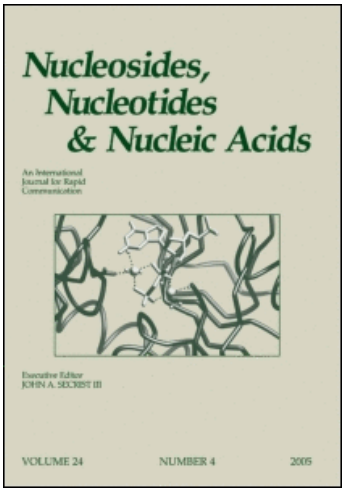

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Galeone, A. , Mayol, L. , Randazzo, A. , Virgilio, A. and Virno, A.(2007) 'Effect of the Introduction of an A-Residue into A Quadruplex Forming Oligonucleotide Containing A 5'-5' Polarity of Inversion Site', Nucleosides, Nucleotides and Nucleic Acids, 26: 8, 1151 — 1154
To link to this Article: DOI: 10.1080/15257770701527034
URL: http://dx.doi.org/10.1080/15257770701527034

Nucleosides, Nucleotides, and Nucleic Acids, 26:1151–1154, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701527034

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EFFECT OF THE INTRODUCTION OF AN A-RESIDUE INTO A QUADRUPLEX FORMING OLIGONUCLEOTIDE CONTAINING A 5′-5′ POLARITY OF INVERSION SITE

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□ *Preliminary NMR studies on structure formed by sequence 3′-TGA-5′-5′-GGT-3′ are described. We proposed the formation of a tetramolecular quadruplex in which strands are equivalent to each other and three G-tetrads are present. The possibility of the occurrence of an A-tetrad also is discussed.*

Keywords G-quadruplex; inversion of polarity site; A-tetrad

The biological importance of G-quadruplex structures mainly lies in two features: their substantiated presence in several regions of genome and their fundamental role as scaffold in a number of aptamer provided with useful biological properties.[1] In the latter frame, the searching of new structural motives is a current research field whose main aim is the improving of the structural stability and the fine tuning of the interaction of aptamers with the target molecules. Recently, we reported our investigations on the G-quadruplex Q55, formed by oligodeoxyribonucleotides containing a 5′-5′ inversion of polarity site, namely 3′-TGA-5′-5′-GGT-3′.[2] The ^{1}H-NMR spectrum of quadruplex Q55 shows four signals in the imino protons spectrum region, implying a four fold symmetry of the complex. Furthermore, its thermal stability is noteworthy higher that its natural counterpart 5′-TGGGGT-3′. In order to investigate the apparent stabilizing properties of a 5′-5′ inversion of polarity site embedded in a G-quadruplex structure, we have undertaken a systematic exploration concerning several sequences containing such backbone modification. Here we report preliminary results about sequence 3′-TGA-5′-5′-GGT-3′. Oligonucleotide 3′-TGA-5′-5′-GGT-3′ was synthesized using standard methods and 3′-phosphoramidites and 5′-phosphoramidites.

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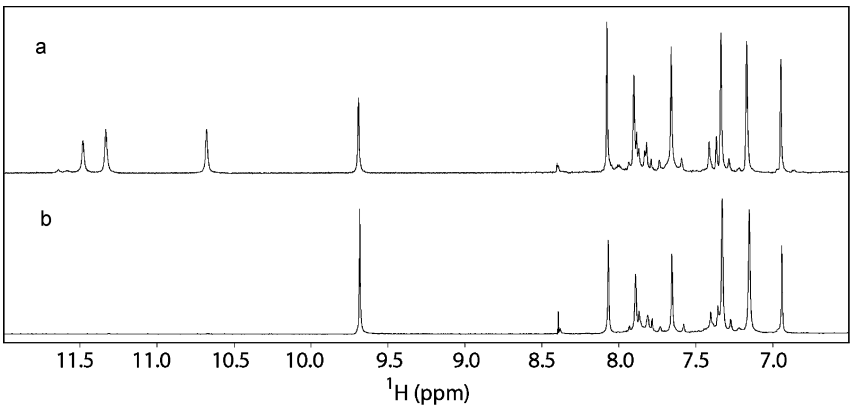

FIGURE 1 ^{1}H NMR spectra of 3′-TGA-5′-5′-GGT-3′ in H_2O (a) and in D_2O (b).

NMR samples were prepared at a concentration of approximatively 6 mM, in 0.6 ml (H_2O/D_2O 9:1) buffer solution having 40 mM KH_2PO_4, 280 mM KCl, 0.2 mM EDTA, pH 7.0. The simple appearance of the ^{1}H-NMR spectrum of 3′-TGA-5′-5′-GGT-3′ (Figure 1a) indicates that, in the conditions used here, the modified oligomer forms mainly a single well-defined hydrogen-bonded conformation. It shows the presence of three well defined signals in the region 10.5–11.5 ppm, distinctive of imino protons involved in Hoogsteen hydrogen bonds of G-quartets. Moreover, six signals in the aromatic region were clearly observable. A further signal at 9.68 ppm is detectable. We assigned this signal to A-H2 consistent with a D_2O exchange study (Figure 1) and NOE connettivities. Taking into account this result, only three signals can be ascribed to imino protons involved in Hoogsteen hydrogen bonds of G-quartets suggesting the possibility of a four fold symmetry for the quadruplex structure adopted by 3′-TGA-5′-5′-GGT-3′. The NOESY spectrum (700 MHz, t = 25°C, mixing time 100 ms) discloses interesting diagnostic information. Particularly, the lack of strong NOEs between any G-H8 and H1′ of the same residue, strongly suggests that all residues are in the *anti* glycosidic conformation (Figure 2). Sequence 3′-TGA-5′-5′-GGT-3′ could, theoretically, arrange in several type of quadruplex structures, among them structures **I** and **II** (Figure 3) are consistent with NMR data, since according to their symmetry properties, they should show only a single set of signals due to the equivalence of the four strands. Structure **I** could enclose an A-tetrad, a planar arrangement of four A residues similar to a G-tetrad, already evidenced in other quadruplex structures.[3–5] On the other hand, structure **II** could be stabilized by two AGAG-tetrads (Figure 3, on the right) sited in the centre of the complex. Although some chemical probing studies on d(G_3TTAG_3) and d($TTAG_3$) units of different lengths suggest the probable formation of AGAG-tetrads in the quadruplex structures,[6] however, other studies question the existence of the AGAG tetrad because of its relatively

