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Structural study of four-stranded quadruplex structures containing 2′-deoxy-8-(propyn-1-yl)adenosine

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Abstract—In this paper, we report the NMR structural study of two quadruplex structures formed by truncations of the human telomeric sequence and containing a modified base, namely d(AprGGGT) and d(TAprGGGT), where Apr indicates 2′-deoxy-8-(propyn-1-yl)adenosines. Both oligonucleotides have been found to form 4-fold symmetric G-quadruplex structures with all strands parallel and equivalent to each other and characterized by higher thermal stabilities than the natural counterparts. The presence of the propynyl groups affects the conformations of the 5′ edge of both quadruplexes in such a way to prevent the formation of one of the two possible H-bond patterns observed for a canonical A-tetrad. The increased thermal stabilities of the modified quadruplexes seem to be mostly due to a prevalent *syn* glycosidic conformation assumed by the Apr residues.

1. Introduction

The ends of chromosomes, termed telomeres, comprise specialized guanine-rich DNA sequences with tandem repeats of simple motifs[1] such as $(TAGGGT)_n$ in *Homo sapiens* and $(TGGGGT)_n$ in the ciliate *Tetrahymena*. These sequences are capable to form highly stable quadruplex structures in vitro.[2,3] The potential role of quadruplex structures in vivo has been highlighted. For example, these structures seem to be involved in the recombination at G-rich sequences,[4] telomere protection[5–7] and elongation[8–10] and transcriptional regulation.[11] Furthermore, quadruplex forming oligonucleotides have resulted to be potent inhibitors of thrombin[12] as well as of HIV-1 integrase,[13] the enzyme responsible for the insertion of viral DNA into the host genome.

The ability to chemically synthesize oligonucleotides and their analogues has opened up the opportunity to produce changes in their structures and activities that occur even upon a single residue substitution.[14] The presence of modified bases into oligonucleotides may indeed produce useful changes in physical and biological properties of the resulting DNA fragments. Recently, we have reported the synthesis of 2′-deoxy-8-(propyn-1-yl)adenosine[15] (Fig. 1) and its incorporation in two different truncations of the human telomeric sequence, namely d(AGGGT) and d(TAGGGT), leading to a remarkable increase of the thermal stabilities of the resulting quadruplex structures. We wish to report here a structural study of the quadruplexes $[d(AprGGGT)]_4$ and $[d(TAprGGGT)]_4$, based on nuclear magnetic resonance (NMR) spectroscopy and molecular mechanic and dynamic calculations.

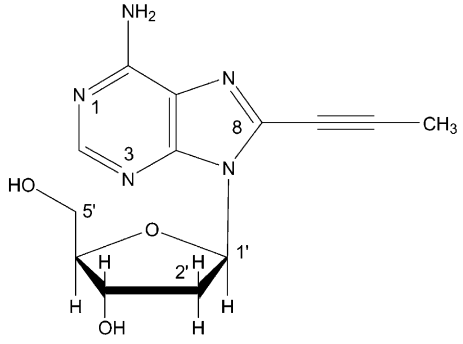

Figure 1. 2′-Deoxy-8-(propyn-1-yl)adenosine.

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2. Results and discussion

The synthesis of the suitably protected Apr phosphoramidite monomer to be used for the preparation of Apr-oligodeoxynucleotides (Apr-ODNs) was performed following a recently proposed synthetic strategy,[15] including bromination of 2′-deoxyadenosine and subsequent coupling with propyne to afford the unprotected 2′-deoxy-8-(propyn-1-yl)adenosine. The monomer was then protected at the exocyclic amino and 5′-OH groups, and activated by coupling with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite. d(AprGGGT) (**1**) and d(TAprGGGT) (**2**) were assembled using the standard phosphoramidite chemistry of the automatized DNA synthesis. The crude oligomers were purified by HPLC and desalted. The NMR samples were prepared at a concentration of 1.0 mM (0.5 mL, 90% H_2O/10% D_2O), having 10 mM potassium phosphate, 70 mM KCl, 0.2 mM EDTA (pH 7.0) buffer.

^{1}H NMR spectra of **1** and **2** were recorded using pulsed-field gradient watergate[16] for H_2O suppression. In a preliminary structural analysis, data from both exchangeable and non-exchangeable protons can provide useful information. For example, the number of slowly exchanging imino protons can substantiate the strand stoichiometry, the number of G-quartet and the symmetry of the quadruplex structure. Furthermore, the range of resonance frequencies may indicate the presence of Watson–Crick or Hoogsteen base-pair as well as the presence of exchange protected imino protons from unpaired residues. Particularly, imino protons chemical shifts in the range 10.5–12 ppm are indicative of guanine NH····O hydrogen bonds found in Hoogsteen arrangement of the G quartets.

