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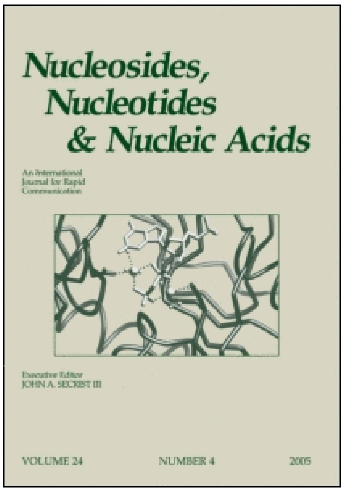

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MOLECULAR MODELLING STUDIES OF FOUR STRANDED QUADRUPLEXES CONTAINING A 3′-3′ OR 5′-5′ INVERSION OF POLARITY SITE

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□ *Recently we reported a preliminarry study on the structure of two novel quadruplex structures, Q33 and Q55, formed by the oligodeoxynucleotides* $^{5'}TGG^{3'}$*-*$^{3'}GGT^{5'}$ *and* $^{3'}TGG^{5'}$*-*$^{5'}GGT^{3'}$*, respectively. Here we report their solution structures at the atomic level. The obtained structures reveal that Q55 and Q33 possess a different stacking among G-quartets and different twist angle (and therefore different helical winding) at the inversion of polarity level.*

Keywords Inversion of polarity; quadruplex; 5′-5′, 3′-3′; DNA

The interest in G-quadruplexes is reflected by hundreds of reports dealing with various aspects of these interesting structures, such as the chemical nature and the topology of the backbone and loops (where present), the conformation of the glycosidic linkage, as well as the influence of structural modifications on the stability of the complexes.[1] Recently we reported a new structural motif in quadruplex structure obtained by introducing an inversion of polarity (3′-3′ or 5′-5′) in the quadruplex forming oligonucleotides.[2] In particular, we reported the preliminary NMR and CD studies of the two quadruplexes named Q33 and Q55, formed by the oligodeoxynucleotides (ODNs) $^{5'}TGG^{3'}$-$^{3'}GGT^{5'}$ and $^{3'}TGG^{5'}$-$^{5'}GGT^{3'}$, respectively. The two quadruplexes were characterized by a different arrangement of the glycosidic angles of the residues. This led to a different symmetry of the two complexes, and to different chemical-physical properties of the two molecules. Particularly, both molecules assume a right-handed helix conformation. Moreover, Q33 is characterized by all G residues in an *anti*

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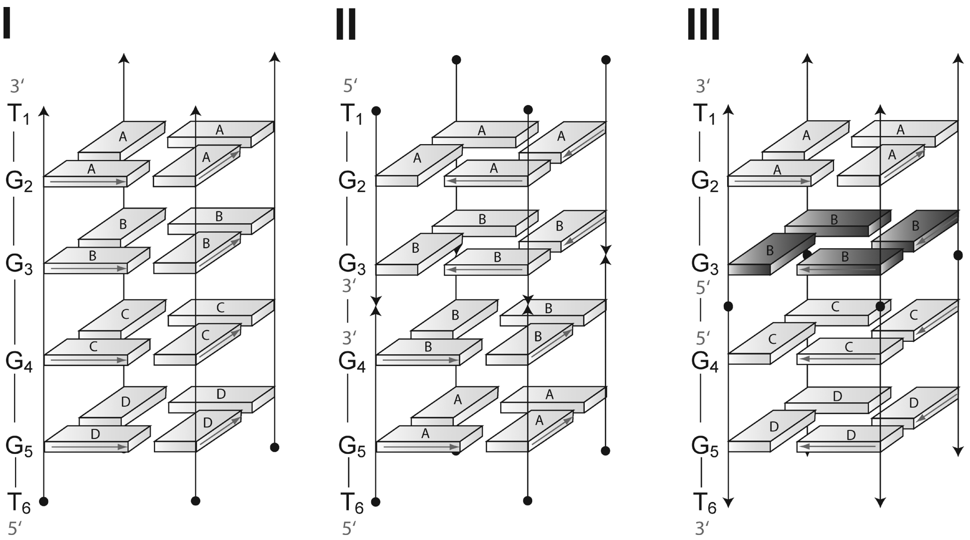

FIGURE 1 Schematic illustration of the structure of $[d(TGGGGT)]_4$, Q33, and Q55. Black arrowheads and circles indicate 5′ and 3′ edges of each subunit/strand, respectively. *Anti* and *syn* guanines are depicted as light and dark gray solids, respectively. Letters (A, B, C, and D) indicate equivalent bases in each complex. Arrows indicate the direction of the proton donors and acceptors in Hoogsteen hydrogen bonds.

