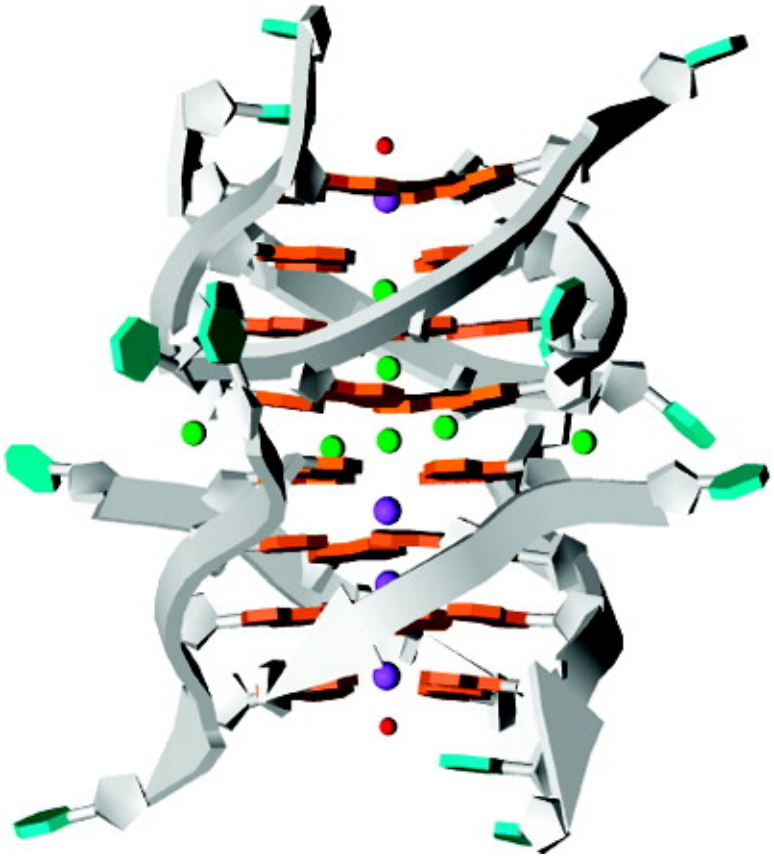
Communication

Observation of the Coexistence of Sodium and Calcium Ions in a DNA G-Quadruplex Ion Channel

Michael P. H. Lee, Gary N. Parkinson, Pascale Hazel, and Stephen Neidle

J. Am. Chem. Soc., **2007**, 129 (33), 10106-10107• DOI: 10.1021/ja0740869 • Publication Date (Web): 28 July 2007



J|A|C|S
COMMUNICATIONS

Published on Web 07/28/2007

Observation of the Coexistence of Sodium and Calcium Ions in a DNA G-Quadruplex Ion Channel

Michael P. H. Lee, Gary N. Parkinson, Pascale Hazel, and Stephen Neidle*

CRUK Biomolecular Structure Group, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, U.K.

Received June 5, 2007; E-mail: stephen.neidle@pharmacy.ac.uk

Guanine-rich sequences of nucleic acids can form a wide variety of inter- or intramolecular four-stranded structures, termed quadruplexes.[1] Such sequences occur at the ends of all eukaryotic chromosomes, in telomeric regions, and comprise tandem repeats of simple motifs such as d(TTAGGG) or d(TGGGG). The building-block of quadruplexes is the in-plane arrangement of four hydrogen-bonded guanine bases, the G-tetrad, which can stack one on another in a quadruplex,[2] as revealed in a number of crystallographic and NMR studies.[3]

