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G-Quadruplex Structure Can Be Stable with Only Some Coordination Sites Being Occupied by Cations: A Six-Nanosecond Molecular Dynamics Study

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G-Quadruplex Structure Can Be Stable with Only Some Coordination Sites Being Occupied by Cations: A Six-Nanosecond Molecular Dynamics Study

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Guanine tetrads are formed spontaneously by guanine rich sequences in the presence of certain cations. Various quadruplex helical structures, stabilized by such tetrads, apparently play an important biological role in vivo. To understand the importance of the cations, a 6 ns molecular dynamics simulation has been performed on a 7-mer G-quadruplex, surrounded by Na^+ counterions and explicit water molecules, but without any ions in the initial structure. Interestingly, the quadruplex structure does not fall apart, but undergoes small structural changes, which enable the solvent molecules, including Na^+ ions, to enter the empty central channel of structure. This channel is fully hydrated within the first 100 ps and two ions move into the central channel between 0.5 and 2 ns of MD simulation, by replacing some of the water molecules. The ions once trapped within the quadruplex channel are not expelled even during 1.5 ns of MD at 400 K. In fact they penetrate deeper into the channel to facilitate entry of additional ions, though all coordination sites within the quadruplex are not occupied even after 6.1 ns of MD simulation. The entry of cations into the central channel leads to a quadruplex structure with more favorable free energy of hydration, which is comparable to that of a fully coordinated quadruplex.

Introduction

In recent years, there has been considerable interest in the formation, topology and stability of higher order DNA helical structure for guanine rich DNA sequences since it was established that, in the presence of cations, these sequences can adopt a four stranded structure.[1,2] Such guanine rich repetitive sequences are observed at the ends of chromosomes,[3−6] immunoglobulin switch regions of higher organisms,[7] mutation hotspots associated with human diseases,[8] "CpG" islands located in the coding and promoter regions of genes,[9] and in triplet repeat sequences associated with fragile X syndrome.[10] A variety of proteins which can bind guanine rich sequences and facilitate formation of G-quadruplex structures have now been identified[9,11−14] and further support the possible biological role for such structures. Fiber diffraction studies[15,16] and crystal structures[17,18] demonstrate that such four stranded structures are stabilized by stacked guanine tetrads (G-tetrads). G-tetrads are formed by the cyclic hydrogen bonding of four guanine bases in a coplanar arrangement (shown in Figure 1). Cations also play a crucial role in stabilizing G-quartets by coordinating eight closely spaced guanine O6 atoms of successive G-tetrads, which form a twisted cube geometry. Recent high-resolution crystal structure[18] has provided definite evidence of Na^+ ion coordination by G-quartets, where dehydrated cations are observed at all the coordination sites between stacked G-quartets, while diffuse electron density corresponding to only one potassium ion has been identified in the antiparallel, hairpin dimer crystal structure[17] of $d(G_4T_4G_4)$. Several experimental studies have revealed that depending upon the cationic concentration and size, different cations selectively stabilize different type of quadruplexes.[19−25] Differences between the stabilizing effect of various cations is also reflected in their NMR spectrum[24−26] and they apparently have different degrees of mobility.[27−31] Molecular dynamics studies have indicated that G-quadruplexes with coordinated cations are very stable.[31−35] However, without any coordinated cations, in the central quadruplex channel of 4-mer quadruplex, the structure is reported to be unstable,[33] with strand slippage occurring at about 0.5 ns.

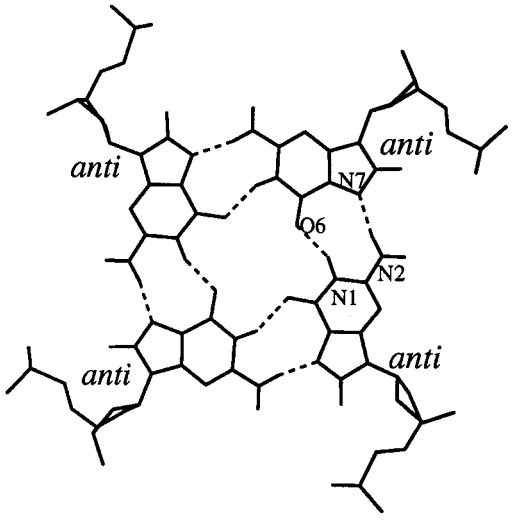

Figure 1. A down the helix axis view of a representative G-tetrad in the parallel crystal structure[18] of $d(TG_4T)$ sequence. The anti glycosidic orientation in the four guanine bases and four equivalent grooves are clearly seen. Dotted lines represent the hydrogen bonds, for which hydrogen to acceptor atom distance is <2.6 Å, with the relevant donor atoms on strand 1 and acceptor atoms in strand 2 being indicated.

We have earlier reported 1.1 ns MD simulations on 7-mer parallel quadruplexes, with coordinated Na^+ and K^+ ions as well as without any coordinated ions.[34] We found that all three structures are stable during MD, albeit with slightly different geometries of G-tetrads and phosphodiester backbones. The

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initial structure without any coordinated ions was stabilized by the entry of water molecules within the first few picoseconds followed by an ion at about 500 ps. To see whether a longer simulation will lead to this structure being disrupted, the ion being expelled or more ions replacing the water molecules in the central channel of the quadruplex, we have carried out an extended 6.1 ns molecular dynamics (MD) simulations on a parallel guanine quadruplex, surrounded by explicit water molecules and Na^+ counterions, but without any initial coordinated ions. The movement of ions from solvent into the central channel and its effect on the quadruplex structure, stability, and solvation patterns around the quadruplex structure have been analyzed. The results of the MD studies clearly show that the quadruplex structure is maintained even when only a few of the available coordination sites are occupied.

