Direct Detection of Monovalent Metal Ion Binding to a DNA G-quartet by ^{205}Tl NMR

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Received October 8, 1999
Revised Manuscript Received January 25, 2000

Monovalent metal cations, such as K^+ and Na^+, are required for the proper folding of several classes of nucleic acids. The best documented example is the monovalent metal ion requirement for G-quartet formation,[1,2] but monovalent cations are also essential for the in vitro activity of catalytic RNAs.[3] The identification of monovalent metal ions within RNA and DNA has been hindered by the lack of direct methods for metal ion observation, though ^{15}N-labeled ammonium has recently been used as a nonmetallic substitute for monovalent cation detection.[4,5] Here we utilize the well characterized DNA G-quartet as a model system to demonstrate that thallous ion (Tl^+) can efficiently substitute for K^+ in the promotion of G-quartet formation and that the bound Tl^+ monovalent metal cations can be directly detected by ^{205}Tl NMR.

The chemical and spectroscopic characteristics of Tl^+ make it an ideal substitute for a K^+ ion.[6] The two metal cations have a similar ionic radii (Tl^+ 1.40 Å; K^+ 1.33 Å), form bonds of similar distance (2.4−2.7 Å), and adopt irregular coordination geometries and bond angles.[7,8] X-ray crystallography of K^+ and Tl^+ macromolecular complexes indicated that they bind equivalently to their ligands.[7] While Tl^+ has found only limited use in nucleic acid systems, it has been used to study monovalent binding sites within protein enzymes.[9] In these systems, Tl^+ usually supports enzymatic activity, and in all cases in which it has been measured, the Tl^+ binds to the ligand at least as well as K^+ ion.[10] Unlike K^+, however, Tl^+ is a spin 1/2 nucleus well suited for NMR applications. The relative receptivity (defined as the sensitivity multiplied by the natural abundance) of ^{205}Tl compared to proton is 0.1355, while that of ^{39}K is only 0.000473.[11] Furthermore, the chemical shift range of ^{205}Tl is extremely large (>3000 ppm) and the resonance frequency is highly sensitive to the chemical environment of the metal ion.[11]

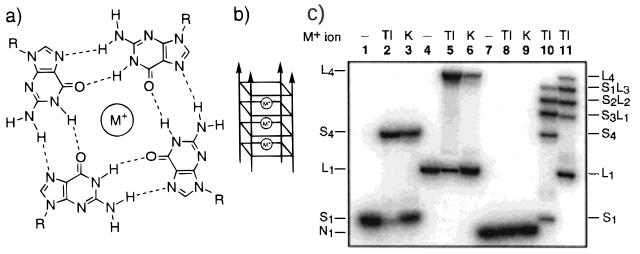

Figure 1. (a) Chemical structure of a G-quartet. (b) Schematic depiction of a parallel tetraplex. (c) Autoradiograph of a non-denaturing polyacrylamide gel demonstrating G-quartet formation in the presence of Tl^+. Samples used for the study were oligonucleotides S [5′−d(TTGGGGTT)-3′] and L [5′−d(TGTTGGGGTTGT)-3′], which contain four contiguous guanosines (underlined), and N [5′−d(GTGTGTG)-3′], an oligonucleotide containing four non-contiguous guanosines. 5′-End radiolabeled samples of each oligonucleotide were mixed with 10 nmol of the cold oligonucleotide, and heated at 95 °C for four minutes in the presence of 10% glycerol, 100 mM Tris-NO_3, pH 7.5, and 0.1 mM EDTA. Samples were cooled to 37 °C, $TlNO_3$ or $K(CH_3COO)$ was added to a final concentration of 100 mM in a total volume of 10 μL, and the solutions were incubated at 37 °C overnight. The mixing experiments were performed in the same manner using 0.5 mM of S and L with either radiolabeled S (lane 10) or L (lane 11). The complexes were resolved by native 20% polyacrylamide gel electrophoresis at 5−6 °C in Tris-borate-EDTA buffer. The metal ion added to each sample is indicated at the top of the autoradiograph, and the molecularity of individual bands are shown on each side, where the subscript indicates the number of strands of a given oligonucleotide.

