Solid-state ^{87}Rb NMR signatures for rubidium cations bound to a G-quadruplex†

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We report the first solid-state ^{87}Rb NMR characterization for rubidium cations bound to G-quartet structures formed by self-association of guanosine 5′-monophosphate and 5′-*tert*-butyldimethylsilyl-2′, 3′-*O*-isopropylidene guanosine.

Alkali metal cations such as Na^+ and K^+ are known to play an important role in stabilizing G-quadruplex structures.[1] Until recently X-ray crystallography has been the only technique available for localizing alkali metal cations in proteins and nucleic acids. In the past several years, solid-state NMR has emerged as a new method for detecting Na^+ and K^+ cations in nucleic acids and related molecular systems.[2–9] For example, we recently used solid-state ^{23}Na NMR to determine the mode of Na^+ binding to an *Oxytricha nova* telomeric DNA repeat, d($G_4T_4G_4$).[9] While K^+ is ubiquitous in biological systems, ^{39}K (spin–3/2, natural abundance = 93.3%) NMR is quite difficult. It is therefore desirable to develop a surrogate nuclear probe for K^+ binding studies. Among alkali metals, Rb^+ has an ionic radius (1.48 Å) slightly larger than that of K^+ (1.33 Å). In many aspects, Rb^+ can be considered to be identical to K^+. Rb^+ has been widely used as a K^+ congener in both solution NMR and magnetic resonance imaging (MRI) studies.[10–13] Rb^+ has also been used in crystallographic studies.[14–16] In the context of G-quadruplexes, Rb^+ has a binding affinity similar to K^+ for the G-quadruplex structure (at both channel and surface sites).[6] It is known that ^{87}Rb (spin–3/2, natural abundance = 27.8%) NMR is about 100 times more sensitive than ^{39}K NMR. With ^{87}Rb isotopic enrichment (up to 99%), the sensitivity improvement of using ^{87}Rb to replace ^{39}K as the NMR probe can be increased by a factor of approximately 400. It is a truly exciting prospect if solid-state ^{87}Rb NMR can be used as a surrogate probe for studying K^+ binding in biological systems. As a first step, here we report solid-state ^{87}Rb NMR characterization for Rb^+ cations bound to several G-quartet structures.

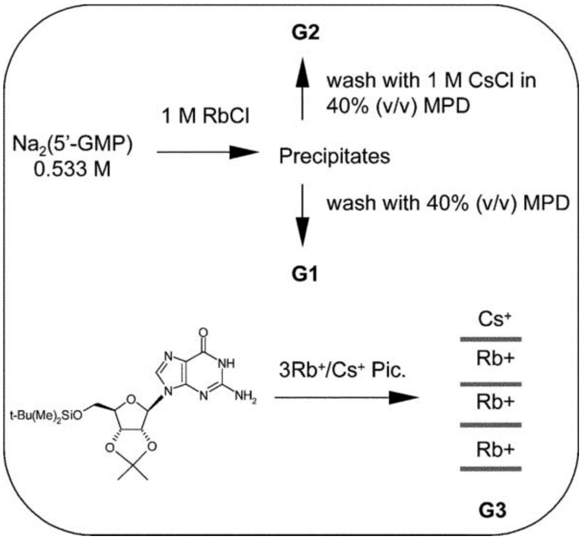

Fig. 1 Illustration of the sample preparation used in this study.

