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Tetramolecular DNA Quadruplexes in Solution: Insights into Structural Diversity and Cation Movement

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Abstract: The present study expands the notion that the simple oligonucleotide sequence d(TG_4T) in solution forms a tetramolecular G-quadruplex having a parallel orientation of the four strands and four G-quartets with all of the residues in the anti orientation. NMR experiments have revealed the equilibrium of two monomeric forms with a ratio between 85:15 and 70:30 in the presence of K^+, Na^+, and $^{15}NH_4^+$ ions. The major form consists of four G-quartets, whereas the minor form exhibits an additional T-quartet at the 5′ end. An analogous oligonucleotide with U at the 5′ end adopts a dimeric structure of G-quadruplex units in the presence of K^+ and $^{15}NH_4^+$ cations but not in the presence of Na^+ ions, where monomeric forms are present. Three $^{15}NH_4^+$ ion binding sites between the four G-quartets within the major monomeric form have been identified, while an additional $^{15}NH_4^+$ ion binding site between the G- and T-quartets at the 5′ end of the minor form has been established. The dimeric d$[(UG_4T)_4]_2$ G-quadruplex exhibits eight $^{15}NH_4^+$ ion binding sites, two of them between the U- and G-quartets. $^{15}NH_4^+$ ions have been shown to move faster between the interior of the tetramolecular structures and the bulk solution in comparison with the monomolecular and bimolecular G-quadruplexes. However, cation movement is slowed by the presence of a T-quartet at the 5′ end.

Introduction

Guanine-rich DNA sequences can fold into four-stranded G-quadruplex structures composed of G-quartets, which are planar arrays of four guanines held together by eight Hoogsteen hydrogen bonds.[1−4] G-rich sequences are overrepresented in the promoter regions of a number of genes, including oncogenes; in rDNAs as well as in telomeric DNA regions; and in immunoglobulin heavy chain switch regions of higher vertebrates.[1,5−7] Formation of G-quadruplexes has been implicated in association with human diseases. Exploration of their stabilization by the binding of diverse types of molecules revealed a role of G-quadruplexes as therapeutic targets in drug design.[8] In addition, G-quadruplexes exhibit great potential in applications utilizing nanomolecular devices because of their well-defined structure and self-recognition properties. Moreover, self-assembly of protected guanine nucleosides may lead to the formation of artificial ion channels.[9,10]

Addition of cations such as K^+ or Na^+ induces formation of G-quadruplexes. Sequence details and the nature of the metal ions play major roles in the formation and stabilization as well as structural diversity of G-quadruplexes.[11] Oligonucleotides containing only a single run of guanines form tetramolecular G-quadruplex structures consisting of four strands. Early NMR spectroscopic studies performed almost 20 years ago revealed that d(TG_4T) in the presence of Na^+ ions forms a tetramolecular G-quadruplex consisting of four G-quartets with all of the guanine residues in the anti conformation.[12,13] X-ray crystallographic studies of d$(TG_4T)_4$ established the formation of the same tetramolecular topology; however, it exhibited head-to-head stacking of two G-quadruplexes in the asymmetric unit.[14−17] A T-quartet involving the 5′ ends has been found at

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(1) *Quadruplex Nucleic Acids*; Neidle, S., Balasubramanian, S., Eds.; Royal Society of Chemistry: Cambridge, U.K., 2006; pp 1−301.
(2) Keniry, M. A. *Biopolymers* **2001**, *56*, 123–146.
(3) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668–698.
(4) Phan, A. T.; Kuryavyi, V.; Patel, D. J. *Curr. Opin. Struct. Biol.* **2006**, *16*, 288–298.
(5) Todd, A. K.; Johnston, M.; Neidle, S. *Nucleic Acids Res.* **2005**, *33*, 2901–2907.
(6) Huppert, J. L.; Balasubramanian, S. *Nucleic Acids Res.* **2005**, *33*, 2908–2916.
(7) Fernando, H.; Sewitz, S.; Darot, J.; Tavare, S.; Huppert, J. L.; Balasubramanian, S. *Nucleic Acids Res.* **2009**, *37*, 6716–6722.
(8) Balasubramanian, S.; Neidle, S. *Curr. Opin. Chem. Biol.* **2009**, *13*, 345–353.
(9) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottareli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060–4067.
(10) Wong, A.; Fettinger, J. C.; Forman, S. L.; Davis, J. T.; Wu, G. *J. Am. Chem. Soc.* **2002**, *124*, 742–743.
(11) Patel, D. J.; Phan, A. T.; Kuryavyi, V. *Nucleic Acids Res.* **2007**, *35*, 7429–7455.
(12) Aboulela, F.; Murchie, A. I. H.; Lilley, D. M. J. *Nature* **1992**, *360*, 280–282.
(13) Aboulela, F.; Murchie, A. I. H.; Norman, D. G.; Lilley, D. M. J. *J. Mol. Biol.* **1994**, *243*, 458–471.
(14) Laughlan, G.; Murchie, A. I. H.; Norman, D. G.; Moore, M. H.; Moody, P. C. E.; Lilley, D. M. J.; Luisi, B. *Science* **1994**, *265*, 520–524.
(15) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171–182.

10.1021/ja104889t

the interface of two G-quadruplex units (PDB entry 1S47).[16] A solution-state NMR study revealed the formation of a U-quartet at the 5′ end in the case of a $d(UG_4T)_4$ quadruplex in the presence of Na^+ ions.[13] Interestingly, in the crystal structure, an U-quartet was found at the 3′ end of an analogous RNA $(UG_4U)_4$ quadruplex.[18]

G-quadruplex structures require cations for their formation, structural integrity, and stabilization. In general, cations are localized along the central cavity of a G-quadruplex between two G-quartets or in the plane of a G-quartet.[19−22] However, cations are not coordinated to their preferred binding sites in a static manner but rather exchange between binding sites and with the bulk solution. ^{15}N-labeled ammonium ion has been utilized as a nonmetallic substitute in combination with 2D NMR spectroscopy to localize cations and study the kinetics of their movement.[23,24] Several studies have shown that $^{15}NH_4^+$ ions are localized between a stacked pair of G-quartet planes.[24−27] The kinetics of cation movement is intrinsically correlated with the structural details and local plasticity of the specific G-quadruplex topology. Our recent studies have demonstrated that the kinetics of $^{15}NH_4^+$ ion movement from the interior of the G-quadruplex core to the bulk solution and back are controlled by the topology of loop orientations and specific interactions of loop residues.[25−28]

The 3D structure of $d(TG_4T)_4$ has been determined by NMR and X-ray crystallographic techniques and represents a model that has been used in various studies of G-quadruplexes (e.g., interactions with small molecules[29,30]). One of the aims of the present study was to scrutinize the apparent discrepancy between the solution and crystal structures in order to widen and deepen our understanding of the structural diversity of $d(TG_4T)$ and thus G-quadruplexes in general. To date, a single form of $d(TG_4T)_4$ in solution has been described, whereas the current NMR study has demonstrated the coexistence of two different forms. In addition, analogues of the parent sequence with substitution of U for T at either the 5′ or 3′ end have proved to be extremely valuable in providing further insights into dimerization and macromolecular association of G-quadruplex units in the presence of K^+, Na^+, and $^{15}NH_4^+$ ions in solution. An additional aspect of the study involves evaluation of the dynamics of cation movement in tetramolecular quadruplexes. $^{15}NH_4^+$ ion movement inside monomolecular G-quadruplex structures is few-fold slower than that within bimolecular analogues.[24−28] The exchange rates, however, are tuned through stacking interactions of loop residues on the G-quartets, which in addition to the G-quadruplex molecularity can make a considerable contribution to the G-quartet's rigidity, thus resulting in smaller rate constants.[24−26] At the outset of the study, movement of $^{15}NH_4^+$ ions through G-quartet planes within tetramolecular structure assemblies was expected to be controlled only by the stiffness of individual G-quartets. $d(TG_4T)_4$ can be considered the simplest model of an ion channel, where cation movement is not affected by the topology of loop orientations or interactions of loop residues. Data on anticipated faster $^{15}NH_4^+$ ion movement with respect to monomolecular and bimolecular G-quadruplexes will provide further insights into the breathing motions of G-quartets.

