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A Lead-Filled G-Quadruplex: Insight into the G-Quartet's Selectivity for Pb^{2+} over K^+

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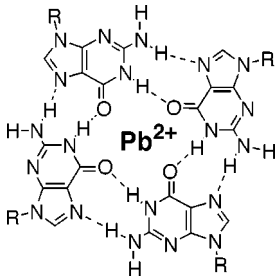
ABSTRACT



The lipophilic nucleoside, G 1, extracts Pb^{2+} picrate from water into organic solvents to give structures based on the hydrogen-bonded G-quartet. Crystal structures indicate important differences between (G 1)$_8$-Pb^{2+} and (G 1)$_8$-K^+. The divalent Pb^{2+} templates a smaller G_8 cage than does K^+, as judged by the M–O6 bond length, O6–O6 diagonal distance, and inter-tetramer separation. The more compact Pb^{2+} octamer correlates with NMR data indicating that N2–N7 hydrogen bonds in (G 1)$_8$-Pb^{2+} are kinetically more stable than in (G 1)$_8$-K^+.

With the increasing activity in supramolecular chemistry,[1] nucleobases have been used to construct some interesting and functional noncovalent assemblies.[2−6] In addition to making new supramolecular architectures, lipophilic nucleobases also serve as valuable models for better understanding the factors that control structure and dynamics in duplex,[7] triplex,[8] and tetraplex[9,10] nucleic acids. Both Gottarelli's group and our group have been studying the cation-templated self-association of lipophilic guanosine derivatives.[10−12] These compounds form self-assembled ionophores that bind cations with affinity and selectivity. In addition to represent-

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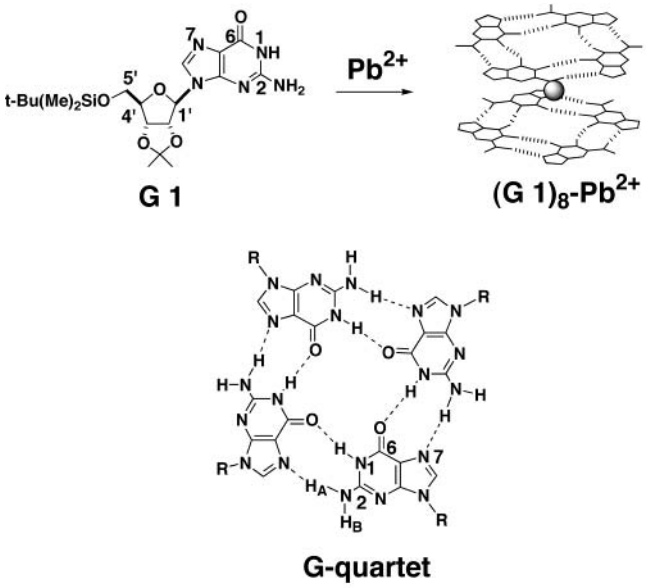
ing a new approach toward ion coordination in organic solvents, our studies should also provide insight into the structural properties of higher-ordered nucleic acid aggregates. Herein, we report solid-state and solution evidence for complexation of Pb^{2+} by the lipophilic guanosine analogue G **1**. The resulting (G **1**)$_8$-Pb^{2+} octamer is a sandwich of two hydrogen-bonded G-quartets (Scheme 1).

Scheme 1. Formation of (G **1**)$_8$-Pb^{2+}



The G-quartet is templated and stabilized by cations.[13] In G-rich DNA, contiguous G-quartets stack to give structures known as G-quadruplexes.[14] In addition to an affinity for the monovalent K^+ and Na^+, the G-quartet also binds divalent cations such as Ba^{2+} and Sr^{2+}.[15,16] Earlier this year, Smirnov and Shafer reported that Pb^{2+} is significantly better than K^+ at inducing G-quartet structure in a DNA oligonucleotide.[17] Since Pb^{2+} (r = 1.29 Å) has an ionic radius similar to but smaller than that of K^+ (r = 1.51 Å),[18] Pb^{2+} should fit into the G_8 cage formed by two stacked G-quartets. It is also reasonable that divalent metal ion coordination might further stabilize the G-quartet's hydrogen bonds. For example, calculations predict that cation coordination to G strengthens hydrogen bonds in G−C and G−G base pairs.[19] This polarization enhancement of hydrogen bond strength is calculated to be greater for divalent cations than for monovalent cations.[20] Such polarization effects have been considered when explaining the G-quartet's monovalent cation selectivity.[21]

