Scientific paper

Is There Any Proton Exchange Between Ammonium Ions localized Within the $d(G_3T_4G_4)_2$ Quadruplex?

Primož Šket,[1,2] Wiktor Koźmiński[3] and Janez Plavec[1,2,4,*]

[1] *Slovenian NMR center, National Institute of Chemistry, Ljubljana, Slovenia*

[2] *EN-FIST Centre of Excellence, Ljubljana, Slovenia*

[3] *Department of Chemistry, University of Warsaw, Poland*

[4] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia*

** Corresponding author: E-mail: janez.plavec@ki.si*

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Dedicated to Prof. Dr. Gorazd Vesnaver on the occasion of his 70th birthday

Abstract

It is generally appreciated that stability, dynamics and function of nucleic acids are affected by nature of cations that are involved in interaction with specific functionalities. DNA G-quadruplexes are unique among nucleic acid structures in their metal ion requirements. Cations inside G-quadruplex structures have been shown to exchange between binding sites and bulk solution. However, in $d(G_3T_4G_4)_2$ G-quadruplex ammonium ions do not exchange through the central G-quartet plane. Passage of larger cations through a G-quartet such as potassium or ammonium ions requires partial opening of G-quartets. The use of HzExHSQC experiment demonstrates that smaller protons move through the central G-quartet plane.

Keywords: Quadruplex, cation, NMR spectroscopy

1. Introduction

DNA molecules can adopt besides the well-known B-type double helix several higher-order structures, including G-quadruplexes.[1–4] G-quadruplexes are secondary structures formed by guanine-rich nucleic acid streches in the presence of cations. G-rich segments are found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells.[5] Formation of G-quadruplexes has been implicated in association with human diseases. G-quadruplexes can be also used as robust building blocks for functional materials and biotechnology due to their structural variability, high temperature stability and the feasibility of controlling their dynamic behavior. The basic building block of G-quadruplex structures are stacks of square-planar arrays called G-quartets, which consist of four guanines that are linked together by eight hydrogen bonds (Fig. 1). G-quadruplex structures require cations for their formation, structural integrity and stabilization.[6,7] In general, cations can be localized along the central cavity of G-quadruplex formed by G-quartets, which are stacked in a regular geometry. The exact nature of interactions of cations with nucleic acids however is far from understood. Furthermore, cations can move between binding sites and bulk solution.[8–13]

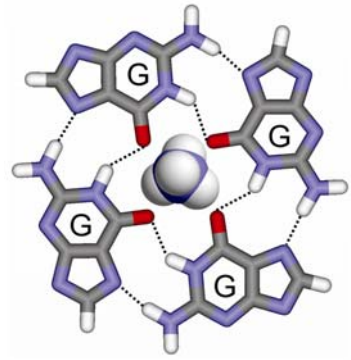

Figure 1: G-quartet with the centrally localized ammonium ion. Note that ammonium ion is out of G-quartet plane.

Due to the critical role that monovalent cations play in G-quadruplex formation considerable efforts have been devoted to their localization using NMR, X-ray and computational techniques. Recently, ^{15}N-labeled ammonium ion has been utilized as a non-metallic substitute in combination with 2D NMR spectroscopy to localize cations inside the interior of G-quadruplex structures as well as provide insight into kinetics of their movement.[8] With this approach we have localized two $^{15}NH_4^+$ ions, that are in slow exchange on NMR time-scale, at the two binding sites between the pairs of G-quartets within $d(G_3T_4G_4)_2$ quadruplex (Fig. 2).[14] NMR experiments have shown that $^{15}NH_4^+$ ions move between the two binding sites, U and L, and bulk solution (B). The quantification of volume integrals of cross-peaks corresponding to U→B and L→B exchange processes has shown that $^{15}NH_4^+$ ion movement into bulk solution is 12-times more frequent from one of the binding site than from the other.[10] The difference has been attributed to steric hindrance imposed by the diagonal compared to the edge-type loop, which requires significant conformational rearrangements for $^{15}NH_4^+$ ions to leave or enter G-quadruplex core.

