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Interaction of a G-DNA quadruplex with mono- and divalent cations
A force field calculation

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Abstract

The formation and stability of four-stranded DNA in solution is specifically dependent on the type of cations present. The interaction potential of a model quadruplex structure with different mono- and divalent ions was determined by force field calculations. Though the electrostatic contribution to the total energy is mainly responsible for the stabilisation of the cations within the quadruplex channel, it is the van der Waals interaction at short distances that determines the specific characteristics of the different cations. An explicit consideration of the solvent indicates that the position of water molecules in close proximity to the DNA channel have a strong influence on the form of the potential, and hence on the capability of the cations for leaving and re-entering the cavity. The effect of cation size, as expressed through their Lennard–Jones parameters, is discussed. © 1997 Elsevier Science B.V.

Keywords: G-DNA; Quadruplex structure; Guanine quartet; Cation interaction; Force field calculations

1. Introduction

Oligonucleotides that contain single or multiple guanine rich segments are known to form specific four-stranded helices in solution [1,2]. This novel structural motif was first related to the highly repetitive ends of chromosomes, known as telomeric DNA [3], but was found later in other naturally occurring stretches of DNA and RNA [4]. The formation and specific conformational characteristics of G-DNA have been investigated by X-ray crystallography [5,6], NMR-spectroscopy [7,8] as well as by chemical probing [3,9] and other spectroscopic techniques [10]. The proposition of such a highly symmetric structure was probably inspired by earlier fiber diffraction analysis of poly(G) that provided strong evidence for a parallel four-stranded orientation of the macromolecule [11]. Solution studies on the self-association of guanosine monophosphate have revealed that there is no polymer backbone necessary to induce self-organisation [12–14].

The central building block of quadruplex structures, the so-called G-quartet, is formed by four guanine bases that arrange in a planar array as shown in Fig. 1a. Each guanine is both a donor and acceptor of two hydrogen bonds. A specific structural motif is the pocket in the centre of the G-quartet, formed by four electronegative carbonyl oxygens. This pocket

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is large enough to act as a coordination site for cations. The specific cation interaction together with the strong hydrogen bonding between the guanine bases explains the stability of the tetrameric arrangement. In G-DNA, mostly four of these guanine quartets are stacked to form helical quadruplex structures as shown in Fig. 1c. The stacking of individual G-quartets results in the formation of a channel within the helical structure that creates another specific interaction site for cations. In addition to the pocket, a cation can occupy the position between two G-quartets. The eight carbonyl O6-oxygen atoms set up a twisted cage due to the helical arrangement of the guanine bases (see Fig. 1b).

A number of structural and thermodynamic studies on different G-DNA molecules have revealed that cations induce and stabilise quadruplex formation in the order $Cs^+ < Rb^+ < K^+ > Na^+ > Li^+$ for monova-