Materials and Methods

Sample Preparation. Oligonucleotides $d(TG_4T)$, $d(TG_4U)$, and $d(UG_4T)$ were purchased from IDT (Leuven, Belgium). Samples were dissolved in 1 mL of H_2O and dialyzed extensively against 10 mM LiCl solution as previously described.[31] The concentrations of the 300 μL NMR samples were between 4.5 and 5.5 mM in strands. $^{15}NH_4Cl$, KCl, and NaCl were titrated into the samples with a pH between 4.5 and 6.0 to a final concentration of 10 mM. We observed only minor spectral changes as a function of pH variation that could be ascribed to protection of the amino groups from exchange with bulk water at lower pH values. The equilibrium between the major and minor forms did not exhibit pH dependence over the pH range from 4.5 to 6.0 used in the current study. The lower pH was used in $^{15}NH_4Cl$-containing samples in order to follow the dynamics of cation movement.

NMR Spectroscopy. All of the NMR spectra were collected on Varian VNMRS 600 and 800 MHz NMR spectrometers. All of the experiments were performed at 298 K. Standard 1D ^1H spectra were acquired with the use of DPFGSE solvent suppression.[32] 2D NOESY and ROESY spectra in H_2O and ^2H_2O were acquired at several mixing times from 40 to 150 ms. Intranucleotide connectivities between imino and aromatic protons were observed by a jump-and-return (JR) HMBC spectrum,[33] which was acquired on natural-abundance samples using 2048 transients. The number of $^{15}NH_4^+$ ion binding sites was determined with the use of $^{15}N-^1H$ HSQC spectra. $^{15}NH_4^+$ ion movement was followed by a series of $^{15}N-^1H$ NzExHSQC spectra[24,34] at mixing times (τ_m) ranging from 13 ms to 7 s. Each binding site is annotated with a single-letter code, except for OG, OG*, OT*, OU, and OU*. In the case of isochronous cross-peaks that correspond to several binding sites, a slash is included (e.g., I2/I2* in Figure 5a). A two-letter code is used to denote the initial and final locations of particular $^{15}NH_4^+$ ions over the course of an NMR pulse sequence (e.g., BI1 and BI2/I2* in Figure 8 for resolved and overlapped cross-peaks, respectively). Diffusion measurements were performed using a spin−echo pulse sequence with gradients.[35]

(16) Caceres, C.; Wright, G.; Gouyette, C.; Parkinson, G.; Subirana, J. A. *Nucleic Acids Res.* **2004**, *32*, 1097–1102.
(17) Creze, C.; Rinaldi, B.; Haser, R.; Bouvet, P.; Gouet, P. *Acta Crystallogr., Sect. D* **2007**, *63*, 682–688.
(18) Deng, J. P.; Xiong, Y.; Sundaralingam, M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13665–13670.
(19) Hud, N. V.; Plavec, J. The Role of Cations in Determining Quadruplex Structure and Stability in Quadruplex Nucleic Acids. In *Quadruplex Nucleic Acids*; Neidle, S., Balasubramanian, S. Eds.; Royal Society of Chemistry: Cambridge, U.K., 2006; pp 100−130.
(20) Engelhart, A. E.; Plavec, J.; Persil, O.; Hud, N. V. Metal Ion Interactions with G-Quadruplex Structures. In *Nucleic Acid−Metal Ion Interactions*; Hud, N. V. Ed.; Royal Society of Chemistry: Cambridge, U.K., 2009; pp 118−153.
(21) Plavec, J. Metal Ion Coordination in G-Quadruplexes. In *Metal Complex−DNA Interactions*; Hadjiliadis, N., Sletten, E. Eds.; Wiley: Chichester, U.K., 2009; pp 55−93.
(22) Hud, N. V.; Plavec, J. *Biopolymers* **2003**, *69*, 144–159.
(23) Hud, N. V.; Schultze, P.; Feigon, J. *J. Am. Chem. Soc.* **1998**, *120*, 6403–6404.
(24) Hud, N. V.; Schultze, P.; Sklenar, V.; Feigon, J. *J. Mol. Biol.* **1999**, *285*, 233–243.
(25) Sket, P.; Plavec, J. *J. Am. Chem. Soc.* **2007**, *129*, 8794–8800.
(26) Podbevsek, P.; Hud, N. V.; Plavec, J. *Nucleic Acids Res.* **2007**, *35*, 2554–2563.
(27) Podbevsek, P.; Sket, P.; Plavec, J. *J. Am. Chem. Soc.* **2008**, *130*, 14287–14293.
(28) Trajkovski, M.; Sket, P.; Plavec, J. *Org. Biomol. Chem.* **2009**, *7*, 4677–4684.
(29) Martino, L.; Virno, A.; Pagano, B.; Virgilio, A.; Di Micco, S.; Galeone, A.; Giancola, C.; Bifulco, G.; Mayol, L.; Randazzo, A. *J. Am. Chem. Soc.* **2007**, *129*, 16048–16056.
(30) Cosconati, S.; Marinelli, L.; Trotta, R.; Virno, A.; Mayol, L.; Novellino, E.; Olson, A. J.; Randazzo, A. *J. Am. Chem. Soc.* **2009**, *131*, 16336–16337.
(31) Crnugelj, M.; Sket, P.; Plavec, J. *J. Am. Chem. Soc.* **2003**, *125*, 7866–7871.
(32) Hwang, T. L.; Shaka, A. J. *J. Magn. Reson., Ser. A* **1995**, *112*, 275–279.
(33) Phan, A. T. *J. Biomol. NMR* **2000**, *16*, 175–178.
(34) Montelione, G. T.; Wagner, G. *J. Am. Chem. Soc.* **1989**, *111*, 3096–3098.
(35) Cevec, M.; Plavec, J. *Biochemistry* **2005**, *44*, 15238–15246.

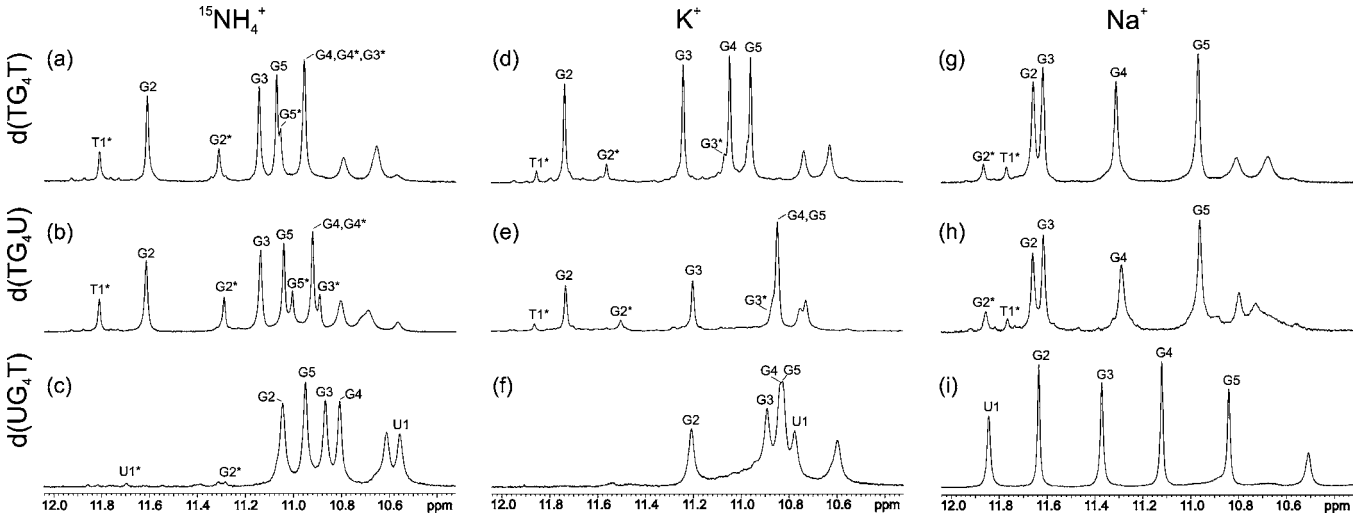

Figure 1. Imino region of the ^{1}H NMR spectra of G-quadruplexes formed by (a, d, g) d(TG_4T), (b, e, h) d(TG_4U), and (c, f, i) d(UG_4T) in the presence of a 10 mM concentration of (a, b, c) $^{15}NH_4Cl$, (d, e, f) KCl, and (g, h, i) NaCl at 25 °C in 5% ^2H_2O. Unlabeled signals between 10.5 and 10.8 ppm correspond to amino protons.

