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A combined QM and MM investigation into guanine quadruplexes

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Abstract

This paper reports on the application of quantum mechanical (QM) energy calculations, QM optimisations and MD simulations to explore the stability of a human telomeric guanine quadruplex, containing potassium and sodium cations. G-quadruplexes are of great biological interest as it has been suggested that they offer a novel path to cancer inhibition. By understanding the stability and geometry of these DNA features gives us the ability to design ligands which can bind and stabilise the G-quadruplex. There are significant structural differences between the potassium containing crystal structure of human telomeric G-quadruplex and the sodium containing NMR structure; in this paper, we investigate the energetics and dynamics of the potassium derived crystal structure and a model for the sodium containing structure. QM investigations upon the 12 G-quadruplex core, extracted from the human potassium quadruplex crystal structure, indicate that replacement of the potassium cations with sodium yields an energetically more favourable structure. However, attempts to geometry optimise both structures at the QM level proved unsuccessful, the structure of the partially optimised potassium containing G-quadruplex retains significant structural integrity with respect to the original crystal structure, whilst the sodium containing G-quadruplex shows significant structural distortion. QM investigation of the 12 G-quadruplex core containing no cations unsurprisingly yields a highly unfavourable energetic structure. MD simulations on the complete quadruplex structure, containing potassium cations, yields a remarkably stable structure after 4 ns of simulation, the most significant deviation from the original crystal structure being the loss of the capping potassium cation from the structure. MD simulation of the sodium containing quadruplex for 4 ns show significant structural reorganisation compared with the original potassium containing crystal structure.

Keywords: G-quadruplex

1. Introduction

The DNA contained in the chromosome of eukaryotes is terminated at the 3′-end with a telomere, a guanine rich sequence of non-coding bases. Telomeres are involved in the protection of the genetic code as well as the regulation of normal cells life span [1,2]. A link has demonstrated between immortality and telomere maintenance in stem and germ line cells [3–5]. Cancerous cells are also immortal, if this attribute could be selectively turned off, the cancerous cells would show signs of aging and eventually die. In 85–90% of proliferating cancerous cells, high levels of telomerase expression and activity have been reported, this is a reverse transcriptase which maintains the length of telomeres [6,7].

Stopping telomerase mediated telomere maintenance has the potential to be an effective treatment for the vast majority of proliferating cancer types whilst sparing normal tissue as well as stem and germ line cells due to their different properties [8]. Telomerase could be either directly inhibited or targeted through its mode of action. The majority of the telomere is double stranded, however, the 3′-end is a single strand of over 200 bases. Telomerase latches onto the end of the single stranded section and aligns the end into a catalytic pocket which allows nucleotide bases to be added on. It continues to add on to the telomere until it is no longer a suitable substrate, which involves the telomere undergoing a structural change the exact form of which is not fully understood.

To stop telomerase extending the single strand, the telomere would have to be occupied in someway to stop the recognition and alignment process. Two ways this could be achieved is to use an external agent to either associate with the telomere making it an unsuitable substrate or by using one to cause and stabilise the formation of a higher order structure in the telomere.

The guanine rich nature of single strand telomeric DNA allows the formation of higher order structures, such as T-loops, which serve to protect the strand end through out most of the cell cycle [9,10]. Guanine residues can efficiently form

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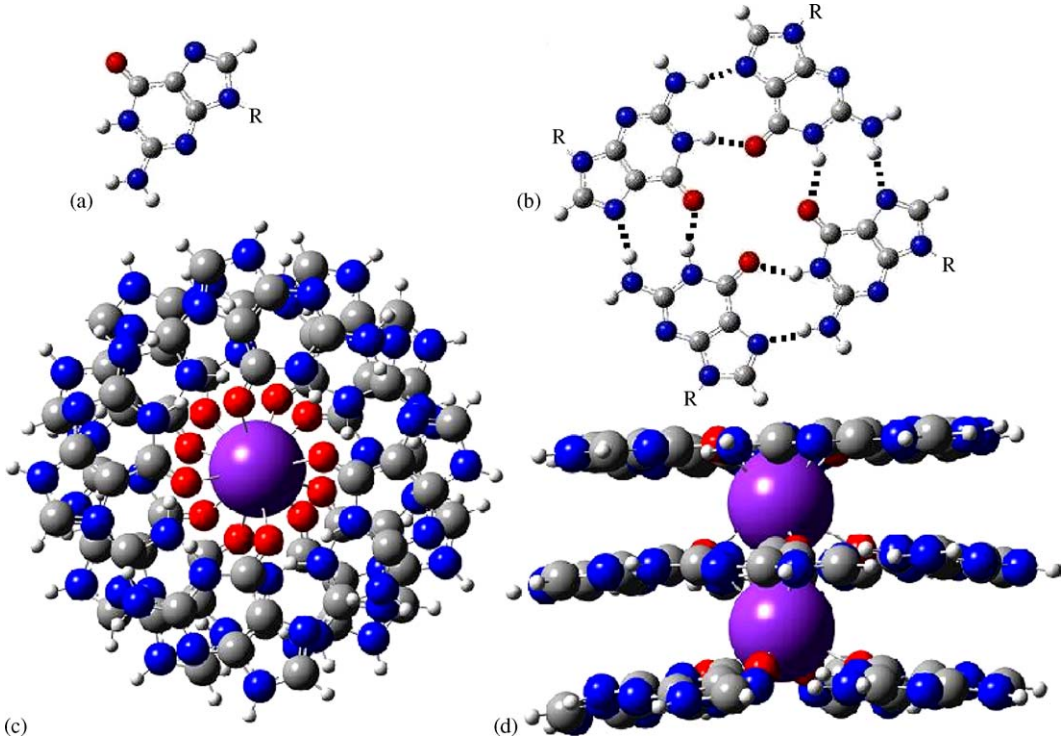

