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Observation of Water Molecules within the Bimolecular $d(G_3CT_4G_3C)_2$ G-Quadruplex

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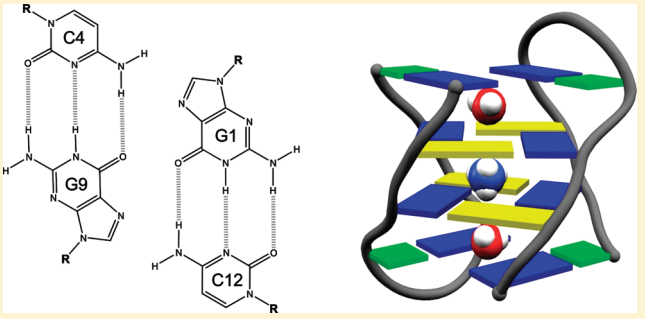
Supporting Information

ABSTRACT: G-Rich oligonucleotides with cytosine residues in their sequences can form G-quadruplexes where G-quartets are flanked by G·C Watson−Crick base pairs. In an attempt to probe the role of cations in stabilization of a structural element with two G·C base pairs stacked on a G-quartet, we utilized solution state nuclear magnetic resonance to study the folding of the $d(G_3CT_4G_3C)$ oligonucleotide into a G-quadruplex upon addition of $^{15}NH_4^+$ ions. Its bimolecular structure exhibits antiparallel strands with edge-type loops. Two G-quartets in the core of the structure are flanked by a couple of Watson−Crick G·C base pairs in a sheared arrangement. The topology is equivalent to the solution state structure of the same oligonucleotide in the presence of Na^+ and K^+ ions [Kettani, A., et al. (1998) *J. Mol. Biol. 282*, 619, and Bouaziz, S., et al. (1998) *J. Mol. Biol. 282*, 637). A single ammonium ion binding site was identified between adjacent G-quartets, but three sites were expected. The remaining potential cation binding sites between G-quartets and G·C base pairs are occupied by water molecules. This is the first observation of long-lived water molecules within a G-quadruplex structure. The flanking G·C base pairs adopt a coplanar arrangement and apparently do not require cations to neutralize unfavorable electrostatic interactions among proximal carbonyl groups. A relatively fast movement of ammonium ions from the inner binding site to bulk with the rate constants of 21 s^{-1} was attributed to the lack of hydrogen bonds between adjacent G·C base pairs and the flexibility of the T_4 loops.



G-Rich DNA can form higher-order structures called G-quadruplexes.[1−5] They are comprised of stacks of four coplanar guanine bases held together by Hoogsteen hydrogen bonds, termed G-quartets. Oligonucleotides containing cytosine residues besides, or within, their G-rich tracts are capable of folding into a G-quadruplex structure in which G-quartets are flanked by couples of G·C Watson−Crick base pairs forming a G·C·G·C quartet.[6−9] Examples of such sequences are oligonucleotides containing the fragile X triplet repeat $d(CGG)_n$.[10−13] While the presence of cations is essential for the formation of G-quartets, their role in stabilization of G·C·G·C quartets has not been studied. There are four closely spaced carbonyl oxygen atoms in the center of each G-quartet whose electrostatic repulsions have to be compensated through coordination of monovalent or divalent cations.[14,15] G·C·G·C quartets exhibit a different layout of functionalities in the center with two guanine carbonyl oxygens and two cytosine amino groups. Cation coordination between two G·C·G·C quartets is unlikely.[6] On the other hand, adjacent G·G·G·G and G·C·G·C quartets form an environment with six carbonyl oxygen atoms that could serve as potential cation binding sites. However, it is unclear whether cation coordination is required for stabilization of such G·C·G·C structures.