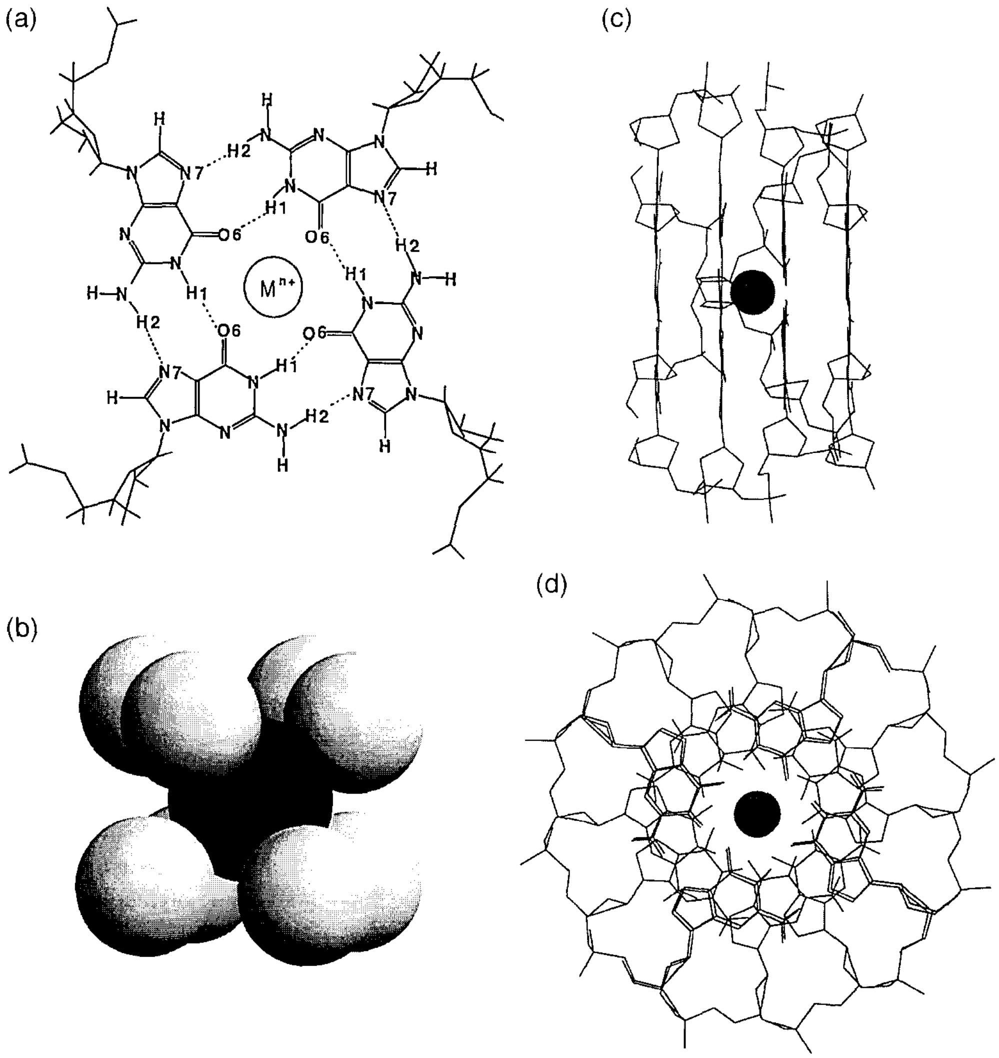

Fig. 1. (a) G-quartet with a metal ion in the pocket, which is formed by four oxygen O6-atoms. (b) Potassium ion in the twisted cage, which is formed by eight O6-atoms. For the oxygen O6-atoms a van der Waals radius of 1.5Å, and for K^+ an ionic radius of 1.65 Å was assumed. Wireframe presentations of the $d[GGGG]_4$ quadruplex structure. (c) view perpendicular to the helical axis; (d) axial view. One ion is shown which is trapped in the central cage.

lent ions and $(K^+) > Ca^{2+} > Mg^{2+}$ for divalent cations [15,16]. Some exceptions from this general sequence have been found. With increasing number of G-segments different polymorphic arrangements from intra- or intermolecular association are possible that can differ in strand stoichiometry, G-quartet and loop geometry or in the local conformation of the sugar residues [2]. Although, no biological role for G-DNA has yet been unequivocally confirmed, it is well conceivable that the cation specificity might be an important regularisation mechanism to switch between these different structures in biological processes.

To understand the extraordinary selectivity of potassium ions for quadruplex formation, Ross and Hardin [17] have performed free energy perturbation calculations to determine the host-guest complex stability as a function of cation size. They found that steric arguments alone cannot fully explain the high selectivity of potassium over sodium ions, and they propose that probably polarisation effects have to be considered to understand the cation specificity. Otherwise, the order of preferential stabilisation of the quadruplex structure, as observed experimentally, is mostly explained by simple geometric arguments. It is assumed that a certain ion with an optimal size with respect to the dimensions of the cage will bind most tightly to the cavity. The size of the cage, as determined by the van der Waals radii of the carbonyl oxygen atoms, is correlated with the ionic radius of the different types of cations [18]. However, the values for the radius of a single cation differs significantly, depending how they were determined [19]. But what is more important, it is expected that the size of the quadruplex cavity should depend on the nature of the trapped particle, and specifically on the coordination number. Hence, it is necessary to calculate the electrostatic as well as the van der Waals contribution to the total interaction energy.

