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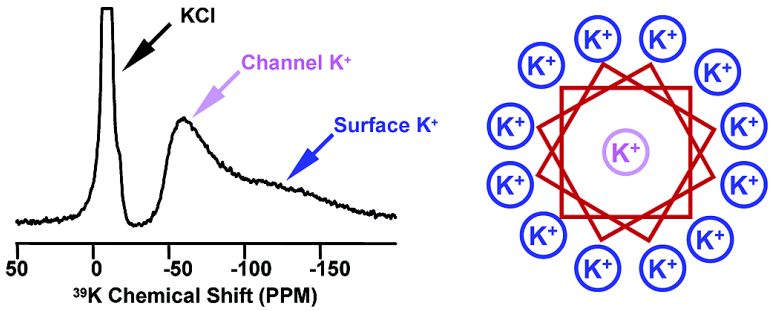

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Direct Detection of Potassium Cations Bound to G-Quadruplex Structures by Solid-State ^{39}K NMR at 19.6 T

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The presence of K^+ ions in living cells is believed to be crucial for the stability of G-quadruplex structures found in telomeric DNA and other G-rich sequences.[1−5] Recent crystallographic studies have yielded reliable information about the K^+ ion coordination geometry in G-quadruplexes,[6−9] confirming an earlier proposal that the K^+ ion is sandwiched between two G-quartets.[10] Because of the critical role that monovalent cations play in G-quadruplex formation, considerable efforts have also been devoted to the development of other spectroscopic techniques for detecting cation binding in G-quadruplexes. Successful NMR applications have been demonstrated to use ^{23}Na (spin $^3/_2$), ^{15}N (spin $^1/_2$), and ^{205}Tl (spin $^1/_2$) as NMR probes to directly study Na^+, NH_4^+, and Tl^+ ions in G-quadruplexes.[11−16] Smirnov et al. recently showed that extended X-ray absorption fine structure (EXAFS) can be used to characterize the Pb^{2+} binding site in G-quadruplexes.[17] In general, the rather weak association between K^+ ions and biological structures renders solution ^{39}K (spin $^3/_2$) NMR spectroscopy to be of limited utility.[10,18] Furthermore, ^{39}K is one of the low-γ quadrupolar nuclides that are extremely difficult to study by NMR at low magnetic fields. Only a few solid-state ^{39}K NMR studies have been reported, and most of those focus on simple inorganic K^+ salts.[19] Until now, crystallography has been the only biophysical technique capable of directly localizing K^+ ions bound to biological structures. Here we report the first solid-state ^{39}K NMR detection of K^+ ions bound to the G-quadruplex structures formed by 5′-*tert*-butyl-dimethylsilyl-2′,3′-*O*-isopropylidene guanosine (G**1**), guanosine (G**2**), and guanosine 5′-monophosphate (G**3**) (Scheme 1).[20] Our strategy for overcoming practical difficulties in studying ^{39}K is to utilize an ultrahigh magnetic field, 19.6 T (830 MHz for ^{1}H), at the National High Magnetic Field Laboratory (NHMFL).

The lipophilic nucleoside G**1** was used as the standard sample for our ^{39}K NMR experiment because G**1** self-associates in the presence of K^+ and Cs^+ picrate to form a crystallographically defined G-quadruplex.[7] As shown in Figure 1, the G-quadruplex formed from G**1** consists of four G-quartets that are stacked on top of one another to give a structure with a central ion channel. This channel is fully occupied by three collinear K^+ ions along its central axis. Each of the K^+ ions is sandwiched by two G-quartets, a structural feature remarkably similar to that recently found in G-rich oligonucleotides.[8,9] As shown in Figure 2, the ^{39}K magic-angle spinning (MAS) spectrum of G**1** exhibits a peak centered at −45 ppm. The detailed features in the line shape suggest an overlap of several central-transition powder spectra. Because there are three crystallographically distinct, yet similar, K^+ sites inside the G**1** quadruplex channel, it is not possible to extract an accurate value