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glycosidic conformation. On the other hand, Q55 is characterized by having one G-quartet (the one adjacent to the inversion of polarity site) containing all residues that adopt a *syn* glycosidic conformation (Figure 1, Ref. [2]). Interestingly, a considerable increase (25°C) in the melting temperature could be determined for Q55 when compared with the natural counterpart, while Q33 resulted to be the least stable complex (Tm = 52°C).

In an attempt to get the solution structures at the atomic level of Q33 and Q55, restrained molecular mechanic and dynamic calculations have been performed. We firstly studied the structure adopted by Q33. Thus, a quantitative estimation of proton-proton distances has been done measuring the cross-peak intensities in 2D NOESY experiments acquired at 700 MHz, both at T = 25°C and T = 5°C (mixing time 100 ms for the experiment acquired in D_2O and 200 ms for the experiment acquired in H_2O). 656 distance restraints (164 for each strand) have been retrieved. Moreover, as suggested by the presence of G imino protons in the 1D ^{1}H-NMR spectrum, 64 supplementary distance restraints (HN1-O6, N1-O6, HN2-N7, N2-N7) for 16 hydrogen bonds (corresponding to the four G-quartets), were also incorporated during the computations.

Further, in agreement with NMR data, glycosidic torsion angles were fixed in the *anti*-domain (the χ angle was kept in a range of $-160°$ to $-70°$).

Therefore, three-dimensional structures which satisfy NOEs were constructed by simulated annealing (SA) calculations. An initial structure of the oligonucleotide was constructed possessing a random conformation and minimized, in order to eliminate any possible source of initial bias in the folding pathway. Restrained simulations were carried out for 500 ps using the CVFF force field as implemented in Discover software (Accelrys, San Diego, CA, USA). The restrained SA calculations started at 1000 K, and, thereafter, the temperature was decreased stepwise down to 273 K. The aim step was to energy-minimize and refine the structures obtained by using the steepest descent followed by the quasi-Newton-Raphson (VA09A) algorithms. A total of 10 structures have been generated. To better characterize the local conformation space of the molecules, each structure was then reconsidered by adding K^+ counterions (there were no constrains for K^+ counterions), and soaking the molecule in explicit H_2O. Thus, each structure was heated to 500 K and kept there for 50 ps, and then cooled to 300

