G-Quadruplexes

International Edition: DOI: 10.1002/anie.201411887
German Edition: DOI: 10.1002/ange.201411887

Flipping a G-Tetrad in a Unimolecular Quadruplex Without Affecting Its Global Fold

*Jonathan Dickerhoff and Klaus Weisz**

Abstract: *A unimolecular G-quadruplex with a hybrid-type topology and propeller, diagonal, and lateral loops was examined for its ability to undergo structural changes upon specific modifications. Substituting 2′-deoxy-2′-fluoro analogues with a propensity to adopt an anti glycosidic conformation for two or three guanine deoxyribonucleosides in syn positions of the 5′-terminal G-tetrad significantly alters the CD spectral signature of the quadruplex. An NMR analysis reveals a polarity switch of the whole tetrad with glycosidic conformational changes detected for all four guanine nucleosides in the modified sequence. As no additional rearrangement of the overall fold occurs, a novel type of G-quadruplex is formed with guanosines in the four columnar G-tracts lined up in either an all-syn or an all-anti glycosidic conformation.*

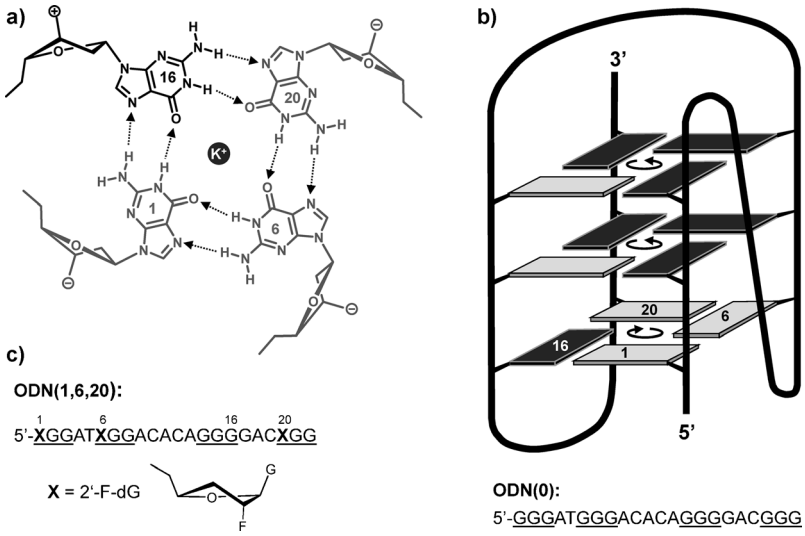

Figure 1. a) 5′-Terminal G-tetrad with hydrogen bonds running in a clockwise fashion and b) folding topology of ODN(0) with *syn* and *anti* guanines shown in light and dark gray, respectively. G-tracts in b) the native ODN(0) and c) the modified ODN(1,6,20) with incorporated 2′-fluoro-dG analogues are underlined.

Guanine-rich DNA and RNA sequences can fold into four-stranded G-quadruplexes stabilized by a core of stacked guanine tetrads. The four guanine bases in the square-planar arrangement of each tetrad are connected through a cyclic array of Hoogsteen hydrogen bonds and additionally stabilized by a centrally located cation (Figure 1 a). Quadruplexes have gained enormous attention during the last two decades as a result of their recently established existence and potential regulatory role in vivo[1] that renders them attractive targets for various therapeutic approaches.[2] This interest was further sparked by the discovery that many natural and artificial RNA and DNA aptamers, including DNAzymes and biosensors, rely on the quadruplex platform for their specific biological activity.[3]

In general, G-quadruplexes can fold into a variety of topologies characterized by the number of individual strands, the orientation of G-tracts, and the type of connecting loops.[4] As guanosine glycosidic torsion angles within the quadruplex stem are closely connected with the relative orientation of the four G segments, specific guanosine replacements by G analogues that favor either a *syn* or *anti* glycosidic conformation have been shown to constitute a powerful tool to examine the conformational space available for a particular quadruplex-forming sequence.[5] There are ongoing efforts aimed at exploring and ultimately controlling accessible quadruplex topologies prerequisite for taking full advantage of the potential offered by these quite malleable structures for therapeutic, diagnostic, or biotechnological applications.