As illustrated in Fig. 1, we prepared two 5′-GMP samples in this study.‡ Results from X-ray powder diffraction, solid-state ^{13}C and ^{23}Na NMR experiments (ESI†) suggest that both of the 5′-GMP samples are 5′-GMP aggregates containing G-quartet stacks with a distance of 3.29 Å between two adjacent stacks. The G-quartet channel is filled with mixed Na^+ and Rb^+ cations. Fig. 2 shows the ^{87}Rb magic-angle spinning (MAS) NMR spectra of **G1** and **G2**.§ In the ^{87}Rb NMR spectrum for **G1**, three groups of signals are observed. The sharp peak at δ_{iso} = 123 ppm is due to residual RbCl. The other two signals are associated with the two types of Rb^+ binding sites in a G-quadruplex structure: channel and surface (or phosphate-bound) sites. This is similar to the situations in 5′-GMP systems containing Na^+ and K^+.[4,7] In solution, only an

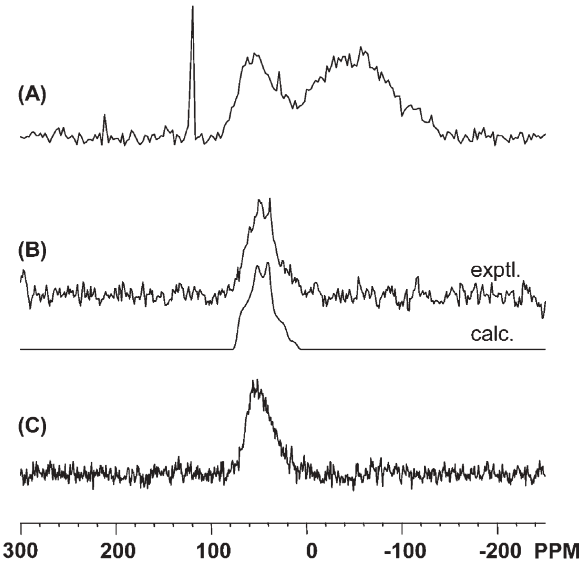

Fig. 2 Solid-state ^{87}Rb MAS NMR spectra of (A) **G1**, (B) **G2**, and (C) **G3**.

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averaged signal was observed in the earlier studies by Laszlo and co-workers.[17] The ^{87}Rb NMR spectrum for **G2** exhibits only one signal having a characteristic line shape caused by the second-order quadrupole interaction. This observation is in agreement with the expectation that all Rb^+ cations in **G2** reside inside the channel. An analysis of the observed line shape yields the following ^{87}Rb NMR signature for the channel Rb^+ cations: $\delta_{iso} = 74 \pm 2$ ppm, $C_Q = 5.1 \pm 0.2$ MHz, and $\eta_Q = 0.6 \pm 0.1$. We also obtained the following estimated parameters for the surface Rb^+ cations: $\delta_{iso} = 5 \sim 10$ ppm and $C_Q = 7.5 \sim 7.7$ MHz.

To confirm the above spectral assignment, we also prepared a G-quadruplex structure using a lipophilic guanosine nucleoside, 5′-*tert*-butyl-dimethylsilyl-2′, 3′-*O*-isopropylidene guanosine. This guanosine nucleoside can self-assemble into a G-quadruplex structure in the presence of alkali metal picrates. For example, in the presence of K^+ and Cs^+ picrates in a 3 : 1 ratio, the self-assembled G-quadruplex structure consists of four stacking G-quartets with three K^+ cations residing inside the channel and one capping Cs^+ cation.[18] The corresponding Rb^+ complex (denoted as **G3**) is expected to be isostructural to the K^+ analog. As seen from Fig. 2, the ^{87}Rb NMR signal observed for **G3** is very similar to that for **G2**. Because there are three crystallographically different Rb^+ cations in **G3**, the ^{87}Rb NMR signal does not show any detailed line shape. Nonetheless, the ^{87}Rb NMR result for **G3** confirms unambiguously the ^{87}Rb NMR spectral assignment.