In this study, we have investigated the interaction of different mono- and divalent cations with a model quadruplex structure by force field calculations. We have obtained a static picture about the energetic situation of the cations at different positions within the quadruplex cavity. Furthermore, we put special emphasise on the exchange of cations between the cavity and the bulk solvent surrounding. Our calculations disclose the importance of solvent molecules that interact with the cations at the end of the channel for this process.

2. Experimental

We have developed a computer program to generate any four-stranded quadruplex structure, consisting of G and T quartets, with variable helical repeat and distance of base planes. The initial model G-DNA structure was build from a single tetramer unit by translation of 3.4 Å along and rotation of 30° about the helical axis. The orientation of the strands was parallel and the glycosidic conformations were all anti. The initial building blocks in our program are G- and T-quartets taken from a refined solution NMR structure published in the Protein Data Bank, PDB (139d) [20].

For our molecular mechanics calculations, we have considered a model quadruplex structure that consists of four guanine quartets ($d[GGGG]_4$) with a total of 396 atoms. Nonpolar hydrogen atoms were treated with carbon as united atoms and no phosphate groups are placed at the ends of the strands. The force field parameters describing the interaction of the DNA atoms were taken from the GROMOS force field [21,22]. As solvent we choose the extended simple point charge model (SPC/E) [23], which reproduces radial distribution functions and dynamic properties of water very well. Lennard–Jones parameters for the cations were taken from Åquist [24], because they are developed with the SPC-model and are available for almost all alkali and alkaline-earth cations. The nonbonded pair interaction energy was calculated according to

$$V(r_c) = \sum_{\text{nonbonded pairs}\,i,c} \left[\frac{C_{12}(i,c)}{r_{ic}^{12}} - \frac{C_6(i,c)}{r_{ic}^{6}} + \frac{q_i q_c}{4\pi\varepsilon_r\varepsilon_0 r_{ic}} \right]$$

where the subscript c denotes the considered cation and i all other atoms (DNA, water-atoms and other cations).

The Lennard–Jones parameters $C_6(i,c)$ and $C_{12}(i,c)$ of atom pairs were obtained from single

Table 1
Force field parameters for metal ions; q in e, $C_6^{1/2}$ in (kcal · mol^{-1} · Å^6)$^{1/2}$, $C_{12}^{1/2}$ in (kcal · mol^{-1} · Å^{12})$^{1/2}$

atom	q_i	$C_6^{1/2}(i,i)$	$C_{12}^{1/2}(i,i)$
O	−0.8476	25.01	421.0 (793.3)
H	0.4238	0	0
ion	q_c	$C_6^{1/2}(c,c)$	$C_{12}^{1/2}(c,c)$
Li^+	+1	2.6	25
Na^+	+1	3.89	143.7
K^+	+1	4.35	522.7
Rb^+	+1	4.64	824.4
Cs^+	+1	5.44	1647.9
Mg^{2+}	+1	8.32	37.0
Ca^{2+}	+1	18.82	264.1
Sr^{2+}	+1	20.54	613.5
Ba^{2+}	+1	24.13	1341.5

atom Lennard–Jones parameters using combination rules

$$C(i,c) = \sqrt{C(i,i)C(c,c)}$$

The force field parameters for water and cations used in the present study are listed in Table 1.

2.1. System A

The initial structure was first energy minimised by the conjugate gradient method, holding the guanine-atoms and the cations rigid and leaving the backbone flexible. The calculations of interaction energies in vacuum were carried out with the obtained structure.