Previously, Patel and co-workers[16,17] determined solution state structures of the G-quadruplexes formed by the $d(G_3CT_4G_3C)$ oligonucleotide in the presence of either Na^+ or K^+ ions. Both structures featured the same head-to-tail fold with two G-quartets. The major difference was in the arrangement of the remaining guanine and cytosine bases. In the presence of smaller Na^+ ions, two G·C·G·C quartets are formed on each side of the G-quadruplex core. Molecular modeling suggested that the Na^+ form can coordinate three cations in the cavities between G·G·G·G and G·C·G·C quartets. The K^+ form was found to accommodate five cations with two of the additional cations in the space between the outer quartets and T_4 loops. However, the large K^+ ions have been found to protrude into the plane of G·C·G·C quartets, which prevents the formation of cross-strand hydrogen bonds. As a result, a sheared arrangement of K^+-bridged G·C Watson−Crick base pairs is formed.[17]

To gain further insight into cation coordination within a G-quadruplex consisting of both G-quartets and G·C base pairs, we utilized ^{15}N-labeled ammonium ions as a nonmetallic replacement to evaluate the nature of proposed cation binding

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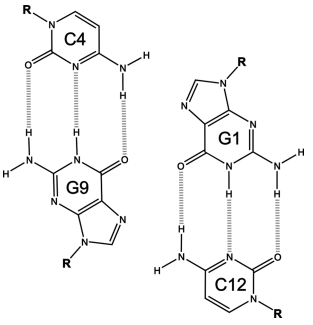

Figure 1. Schematic representation of two sheared G·C base pairs in the arrangement observed in the structure of the $d(G_3CT_4G_3C)_2$ quadruplex in the presence of ammonium ions. Dashed lines represent H-bonds. R is 2′-deoxyribose.

sites. $^{15}NH_4^+$ ions allow the probing of cation binding sites directly with the use of heteronuclear NMR.[18,19] In this study, we describe the topology of the $d(G_3CT_4G_3C)_2$ G-quadruplex in the presence of $^{15}NH_4^+$ ions determined by solution state NMR. Ammonium ions exhibit an ionic radius that is larger in comparison to those of K^+ and Na^+ ions. A single $^{15}NH_4^+$ ion binding site was identified between two G·G·G·G quartets in the center of the G-quadruplex core. Interestingly, the two cavities between G·G·G·G quartets and G·C base pairs are occupied by water molecules. In the past, considerable interest was devoted to the studies of DNA hydration patterns, because of the role of water molecules in the conformational stability of DNA secondary structures and in mediation of protein−DNA interactions.[20−24] Relaxation dispersion and nuclear Overhauser effect spectroscopy were used to evaluate the dynamics of bound water.[25−31] Water activity was found to play an important role in G-quadruplex stability.[32,33] However, to the best of our knowledge, this is the first observation of water molecules residing within a G-quadruplex structure. Herein, we discuss the implications of a sheared arrangement of the G·C base pairs (Figure 1), revealed by NOE connectivities, in the context of water localization.

MATERIALS AND METHODS

The oligonucleotide $d(G_3CT_4G_3C)$ was synthesized on an Expedite 8909 synthesizer using standard phosphoramidite chemistry following the manufacturer's protocol. The oligonucleotides were cleaved from the solid support and deprotected with concentrated aqueous ammonia overnight at 55 °C. DNA was desalted on a Sephadex G15 column. Only fractions containing full-length oligonucleotides were pooled and lyophilized. The DNA was dissolved in 0.3 mL of a 95% H_2O/5% ^2H_2O mixture. LiOH or HCl was added to adjust the pH of the sample to 7.0. The final oligonucleotide concentration was 3 mM per strand.

NMR data were collected on Varian VNMRS 600 and 800 MHz NMR spectrometers. All one-dimensional and two-dimensional (2D) spectra were recorded at 25 °C with DPFGSE solvent suppression. 2D NOESY spectra (τ_m = 80, 150, and 250 ms) were utilized to assign ^1H resonances. The number of ammonium ion binding sites was determined with the use of a $^{15}N-^1H$ HSQC spectrum. Ammonium ions and water molecules were localized within the G-quadruplex structure using 2D ROESY spectra (τ_m = 80, 150, and 250 ms). The movement of ammonium ions was evaluated with a series of $^{15}N-^1H$ NzExHSQC experiments with mixing times ranging from 13 ms to 2 s. Data were analyzed with VNMRJ, Sparky, and Origin.

