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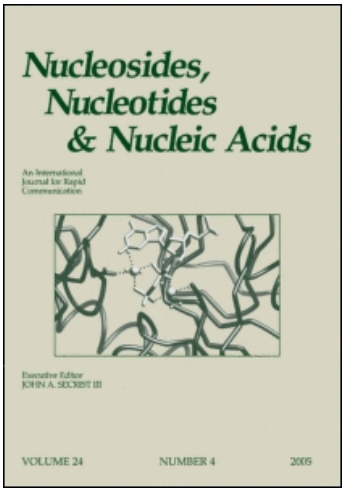

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Structural insight into the *h TERT* intron 6 sequence d(GGGGTGAAAGGGG) from ^{1}H-NMR study

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STRUCTURAL INSIGHT INTO THE *hTERT* INTRON 6 SEQUENCE d(GGGGTGAAAGGGG) FROM ^{1}H-NMR STUDY

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□ *The interest in DNA quadruplex structures has been fueled by the recognition that telomeres, the 3′ single stranded guanine-rich overhangs found at the termini of chromosomes, are likely to form G-tetrads type structures important in cell senescence and cancer. In addition to their presence in telomeres, where they may play a role in maintaining the stability and integrity of chromosomes, guanine-rich regions are found in other region of the genome, amongst these is intron 6 of hTERT a gene codifying for the enzyme telomerase. Interestingly, the formation of G-quadruplexes in this region is involved in the down-regulation of telomerase activity caused by an alteration of the hTERT splicing pattern. Therefore, we have analyzed several sequences of that intron by* ^{1} *H-NMR and CD spectroscopy, and we have found that the sequence d(GGGGTGAAAGGGG) is able to fold in a single well-defined antiparallel quadruplex structure consisting of four G-tetrads, possessing a twofold symmetry, and containing four Gs in a syn glycosidic conformation.*

Keywords *hTERT*; intron 6; NMR; quadruplex

For over 30 years, it has been known that DNA guanine rich sequences form quadruplex structures containing G-quartets.[1] G-quartets are characterized by cyclic hydrogen bonding of four guanine bases in a coplanar

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arrangement. Strand stoichiometry variation allows G-quadruplexes to be formed by association of one, via intramolecular folding, two, by dimerization of a folded-back hairpin, or four separate strands. Differences in stoichiometry and topology are reflected in the widths of the four grooves of the tetrad's structure. dGn segments can form a tetramolecular complex with all G bases in the *anti* conformation and all strands parallel to each other leading to the formation of four identical grooves of width similar to the one of the duplex B-DNA minor groove (defined as medium width). The folded-back dimeric quadruplex is instead characterized by alternating *syn* and *anti* nucleotide conformations along each strand and two pairs of adjacent parallel strands, which results in a quadruplex of alternative wide, medium, narrow, medium width grooves between strands. Oligonucleotides forming an unimolecular quadruplex show the residues of the tetrads in an *anti-syn-anti-syn* conformation with alternating antiparallel strands. This kind of quadruplex results in a rectangular G-tetrad core with grooves of alternating wide-narrow-wide-narrow widths. Several important regulatory proteins bind to G-tetrads *in vitro*, selecting specific topological forms of quadruplex structures. This, and a plethora of indirect functional data has led to the proposal that G-quartets have functional roles *in vivo*. Indeed, in addition to their presence in telomeres,[2,3] where they may play a role in maintaining the stability and integrity of chromosomes, guanine-rich regions are also involved in recombination and mutation in "hot spots"[4–8] and gene regulation,[9] and they have been linked to important human diseases.[10,11] It is known that the intron 6 of the hTERT gene contains several repeated GGG motifs that are able to form G-quadruplexes.[12] Interestingly, it has been shown that the formation of these G-quadruplexes is involved in the down-regulation of telomerase activity caused by an alteration of the hTERT splicing pattern, in which the inactive $-\beta$ transcript becomes overexpressed. In this frame, we have analyzed several sequences of that intron by ^{1}H-NMR spectroscopy and CD spectroscopy. Particularly we have studied the sequences d(GGGGTGAAAGGGGCCCCTGGGCTTGGG), d(GGGGGCCTTGGGGCTCGGCAGGGGTGAAAGGGG), d(GGGGTAGG TGGGGATCTGTGGGATTGG) and a shorter sequence d(GGGGTGAAAG GGG). All samples were prepared at a concentration of approximatively 2 mM, in 0.6 ml (H_2O/D_2O 9:1) buffer solution having 10 mM KH_2PO_4, 70 mM KCl, 0.2 mM EDTA, pH 7.0. All of them were heated for 10 minutes at 80°C and slowly cooled down to room temperature. ^{1}H-NMR spectra were recorded by using pulsed-field gradient WATERGATE[13] for H_2O suppression. Each DNA sequence folds in a number of different quadruplex structures as indicated by the presence of a very high number of imino proton signals in the region between 11 and 12.5 ppm. Neither changing the buffer solution nor varying the temperature were able to select a preferential conformation. However, only the sequence d(GGGGTGAAAGGGG) (**1**) turned out to be able to fold in a single well-defined quadruplex. In fact, the