Data Analysis. Volumes of cross-peaks in the $^{15}N-^1H$ HSQC and NzExHSQC spectra were integrated with Varian VNMRJ 2.1B software. Iterative least-squares fitting was done with Origin 7.5 software (www.originlab.com). Determinations of exchange rate constants for $^{15}NH_4^+$ ion movements were done with the use of the following equation: $V_c(\tau_m) = A[e^{-(\tau_m/T_{1c})}(1 - e^{k_N\tau_m})]$, where V_c represents the volume integral of a cross-peak, T_{1c} is the corresponding spin−lattice relaxation time, τ_m is the mixing time of the 2D NzExHSQC experiment, and k_N corresponds to the rate constant for $^{15}NH_4^+$ ion movement.[25] The accuracy of the cross-peak volume integrations was hampered by the huge signal from bulk ammonium ion.

Native PAGE. Aliquots of NMR samples of d(TG_4U) and d(UG_4T) in the presence of NaCl and KCl, respectively, together with a GeneRuler Ultra Low Range DNA ladder with 10−300 base pairs (Fermentas) were loaded and resolved on a 20% native PAGE gel and stained with Stains All (Sigma-Aldrich) according to the manufacturer's instructions. The concentrations of oligonucleotides and cations were ∼0.2 and 10 mM, respectively.

Results

Formation of T- and U-Quartets. d(TG_4T) and its U analogues were folded into G-quadruplex structures upon titration of $^{15}NH_4Cl$, KCl, and NaCl into aqueous solutions of carefully dialyzed oligonucleotides. Increasing the concentration of the three monovalent cations to 10 mM resulted in well-resolved imino, aromatic, and other proton resonances in the ^{1}H NMR spectra (Figure 1). In order to allow sufficient time for G-quadruplex formation, we took special care with the kinetics of folding because of the notoriously slow formation of tetramolecular G-quadruplexes.[36−38] No apparent spectral changes could be observed 2−3 weeks after the addition of cations. In the case of d(TG_4T) and d(TG_4U), two sets of ^{1}H NMR signals indicated the formation of two species with different populations in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions (Figure 1). The ratio between the major and minor forms of the d$(TG_4T)_4$ quadruplex in the presence of $^{15}NH_4^+$ ions was 70:30, while it was 85:15 in the presence of K^+ or Na^+ ions (Figure 1a,d,g). During the course of the folding process, we observed both the major and minor forms in the early stages of structure formation. Interestingly, the relative ratio of the two forms did not change with time, while the appearance of G-quadruplex forms was more and more pronounced over a few weeks of formation. Replacement of T with U at the 3′ end did not influence the ratio between the major and minor forms considerably (Figure 1b,e,h). In contrast, substitution of T with U at the 5′ end resulted in a well-resolved imino signal corresponding to almost exclusive (>98%) formation of a single species in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions (Figure 1c,f,i). It is interesting to note that the imino chemical shift range of the major form of the G-quadruplex adopted by d(UG_4T) is narrower for $^{15}NH_4^+$ and K^+ ions than for Na^+ ions. In contrast to its 5′-T analogues, for which only the minor form showed observable thymine imino signals, d$(UG_4T)_4$ exhibited sharp uracil imino signals for the major form (Figure 1c,f,i). Comparison of the chemical shifts of the imino protons of the T or U residues in the three oligonucleotides suggests the formation of different G-quadruplex structures. Interestingly, the chemical shifts of the T1*(H3) protons (11.76−11.84 ppm) of the minor forms of d$(TG_4T)_4$ and d$(TG_4U)_4$ in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions were comparable to the chemical shifts observed for the U1(H3) protons (11.83 ppm) of the G-quadruplex formed by d(UG_4T) in the presence of Na^+ ions. In contrast, the chemical shifts of the U1(H3) protons involved in U-quartets of the G-quadruplex formed by d(UG_4T) in the presence of $^{15}NH_4^+$ and K^+ ions were shifted significantly upfield, implying the existence of different local environments correlated with the different structures of the respective quadruplexes (Figure 1c, f).

The aromatic−anomeric region of the 2D NOESY spectra of the G-quadruplexes formed by d(TG_4T) and d(TG_4U) clearly revealed two continuous 5′−3′ sequential NOE connectivities in the presence of all three cations. As can be seen in Figure 2a, the sequential connectivities in the major and minor forms of d(TG_4T) were typical for residues in the anti conformation. Analogously, the G-quadruplexes formed by d(UG_4T) in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions exhibited continuous 5′−3′ sequential NOE connectivities from U1 to T6 in the aromatic−anomeric region of the NOESY spectra (Figure 2b−d).

(36) Mergny, J. L.; De Cian, A.; Ghelab, A.; Sacca, B.; Lacroix, L. *Nucleic Acids Res.* **2005**, *33*, 81–94.
(37) Bardin, C.; Leroy, J. L. *Nucleic Acids Res.* **2008**, *36*, 477–488.
(38) Rachwal, P. A.; Fox, K. R. *Methods* **2007**, *43*, 291–301.

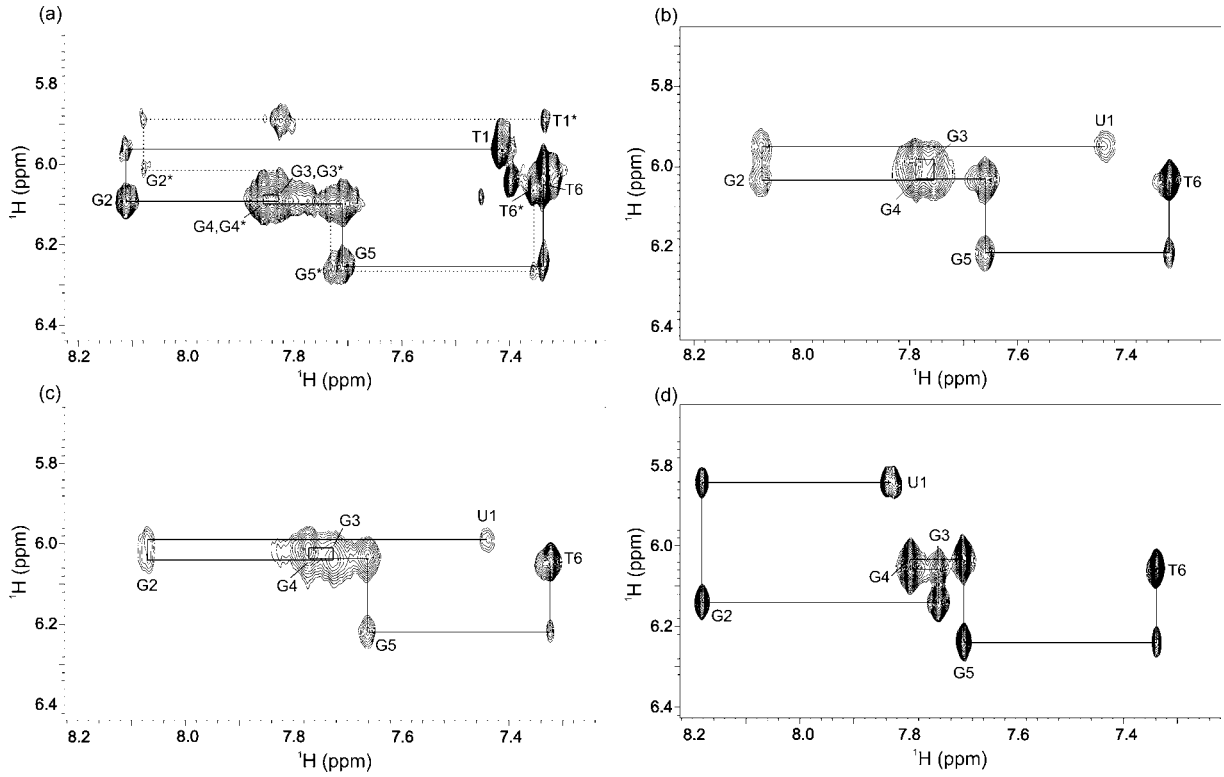

Figure 2. Aromatic−anomeric region of the NOESY spectra (τ_m = 150 ms) of (a) d(TG$_4$T) in the presence of 10 mM $^{15}NH_4^+$ ions and (b−d) d(UG$_4$T) in the presence of 10 mM (b) $^{15}NH_4^+$, (c) K^+, and (d) Na^+ ions at 25 °C in 100% ^2H_2O. Sequential aromatic−H1′ connectivities are labeled by solid lines. In panel (a), dotted lines represent aromatic−H1′ sequential connectivities for the minor form.