Methods

The uniform quadruplex helical structure, with all four chains in parallel orientation, as proposed from fiber diffraction studies was chosen as the starting structure.[15] This was chosen, rather than the high-resolution crystal structure, which has coordinated ions present in the central channel, to see whether during MD, the quadruplex converges toward the latter structure. The 7-mer quadruplexes were surrounded by 24 Na^+ counterions to neutralize the negative charges on the phosphate groups. The counterions were placed 6 Å from each of the phosphorus atoms, along the bisector of the two pendant oxygens, using the EDIT module of AMBER. DNA and counterions were then placed in a preequilibrated box of TIP3P water molecules. The periodic box of water was extended to a distance of 5 Å from DNA and counterions, thus effectively covering a distance of ~10 Å from the phosphate cylinder (1103 water molecules solvate the system). Molecular dynamics was performed under NPT condition with SANDER module of AMBER 4.1 program[36] using the PARM 94 all atom force field and particle mesh Ewald method (PME) was used for the calculation of electrostatic interactions.[37] This is a fast implementation of the Ewald summation method for calculating the full electrostatic energy of a unit cell in a macroscopic lattice of repeating images. The PME method has been successfully used in several other MD studies of DNA and stable trajectories are obtained.[38−45] The PME grid spacing was ~1.0 Å. It was interpolated on a cubic B-spline, with the direct set tolerance set to 0.000001. Periodic boundary conditions were imposed in all directions. The long-range electrostatic interactions have been calculated without any truncation, while a 9 Å cutoff was applied to Lennard-Jones interactions. The nonbonded pair list was updated every 20 steps and the SHAKE algorithm was applied to constrain the covalent bonds. A time step of 2 fs was used and the structures were saved after every 100 steps, i.e., at every 0.2 ps interval, for the entire duration of the MD run. To minimize the "flying ice cube" problem,[46−47] the center of mass velocity was removed periodically during the production simulation at intervals of 10 ps.

Initial systems were energy minimized to a root-mean-square (rms) gradient of 0.1 kcal/mol Å. To equilibrate the solvent molecules, all the waters and surrounding counterions were subjected to 20 ps dynamics at 100 K, keeping DNA fixed, followed by an energy minimization of the entire system. The quenched system was then heated slowly, from 0 to 300 K, by coupling to a heat bath whose temperature was raised at the rate of 50 K for every 2 ps of MD run. The system was equilibrated for another 88 ps and the dynamics run continued for a further 3 ns during which the structures were coupled to a heat bath at 300 K with a coupling constant of 0.1 ps. To test the quadruplex stability at higher temperature and facilitate the movement of counterions, the system temperature is raised to 400 K and the MD simulation is continued up to 4.6 ns. Then the system is quenched to 300 K, and the simulation is continued for another 1.5 ns. Thus, the total duration of the entire simulation is 6.1 ns. Two MD average structures are obtained from the coordinates saved between 2.1 and 3.1 ns and from 5.1 to 6.1 ns. All the structural parameters are calculated using the program NUPARM[48−49] and the trajectory plots are generated using MATLAB package. In our free energy analysis, cavity detection, solvent accessible surface (SAS) area, and volume calculations were performed using the MSP suite of programs of Connolly[50] with a probe radius of 1.4 Å.

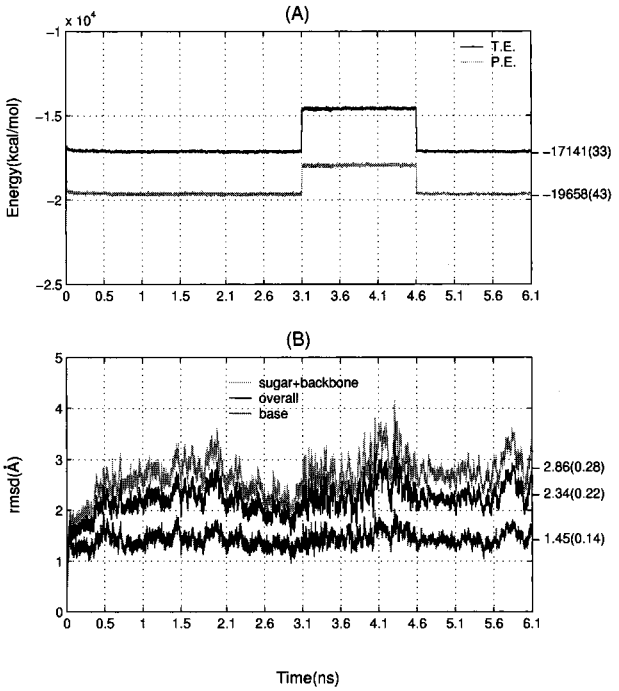

Figure 2. (A) Trajectories showing the potential (PE) and total energy (TE. = potential + kinetic) profiles during the 6.1 ns MD simulation. The upper plot corresponds to total energy, while the bottom plot indicates potential energy. Time (in nanosecond) is along the *x*-axis and energy (in kcal/mol) is along the *y*-axis. (B) Root-mean-square (rms) deviation profiles, during the MD simulation of d(G7) parallel quadruplex, with respect to the initial energy minimized quadruplex structure. The mean value of rms deviations between 5.1 and 6.1 ns interval is indicated on the right-hand side of each plot, while the standard deviation is given within parentheses. The three plots, from top to bottom, correspond to the rmsd for the phosphodiester backbone along with sugar atoms, all the atoms and the guanine bases alone, respectively. The rms deviations for base atoms are smallest, while they are largest for sugar and backbone atoms, particularly during the elevated temperature simulation (3.1−4.6 ns).