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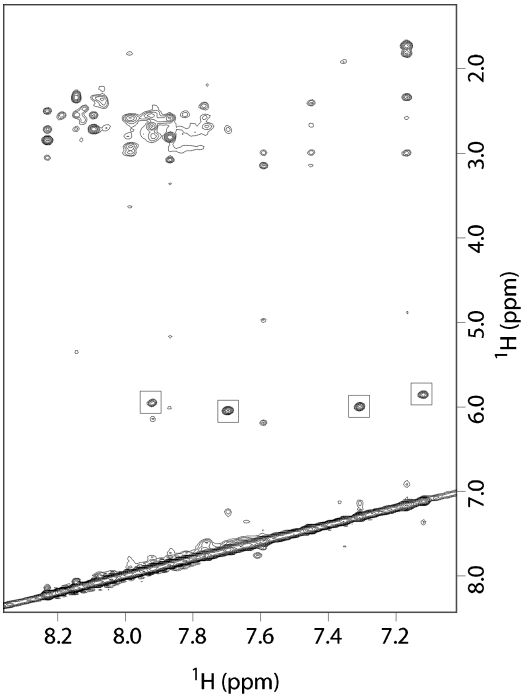

FIGURE 1 Expanded region of NOESY spectrum of **1** (800 MHz, T = 25°C, mt = 100 ms) correlating G-H8, H2′-H2″, and H1′ protons. Strong G-H8/H1′ NOEs indicating a *syn* glycosidic conformation are boxed.

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^{1}H-NMR spectrum of **1** (700 MHz, T = 25°C) shows the presence of eight well defined signals in the region 11.5–12.5 ppm, attributable to imino protons involved in Hoogsteen hydrogen bonds. Moreover, sixteen signals in the aromatic region due to the presence of nine guanine H8, one thymine H6, and six adenine H8/H2 protons were clearly observable. These data indicate that the quadruplex must be symmetric. Additional structural information was obtained from the analysis of 2D NOESY (800 MHz, 25°C) and TOCSY spectra (800 MHz, 25°C). Particularly, the intensities of NOE cross-peaks at between the H8 proton bases and sugar H1′ resonances (mixing time 100 ms) indicate that four out of nine Gs adopt a *syn* glycosidic conformation (Figure 1). Then, four *anti*-Gs have classical H8/H2′-H2″ sequential connectivities to 5′ neighboring *syn*-Gs, indicating that those residues are involved in the formation of a right-handed helical structure. The presence of an alternation of *syn* and *anti* G residues within each G-tract, along with the above observations, suggests that **1** folds into a bimolecular fold-back quadruplex, characterized by four G-tetrads. Consistently with this hypothesis, a number of unusual NOE connectivities were observed for A and T residues, indicating that the residues connecting the G-tracts are forming partially structured loops.