The three-dimensional NMR structure of the artificially designed intramolecular quadruplex ODN(0) was recently reported.[6] Remarkably, it forms a (3 + 1) hybrid topology with all three main types of connecting loops, that is, with a propeller, a lateral, and a diagonal loop (Figure 1 b). We wanted to follow conformational changes upon substituting an *anti*-favoring 2′-fluoro-2′-deoxyribo analogue 2′-F-dG for G residues within its 5′-terminal tetrad. As shown in Figure 2, the CD spectrum of the native ODN(0) exhibits positive bands at $\lambda = 290$ and 263 nm as well as a smaller negative band at about 240 nm typical of the hybrid structure. A 2′-F-dG substitution at *anti* position 16 in the modified ODN(16) lowers all CD band amplitudes but has no noticeable influence on the overall CD signature. However, progressive substitutions at *syn* positions 1, 6, and 20 gradually decrease the CD maximum at $\lambda = 290$ nm while increasing the band amplitude at 263 nm. Thus, the CD spectra of ODN(1,6), ODN(1,20), and in particular of ODN(1,6,20) seem to imply a complete switch into a parallel-type quadruplex with the absence of Cotton effects at around $\lambda = 290$ nm but with

[*] J. Dickerhoff, Prof. Dr. K. Weisz
Institut für Biochemie
Ernst-Moritz-Arndt-Universität Greifswald
Felix-Hausdorff-Strasse 4, 17487 Greifswald (Germany)
E-mail: weisz@uni-greifswald.de

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201411887.

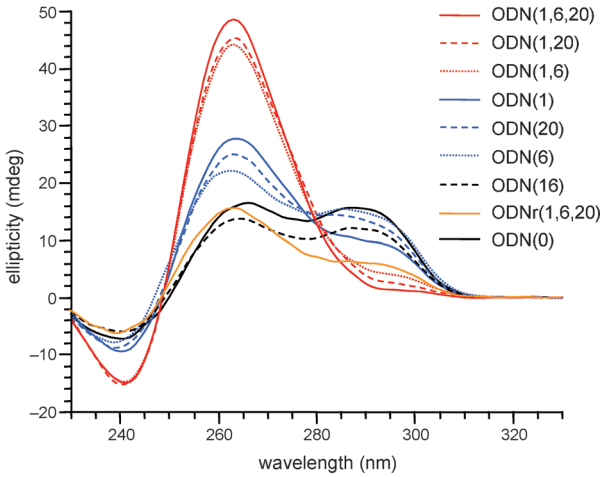

Figure 2. CD spectra of native ODN(0), rG-modified ODNr(1,6,20), and 2′-F-dG modified ODN sequences at 20 °C in 20 mM potassium phosphate, 100 mM KCl, pH 7. Numbers in parentheses denote sites of substitution.

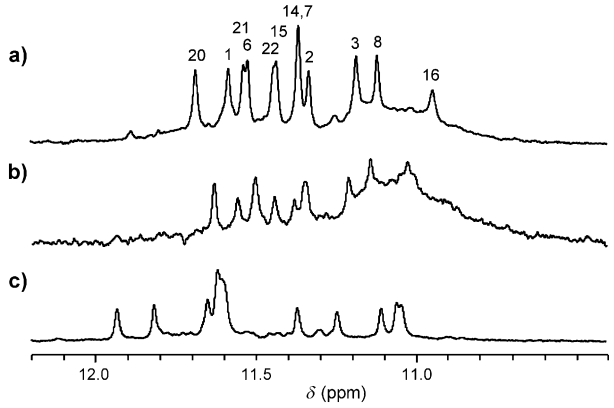

Figure 3. ^{1}H NMR spectra showing the imino proton spectral region for a) ODN(1,6,20), b) ODN(1,20), and c) ODN(0) at 25 °C in 10 mM potassium phosphate (pH 7). Peak assignments for the G-tract guanines are indicated for ODN(1,6,20).

a strong positive band at 263 nm and a negative band at 240 nm. In contrast, ribonucleotides with their similar propensity to adopt an *anti* conformation appear to be less effective in changing ODN conformational features based on the CD effects of the triply rG-modified ODNr(1,6,20) (Figure 2).

Resonance signals for imino groups detected between $\delta =$ 10.8 and 12.0 ppm in the ^{1}H NMR spectrum of unmodified ODN(0) indicate the formation of a well-defined quadruplex as reported previously.[6] For the modified sequences ODN-(1,20) and ODN(1,6,20), resonance signals attributable to imino groups have shifted but the presence of a stable major quadruplex topology with very minor additional species is clearly evident for ODN(1,6,20) (Figure 3). Likewise, only small structural heterogeneities are noticeable for the latter on gels obtained with native PAGE, revealing a major band together with two very weak bands of lower mobility (see Figure S1 in the Supporting Information). Although two-dimensional NOE experiments of ODN(1,20) and ODN(1,6,20) demonstrate that both quadruplexes share the same major topology (Figure S3), the following discussion will be restricted to ODN(1,6,20) with its better resolved NMR spectra.