In addition to providing general insight into G-quartet−cation interactions, studying Pb^{2+} binding to nucleobases may result in a better understanding of the molecular basis for lead's genotoxicity.[22] While lead's coordination chemistry is well understood,[23] there are few structural details regarding binding of Pb^{2+} to nucleic acids. X-ray crystallography has shown that tRNA and RNA leadzymes have specific Pb^{2+} binding sites that utilize both nucleobase and phosphate ligands.[24,25] Shafer's finding that Pb^{2+} promotes folding of a DNA G-quadruplex[17] focused our attention on the interaction of Pb^{2+} with the lipophilic G **1**.

The nucleoside G **1** is an excellent model compound for obtaining molecular level details about G-quartets. We recently reported a G-quadruplex crystal structure formed from G **1** and K^+ picrate.[10a] The G-quadruplex was composed of two coaxial (G **1**)$_8$-K^+ octamers with K^+ cations sandwiched between G-quartet layers. We have now located Pb^{2+} cations within a similar G-quadruplex. Our current solid-state and solution data confirm that Pb^{2+} is better than K^+ at stabilizing the G-quadruplex.

Solid State Structure. The lipophilic G **1** extracted Pb^{2+} picrate into $CDCl_3$ from water containing a 1:2 molar ratio of $PbCl_2$ and K^+ picrate.[26] Integration of ^{1}H NMR signals for G **1** and picrate indicated an octameric stoichiometry. Solvent evaporation gave a solid whose elemental analysis was consistent with (G **1**)$_8$-Pb^{2+}(pic)$_2$.[27] Single crystals, from $CH_3CN/CHCl_3$, had unit cell dimensions that were macromolecular: a = 25.5691(13) Å, b = 44.385(2) Å, and c = 83.840(4) Å.[28] This cell contained four G-quadruplexes, representing over 4500 non-hydrogen atoms. Each G-quadruplex was formed from two coaxial (G **1**)$_8$-Pb^{2+}

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(26) Competitive extraction experiments indicate that G **1** has at least a 100:1 extraction selectivity for Pb^{+2} picrate over K^+ picrate.

(27) Calculated for $C_{164}H_{252}N_{46}O_{54}Si_8Pb$: C, 47.32; H, 6.06; N, 15.48; Pb, 4.98. Found: C, 47.19; H, 6.18; N, 15.42; Pb, 5.01.

(28) Crystal data for (G **1**)$_8$·Pb(pic)$_2$·$CH_3CN_{2.6}$·$[CHCl_3]_{3.6}$·$H_2O_{4.6}$: $C_{169.81}H_{270.06}N_{48.63}O_{58.63}Cl_{1.31}Si_8Pb$, MW = 4409.35, orthorhombic, space group $P2_12_12_1$; a = 25.5691(13), b = 44.385(2), and c = 83.840(4) Å; α = 90°, β = 90°, γ = 90°; V = 95, 149(8) Å^3; Z = 16, μ(Mo K$_\alpha$) = 0.845 mm^{-1}. Data were collected at 193(2) K on a Bruker SMART1000 CCD diffractometer. The structure was determined by direct methods and refined using SHELXL.[36] The structure was refined to convergence with R(F) = 16.19%, wR(F^2) = 24.35%, and GOF = 1.037 for 88,730 independent reflections [R(F) = 8.75%, wR(F^2) = 21.40% for those 54223 data with $F_o > 4(F_o)$]. Crystal data (excluding structure factors) are deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-146884. Data can be obtained free from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: (+44)1223-336-033. e-mail: deposit@ccdc.cam.ac.uk).

octamers (Figure 1A). With individual G-quartets twisted 30° relative to each other, each Pb^{2+} cation coordinates eight