Results and Discussion

Parallel Quadruplex Retains Tetrameric Form Even at 400 K. The potential energy, as well as total energy (Figure 2A) of the simulated system at 300 K, approaches equilibrium within 100 ps. As expected, the potential as well as total energy increases during the elevated temperature dynamics (between 3.1 and 4.1 ns) and decreases on lowering temperature to 300 K. The rms deviation values, with respect to the initial energy minimized structure, were calculated for all the atoms in the 7-mer DNA quadruplex, as well as for the guanine base atoms and sugar−phosphate backbone atoms separately (shown in

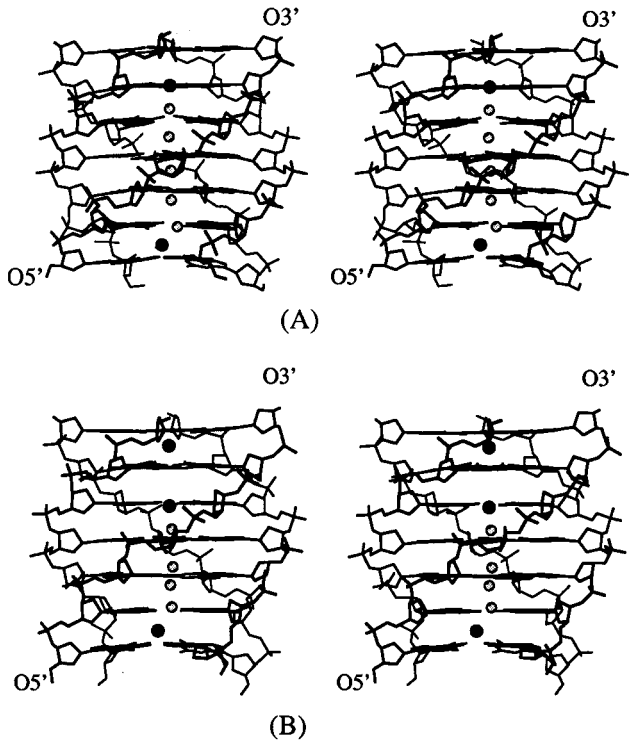

Figure 3. Stereo diagram showing the MD average quadruplex structure obtained during (A) 2.1−3.1 ns (B) 5.1−6.1 ns simulation. The mean positions of Na^+ counterions and water molecules, which have moved from the solvent to locations within the quadruplex channel, are also shown. The water molecules are represented by open circles and Na^+ counterions are shown as filled circles.

Figure 2B). It is observed that, due to the movement of the Na^+ ions and water molecules through the quadruplex channel, the rms deviation gradually increases during the first 2 ns of dynamics, but subsequently stabilizes at around 2.0 ± 0.2 Å (between 2.1 and 3.1 ns). Interestingly, during the entire simulation (including elevated temperature simulation), the rms deviation of guanine bases with respect to initial energy minimized fiber structure oscillates between 1.4 and 1.5 Å, indicating that the G-tetrads are really robust structural entities. The rms deviation of backbone atoms is always greater than that of the bases and increases slightly during the 400 K dynamics, which leads to the rms deviation of the whole 7-mer quadruplex structure also increasing during this interval, but the quadruplex structure maintains its integrity. Stereo diagrams of MD average quadruplex structures along with average water and ion positions within the quadruplex channel obtained during 2.1−3.1 ns and 5.1−6.1 ns of dynamics (i.e., before and after the elevated temperature dynamics) are shown in Figure 3A and B, respectively. It is seen that water molecules as well as Na^+ counterions occupy the coordination sites inside the quadruplex structure and presumably help maintain the quadruplex structure even at high temperatures. Two sodium ions and five water molecules have occupied the quadruplex channel at the end of 3.1 ns simulation, while three ions and four water molecules are present within the channel after the 400 K dynamics. The structure during the last 1 ns of dynamics, is not very different from the quadruplex before the heating phase (rmsd between the two MD average structures being 0.98 Å). However, during the dynamics at 400 K, the backbone orientation undergoes some changes. In particular, two backbone torsion angles (α and γ) in the second nucleotide in the second strand undergo transition from g^-, g^+ regions to g^+, t regions, respectively. This leads to the groove becoming wider in that region, a feature which is subsequently retained (bottom of Figure 3B). A detailed analysis has been carried out on water and ion positions in the grooves and movement of water and ions through the quadruplex channel.