Fig. 1. (a) Guanine, (b) G-quartet, (c) and (d) the stacked G-quartets with potassium ions. Oxygens are shown in red, carbons in grey, nitrogen in blue, hydrogen in white, potassium in purple.

self complimentary hydrogen bonds. The most stable form of these is seen in G-quartets (Fig. 1) [11–13]. Guanine can also interact through pi stacking, the combination of these properties results in stacked quartets giving an electronegative channel down the centre of the structure (Fig. 1) [14]. Small cations such as Na^+ and K^+ can reside in the central channel to stabilise the structure and water molecules can also increase stability through the creation of hydrogen bond bridges [15]. When DNA associates to give stacked G-quartets, the resulting structure is referred to as a G-quadruplex. In the late 1980s, it was proposed that G-quadruplexes are important secondary structural features which regulate biochemical processes in the telomeric region, and since then a large amount of interest and work has been carried out into the fields of telomeres and their formation of G-quadruplexes [14,16].

The introduction of an external agent to encourage the formation of higher order structures can take advantage of the naturally occurring phenomenon of guanine self association. The exact structure of telomeric G-quadruplexes depends on four main factors:

(1) The number of constituent DNA strands, in vitro structures from 4, 2 and 1 strands have been observed [14,17–19].
(2) The strand orientation, parallel or anti-parallel.
(3) The exact base sequence of the strands involved.
(4) The metal ions associated with the structure (Fig. 2).

This work set out to probe the stability of the G-quadruplex formed by human DNA with a view to investigate the binding of small molecules to stabilise the structure. The human potassium containing telomeric structure is used in this work.

2. Methodology

The initial structure for all of the work carried out was taken from a 2.1 Å resolution X-ray crystal structure gained of a human telomeric sequence crystallised with potassium ions, protein data bank 1KF1 [20]. The structure was taken either as a whole or considered as the guanine core (Fig. 3).

2.1. Single point QM

For the single point QM calculations, the DNA structure was stripped down to the central 12 base guanine core containing two metal ions, dangling bonds were terminated with hydrogen atoms leaving a total of 194 atoms. The calculations were not only carried out for the core containing potassium ions but also where the geometry was maintained but the ions replaced with sodium ions or simply deleted.

The QM calculations were carried out using Gaussian 98 and Gaussian 03 [21,22] on a P4 2.8 GHz processor, a 16 node 1.4 GHz processor opteron cluster and a four node 1.3 GHz processor Itanium server. GaussView was used to prepare simple input files and visualise the structures. The calculations were carried out at HF and DFT B3LYP levels of theory using the 3–21G* and 6–31G** basis sets on the P4. The system of 194 atoms at the 6–31G** level consisted of 1354 basis functions. Even without calculating the counterpoise correction for these systems an energy calculation for 194 atoms with DFT

