexes.[21] This change in CD signature was recently reported by Dickerhoff and Weisz for another G-quadruplex with an inverted G-tetrad.[15]

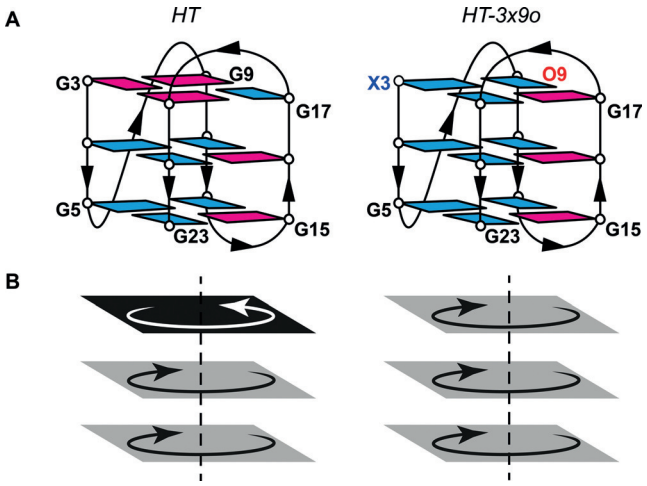

Figure 3. Comparison between the *HT* (left) and *HT-3x9o* (right) G-quadruplexes. A) Schematic structures of *HT* and *HT-3x9o*. *Syn* and *anti* guanines (or analogues) are colored in magenta and cyan, respectively. B) Schematic representation of the hydrogen-bond directionality of G-tetrads in *HT* and *HT-3x9o*. Each tetrad is represented by a plane, with the hydrogen-bond directionality indicated by an arrow. The reversal in directionality and base faces is represented by an inversion of the arrow direction and color, respectively.

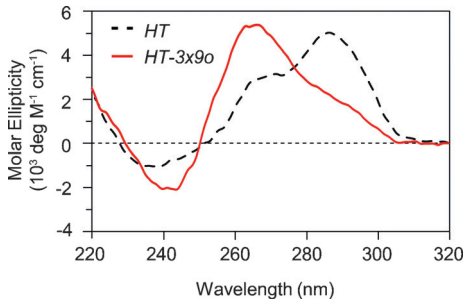

Figure 4. CD spectra of *HT* and *HT-3x9o* G-quadruplexes in buffer containing 70 mM KCl and 20 mM KPi (pH 9.0) at 20 °C.

The intensities of intraresidue H8–H1′ NOEs in the NOESY spectrum (Figure S4) indicate that the G17·G21·X3·O9 tetrad adopts a *syn·anti·anti·anti* configuration. This is in contrast to the reported structure of *HT*, in which the top tetrad G17·G9·G3·G21 adopts an *anti·syn·syn·syn* configuration.[19] This difference further substantiates the inversion of hydrogen-bond directionality within the modified G-tetrad, since a flip of glycosidic torsion angles is required to accommodate such an inversion (Figure 1B and 3).

The resulting G-quadruplex scaffold adopted by *HT-3x9o* positions O9 in an unfavorable *anti* conformation,[22] leading to an approximately 12 °C decrease in the melting temperature of *HT-3x9o* compared to that of the original structure *HT* (Table 1). To investigate the possibility of inverting the G-tetrad polarity in a manner that allows O to adopt a more favorable *syn* glycosidic conformation, X and O were incorporated at positions 9 and 17 respectively, to give the *HT-9x17o* sequence (Table 1). *HT-9x17o* demonstrates the characteristic NMR spectral pattern of a major G-quadruplex form containing a G·G·X·O tetrad (presumably with the top tetrad being inverted compared to that in *HT*), which co-exists with other G-quadruplex form(s) (Figure S1). This conclusion is consistent with the CD spectra observed for *HT-9x17o* (Figure S6 and S7). The melting temperature of the major form of *HT-9x17o*, presumably a fold with O adopting a *syn* conformation, is approximately 6 °C higher than that of *HT-3x9o*, in which O adopts an *anti* conformation (Table 1 and Figure S8).

In conclusion, we have demonstrated that a judicious placement of two base-modified guanine analogues, X and O, within a G-tetrad can drive the reversal of G-tetrad polarity (Figure 3), while conserving the overall fold of the unmodified G-quadruplex. This approach can thus be applied as a tool to program the hydrogen-bond directionality within individual G-tetrads. The X·O modification could facilitate fundamental studies of the G-quadruplex structure and may have potential for use in therapeutic or nanomaterials applications. The application of X and O within the G-quadruplex scaffold has the additional benefit of possessing a highly electronegative groove, which may modulate its properties.[18]

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