In our case, one dimensional proton spectrum of d(AprGGGT) (**1**) 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 Apr-H2 protons were present in the region between 7 and 8.5 ppm. These data are consistent[2,3] with the formation of highly symmetric G-quadruplex structures containing three G-tetrads and possessing a 4-fold symmetry with all strands equivalent to each other.

Analogously, proton spectrum of d(TAprGGGT) (**2**) shows that only one form of quadruplex is present in solution since there are six signals in the aromatic region and three signals in the region between 10.5 and 12 ppm. The exchange rates of the imino protons of **1** and **2** with solvent were qualitatively estimated by partially drying the samples in water and reconstituting them in D_2O. Periodic examination of the imino protons signals shows that they are significantly inaccessible to the solvent, in agreement to what observed for other quadruplex structures.[17] Moreover, circular dichroism (CD) data further inferred the formation of parallel quadruplexes. In fact, the presence of a maximum and minimum Cotton effect at 263 and 246 nm, respectively, is typical of quadruplex involving four parallel strands.[18]

Unfortunately, ^{1}H NMR spectra of both d(AprGGGT) (**1**) and d(TAprGGGT) (**2**) were affected by severe line broadening, especially for the residues at the 5′ edge, where Apr bases are present. On the other hand, the appearance of the spectra could not be improved by altering the temperature and the buffer of the system. This prevented us from fully assigning the residues Apr-1 in **1** and T-1 and Apr-2 in **2**. So, proton signals for **1** and **2** have been only partially assigned on the basis of NOESY and TOCSY data obtained at 500 MHz (T = 300 K) (see Experimental). The NOEs between H2′/H2″ and H6/H8 are firstly 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, suggests that the backbone conformations for both **1** and **2** are similar to that of regular parallel quadruplex DNA.[19] 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.[2] 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.

2D NOESY spectrum (mixing time = 100 ms) was used to extract distance constraints in order to determine the three-dimensional structures of **1**. The Overhauser effect intensities were converted into distances by the tools of the program CYANA.[20] Pseudo-atoms were introduced where needed. 192 upper distance restraints were calculated and reduced to 68 after removal of irrelevant restraints. The NOE restraints were supplemented by 48 distance restraints (HN1–O6, N1–O6, HN2–N7, N2–N7) for 24 hydrogen bonds of the three G-quartets obtained from NH deuterium exchange study. Furthermore, backbone torsion angles were restricted to be in a range of $\pm 20°$ of helical values of the natural quadruplex [d(AGGGT)]$_4$. Glycosidic torsion angles for all guanines were kept in a range of $-157°/-137°$ (*anti* conformation).

The structure determinations were performed using the program CYANA.[20] The calculation started with 400 randomised conformers. The ten structures with the lowest CYANA target functions resulting from van der Waals and restraints violations were analysed. Thus, these structures were subjected to restrained energy minimization using the CVFF forcefield as implemented in the program Discover (Molecular Simulations, San Diego, CA, USA). In particular, the superimposition of the best ten structures is characterized by average RMSD values of 1.02 ± 0.34 and 0.94 ± 0.30 for the backbone and all heavy atoms, respectively. Overall, the RMSD values suggest that the structure of **1** is in a good agreement with experimentally determined restraints. As expected, the structure of the GGGT segment is basically similar to the corresponding segment

