

Direct NMR detection of the “invisible” alkali metal cations tightly bound to G-quadruplex structures

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Abstract

We report the first direct solution NMR detection of the alkali metal cations ($^{23}\text{Na}^+$, $^{39}\text{K}^+$, and $^{87}\text{Rb}^+$) residing inside G-quadruplex channel structures formed by guanosine 5'-monophosphate and a DNA oligomer, d(TG₄T). In solution, these channel alkali metal cations are tightly bound to the G-quadruplex structure and have been considered to be “invisible” to NMR spectroscopy for many years. Our finding that it is possible to directly observe these alkali metal cations by NMR spectroscopy provides a new tool for studying cation binding affinity and dynamics in G-quadruplex DNA.

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Until now detection of alkali metal cations in G-quadruplexes has relied on either direct solid-state techniques such as X-ray crystallography and solid-state NMR [1–15], or indirect methods using surrogate spin-1/2 probes such as $^{15}\text{NH}_4^+$ and $^{205}\text{Tl}^+$ in solution NMR experiments [16–18]. On the basis of these studies, two types of alkali metal cations are generally found to be bound to a G-quadruplex structure: one type being loosely coordinated to phosphate groups and the other residing inside the G-quadruplex channel. This latter type of alkali metal cations (referred to as channel cations in this study) is tightly bound to the G-quadruplex structure and has long been considered to be “invisible” to NMR spectroscopy in solution [19,20]. As a result, although a number of crystal and NMR structures for G-quadruplex DNA are available in the literature, little is known about the alkali metal cation transport dynamics in this important class of nucleic acid structures. Recently, we have successfully obtained NMR signatures for Na^+ , K^+ , and Rb^+ cations bound to a G-quadruplex structure using solid-state NMR spectroscopy

[11,12,15]. Because cation movement is greatly restricted in the solid state, it is straightforward to observe solid-state NMR signals for channel Na^+ , K^+ , and Rb^+ cations. Of course, the limitation of the solid-state NMR approach is that it is not possible to study cation transport dynamics. The primary rationale for the “invisibility” of tightly bound channel alkali metal cations in the solution state is that the quadrupole relaxation for ^{23}Na ($S = 3/2$), ^{39}K ($S = 3/2$), and ^{87}Rb ($S = 3/2$) nuclei would be so rapid that the NMR signals become too broad to be observed. However, our solid-state NMR characterization of the channel alkali metal cations has led us to challenge this commonly accepted viewpoint. Here we report the first solution NMR observation for these so-called “invisible” alkali metal cations ($^{23}\text{Na}^+$, $^{39}\text{K}^+$, and $^{87}\text{Rb}^+$) in two G-quadruplex structures: guanosine 5'-monophosphate (5'-GMP) and a DNA oligomer, d(TG₄T). The new finding also provides new insight into alkali metal cation transport through a G-quadruplex channel.

Materials and methods

Sample preparation. Hydrated sodium salt of guanosine 5'-monophosphate (5'-GMP, >99% purity) and sodium chloride were purchased

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from Sigma–Aldrich (Ontario, Canada). The DNA oligonucleotide used in this study, d(TG₄T), was synthesized by Cortec DNA Service Laboratories (Kingston, Ontario, Canada). The oligonucleotide was synthesized on a 12-channel oligonucleotide synthesizer (Northwest Engineering Inc.) using standard CE phosphoramidite chemistry. The oligonucleotide was cleaved from the CPG support and protecting groups were removed using a 50/50 mixture of ammonium hydroxide and methylamine (AMA) for 5 h at room temperature. Prime pure oligonucleotide purification Cartridges were used to purify the oligonucleotide according to the manufacturer's protocol. The purified oligomer was dialyzed against NaCl(aq) for two hours at each of the following concentrations: 250, 100, and 1 mM. A microdialyzer and a membrane of 1000 MW cutoff (Sialomed, Inc.) were used in the dialysis experiment. The oligomer was then lyophilized using a freeze dryer (Labconco Freezone 4.5) operating at $480 \pm 5 \times 10^{-3}$ mbar and -51 ± 1 °C. The DNA oligomer was redissolved in 0.4 mL of 10 mM sodium phosphate buffer (pH 7.1) with 100 mM NaCl(aq) for NMR experiments.