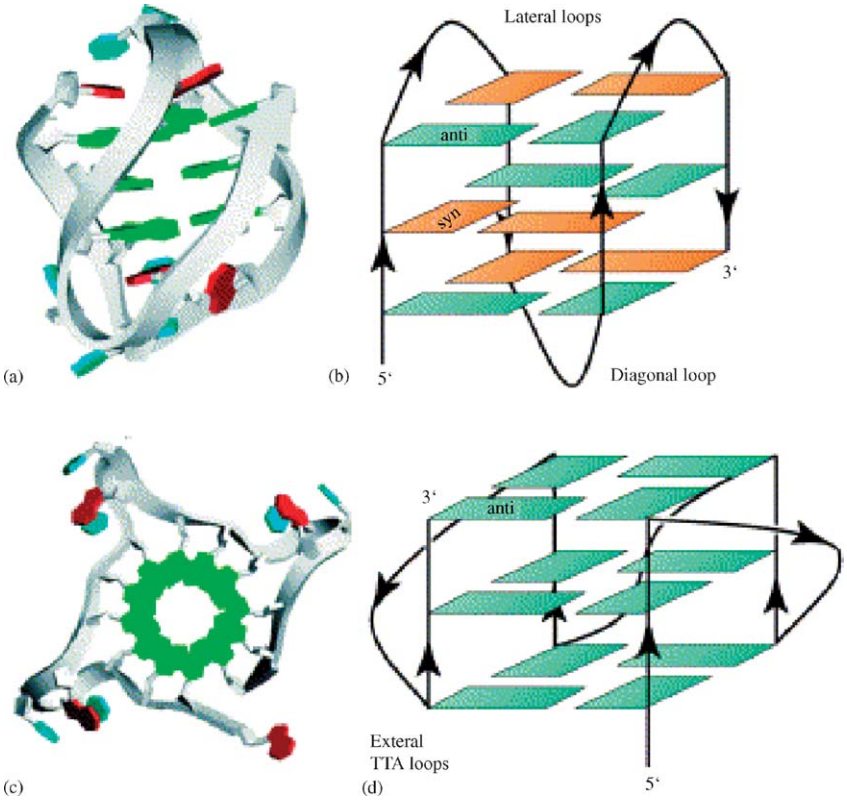

Fig. 2. The binding of different metal ions results in the formation of different loop structures. (a) NMR structure of human telomeric sequence bound with sodium, (b) its loop structure, (c) crystal structure of human telomeric sequence bound with potassium, (d) its loop structure [1].

B3LYP 3–21G* took ca. 18 h on the P4 processor. The counterpoise corrected calculations were also carried out, each of the quartets and metal ions were treated as individual fragments to calculate the counterpoise correction. The files were generated from GaussView fragment files and the files were changed to the correct format for counterpoise correction calculations.

2.2. QM optimisations

The quantum mechanical optimisations were started as the next step in this work with the intention of locating the geometry of minimum energy for the central guanine core. The initial structures used were based on the central guanine core from the X-ray crystal structure. The ions of the structure again were potassium, sodium or deleted depending on the specific calculation.

The QM optimisations were carried out using Gaussian03 [22] on a 16 processor opteron cluster (1.4 GHz) and on a 4 processor Itanium system. GaussView was used to prepare the input files and visualise the structures on a P4 2.8 GHz processor machine. The calculations were carried out at the DFT B3LYP and HF levels of theory using the 3–21G* and 6–31G** basis sets.

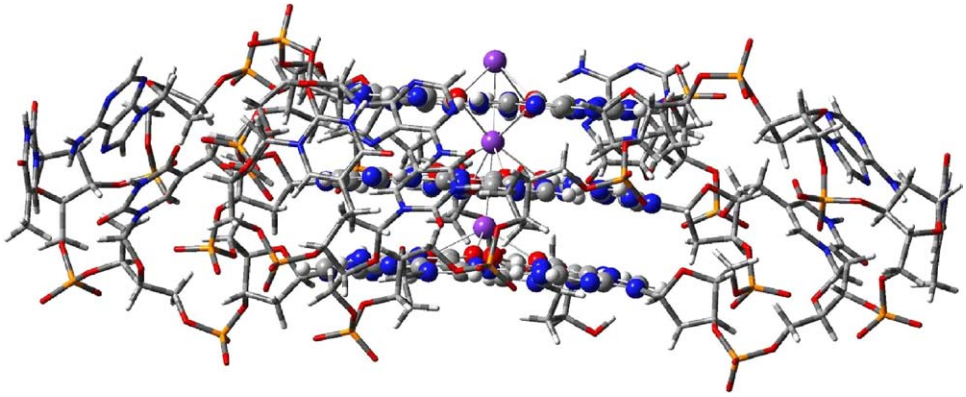

Fig. 3. The structure gained from X-ray crystallization of the human telomeric sequence with potassium ions. The guanine core is high lighted as ball and stick and the rest as tubes, phosphorous atoms are shown in orange.

2.3. MD simulations

The molecular dynamics simulations were carried out using the AMBER8 suite of programs with the Cornell force field [23,24]. The MD simulations used the whole potassium structure gained from the X-ray crystallography and were carried out on the potassium structure and where sodium was substituted [20]. Xleap was used to import the pdb and add 18 sodium atoms to charge neutralise the structure. An 8 Å truncated octahedron of TIP3P water was added to solvate the structure giving a total system of 10918 atoms. The atoms in the simulation were given a 10 Å cut-off and the particle mesh Ewald method was implemented to treat the long range electrostatics and to reduce the negative effects of the introduction of a cut-off. SANDER was used to carry out the minimisations and the molecular dynamics runs. VMD was used to view graphical representations of the optimisation geometries [25]. These calculations were run on a 4 processor Itanium system and the visualizations carried out on a 2.8 GHz P4 processor machine. The water was minimised while the DNA was restrained for 4000 steps, then the free system was minimised for a further 5000 steps both times using a combination of steepest descent and conjugate gradient methods. The structure was then heated from 0 to 300 K using a Langevin temperature equilibration scheme and a constant volume regime for 20ps with 2fs steps as SHAKE was used to restrain hydrogen bonds.