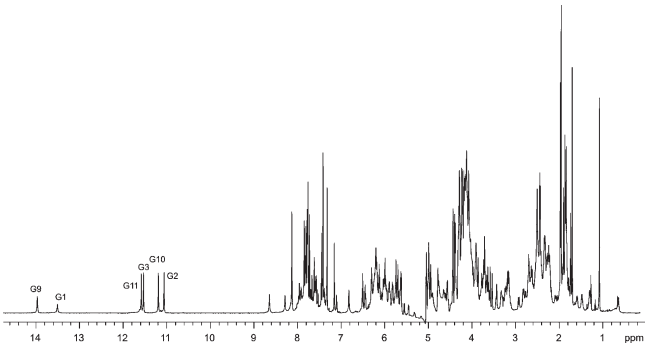

Figure 2. ^1H NMR spectrum of $d(G_3CT_4G_3C)$ in the presence of 100 mM $^{15}NH_4Cl$ in 95% H_2O at pH 7 and 25 °C.

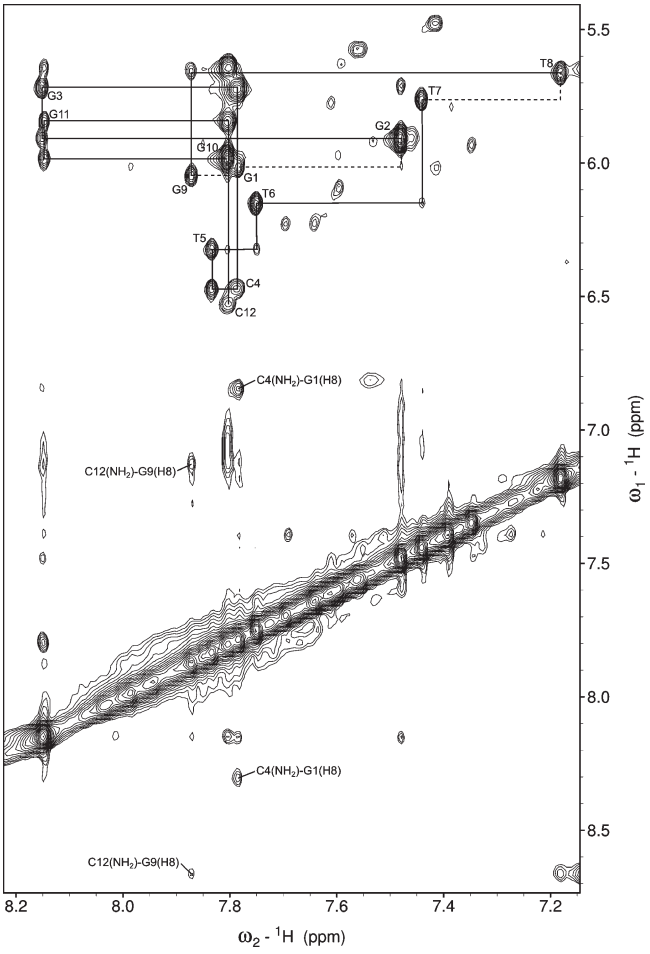

Figure 3. 2D NOESY spectrum (τ_m = 150 ms) of $d(G_3CT_4G_3C)$ in the presence of a $^{15}NH_4^+$ ion concentration of 100 mM. The sequential walk in the aromatic−anomeric region is shown as lines (dashed lines represent broken connectivities). Cross-peaks correlating aromatic protons of G1 and G9 with both cross-strand amino protons indicating a sheared arrangement of Watson−Crick base pairs are labeled.

RESULTS

A study of folding of $d(G_3CT_4G_3C)$ into a G-quadruplex was started from an aqueous solution of the oligonucleotide devoid of any quadruplex-stabilizing cations. No imino resonances, characteristic of G-quadruplex formation, could be observed in ^1H

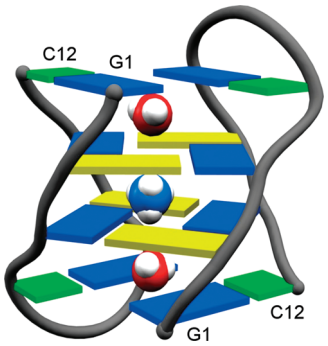

Figure 4. Topology of a bimolecular $d(G_3CT_4G_3C)_2$ quadruplex in the presence of $^{15}NH_4^+$ ions. The *anti* guanine, *syn* guanine, and cytosine residues are shown as blue, yellow, and green rectangles, respectively. For the sake of clarity, thymine bases are not shown. Inner and outer binding sites are occupied by $^{15}NH_4^+$ ions and water molecules, respectively.