We have selected the four-stranded DNA G-quartet structure as a simple system to develop ^{205}Tl NMR methods for the analysis of monovalent metal binding to nucleic acids. G-quartets are a noncanonical base pairing motif in which four guanine bases form a cyclic and coplanar hydrogen bonding array (Figure 1a).[12] These four-stranded structures are exceptionally stable and include both parallel and anti-parallel alignments of the G-rich strands (Figure 1b).[2] G-quartets can be formed from DNA or RNA sequences of several different G-rich variations that are biologically relevant.[2,12−14]

All G-quartets are stabilized by specifically bound monovalent metal ions.[2] The cations are positioned between the planes of the G-quartets and each is coordinated to eight different G carbonyl groups that line the interior channel of the quartet.[5,15,16] In this arrangement, the number of metal ions is one less than the number of G-quartets in the complex. K^+ cations form the most stable quartets, but Na^+ and Rb^+ also support quartet formation.[17] However, to our knowledge there is no report in the literature on the ability of Tl^+ to promote G-quartets.

To explore this question, we utilized the short oligonucleotide d(TTGGGGTT) (designated S for short), which is related to the telomeric repeat sequence found at the end of *Tetrahymena* chromosomes.[18] In the presence of K^+ the *Tetrahymena* telomeric repeat has been reported to form a parallel stranded G-quartet

(1) Sen, D.; Gilbert, W. *Nature* **1988**, *334*, 364−366.
(2) Williamson, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703−730.
(3) Pyle, A. M. *Catalytic reaction mechanisms and structural features of group II intron ribozymes*; Eckstein, F., Lilley, D. M. J., Eds.; Springer: New York, 1996; Vol. 10, pp 75−107; Nolan, J. M.; Pace, N. R. *Structural analysis of the bacterial ribonuclease P RNA*; Eckstein, F., Lilley, D. M. J., Eds.; Springer: New York, 1996; Vol. 10, pp 109−128; Wang, Y.-W.; Lu, M.; Draper, D. E. *Biochemistry* **1993**, *32*, 12279−12282; Miskin, R.; Zamir, A.; Elson, D. *J. Mol. Biol.* **1970**, *54*, 355−378; Zamir, A.; Miskin, R.; Elson, D. *J. Mol. Biol.* **1971**, *60*, 347−364.
(4) Hud, N. V.; Schultze, P.; Feigon, J. *J. Am. Chem. Soc.* **1998**, *120*, 6403−6404; Hud, N. V.; Sklenar, V.; Feigon, J. *J Mol. Biol.* **1999**, *286*, 651−660.
(5) Hud, N. V.; Schultze, P.; Sklenar, V.; Feigon, J. *J. Mol. Biol.* **1999**, *285*, 233−243.
(6) Douglas, K. T.; Bunni, M. A.; Baindur, S. R. *Int. J. Biochem.* **1990**, *22*, 429−438.
(7) Basu, S.; Rambo, R. P.; Cate, J. H.; Ferre-D Amare, A. R.; Strobel, S. A.; Doudna, J. A. *Nat. Struct. Biol.* **1998**, *5*, 986−992.
(8) Brown, I. B. *Acta Crystallogr., Sect. B* **1988**, *44*, 545−553.
(9) Reuben, J.; Kayne, F. J. *J. Biol. Chem.* **1971**, *246*, 6227−6234; Grisham, C. M.; Gupta, R. K.; Barnett, R. E.; Mildvan, A. S. *J. Biol. Chem.* **1974**, *249*, 6738−6744; Villeret, V.; Huang, S.; Fromm, H. J.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8916−8920; Loria, J. P.; Nowak, T. *Biochemistry* **1998**, *37*, 6967−6974; Pedersen, P. A.; Nielsen, J. M.; Rasmussen, J. H.; Jorgensen, P. L. *Biochemistry* **1998**, *37*, 17818−17827.
(10) Hinton, J. F.; Whaley, W. L.; Shungu, D.; Koeppe, R. E.; Millett, F. S. *Biophys. J.* **1986**, *50*, 539−544; Hinton, J. F.; Turner, G.; Millett, F. S. *Biochemistry* **1982**, *21*, 651−654.
(11) Hinton, J. F.; Metz, K. R.; Briggs, R. W. *Thallium NMR spectroscopy*; Emsley, J. W., Feeney, J., Sutcliffe, L. H., Eds.; Pergamon Press: 1988; Vol. 20, pp 423−513.
(12) Williamson, J. R.; Raghuraman, M. K.; Cech, T. R. *Cell* **1989**, *59*, 871−880.
(13) Kim, J.; Cheong, C.; Moore, P. B. *Nature* **1991**, *351*, 331−332.
(14) Wang, K. Y.; McCurdy, S.; Shea, R. G.; Swaminathan, S.; Bolton, P. H. *Biochemistry* **1993**, *32*, 1899−1904.
(15) Kang, C.; Zhang, X.; Ratliff, R.; Moyzis, R.; Rich, A. *Nature* **1992**, *356*, 126−131.
(16) 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.
(17) Hardin, C. C.; Watson, T.; Corregan, M.; Bailey, C. *Biochemistry* **1992**, *31*, 833−841.
(18) Cherry, J. M.; Blackburn, E. H. *Cell* **1985**, *43*, 747−758.
(19) Wang, Y.; Patel, D. J. *Biochemistry* **1992**, *31*, 8112−8119.