NMR spectroscopic assignments were obtained from standard experiments (for details see the Supporting Information). Structural analysis was also facilitated by very similar spectral patterns in the modified and native sequence. In fact, many nonlabile resonance signals, including signals for protons within the propeller and diagonal loops, have not significantly shifted upon incorporation of the 2′-F-dG analogues. Notable exceptions include resonances for the modified G-tetrad but also signals for some protons in its immediate vicinity. Fortunately, most of the shifted sugar resonances in the 2′-fluoro analogues are easily identified by characteristic ^{1}H–^{19}F coupling constants. Overall, the global fold of the quadruplex seems unaffected by the G→2′-F-dG replacements. However, considerable changes in intranucleotide H8–H1′ NOE intensities for all G residues in the 5′-terminal tetrad indicate changes in their glycosidic torsion angle.[7] Clear evidence for guanosine glycosidic conformational transitions within the first G-tetrad comes from H6/H8 but in particular from C6/C8 chemical shift differences detected between native and modified quadruplexes. Chemical shifts for C8 carbons have been shown to be reliable indicators of glycosidic torsion angles in guanine nucleosides.[8] Thus, irrespective of the particular sugar pucker, downfield shifts of $\delta = 2$-6 ppm are predicted for C8 in guanosines adopting the *syn* conformation. As shown in Figure 4, resonance signals for C8 within G1, G6, and G20

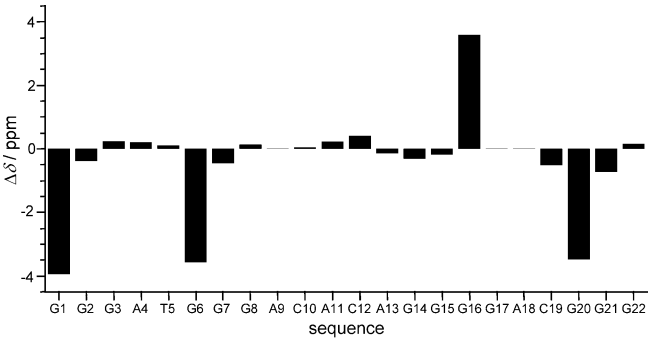

Figure 4. ^{13}C NMR chemical shift differences for C8/C6 atoms in ODN(1,6,20) and unmodified ODN(0) at 25 °C. Note, base protons of G17 and A18 residues within the lateral loop have not been assigned.

are considerably upfield shifted in ODN(1,6,20) by nearly $\delta =$ 4 ppm. At the same time, the signal for carbon C8 in the G16 residue shows a corresponding downfield shift whereas C6/C8 carbon chemical shifts of the other residues have hardly changed. These results clearly demonstrate a polarity reversal of the 5′-terminal G-tetrad, that is, modified G1, G6, and G20 adopt an *anti* conformation whereas the G16 residue changes from *anti* to *syn* to preserve the cyclic-hydrogen-bond array.

Apparently, the modified ODN(1,6,20) retains the global fold of the native ODN(0) but exhibits a concerted flip of glycosidic torsion angles for all four G residues within the

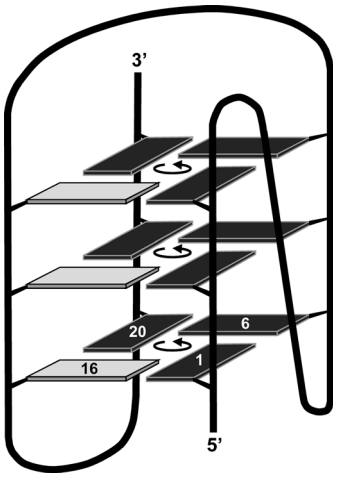

Figure 5. Schematic representation of the topology and glycosidic conformation of ODN(1,6,20) with *syn*- and *anti*-configured guanosines shown in light and dark gray, respectively.