2.2. System B

For the calculations in solution, the solute was placed in a cubic box of 1253 water molecules. The cutoff radius for nonbonded interaction in the MD-Simulation is 12 Å/16 Å, (twin range method), while in the pair interaction calculations no cutoff was applied. The bond length were constrained during the simulation, using the SHAKE procedure [25]. The initial energy minimisation (steepest descent) includes only the positions of the water molecules, while the DNA atom positions were held fixed by adding a harmonic potential (force constant = 5000 kJ mol^{-1} nm^{-2}). In order to obtain a neutral system, 12 water molecules were replaced by potassium ions, and a second energy minimisation was performed. This minimised structure provides the initial geometry for the MD simulation in the NPT ensemble. Temperature (300 K) and pressure (1 atm) were held constant by weak coupling to an external bath [26]. After short equilibration of the system (1.9 ps) with increasing time steps (from 0.8 fs to 1.5 fs), the production phase of MD simulations starts with all atoms flexible. Snapshots were taken at the beginning of the production period, before the system had fully reached equilibration.

3. Results

The influence of various alkali and alkaline-earth cations on the formation and stabilisation of four-stranded DNA was investigated. First we calculated the potential energy of the single cation in a model quadruplex structure d[G_4]$_4$ under vacuum conditions. In a next step, we then explicitly included the surrounding solvent in our force field calculations. We have characterised changes in the form of the potential along, and especially at the channel exit, in dependence on the location of water molecules. We also considered additional cations within the quadruplex cavity, because it is known from X-ray crystallography that three to four cations can be found within the cavity at the same time.

3.1. Force field calculations in vacuum

To obtain a more detailed picture about the local atomic interaction in the quadruplex cavity, we have first calculated the pair potential between a cation and a single carbonyl oxygen atom. For potassium Fig. 2a clearly indicates that the strong electrostatic attraction is counterbalanced by the repulsive part of the Lennard-Jones potential at short distances, and the addition of both forces leads to a distinct energy minimum. The corresponding potentials for different alkali cations are shown in Fig. 2b. As is known from X-ray crystallography, the characteristic oxygen O6-cation distance in the quadruplex channel is in the range of 2–3 Å (see Fig. 5). Fig. 2b indicates that, due to the short distance between these atoms,

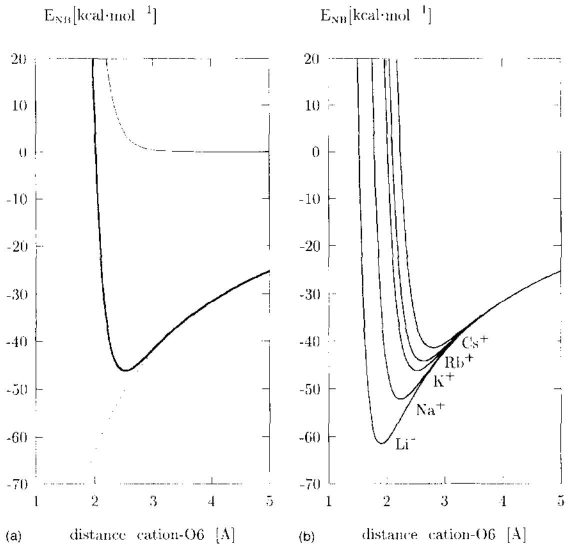

Fig. 2. Individual Coulomb (· · ·), and Lennard–Jones (thin line) contributions to the total energy (thick line). Nonbonded energy for pairwise interaction of different alkali metal ions with carbonylic O6-atoms.

both the Coulombic and the van der Waals forces contribute significantly to the total interaction potential. As expected, the potential well depth increases with increasing charge density of the cations, and likewise, the position is shifted to smaller distances between the ion and the O6-atom.

However, it is obviously not the interaction of the cation with an individual electronegative oxygen atom but the caging effect of all nearest neighbours that will determine the cation specific stability of quadruplex structures. Therefore, we have to consider the interaction of the cation with all neighbouring O6-oxygen atoms, as well as with all other atoms of the DNA molecule. Hence, a three dimensional potential surface is necessary to describe the interaction between the cations and the oligonucleotide. In the following, we will limit our consideration to a single co-ordinate along the helical axis of the quadruplex structure. It is this path along the main axis of the molecule, equivalent to the centre of the channel, the cation has to follow to escape and possibly re-enter the cavity.