10.1021/ja993614g CCC: $19.00
Published on Web 03/21/2000

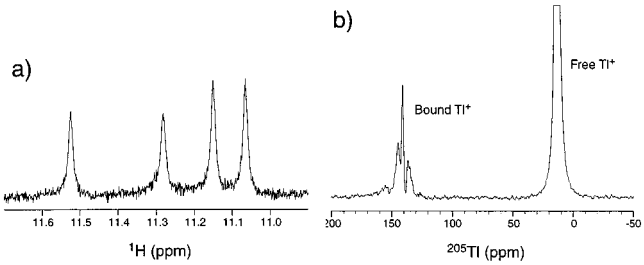

Figure 2. ^{1}H and ^{205}Tl spectra of reverse phase HPLC purified 2.5 mM d(TTGGGGTT) at 30 °C in 90% H_2O/10% D_2O containing 50 mM $TlNO_3$, 50 mM Tris-NO_3, pH 7.5, and 0.1 mM EDTA. (a) Imino proton region of ^{1}H spectrum. (b) ^{205}Tl spectrum processed with 200 Hz line broadening. The free and bound Tl^+ resonances are labeled. Spectra were referenced to zero using an external sample of 10 mM $TlNO_3$ in H_2O at 30 °C. ^{205}Tl spectra were collected on a Varian Inova 800 MHz (461 MHz ^{205}Tl) spectrometer at the Yale W. M. Keck High Field NMR facility using a probe with a broad band channel tuned to the ^{205}Tl frequency 461 MHz and home-built ^{205}Tl frequency amplifiers. ^{205}Tl data were collected with a spectral width of 200 kHz (10 Hz/point) with the offset near the bond ^{205}Tl peaks. Proton spectra were collected on a Varian Unity 500 MHz spectrometer with a spectral width of 8 kHz (1 Hz/point), using a WATERGATE[25] solvent suppression sequence, and 256 FIDs were accumulated. NMR data were processed using NMRPipe.[26]

with all the guanine bases in an *anti* configuration.[19] In the absence of monovalent metal ion, oligonucleotide S does not form a G-quartet as determined by a native gel electrophoretic mobility shift assay (Figure 1c, lane 1); however, the addition of either 100 mM K^+ or 100 mM Tl^+ to the hybridization buffer resulted in a new band with substantially slower mobility, consistent with G-quartet formation (Figure 1c, lanes 2 and 3). The formation of a slower mobility species is specific to oligonucleotides containing a contiguous G-rich sequence, as a longer oligonucleotide with a G-rich sequence, d(TGTTGGGGTTGT) (designated L for long), showed a similar mobility pattern as S, while the mixed oligonucleotide d(GTGTGTG) (designated N for non-G quartet) demonstrated no change in mobility upon addition of K^+ or Tl^+ (Figure 1c, lanes 4−9). To confirm that the Tl^+ induced complex is tetrameric, we mixed 0.5 mM each of unlabeled S and L with either ^{32}P-labeled S or ^{32}P-labeled L (Figure 1c, lanes 10 and 11). As visualized by autoradiography, this resulted in four bands of mobility, consistent with a set of 4-stranded heteromeric complexes containing zero to three strands of the unlabeled oligonucleotide and one to four strands of the labeled oligonucleotide.

The efficiency of quartet formation by oligonucleotides S and L appeared to be greater with Tl^+ than with K^+ (Figure 1c). At 1 mM d(TTGGGGTT), a Tl^+ concentration of 23 mM provided 50% tetraplex formation, while about 3 times as much K^+ (73 mM) and 6 times as much Na^+ (132 mM) was required to yield the same amount of complex.

The Tl^+ induced tetrameric S complex was further investigated by proton NMR spectroscopy. A 2.5 mM sample of d(TTGGGGTT) containing 50 mM Tl^+ in H_2O was used to collect 1-D proton spectra (Figure 2a) and a NOESY spectrum with a mixing time of 500 ms that allowed assignment of the G quartet imino, aromatic, and many of the sugar proton resonances (data not shown).[20] As expected for a G quartet complex, the four imino protons that belong to the guanosine residues are protected from solvent exchange (Figure 2a), even at temperatures as high as 80 °C (data not shown). In addition, the pattern of observed nuclear Overhauser effects (NOEs) is indicative of a 4-fold symmetric right-handed parallel stranded complex with all the guanosine bases in the *anti* conformation, as previously reported for similar G quartets.[16,19,20]