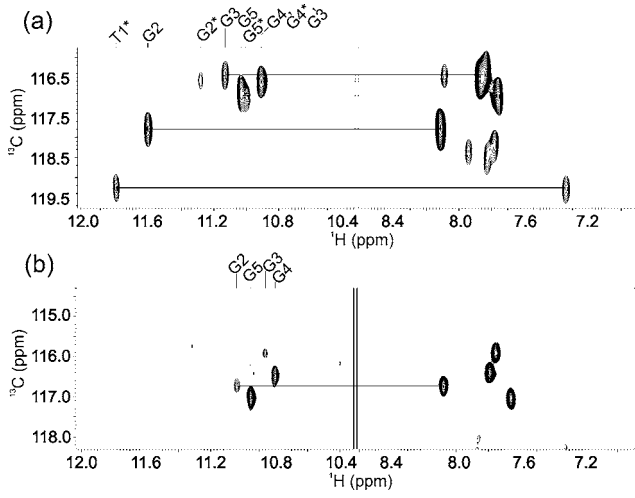

Figure 3. Through-bond correlations between imino and aromatic protons in the JR-HMBC spectra of G-quadruplexes formed by (a) d(TG$_4$U) and (b) d(UG$_4$T) in the presence of 10 mM $^{15}NH_4^+$ ions at 25 °C.

The intraresidual correlations of imino and aromatic protons by the use of a JR-HMBC experiment enabled their complete and unequivocal assignment in both the major and minor forms (Figure 3).

Formation of Dimers. The hydrodynamic dimensions of the G-quadruplexes formed by d(TG$_4$T) and its U analogues were assessed by NMR diffusion measurements. Translational diffusion coefficients (D_t) were determined from the slopes of Stejskal−Tanner plots (Figure S1 in the Supporting Information).[39] The D_t values for the G-quadruplexes formed by d(TG$_4$T) and d(TG$_4$U) in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions are 1.46 (±0.04) × 10^{-6} cm^2 s^{-1}. These values are in excellent agreement with the published data for d(TG$_4$T) in the presence of Na^+ ions.[40] Calculations utilizing a spherical model afforded a hydrodynamic diameter of 27.5 Å (without a hydration layer of 2 × 2.8 Å), which is in reasonable agreement with the length and diameter (∼21 and ∼25 Å, respectively) of one of the molecules from the X-ray structure of d(TG$_4$T)$_4$ with the 5′-T quartet (PDB entry 1S47).[16] Attempts to utilize the oblate ellipsoid model did not result in a better representation of the hydrodynamic behavior of the quadruplex structures in solution under the cationic conditions used.

The translational diffusion coefficient of d(UG$_4$T)$_4$ in the presence of Na^+ ions is 1.47 (±0.04) × 10^{-6} cm^2 s^{-1}, which is equivalent to the values for d(TG$_4$T)$_4$ and d(TG$_4$U)$_4$ and therefore suggests a similar size for the two G-quadruplex structures. In contrast, the D_t values for d(UG$_4$T) folded in the presence of $^{15}NH_4^+$ and K^+ ions were 1.17 (±0.04) × 10^{-6} and 1.15 (±0.04) × 10^{-6} cm^2 s^{-1}, respectively. The smaller D_t values indicate the formation of a larger species. Calculation of the length of the G-quadruplex structure using a cylindrical model with the assumption of a hydrodynamic diameter of 30.8 Å across the G-quartet (25.2 Å for a G-quartet[16] + 2 × 2.8 Å for the hydration layer) gave lengths of 32−34 Å (without the hydration layer) in the presence of $^{15}NH_4^+$ and K^+ ions. However, these relative values of diameter and length do not warrant the use of a cylindrical model. Calculations using a spherical model afforded hydrodynamic diameters of 35.7 and 36.5 Å (without hydration layer) for solutions containing $^{15}NH_4^+$ and K^+ ions, respectively, suggesting formation of a dimeric

(39) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288–292.

(40) Ida, R.; Wu, G. *J. Am. Chem. Soc.* **2008**, *130*, 3590–3602.

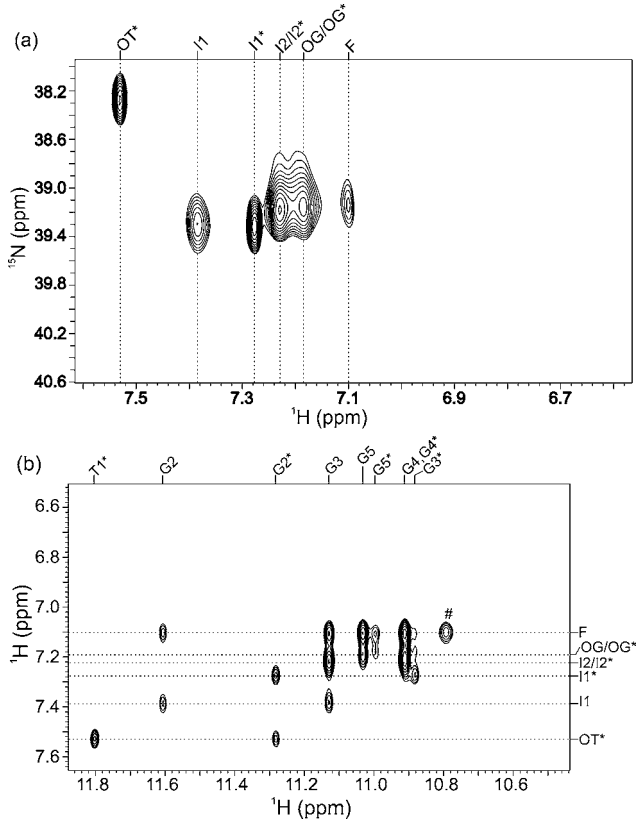

Figure 4. (a) ^{15}N−^{1}H HSQC spectra and (b) ROESY spectra (τ_m = 80 ms) of G-quadruplexes adopted by d(TG$_4$U) in the presence of 10 mM $^{15}NH_4^+$ ions at 25 °C. Cross-peaks corresponding to the minor forms in (a) and (b) are labeled with stars. The signal labeled with F in (a) corresponds to $^{15}NH_4^+$ ions involved in a very fast exchange. The ROESY spectrum in (b) shows cross-peaks between the bound $^{15}NH_4^+$ ions and the nearby imino protons. The cross-peak labeled with # in (b) is attributed to exchange.

structure. The values are in very good agreement with the length of ~35 Å from the X-ray crystal structure of a dimer of a tetramolecular G-quadruplex (PDB entry 1S45).[16] It is interesting to note that minor signals in the case of d(UG$_4$T) in the presence of $^{15}NH_4^+$ ions exhibit a D_t of 1.46 (±0.04) × 10^{-6} cm^2 s^{-1}, which corresponds to monomeric forms. In order to confirm the formation of a larger species, we also performed native PAGE (Figure S2 in the Supporting Information). To further explore the dimensions of the G-quadruplexes, we utilized the Hydropro program[41] using coordinates from the X-ray structure of d(TG$_4$T)$_4$ with the 5′-T quartet (PDB entry 1S47)[16] and the corresponding dimeric structure (PDB entry 1S45).[16] The calculated D_t values of 1.34 × 10^{-6} and 1.10 × 10^{-6} cm^2 s^{-1} were in very good agreement with our experimental data, affirming the formation of monomeric and dimeric forms.