The structures were checked after the equilibration stage and both the potassium and sodium G-quadruplexes were intact. The plots of parameters such as density, potential energy and bond angle all showed a variance about a mean value so the structure was taken as an equilibrated system onto a production run. The conditions from the final equilibrium stage were used but in a constant temperature and pressure regime with 2,000,000 steps being taken to a give 4 ns production run.

3. Results

3.1. Single point QM

3.1.1. Potassium filled G-quartet stacks

The potassium filled structure was considered in three ways: (1) a three tier stack with 12 guanines and two potassium ions; (2) the top pair of quartets with eight guanines and a potassium ion; (3) the bottom pair of quartets with eight guanines and one potassium ion. The interaction energies of the three structures were calculated (Table 1) and their stability was considered with respect to individual optimised guanines (Table 2) and with respect to the individual quartets (Table 3). All the stacks were found to be more energetically stable than the individual molecules and the separate quartets at both levels of theory and basis set used. It was found that the top and bottom pairs are energetically non-equivalent, which is expected as the individual quartets have different geometries. Using HF, the bottom pair was seen to be more stable than the top pair, but with DFT, the top pair was found to be more energetically stable than the bottom pair.

Table 2
Comparison of the energies of the potassium guanine structures to individual guanine residues and ions at varying levels of theory kcal/mol

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	−532.54	−292.63	−573.51	−402.15
Top pair	−230.86	−90.97	−404.41	−257.58
Bottom pair	−270.84	−131.72	−353.67	−207.01

When the counterpoise correction for the individual layers is considered, the triple stack and bottom pair were found to be stable with respect to the separate quartets and potassium ions at all levels (Table 4). The top pair of quartets when considered at HF 6–31G** appeared to be an unfavourable stacking; however, with the other basis sets and levels of theory, the stacking of the top pair was found to be energetically favourable though to a lesser extent than the bottom pair. The inclusion of the counterpoise correction has caused a change in behavior as now both HF and DFT predict that the bottom pair is more stable.

3.1.2. Sodium filled G-quartet stacks

The stacked structures containing sodium ions were investigated with DFT B3LYP 6–31G** and compared to their constituent quartets and ions showing that the stacked structures are more energetically stable than the individuals parts (Table 5). The pairs were found to be about 20 kcal/mol more stable than for the potassium form and the quadruplex was about 40 kcal/mol more stable, even though the structure was based on the geometry from the potassium crystallised form of the quadruplex.

The counterpoise correction for the layers of the sodium structure were calculated and compared with the potassium case at B3LYP 6–31G**. The sodium energies for the pairs are still about 20 kcal/mol more stable than the potassium structures and the order of stability for the pairs switches for both ions with the inclusion of the counterpoise correction (Table 6).

Table 1
Energies of potassium stacked guanine structures Hartrees

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	−7629.245262	−7671.325741	−7668.836801	−7710.821095
Top pair	−4887.296686	−4914.409838	−4913.678932	−4940.622301
Bottom pair	−4887.360414	−4914.458857	−4993.598073	−4940.541713

Table 3
Comparison of the energies of the potassium stacked structures with the constituent quartets kcal/mol

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	−305.88	−175.81	−248.42	−201.45
Top pair	−80.61	−11.1	−187.53	−124.02
Bottom pair	−119.47	−56.14	−137.01	−73.52

Table 4
Comparison of potasium filled structures with the five constituent layers including the counterpoise correction to the layers kcal/mol

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	−166.83	−146.26	−164.21	−147.2
Top pair	−10.59	7.25	−17.38	−13.36
Bottom pair	−51.3	−38.23	−54.64	−46.39

3.1.3. Unfilled G-quartet stacks

It was found that all the unfilled structures studied were more energetically stable than individually separated guanine molecules, at all levels of theory and basis set used (Table 7). For the comparison of the triple stacked structure to its constituent quartets, the energies were also calculated at the MP2 level with a 3–21G basis set. The unfilled stacked structures were found to be energetically unfavourable compared to their individual quartets (Table 8). The instability of the stacked structures is related to the removal of the cation from the structures under consideration. This result agrees with past QM work on G-quartet tubes that were assembled from individually optimised guanine residues and showed unfavourable stacking energies when considered without potassium ions [26].

When the counterpoise correction for the individual layer is considered, the stacked structures were found to still be unstable with respect to the separate quartets, including at MP2 3–21G* (Table 9).

The instability of the triple stacked structure here was found to be equivalent to 0.79 eV per quartet at the DFT 6–31G** level compared with 1.4 eV per quartet which Calzolari et al. found using plane wave psuedopotential DFT [26]. The stability gain for the inclusion of potassium ions compared with the empty structure in this work was found to be 2.3 eV per G-quartet at the DFT 6–31G** level where as Calzolari et al. reported a gain of only 1.8 eV per G-quartet when plane wave psuedopotential DFT was used [26].