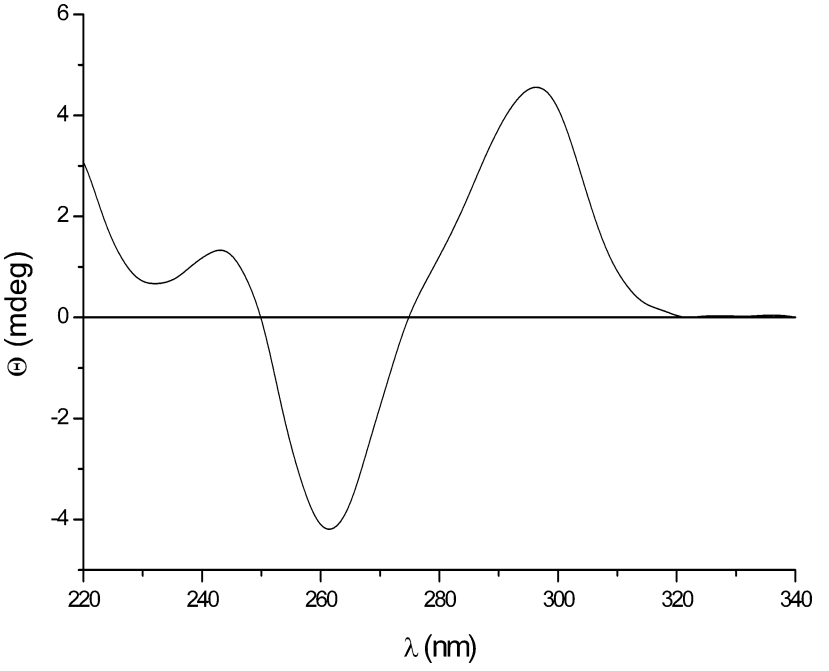

FIGURE 2 CD spectra of **1** at 20°C.

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Support to this preliminary analysis is provided by the analysis of the CD spectrum of 1 (Figure 2). At 20°C, the spectrum exhibits two positive bands at 243 and 295 nm and a negative one at 261 nm, typical of anti-parallel quadruplex structures, containing residues in *syn*-glycosidic conformations. From this preliminary results, we can conclude that the structure adopted by **1** closely resemble that adopted by $[d(GGGGTTTTGGGG)]_2$.[14,15] Further experiments to fully characterize the structure at the atomic level are in progress in our laboratory.

REFERENCES

1. Davis, J.T. G-Quartets 40 years later: From 5′-GMP to molecular biology and supramolecular chemistry. *Angew. Chem. Int. Ed.* **2004**, 43, 668–698.
2. Henderson, E.R.; Blackburn, E.H. An overhanging 3′ terminus is a conserved feature of telomeres. *Mol. Cell. Biol.* **1989**, 9, 345–348.
3. Blackburn, E.H. Structure and function of telomeres. *Nature (London)* **1991**, 350, 569–573.
4. Nikaido, T.; Yamawaki-Kataoka, Y.; Honjo, T.; Nucleotide sequences of switch regions of immunoglobulin C epsilon and C gamma genes and their comparison. *J. Biol. Chem.* **1982**, 257, 7322–7329.
5. Jeffreys, J.; Wilson, V.; Thein, S.L. Hypervariable 'minisatellite' regions in human DNA. *Nature (London)* **1985**, 314, 67–73.
6. Collier, D.A.; Griffin, J.A.; Wells, R.D. Non-B right-handed DNA conformations of homopurine.homopyrimidine sequences in the murine immunoglobulin C alpha switch region. *J. Biol. Chem.* **1988**, 263, 7397–7405.

7. Hastie, N.D.; Allshire, R.C. Human telomeres: fusion and interstitial sites. *Trends Genet.* **1989**, 5, 326–331.
8. Akman, S.A.; Lingeman, R.G.; Doroshow J.H.; Smith, S.S. Quadruplex DNA formation in a region of the tRNA gene supF associated with hydrogen peroxide mediated mutations. *Biochemistry* **1991**, 30, 8648–8653.
9. Nickol, J.M.; Felsenfeld, G. DNA conformation at the 5′ end of the chicken adult beta-globin gene. *Cell* **1983**, 35, 467–477.
10. Simonsson, T.; Pecinka, P.; Kubista, M. DNA tetraplex formation in the control region of c-myc. *Nucleic Acids Res.* **1998**, 26, 1167–1172.
11. Hammond-Kosack, M.C.; Kilpatrick, M.W.; Docherty, K.; Analysis of DNA structure in the human insulin gene-linked polymorphic region in vivo. *J. Mol. Endocrinol.* **1992**, 9, 221–225.
12. Gomez, D.; Lemarteleur, T.; Lacroix, L.; Mailliet, P.; Mergny, J.L.; Riou, J.F. Telomerase downregulation induced by the G-quadruplex ligand 12459 in A549 cells is mediated by hTERT RNA alternative splicing. *Nucleic Acids Res.* **2004**, 32, 371–379.
13. Piotto, M.; Saudek, V.; Sklenar, V.J. Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions. *J. Biomol. NMR* **1992**, 2, 661–665.
14. Schultze, P.; Flint, W.S.; Feigon, J. Refined solution structure of the dimeric quadruplex formed from the Oxytricha telomeric oligonucleotide d(GGGGTTTTGGGG). *Structure* **1994**, 2, 221–233.
15. Smith, F.W.; Feigon, J. Quadruplex structure of Oxytricha telomeric DNA oligonucleotides. *Nature* **1992**, 356, 164–168.

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