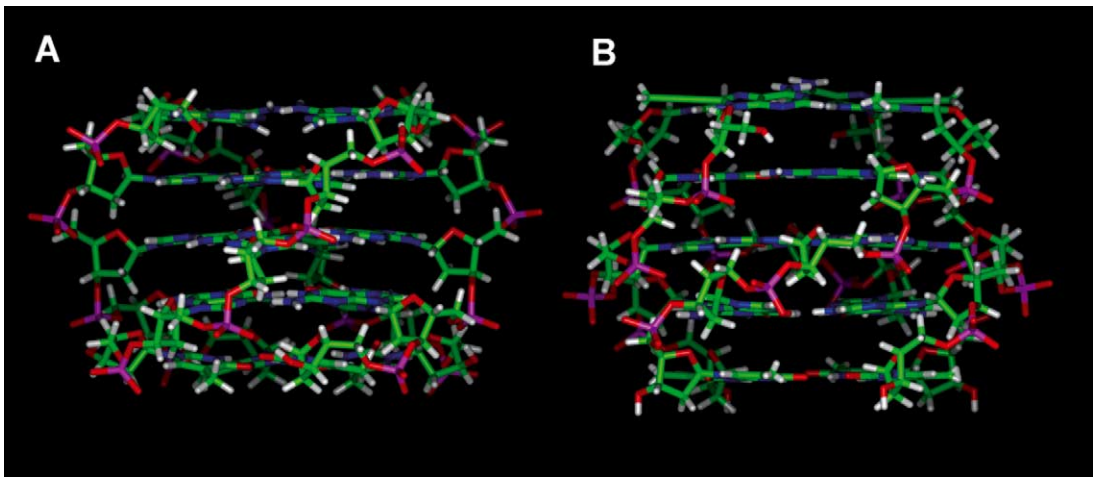

Figure 2. (a) Most representative structure of the quadruplex $[d(AGGGT)]_4$,[2] (b) Final average structure for $[d(AprGGGT)]_4$.

of the quadruplex $[d(AGGGT)]_4$,[2] whereas main differences could be observed at the 5′-end of the molecule (Fig. 2). Thus, differently from that observed in the unmodified quadruplex, the six-membered rings of all Apr residues stack only partially over the six-membered rings of the underneath guanines. As in the unmodified quadruplex, all Apr residues assume a planar arrangement and adopt a *syn* conformation around the glycosidic bonds. The propynyl groups are almost perpendicular to each other and they point outward the quadruplex structure (Fig. 3). It is interesting to note that the π orbitals of the propynyl groups do not stack over the underneath guanines in any of the 10 best structures. Moreover, as observed in $[d(AGGGT)]_4$,[2] all structures could be characterized by a H-bond between the 5′ hydroxyl of Apr and its own N3 atom. It is interesting to note that the structure of the unmodified $[d(AGGGT)]_4$ is also characterized by a novel A-tetrad, in which four adenosines in *syn* glycosidic conformation are held together by two possible patterns of H-bonds.[2] Particularly, one is characterised by hydrogen bonds between hydrogen H6 of one base and nitrogen N1 of the adjacent one (pattern N61; Fig. 4), whereas, in the other one, hydrogen H6 is involved in the H-bond with nitrogen N7 (pattern N67; Fig. 4). In our case, at least in the 10 best structures obtained for $[d(AprGGGT)]_4$, neither pattern N61 nor N67 could be observed.

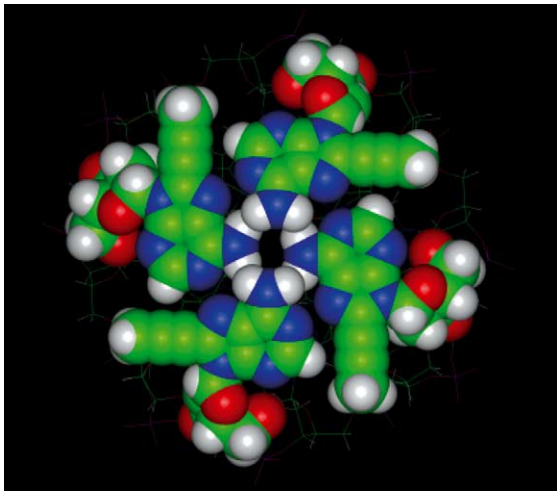

Figure 3. Top view of the average structure for $[d(AprGGGT)]_4$ in CPK model.

