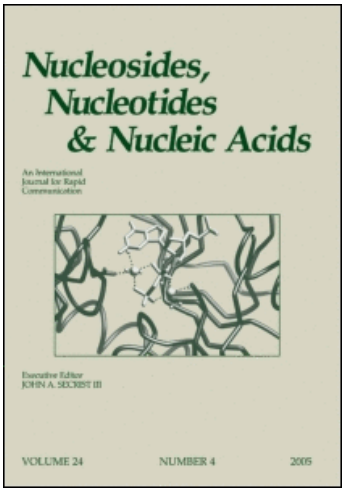

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MOLECULAR MODELING STUDIES OF A PARALLEL STRANDED QUADRUPLEXES CONTAINING A 8-BROMOADENOSINE

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▫ *Truncated sequences of human telomeric DNA can readily assemble to form parallel stranded quadruplexes containing A- and G-tetrads. The formation of an A-tetrad is highly context-dependent and the relationship between the formation of an A-tetrad and the glycosidic torsion angle of the adenosine residues implicated has not been completely clarified so far. In order to give a further insight in this issue we synthesized the modified oligomers d(ABrGGGT) and d(TABrGGGT), two different truncations of the human telomeric sequence containing a 8-bromoadenosine residue, named ABr. NMR data show that both the modified oligomers are able to perfectly fold into highly symmetric quadruplexes with all strands parallel to each other. Molecular modeling studies were performed on both* $[d(ABrGGGT)]_4$ *and* $[d(TABrGGGT)]_4$*, indicating that a bulky substituent, such as a bromine atom at the C8 position of adenines, can force the glycosidic bond to adopt a syn conformation, stabilizing the resulting quadruplexes.*

Keywords DNA, Quadruplex, A-Tetrad, 8-Bromoadenosine

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INTRODUCTION

Guanine-rich DNA sequences have been identified at telomeric repeats level at the end of linear chromosomes[1] and are able to form unusual structures, called G-quadruplex DNA, in presence of monovalent cations.[2–4] Particularly, in human cells, the telomeric DNA is usually characterized by 5–15 kb of tandem repeats of guanine-rich segments $[d(T_2AG_3)_n]$ in double-helix form, with 130–210 bases at 3′-edge forming a single strand overhang in order to allow the complete chromosomal DNA replication.[5] An evolutionarily conserved property of this G-rich telomeric overhang is the ability to form G-quadruplex structures, that play an important role in interfering with telomerase activity.[2,6,7] Telomerase is a ribonuclear protein complex responsible of the addition of G-rich repeats to the end of chromosomes and its activity is related to aging and diseases like cancer.[2,6,7] Therefore, in recent years, the structure of truncated sequences of human telomeric DNA has been object of great interest. Particularly, it has been found that these segments can readily assemble to form parallel stranded quadruplexes containing A- and G-tetrads.[8] The formation of an A-tetrad is highly context-dependent and the relationship between the formation of an A-tetrad and the glycosidic torsion angle of the adenosine residues implicated is still obscure at the present.[8–10] In order to clarify this point and investigate the effects of a bulky substituent, such as a bromine in 8 position of adenosine, on the resulting structures, we synthesized the modified oligomers d(ABrGGGT) and d(TABrGGGT), two different truncations of the human telomeric sequence containing a 8-bromoadenosine residue, named ABr.

