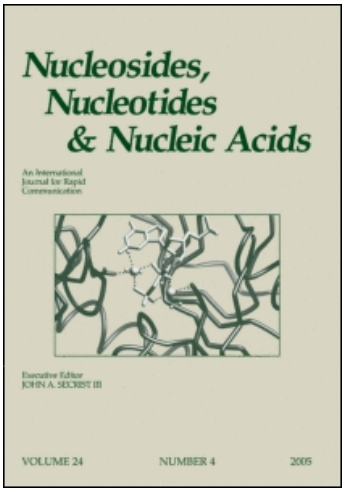

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EFFECTS OF A 8-OXOADENOSINE INCORPORATION ON QUADRUPLEX STRUCTURES: THERMAL STABILITIES AND STRUCTURAL STUDIES

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▫ *The effects of incorporation of 8-oxoadenosine in two different truncations of human telomeric sequence forming quadruplex structures are reported. In order to characterise their structures, a combination of NMR and UV spectroscopy and computational techniques were used. Both oligonucleotides have been found to form fourfold symmetric quadruplex structures. As a tautomeric equilibrium between keto and enol forms of 8-oxoadenosine may establish in solution and intrinsic stabilities effects, such as internal H-bonds, for example, may determine the predominance of some particular tautomer, molecular modelling studies were performed on quadruplex structures containing both the tautomeric forms. Both molecules resulted to be thermally less stable than the natural.*

Keywords DNA, Quadruplex, A-Tetrad, 7,8-Dihydro-8-Oxoadenine

INTRODUCTION

Oxidative damage to DNA bases commonly results in the formation of oxidized purines, such as 7,8-dihydro-8-oxoguanine (G^{oxo}) and 7,8-dihydro-8-oxoadenine (A^{oxo}).[1] It has been demonstrated that 8-oxopurines in DNA are involved in mutagenic, carcinogenic and aging processes.[2–4] T_2AG_3 repeat characterizes the extreme 3′-end of the human telomeric DNA overhang. Particularly, it has been found that these segments can readily assemble to form parallel stranded quadruplexes forming A- and G-tetrads.[5,6] Thus, we focused our attention on effects of incorporation of A^{oxo} in two different truncations of this sequence, namely d(A^{oxo}GGGT) (**1**) and d(TA^{oxo}GGGT) (**2**).

One-dimensional proton spectra of both quadruplex complexes show the presence of three imino peaks in the region 11–12 ppm and five and six signals,

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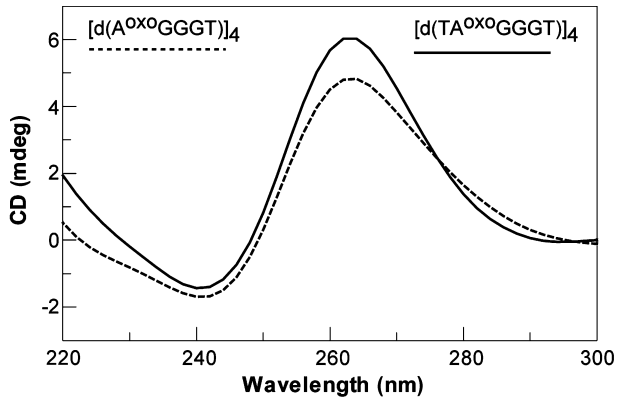

FIGURE 1 CD spectra of $[d(A^{oxo}GGGT)]_4$ and $[d(TA^{oxo}GGGT)]_4$.

respectively, belonging to three G-H8 and to one or two T-H6 and A^{oxo}-H2 protons, in the region between 7 and 8.5 ppm, suggesting in both cases the presence in solution of a single well-defined species. These data are also consistent with the formation of highly symmetric G-quadruplex structures containing three G-tetrads and possessing a fourfold symmetry with all strands equivalent to each other. This hypothesis was also inferred by circular dichroism (CD) data (Figure 1). In fact CD spectra of both molecules are typical of quadruplexes involving four parallel strands (maximum and minimum Cotton effect at 263 nm and 246 nm, respectively).[7] Proton signals for both quadruplexes have been only partially assigned on the basis of NOESY and TOCSY data obtained at 500 MHz (T=300 K). The relative intensities of NOEs observed between G H8 and ribose H2′ compared with the NOEs observed between G H8/T H6 and H1′ demonstrates that all G and T residues are in an *anti* conformation and the polarity connectivities (G H8 to ribose protons on the 5′ side only) is indicative of a right-handed helix, as expected for parallel quadruplexes.[5] PE-COSY spectra analysis indicates that H1′/H2′ coupling constants are reasonably large, suggesting that the sugar geometries are