Thus, all 10 structures obtained from structural calculations are not able to explain the positive effect of the propynyl moiety on the thermal stability of the modified quadruplex in comparison to the unmodified ones. However, it should be noted that the lack of a sufficient number of constraints at the 5′-end of the molecule and the line broadening suffered by the signals belonging to Apr residues could have prevented the right definition of that part of the molecule. So, molecular modelling studies were performed on the quadruplex $[d(AprGGGT)]_4$. In particular, we wanted to test the possibility for Apr residues to organize in either N61 or N67 hydrogen arrangements and to evaluate whether the increment of thermal stability could be justified by an increase of the stacking over the underneath guanines caused by the π orbitals of the propynyl groups. Therefore, we have generated four models containing all the experimental constraints (interproton distances) and the following structural features deduced from the structural NMR studies: (a) the strands are parallel and adopt a right-handed helical twist; (b) all the Gs and T-5 adopt an *anti* glycosidic torsion angle; (c) the guanine imino and amino protons are hydrogen bonded around the G-tetrad accordingly to what observed for other G-quadruplexes. The four models are different in the conformation of the Apr-tetrads: the first two models (**M1** and **M2**) are both characterised by an *anti* glycosidic conformation of the modified adenines and differ from the pattern of H-bond imposed in the calculation (N61 and N67, respectively). The second two models (**M3** and **M4**), again built imposing respectively N61 and N67 patterns of H-bond, are instead both characterised by a *syn* glycosidic conformation of the modified adenines. Models of **M2** and **M4** clearly suggest that the presence of the propynyl groups makes the formation of the hydrogen bonds of the type N67 impossible, unless upon brutal distortion of the backbone and lost of the planarity for the four modified adenines. In fact, as shown by the top view of the CPK model of **1** in Figure 5, steric hindrance between the propynyl groups and the

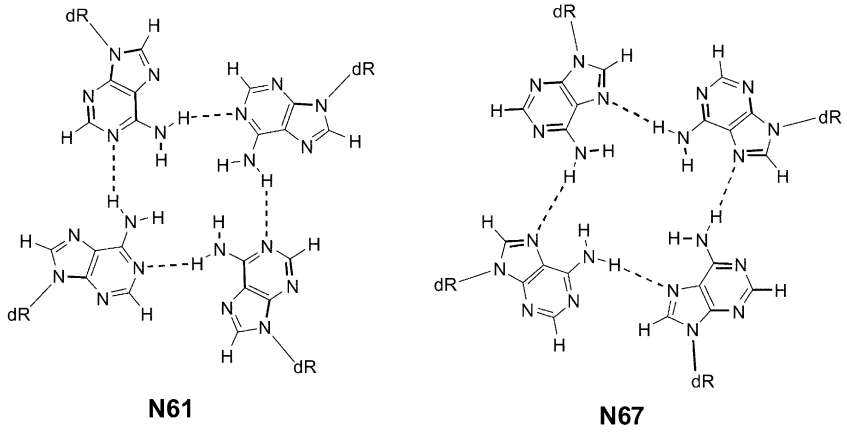

Figure 4. N61 and N67H-bonds arrangements for an A-tetrad. The H-bonds are indicated by dotted lines.

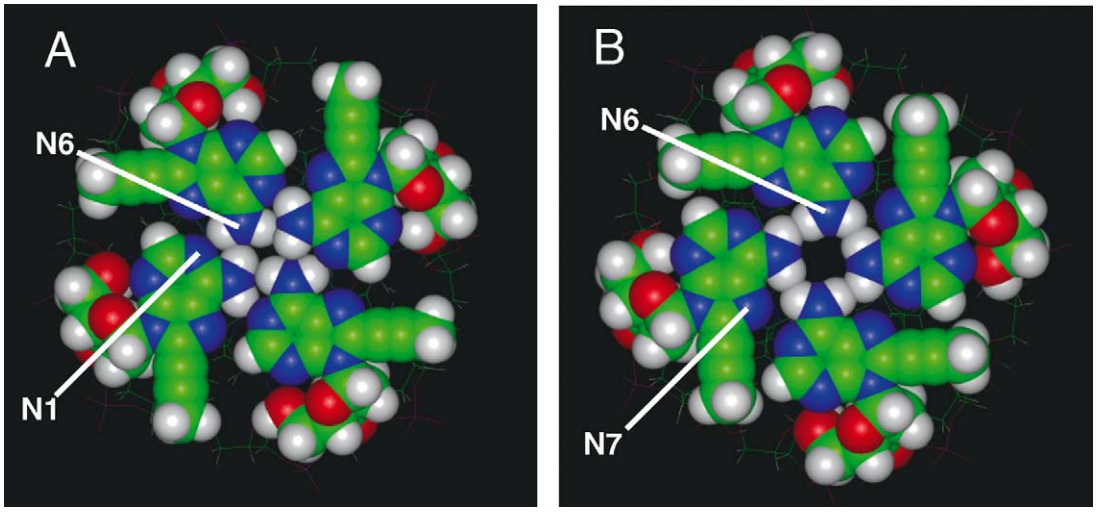

Figure 5. Top view of the CPK models for $[d(AprGGGT)]_4$ in N61 (A) and N67 (B) arrangement.