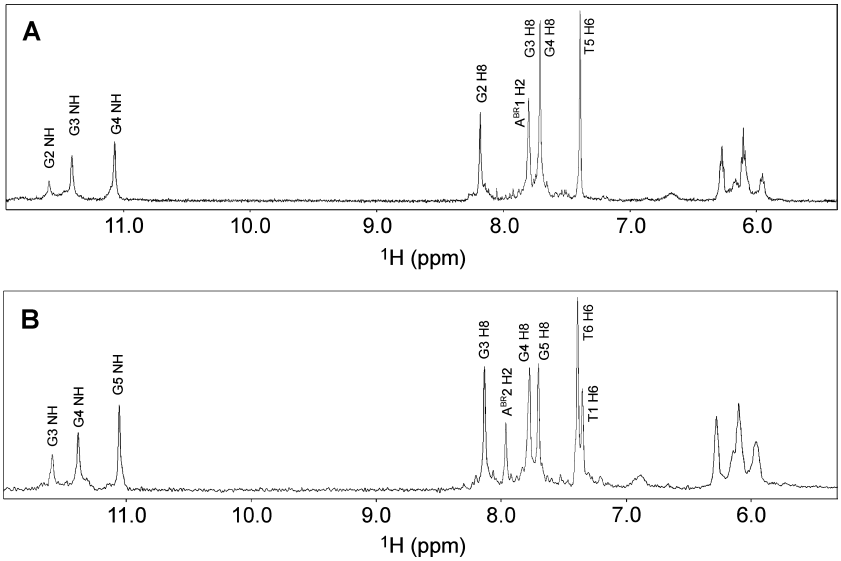

FIGURE 1 Imino and base proton NMR spectrum (500 MHz, T=300 K) of $[d(ABrGGGT)]_4$ (A) and $[d(TABrGGGT)]_4$ (B). The guanine imino protons involved in G-tetrad formation resonate between 11.0 and 12.0 ppm. The narrow non-exchangeable base protons resonate between 7.0 and 8.0 ppm.

One-dimensional proton spectrum of d(ABrGGGT) (Figure 1A) shows the presence of three imino peaks in the region 11–12 ppm, thus suggesting the presence of Hoogsteen hydrogen bonds of G quartets. Moreover, five signals belonging to three G-H8 and to T-H6 and ABr-H2 protons were present in the region between 7 and 8.5 ppm. These data are 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. Analogously, proton spectrum of d(TABrGGGT) (Figure 1B) shows the presence of six signals in the aromatic region and three signals in the region between 10.5–12 ppm. The whole of these data suggest the absence of conformational heterogeneity for both samples.

Partial assignments have been accomplished on the basis of NOESY and TOCSY data obtained at 500 MHz (T=300 K). The NOEs between H2′/H2″ and H6/H8 are first used for peak assignment. The observation of an unbroken path of NOE connectivities along the strands, in contrast to what observed for antiparallel quadruplex structures,[11] suggests that the backbone conformations for both $[d(ABrGGGT)]_4$ and $[d(TABrGGGT)]_4$ are similar to that of regular parallel quadruplex DNA. The relative intensities of NOEs observed between G H8 and ribose H2′ compared with the NOEs observed between G H8 and H1′ indicates that glycosidic torsion angle in all G 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 a parallel quadruplex.[8] PE-COSY spectra analysis indicates that H1′/H2′ coupling constants are reasonably large. This suggests that the sugar geometries are predominantly S-type and consequently the strand structure may be taken to be similar to B-form rather than A-form duplex DNA. The 1-D proton spectra of both $[d(ABrGGGT)]_4$ and $[d(TABrGGGT)]_4$ (Figure 1), are characterized by a slight line broadening of the signals belonging to ABr residues and the neighbor residues, which indicates even in this case a higher flexibility of this portion of the complex in comparison to their natural counterparts. Unfortunately, it was not possible to detect any A-tetrad for $[d(ABrGGGT)]_4$ and $[d(TABrGGGT)]_4$.

The structure calculations on the two molecules $[d(ABrGGGT)]_4$ and $[d(TABrGGGT)]_4$ were undertaken by using restrained distance geometry calculations (CYANA).[12] The 10 out of 100 structures with the lowest CYANA target functions resulting from van der Waals and restraints violations were analysed in both cases and subjected to restrained energy minimization by using the forcefiled CVFF. As expected, each strand of the complexes possesses a right-handed helical backbone geometry and the same guanines from each of the four strands align in a plane to form three G-tetrads. As for ABr residues arrangement, they assume an almost planar arrangement and adopt a *syn* conformation around the glycosidic bonds. Interestingly, while in the unmodified $[d(AGGGT)]_4$[8] two different patterns of H-bonds could be observed between H6 and N1 (named N61) and alternatively between H6 and N7 (named N67) (Figure 2), the ABr residues seem to be not characterized by either pattern N61 or N67 in both complexes.