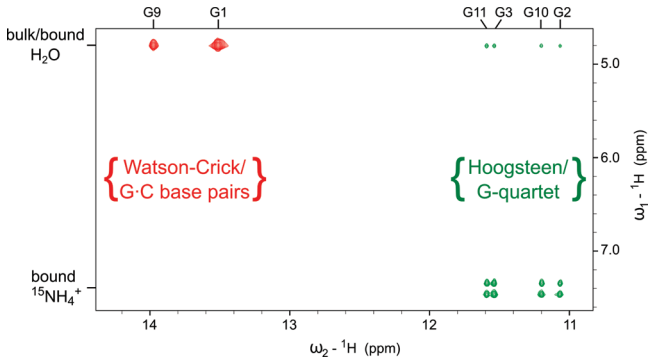

Figure 5. Imino region of a 150 ms 2D ROESY spectrum of d-$(G_3CT_4G_3C)$ in the presence of a $^{15}NH_4^+$ ion concentration of 100 mM. Cross-peaks correlating water protons with G1 and G9 imino protons have a positive sign indicating chemical exchange. Cross-peaks correlating G2, G3, G10, and G11 imino protons with water or ammonium protons have a negative sign indicating cross-relaxation. Positive and negative cross-peaks are colored red and green, respectively.

NMR spectra in the absence of cations. Upon gradual titration with an aqueous solution of $^{15}NH_4Cl$, the oligonucleotide was folded into a single G-quadruplex species. Sharp and well-resolved proton resonances were observed at a $^{15}NH_4^+$ ion concentration of 100 mM. No intermediate states could be observed during titration. The ^1H spectrum revealed a G-quadruplex structure with four hydrogen-bonded imino resonances in the range of δ 11.0−11.6 ppm, which corresponded to Hoogsteen-type H-bonds (Figure 2). Two upfield imino resonances were assigned to *syn* guanines, while the downfield resonances belonged to *anti* guanines. Additionally, two hydrogen-bonded imino resonances in Watson−Crick geometry could be identified at δ 13.5 and 14.0.

All imino, aromatic, and anomeric resonances could be assigned through analysis of NOESY spectra (Figure 3). Two strong H8−H1′ intranucleotide cross-peaks were identified and attributed to G2 and G10, which exhibit a *syn* conformation along their glycosidic bonds. The glycosidic bond conformations along the oligonucleotide sequence are *anti-syn-anti-anti-*T_4*-anti-syn-anti-anti*. As a result, the complete sequential walk cannot be observed throughout the sequence because of large distances in the G1 (*anti*)−G2 (*syn*) and G9 (*anti*)−G10 (*syn*) steps. Furthermore, the sequential walk is interrupted between T7 and T8, which implies their spatial relationship and structural characteristics of the loops.