Table 5
The relative energy of the sodium and potassium stacked structures compared with the constituent quartets and metal ions kcal/mol

Structure	Sodium ion	Potassium ion
Triple stack	−243.22	−201.45
Top pair	−143.02	−124.02
Bottom pair	−95.8	−73.52

Table 6
The relative energy of the counterpoise corrected structure compared to the constituent quartets and ions for both the sodium and potassium filled structures kcal/mol

Structure	Sodium ion	Potassium ion
Triple stack	−191.12	−147.20
Top pair	−32.02	−13.36
Bottom pair	−69.56	−46.39

3.2. QM energy optimisations

3.2.1. Potassium filled guanine core

The optimisation was initially considered at the DFT B3LYP 6–31G** level, but due to the computational demand, it was determined that the optimizations should proceed using the 3–21G* basis set. After a significant number of optimization cycles, it was noted that the convergence parameters had begun to oscillate with respect to the RMS displacement of the atoms. The sequence of structures generated during the optimisation showed that the stacked structure was still present so the calculation was stopped and restarted with a new initial Hessian in an attempt to overcome this issue. Unfortunately, this did not solve the problem and within a few hundred optimization cycles, the oscillating convergence problem reoccurred.

The full optimisation of the potassium filled structure was not successful. The geometry at the termination of the run is shown in Fig. 4; it can be seen from this that the essential structure of the G-triple stack retains the planarity of the original crystal structure (Fig. 3).

3.2.2. Sodium filled guanine core

Optimisation of the sodium filled G-quadruplex was attempted at the B3LYP level of theory using the 3–21G* basis set. During the course of the optimisation, the geometry files were investigated and it was noticed that the integrity of the stacked structure were becoming compromised. The top and bottom quartets were acting as pairs of dimers that were tipping at an angle to each other, much like the base twisting observed in G-quartets by Meyer and Sühnel when they studied different metal ions with isolated G-quartets (Fig. 5a and b) [27]. The bottom quartet has bent along the *x*-axis pointing the carbonyl oxygens towards the sodium whilst tipping the rest of the guanine downward. The top quartet has bent along the *y*-axis pointing the carbonyl oxygens towards the sodium ion and tipping the rest of the guanine up. The middle quartet appears to be acting as four separate guanines, bending in both the *x*- and the *y*-plane to keep the most stacking with the outer two

Table 7
Comparison of unfilled structures to individual guanines kcal/mol

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	−211.04	−73.69	−322.29	−176.66
Top pair	−142.10	−55.59	−216.06	−121.72
Bottom pair	−143.32	−52.31	−160.06	−70.17

Table 8
Comparison of unfilled structures to the individual quartets kcal/mol

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	15.62	43.13	2.8	24.04
Top pair	8.15	24.28	0.82	11.84
Bottom pair	8.05	23.27	56.6	63.31

quartets, appearing to bend in the same direction as the bottom quartet in (a) and the top quartet in (b). The top and bottom quartets have rotated to the point where the guanines are aligned, so only eight carbonyl oxygens are visible from above (Fig. 5c).

Unfortunately, the optimisation did not produce a stable G-quadruplex structure, so the calculation was stopped. The instability of the sodium filled quadruplex could be due to the fact that the geometry was based on the X-ray structure of the potassium G-quadruplex and the possibility exists that there is a significant difference between structures containing sodium as opposed to potasssium. It has been suggested that the sodium form of a G-quadruplex appears different to the potassium form as the smaller ions can lie within the plane of the quartet as well as between them [27]. The observed patterns in X-ray crystal structures including sodium ions has shown the ions to lie alternately in the quartet plane and then between them meaning the separation between ions is one and half times larger than used here [28].

3.2.3. Non-cation filled guanine core

To investigate the need for a cation to hold the structure together, three systems were investigated: (1) one of the potassium ions was replaced by a water molecule while the other was retained; (2) both potassium ions where replaced with water molecules; (3) both the potassium ions were deleted. The calculations for the water structures were carried out using HF with a 3–21G* basis set and the guanine core was initially frozen and a short optimisation of only the water molecules was carried out before the main optimisation of the whole structure.

The first case where the bottom ion was replaced with a water molecule showed after only 38 steps the bottom quartet breaking up, with one of the bases tilting the carbonyl oxygen upwards (Fig. 6a). The top pair that surrounded the potassium ion remained stable. Where both the ions were replaced by water molecules, the quartets have began to slide apart and the individual quartets appeared to twist with the most buckling occurring in the bottom quartet (Fig. 6b and c). In Fig. 6c, the drifting of the quartet layers can be seen as the previously neat central carbonyl channel has been lost.