Localization of $^{15}NH_4^+$ Ions within Cation Channels of d(TG$_4$T)$_4$ and d(TG$_4$U)$_4$. 2D NOESY, ROESY, and ^{15}N−^{1}H HSQC experiments were used to establish $^{15}NH_4^+$ ion binding sites within G-quadruplexes formed by d(TG$_4$T) and its 3′-U analogue. Analysis of the ^{15}N−^{1}H HSQC spectra revealed six resolved cross-peaks corresponding to $^{15}NH_4^+$ ions in distinct chemical environments inside the G-quadruplexes formed by d(TG$_4$T) and d(TG$_4$U) (Figure 4a). It is interesting to note that one of the cross-peaks was observed at the proton chemical shift of bulk ammonium ions (labeled F in Figure 4a). ROESY

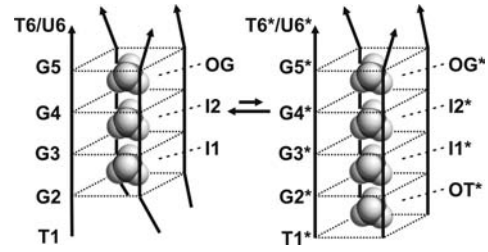

Figure 5. Equilibrium between the (left) major and (right) minor G-quadruplex structures adopted by d(TG$_4$T)$_4$ and d(TG$_4$U)$_4$. Individual residues are noted in one strand of each structure; stars indicate the minor structure. $^{15}NH_4^+$ ion binding sites in the interior of each form are labeled using dotted lines.

spectra were used to assign and localize the $^{15}NH_4^+$ ions within the G-quadruplexes (Figure 4b). $^{15}NH_4^+$ ions resonating at 7.39 ppm (binding site I1) showed cross-peaks to imino protons of G2 and G3 residues that belong to the major form of the G-quadruplex formed by d(TG$_4$T) and d(TG$_4$U) (Figure 4b). $^{15}NH_4^+$ ions resonating at 7.23 ppm were localized between the G3 and G4 quartets in both the major and minor forms (I2/I2*). $^{15}NH_4^+$ ions at binding sites OG and OG* in the major and minor forms resonated at 7.19 ppm and showed cross-peaks with the imino protons of G4 and G5 in the major form and of G4* and G5* in the minor form. The similarity of the ^{1}H and ^{15}N chemical shifts of the cross-peaks corresponding to I2 and I2* as well as OG and OG* ammonium binding sites indicates a close resemblance of the (de)shielding in the interiors of the major and minor forms of the G-quadruplexes formed by d(TG$_4$T) and d(TG$_4$U). $^{15}NH_4^+$ ions resonating at 7.53 ppm (OT*) and 7.28 ppm (I1*) both showed cross-peaks to imino protons of G2* in d(TG$_4$T)$_4$ and d(TG$_4$U)$_4$. In addition, $^{15}NH_4^+$ ions at binding site OT* exhibited a cross-peak with the imino protons of T1*, while $^{15}NH_4^+$ ions localized at binding site I1* displayed a cross-peak to G3* (Figure 4b). Three $^{15}NH_4^+$ ion binding sites are thus localized between the pairs of adjacent G-quartets in the major forms of d(TG$_4$T)$_4$ and d(TG$_4$U)$_4$ (Figure 5 left). There is an additional binding site, OT* in the minor forms of d(TG$_4$T)$_4$ and d(TG$_4$U)$_4$, that is closed by a T-quartet at the 5′ end (Figure 5 right). It is interesting to note that ROESY cross-peaks to the proton chemical shift of bulk $^{15}NH_4^+$ ions were observed for all of the imino protons of the major and minor forms except T1* and G2* of the minor form (see below).

$^{15}NH_4^+$ Ion Localization within d[(UG$_4$T)$_4$]$_2$. The ^{15}N−^{1}H HSQC spectrum of the d[(UG$_4$T)$_4$]$_2$ G-quadruplex in the presence of $^{15}NH_4^+$ ions exhibited three resolved cross-peaks corresponding to $^{15}NH_4^+$ ions at different binding sites inside the major dimeric structure and three weaker cross-peaks corresponding to $^{15}NH_4^+$ ions inside the minor monomeric form of d(UG$_4$T)$_4$ with the T-quartet at the 5′ end (Figure 6a).

The ratio of the volumes of the HSQC cross-peaks corresponding to the I1:I2/OG:OU binding sites for the dimeric form was 2.0:3.8:1.8, while the relative volume ratio for the OU*:I1*:I2*/OG* cross-peaks of the monomeric form was 0.7:0.9:2.0 (with the actual fraction of 2% for the monomeric form). The ROESY spectrum of d[(UG$_4$T)$_4$]$_2$ exhibited contacts of $^{15}NH_4^+$ ions resonating at an ^{1}H chemical shift of 6.65 ppm (OU) with the U1 and G2 imino protons (Figure 6b). $^{15}NH_4^+$ ions at 7.12 ppm (I1) showed cross-peaks with the imino protons of residues G2 and G3. $^{15}NH_4^+$ ions resonating at 7.09 ppm (I2/OG) showed contacts to imino protons of G3, G4, and G5 (Figures 6b). Examination of the above and other NMR data suggested that d(UG$_4$T) adopts a dimeric structure in which two

(41) Fernandes, M. X.; Ortega, A.; Martinez, M. C. L.; de la Torre, J. G. *Nucleic Acids Res.* **2002**, *30*, 1782–1788.

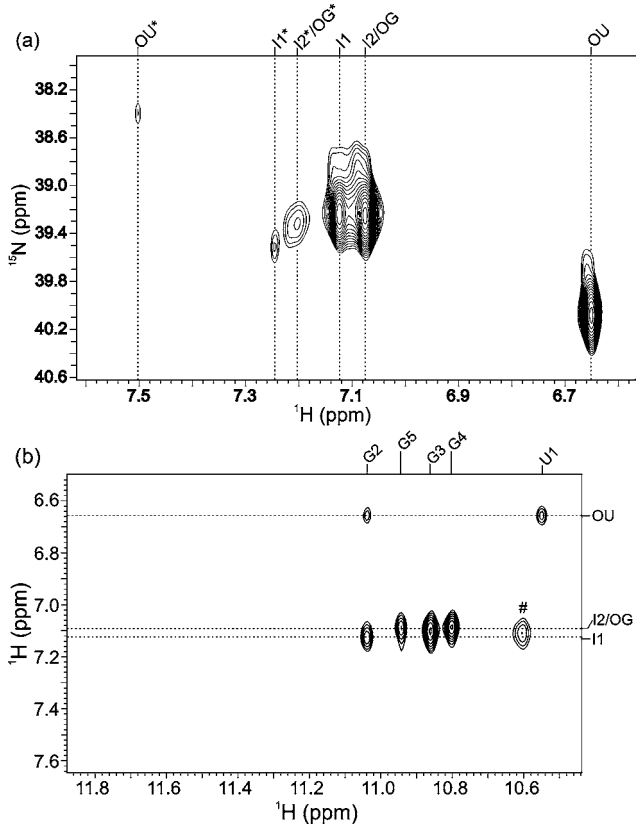

Figure 6. (a) $^{15}N-^{1}H$ HSQC spectra and (b) ROESY spectra ($\tau_m = 40$ ms) of the G-quadruplexes adopted by $d(UG_4T)$ in the presence of 10 mM $^{15}NH_4^+$ ions at 25 °C. Residues corresponding to the minor form in (a) are labeled with stars. The ROESY spectrum in (b) shows cross-peaks between the bound $^{15}NH_4^+$ ions and the nearby imino protons. The cross-peak labeled with # in (b) is attributed to exchange.