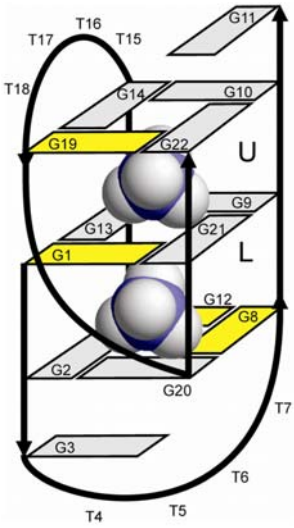

Figure 2: Location of ammonium ions within the dimeric fold-back structure adopted by $d(G_3T_4G_4)_2$. G1-G11 and G12-G22 indicate residues in the two strands. The guanine bases are shown as rectangles, where yellow rectangles represent *syn* nucleobases. Thymine bases are omitted for clarity. Labels U and L indicate two $^{15}NH_4^+$ ion binding sites.

In addition, NMR data undoubtedly showed the absence of $^{15}NH_4^+$ ion movement between the two binding sites along the central axis of G-quadruplex, which was unexpected observation that might have important implications in nanotechnological applications. Observation of ammonium ion (non)movement within $d(G_3T_4G_4)_2$ prompted us to extend our studies regarding ion movements through the central G-quartet of $d(G_3T_4G_4)_2$ G-quadruplex and to explore potential for movement of smaller protons inside the G-quadruplex.

2. Experimental

Oligonucleotides were synthesized on an Expedite 8909 synthesizer using phosphoramidite chemistry following the manufacturer's protocol and deblocked with concentrated aqueous ammonia. DNA was purified on 1.0 m Sephadex G25 column. Fractions containing only full-length oligonucleotide were pooled, lyophilized, redissolved in 1 mL H_2O and dialyzed extensively against LiCl solution. Sample concentration was 3 mM in strand (1.5 mM in G-quadruplex) with a pH of 4.5, which was adjusted with LiOH or HCl. $^{15}NH_4Cl$ was titrated into the sample to 50 mM concentration. All NMR spectra were collected on a Varian VNMRS 600 MHz NMR spectrometer using a triple resonance probe at 25 °C. The mixing times of 2D ^{15}N-^1H HzExHSQC spectra were set between 14 ms and 1.9 s, with 128 increments, 8 scans per increment and spectral widths of 12 kHz (^1H) and 2 kHz (^{15}N). Volumes of cross-peaks in 2D ^{15}N-^1H HzExHSQC spectra were integrated with Varian VNMRJ 2.1B software. If not stated otherwise, volume integrals were expressed relative to the autocorrelation peak U at mixing time of 14 ms, which was assigned an arbitrary volume of 100 units. Iterative least-squares fitting was done with Origin 7.5 software (www.originlab.com).

3. Results and Discussion

In our previous studies[10] the kinetics of exchange of ammonium ions has been evaluated with the use of 2D ^{15}N-^1H NzExHSQC experiment, which is related to a standard heteronuclear 2D HSQC experiment with additional block in its pulse sequence that enables detection of physical movement of ^{15}N isotopically labeled ammonium ions. Since magnetization is stored on nitrogen atom (*e.g.* Nz state) during the mixing time the resulting cross-peaks in NMR spectra reflect movement of nitrogen atoms that are part of ammonium ions from the initial to the final position (Fig 3a). Herein, we have modified the experiment in such a way to monitor anticipated jumps of protons from one ammonium ion to another. In resulting 2D ^{15}N-^1H HzExHSQC experiment the magnetization is stored on protons during the mixing time (*e.g.* in Hz state), since mixing time occurs after nitrogen chemical shift evolution and transfer of magnetization from nitrogen back to proton (Fig. 4).

A series of 2D ^{15}N-^1H HzExHSQC experiments with mixing times in the range from 14 ms to 1.9 s have been recorded at 25 °C on $d(G_3T_4G_4)_2$ folded in the presence of $^{15}NH_4^+$ ions (Fig. 3b).