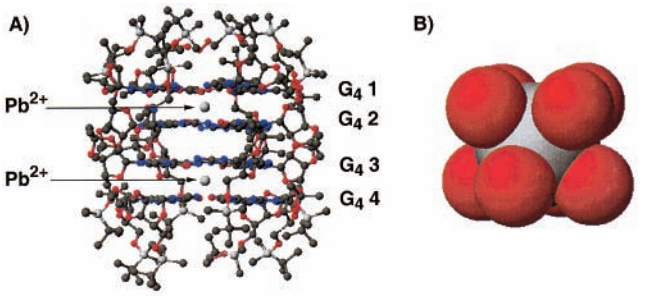

Figure 1. (A) Ball-and-stick representation of the lead-filled G-quadruplex. This G-quadruplex is composed of two coaxial (G **1**)$_8$-Pb^{2+} octamers. The individual G-quartets, G_4 1−G_4 4, are labeled. The picrate anions are removed for clarity. (B) This space-filling representation of the octamer (G **1**)$_8$-Pb^{2+} shows the eight oxygen atoms in the twisted G_8 cage coordinated to Pb^{2+}. Average hydrogen bond distances, Pb^{2+}−O6 distances, and G_4−G_4 inter-quartet distances for (G **1**)$_8$-Pb^{2+} units are listed in Table 1.

O6 atoms in a geometry intermediate between a cube and a square antiprism (Figure 1B). Overall, the crystal structures for the K^+ [10a] and Pb^{2+} quadruplexes are quite similar, raising the issue of whether the genotoxicity of Pb^{2+} may be due to its ability to substitute for K^+ in nucleic acid structures.

Despite their similarities, the (G **1**)$_8$-Pb^{2+} and (G **1**)$_8$-K^+ units have some key structural differences consistent with Pb^{2+} forming the more stable octamer (Table 1). First, the

Table 1. Mean Distances (Å) in the (G 1)$_8$ Octamer Units from X-ray Crystal Structures of the Pb^{2+} and K^+ G-Quadruplexes[a,b]

	(G **1**)$_8$-Pb^{2+}	(G **1**)$_8$-K^+
M−O6	2.66 ± 0.05	2.80 ± 0.06
O6−O6	4.46 ± 0.05	4.58 ± 0.06
between (G **1**)$_4$ planes	3.22 ± 0.01	3.31 ± 0.03
N1−O6 H-bond	2.86 ± 0.03	2.88 ± 0.02
N2−N7 H-bond	2.82 ± 0.02	2.90 ± 0.01

[a] Values for (G **1**)$_8$-K^+ are mean distances for the unit cell's four G-quartets, see ref 10a. The standard deviations are those observed for the set of distances in the four G-quartets. [b] Values for (G **1**)$_8$-Pb^{2+} are mean distances for the structure's 16 unique G-quartets. The standard deviations are those observed for the set of distances in the 16 G-quartets.

mean cation−G O6 distances are 0.14 Å shorter in (G **1**)$_8$-Pb^{2+} than in (G **1**)$_8$-K^+. Second, the mean O6−O6 diagonal, a measure of G-quartet diameter,[29] is 0.12 Å shorter for (G **1**)$_8$-Pb^{2+} than for (G **1**)$_8$-K^+. Third, vertical separation of G-quartets in (G **1**)$_8$-Pb^{2+} is approximately 0.10 Å less than in (G **1**)$_8$-K^+. In short, the divalent Pb^{2+} templates a smaller G_8 cage than does K^+. Hydrogen bond lengths for the N2 H_A−N7 pair also become shorter as the octamer cage shrinks. As described below, a more compact octamer correlates well with NMR data indicating that the N2 H_A−N7 hydrogen bonds in (G **1**)$_8$-Pb^{2+} are kinetically more stable than in (G **1**)$_8$-K^+.

Solution NMR Studies. We used both ^{207}Pb and ^{1}H NMR to show that the (G **1**)$_8$-Pb^{2+} is also stable in solution. Previous heteronuclear NMR studies using $^{23}Na^+$,$^{15}NH_4^+$, and $^{81}Tl^+$ have directly demonstrated cation binding by DNA G-quartets.[30−32] Lead-207, a spin $^1/_2$ nucleus of 22% natural abundance, has a large chemical shift range (16 000 ppm) that makes its NMR spectra exquisitely sensitive to the coordination environment.[33] After extraction of Pb^{2+} picrate by G **1**, a sharp ^{207}Pb NMR signal in $CDCl_3$ was observed at δ −3029, relative to $PbMe_4$ (see Supporting Information). The same ^{207}Pb NMR peak was observed when crystals of the Pb^{2+} complex were dissolved in $CDCl_3$. This ^{207}Pb NMR peak is strong evidence for cation coordination by G **1**, since Pb^{2+} picrate itself is insoluble in $CDCl_3$.