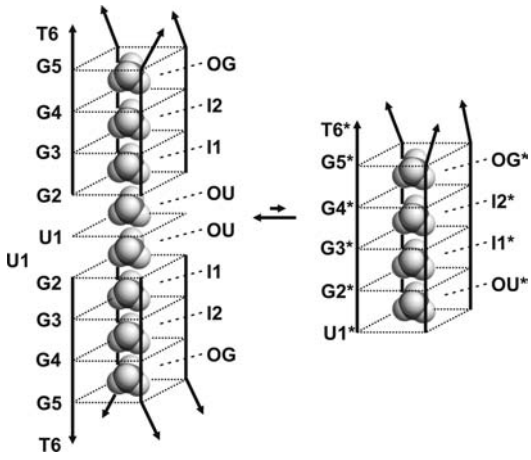

Figure 7. Equilibrium between the dimeric and monomeric forms of $d(UG_4T)_4$. Individual residues are noted in one strand of each structure; stars indicate the minor structure. $^{15}NH_4^+$ ion binding sites in the interior of each forms are labeled using dotted lines.

$d(UG_4T)_4$ G-quadruplex units interact in a head-to-head fashion by sharing a common U1-quartet at the 5′ ends (Figure 7). A single U1(H3) resonance corresponding to four H-bonded imino protons involved in a U-quartet was observed. The volume integral ratios of the cross-peaks in the HSQC spectrum together with the cross-peaks in the ROESY spectrum suggest that there are six $^{15}NH_4^+$ ion binding sites between the eight G-quartets and an additional two at the interfaces of the two $d(UG_4T)_4$ quadruplex units (Figure 7). The latter two binding sites involve a G2-quartet and a U1-quartet shared by two G-quadruplexes. The two OU binding sites are symmetry-related, and the ^{1}H and ^{15}N chemical shifts of the $^{15}NH_4^+$ ions residing at these sites were isochronous.

The T-Quartet in $d(TG_4T)_4$ and $d(TG_4U)_4$ Controls the Kinetics of Cation Movements. The appearance of cross-peaks in addition to autocorrelation peaks in the 2D $^{15}N-^{1}H$ NzExH-SQC spectra clearly demonstrates that $^{15}NH_4^+$ ions move from one binding site to another as well as into the bulk solution and back (Figure 8). The BI1 cross-peak demonstrates $^{15}NH_4^+$ ion movement from the bulk solution into binding site I1 in the major forms of the G-quadruplexes adopted by $d(TG_4T)$ and $d(TG_4U)$ (Figures 5 and 8a). The cross-peaks BOT* and OT*B indicate $^{15}NH_4^+$ ion exchange between the bulk solution and the OT* binding site in the minor forms. In addition, $^{15}NH_4^+$ ions move from the bulk solution into the binding sites OG and OG* at the other end of the G-quadruplex structure (BOG/OG* cross-peak, Figure 8a). The cross-peak BI2/I2* in Figure 8a is a result of a double $^{15}NH_4^+$ ion movement, the first from the bulk solution into the binding sites OG/OG* and the second from OG/OG* to the binding sites I2/I2* of the G-quadruplex, which can be observed only at mixing times longer than 40 ms. A single cross-peak was observed for the movement of $^{15}NH_4^+$ ions from the G-quadruplex into the bulk solution (i.e., I1B, I2/I2*B, OG/OG*B, and I1*B). The cross-peaks OT*I1* and I1*OT* demonstrate $^{15}NH_4^+$ ion movement within the G-quadruplex.

The intensities of the cross-peaks are directly related to the number of $^{15}NH_4^+$ ions that move from one binding site to the other during the (user-defined) mixing time. Comparison of the intensities of the BI1 and BOT* cross-peaks clearly shows that movement of $^{15}NH_4^+$ ions from the bulk solution into the $d(TG_4T)_4$ and $d(TG_4U)_4$ quadruplexes is by far more effective in the absence of a T-quartet (Figure 8a). Quantitative analysis of the cross-peak volumes as a function of mixing time afforded rate constants. The movement of $^{15}NH_4^+$ ions from the bulk solution into binding site I1 in the major forms of $d(TG_4T)_4$ and $d(TG_4U)_4$ is characterized by the rate constant $k_{BI1} = 48 \pm 26\ s^{-1}$. The large error limits are a result of the restricted number of experimental points at short mixing times. For comparison, the rate constant k_{BOT*} for the movement from the bulk solution into binding site OT* in the minor forms of $d(TG_4T)_4$ and $d(TG_4U)_4$ is $2.1 \pm 0.5\ s^{-1}$ (Figure 9).

The reverse movement from OT* to the bulk solution is characterized by the rate constant $k_{OT*B} = 2.1 \pm 0.2\ s^{-1}$ in the minor forms of $d(TG_4T)_4$ and $d(TG_4U)_4$. Quantitative analysis of the BOG/OG* cross-peak corresponding to $^{15}NH_4^+$ ion movement from the bulk solution into the G-quadruplex through the 3′ ends (i.e., the G5-quartet) of the major and minor forms provided the apparent rate constant $k_{BOG/OG*} = 43 \pm 14\ s^{-1}$ (Figure 9). It is interesting to note that $k_{BOG/OG*}$ is similar to the rate constant k_{BI1} corresponding to the movement of $^{15}NH_4^+$ ions from the bulk solution into the major form of the $d(TG_4U)_4$ quadruplex through the 5′ end (i.e., the G2-quartet). Quantification of a cross-peak corresponding to I1B, I2/I2*B, and OG/OG*B movements afforded an apparent rate constant of $39 \pm 4\ s^{-1}$. Unfortunately, the almost identical chemical shifts of the autocorrelation peaks for I1, I1*, I2/I2*, and OG/OG* along the ^{15}N dimension prevented quantitative analysis of the $^{15}NH_4^+$ ion movement among the I1, I2, and OG sites in the major form and the I1*, I2*, and OG* sites in the minor form. $^{15}NH_4^+$ ion movement between the OT* and I1* binding sites in the minor

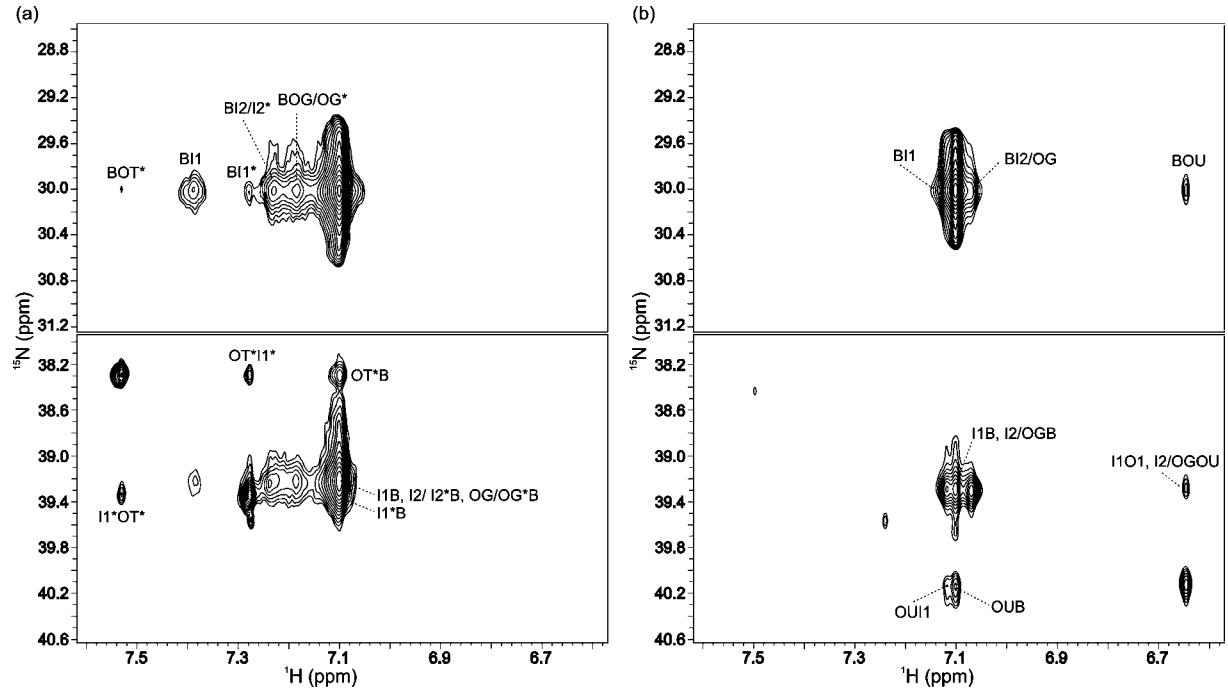

Figure 8. Plots of 2D ^{15}N−^{1}H NzExHSQC spectra of the G-quadruplexes adopted by (a) d(TG$_4$U) at a mixing time of 300 ms and (b) d(UG$_4$T) at a mixing time of 120 ms at 25 °C and pH 4.5 in 5% $^{2}H_2O$. The cross-peaks are labeled with two letters, where the first indicates the initial and the second the final binding site. Slashes indicate cross-peaks with isochronous chemical shifts in the ^{1}H and ^{15}N dimensions. The oligonucleotide concentration was 4.9 mM in strands, while the concentration of $^{15}NH_4Cl$ was 10 mM.