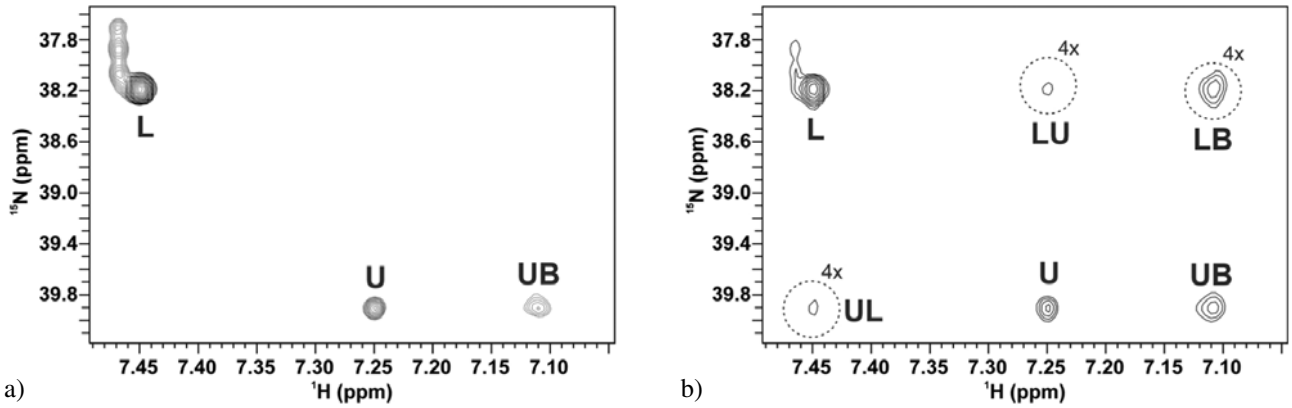

Figure 3: Comparison of plots of 2D ^{15}N-^{1}H NzExHSQC (a) and 2D ^{15}N-^{1}H HzExHSQC spectra (b) of d$(G_3T_4G_4)_2$ at 25 °C and pH 4.5 in 10% $^{2}H_2O$ at mixing time of 300 ms. Autocorrelation cross-peaks are labeled with single letter, while cross-peaks indicating exchange are labeled with two letters, where the first indicates the initial and the second the final position. Intensity of cross-peaks marked with dashed circles in (b) has been increased by a factor of four compared to the other signals. The large autocorrelation peak of bulk $^{15}NH_4^+$ ions (δ_{1H} = 7.11 and δ_{15N} = 30 ppm) is outside the shown region. The oligonucleotide concentration was 3.0 mM in strand, while the concentration of $^{15}NH_4Cl$ was 50 mM.