Considering all pair interactions of the cation with the atoms of the DNA, it is again the Coulomb interaction with the electronegative oxygen atoms that is mainly responsible for the strong attraction. For K^+, Fig. 3 reveals a shallow minimum within the plane of the G-quartets for the electrostatic energy. The position of the planar tetrameric units is indicated by lines parallel to the ordinate. But it is the Lennard–Jones contribution that determines the characteristic form of the potential energy with distinct minima, now located in the cavity between the tetramer units. Moreover, Fig. 3 indicates that the structural features of the potential is mainly determined by the interaction of the cation with the O6-carbonyl atoms. The contribution of all other DNA atoms results in an overall decrease of the stability but does not significantly alter the form of the potential (data not shown).

In Fig. 4, we have compared the potential energy along the main axis of the quadruplex structure for the different monovalent and divalent cations. As expected, the global depth of the potentials depends first of all on the charge of the different particles. A closer inspection of the different potentials reveals

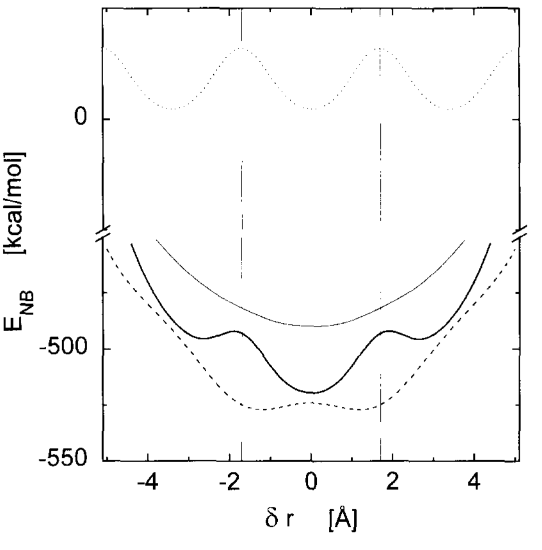

Fig. 3. Nonbonded energy for K^+ in the channel of 16 O6-atoms in dependence on the displacement of the ion from the central position. Motion proceeds through the center of the planes, along the helical axis and describes the escape from the channel. (The position of the planes is indicated by vertical lines.) Coulomb (—) Lennard–Jones (· · ·) and total energy (thick line). The thin line shows the total energy in a T-cage of 16 O4-oxygen atoms.

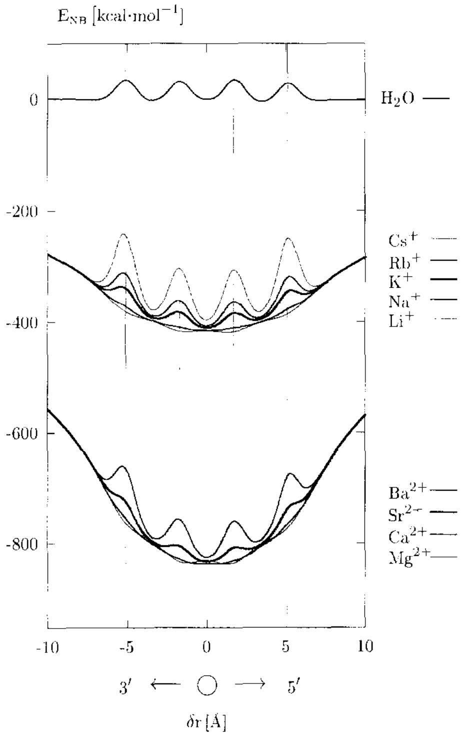

Fig. 4. Pairwise interaction of alkali, alkaline-earth metal ions, and water with all DNA-atoms along the helical axis.