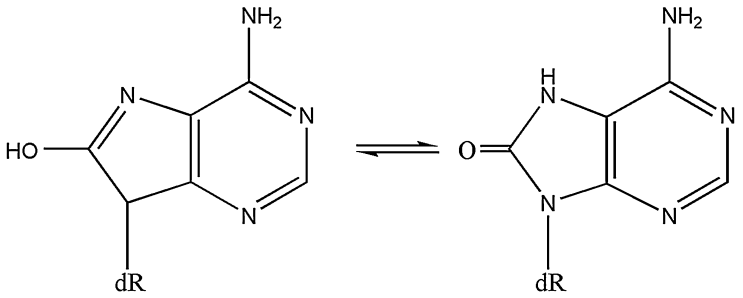

FIGURE 2 Tautomeric equilibrium between keto and enol forms of 8-oxoadenosine.

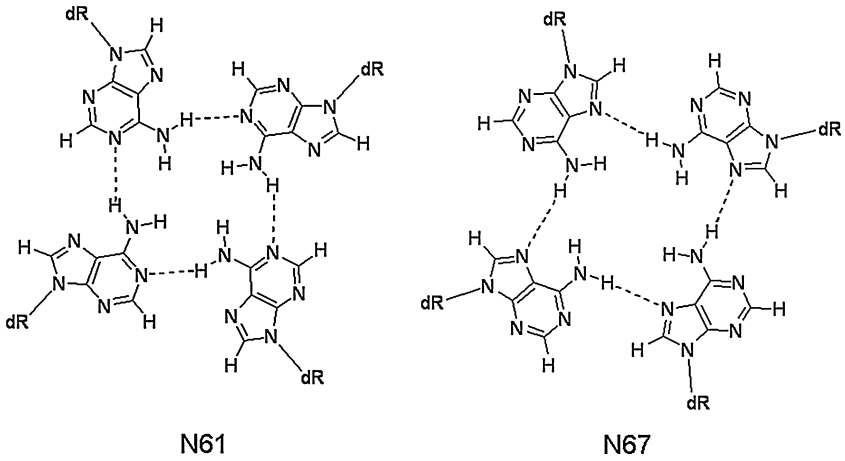

FIGURE 3 N61 and N67 H-bond arrangements for an A-tetrad. The H-bonds are indicated by dotted lines.

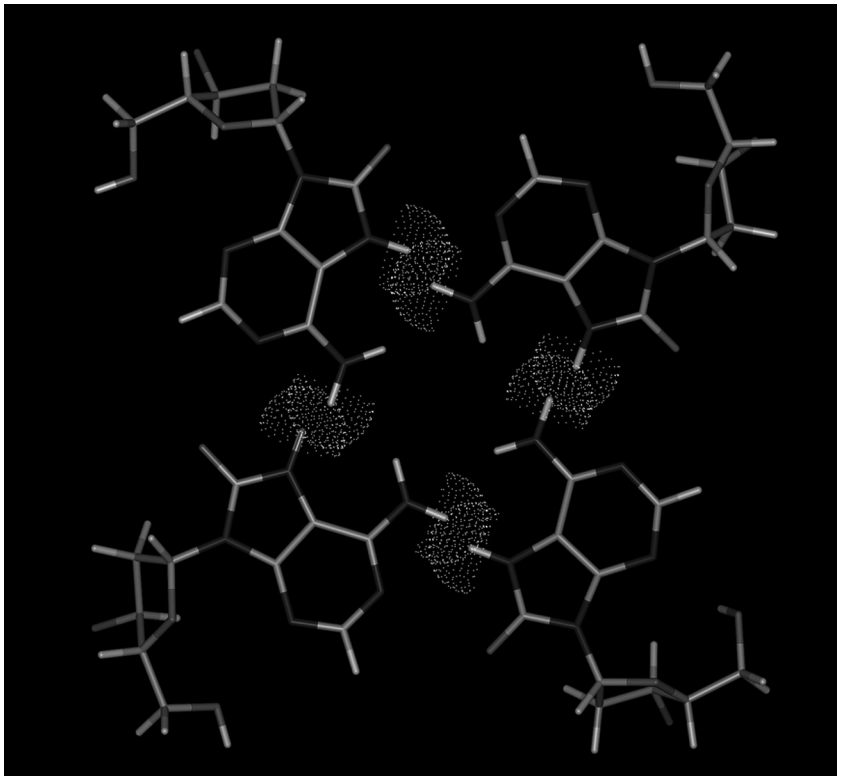

FIGURE 4 Top view of the model of $[d(A^{oxo}GGGT)]_4$ characterized by N67 pattern of H-bond for the A^{oxo}-tetrad. Steric hindrance between A^{oxo} amino group and N7 proton of the adjacent modified base prevents the formation of the hydrogen bonds.