All quadruplexes require the presence of metal ions for stability. These form a channel in the center of the molecule.[2] Crystallographic studies on Na^+- and K^+-containing quadruplexes have shown that they always coordinate to the O6 atoms of guanine,[3b,4] in accord with a number of NMR studies.[5] These observations have shown that octahedral coordination is common, especially when an ion is midway between G-tetrads, as is the case with K^+ ions. Thus, ionic radius appears to be an important determinant of the ability of a particular ion to stabilize a quadruplex. The accepted order of stabilization[2b,5a] is $K^+ > Na^+ > NH_4^+$, although other ions such as Ca^{2+}, Mn^{2+}, Sr^{2+}, and Pb^{2+} have also been shown[6−9] to impart quadruplex stabilization. Tl^+ ions have been found to participate in the channel in the bimolecular quadruplex formed by two strands of the *Oxytricha nova* quadruplex d($G_4T_4G_4$), at occupancies in the range 0.3−0.7, with a Na^+ ion at the extremity of the quadruplex, between a terminal G-tetrad and thymine bases.[10] The simpler tetramolecular quadruplex, formed by four strands of d(TG_4T), has been crystallized in the presence of Na^+, Li^+, or Tl^+ ions.[11−13] This versatility is in accord with NMR studies[5] which have also directly shown the presence of sodium, potassium, or rubidium ions in the d(TG_4T) channel.[14] No crystal structure of a DNA quadruplex with a divalent channel cation has been reported to date, although the ability of Ca^{2+} and other ions to induce a transition from antiparallel to parallel topology suggests that they may have significant structural roles. The crystal structure of the analogous (UG_4U) RNA quadruplex with Sr^{2+} ions shows them occupying alternating positions in the channel.[8a]

We report here on a new crystal form for the intermolecular quadruplex formed by four parallel strands of the sequence d(TG_4T). Previous reports have shown Na^+ ions occupying all positions in the G-tetrad channel[11] and, most recently, thallium[14] ions in the region between two adjacent stacked quadruplexes. We have now crystallized this sequence in a mixed Ca^{2+} and Na^+ ion environment, and unexpectedly find an arrangement involving both ions, that has not been previously reported.

The crystal structure was solved to 1.55 Å by molecular replacement methods and refined to an R of 16.3% and an R_{free} value of 20.8% using data collected on a laboratory X-ray source. The crystallographic asymmetric unit in the space group $P\bar{1}$, contains two stacked d(TG_4T) quadruplexes, related by a pseudo-two-fold axis. Fourier and difference electron density maps at an early stage in the refinement showed strong peaks in the channel, between each G-tetrad within the quadruplexes, and one between the two quadruplexes, which were initially assigned as Na^+ ions. Correct assignment of atom types to these peaks was facilitated by calculation of an anomalous difference Fourier map, using the full sphere of diffraction data. Calcium has an anomalous signal at the wavelength used for data collection (mirror-monochromatic Cu Kα radiation at 1.54178 Å), with a $\Delta f''$ of 1.286 e, whereas Na^+ does not have a significant signal (0.124 e) at this wavelength. Thus, we reasoned that, in the absence of any other metal ions, any significant peaks on the anomalous difference map must be due to Ca^{2+} ions. This map shows high density at three positions in the channel (Figure 1), although these are markedly asymmetric with respect to the quadruplex dimer. These three peaks were assigned at full-occupancy Ca^{2+} ions, and other peaks found in difference electron density maps have been assigned as Na^+ ions (although

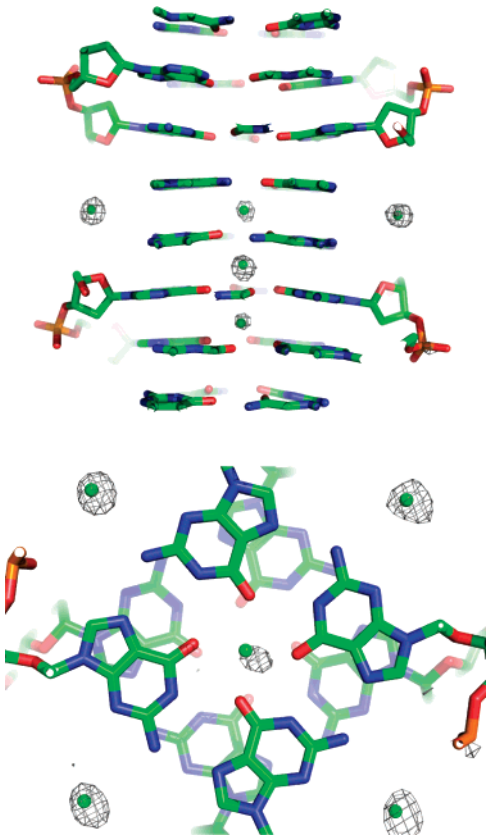


Figure 1. Anomalous difference maps, with electron density contoured at 2.5σ. The top figure shows a view along the plane of the G-tetrads, with most of the backbone removed to enhance clarity. The three horizontal peaks are at the interface between the two crystallographically independent quadruplexes. The bottom figure shows a projection onto the plane of the two tetrads at the interface, showing the four groove-bound Ca^{2+} ions.