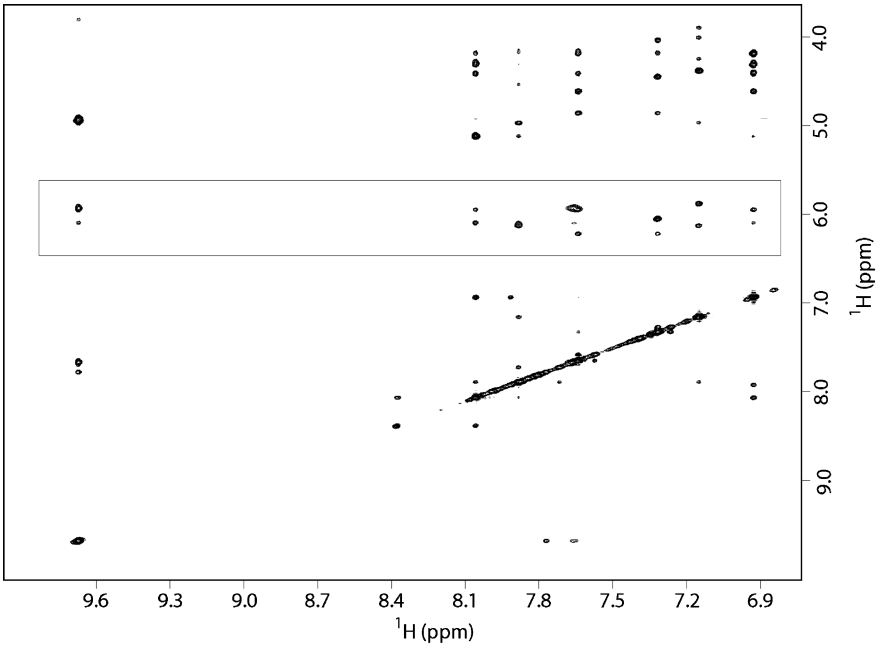

FIGURE 2 Expanded region of the NOESY spectrum of 3′-TGA-5′-5′-GGT-3′. Section correlating G-H8 and H1′ protons is boxed.

high energy and the lacking of oxygen atoms required for the formation of the cation site.[7,8] Finally, a theoretical investigation on structures and properties of mixed DNA bases tetrads[9] revealed that a AGAG-tetrad would be characterized by a V-shaped structure suggesting that it cannot be stacked

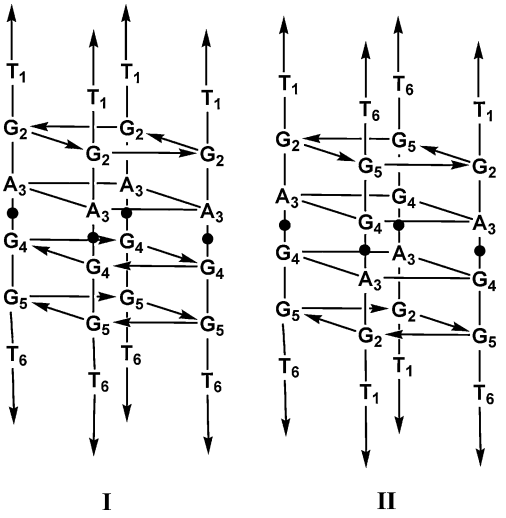

FIGURE 3 Possible structures for the quadruplex adopted by 3′-TGA-5′-5′-GGT-3′. Equivalent residues are similarly labeled.

with the G-tetrads in the stem of quadruplexes and hence, that this tetrad arrangement may not be important in the structure stabilization, particularly in presence of cations as Na^+ or K^+.

Concerning oligonucleotide 3′-TGA-5′-5′-GGT-3′ the sequential NOE connectivity pathway along the sequence is broken in the 5′-5′ inversion of polarity site. However, in the tract 3′-TGA-5′ it gives patters and intensities of NOE consistent with the adenine base stacking within the quadruplex with all purine glycosidic torsion angles in the anti range. The most probable arrangement of the A residues should involve a putative interstrand hydrogen bonding between the amino group and N1. In fact, this brings the adenine H2 of one strand in close proximity to the N7 of an adjacent adenine, potentially accounting for the unusual downfield shift observed for A-H2, as reported by other authors.[5] The occurrence of a crosspeak between adenine H2 and H8 further suggests a planar arrangement of the four adenine bases. According to the above results and considerations, structure I was supposed to be the most probable one, although further NMR investigations are in progress.

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