Movement of Na^+ Ions and Water Molecules along the Quadruplex Channel. During MD simulation of the G-quadruplex without any coordinated ion in the initial structure, the quadruplex structure is quite stable at 300 K, with the central channel retaining its shape as observed by the diagonal O6−O6 distances within the G-tetrads (data not shown). The quadruplex channel was hydrated within 100 ps, with an average of six water molecules occupying the six coordination sites between the seven G-tetrads. As the simulation progresses, between 500 and 600 ps, one Na^+ ion was seen to move from the solvent to an in-plane location at 550 ps, and subsequently to the coordination site between the two G-tetrads at the 3′ end of the DNA quadruplex. Similarly, between 1.2 and 1.6 ns, another counterion enters the quadruplex channel through the 5′ end, after displacing one water molecule from the channel and occupies the coordination site between the first and second tetrads. At the end of 3.1 ns dynamics (i.e., after another 1.5 ns of simulation), the same five water molecules and two Na^+ counterions occupy the quadruplex channel and stabilize the structure. We therefore raised the simulation temperature to 400 K to see whether this would expel any of the waters or ions from the quadruplex channel. On the contrary, during the elevated temperature MD, the Na^+ ion, at the 3′ end of the channel moves toward the 5′ end of the quadruplex. Subsequently, between 3.4 and 3.7 ns, one more counterion enters the quadruplex channel through the 3′ end and both the Na^+ ions slowly move from the 3′ end of quadruplex toward the 5′ end, pushing out one water molecule from the channel, without any strand dissociation. Since the 5′ end of the quadruplex channel is blocked by a Na^+ ion (Figure 3A), the water molecule inside the channel is squeezed out between the G-tetrads and into the grooves of the quadruplex. The lateral movement of water molecule is facilitated by the guanine bases (in the second, third, and fourth G-tetrads) undergoing oscillations along the *z*-axis, leading to buckling of the G-tetrads, as shown in Figure 4A. The water molecule is squeezed out between tetrads 3 and 4 and both these G-tetrads show buckling, while the third guanine tetrad also opens up transiently to allow the passage of water molecules toward the groove (Figure 4B). Interestingly, this water molecule stays within the groove for another ∼100 ps and interacts with the N7 atoms in the third and fourth G-tetrads, through hydrogen bonds (Figure 4C shows the interaction with the third tetrad) before moving away. The quadruplex channel at 3.9 ns is thus occupied by three counterions and four water molecules. The positions of ions and water molecules inside the channel change very little even after an additional 700 ps of MD at 400 K, as well as 1.5 ns of MD at 300 K, as seen in Figure 3B. Movement of an ion from solvent into the quadruplex channel, without disrupting the G-tetrads is clearly seen from Figure 5, wherein 7 snapshots of the four terminal nucleotides in the quadruplex structure are shown superimposed, along with the first Na^+ counterion which enters the quadruplex through the 3′ end and traverses along the central channel. Thus, it is clear that, during the MD simulation, Na^+ ions in the solvent can travel a considerable distance and enter the quadruplex core through the ends, thereby displacing the water molecules already present there. It is also found that once the ion is trapped inside the quadruplex, its movement is considerably slower, though it continues to move

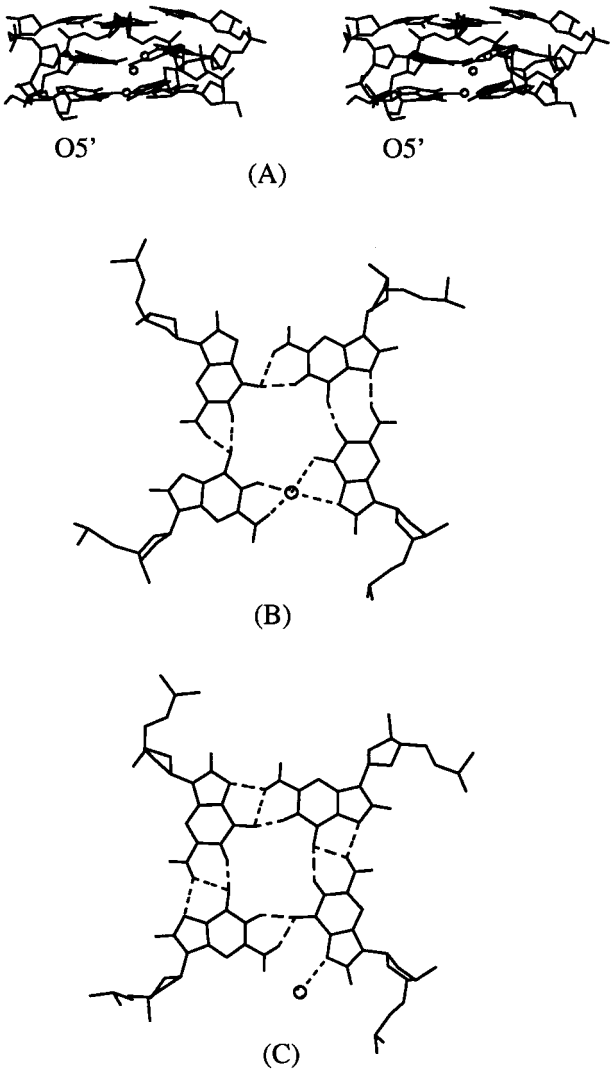

Figure 4. (A) Snapshot at 3580 ps showing the second, third and fourth G-tetrads in an intermediate structure during the movement of water molecules from inside the channel to an outside position. The buckling of the G-tetrads is clearly seen in this stereo figure. The water oxygen is shown as an open circle. (B) A view down the helix-axis of the third G-tetrad at 3580 ps. Dotted lines represent the hydrogen bonds, for which hydrogen to acceptor atom distance is <2.6 Å. The G-tetrads are considerably distorted leading to disruption of the hydrogen bonds between the guanines. (C) The third G-tetrad after a further 90 ps of MD (at 3670 ps). The water molecule shown in part B is now located in the groove, being still hydrogen bonded to the N7 atoms in third and fourth G-tetrads, while cyclic hydrogen bonds within the G-tetrad are reformed.

along the central channel of the quadruplex, thereby facilitating the entry of more ions.