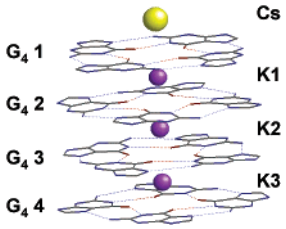
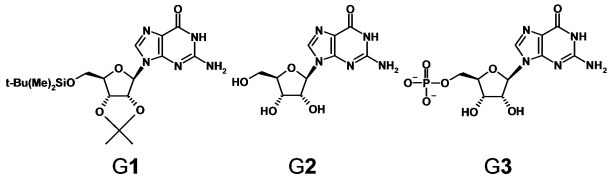


Figure 1. Diagram illustrating the cation binding environment in the G-quadruplex structure formed by G**1** self-association.[7]

Scheme 1



for the ^{39}K nuclear quadrupole coupling constant (C_Q) for each of the K^+ sites. On the basis of the ^{39}K spectra obtained at 19.6 and 11.7 T, we obtained the following estimates: $\delta = -42$ ppm and $C_Q < 0.7$ MHz. G**2** forms a viscous gel in water in the presence of KCl, indicating formation of a highly ordered molecular assembly. An earlier X-ray fiber diffraction study confirmed that the G**2** aggregates have a quadruplex structure.[21] The ^{39}K MAS spectrum of G**2** exhibits a peak with $\delta = -45$ ppm and $C_Q \approx 0.7-0.8$ MHz. An additional sharp peak at −9 ppm arises from a small excess of KCl. These observations suggest that, in the self-aggregates of G**2**, the K^+ ions reside exclusively inside the quadruplex channel in a fashion similar to those in the G**1** quadruplex. G**3** is among the earliest examples examined by Gellert et al., who first proposed the G-quartet model.[22] Subsequent X-ray diffraction studies confirmed that the self-assembly of G**3** forms a right-handed quadruplex helix.[23] In contrast to nucleosides G**1** and G**2**, G**3** is a mononucleotide where the negatively charged phosphate group is another potential K^+ binding site. Consequently, in addition to the large KCl signals (−9 ppm and associated spinning sidebands) and the signal attributable to the channel K^+ ions, the ^{39}K MAS spectrum of G**3** shows another peak with a line width 3 times greater than that of the signal from the channel K^+ ions. This broad signal ($\delta \approx -60$ ppm) can be assigned to the K^+ ions bound to the phosphate group. The ratio between the signal areas for the phosphate-bound and channel K^+ ions is approximately 3:2, much smaller than the 8:1 ratio expected for a G**3** quadruplex saturated with K^+ ions. Our previous study showed that the G-quadruplex channel strongly favors K^+ ions, whereas the doubly charged phosphate group of G**3** prefers Na^+ ions over K^+ ions.[13] As the G-quadruplex sample containing G**3** was prepared in the presence of both K^+ and Na^+

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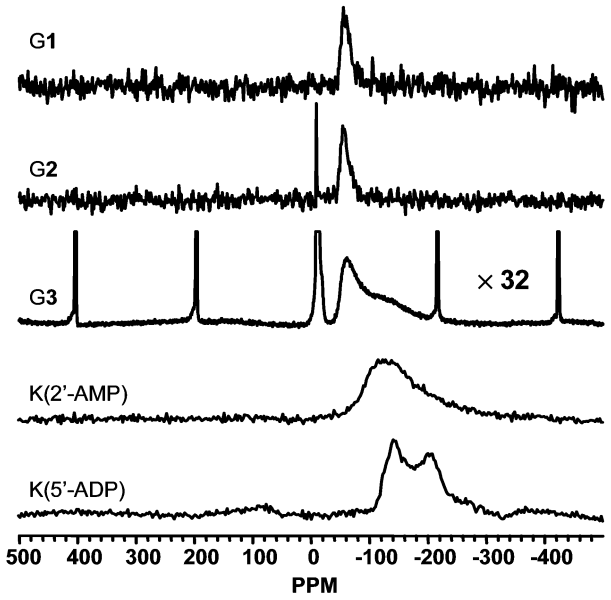