Our NMR data demonstrated that the fold of the G-quadruplex adopted by $d(G_3CT_4G_3C)_2$ in the presence of $^{15}NH_4^+$ ions is identical to the fold adopted in the presence of Na^+ or K^+ ions determined previously by Patel and co-workers.[16,17] To assess the stabilizing effect of different cations, we performed UV melting experiments with the $d(G_3CT_4G_3C)_2$ quadruplex folded in the presence of Na^+, NH_4^+, and K^+ ions. Single hyperchromic transitions were observed at 24, 29, and 52 °C for Na^+, NH_4^+, and K^+ ions, respectively. The bimolecular $d(G_3CT_4G_3C)_2$ quadruplex exhibits a head-to-tail topology with two G-quartets and two edge-type T_4 loops that connect the adjacent antiparallel strands (Figure 4). The flanking guanine and cytosine bases stack on the G-quartets; however, they do not form a G·C·G·C quartet. The downfield cytosine amino resonances at δ 8.3 and 8.7 ppm were assigned to Watson−Crick hydrogen-bonded amino protons of C4 and C12, respectively (Figure 3). The remaining cytosine amino protons could be involved in cross-strand hydrogen bonds. However, their chemical shifts of δ 6.8 and 7.1 ppm for C4 and C12, respectively, indicate that this is not the case. The lack of cross-strand hydrogen bonding suggests that couples of G·C Watson−Crick base pairs stack on top of the G-quartets in a sheared arrangement. In full support, we observed NOE contacts between aromatic protons of G1 and G9 with both cross-strand amino protons of C4 and C12 [i.e., G1(H8)−C4(NH_2) and G9(H8)−C12(NH_2)]. The relative positions of G and C residues schematically presented in Figure 1 result in large distances between cytosine H5 protons and cross-strand guanine aromatic protons [i.e., C4(H5)−G1(H8) and C12(H5)−G9(H8)], which are in accordance with the absence of respective NOE connectivities. Additionally, the H5 chemical shifts of C4 and C12 are δ 5.73 and 5.65 ppm, respectively, which are closer to those observed for the K^+ form with a sheared arrangement of G·C base pairs (δ 5.65 and 5.76 ppm) than those of the Na^+ form, which exhibits actual G·C·G·C quartets (δ 5.27 and 5.24 ppm).[16,17]

The topology of the $d(G_3CT_4G_3C)_2$ quadruplex implies the existence of three cation binding sites: an inner cation binding site between adjacent G-quartets and two outer binding sites between G-quartets and flanking Watson−Crick G·C base pairs. To our surprise, only a single cross-peak for bound $^{15}NH_4^+$ ions could be observed in $^{15}N-^1H$ HSQC spectra. We were able to observe a weak cross-peak corresponding to bulk ammonium ions in an HSQC spectrum recorded at pH 6, but no additional cross-peak that would indicate the presence of $^{15}NH_4^+$ ions at some other site (Figure S1 of the Supporting Information). Even after careful further lowering of the pH, the G-quadruplex structure disintegrated. There is a possibility of partial and transient occupancy of $^{15}NH_4^+$ ions at the outer binding sites, because they have to pass through the outer binding site to exchange between the inner binding site and bulk solution. However, because of the absence of a resolved HSQC cross-peak(s), the lifetime of $^{15}NH_4^+$ ions at the outer binding sites and possibly loops is short on the NMR time scale. These sites are possibly partially occupied by water and $^{15}NH_4^+$ ions. Cross-relaxation ROESY cross-peaks between ammonium protons at δ 7.20 ppm and all eight imino protons of the two G-quartets allowed us to localize $^{15}NH_4^+$ ions at the inner binding site (Figure 5). On the other hand, water protons exhibit cross-peaks to all guanine

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imino protons, including those involved in Watson−Crick base pairs. Cross-peaks correlating water protons with G-quartet imino protons exhibit a negative sign, which is the result of dipole−dipole interactions and indicates that water molecules are spatially close to G-quartet imino protons. This is only possible if water molecules are residing at the outer binding sites for a sufficient period of time to allow for cross-relaxation to build up. However, the chemical shift of bound water is identical to the chemical shift of bulk water, which suggests that exchange between two chemical environments is fast on the NMR time scale. On the other hand, cross-peaks correlating water protons with imino protons involved in Watson−Crick base pairs show a positive sign and arise because of chemical exchange (with diagonal phased positive). Interestingly, volume integrals of the positive cross-peaks corresponding to G1 and G9 imino resonances differ considerably and indicate an asymmetric proton exchange with the solvent. The G1 cross-peak in a ROESY spectrum (τ_m = 150 ms) is more intense, which can be attributed to a more efficient exchange of the G1 imino proton because of its greater exposure to bulk water being the terminal 5′ residue, while the G9 imino proton is more protected from exchange by the T_4 loop. The ratio between the volumes of imino G1 and G9 ROESY cross-peaks with water is 2.4. No additional ammonium or water molecules could be localized between the G·C base pairs and T_4 loops. If the negative ROE cross-peaks were a result of dipole−dipole interaction between G-quartet imino protons and exchanging imino protons involved in Watson−Crick base pairs, their volumes would be modulated by the interproton distances. The distances in the high-resolution structure determined in the presence of KCl (Protein Data Bank entry 1A8W) between imino protons of G3 and G9 and between those of G11 and G1 are 4.1 and 4.0 Å, respectively, while the distances between imino protons of G10 and G9 and between those of G2 and G1 are 5.4 and 5.6 Å, respectively. Considering the r^{-6} relation between the interproton distance and the efficiency of dipole−dipole transfer, the cross-peak volumes would differ by 1 order of magnitude. However, the imino−water ROESY cross-peak volumes are −100, −92, −82, and −78 arbitrary units for G11, G3, G10, and G2, respectively. Distances between different G-quartet imino protons and cytosine amino protons in the high-resolution structure differ even more, which rules out the possibility of negative ROE being the result of exchange-dipolar transfer via neighboring quickly exchanging protons.