Effect of Ions on the G-Tetrad Stacking Energy. Occurrence of both water molecules and ions in the quadruplex channel, allows us to analyze their effect on the relative stability of the G-quadruplex structure in their presence. Interaction energy within a G-tetrad, as well as stacking energy between successive G-tetrads depends primarily on the orientation of O6 atoms within the quadruplex structure. Close clustering of O6 atoms in stacked G-tetrads leads to unfavorable electrostatic energy (about $+10 \pm 3$ kcal/mol) between the neighboring G-tetrads in the quadruplex structures, even in the absence of any coordinated ions. However, favorable van der Waals component of base stacking energy ($\sim -40 \pm 2$ kcal/mol) makes the total stacking energy favorable ($\sim -30 \pm 3$ kcal/mol). Intrusion of Na^+ counterions into the quadruplex channel affects the stacking energy values. If the contributions due to the coordinated water molecules and ions are included, the van der Waals components of stacking energies in all the tetrads remain around ~ -40.0 kcal/mol, while electrostatic component of stacking energy depends on the nature of the coordinated molecules. The interactions between water molecules and the G-tetrads, reduce the unfavorable electrostatic energy component to $\sim +1 \pm 3$ kcal/mol, which makes the overall stacking energy value to be $\sim -39 \pm 3$ kcal/mol, for stacked G-tetrads with water in the central channel. In the case of Na^+ ion coordinated tetrads, electrostatic energy component of stacking energy is around ~ -20.0 kcal/mol,[34] giving a total stacking energy of ~ -60 kcal/mol for G-tetrads, if the interaction involving the coordinated Na^+ ions is included. Hence it is clear that coordinated Na^+ ions contribute considerably higher stabilization energy to the G-quadruplex as compared to water molecules. It is worth mentioning that the interaction energy (with all other atoms) of a channel bound cation is more favorable than the interaction energy of the ion in the solvent.

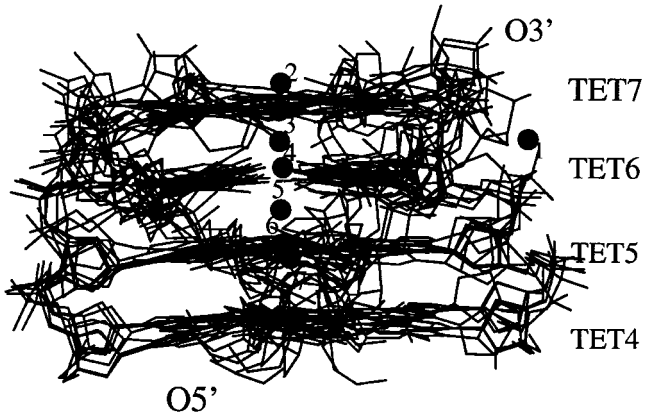

Figure 5. Seven representative snapshots of the fourth, fifth, sixth, and seventh G-tetrads in the quadruplex structure are shown superimposed, alongwith a particular Na^+ ion (filled circles) which moves from the position "1" in the solvent to position "7", which is well within the channel formed by the stacked G-tetrads. The snapshots correspond to the structures at 0.2, 500, 540, 590, 1100, 3420, and 6108 ps during the 6.1 ns MD simulation.

Hydrogen Bond Patterns in G-Tetrads. The starting fiber model,[15] as well as high-resolution crystal structure[18] of parallel quadruplex with coordinated Na^+ ions, contain N1−H1···O6 and N2−H2···N7 (Hoogsteen type) hydrogen bonds between adjacent guanines, leading to eight hydrogen bonds per G-tetrad (as seen in Figure 1). It is observed that during the molecular dynamics simulation, hydrogen bonding scheme within a G-tetrad depends on the electrostatic interaction between the polar atoms of guanine bases and the coordinating molecules. During the initial phase of the simulation (between 100 and 600 ps), in the absence of any coordinated ion, even though water molecules are present in the quadruplex channel, the bases in the G-tetrads undergo in-plane rotation, to minimize the electrostatic repulsion between closely spaced O6 atoms. The adjacent O6 atoms move apart and sit almost midway between N1 and N2 atoms of the adjacent guanine (as shown in Figure 6) leading to the formation of bifurcated N1−H1···O6 and N2−H2···O6 hydrogen bonds, while a few N2−H2···N7 hydrogen bonds are broken. In-plane rotation of the guanine bases is also confirmed by the distances between O6 atoms on adjacent ($\sim$4.2 $\pm$ 0.4 Å) and diagonal ($\sim$6.1 $\pm$ 0.4 Å) guanines in the G-tetrads which at this stage show much larger values, than the corresponding distances in the crystal structure ($\sim$3.2 and $\sim$4.5 Å,