We next examined the d(TTGGGGTT) sample by ^{205}Tl NMR (Figure 2b) using a Varian Inova 800 MHz NMR spectrometer with a broadband channel probe tunable to the ^{205}Tl frequency (461 MHz). Unfortunately, the amplifiers provided by the manufacturer did not cover the required midrange frequencies, so it was necessary to add a thallium amplifier and preamp to the console.[21] A 1-D ^{205}Tl spectrum was collected in 14 h with a total of 200 000 transients using a simple pulse-acquire sequence with a recycle time of 0.2 s. A cluster of three thallium peaks were detected approximately 120 ppm downfield of the free $TlNO_3$ peak. The bound peaks have line widths of 1460, 660, and 1480 Hz, respectively, suggesting that the exchange properties of the middle peak are substantially different from those of the other two. No bound Tl^+ peaks were observed in the absence of DNA or in the presence of 2.5 mM control oligonucleotide N (data not shown). Because this parallel-stranded G-quartet does not have a 2-fold symmetry axis perpendicular to its length, the monovalent ions should not be symmetry related. Thus, the observation of three distinct ^{205}Tl peaks of approximately equal areas is consistent with a tetramer in which three Tl^+ ions sit between the layers of the four G-quartets, analogous to that observed with K^+, Na^+, and NH_4^+ stabilized quartets.[4,5,15,16] The narrow middle bound peak in the ^{205}Tl spectrum likely belongs to the middle ion of the G-quartet complex, which would be expected to exchange with free Tl^+ more slowly than the flanking ions. The approximately 120 ppm separation of the free and bound peaks demonstrates that the Tl^+ ions have a bound lifetime limit of at least 3 μs, although measurements of other monovalent cations binding to G quartets suggest that the lifetime may be substantially longer.[5,22]

Recently, ^{15}N-labeled ammonium ions have been used as an ^{1}H NMR spectroscopic probe of monovalent cation binding to duplex DNA and to the G-quartet formed by d(GGGGTTTTGGGG).[4,5] In those 2-fold symmetric, bimolecular G-quartets, two ammonium proton peaks were observed with relative intensities of 2:1, corresponding to two outer and one inner bound ammonium ion.[5] The ^{205}Tl NMR data reported here demonstrate that, like the nonmetallic ammonium cation, Tl^+ can substitute for K^+ within DNA G-quartets and that the bound Tl^+ ions can be directly detected by ^{205}Tl NMR. Thallium NMR may offer some advantage over the ^{15}N-labeled ammonium in that the ^{205}Tl chemical shifts are very sensitive to the chemical environment of the binding site,[11] and the large chemical shift dispersion of ^{205}Tl NMR may allow observation of ions with bound lifetimes shorter than those observable by proton NMR.[23]

Previous biochemical and crystallographic experiments have shown that Tl^+ can also substitute for K^+ within the tetraloop receptor, a complex folding motif present in all three classes of large catalytic RNAs.[7,24] The present study suggests that the spectroscopic properties of ^{205}Tl will provide a useful tool for the analysis of monovalent metal ion binding sites within complex RNA and DNA structures.

Acknowledgment. We thank Jason Rife, Kurt Zilm, and Xiaoling Wu for helpful discussions and for assistance with NMR instrumentation, and Ben Bangerter for construction of the ^{205}Tl frequency amplifiers. This work was supported by NSF CAREER award CHE-9701787 and NIH Grant GM 54839.

JA993614G

(20) Aboul-ela, F.; Murchie, A. I.; Lilley, D. M. *Nature* **1992**, *360*, 280−282.

(21) The amplifier was assembled from a Mirage D24, 430−450 MHz unit (Mirage Communications Equipment, Morgan Hill, CA 95037) and had an output at 461 MHz of 15 W.

(22) Deng, H.; Braunlin, W. H. *J. Mol. Biol.* **1996**, *255*, 476−483.

(23) For example, the chemical shift separation (60 000 Hz) in the ^{205}Tl spectrum shown here allows detection of ions with minimum bound lifetimes of 3 μs compared to 1 ms (150 Hz separation) in a proton spectrum such as that in ref 5.

(24) Costa, M.; Michel, F. *EMBO J.* **1995**, *14*, 1276−1285.

(25) Piotto, M.; Saudek, V.; Sklenar, V. *J Biomol NMR* **1992**, *2*, 661−665; Altieri, A.; Byrd, R. A. *J. Magn. Reson. B* **1995**, *107*, 260−266.

(26) Delaglio, F.; Grzesiek, S.; Vuister, G.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277−293.