The new solid-state ^{87}Rb NMR result, coupled with previously known NMR signatures for $^{23}Na^+$ and $^{39}K^+$ cations bound to a G-quadruplex, provides us with an excellent opportunity to examine the NMR parameters for these alkali metal cations on a common ground. It is interesting to note from Fig. 3 that, whereas the channel Na^+ has a smaller chemical shift than does the surface Na^+, the chemical shifts for K^+ and Rb^+ show an opposite trend. This observation is consistent with results for alkali metals in ionophore complexes.[19] As expected, among the three alkali metals, the heaviest cation $^{87}Rb^+$ exhibits the largest chemical shift difference between channel and surface binding sites, *ca.* 60 ppm. This demonstrates the remarkable sensitivity of ^{87}Rb chemical shielding to the chemical environment at the binding site. It is also worth noting that, even at a moderate magnetic field, 11.75 T, the observed ^{87}Rb NMR sensitivity and resolution are already higher than those of the ^{39}K experiment at 19.6 T.[7]

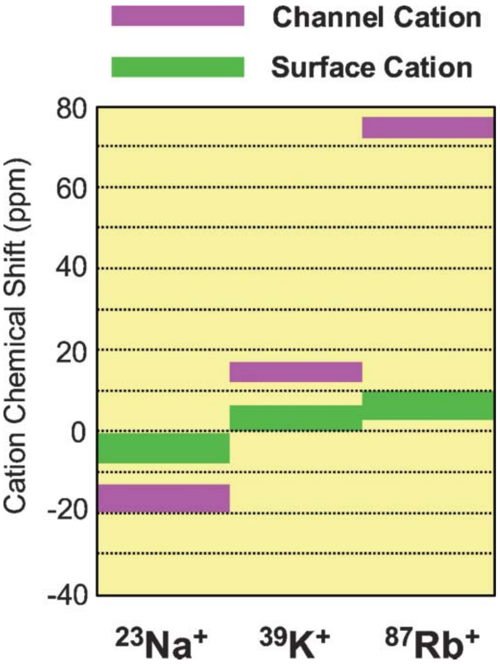

Fig. 3 Comparison of solid-state NMR signatures for alkali metal cations bound to a G-quadruplex structure.

In summary, our new solid-state ^{87}Rb NMR results have demonstrated the feasibility of this new NMR probe for studying cation binding in G-quadruplexes. Because ^{87}Rb has a much higher NMR sensitivity than does ^{39}K, we believe that ^{87}Rb will be a useful surrogate NMR probe for detecting K^+ cation binding in nucleic acids and ion channel proteins. Research in this direction is under way in this laboratory.

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Notes and references

‡ The two 5′-GMP samples were prepared as follows. To 1 mL 0.533 M Na_2(5′-GMP) solution was added 121 mg RbCl (1 mmol). The mixture was heated until complete dissolution. The solution was then allowed to cool to 5 °C and white wax-like precipitates formed. The precipitates were gently washed with 40% (v/v) 2-methyl-2,4-pentanediol (MPD) aqueous solution (3 × 0.5 mL) to remove excessive free RbCl. This sample is denoted as **G1**. The second 5′-GMP sample (denoted as **G2**) was prepared by washing the aforementioned precipitates by 1 M CsCl in 40% (v/v) MPD aqueous solution (3 × 0.5 mL) to remove both free RbCl and phosphate-bound Rb^+ cations. Because Cs^+ is known to be too large to enter the G-quadruplex channel, the channel cations (both Na^+ and Rb^+) are expected to remain unperturbed in **G2**, as compared to the situations in **G1**. 5′-*tert*-Butyl-dimethylsilyl-2′, 3′-*O*-isopropylidene guanosine and **G3** microcrystals were prepared according to a procedure described previously, except rubidium picrate was used.[5]

§ Solid-state ^{87}Rb MAS NMR spectra were recorded on a Bruker Avance-500 spectrometer operating at 500.03 and 163.62 MHz for ^{1}H and ^{87}Rb nuclei, respectively. The sample spinning frequency was 15,000 ± 4 Hz. The RF field strength at the ^{87}Rb Larmor frequency was approximately 90 kHz. All ^{87}Rb chemical shifts are referenced to Rb^+(aq) at $\delta = 0$ ppm.

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