some interesting features. For the small ions Li^+, and Mg^{2+} it seems that the electrostatic interaction is also responsible for the form of the potential which gives a shallow minimum at the pocket positions of the quadruplex (see for comparison Fig. 3). Similarly, the potential of Na^+ and Ca^{2+} appears rather smooth. For the larger cations however, we observe distinct minima, located at the centre of the cavities between the planar tetramer units. The height of the potential barrier increases with increasing size of the cations, while the minimum energy value does not vary significantly. This rather simple energetic model is in good qualitative agreement with findings from X-ray crystallography concerning the position of different cations in the cylindrical cavity of quadruplex structures. Laughlan et al. [5] have solved the crystal structure of $d[TG_4T]_4$ with very high resolution (1.2 Å), and they found that three to four Na^+-ions per quadruplex are partially located in the tetrameric plane as well as between neighbouring G-quartets. On the other hand, a study by Kang et al. [6], unfortunately with lower resolution (2.5 Å), suggest that the potassium ions reside in the cage formed by eight O6-atoms. However, at this point our simple model does not explain the decreasing stability effect of the larger cations Rb^+ and Cs^+. The increasing height of the potential barrier suggests a tighter binding and a longer residence time for these cations. On the other hand, this potential barrier also exists at the outer G-quartets, and might prevent the larger cations from entering the quadruplex channel.

So far, the channel width, as determined by the distance of the carbonyl O6-atoms, was considered as being almost constant in our force field calculations. Only slight changes from the initial size of the starting structures during the short equilibration might have occurred. The influence of the cavity dimensions becomes evident, if we consider the potential of a potassium ion in a cage build from thymine residues. It is known that thymine can also organise to quartet structures, but these structures are less stable, partly because only four hydrogen bonds are involved in tetramer formation. The significant larger width of the quadruplex channel in a T_4-unit is shown in Fig. 5. The typical average distance between the O4 oxygen atoms within a thymine quartet

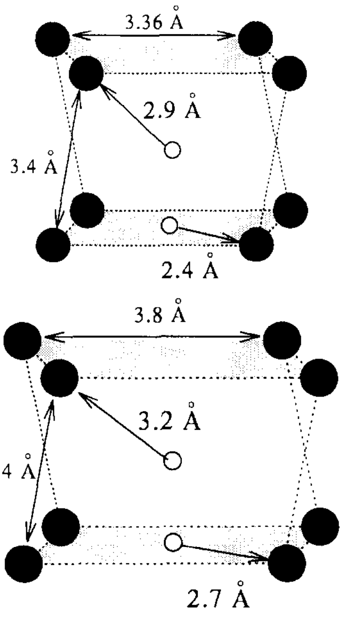

Fig. 5. Average atomic distances in a G-cage (O6–O6 and ion–O6) and in a T-cage (O4–O4 and ion–O4).

is 3.8 Å compared to 3.36 Å for a G-quartet. As a consequence, for K$^+$ the minima in the potential energy at the pocket position disappear, and the overall form of the potential is similar to the situation for the smaller Li$^+$-ions in a guanine structure (see Fig. 3). Only the very large caesium ions cause slight maxima (data not shown). Our MD simulations (to be published) have shown that the missing energy barrier leads to very short residence times for the cations in thymine cages, and is probably responsible for the lower stability of these structures.

3.2. Force field calculations in solution

So far, we have considered only the interactions between the oligonucleotide and the cations in our in vacuum molecular mechanics calculations. In a next step, we took into account the effect of water molecules around the quadruplex structure (system B). The model G-DNA was solvated in a box of SPC/E water, and the system was equilibrated as explained in Section 2.2. We then performed short molecular dynamics simulations to generate different conformations for the energy considerations. From our simulations (to be published) we know that water molecules located at both ends of the quadruplex reveal an exceptionally long residence time at these positions in comparison to bulk water, especially if there is a cation located at the end of the cylindrical channel. In this case, the oxygen atom of the water molecule occupies one of the coordination positions for the cation which explains the long residence time of the water molecules. The distance between the oxygen atom of the water molecule and the cations amounts to 2.6–2.8 Å in an equilibrated system. Inclusion of the water molecules at the end of the channel in our molecular mechanics computations results in a much stronger increase in the potential energy at the end of the cavity in comparison to the calculations in vacuum. This effect, as shown in Fig. 6, is due to the strong repulsive Lennard–Jones interaction, if the cation approaches the water molecule. However, we know from our molecular dynamics simulations that, in spite of the long residence time (compared to bulk water), the water molecule is able to move away from the exit of the channel. Hence, during this process, we observed for the G-DNA structure with potassium ions in the channel that the steep rise in the potential shifts away from the quadruplex, creating a shallow minimum in the transition regime to the solvent. Force field calculations at different times during the simulation, reveal that the potential at the end of the different quadruplex conformations changes significantly, depending on the position of the water molecule. As shown in Fig. 6, if the water molecule moves away from the DNA, the minimum of the potential well at the end of the cavity becomes more distinct. In addition, the potential barrier located at the position of the outer G-quartet almost disappears, which might reflect that the cation starts to interact with other water molecules in the vicinity, building its own hydration shell when leaving the quadruplex structure.