predominantly S-type and consequently the strand structure may be taken to be similar to B-form rather than A-form duplex DNA.

As a tautomeric equilibrium between keto and enol forms of 8-oxoadenosine may establish in solution (Figure 2) and intrinsic stabilities effects, such as internal H-bonds for example, may determine the predominance of some particular tautomer, molecular modeling studies were performed on quadruplex structures containing both the tautomeric forms. Therefore, we have generated eight models for each molecule (**1** and **2**), containing all the experimental constraints (inter-proton distances), extracted from 2D NOESY spectra (mixing time=100 ms), and the following structural features deduced from the structural NMR studies: 1) the strands are parallel and adopt a right-handed helical twist; 2) all the Gs and Ts adopt an *anti* glycosidic torsion angle; 3) the guanine imino and amino protons are hydrogen bonded around the G-tetrad accordingly to what observed for other G-quadruplexes. The eight models obtained for the quadruplex structures adopted by **1** and **2** differ 1) for the pattern of H-bonds imposed for A-modified tetrads, namely N61 and N67, respectively, as reported in precedent papers[5] (Figure 3); 2) for the glycosidic conformation of modified adenosines (*anti* and *syn*, respectively), and 3) for the tautomeric form examined (keto A^{oxo} and enol A^{OH} forms, respectively).

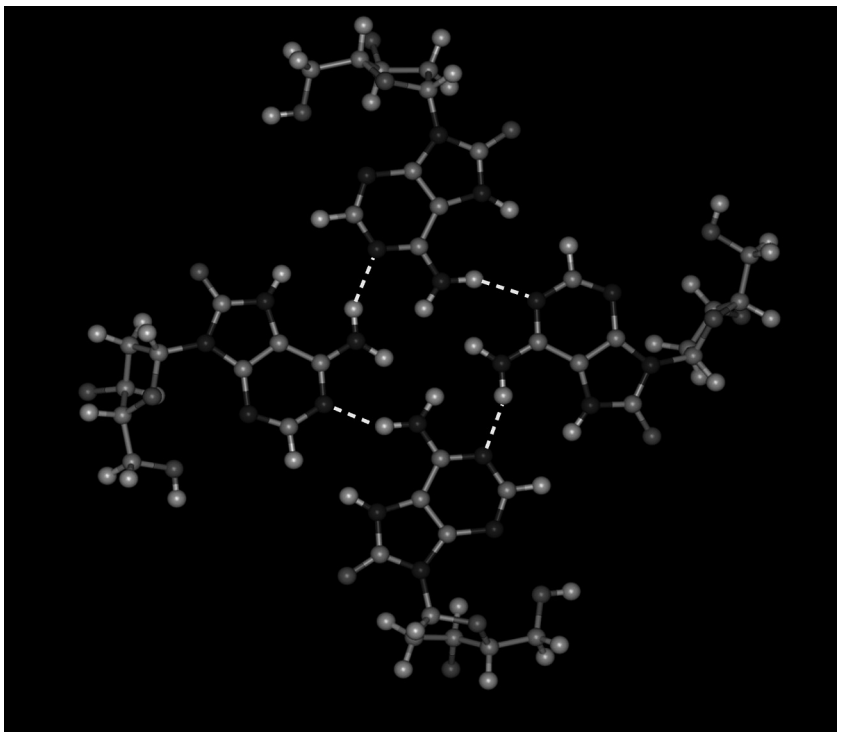

FIGURE 5 Top view of the model of $[d(A^{oxo}GGGT)]_4$ characterized by N61 pattern of H-bond and *syn* glicosidic conformation for A^{oxo} residues.