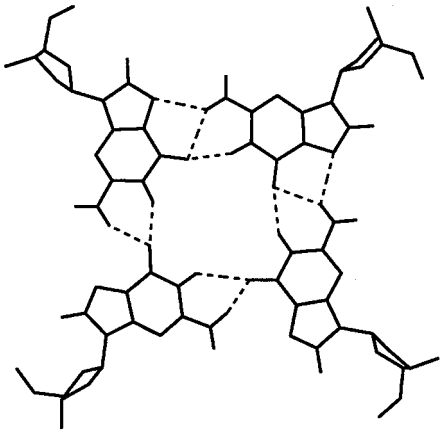

Figure 6. Hydrogen bonding scheme in a representative G-tetrad during the initial phase (100−600 ps) of MD simulation. Dotted lines indicate hydrogen bonds with hydrogen to acceptor distance of <2.6 Å.

respectively). Recent ab initio study on G-tetrad[51] also suggested that, in the absence of a coordinated cation, G-tetrad is stabilized by bifurcated hydrogen bonds between N1−H1···O6 and N2−H2···O6 atoms, whereas the MD simulation of the 4-mer parallel quadruplex structure without any coordinated cations[33] reported disruption of the G-tetrad geometry due to strand slippage. During the MD simulation between 0 and 3.1 ns, two counterions enter the quadruplex channel (between 0.5 and 0.6 ns and between 1.2 and 1.6 ns) through the 3′ or 5′ ends and occupy the coordination site between the sixth and seventh tetrads as well as between the first and second tetrads, respectively. Because of the strong attractive force between the Na^+ ion and O6 atoms of guanine, O6 atoms within these G-tetrads overcome mutual repulsion and come close to one another by in-plane rotational motion. Some of the G-tetrads are now stabilized by standard Hoogsteen type hydrogen bonds, as shown in Figure 1, while other G-tetrads in the 7-mer quadruplex structure retain the bifurcated hydrogen bond pattern (Figure 6). During the elevated temperature dynamics, one water molecule is expelled while one more Na^+ ion enters the channel (3.1−4.6 ns). The occurrence of rotational motion of guanine bases within a G-tetrad during this period is indicated by the large fluctuations seen in the time trajectories of distance between diagonal O6 atoms in some of the G-tetrads (data not shown). During the final 1 ns of dynamics at 300 K, four of the tetrads are stabilized by normal Hoogsteen type hydrogen bonds while tetrads 2, 3, and 4 are held together by bifurcated hydrogen bonds because only water is present in the central channel near these tetrads (Figure 3B). All these movements of guanine bases are accompanied by some conformational transitions in the phosphodiester backbone torsion angles, as well as in minor repuckering of several deoxyribose sugar rings.

The starting quadruplex fiber model[15] as well as high-resolution crystal structure[18] with coordinated ion has a twist value around 30°. During the initial phase of simulation (100−600 ps), the structure without any coordinated ion shows unwinding without affecting the helical "rise", with the average twist value in the middle guanine steps being around 26° at this stage. However, during the final 1 ns of simulation, the partially ion coordinated MD average structure has an average twist value of 28° ± 2°. The average propeller twist and buckle values (−3.9° ± 3.1° and −3.9° ± 3.9°) in the middle segment of this structure are closer to crystal structure values (−1.3° ± 4.1° and −12.4° ± 3.7°) rather than the starting fiber model values (4.5° and 12°), while the values for average rise and displacement " d*x*" from the helix axis are 3.3 ± 0.1 Å and

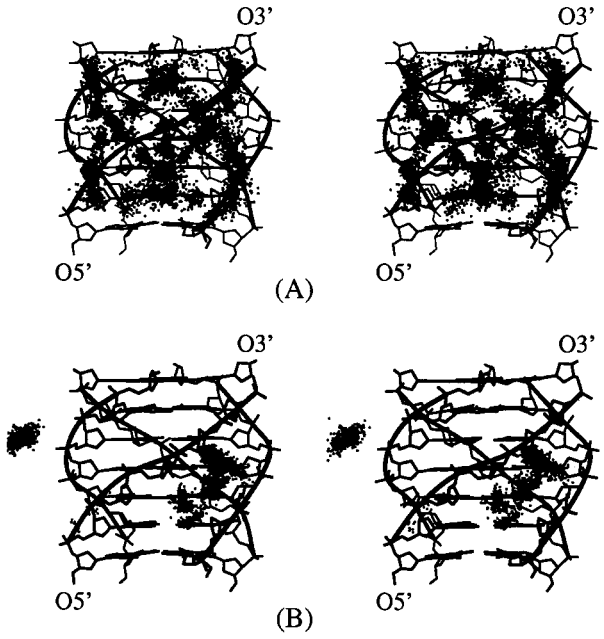

Figure 7. (A) Stereo diagram showing MD average structure during last 1 ns of simulation (between 5.1 and 6.1 ns) along with the hydration pattern around the middle five base tetrads. Black dots represent positions of water molecules (oxygen atom of water molecule), which stay within 3 Å of guanine base atoms (between 5.1 and 6.1 ns). Positions of water molecules at intervals of 5 ps between 5.1 and 6.1 ns of dynamics are superimposed on the MD average quadruplex structure. (B) Same as in part A but showing the positions of Na^+ counterions located within 3.5 Å of DNA atoms, for more than 50% of dynamics time (between 5.1 and 6.1 ns). A ribbon is drawn through the phosphodiester backbone.