2D NzExHSQC experiments were used to follow the movement of $^{15}NH_4^+$ ions from the interior of the G-quadruplex into bulk solution. The NzExHSQC experiment is a variant of the HSQC experiment with additional mixing time in the pulse sequence.[19,34] During the mixing time, the magnetization is on nitrogen atoms (i.e., Nz), which allows us to observe physical movement of $^{15}NH_4^+$ ions between different chemical environments. The movement manifests itself as a resolved cross-peak with δ ^{15}N and δ ^{1}H of the initial and final locations, respectively. However, no exchange cross-peaks corresponding to $^{15}NH_4^+$ ions could be observed at pH 7. In $d(G_3CT_4G_3C)_2$, ions move from the inner binding site directly into bulk where they are exposed to efficient proton exchange with water, which results in the broadening of $^{15}NH_4^+$ ion resonances to baseline. All attempts to lower the pH to <6 resulted in the broadening of all G-quadruplex resonances. The extreme pH sensitivity of the sample was most likely caused by the protonation of cytosine bases, which led to the disintegration of the G-quadruplex structure. Analysis of intensities of autocorrelation NzExHSQC cross-peaks at pH 7 as a function of mixing time allowed us to estimate the exchange rates for the movement of ammonium ions from the inner binding site. The magnitude of the autocorrelation cross-peak corresponding to bound ammonium ions decreases with the increasing mixing time of the NzExHSQC experiment. The decrease can be described as a double exponential with the rate constants related to the movement of ammonium ions from the binding site into solution and ^{15}N T_1 relaxation time.[35,36] Eleven data points (τ_m = 0.13−2.00 s) were collected at 25 °C, and a good fit was obtained through iterative fitting (Figure S2 of the Supporting Information). The two decay constants corresponding to the exchange rate constant for the movement of $^{15}NH_4^+$ ions from the inner binding site and the ^{15}N relaxation time are 21 ± 5 s^{-1} and 1.1 ± 0.1 s, respectively. The high experimental error of the exchange rate constant is due to fast movement of $^{15}NH_4^+$ ions and limitations of the pulse sequence used to record NzExHSQC spectra with very short mixing times, which limits the number of data points and thus the accuracy of the determined rate constants.

DISCUSSION

The $d(G_3CT_4G_3C)$ oligonucleotide was folded into a single G-quadruplex structure in the presence of ammonium ions. The fold of the quadruplex is in agreement with the fold of the same oligonucleotide in the presence of Na^+ or K^+ ions, which has been determined previously by Patel and co-workers.[16,17] While the presence of different cation species appears to have little effect on the overall topology of the G-quadruplex, subtle differences in the structure of the guanine and cytosine bases flanking the two central G-quartets can be observed. Additionally, molecular modeling studies suggested that the number of cations located within the G-quadruplex structure varied with changes in the nature of cations.[16,17] The Patel group proposed a total of five potassium ion binding sites, which were located between G·G·G·G quartets, G·C base pairs, and T_4 loops. On the other hand, because of the different orientation of the T_4 loops, only three binding sites between G·G·G·G and G·C·G·C quartets were suggested for sodium ions. It was proposed that smaller Na^+ ions located at the outer binding sites stabilize G·C·G·C quartets.[16] In contrast, larger K^+ ions located at the outer binding sites penetrate into the plane of the two G·C base pairs, which interferes with the formation of a G·C·G·C quartet.[17] The presence of K^+ ions results in a sheared arrangement of two G·C base pairs, which are bridged by the K^+ ion located at the outer binding site.