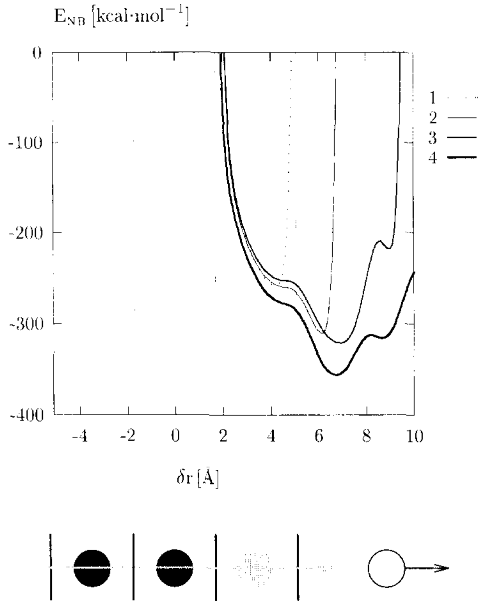

Fig. 6. Pairwise interaction of K$^+$ for different water configurations (1 to 4). Two additional potassium cations in the neighbouring cages were considered (black spheres). Simulation-period: ca. 2 ps.

The specific behaviour of potassium ions with respect to the other alkali cations becomes evident in Fig. 7. We have compared the potential energy for the different cations in the transition regime from the quadruplex cavity to the free solvent (for conformation 3 in Fig. 6). For the smaller Li$^+$-ion, the poten-

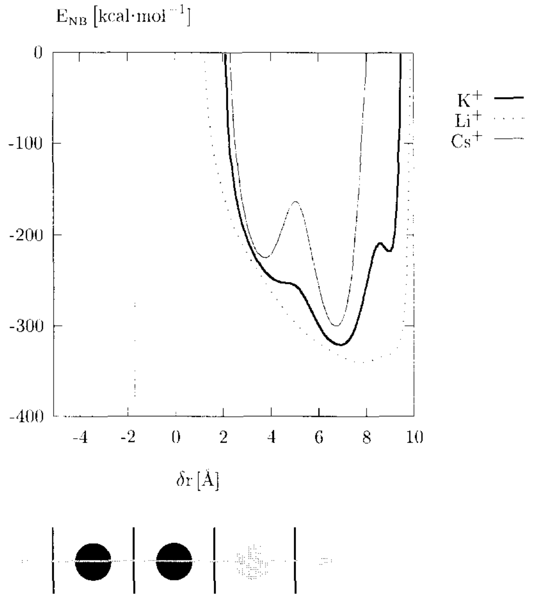

Fig. 7. Comparison of the pair interaction of Li$^+$-, K$^+$-, and Cs$^+$ ions.

tial energy decreases steadily, suggesting a high mobility for this ion as already obtained from our static picture. On the other hand, for Cs$^+$ the potential barrier at the outer tetramer plane is maintained, even if the water molecule moves further away from the cavity. This situation could prevent the Cs$^+$-ion both from leaving the quadruplex cavity as well as entering it from the bulk solvent regime, as we already suspected from the vacuum calculations.

4. Discussion

Our simple force field calculations can provide some insight into the behaviour of different cations with quadruplex structures. We have shown that Coulomb as well as the Lennard–Jones forces are relevant to understand the cation–quadruplex interaction. The electrostatic contribution to the total interaction energy is identical for all cations of the same charge. The attractive Coulomb interaction with the oxygen atoms can account for the binding of cations within the channel. However, our study indicates that it is the modification by the van der Waals forces that is probably responsible for the high specificity of cation stabilisation.