−4.9 ± 0.9 Å, respectively, which are close to those in the fiber model[15] as well as those in the crystal structure.[18]

Hydration and Counter Ions within Grooves. There is considerable experimental and theoretical evidence, which indicates the existence of a spine of water molecules in the grooves of DNA helices, hydrogen bonded to DNA base atoms in duplex DNA structures.[40,52−55] The parallel quadruplex structure has four symmetrical grooves (the shortest P−P separation between neighboring strands is ∼10 Å in the fiber model), and the grooves are shallower than in the duplex structure. The mean groove width in the MD average structure is 10.6 ± 2.0 Å. We have analyzed the hydration patterns of the grooves, in the quadruplex structure, and have found that several water molecules are observed within each of the four grooves, hydrogen bonded to some of the solvent exposed base atoms. Hydration pattern around the base atoms of middle five tetrads (within 3 Å of any guanine base atom) is shown in Figure 7A for the last 1 ns of molecular dynamics. Water molecules are seen to be clustered near the 2-amino groups and N3 atoms of guanines (<3 Å) with these positions being occupied for more than 50% of the dynamics time. The 2-amino groups in guanine bases are better water-binding sites than the N3 atoms, as observed in the crystal structure of the 4-mer parallel quadruplex[18] as well as Z-DNA crystal structures.[55] Due to the in-plane base rotation, the N7 atoms in some of the middle G-tetrads are also accessible to solvent and a few water molecules are observed within 3 Å of these N7 atoms, for more than 50% of dynamics time. The hydration patterns around the guanine base atoms are not sensitive to groove width variation and are quite similar to that observed in our previous MD study[34] of 7-mer parallel quadruplexes with six Na^+ or K^+ coordinated ions.

A few counterions are also found to be located within the quadruplex grooves. During the final 1 ns of dynamics, apart from three channel-bound counterions, three more Na^+ ions are close to DNA (within 3.5 Å of any DNA atom) for more than 50% of dynamics time, as shown in Figure 7B. Two of the ions are bound in the grooves between strands 1, 2, and 3, while the third ion is close to a 5′ phosphate of G5 in the fourth strand. These groove bound counterions mainly interact simultaneously (viz., <5.5 Å) with the pendent oxygen atoms as well as the phosphodiester (O5′ and O3′) atoms on neighboring strands. The groove bound counterion between strands 1 and 2 interacts with the phosphate group of G5 in first strand and phosphate group of G4 in second strand, while the counterion between strands 2 and 3 interacts with the phosphate group of G6 in second strand and phosphate group of G5 in third strand. The presence of tightly bound ions leads to a narrowing of the grooves (∼9 Å) between strands 1, 2 and 3, toward the 3′-end of the quadruplex (Figures 3 and 7). Interestingly, the same grooves are much wider at the 5′-end of the molecule (shortest P−P distance of ∼14 Å). We do not observe any counterions interacting with DNA atoms via water molecules, over any significant length of time (viz., more than 100 ps of dynamics time).

We have also analyzed the change in solvent accessible surfaces of the quadruplex molecules, due to the intrusion of ions into the central channel, using the methods developed by Lee and Richards[56] and Connolly.[57] As mentioned above, due to the electrostatic repulsion between closely clustered O6 atoms, the four strands of quadruplex structure, without any coordinated ions, tend to stay apart from each other. Consequently, the central channel as well as whole of the MD average parallel quadruplex structure, obtained from snapshots during 100−600 ps of dynamics (before the counterion intrusion) has a larger volume than the volumes of the average structures obtained during 2.1−3.1 and 5.1−6.1 ns of dynamics, although the latter structures have larger solvent accessible surface area due to the grooves being more well-defined. The quadruplex structure without any coordinated ion (average structure between 100 and 600 ps) has a central cavity volume of 163 $Å^3$, which is considerably larger than the cavity present in the average structure (between 2.1 and 3.1 ns) with two coordinated cations (90 $Å^3$), as well as the average structure (between 5.1 and 6.1 ns) with three coordinated cations (68 $Å^3$). The SAS program of Connolly[57] and atomic solvation parameters (ASP) of Eisenberg and MacLachlan[58] have been used to quantitatively analyze the differences in free energy of hydration (ΔG_H) of guanine nucleotides between these two structures. The free energy for each structure is calculated using relation given by Kagawa et al.[59,60] ($\Delta G_H = \sum ASP_i SAS_i$ for each atom type "i"). This method of calculating solvent free energies has been successfully used in predicting the relative stability of the different forms of duplex DNA structures for various base sequences.[59−61] A free energy difference of 0.5 kcal/mol/base pair in the solvent free energy of A- and B-DNA models was used as the criterion to distinguish between A- and B-DNA favoring sequences.[61] We have calculated the free energy of hydration values for two MD average structures, one with only waters present in the channel and the other with both ions and water molecules occupying the channel (i.e., the MD average quadruplex structures obtained from the structures saved between 100 and 600 ps and from 5.1 to 6.1 ns of dynamics). The ΔG_H values for the quadruplex fragment consisting of the middle five G-tetrads show that the hydration free energy of MD average structure during 5.1−6.1 ns of dynamics (−21.4 kcal/mol per G-tetrad) is more favorable than that of the initial hydrated structure without any coordinated cation (−20.6 kcal/mol per G-tetrad). Interestingly, the hydration free energy of quadruplex structure with 3 coordinated ions (MD average structure during 5.1−6.1 ns) approaches the hydration free energy of MD average quadruplex structure with six coordinated ions (−21.8 kcal/mol per G-tetrad).[34] Thus, the free energy of hydration in a partially ion coordinated parallel quadruplex structure is comparable to a fully coordinated quadruplex structure, while both are more favorable than the structure with only waters in the central channel.