Table 9
Comparison of the unfilled structures with the individual quartets including the counterpoise correction to the layers kcal/mol

Structure	HF 3–21G*	HF 6–31G**	B3LYP 3–21G*	B3LYP 6–31G**
Triple stack	64.58	71.39	55.62	53.89
Top pair	32.9	43.94	27.17	28.91
Bottom pair	31.29	37.24	24.43	27.53

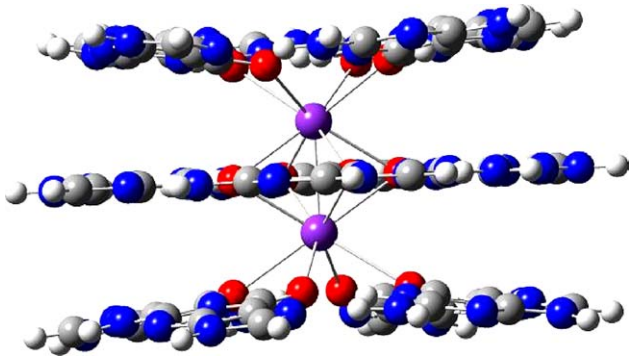

Fig. 4. Structure of partially optimised potassium filled G-triple stack used in the frequency calculation.

The optimisation of the empty G-core structure was run using DFT B3LYP 6–31G**. The optimisation was stopped when the sequence of geometries showed that the trend was towards the structure drifting apart and not holding together. By 111 steps of optimisation, the outer two quartets had started to buckle, moving the electronegative carbonyl oxygens away from each other. The quartets did, however, remain vertically aligned even though the actual quartets were buckling, the resulting structure looked ball like (Fig. 7).

These results support the QM single point calculations that without the cation present the structure is not stable due to the repulsion of the electronegative oxygen channel in the centre of the G-core. These QM optimizations indicate that the non-potassium G-core structures are not energetically favourable when considered without the rest of the DNA strand. Past MD work indicates that G-quadruplex structures that do not have cations present are not stable, agreeing with this QM optimisation of the empty stacked guanine quartets [29]. However, it has been shown that when non-classical hydrophobic effects are included in the aromatic stacking calculations empty G-quartets have favourable stacking energies, so the instability observed could be due to the absence of consideration for the non-classical hydrophobic effect not the absence of a cation [14,30].

3.3. MD simulations of the DNA quadruplex structure

3.3.1. Potassium filled G-quadruplex

Analysis of the backbone carbon, oxygen and phosphorous atoms of the 25 residues that make up the DNA strand from the 4 ns trajectory were compared with that of the original X-ray crystal structure and yielded an RMSd of only 2.8 Å. Inspection of the RMSd on a per residue basis indicated that substantial displacements occurred at the end residues which do not actually form the G-quadruplex and so are free to move about. Excluding the end two residues from the fit resulted in an RMSd compared to the original X-ray structure of only 2.2 Å. The G-quadruplex in the centre still appears intact although the quartets were slightly distorted compared with the original X-ray crystal structure.

The potassium ion that was originally above the stack moved away early in the equilibration stage and was not replaced by

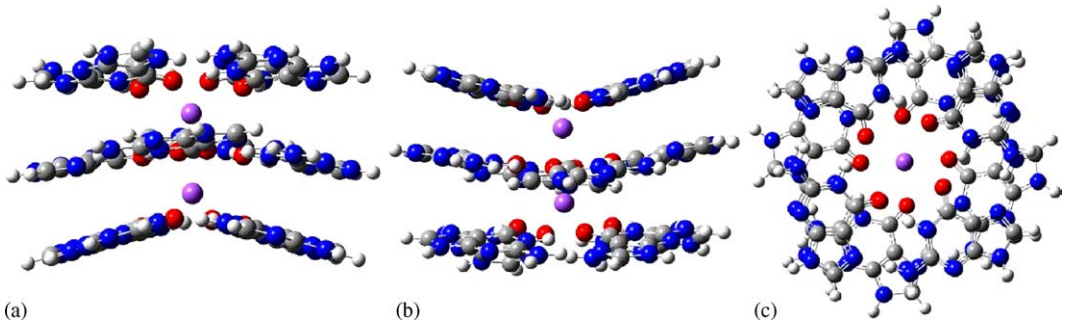

Fig. 5. Structures resulting from the partial optimisation of the sodium filled quadruplex (a) side view showing the bottom quartet bending, (b) side view rotated 90° to (a) showing the bending of the top quartet, (c) top view showing that the top and bottom quartet have rotated and aligned directly above each other.

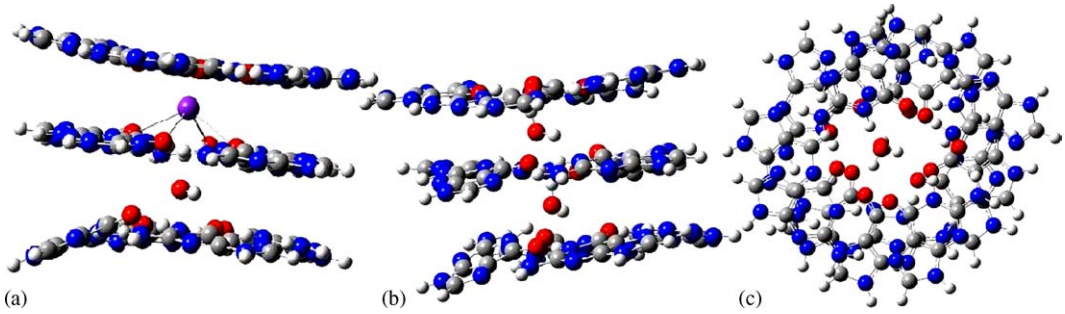

Fig. 6. Resulting geometries from two of the attempted optimisations of non-cation only G-core structures. (a) Side view for the bottom potassium ion replaced with a water molecule, (b) side view of the replacement of both ions with water molecules, (c) top view of the replacement of both ions with water molecules.

