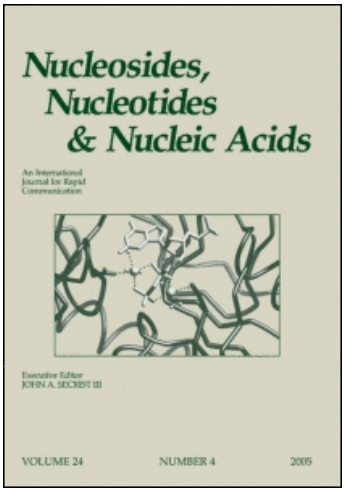

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^{1}H-NMR Study of the Quadruplex [d(TGGGT)]$_4$ Containing a Modified Thymine

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ABSTRACT

A NMR structural study of quadruplex [d(TGGGT)]$_4$ containing a modified thymine is reported. The three dimensional structure of the complex is very similar to those of other parallel stranded quadruplexes. The modified thymines (T*) are able, at least in the minimised structures, to form a tetrad containing extra H-bonds through the hydroxyl groups. Nevertheless, in this new tetrad the modified thymines are slightly open towards the solvent respect to the unmodified T-tetrad.

Key Words: T-tetrad; Modified thymine; Quadruplex; NMR.

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It is well known that, at physiological concentrations of monovalent ions, G-rich oligonucleotides may adopt four-stranded structures called G-quadruplexes.[1] G-quadruplex structures comprise stacked tetrads in which four guanines are arranged in a square-planar array and each guanine serves as both hydrogen bond acceptor and donor in a reverse Hoogsteen base pair. Several biologically important genomic regions such as telomeres,[2] the immunoglobulin switch regions,[3] the promoter regions of genes[4] and recombination sites[5] were found to have the propensity to form G-quadruplex structures, making these molecules an attractive topic of a large number of researches ranging from chemistry to molecular biology and pharmacology. Furthermore, a number of quadruplex forming oligonucleotides have resulted to be potent inhibitors of thrombin[6] as well as of HIV-1 integrase,[7] the enzyme responsible for the insertion of viral DNA into the host genome.

The ability to chemically synthesize biomolecule analogues has opened up the opportunity to observe changes in structure and activity that occur even upon single residue substitution. The incorporation of modified bases into oligonucleotides may indeed produce useful changes in physical and biological properties of the resulting DNA fragments. In this frame, we have synthesized 5-hydroxymethyl-2′-deoxyuridine-containing oligonucleotide (T*-ODN). In particular, $[d(T^*GGGT)]_4$ was prepared and its structure was investigated by ^{1}H-NMR and CD spectroscopy.

The synthesis of modified thymine (T*) was carried out using the fully protected 5-hydroxymethyl-2′-deoxyuridine phosphoramidite as synton as described before.[8] The oligonucleotide 5′-T*GGGT-3′ was synthesised on a Millipore Cyclon Plus DNA synthesiser, using solid phase β-cyanoethyl phosphoramidite chemistry. NMR measurements were performed at a concentration of 1.0 mM (0.5 mL, 90% H_2O/ 10% D_2O), having 10 mM potassium phosphate, 1 M KCl, 0.1 mM EDTA (pH 7.0). The ^{1}H-NMR spectrum was recorded using pulsed-field gradient WATERGATE[9] for H_2O suppression. The presence of 5 signals from three G-H8 and T-H6 and T*-H6 protons in the aromatic region and the presence of three imino peaks resonating at 11-12 ppm indicate the formation of a G-quadruplex structure, consisting of three G-tetrads and possessing a fourfold symmetry with all strands parallel to each other. CD spectra for $[d(TGGGT)]_4$ and $[d(T^*GGGT)]_4$ were also recorded and resulted to be very similar. Particularly, they are characteristic of parallel-stranded quadruplex structures with a positive band at 263 nm and a negative band at 245 nm, although they show slight differences attributable to non equivalent conformation in solution.