Comparison of MD Results with Previous Studies. The movement of ions through the quadruplex channel has been demonstrated using ammonium ions by NMR study,[30] since the dehydrated Na^+ ions within the channel are not detectable by NMR experiments.[28] Our MD study demonstrates that the Na^+ ions from the solvent can move into the empty quadruplex channel without any strand dissociation and can also travel through the channel, although their movement inside the channel is much slower than that in the solvent. Relocation of Na^+ and K^+ ions through the terminal GGGG and GCGC tetrads have also been observed in the recent MD studies of 4-mer quadruplexes with mixed guanine/cytosine tetrads.[62]

An ab initio study[51] on a Hoogsteen hydrogen bonded G-tetrad, without any cation suggesting that G-tetrads are quite stable, with a stabilization energy of −62 kcal/mol at the HF/6-311G(d,p) level. The optimized structure showed that the G-tetrad is stabilized by bifurcated N1−H1···O6 and N2−H2···O6 hydrogen bonds. In agreement with ab initio study, in the absence of coordinated Na^+ ions at the coordination sites, G-tetrads are stabilized by N1−H1···O6 and N2−H2···O6 hydrogen bonds and the interaction energy within the G-tetrad is calculated to be $\sim -64 \pm 4$ kcal/mol, by the AMBER force field. However, normal Hoogsteen type hydrogen bonds in the G-tetrad are restored in the presence of Na^+ ions at the coordination sites, inside the quadruplex channel, as observed in the Na^+ ion coordinated crystal structure of $d(TG_4T)$. It is interesting to note that during the MD simulation, the quadruplex structure with only partial coordination, has converged toward the crystal structure, with an rms deviation between the four nucleotides of MD average structure (between 5.1 and 6.1 ns) and a crystal structure being 0.8 Å, while the rms deviation is 1.0 Å between the MD average structure and the initial fiber model.

Recent molecular dynamics simulations[33] by Spackova et al. on a parallel 4-mer G-quadruplex without coordinated ions showed that even though the channel was fully hydrated within the first 100 ps, the quadruplex structure is subsequently unstable, with dissociation of G-tetrads and strand slippage occurring at 0.5 ns followed by a further rearrangement taking place at 1.8 ns. We have reconfirmed this destabilization of a 4-mer G-quadruplex structure without any coordinated ion, by carrying out a 1.1 ns simulation (unpublished data). However, in our simulation of a 7-mer quadruplex structure, water molecules first occupy the channel (in <100 ps) followed by entry of two Na^+ ions from the surrounding medium (at ∼0.55 and ∼1.5 ns) and the quadruplex structure does not undergo any major distortion or strand dissociation. During the extended simulation, at elevated temperature, only one more counterion entered the quadruplex channel. Thus, it appears that, while coordinated Na^+ ions are essential for the stability of 4-mer quadruplex structure,[18] the larger number of stacked G-tetrads in the longer fragment prevent dissociation of the 7-mer parallel quadruplex structure during the initial phase of simulation, when there are no coordinated ions. However, the presence of some coordinated ions inside the quadruplex channel increases the

stability of a quadruplex structure. This is confirmed by the fact that even the larger K^+ ions, positioned exactly above an empty channel gate, swiftly move into quadruplex channel (within 50 ps), but then remain almost stationary between the terminal G-tetrads for at least 1 ns (unpublished data).

Conclusions

An extended molecular dynamics simulation of parallel four stranded $d(G)_7$ structure indicates that the guanine quadruplex is stable, even in the absence of coordinated cations. Water molecules can occupy the empty coordination sites in this situation. However, some Na^+ counterions from the surrounding solvent medium enter the quadruplex channel, by replacing the bound water molecules, thus indicating that the monovalent Na^+ ions are the preferred ligands at these coordination sites.

In the absence of any coordinated ion, due to strong O6−O6 repulsion, adjacent guanines in the tetrad undergo rotation, so as to move the O6 atoms further apart and the G-tetrad is stabilized by bifurcated hydrogen bonds similar to that reported in an ab initio study. However, after the entry of Na^+ ions, a strong attractive force between O6 atoms and the ion restores the standard Hoogsteen type hydrogen bonded G-tetrads.

Our MD studies clearly indicate that sodium ions can enter a preformed quadruplex through the ends and travel within the quadruplex channel without significantly distorting the G-tetrad geometry, while water molecules can exit the channel through the ends as well as through the grooves. The grooves in the G-quadruplex are well hydrated during the entire simulation with water molecules being tightly bound, through hydrogen bonds to the exposed 2-amino groups and N3 atoms of guanine bases. A few counterions are found to strongly interact with the oxygen atoms of the phosphate groups. The free energy of hydration for the partially coordinated quadruplex structure is more favorable than that of the structure without any coordinated ion and comparable to that of a fully coordinated quadruplex. Thus, in the presence of a few channel bound cations, the 7-mer quadruplex structure retains its tetrameric form even during MD at 400 K, indicating that all coordination sites need not be occupied for the formation of a stable G-quadruplex structure. The robust nature of the longer G-quadruplex structure and its ability to allow relatively free and rapid movement of cations (on nanosecond time scale) leads one to speculate that such structures could be examined as potential candidates for designing nanostructures with specific ion conduction properties.

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