For the small Li$^+$-ion we did not observe any preferential binding position with respect to the O6-atoms within the quadruplex cavity. Furthermore, the calculations in solution suggest that this cation probably can easily leave the channel and build up a hydration shell for stabilisation. This observation is in agreement with the experimental finding that lithium does not significantly stabilise quadruplex structures [3], and also with our molecular dynamics simulation. For sodium ions our energy calculations gave very similar results, although the cation is known for a strong influence on quadruplex formation [2].

In contrast, the larger cations like K$^+$ are strongly trapped between neighbouring planar tetramer units build from guanine bases of the four DNA strands. These cations are coordinated to eight oxygen atoms of neighbouring G-quartets in an almost bipyramidal antiprismatic arrangement. Our static and purely energetic considerations are in agreement with findings from X-ray crystallography which indicate that the potassium and sodium ions can occupy different positions within the cavity. While K$^+$ is found between the tetramer planes, Na$^+$ is also found in the pocket position within a G-quartet.

The calculations in solution show that the cations at the end of the quadruplex structure strongly interact with a water molecule located near the cavity. Snapshots taken at different times of a molecular dynamic simulation provide geometric structures which differ mainly in the position of this water molecule with respect to the oligonucleotide and the trapped cation. Our molecular mechanics calculations indicate that the motion of the water molecule away from the oligonucleotide is correlated with the escape of the potassium ion from the channel. For the larger Cs$^+$-ions however, the outer planar tetramer units represent a high energy (potential) barrier, preventing the cations from leaving but probably also from entering the quadruplex cavity. This finding provides evidence for the exceptional capability of potassium ions to stabilise quadruplex structures. At this point, one has to keep in mind that the form of the potential is very much dependent on the cation-O6 distance. That means, increasing the width of the cavity (as we have observed at longer times in our MD simulation) will also decrease the potential barrier for passing a G-quartet. This change in the DNA conformation will weaken the hydrogen bonding between the guanine bases, and hence decrease the stability of the intermolecular complex. This might explain the experimental results for the larger cations which reveal a much weaker complexation. Going one step further, the preferential location of the potassium cation between two planar tetramer units might suggest that a K^+-ion can bridge quadruplexes, leading to supramolecular structures often observed experimentally [27].

Though there is a lot of experimental evidence that sodium ions promote quadruplex formation, we did not find unequivocal evidence from purely energetic considerations. This conclusion manifests the shortcomings of our molecular mechanics calculations. Looking at static structures from an energetic point of view surely neglects the importance of the dynamics on the stability effects of different cations. We have observed that the dynamics of water molecules, and hence their position relative to the channel entrance, play an important role for the interaction potential. The same arguments hold most likely for the flexibility of the DNA molecules that was neglected in this study. We know from our detailed MD simulations in the presence of K^+-ion that the entering into, but also the escape from the quadruplex channel, is accompanied by a significant change in the O6 distances, indicating a re-organisation of the local structure of the four-stranded DNA molecule. As expected, for Li^+ we observe a contraction of the quadruplex cavity, while larger cations like Rb^+ or Cs cause a significant deformations of the DNA, leading to an overall destabilisation of the quadruplex conformation. The importance of the cage size dimensions can already be assessed from Figs. 3 and 5. For thymine residues the oxygen–oxygen distance is significantly larger than for guanine (see Fig. 5) which has a dramatic influence on the interaction potential of K^+ within the quadruplex channel. The same effect is feasible in a G-cage through cooperative motion of the bases in such a way that a widening of the cage dimensions results.

Similarly, our calculations indicate that one has to consider the influence of the hydration shell for the different cations. Before a certain cation can enter the cavity, it has to loose its hydration water, and leaving the cavity assumes that the hydration shell around the cations is restored. Therefore, a detailed molecular dynamics simulation should provide a better understanding about the different dynamic aspects that are of importance for quadruplex stabilisation. Corresponding investigations are currently under way in our laboratory.

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