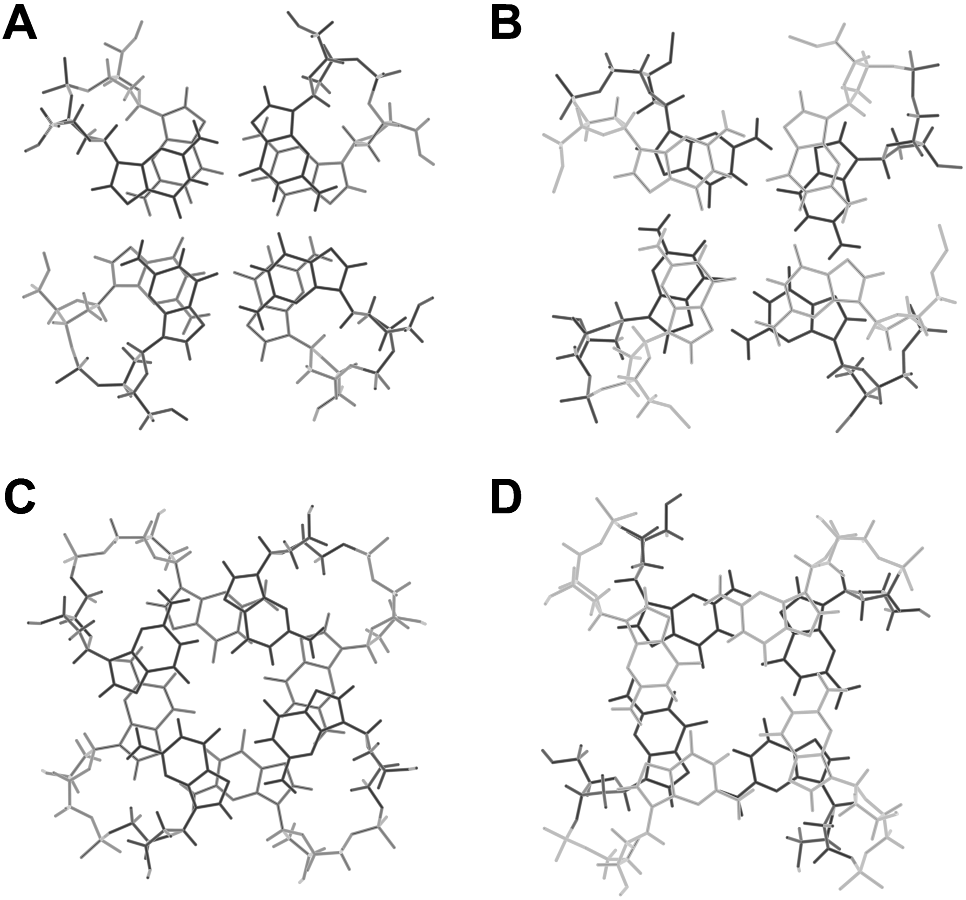

FIGURE 2 Top view of the stacking observed between the G-quartets (G3- and G4-quartet) comprising the inversion of polarity site of Q33 (A) and Q55 (C), and stacking observed between the G2- and G3-quartet of Q33 (B) and Q55 (D).

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K and the system was maintained there for other 50 ps. All 10 structures obtained were subsequently energy minimized using a combination of steepest descent and quasi-Newton-Raphson (VA09A) algorithms. It was possible to obtain a good superimposition of the 10 structures with RMSD values of 0.48 ± 0.16 for heavy atoms.

Concerning Q55, it was subject to the same procedure described for Q33. In this case, 520 distances (130 for each strand) have been used in the calculations, and, as suggested by the presence of G imino protons in the 1D ^{1}H-NMR spectrum, 64 supplementary distance restraints (HN1-O6, N1-O6, HN2-N7, N2-N7) for 16 hydrogen bonds (corresponding to the four G-quartets), were also incorporated during the computations. It is interesting to note that the χ angles of twelve out of sixteen guanines involved in the formation of the four G-quartets were fixed in the *anti*-domain (−160° to −70°), whereas a range of 10° to 100° (*syn*-conformation) was used for the remaining four G residues. At the end of the calculations, also for Q55, it was possible to observe a good superimposition of the 10 structures generated, with RMSD values of 0.92 ± 0.32 for heavy atoms.

The obtained structures reveal that Q55 and Q33 possess a different stacking (Figure 2) and different twist angle (and, therefore, different helical winding) at the inversion of polarity level (Figure 3).

Surprisingly Q33 is characterized by a better stacking between the two G-quartets (i.e., G3 and G4) adjacent the inversion of polarity site. Thus, the higher thermal stability of Q55 could be ascribed to a more efficient stacking between G2- and G3-quartets. However, the higher flexibility of the inversion of polarity site of Q55, also may be responsible of the different melting behavior. Another possible explanation could be ascribed to the different bases orientation in the two complexes, that, in principle, could

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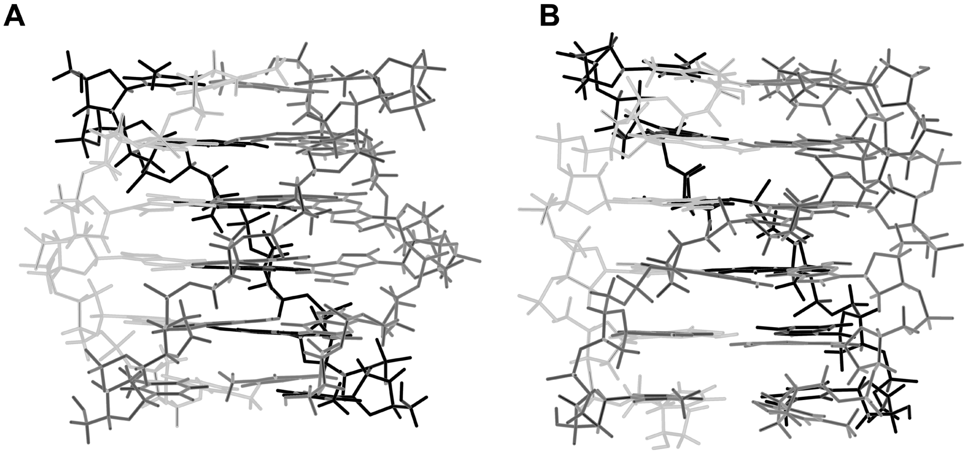

FIGURE 3 Side view of average structures of the best 10 structures of Q33 (A) and Q55 (B) quadruplexes.

lead to a different efficiency in the coordination of K^+ ions. For this reason an in-depth thermodynamic study will be performed.

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