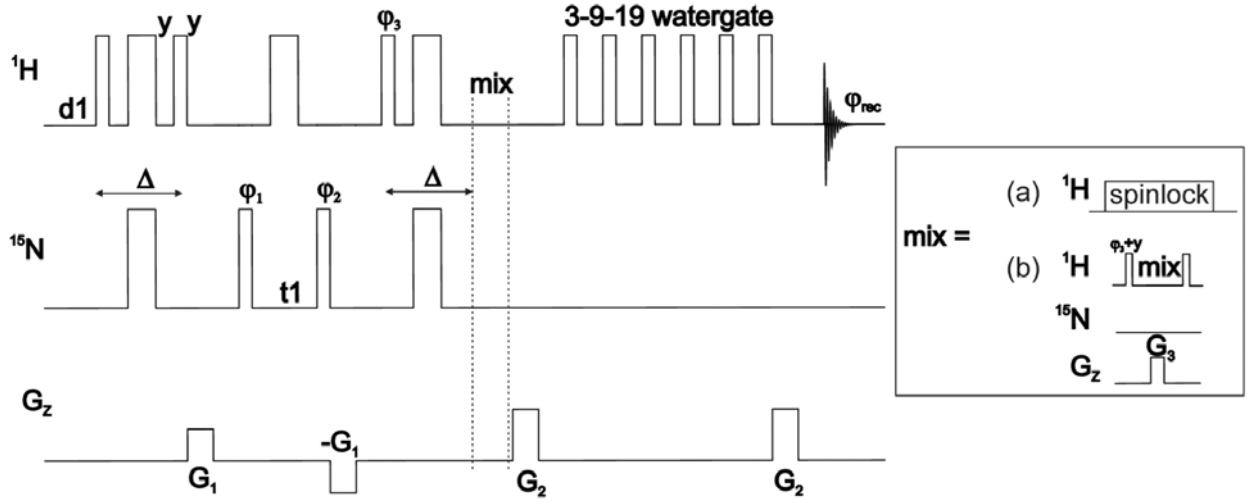

Figure 4: ^{15}N-^{1}H HzExHSQC pulse sequence. (a) Rotating frame exchange and (b) HzExchange. d1 is relaxation delay, t1 is evolution time and mix is mixing time (τ_m). Water signal is suppressed with 3-9-19 watergate pulse sequence. Pulse field gradients are applied along z axis with durations of G1, G2 and G3. Δ is set according to $1/2J_{NH}$, where J has been 75 Hz. φ_1 = x, x, φ_2 = 2x, 2(–x), φ_3 = 4x, 4(–x) and $\varphi_{rec} = \varphi_1 + \varphi_2 + \varphi_3$.

In ^{15}N-^{1}H HzExHSQC spectra autocorrelation cross-peaks labeled with U, L and B correspond to protons that belong to ammonium ions at individual binding sites and bulk solution that did not change their position during the mixing time of the experiment. In addition to autocorrelation peaks we have observed cross-peaks labeled with UB, LB and BU (outside the spectral region shown in Fig. 3b) that denote proton exchange between ammonium ions within d$(G_3T_4G_4)_2$ G-quadruplex and ammonium ions in bulk solution. Since exchange of ammonium ions between bulk solution and binding site U is much faster than between bulk solution and binding site L the corresponding cross-peaks are of different intensities in NzExHSQC experiments. In full accordance, cross-peak BL has not been observed in NzExHSQC experiments, which has been attributed to a very slow movement.[10] The expected cross-peak BL in HzExHSQC experiment that would indicate exchange of protons from ammonium ions in bulk solution to ammonium ions at binding site L was not observed as its intensity was below detection limit. As a consequence, hypothetical double stage movement (U→B→L) is well beyond detection limits and can be safely excluded. In contrast to ^{15}N-^{1}H NzExHSQC spectra where no cross-peaks indicating movement of ammonium ions between U and L binding sites was observed, ^{15}N-^{1}H HzExHSQC spectra clearly showed two, albeit weak cross-peaks marked with UL and LU that indicated exchange of protons between ammonium ions residing at U and L binding sites.

Decrease of autocorrelation cross-peaks as a function of mixing time in ^{15}N-^{1}H NzExHSQC spectra could only be adequately accounted for by using biexponential

function. Analogous decrease in ^{15}N-^{1}H HzExHSQC experiments could be described by a single-exponential function (eqn. 1).

$$V_a(\tau_m) = A\left[e^{-B\tau_m}\right] = A\left[e^{-(k_H+1/T_{1a})\tau_m}\right] \quad (1)$$

where V_a represents volume integral of an autocorrelation peak, A is the scaling factor, B is the rate constant and τ_m is the mixing time used in 2D HzExHSQC experiments. As suggested by the right-hand side of eqn. 1, the rate constant B can be dissected into the sum of the rate constant for the movement of protons (k_H) and the reciprocal of spin-lattice relaxation time (*i.e.* longitudinal relaxation rate) for autocorrelation peak (T_{1a}). The best fit of experimental data corresponding to volumes of autocorrelation cross-peaks U and L to parameters in eqn. 1 is shown in Fig. 5.