adjacent modified bases prevents the right orientation of the hydrogens H6 and the nitrogen N7 required for the formation of the hydrogen bonds. Furthermore, as expected,[21] models **M1** and **M2**, where Apr residues adopt an *anti* glycosidic conformation, are affected by the presence of steric effects between propynyl groups and the backbone. Therefore, the only model able to form an Apr-tetrad in **1** seems to be **M3**, where all adenine residues adopt a *syn* conformation, and this is in agreement to what observed in the natural quadruplex $[d(AGGGT)]_4$. In any model, including **M3**, π orbitals of the propynyl groups are not able to stack over the underneath guanines. Therefore, even assuming the formation of the only possible H-bond pattern (N61), the increased thermal stability of the modified quadruplexes in comparison to the natural ones could be explained taking into account the steric effects of the propynyl moieties. These in fact force the Apr residues to assume a prevalent *syn* glycosidic conformation, which seems to be a prerequisite for the formation of the A-tetrads.[2] This hypothesis was substantiated by the study of the *ad hoc* synthesised oligonucleotides d(AbrGGGT) and d(TAbrGGGT), where Abr indicates 2′-deoxy-8-bromoadenosines. In fact, a bulky substituent such as the bromine atom at the C8 position of purines, destabilizes the normal *anti* orientation of the base, thus forcing the glycosidic bond to adopt a *syn* conformation.[21] The oligonucleotide was dissolved in the same buffer used for **1** and **2**, annealed and slowly cooled down to room temperature. ^{1}H NMR data clearly indicate that both d(AbrGGGT) and d(TAbrGGGT) were able to fold perfectly into parallel quadruplex structures. The thermal stability of the quadruplexes adopted by d(AbrGGGT) and d(TAbrGGGT) were determined by CD thermal denaturation experiments. Both the molecules showed sharp transitions and well-shaped sigmoid curves, with midpoint temperatures, T_m, of 68 and 73 °C respectively (Fig. 6), that are basically identical to the melting points of the quadruplex formed by **1** and **2** (68 and 72 °C, respectively).

The structure determination of **2** was performed as for **1**. However, this study resulted to be dramatically affected by the lack of assignment for the residues T-1 and Apr-2. This led to a smaller number of NOEs used in the structural calculation, and consequently, to a worse definition of the structure of the molecule at the 5′ edge.

Therefore, we generated four other models for the quadruplex $[d(TAprGGGT)]_4$, using the same rationale

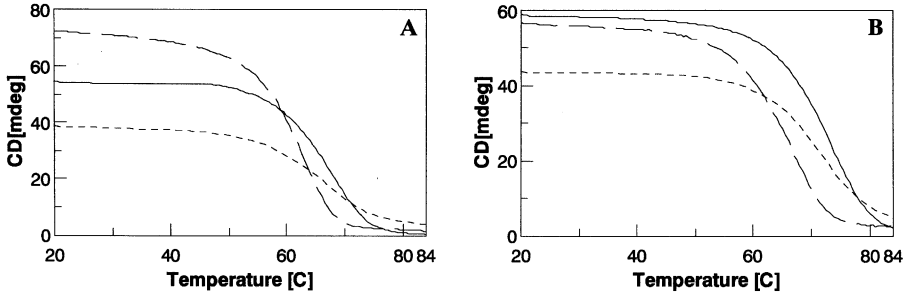

Figure 6. CD thermal denaturation spectra of (A) $[d(AGGGT)]_4$ (– –), $[d(AprGGGT)]_4$ (- - - -), and $[d(AbrGGGT)]_4$ (—); (B) $[d(TAGGGT)]_4$ (– –), $[d(TAprGGGT)]_4$ (- - - -), and $[d(TAbrGGGT)]_4$ (—).

used for **M1**–**M4**. Analogously to what observed with the previous models, it was not possible to fit the Apr bases to form the hydrogen bonds as in pattern N67 and the steric effects in the *anti* conformers were even greater than those observed for $[d(AprGGGT)]_4$. Thus, the most suitable model to represent the quadruplex $[d(TAprGGGT)]_4$ seems to be, as in **1**, the one containing all modified adenines in a *syn* conformation and with a N61 pattern for hydrogen bonds. However, even in this model, propynyl groups seem to suffer from steric effects due to their interaction with the deoxyribose rings of the previous bases of each strand. This observation could explain the larger line broadening observed in **2** than in **1**. Even in this case, no significant stacking between propynyl groups and the underneath bases could be observed.