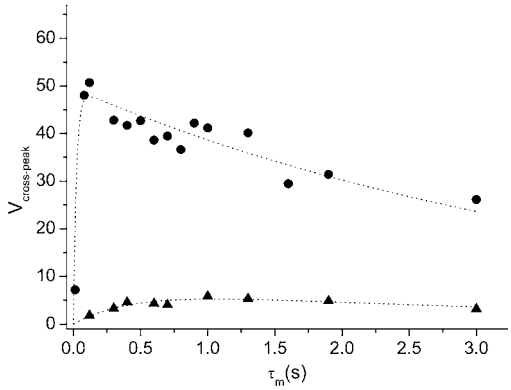

Figure 9. Relative volumes of BOG/OG* (●) and BOT* (▲) cross-peaks as a function of mixing time (τ_m) for d(TG$_4$U)$_4$ at 25 °C. Iterative least-squares fits (shown as dotted lines) afforded the rate constant values $k_{BOG/OG*} = 43 \pm 14$ s^{-1} and $k_{BOT*} = 2.1 \pm 0.5$ s^{-1}. The least-squares fits of $V_{BOG/OG*}$ and V_{BOT*} are characterized by rmsd's of 5.7 ($R = 0.847$) and 0.5 ($R = 0.911$) arbitrary volume units, respectively.

form is characterized by the rate constants $k_{OT*I1*} = 0.5 \pm 0.1$ s^{-1} and $k_{I1*OT*} = 0.6 \pm 0.1$ s^{-1}. Cation movement within a G-quadruplex with a T-quartet at the 5′ end as well as exchange with the bulk solution proceeds in two different rate regimes. The slower regime occurs between binding sites OT* and I1* as well as with the bulk solution at the 5′ end, while the faster regime is observed between binding sites I2* and OG* and the bulk solution at the 3′ end. The fast-movement regime in the major and minor forms is supported by cross-peaks between $^{15}NH_4^+$ ions marked with F and the imino protons in the ROESY spectra (Figure 4b). It is noteworthy that the imino protons of residues T1* and G2* in the minor form do not give ROE contacts with the $^{15}NH_4^+$ ions marked with F.

$^{15}NH_4^+$ Ion Movement Inside the Dimeric G-Quadruplex. The cross-peaks BI1, BI2/OG, and BOU demonstrate ion movement from the bulk solution into the d[(UG$_4$T)$_4$]$_2$ G-quadruplex (Figure 8b). The cross-peak OUB was observed for movement from binding site OU into the bulk solution. Ion movements from binding sites I1, I2, and OG to the bulk solution are evidenced by a single cross-peak denoted as I1B, I2/OGB. $^{15}NH_4^+$ ion movement inside the central cavity is evidenced by the single cross-peak OUI1 indicating ion movement from binding site OU to binding site I1. The single cross-peak I1OU, I2/OGOU was observed for the $^{15}NH_4^+$ ion movement from I1 to OU as well as from I2 and OG to OU. Only the former is compatible with a single-step $^{15}NH_4^+$ ion movement. Subsequent quantitative analysis of the resolved cross-peaks corresponding to the movement of $^{15}NH_4^+$ ions between the bulk solution and binding site OU afforded a value of 5.7 ± 2 s^{-1} for rate constants k_{OUB} and k_{BOU}. However, it is noteworthy that in the dimeric structure shown in Figure 7, OUB and BOU represent four-step movements. In full support of the four-step movement, the ROESY spectra at mixing times longer than 80 ms show contacts of $^{15}NH_4^+$ ions with a ^{1}H chemical shift of 6.65 ppm (OU) to the imino protons of all of the guanine residues in the four intervening G-quartets.

Discussion

Earlier NMR studies have shown that d(TG$_4$T) in the presence of Na^+ ions in solution adopts a tetramolecular G-quadruplex structure in which all of the strands are parallel and all of the guanine residues are in the anti conformation.[12,13] In the current study, solution-state NMR spectroscopy was used to deepen our understanding of the structural diversity of d(TG$_4$T) and its U analogues and evaluate the role of $^{15}NH_4^+$, K^+, and Na^+ ions in controlling their folding into tetramolecular G-quadruplexes and in further association. Our data clearly show that d(TG$_4$T)$_4$ is involved in the equilibrium between two monomeric units; the minor form consists of a T-quartet at the 5′ end in addition to the four G-quartets, and the major form is the well-known

tetramolecular quadruplex consisting of only four G-quartets. The ratio of the two forms varies slightly with the choice of cation and is between 85:15 and 70:30. Replacement of T with U at the 3′ end exerted no apparent effect on the formation and structural equilibrium between the two monomeric quadruplex units. On the other hand, when T was replaced with U at the 5′ end, dimeric forms were established in the presence of $^{15}NH_4^+$ and K^+ ions, while a monomeric form containing a U-quartet at the 5′ end predominated in the presence of Na^+ ions. The dimeric $d[(UG_4T)_4]_2$ quadruplex involves a common U-quartet that is flanked in a symmetric manner by the four G-quartets on each side. It is interesting to note that dimerization of tetramolecular G-quadruplexes was observed only with U at the 5′ end of the sequence and with relatively larger $^{15}NH_4^+$ and K^+ ions. As T differs from U only in the presence of a methyl group at C5 instead of a proton, steric interactions of the methyl group might be one of the reasons that the dimerization of $d(TG_4T)_4$ quadruplex units is hampered. Oligonucleotides containing a stretch of consecutive guanines without pyrimidine residues at the 5′ or 3′ ends were shown to form higher-order structures.[42−45] Although the details of such structures are not well-known at the molecular level, formation of higher aggregates can be hampered by the presence of a phosphate group at the 5′ end as a result of unfavorable charge−charge repulsions among the negatively charged groups, which disrupt the stacking interactions of the two monomeric units.[46,47] One of the driving forces of dimerization and possibly oligomerization of G-quadruplexes is most likely hydrophobic stabilization, which leads to the exclusion of a great number of solvent molecules and exposure of a smaller number of aromatic quartet faces to the bulk solvent.

Our data have established that T- and U-quartets can form at the 5′ ends of a G-quadruplex core, whereas only a U-quartet was found at the interface of two quadruplex units. ^{1}H NMR chemical shifts of the imino protons of T and U provide information on the vicinity and spatial relationship with respect to the nearby G-quartet, since protons experience strong shielding by ring-current effects. The U1(H3) protons of the G-quadruplexes formed by $d(UG_4T)$ in the presence of $^{15}NH_4^+$ and K^+ ions are shifted upfield by ∼1 ppm with respect to the chemical shifts observed for the T1(H3) protons of $d(TG_4T)_4$ and $d(TG_4U)_4$ in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions. In contrast, the U1(H3) protons of $d(UG_4T)$ in the presence of Na^+ ions exhibit a downfield chemical shift of 11.83 ppm. The deshielding of the imino protons of U and T is in accordance with formation of terminal U- and T-quartets, while the shielding of U1(H3) suggests that the U-quartet is placed between two G-quartets in the interior of $d[(UG_4T)_4]_2$. In complete agreement with the above observations, the imino protons of thymine residues involved in the middle T-quartet of the G-quadruplex adopted by a telomere repeat of *Saccharomyces cerevisiae*, d(TGGTGGC), exhibit strong shielding ($\delta \approx 10$ ppm).[48] The chemical shifts of the imino protons of pyrimidine residues could represent a simple and efficient method for assessment of the character of stacking of respective T- or U-quartets.