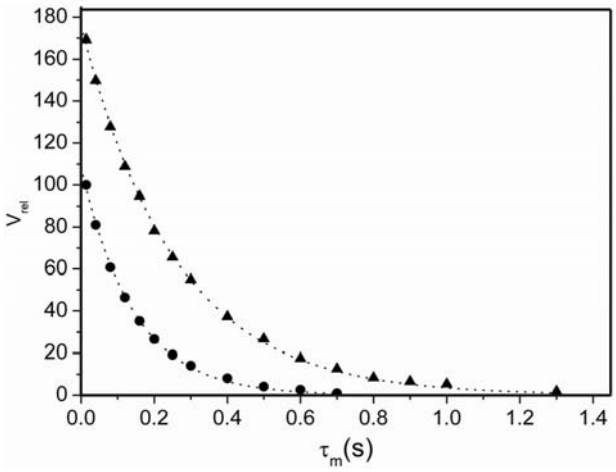

Figure 5: Relative volumes of autocorrelation cross-peaks U (circles) and L (triangles) as a function of mixing time (τ_m) in HzExHSQC experiment for $d(G_3T_4G_4)_2$ at 25 °C. Dotted curves represent the best fits of the experimental data to eqn. 1. The parameters that best fit the experimental data for U are A = 109 ± 1, B = 7.0 ± 0.1 s^{-1} and for L are A = 176 ± 1, B = 3.9 ± 0.1 s^{-1}.

Cross-peak volumes resulting from proton jumps between ammonium ions are expected to increase as a function of mixing time as more protons move from one ammonium ion to another during longer time. Simultaneously their volumes decrease due to longitudinal relaxation. In the simplest case, the volume integral of a cross-peak is defined by a combination of the two effects described above, which can be expressed by the following equation, eqn. 2:

$$V_c(\tau_m) = A[e^{-\frac{\tau_m}{T_{1c}}}(1-e^{-k_H\tau_m})] \quad (2)$$

where *Vc* represents volume integral of individual cross-peak, T_{1c} is spin-lattice relaxation time, τ_m is mixing time of the 2D HzExHSQC experiment and k_H corresponds to the rate constants for the proton movement. Analyses of

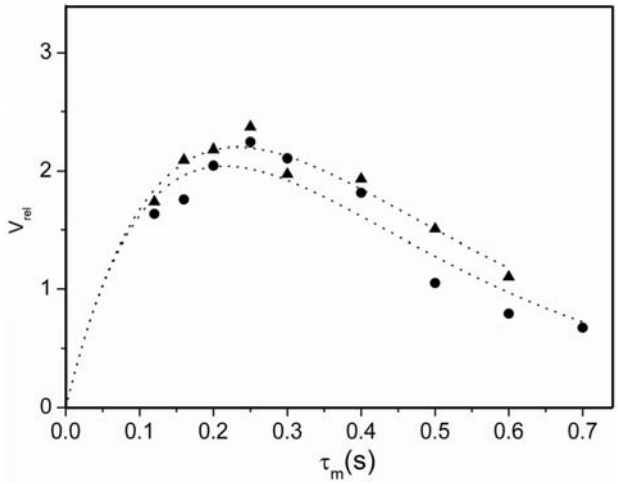

Figure 6: Relative volumes of cross-peaks UL (circles) and LU (triangles) as a function of the mixing time (τ_m) for $d(G_3T_4G_4)_2$ at 50 mM $^{15}NH_4Cl$ and 25 °C. Dotted curves represent the best fits of the experimental data to eqn. 2.

the volumes of cross-peaks UL and LU indicating the movement of protons within G-quadruplex core revealed their increase up to a mixing time of 250 ms (Fig. 6). At longer mixing times relaxation predominates and cross-peaks' volumes decrease.

The movement of protons from ammonium ions at binding site U to ammonium ions at binding site L is characterized by the rate constant k_{UL} of 1.7 ± 0.6 s^{-1}. On the other hand, the rate constant for the reverse movement of protons k_{LU} is 0.9 ± 0.5 s^{-1}. Unfortunately, differences in both rate constants are within the experimental error. On the other hand, overall interpretation should consider that ammonium ions at binding sites are not static and are simultaneously exchanging with other ions in bulk solution during the mixing time. The actual exchange rates of protons should be higher than estimated from the best fits using eqn. 2 presented in Fig. 6. Nevertheless, with HzExHSQC experiment we were able to get direct evidence on exchange of protons through the central G-quartet in $d(G_3T_4G_4)_2$ quadruplex, while larger ammonium ions could not exchange through that G-quartet.