another cation from the system, the third potassium ion can be seen off to the top left of Fig. 8. The tail of the DNA strand moved round during the production run to allow the stacking of the tail adenine above the G-quadruplex where the potassium had originally resided. This would imply that the decision not to include the third potassium ion in QM calculations was the correct one as it does not appear to be an essential part of the quadruplex.

The output from the simulation was analysed to create visual representations of the properties throughout the run. The energy of the system and the temperature varied less than 1% throughout the simulation. The energy associated with the angle and bond terms varied to a greater degree.

3.4. Sodium filled G-quadruplex

The last geometry from the 2 ns equilibration run was investigated and the RMSd of the back bone compared with that of the original X-ray structure was found to be 2.4 Å. Repeating the analysis excluding the outer two residues, as for the potassium containing structure, the RMSd was found to be 2.0 Å. As in the potassium case, the third cation, which had been located directly above the quadruplex core, moved away even before the end of the equilibration stage (Fig. 9a). The backbone carbon, oxygen and phosphorous atoms of the 25 residues in the final geometry from the 4 ns production run was compared with that of the original X-ray crystal structure

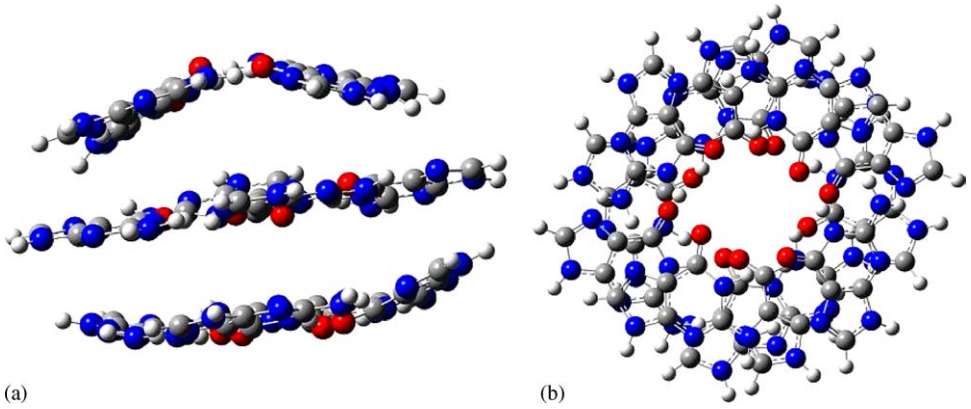

Fig. 7. Structure gained after 111 steps of optimisation for the unfilled guanine core (a) side view, (b) top view.

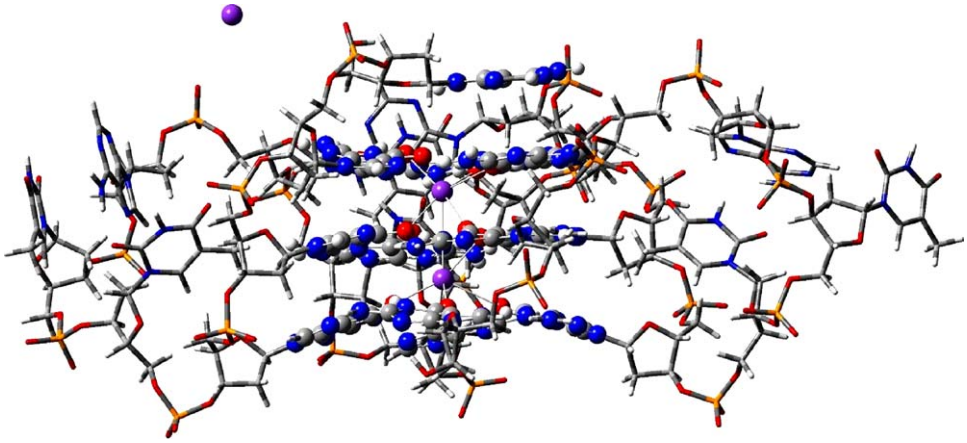

Fig. 8. Final structure from the MD simulation of the potassium filled G-quadruplex.