Our NMR spectra provide unequivocal evidence of the localization of ammonium ions within the G-quadruplex structure. ^{15}N−^{1}H HSQC spectra revealed a single ammonium ion binding site within the architecture of the $d(G_3CT_4G_3C)_2$ quadruplex. Localized $^{15}NH_4^+$ ions are sandwiched between two G-quartets that form the core of the G-quadruplex. The remaining guanine and cytosine bases form two side-by-side couples of G·C Watson−Crick base pairs on each end of the G-quadruplex core. NMR data indicate that there are no hydrogen bonds between two G·C base pairs that would contribute to cross-strand interactions. Neighboring G·C base pairs are aligned in the same way as in the presence of K^+ ions. However, no ammonium ions could be found at the outer binding sites between G-quartets and G·C base pairs. Because the relatively large potassium ions affect the structure of G·C·G·C quartets, the presence of slightly larger ammonium ions would be even more destabilizing. Interestingly, the cavity between G-quartets

and G·C base pairs is occupied by water molecules. The values of the K^+ ionic radius and the van der Waals radius of water molecules are almost identical, which appears to have a similar effect on the hydrogen bonding and structure of flanking guanine and cytosine bases. It appears that there is no need to counteract the electrostatic repulsion of the guanine carbonyl groups by a positively charged ion. The carbonyl groups are spatially closer in G·C·G·C quartets (Na^+ form) than in sheared G·C base pairs (K^+ form).[16,17] The large distance between guanine C6 carbonyl groups could reduce or even eliminate the need for cation-mediated neutralization of unfavorable electrostatic interactions.

The water molecules localized at the outer binding sites are highly mobile. Wüthrich et al. studied the exchange rates of water molecules located in the grooves of double-helical DNA.[25] They found that negative ROESY and positive NOESY water cross-peaks indicate that the residence lifetimes of bound water molecules are longer than 10^{-9} s. However, we believe that because of the cavity-like environment embracing the water molecules localized at the outer binding sites of the $d(G_3CT_4G_3C)_2$ quadruplex, the lifetimes are considerably longer. Observation of identical ^1H NMR chemical shifts corresponding to bound and bulk water can be used to set the upper limit of the bound water lifetime at 10^{-3} s.

Ammonium ions are not statically localized within the G-quadruplex structure and exchange with ions in bulk solution. A relatively fast movement of ions from the inner binding site with a rate constant of 21 s^{-1} was detected using $^{15}N-^1H$ NzExHSQC experiments. The ammonium ions at the inner binding site can exchange with bulk solution by movement through the G-quartets. Because no NOE−ROE contacts between ammonium protons and imino protons of the G·C base pairs could be observed, the movement of ions from the outer binding sites into bulk solution is fast on the NMR time scale. The large ionic radius of ammonium ions prevents their free movement between binding sites and couples it with partial opening of G-quartets. The fast movement of ammonium ions from the inner binding site indicates that the G-quartets are able to undergo partial opening with relative ease. In comparison, in $d(G_4T_4G_4)_2$, a G-quadruplex with bimolecular fold-back topology comprised solely of G·G·G·G quartets, the ammonium ions move from the inner binding site at ∼6-fold slower rate (i.e., 3.7 s^{-1}), while no movement at all could be observed in $d(G_4T_3G_4)_2$ at room temperature.[36,37] However, as we suggested recently, the movement of $^{15}NH_4^+$ ions is ultimately controlled by the ability of cations to move into bulk solution and therefore the stability of the terminal quartets and loop conformations.[35,36,38] In the case of $d(G_3CT_4G_3C)_2$, the couples of G·C base pairs are involved in a rather loose mutual interaction and represent little if any restriction for the movement of $^{15}NH_4^+$ ions. One guanine and one cytosine base are involved in a stable Watson−Crick base pair; however, because of the lack of cross-strand hydrogen bonding, G·C base pairs can simply slide to the side and allow the passage of ammonium ions or water molecules. The edge-type T_4 loops, which connect the Watson−Crick bonded pair, do not provide any cross-strand interactions and thus do not contribute to the stabilization of the stacked G·C·G·C structure. The T_4 loops appear to be flexible, because NOEs between loop protons and G·C base pair protons are scarce. Furthermore, the asymmetry in the exchange of G1 and G9 imino protons with solvent indicates that there is no stable capping-type structure stacked on the two G·C base pairs. In comparison, G-quadruplex adopted by the thrombin binding aptamer also exhibits a single ammonium ion binding site where $^{15}NH_4^+$ ions are sandwiched between two adjacent G-quartets. However, because of the efficient stacking of flanking guanine and thymine residues, the exchange rate of the ammonium ions at 15 °C is 20 times slower in the case of the thrombin binding aptamer than in the case of the $d(G_3CT_4G_3C)_2$ quadruplex.[39]