3. Conclusion

In a previous paper, we showed that the substitution of a canonical A in two different truncations of the human telomeric sequence, namely d(AGGGT) and d(TAGGGT), with a 2′-deoxy-8-(propyn-1-yl)adenosine (Apr)[15] increases the stability of both quadruplexes when compared to the natural counterparts. In the present paper, we have described the NMR investigation of these two oligonucleotide analogues which have been found to form highly symmetric G-quadruplex structures containing three G-tetrads and possessing a 4-fold symmetry with all strands parallel and equivalent to each other. All G residues are in an *anti* conformation and the sugar geometries are predominantly S-type. Molecular mechanic and dynamic calculations indicate that the presence of the propynyl groups determines the choice between the two possible H-bond pattern, namely N61 and N67, preventing the formation of the latter due to steric effect with the adjacent adenines, all of them possessing a *syn* glycosidic conformation. Nevertheless, these molecular modelling outcomes could not be fully confirmed by NMR data due to the severe line broadening of the spectra which prevented us from clearly observing the signals in the regions of interest. Moreover, π orbitals of the propynyl groups are not able to stack over the underneath guanines in any of the structures and models obtained. Therefore, we believe that the increased thermal stability of the modified quadruplexes in comparison to the natural ones, is most likely due to the fact that Apr residues are forced to assume a prevalent *syn* glycosidic conformation, which seems to be a prerequisite for the formation of the A-tetrads.[2] This hypothesis has been supported by preliminary ^1H NMR and CD thermal denaturation experiments performed on the quadruplex forming oligonucletides d(AbrGGGT) and d(TAbrGGGT), where Abr indicates 2′-deoxy-8-bromoadenosines. In order to give further insight into the above conformational features, researches dealing with design, synthesis and structural studies of new modified A containing quadruplexes, are currently in progress in our laboratories. In-depth thermodynamic analysis on the whole of the synthesised oligonucleotide analogues will be also carried out.

4. Experimental

The oligonucleotides were synthesized on a Millipore Cyclon Plus DNA synthesizer, using solid phase β-cyanoethyl phosphoramidite chemistry. Oligomers **1**–**2** were detached from the support, washed with H_2O and purified by standard procedures. The combined filtrates and washings were concentrated in vacuo, redissolved in H_2O and analysed and purified by HPLC. HPLC purifications were carried out by a Waters 515 Pump equipped with a UV detector (Waters 2487). A Nucleogel SAX column (Macherey-Nagel, 1000-8/46) was used; buffer A: 20 mM KH_2PO_4 aq solution, pH 7.0, containing 20% (v/v) CH_3CN; buffer B: 1 M KCl, 20 mM KH_2PO_4 aq solution, pH 7.0, containing 20% (v/v) CH_3CN; a linear gradient from 0 to 100% B in 30 min. and flow rate 1 mL/min were used. The isolated oligomers have the following retention times: **1** = 10.1 min (as single strand), 20.2 min (as quadruplex); **2** = 11.2 min (as single strand), 21.1 min (as quadruplex). They were collected and successively desalted by Sep-Pak cartridges (C18). The isolated oligomers resulted to be more than 98% pure (NMR).

4.1. Nuclear magnetic resonances

NMR samples were prepared at a concentration of approximatively 1 mM, in 0.5 mL (H_2O/D_2O 9:1) buffer solution having 10 mM potassium phosphate, 70 mM KCl, 0.2 mM EDTA, pH 7.0. For D_2O experiments, the H_2O was replaced with D_2O by drying down the sample, lyophilization and redissolution in D_2O alone. NMR spectra were recorded with a Bruker AMX 500

spectrometer. 1D proton spectra of samples in H_2O were recorded using pulsed-field gradient watergate[16] for H_2O suppression. Phase sensitive NOESY spectra[22] were recorded with mixing times of 100 and 200 ms ($T = 300$ K). Pulsed-field gradient watergate was used for NOESY spectra in H_2O. TOCSY spectra[23] with mixing times of 120 ms were recorded with D_2O solutions. NOESY and TOCSY were recorded using TPPI[24] procedure for quadrature detection. In all 2D experiments the time domain data consisted of 2048 complex points in t_2 and 400–512 fids in t_1 dimension. The relaxation delay was kept at 1.2 s for all experiments. The NMR data were processed on a SGI Octane workstation using FELIX 98 software (Byosym, San Diego, CA, USA).