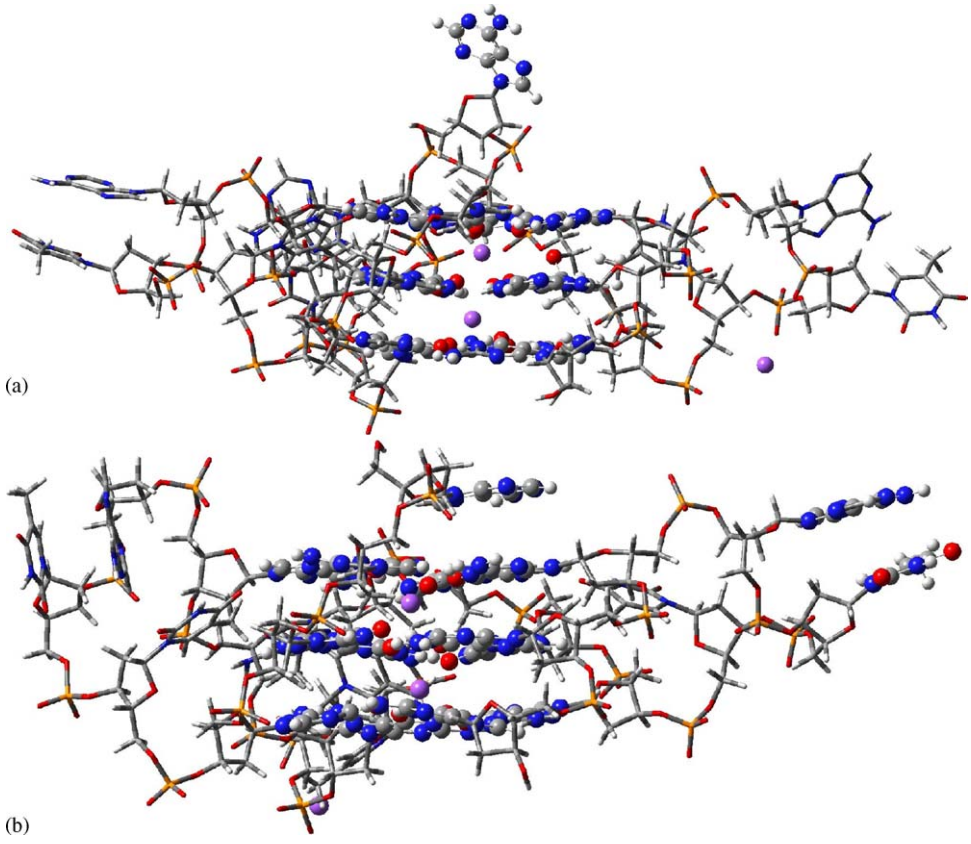

Fig. 9. Final structures from the MD simulations of the sodium filled G-quadruplex: (a) after the 2ns equilibration, (b) after the 4 ns production run.

showed an RMSd of only 2.7 Å. As for the potassium case, substantial movement was observed from the end residues not involved with G-quadruplex, excluding these two end residues resulted in an RMSd of 2.3 Å. The RMSd of all the atoms, not including water and added cations, in the sodium filled G-quadruplex productions run was found to be 4.0 Å which is about 30% greater than for the potassium filled G-quadruplex (3.3 Å); this is unsurprising when the integrity of the G-quartets is compared.

Several changes can be seen between the 2 ns equilibration and the structure after a further 4 ns of simulation (Fig. 9a and b). During the 4 ns production run, the tail of the DNA strand had rotated round to allow the stacking of the adenine above the G-quadruplex. The bases on the two external loops have rotated round to base stack with each other, the loop on the left has bases running almost vertically and the one on the right with the bases running horizontally (Fig. 9b). A similar rotation and alignment of the bases on the left external loop can also be seen in the potassium filled G-quadruplex case after 4 ns of production run (Fig. 8). The G-quartets after 2ns of equilibration still look like the quartets starting structure; however, after the 4 ns production run, the guanine bases in the

quartets have tilted in a very similar way to that seen in the partial QM optimisation of the G-quadruplex core (Fig. 5).

4. Conclusions

The sodium filled guanine core may appear to be energetically more stable than for the potassium case from the QM calculations, but the partial QM optimisation indicates that the guanine core is not stable and the MD simulations show that even with the DNA structure present, the core does not remain stable. This could all be due to the fact that the structure was based on the potassium form and not the sodium form which has a different strand pattern and the bases are a mixture of syn and anti rather than just anti. Further QM/MM optimisations of the potassium filled G-quadruplex would ideally be carried out.

Even the QM calculations on the unfilled structure revealed some interesting questions which are being investigated: the change in stability behaviour between the basis sets indicates that the energy of the quartets and the stacks may be very close. Ideally, energy calculations on the central guanine core would be carried out at the MP2 6–31G** level with counterpoise corrections; as the literature indicates that this level of theory is sufficient to provide accurate energies for base stacking interactions. The fact that even the unfilled structure is close to being stable means the introduction of a stabilising ligand may well tip the balance in favour of quadruplex formation especially if physiological concentrations of ions were present.

Small molecules designed to stabilize G-quadruplexes have already shown efficacy against human breast cancer cells [31]. Further work into how ligands bind to the quadruplex and the degree of stabilisation imparted by them is on going with an aim to improve selectivity for cancer treatment.

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