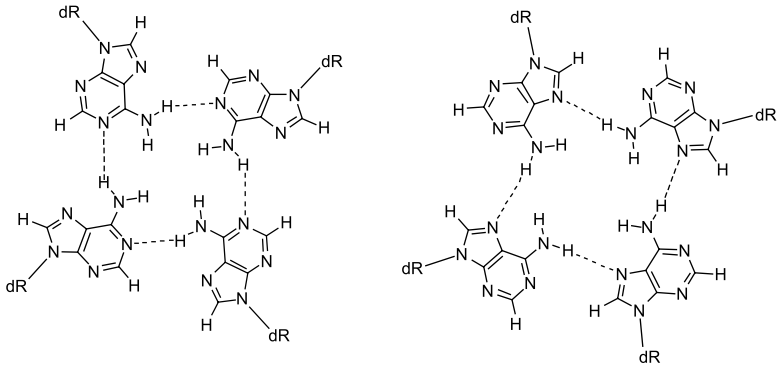

FIGURE 2 N61 and N67 H-bond arrangements for an A-tetrad. The H-bonds are indicated by dotted lines.

In order to better understand why no H-bond pattern has been selected by the modified bases, we performed further molecular modelling studies on both molecules. Particularly, we have generated a total of four models for each complex taking into account all the experimental constraints (interproton distances) and several structural features deduced from NMR studies, such as 1) the 4-fold symmetry and 2) right-handed helicity of the overall structures, 3) the *anti* glycosidic conformation of all G and T residues, and 4) the presence of three G-quartets in a planar hydrogen bonded arrangement. The four models are different in the conformation of the ABr-tetrads: the first two models are both characterized by an *anti* glycosidic conformation of the modified adenines and differ from the pattern of

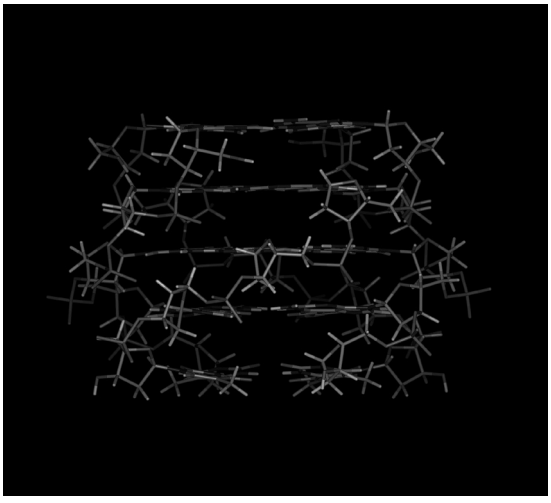

FIGURE 3 Side view of the model of $[d(ABrGGGT)]_4$ characterized by N61 pattern of H-bond and *syn* glycosidic conformation for ABr residues.

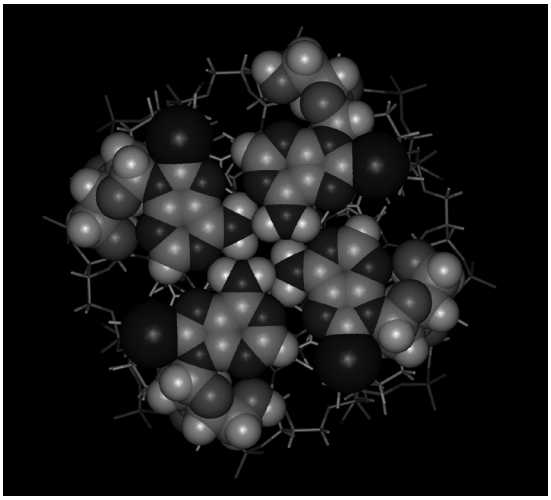

FIGURE 4 Top view of the model of $[d(ABrGGGT)]_4$ characterized by N61 pattern of H-bond and *syn* glycosidic conformation for ABr residues.

H-bond imposed in the calculation (N61 and N67, respectively). On the contrary, the second two models, again built imposing, respectively, N61 and N67 patterns of H-bond, are both characterised by a *syn* glycosidic conformation of the modified adenines. As expected, both models for $[d(ABrGGGT)]_4$ and $[d(TABrGGGT)]_4$ where ABr residues adopt an *anti* glycosidic conformation, are affected by the presence of steric effects between bromine and the backbone. Nevertheless, models characterized by *syn* glicosidic conformation and N67 H-bond pattern clearly suggest that, due to slight steric effect with the adjacent base, the presence of the bromine makes the formation of the hydrogen bonds of the type N67 more difficult. Therefore, the models able to form an ABr-tetrad with no distorsion seem to be those with a *syn* glycosidic conformation of the modified adenines and characterized by N61 pattern of H-bond (Figures 3 and 4). These data seem to indicate that the presence of ABr residues in the truncated sequences of the human telomere significantly disturbs the formation of the ABr-tetrad.

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