Two sets of ^{1}H NMR signals in a 1:1 ratio and diagnostic NOEs revealed that (G **1**)$_8$-Pb^{2+} forms in $CDCl_3$ by head-to-tail stacking of G-quartets.[34] Amide N1 H (δ 11.80 and 11.41) and amino N2 H_A (δ 9.97 and 9.20) resonances were downfield shifted, as expected for hydrogen-bonded protons. These resonances were present only after Pb^{2+} extraction, again strong evidence that the cation templates the G-quartet's structure.

In the ^{1}H NMR spectrum of a G_8 octamer, there are two sets of amino resonances. Each set contains a hydrogen-bonded resonance (N2 H_A) and a non-hydrogen-bonded resonance (N2 H_B). The ^{1}H NMR spectra revealed that Pb^{2+}, as compared to K^+, forms a G-quartet with kinetically stronger N2 H_A-N7 hydrogen bonds. Specifically, C2−N2 bond rotation was slower in (G **1**)$_8$-Pb^{2+} than in (G **1**)$_8$-K^+.

All four amino NH_2 resonances in (G **1**)$_8$-Pb^{2+} were sharp and distinct at 25 °C (Figure 2). Coalescence of these amino signals did not occur even at 50 °C, indicating a significant barrier for C2−N2 bond rotation in (G **1**)$_8$-Pb^{2+}. In marked contrast, amino resonances for (G **1**)$_8$-K^+ were broadened into the baseline at temperatures above 10 °C, indicating

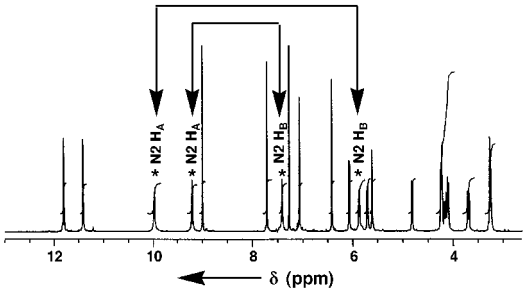

Figure 2. A region of the 500 MHz ^{1}H NMR spectra of (G **1**)$_8$-Pb^{2+}(pic)$_2$ (5 mM) in $CDCl_3$ at 25 °C. The two sets of separate resonances for the N2 H_A and N2 H_B amino protons (marked by asterisks) indicate a significant barrier for C2−N2 bond rotation in (G **1**)$_8$-Pb^{2+}.

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much faster C2−N2 bond rotation in the K^+ octamer. A conservative estimate indicates that $\Delta G^{\ddagger}_c$ for C−N bond rotation is at least 1.5 kcal/mol greater for the Pb^{2+} complex as compared to the K^+ complex.[35] These results, showing that the C−N bond rotation barrier is significantly higher for (G **1**)$_8$-Pb^{2+} relative to (G **1**)$_8$-K^+, indicate that the divalent cation stabilizes the G-quartet's hydrogen bonds more than a monovalent cation.

Conclusion. Both the solid state and solution evidence show that the smaller and more highly charged Pb^{2+} cation templates a smaller G_8 octamer cage than does K^+. This tighter coordination geometry kinetically stabilizes the G-quartet's N2 H_A−N7 hydrogen bonds. These experimental results, including data from the first crystal structure of a G-quadruplex bound to a divalent cation, are consistent with calculations that predict the polarization enhancement of DNA base pairing upon cation binding.[19−21] While it remains to be seen if Pb^{2+} binding to DNA G-quartets has a role in the cause and effect of lead's genotoxicity, these studies with G **1** provide a firm rationale for why Pb^{2+} binds more tightly to a G-quadruplex than does K^+.

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Supporting Information Available: Crystallographic tables, final coordinates and thermal parameters, selected bond lengths and angles, and ^1H and ^{207}Pb NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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