The models, where A^{oxo} and A^{OH} residues adopt an *anti* glycosidic conformation, showed heavy distortions of the backbone and disagreed slightly with experimental data. Therefore, they cannot be considered as representative models of the structures in solution. Concerning the models of the 5-mer and the 6-mer containing the oxo form of the A residue, N67 hydrogen bond pattern showed steric hindrance between A^{oxo} amino group and N7 proton of the adjacent modified base, preventing the interaction between the hydrogens H6 and the nitrogen N7 required for the formation of the hydrogen bonds (Figure 4). Therefore, the only model able to form an A^{oxo}-tetrad seems to be the one where all adenine residues adopt a *syn* conformation and a N61 hydrogen bond pattern (Figure 5). However, it is interesting to note that, in the latter conformation, the model for $[d(TA^{oxo}GGGT)]_4$ resulted to be characterized by two additional H-bonds between the 5′ hydroxyl of T1 and 8-cheto group of underneath A^{oxo} residues. As for the models containing the enol form of the 8-oxoadenosine (A^{OH}) in *syn* conformation, the formation of both N61 and N67 patterns of H-bond is equally realizable. Moreover, in the case of N67 A^{OH}-tetrad, the formation of two supplementary H-bonds

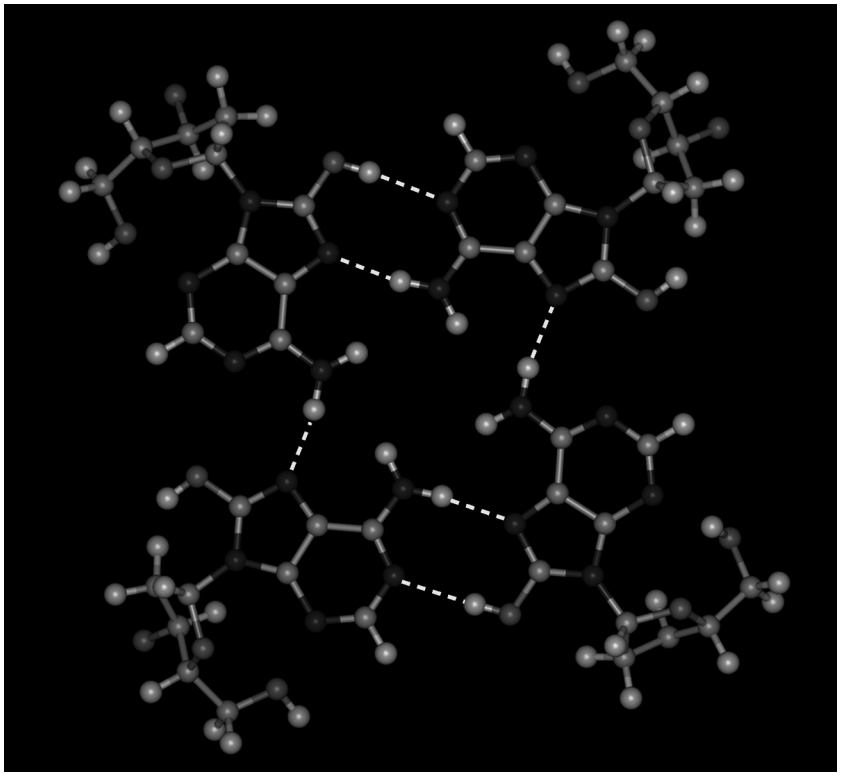

FIGURE 6 Top view of the A^{OH}-tetrad, where the formation of two supplementary H-bonds between the 8-enol function of A^{OH} residue and N1 of the adjacent one seems to be possible both for $[d(A^{OH}GGGT)]_4$ and $[d(TA^{OH}GGGT)]_4$.

between the 8-enol function of A^{OH} residue and N1 of the adjacent one seems to be possible both for $[d(A^{OH}GGGT)]_4$ and $[d(TA^{OH}GGGT)]_4$ (Figure 6).

Thermal stability of quadruplexes adopted by **1** and **2** was determined by UV thermal denaturation experiments. Both the molecules showed sharp transitions and well-shaped sigmoid curves, with midpoint temperatures, T_m, of 54°C and 64°C, respectively, that are basically lower than the melting points of quadruplexes formed by the natural counterparts $[d(AGGGT)]_4$ and $[d(TAGGGT)]_4$ (68°C and 72°C, respectively).

Unfortunately, the molecular modeling studies were not able to justify the lower thermal stabilities of the modified quadruplexes. Thus, in order to give further insight into the above conformational features and the thermal stability data additional physicochemical studies are currently in progress in our laboratories.

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