Supplementary information

Topology variation and loop structural homology in crystal and simulated structures of a bimolecular DNA quadruplex

Pascale Hazel, Gary N. Parkinson and Stephen Neidle[*]

Cancer Research UK Biomolecular Structure Group
The School of Pharmacy, University of London
29-39 Brunswick Square, London WC1N 1AX, UK

Modelling methods

The AMBER 8 program[1] was used for all simulations. The systems, including channel K^+ ions, were neutralised with K^+ ions using the LEAP module of AMBER, and were solvated in a pre-equilibrated box of TIP3P water. The box size depended on the system, but always extended at least 10 Å from the solute in every direction. The equilibration procedure consisted of 10 steps, beginning with 1000 steps of minimisation and 25 ps of dynamics of the solvent only. The whole system was then minimised for 1000 steps, followed by 3 ps of dynamics with a restraint of 25 kcal.mol^{-1} on the DNA. The DNA restraint was lowered by 5 kcal.mol^{-1} during each of the next 5 1000-step minimisations. Finally, the system was heated slowly to 300 K over 20 ps, with no further restraints. MD simulations were carried out at 300 K, using a 2 fs time step, with SHAKE applied to constrain the bonds containing hydrogen. The PME method was used to deal with long range electrostatic interactions. A charge grid spacing of approximately 1.0 Å in every direction was used, with

Lennard-Jones interactions were cut off at 10 Å.

The MM-PBSA method within AMBER was used to calculate approximate free energies. Snapshots were collected every 20 ps for energetic analysis. The electrostatic contribution to the solvation free energy was calculated using the Delphi II program[2]. Dielectric constants of 1.0 and 80.0 were assigned to solute and solvent, respectively. A grid spacing of 0.5 Å was chosen, with the longest linear dimension of the molecule occupying 80% of this grid. The AMBER parm99 charge set and BONDI radii were used. All MM-PBSA calculations included the three K^+ ions within the quadruplex channel explicitly. The solute entropic contribution was estimated with the NMODE program, using snapshots collected every 200 ps. Each snapshot was minimised in the gas phase, using a distance-dependent dielectric of $\varepsilon = 4r$, before calculation of the vibrational mode frequencies. The minimisations caused some distortion to the structures. However this did not have a significant effect on the calculated entropies.

Results

G_{total} was decomposed into individual loop contributions, according to the method described by Fadrna *et al*[2], and G_{loop1} and G_{loop2} are shown in Table

3. The stabilisation gained by the head-to-head dimers upon formation of an eight G-quartet stack can be estimated by comparing the free energies of the separate dimers, and the energy of the two stacked dimers. The two-quadruplex stack is more stable than the sum of the two individual dimers (G_{total} = -9159 kcal.mol^{-1} for the dimer stack, and individual dimer energies taken from Table 3). Most of the increased stabilisation comes from the extra K$^+$ ion located between the two G-quartet dimers, whereas only three K$^+$ ions were included in each individual G-quadruplex calculation. We have not compared energies in detail for dimer vs monomer in view of the difference in ion number. Table 3 also shows the free energy of the two head-to-head dimers simulated together as a stack of dimers. During this simulation, the C, D head-to-head dimer was much less favourable than during its simulation alone in solution. This was reflected in both the G-stem and loop energies. On the other hand, the E, F dimer was more favourable when simulated as part of the stack, although the 4 kcal.mol^{-1} energy difference is within the MM-PBSA calculation error margin. However, for both dimers, G_{stem} was much less favourable when the dimer stack was simulated. This could be due to the increased rigidity caused by the greater number of G-quartet stacks, which does not enable the G-stems to equilibrate as well as when the dimers are separate in solution. However, no difference in the entropic contribution was found between the separate and stacked simulations.

According to the MM-PBSA calculations, the difference between the head-to-head and head-to-tail dimers is due mostly to the loop energies, as G-stem energies are very similar. This suggests that the energy difference between alternating *syn-anti-syn-anti* and *syn-syn-anti-anti* G-glycosidic

angles around the G-quartets is very small. Loops in the type 1 conformation over the wide quadruplex groove were the most favourable, as highlighted in magenta in Table 3, which is consistent with their frequent occurrence in the various crystal structures.

Problems associated with the simulation and prediction of quadruplex loop conformations have been previously highlighted in the study of T_4 loops[2]. Not only was it not possible in this study to predict the experimental diagonal loop conformation, but the experimental loop structure was also unstable during MD simulations. In contrast to the T_4 diagonal loops, no ions were observed to bind within the T_3 loops in the crystal structures reported here. Simulations of the experimental loop conformations were found to be stable in most cases, with only minor loop fluctuations occurring.

References

1. Case, D. A. et al. AMBER 8. University of California, San Francisco, 2004.

2. Rocchia, W.; Sridharan, S.; Nicholls, A.; Alexov, E.; Chiabrera A.; Honig, B. *J. Comp. Chem.* **2002***, 23*, 128-137.

3. Fadrna, E.; Spackova, N.; Stefl, R.; Koca, J.; Cheatham, T. E.; Sponer, J. *Biophys. J.* **2004**, *87*, 227-242.

Table 1. G_{total} values (kcal.mol^{-1}) calculated using MM-PBSA for the crystal structure simulations. All values were calculated over the final 2 to 4 ns of dynamics. Values for the head-to-head dimers (strands C, D and strands E, F) were calculated both alone in solution and as part of the stacked dimer of dimers. The ^{Br}U residues in the $(G_4{}^{Br}UTTG_4)_2$ *P2$_1$* structure were mutated to T

residues prior to simulation. Type 1 loops are highlighted in red. Standard errors of the mean are in brackets.

dimer	strands	G_{total}[a]	G_{stem}	G_{loop1}	G_{loop2}	$-TS_{total}$
C222 head-to-tail	A, A	-4556 (4)	-3639 (4)	-455 (4)	-462 (4)	-582 (1)
separate simulations						
P2₁ head-to-tail	A, B	-4551 (4)	-3634 (4)	-457 (4)	-460 (4)	-581 (1)
P2₁ head-to-head	C, D	-4545 (4)	-3635 (4)	-447 (4)	-456 (4)	-579 (1)
P2₁ head-to-head	E, F	-4523 (5)	-3637 (4)	-445 (4)	-441 (4)	-585 (2)
simulation of the stacked dimers						
P2₁ head-to-head	C, D	-4520 (4)	-3629 (4)	-440 (4)	-448 (4)	-579 (1)
P2₁ head-to-head	E, F	-4527 (4)	-3629 (4)	-447 (4)	-445 (4)	-583 (2)