Table 1. Non-exchangeable proton chemical shifts for $[d(T^*GGGT)]_4$ in 10 mM KH_2PO_4, 70 mM KCl, 0.2 mM EDTA (pH 7.0, T = 300 K).

Base (5′-3′)	H8/H6	H1′	H2′/H2″	H3′	H4′	H5′/H5″	H2/Me
T*1	7.82	6.04	2.28–2.60	4.80	4.53	4.17	3.97–4.01
G2	8.20	6.14	2.78–3.05	5.08	4.47	4.28	–
G3	7.90	6.06	2.75	5.10	4.53	4.27–4.14	–
G4	7.71	6.27	2.57–2.69	4.93	4.51	4.23–4.08	–
T5	7.37	6.08	2.17	4.98	4.48	4.05–4.21	1.64

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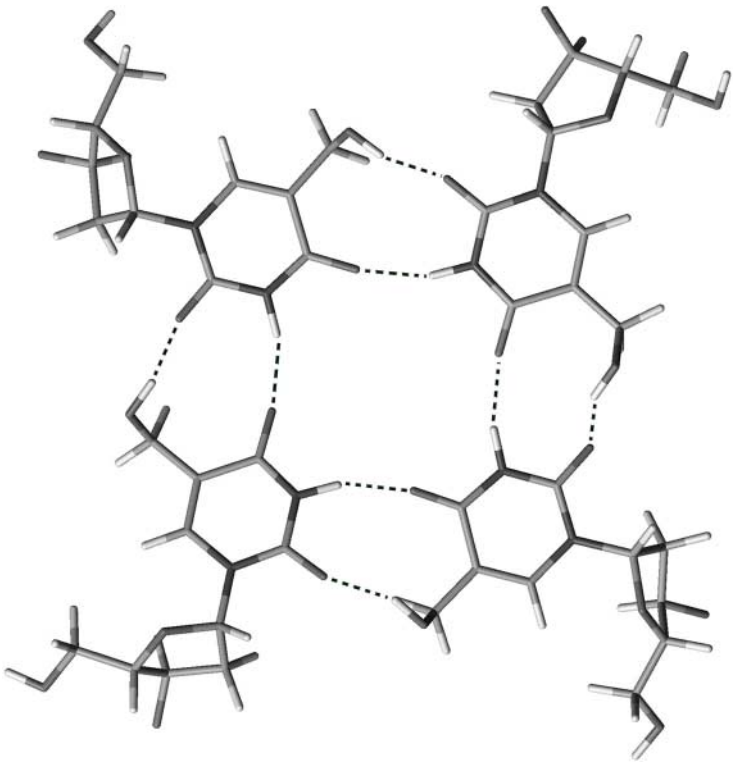

Figure 1.

A nearly complete set of ^{1}H NMR assignments was obtained using 2D homonuclear experiments such as NOESY and TOCSY (table 1). A 2D NOESY (mixing time = 100 ms) was used to extract distance constraints in order to determine the three-dimensional structure of [d(T*GGGT)]$_4$. The NOE restraints were supplemented by 48 distance restraints (HN1-O6, N1-O6, HN2-N7, N2-N7) for 24 hydrogen bonds between Gs obtained from NH deuterium exchange study.

The structure determination was performed using the program CYANA.[10] The calculation started with 100 randomised structures. The 10 structures with the lowest CYANA target functions were subjected to restrained energy minimization using the CVFF forcefield as implemented in the program Discover (Molecular Simulations, San Diego, CA, USA).

The three dimensional structures obtained, as expected, are very similar to other parallel stranded quadruplexes.[1] In particular, the resulting models indicate that the modified thymines are able to form an extra H-bonds through the hydroxyl groups (Fig. 1). Hence, this new tetrad is characterised by four additional H-bonds respect to the unmodified T-tetrad, at least in the energy minimised structure. On the other hand, in order to form these H-bonds the carbonyl groups of thymine residues are forced outside the plane, consequently, the T*-tetrad appears slightly more open towards the solvent respect to the unmodified T-tetrad.

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