10.1021/ja0740869 CCC: $37.00

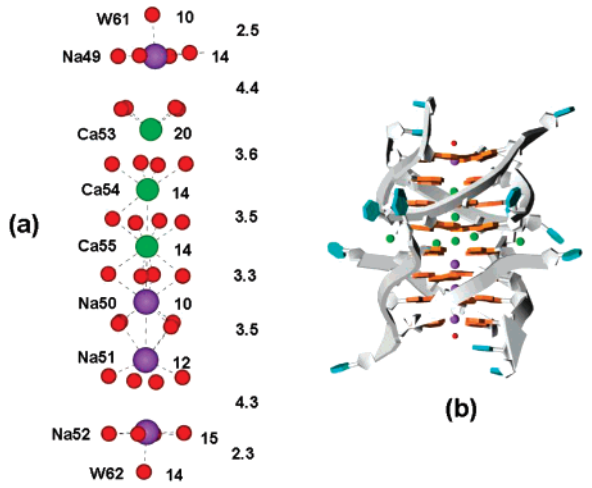


Figure 2. (a) View of the channel of Ca^{2+} (green) and Na^{+} (mauve) ions and oxygen atoms (red). The distance between each atom in the channel is shown on the right-hand side, and each isotropic temperature (*B*) factor, in Å^2, immediately to the right of each channel atom. Water oxygen atoms coordinating to the terminal metal ions are shown, together the O6 guanine atoms belonging to each G-tetrad, with hydrogen bonds to ions as dashed lines. (b) View of the crystallographic unit of two d(TG_4T) molecules stacked end-to-end, with the DNA shown in schematic form. The ions and water molecules constituting the channel are shown, with color scheme the same as that used in (a). Guanine bases are colored orange, and thymines are cyan.

they could also be NH_4^+ ions). Figure 2a shows the final arrangement of ions in the channel, together with coordinated O6 guanine oxygen atoms and terminal water molecules.

The channel that extends along the center of the quadruplex dimer is thus asymmetrically populated by ions. The lower half (Figure 2a,b) has three Na^+ ions, irregularly positioned with respect to the G-tetrad planes, so that the outermost Na^+ is almost coplanar with a G-tetrad, and the inner one is almost equidistant from two G-tetrads. A Ca^{2+} ion sits at the dimer interface (itself in plane with four other Ca^{2+} ions each in a groove (Figures 1, 2b). This Ca^{2+} ion is followed by two more in the upper half of the channel, both of which are approximately midway between G-tetrads. Each ion channel is capped by a water molecule. The increased mobility (Figure 2a) of the outermost Ca^{2+} ion is paralleled by its reduced level of water coordination compared to that of the other ions. Its higher than average *B* factor combined with its reduced electron density suggest that this ion may be <100% Ca^{2+} occupancy. No significant distortion of the quadruplex has taken place, compared to the Na^+ structure.[11]

The implication of finding mixed cations within a single quadruplex is that ion mobilities in the central channel of a parallel DNA G-quadruplex are greater than has been assumed. We have attempted to model this by means of molecular dynamics simulations. These initially led to rapid diffusion of Ca^{2+} ions out of the channel. HF and DFT calculations indicated that the charges on the Ca^{2+} ions were somewhat delocalized within the G-tetrads. Accordingly, once the +2 charge was reduced to +1.5, the Ca^{2+} mobility decreased, and averaged structures from dynamics simulations corresponded more closely with the crystal structure, although some ion diffusion out of the channel did still occur in the early stages of the 4 ns simulation.

The surprising finding here, of an asymmetric quadruplex dimer with a very unequal distribution of Ca^{2+} ions in the channel running through the dimer, lends further support to the concept that the Ca^{2+} and Na^+ ions in this quadruplex channel are mobile and can readily interchange positions since their ionic radii are closely similar (0.99 and 0.97 Å).

Acknowledgment. We are grateful to Cancer Research UK for Programme Grant support (S.N.) at the School of Pharmacy, and to the Association for International Cancer Research (studentship to P.H.).