NMR assignment for $[d(AprGGGT)]_4$: ^1H NMR (D_2O, $T = 300$ K) δ 7.68 (Apr2, H2), 6.33 (Apr1, H1′); 2.39, 2.55 (Apr1, H2′, H2″); 2.21 (Apr1, CH_3); 8.19 (G2, H8); 6.16 (G2, H1′); 5.08 (G2, H3′); 3.06, 2.85 (G2, H2′, H2″); 7.76 (G3, H8); 5.95 (G3, H1′); 5.05 (G3, H3′), 2.67 (G3, H2′, H2″); 7.70 (G4, H8); 6.28 (G4, H1′); 4.93 (G4, H3′); 4.52 (G4, H4′); 4.29 (G4, H5′, H5″); 2.54, 2.69 (G4, H2′, H2″); 7.39 (T5, H6); 6.11 (T5, H1′); 4.50 (T5, H3′); 4.09 (T5, H4′); 2.20 (T5, H2′, H2″); 1.65 (T5, CH_3).

NMR assignment for $[d(TAprGGGT)]_4$: ^1H NMR (D_2O, $T = 300$ K) δ 7.20 (T1, H6); 5.97 (T1, H1′); 4.58 (T1, H3′); 2.83, 1.95 (T1, H2′, H2″); 1.57 (T1, CH_3); 7.73 (Apr2, H2), 6.30 (Apr2, H1′); 2.41, 2.53 (Apr2, H2′, H2″); 2.20 (Apr2, CH_3); 8.07 (G3, H8); 6.08 (G3, H1′); 5.03 (G3, H3′); 4.47 (G4, H3′); 4.19 (G3, H4′); 2.98, 2.70 (G3, H2′, H2″); 7.78 (G4, H8); 5.97 (G4, H1′); 5.02 (G4, H3′), 2.66 (G4, H2′, H2″); 7.70 (G5, H8); 6.26 (G5, H1′); 4.94 (G5, H3′); 4.53 (G5, H4′); 2.53, 2.67 (G5, H2′, H2″); 7.37 (T6, H6); 6.08 (T6, H1′); 4.48 (T6, H3′); 4.07 (T6, H4′); 2.16 (T6, H2′, H2″); 1.62 (T6, CH_3).

4.2. Structural calculations

The structure calculations were performed with the program CYANA[20] starting from 400 random conformations. Upper limit distance constraints for both exchangeable and non-exchangeable hydrogens were classified according to their intensity in the NOESY spectra (mt = 100 ms) with the CALIBA tool of the program CYANA.[20] 192 upper distance restraints were calculated and reduced to 68 after removal of irrelevant restraints. Pseudo-atoms were introduced where needed. Hydrogen bonds constraints (16 upper and 16 lower limit constraints/G-tetrad) were used: upper and lower distance limits of 2.0 and 1.7 Å for hydrogen-acceptor distance, and 3.0 and 2.7 Å for donor-acceptor distance, respectively. These constraints for H-bonds did not lead to an increase in residual constraints violation. Backbone torsion angles were restricted to be in a range of ±20° of the helical values of natural quadruplexes.[2] Glycosidic torsion angles for all guanines were kept in a range of −157°/−137° (*anti* conformation). The input for final CYANA structure calculations also included constraints to close the sugar rings (C4′–O4′: 1.41 Å, C4′–C1′: 2.40 Å, C5′–C4′: 2.39 Å, H4′–O4′: 2.12 Å). The dynamics run for 35,000 steps (highsteps = 7000; minsteps = 7000). The 10 structures with the lowest CYANA target functions were subjected to energy minimization by conjugate gradient methods as implemented in the program DISCOVER (Molecular Simulations, San Diego, CA, USA), using CVFF force field. During energy minimization, interproton distances and H-bond constraints involving G-tetrads were used with a force constant of 20 and 100 kcal mol^{-1} $Å^{-2}$, respectively. Illustrations of structures were generated with INSIGHTII program, version '98 (Biosym Technologies Inc.). All the calculation have been performed on a SGI Octane workstation.