5′-terminal tetrad, thus reversing its intrinsic polarity (see Figure 5). Remarkably, a corresponding switch in tetrad polarity in the absence of any topological rearrangement has not been reported before in the case of intramolecularly folded quadruplexes. Previous results on *anti*/*syn*-favoring guanosine replacements indicate that a quadruplex conformation is conserved and potentially stabilized if the preferred glycosidic conformation of the guanosine surrogate matches the original conformation at the substitution site. In contrast, enforcing changes in the glycosidic torsion angle at an "unmatched" position will either result in a severe disruption of the quadruplex stem or in its complete refolding into a different topology.[9] Interestingly, tetramolecular all-*trans* quadruplexes $[TG_{3\text{-}4}T]_4$ lacking intervening loop sequences are often prone to changes in tetrad polarity through the introduction of *syn*-favoring 8-Br-dG or 8-Me-dG analogues.[10]

By changing the polarity of the ODN 5′-terminal tetrad, the antiparallel G-tract and each of the three parallel G-tracts exhibit a non-alternating all-*syn* and all-*anti* array of glycosidic torsion angles, respectively. This particular glycosidic-bond conformational pattern is unique, being unreported in any known quadruplex structure, expanding the repertoire of stable quadruplex structural types as defined previously.[11,12] The native quadruplex exhibits three 5′-*syn–anti–anti* segments together with one 5′-*syn–syn–anti* segment. In the modified sequence the four *syn–anti* steps are changed to three *anti–anti* and one *syn–syn* step. UV melting experiments on ODN(0), ODN(1,20), and ODN(1,6,20) showed a lower melting temperature of approximately 10 °C for the two modified sequences with a rearranged G-tetrad. In line with these differential thermal stabilities, molecular dynamics simulations and free energy analyses suggested that *syn–anti* and *syn–syn* are the most stable and most disfavored glycosidic conformational steps in antiparallel quadruplex structures, respectively.[13] It is therefore remarkable that an unusual all-*syn* G-tract forms in the thermodynamically most stable modified quadruplex, highlighting the contribution of connecting loops in determining the preferred conformation. Apparently, the particular loop regions in ODN(0) resist topological changes even upon enforcing *syn*↔*anti* transitions.

CD spectral signatures are widely used as convenient indicators of quadruplex topologies. Thus, depending on their spectral features between $\lambda = 230$ and 320 nm, quadruplexes are empirically classified into parallel, antiparallel, or hybrid structures. It has been pointed out that the characteristics of quadruplex CD spectra do not directly relate to strand orientation but rather to the inherent polarity of consecutive guanine tetrads as defined by Hoogsteen hydrogen bonds running either in a clockwise or in a counterclockwise fashion when viewed from donor to acceptor.[14] This tetrad polarity is fixed by the strand orientation and by the guanosine glycosidic torsion angles. Although the native and modified quadruplex share the same strand orientation and loop conformation, significant differences in their CD spectra can be detected and directly attributed to their different tetrad polarities. Accordingly, the maximum band at $\lambda =$ 290 nm detected for the unmodified quadruplex and missing in the modified structure must necessarily originate from a *syn–anti* step giving rise to two stacked tetrads of different polarity. In contrast, the positive band at $\lambda = 263$ nm must reflect *anti–anti* as well as *syn–syn* steps. Overall, these results corroborate earlier evidence that it is primarily the tetrad stacking that determines the sign of Cotton effects. Thus, the empirical interpretation of quadruplex CD spectra in terms of strand orientation may easily be misleading, especially upon modification but probably also upon ligand binding.

The conformational rearrangements reported herein for a unimolecular G-quadruplex are without precedent and again demonstrate the versatility of these structures. Additionally, the polarity switch of a single G-tetrad by double or triple substitutions to form a new conformational type paves the way for a better understanding of the relationship between stacked tetrads of distinct polarity and quadruplex thermodynamics as well as spectral characteristics. Ultimately, the ability to comprehend and to control the particular folding of G-quadruplexes may allow the design of tailor-made aptamers for various applications in vitro and in vivo.