4. Conclusion

HzExHSQC pulse sequence enables direct observation of exchange of protons between several binding sites and more specifically through the G-quartet plane. The NMR experiment was tested on $d(G_3T_4G_4)_2$ quadruplex where larger ammonium ions did not exchange through the central G-quartet. NMR data undoubtedly showed that smaller protons do exchange from one ammonium ion to another. Such knowledge can have important implications in design of artificial ion channels based on G-quadruplex structures.

5. Acknowledgment

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6. References

1. S. Burge; G. N. Parkinson; P. Hazel; A. K. Todd; S. Neidle, *Nucleic Acids Res.* **2006,** *34*, 5402–5415.
2. A. T. Phan; V. Kuryavyi; D. J. Patel, *Curr. Opin. Struct. Biol.* **2006,** *16*, 288–298.
3. M. A. Keniry, *Biopolymers* **2001,** *56*, 123–146.
4. J. T. Davis, *Angew. Chem.-Int. Edit.* **2004,** *43*, 668–698.
5. S. Neidle; S. Balasubramanian, *Quadruplex Nucleic Acids*. The Royal Society of Chemistry: Cambridge, **2006**; p 301.
6. N. V. Hud; J. Plavec, The Role of Cations in Determining Quadruplex Structure and Stability in Quadruplex Nucleic Acids. In *Quadruplex Nucleic Acids*, Neidle, S.; Balasubramanian, S., Eds. The Royal Society of Chemistry: Cambridge, **2006**; pp 100–130.
7. J. Plavec, Metal Ion Coordination in G-Quadruplexes. In *Metal Complex-DNA Interactions*, Hadjiliadis, N.; Sletten, E., Eds. John Wiley & Sons Ltd: Chichester, **2009**; pp 55–93.
8. N. V. Hud; P. Schultze; V. Sklenar; J. Feigon, *J. Mol. Biol.* **1999,** *285*, 233–243.
9. P. Sket; M. Crnugelj; W. Kozminski; J. Plavec, *Org. Biomol. Chem.* **2004,** *2*, 1970–1973.
10. P. Sket; J. Plavec, *J. Am. Chem. Soc.* **2007,** *129*, 8794–8800.
11. P. Podbevsek; N. V. Hud; J. Plavec, *Nucleic Acids Res.* **2007,** *35*, 2554–2563.
12. P. Podbevsek; P. Sket; J. Plavec, *J. Am. Chem. Soc.* **2008,** *130*, 14287–14293.
13. J. Zavasnik; P. Podbevsek; J. Plavec, *Biochemistry* **2011,** *50*, 4155–4161.
14. P. Sket; M. Crnugelj; J. Plavec, *Nucleic Acids Res.* **2005,** *33*, 3691–3697.

Povzetek

Znano je, da na stabilnost, dinamiko in funkcijo nukleinskih kislin vpliva narava kationov, ki so udeleženi v raznih interakcijah. DNA G-kvadrupleksi so edinstveni med strukturami nukleinskih kislin zaradi njihove potrebe po prisotnosti kovinskih ionov. Pokazano je bilo, da kationi znotraj G-kvadrupleksnih struktur ne mirujejo, ampak se izmenjujejo med vezavnimi mesti kakor tudi s kationi iz okoliške raztopine. V primeru $d(G_3T_4G_4)_2$ G-kvadrupleksa je bilo ugotovljeno, da se amonijevi ioni ne izmenjujejo skozi osrednjo G-kvartetno ravnino. Za prehod večjih kationov, kot sta npr. kalijev in amonijev ion, je namreč potrebno, da se G-kvarteti delno razprejo. S pomočjo uporabe HzExHSQC eksperimenta smo pokazali, da manjši protoni lahko prehajajo skozi osrednjo G-kvartetno ravnino.