UV melting studies were used to evaluate the stabilizing effects of bound water molecules. Interestingly, the T_m values of the $d(G_3CT_4G_3C)_2$ quadruplex in the presence of Na^+ and NH_4^+ ions are similar. While the Na^+ structure contains three ion binding sites, there is only one NH_4^+ ion binding site within the $d(G_3CT_4G_3C)_2$ quadruplex. If we consider that the relative G-quadruplex stabilizing abilities of Na^+ and NH_4^+ ions are comparable, the localized water molecules must contribute to the overall stability of the G-quadruplex. On the other hand, the $d(G_3CT_4G_3C)_2$ quadruplex in the presence of K^+ ions exhibits noticeably higher stability (T_m values of 52 °C for K^+ and 24 and 29 °C for Na^+ and NH_4^+ ions, respectively). While this could be solely due to the better stabilizing properties of the K^+ ions, it is more likely the result of loop stabilization by two proposed additional K^+ ions. It is interesting that a bimolecular G-quadruplex adopted by $d(G_4T_4G_4)$ exhibits T_m values of 57 and 48 °C in the presence of Na^+ and K^+ ions, respectively.[40] It should be noted, however, that topologies of the two four-quartet quadruplex structures are different, and their thermodynamic stabilities are in addition to a mere number and nature of quartets greatly affected by the orientation and stacking of the loop residues.

CONCLUSIONS

The $d(G_3CT_4G_3C)$ oligonucleotide was folded into a single G-quadruplex species upon addition of $^{15}NH_4^+$ ions. The fold of the bimolecular antiparallel quadruplex with edge-type loops is in agreement with structures established previously in the presence of K^+ or Na^+ ions. Two G-quartets comprise the core of the G-quadruplex, while the flanking guanine and cytosine bases form couples of Watson−Crick bonded G·C base pairs that stack on top of the G-quartets. The adjacent G·C base pairs are in a sheared arrangement with no cross-strand hydrogen bonds. Only one ammonium ion binding site could be identified between adjacent G-quartets. A relatively fast movement of ammonium ions from the inner binding site to bulk was detected with the use of heteronuclear NMR. The cavity between G-quartets and G·C base pairs is occupied by water molecules that are in fast exchange with bulk solution. The lack of cross-strand interactions between G·C base pairs and the poorly defined structure of the T_4 loops pose little restriction on the movement of ammonium ions through the central cavity of the G-quadruplex and fast exchange of bound water molecules with bulk water.

ASSOCIATED CONTENT

Supporting Information. $^{15}N-^1H$ HSQC spectrum and relative volumes of autocorrelation peaks as a function of mixing time of the $^{15}N-^1H$ NzExHSQC experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

NMR, nuclear magnetic resonance; DPFGSE, double pulse field gradient spin echo; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotating frame Overhauser effect spectroscopy; HSQC, heteronuclear single-quantum coherence; NzExHSQC, Nz exchange heteronuclear single-quantum coherence.

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