4.3. Molecular modelling

Models of the quadruplex structures $[d(AprGGGT)]_4$ and $[d(TAprGGGT)]_4$ were performed with the program CYANA starting from 400 random conformations as described in Section 4.2. The G and T nucleotides were kept in *anti* glycosidic conformation, whereas the Apr residues were kept in *anti* (for **M1** and **M2**) and *syn* (for **M3** and **M4**). The same has been performed for $[d(TAprGGGT)]_4$. The eight models (four for the pentamer and four for the hexamer) with the lowest CYANA target function were then subjected to energy minimization by conjugate gradient methods as implemented in the program DISCOVER (Molecular Simulations, San Diego, CA, USA), using CVFF force field. During energy minimization, interproton distances and H-bond constraints involving G-tetrads were used with a force constant of 20 and 100 kcal mol^{-1} $Å^{-2}$, respectively. Illustrations of models were generated with INSIGHTII program, version '98 (Biosym Technologies Inc.).

4.4. CD melting experiments

CD melting curves were registered on a Jasco 715 circular dichroism spectrophotometer in temperature mode at 263 nm, from 20 to 90 °C, in a 0.1 cm pathlength cuvette, with a scan rate of 1.0 K min^{-1}. Thermal unfolding curves were recordered in the same buffer used for NMR experiments in a 0.1 cm pathlength cuvette.

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References and notes

1. Blackburn, E. H. *Nature* **1991**, *350*, 569.
2. Patel, P. K.; Koti, A. S. R.; Hosur, R. V. *Nucleic Acids Res.* **1999**, *27*, 3836.

3. (a) Aboul-ela, F.; Murchie, A. I. H.; Norman, D. G.; Lilley, D. M. J. *J. Mol. Biol.* **1994**, *243*, 458. (b) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171.
4. Sun, H.; Yabuki, A.; Maizels, N. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12444.
5. Henderson, E.; Hardin, C. C.; Walk, S. K.; Tinoco, I., Jr.; Blackburn, E. H. *Cell* **1987**, *51*, 899.
6. Veselkov, A. G.; Malkov, V. A.; Frank-Kamenetskll, M. D.; Dobrynin, V. N. *Nature* **1993**, *364*, 496.
7. Sundquist, W. I.; Klug, A. *Nature* **1989**, *342*, 825.
8. Salazar, M.; Thompson, B. D.; Kerwin, S. M.; Hurley, L. H. *Biochemistry* **1996**, *35*, 16110.
9. Zahler, A. M.; Williamson, J. R.; Cech, T. R.; Prescott, D. M. *Nature* **1991**, *350*, 718.
10. Sun, D.; Thompson, B.; Cathers, B. E.; Salazar, M.; Kerwin, S. M.; Trent, J. O.; Jenkins, T. C.; Neidle, S.; Hurley, L. H. *J. Med. Chem.* **1997**, *40*, 2113.
11. Simonsson, T.; Pecinka, P.; Kubista, M. *Nucleic Acids Res.* **1998**, *26*, 1167.
12. Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564.
13. Jing, N.; Hogan, M. E. *J. Biol. Chem.* **1998**, *273*, 34992.
14. He, G.; Krawczyk, S. H.; Swaminathan, S.; Shea, R. G.; Dougherty, J. P.; Terhorst, T.; Law, V. S.; Griffin, L. C.; Coutré, S.; Bischofberger, N. *J. Med. Chem.* **1998**, *41*, 2234.
15. Catalanotti, B.; Galeone, A.; Gomez-Paloma, L.; Mayol, L.; Pepe, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2005.
16. Piotto, M.; Saudek, V.; Sklenar, V. J. *J. Biomol. NMR* **1992**, *2*, 661.
17. Wang, K. Y.; McCurdy, S.; Shea, R. G.; Swaminathan, S.; Bolton, P. H. *Biochemistry* **1993**, *32*, 1899.
18. Jin, R.; Gaffney, B. L.; Wang, C.; Jones, R. A.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8832.
19. Smith, F. W.; Feigon, J. *Nature* **1992**, *356*, 164.
20. Guntert, P.; Mumenthaler, C.; Wuthrich, K. *J. Mol. Biol.* **1997**, *273*, 283.
21. (a) Dias, E.; Battiste, J. L.; Williamson, J. R. *J. Am. Chem. Soc.* **1994**, *116*, 4479. (b) Uesugi, S.; Ikehara, M. *J. Am. Chem. Soc.* **1977**, *99*, 3250. (c) Jordan, F.; Niv, H. *Biochim. Biophys. Acta* **1977**, *476*, 265. (d) Uesugi, S.; Ohkubo, M.; Urata, H.; Ikeara, M.; Kobayashi, Y.; Kyogoku, Y. *J. Am. Chem. Soc.* **1984**, *106*, 3675.
22. Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.
23. Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521.
24. Marion, D.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 967.