Figure 2. Experimental ^{39}K MAS NMR spectra at 19.6 T. All solid-state NMR experiments were performed with a narrow-bore magnet (31 mm) and a Bruker Avance console at NHMFL operating at 38.72 MHz for ^{39}K nuclei. A home-built MAS probe equipped with a 4-mm stator was used. Reported ^{39}K chemical shifts were referenced to KBr(s), $\delta = 0$ ppm. Other experimental details: G**1**, 10-kHz spinning, 7500 transients, 2-s recycle delay; G**2**, 10-kHz spinning, 12 864 transients, 0.5-s recycle delay; G**3**, 8-kHz spinning, 100 000 transients, 1-s recycle delay; K(2′-AMP), 8-kHz spinning, 34 956 transients, 2-s recycle delay; K(5′-ADP), 8-kHz spinning, 45 364 transients, 2-s recycle delay.

ions,[20] the central channel is clearly filled with K^+ ions; however, there are likely to be a considerable amount of Na^+ ions that remain bound to the phosphate groups. To further confirm this spectral assignment, we obtained ^{39}K MAS spectra for hydrated K salts of adenosine 2′-monophosphate and adenosine 5′-diphosphate, K(2′-AMP)·1.5H_2O and K(5′-ADP)·2H_2O. The K^+ ion in K(2′-AMP) is coordinated to two phosphate oxygens, two water molecules, and two hydroxyl groups from the ribose.[24] The ^{39}K spectrum of K(2′-AMP) exhibits a broad peak with $\delta = -55$ ppm and $C_Q = 1.85$ MHz, in excellent agreement with that observed for the phosphate-bound K^+ ions in G**3**. The K^+ ion in K(5′-ADP) is coordinated to seven neighbors: four phosphate oxygens, one water, one hydroxyl, and an N atom from the adenine base.[25] The ^{39}K MAS spectrum of K(5′-ADP) exhibits a clear second-order quadrupole line shape, with $\delta = -105$ ppm and $C_Q = 2.05$ MHz. These parameters are quite different from those observed for the K^+ ions in G-quadruplexes, reflecting the unusual K^+ coordination in K(5′-ADP).

In summary, we have obtained unambiguous ^{39}K NMR signatures for the K^+ ions bound to G-quadruplex structures. This is an important first step toward solid-state ^{39}K NMR studies of telomeric DNA. The rich spectral information in the ^{39}K NMR spectra of K-nucleotide systems suggests that solid-state NMR is a viable new method for detecting K^+ ions in biomolecular systems.

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(20) G**1** was prepared as previously described.[7] G**1** (40 mg) was dissolved in chloroform (5 mL) and stirred for 2 h with a 5.0-mL aqueous solution of K^+ and Cs^+ picrate (75:25 mM). The chloroform layer was separated and dried under a stream of N_2(g) to yield a yellow powder. Yellow, cubelike crystals of $[G\mathbf{1}]_{16}$·[3K/$CsPic_4$] were obtained upon crystallization from acetonitrile solution. The unit cell parameters of the cubelike crystals used for NMR experiments were identical to those reported in the X-ray study.[7] The self-assembly of G**2** was achieved as follows. To a 30-mL aqueous solution of G**2** (250 mg) was added 5.75 mL of 3.5 M KCl(aq) under heating. Upon cooling of the solution to room temperature, a white gel was formed. The gel was washed several times with cold water to remove excessive KCl and dried in a vacuum desiccator. The self-assembly of G**3** was achieved in the following manner. To an aqueous solution of Na_2(5′-GMP) (300 mg) at pH 8 was added 2.0 mL of 1.0 M KCl(aq). A white gel was formed upon standing. The gel was carefully washed three times with a cold 1:1 mixture of ethanol and water and dried in air.

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