Three $^{15}NH_4^+$ ion binding sites between the four G-quartets in the major forms of $d(TG_4T)_4$ and $d(TG_4U)_4$ were identified. An additional $^{15}NH_4^+$ ion binding site was established in the minor forms of the $d(TG_4T)_4$ and $d(TG_4U)_4$ quadruplexes between G- and T-quartets at the 5′ end. The dimeric $d[(UG_4T)_4]_2$ G-quadruplex with a common U-quartet that is shared by two G-quadruplex units exhibits eight $^{15}NH_4^+$ ion binding sites. Two of the cation binding sites are localized between the U-quartet and the adjacent G-quartets. In contrast, seven sodium ions were found between the eight G-quartets in several X-ray crystallographic structures of $d[(TG_4T)_4]_2$.[14,15,17] However, in one of these structures (PDB entry 352D), four calcium ions were found in the region between two stacked quadruplexes in addition to seven sodium ions.[15] A related study on crystallization of $d(TG_4T)$ in a mixed Ca^{2+}/Na^+ environment led to a structure with seven cations that are asymmetrically distributed along the central cation cavity of a quadruplex dimer.[49] One of the X-ray structures formed by $d(TG_4T)$ showed that the G-quartets were stabilized by two Na^+ and five Tl^+ ions in a dimeric form (a total of seven cations per 2 × 4 G-quartets).[16] The trimeric form involving a T-quartet exhibits six Na^+ and six Tl^+ ions (a total of 12 cations per 3 × 4 G-quartets + 1 T-quartet).[16] A single resonance corresponding to monovalent cations (e.g., Na^+, K^+, and Rb^+) residing inside the channel of $d(TG_4T)_4$ G-quadruplexes was observed by solution-state NMR spectroscopy.[40,50] Unfortunately, direct NMR measurements of metal ions do not offer sufficient resolution on details of cation localization within a G-quadruplex core. A study of a related tetramolecular $d(T_2G_4T_2)_4$ structure by solution-state ^{205}Tl NMR spectroscopy revealed three resolved peaks having approximately equal intensities corresponding to three Tl^+ ions bound between the four G-quartets.[51]

Our data show that cations inside G-quadruplexes are not static but rather are mobile and exchange between binding sites and the bulk solution in a way that is reminiscent of ion channels. Earlier studies of mono- and bimolecular systems established that G-quartets have to open partially for $^{15}NH_4^+$ ions to come through. Therefore, the stiffness of G-quadruplex structures has been predicted to play a major role in cation transport along the central cation cavity. The data presented in this study unequivocally prove that $^{15}NH_4^+$ ions move between binding sites and the bulk solution more rapidly in tetramolecular structures than in monomolecular and bimolecular G-quadruplexes.[24–27] However, when a T-quartet is present at the 5′ end of a tetramolecular G-quadruplex core, such as in the minor forms of $d(TG_4T)_4$ and $d(TG_4U)_4$, the exchange rate of $^{15}NH_4^+$ ion movement from the bulk solution into the G-quadruplex decreases by a factor of 20. It is fascinating that no change in the rate of $^{15}NH_4^+$ ion movement in the above quadruplexes was noticed at their 3′ ends. A T-quartet at the 5′ end locally restricts the flexibility and breathing motions of tetramolecular structures. Examination of the differences in exchange rate constants of $^{15}NH_4^+$ ions from different binding sites suggests that binding of a cation is stronger between $T_4||G_4$-quartets than between $G_4||G_4$-quartets or, alternatively, that there

(42) Sen, D.; Gilbert, W. *Biochemistry* **1992**, *31*, 65–70.
(43) Guo, Q.; Lu, M.; Kallenbach, N. R. *Biochemistry* **1993**, *32*, 3596–3603.
(44) Marsh, T. C.; Henderson, E. *Biochemistry* **1994**, *33*, 10718–10724.
(45) Krishnan-Ghosh, Y.; Liu, D. S.; Balasubramanian, S. *J. Am. Chem. Soc.* **2004**, *126*, 11009–11016.
(46) Uddin, M. K.; Kato, Y.; Takagi, Y.; Mikuma, T.; Taira, K. *Nucleic Acids Res.* **2004**, *32*, 4618–4629.
(47) Biyani, M.; Nishigaki, K. *Gene* **2005**, *364*, 130–138.
(48) Patel, P. K.; Hosur, R. V. *Nucleic Acids Res.* **1999**, *27*, 2457–2464.
(49) Lee, M. P. H.; Parkinson, G. N.; Hazel, P.; Neidle, S. *J. Am. Chem. Soc.* **2007**, *129*, 10106–10107.
(50) Wong, A.; Ida, R.; Wu, G. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 363–366.
(51) Basu, S.; Szewczak, A.; Cocco, M.; Strobel, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 3240–3241.

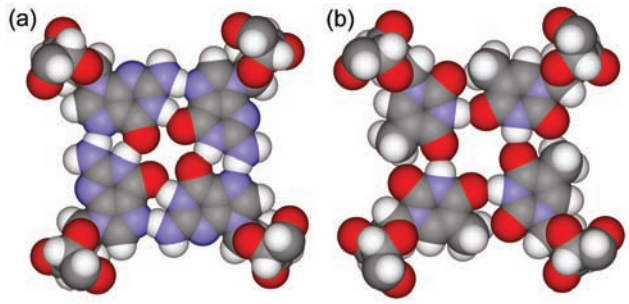

Figure 10. Comparison of space-filling models of (a) G-quartets and (b) T-quartets from the structure of $d(TGGTGGC)_4$ (PDB entry 1EMQ).[48]

is a higher energy barrier for their movement through a T-quartet. Perusal of space-filling models of G- and T-quartets indicates a bigger central hole in the latter, which suggests that movement of $^{15}NH_4^+$ ions through a T-quartet could be faster (Figure 10). As the experimental data clearly disprove this prediction, the size of a quartet's central hole is not the major determinant in controlling cation movement. The electrostatic interactions between oxygen atoms and the cations probably hinder their movement by strengthening H-bonds and/or stacking interactions. Formation of T-quartets might find applications in the design of artificial ion channels to serve as regulators for cation transport.

Conclusions

Solution-state NMR spectroscopy has been used to deepen our understanding of the structural diversity of a simple oligonucleotide $d(TG_4T)$ as well as its U analogues in the presence of $^{15}NH_4^+$, K^+, and Na^+ ions. The NMR experiments have demonstrated the existence of an equilibrium between two monomeric forms of $d(TG_4T)_4$ quadruplexes in the presence of all three cations that is slow on the NMR time scale. The minor monomeric form consists of a T-quartet at the 5′ end in addition to four G-quartets. The major form is the well-known tetramolecular G-quadruplex consisting of only four G-quartets. Replacement of T with U at the 3′ end of the parent sequence has only a slight influence on the above equilibrium between the two forms. On the other hand, replacement of T with U at the 5′ end leads to dimerization of the G-quadruplex units in the presence of K^+ and $^{15}NH_4^+$ ions. In contrast, the presence of Na^+ ions promotes the formation of a monomeric G-quadruplex with a U-quartet at the 5′ end. The use of ^{15}N-labeled ammonium ions enabled us to localize three cation binding sites within the major form of $d(TG_4T)_4$ and four within the minor form. The additional $^{15}NH_4^+$ ion binding site in the minor form has been established between the G- and T-quartets at the 5′ end. Eight $^{15}NH_4^+$ ion binding sites were established within the dimeric $d[(UG_4T)_4]_2$ G-quadruplex, which exhibited a U-quartet at the interface of two quadruplex units. The NMR experiments have unequivocally proved that $^{15}NH_4^+$ ion movement inside the central ion cavities of tetramolecular G-quadruplexes is ~10 times faster than in bimolecular and monomolecular G-quadruplexes. However, a T-quartet at the 5′ end within the monomeric form decelerates cation movements.

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Supporting Information Available: Pulse field gradient diffusion experiment results and native PAGE data. This material is available free of charge via the Internet at http://pubs.acs.org.

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