Supporting Information Available: Details of crystallographic experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Burge, S.; Parkinson, G. N.; Hazel, P.; Todd, A. K.; Neidle, S. *Nucleic Acids Res.* **2006**, *34*, 5402−5415.
(2) (a) Williamson, J. R. *Ann. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703−730. (b) Davies, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668−698. (c) Hud, N. V.; Plavec, J. In *Quadruplex Nucleic Acids*; Neidle, S., Balasubramanian, S., Eds.; Royal Society of Chemistry Publishing: Cambridge UK, 2006.
(3) See for example: (a) Smith, F. W.; Feigon, J. *Nature* **1992**, *356*, 164−168. (b) Parkinson, G. N.; Lee, M. P. H.; Neidle, S. *Nature* **2002**, *417*, 876. (c) Wang, Y.; Patel, D. J. *Structure* **1993**, *1*, 263−282. (d) Phan, A. T.; Modi, Y. S.; Patel, D. J. *J. Am. Chem. Soc.* **2004**, *126*, 8710−8716. (e) Ambrus, A.; Chen, D.; Dai, J.; Jones, R. A.; Yang, D. *Biochemistry* **2005**, *44*, 2048−2058. (f) Ambrus, A.; Chen, D.; Dai, J.; Bialis, T.; Jones, R. A.; Yang, D. *Nucleic Acids Res.* **2006**, *34*, 2723−2735.
(4) Haider, S.; Parkinson, G. N.; Neidle, S. *J. Mol. Biol.* **2002**, *320*, 189−200.
(5) For example: (a) Schultze, P.; Hud, N. V.; Smith, F. W.; Feigon, J. *Nucleic Acids Res.* **1999**, *27*, 3018−3028. (b) Hud, N. V.; Smith, F. W.; Anet, F. A. L.; Feigon, J. *Biochemistry* **1996**, *35*, 15383−15390. (c) Sket, P.; Crnugelj, M.; Plavec, J. *Nucleic Acids Res.* **2005**, *33*, 3691−3697. (d) Gill, M. L.; Strobel, S. A.; Loria, J. P. *J. Am. Chem. Soc.* **2005**, *127*, 16723−16732. (e) Kwan, I. C.; Mo, X.; Wu, G. *J. Am. Chem. Soc.* **2007**, *129*, 2398−2407. (f) Ida, R.; Kwan, I. C. M.; Wu, G. *Chem. Commun.* **2007**, 795−797.
(6) Miyoshi, D.; Nakao, A.; Sugimoto, N. *Nucleic Acids Res.* **2003**, *31*, 1156−1163.
(7) Marathias, V. M.; Wang, K. Y.; Kumar, S.; Pham, T. O.; Swaminanathan, S.; Bolton, P. H. *J. Mol. Biol.* **1996**, *260*, 378−394; Blume, S. W.; Guarcello, V.; Zacharias, W.; Miller, D. M. *Nucleic Acids Res.* **1997**, *25*, 617−625.
(8) (a) Deng, J.; Xiong, Y.; Sundaralingam, M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13665−13670. (b) Chen, F. M. *Biochemistry* **1992**, *31*, 3769−3776.
(9) (a) Smirnov, I.; Shafer, R. H. *J. Mol. Biol.* **2000**, *296*, 1−5. (b) Smirnov, I. V.; Kotch, F. W.; Pickering, I. J.; Davies, J. T.; Shafer, R. H. *Biochemistry* **2002**, *41*, 12133−12139.
(10) Gill, M. L.; Strobel, S. A.; Loria, J. P. *Nucleic Acids Res.* **2006**, *34*, 4506−4514.
(11) Laughlan, G.; Murchie, A. I.; Norman, D. G.; Moore, M. H.; Moody, P. C.; Lilley D. M. J.; Luisi, B. *Science* **1994**, *265*, 520−524; Phillips, K.; Dauter, Z.; Murchie, A. I.; Lilley D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171−182.
(12) Creze, C.; Rinaldi, B.; Haser, B.; Bouvet, P.; Gouet, P. *Acta Crystallogr.* **2007**, *63*, 682−688.
(13) Caceres, C.; Wright, G.; Gouyette, Parkinson, G.; Subirana, J. A. *Nucleic Acids Res.* **2005**, *32*, 1097−1102.
(14) Wong, A.; Ida, R.; Wu, G. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 363−366.
(15) Crystallographic data are available from the Protein Data Bank as entry 2GW0.

JA0740869