Keywords: circular dichroism · conformational analysis · glycosides · G-quadruplexes · NMR spectroscopy

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 5588–5591
Angew. Chem. **2015**, *127*, 5680–5683

[1] E. Y. N. Lam, D. Beraldi, D. Tannahill, S. Balasubramanian, *Nat. Commun.* **2013**, *4*, 1796.
[2] S. M. Kerwin, *Curr. Pharm. Des.* **2000**, *6*, 441–471.
[3] J. L. Neo, K. Kamaladasan, M. Uttamchandani, *Curr. Pharm. Des.* **2012**, *18*, 2048–2057.
[4] a) S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd, S. Neidle, *Nucleic Acids Res.* **2006**, *34*, 5402–5415; b) M. Webba da Silva, *Chem. Eur. J.* **2007**, *13*, 9738–9745.
[5] a) Y. Xu, Y. Noguchi, H. Sugiyama, *Bioorg. Med. Chem.* **2006**, *14*, 5584–5591; b) J. T. Nielsen, K. Arar, M. Petersen, *Angew. Chem. Int. Ed.* **2009**, *48*, 3099–3103; *Angew. Chem.* **2009**, *121*,

3145 – 3149; c) A. Virgilio, V. Esposito, G. Citarella, A. Pepe, L. Mayol, A. Galeone, *Nucleic Acids Res.* **2012**, *40*, 461 – 475; d) Z. Li, C. J. Lech, A. T. Phan, *Nucleic Acids Res.* **2014**, *42*, 4068 – 4079.

[6] M. Marušič, P. Šket, L. Bauer, V. Viglasky, J. Plavec, *Nucleic Acids Res.* **2012**, *40*, 6946 – 6956.

[7] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, John Wiley and Sons, New York, **1986**, pp. 203 – 223.

[8] a) K. L. Greene, Y. Wang, D. Live, *J. Biomol. NMR* **1995**, *5*, 333 – 338; b) J. M. Fonville, M. Swart, Z. Vokáčová, V. Sychrovský, J. E. Šponer, J. Šponer, C. W. Hilbers, F. M. Bickelhaupt, S. S. Wijmenga, *Chem. Eur. J.* **2012**, *18*, 12372 – 12387.

[9] a) C.-F. Tang, R. H. Shafer, *J. Am. Chem. Soc.* **2006**, *128*, 5966 – 5973; b) A. T. Phan, V. Kuryavyi, K. N. Luu, D. J. Patel, *Nucleic Acids Res.* **2007**, *35*, 6517 – 6525; c) D. Pradhan, L. H. Hansen, B. Vester, M. Petersen, *Chem. Eur. J.* **2011**, *17*, 2405 – 2413; d) C. J. Lech, Z. Li, B. Heddi, A. T. Phan, *Chem. Commun.* **2012**, *48*, 11425 – 11427.

[10] a) A. Virgilio, V. Esposito, A. Randazzo, L. Mayol, A. Galeone, *Nucleic Acids Res.* **2005**, *33*, 6188 – 6195; b) P. L. T. Tran, A. Virgilio, V. Esposito, G. Citarella, J.-L. Mergny, A. Galeone, *Biochimie* **2011**, *93*, 399 – 408.

[11] a) M. Webba da Silva, M. Trajkovski, Y. Sannohe, N. Ma'ani Hessari, H. Sugiyama, J. Plavec, *Angew. Chem. Int. Ed.* **2009**, *48*, 9167 – 9170; *Angew. Chem.* **2009**, *121*, 9331 – 9334; b) A. I. Karsisiotis, N. Ma'ani Hessari, E. Novellino, G. P. Spada, A. Randazzo, M. Webba da Silva, *Angew. Chem. Int. Ed.* **2011**, *50*, 10645 – 10648; *Angew. Chem.* **2011**, *123*, 10833 – 10836.

[12] There has been one report on a hybrid structure with one all-*syn* and three all-*anti* G-tracts suggested to form upon binding a porphyrin analogue to the *c-myc* quadruplex. However, the proposed topology is solely based on dimethyl sulfate (DMS) footprinting experiments and no further evidence for the particular glycosidic conformational pattern is presented. See: J. Seenisamy, S. Bashyam, V. Gokhale, H. Vankayalapati, D. Sun, A. Siddiqui-Jain, N. Streiner, K. Shin-ya, E. White, W. D. Wilson, L. H. Hurley, *J. Am. Chem. Soc.* **2005**, *127*, 2944 – 2959.

[13] X. Cang, J. Šponer, T. E. Cheatham III, *Nucleic Acids Res.* **2011**, *39*, 4499 – 4512.

[14] S. Masiero, R. Trotta, S. Pieraccini, S. De Tito, R. Perone, A. Randazzo, G. P. Spada, *Org. Biomol. Chem.* **2010**, *8*, 2683 – 2692.

Received: December 10, 2014
Revised: February 22, 2015
Published online: March 16, 2015