NMR spectroscopy. All ^{23}Na , ^{39}K , and ^{87}Rb NMR experiments were performed on Bruker Avance-400, Avance-500 and Avance-600 spectrometers. For ^{23}Na NMR experiments, a 5-mm quartz NMR tube (NE-HQ5-7, New Era Enterprises, Inc.) was used to reduce the ^{23}Na background signal. Detailed experimental parameters are given in figure captions.

Results and discussion

Fig. 1 shows ^{23}Na NMR spectra for 0.80 M Na₂(5'-GMP) in an aqueous solution at pH 8. The signal centered at 0 ppm exhibits a bi-Lorentzian line shape, which is due to a slow exchange of Na⁺ cations between phosphate-bound and free states. This observation is in agreement with previous ^{23}Na NMR studies [21–23]. In this study, we refer to this type of alkali metal cations as surface/free

cations. The small peak at –17 ppm is due to the Na⁺ cations residing inside the 5'-GMP channel. This ^{23}Na chemical shift value is in excellent agreement with the NMR signature obtained for channel Na⁺ cations by solid-state ^{23}Na NMR [10,11]. The signal intensity for the channel Na⁺ cations decreases as the sample temperature is increased from 278 to 308 K, an indication of “melting” of the 5'-GMP aggregates. This melting behavior can be monitored using both ^1H and ^{23}Na NMR spectra (see [supporting materials](#)).

To examine whether the same type of channel ^{23}Na NMR signals can also be detected for a true DNA G-quadruplex, we prepared a DNA oligomer sample, d(TG₄T). In the presence of Na⁺, d(TG₄T) is known to fold into a parallel-stranded G-quadruplex structure in solution [24]. The ^1H NMR spectra obtained for our d(TG₄T) sample were in excellent agreement with those reported in the literature [24], confirming the formation of the G-quadruplex. As seen from Fig. 1, the ^{23}Na NMR spectra of d(TG₄T) also show the channel Na⁺ signal at –17 ppm. Interestingly, the total integrated area for this signal remains approximately unchanged between 278 and 293 K, indicating that the G-quadruplex structure of d(TG₄T) does not melt at 293 K. We have observed similar ^{23}Na NMR spectra for d(TG₄T) at two other magnetic fields, 9.4 and 11.7 T. At 9.4 T, a 17-ppm separation between the channel and surface/free Na⁺ cations corresponds to a frequency separation of approximately 1800 Hz. The fact that two well-defined ^{23}Na NMR signals are observed for the channel and surface/free Na⁺ cations for d(TG₄T) at 298 K immediately suggests that the residence time for Na⁺ cations inside the G-quadruplex channel must be much longer than $1/1800 \text{ Hz} = 0.556 \text{ ms}$. This means that Na⁺ cations should move through a G-quadruplex channel at a rate much slower than that previously suggested by Deng and Braunlin [20]. We are currently in the process of determining this Na⁺ cation transport rate accurately and the results will be published elsewhere.

Here we briefly comment on the reasons as to why previous researchers failed to observe the ^{23}Na NMR signal for channel Na⁺ cations. First, early cation NMR studies by Laszlo and colleagues [21] on 5'-GMP were carried out at low magnetic fields (<5.8 T), which made it impossible to separate the signals for channel and free cations. Second, a common belief that alkali metal cations at a strong binding site must exhibit severe quadrupole line broadening, coupled with a lack of chemical shift information for the channel cations at the time, led Braunlin and colleagues [19] to conclude incorrectly that the channel alkali metal cations in G-quadruplexes are “invisible” to NMR in solution.

Fig. 2 shows ^{39}K NMR spectra for 0.53 M Na₂(5'-GMP) in the presence of 0.1 M K⁺. Because K⁺ cations are strongly favored by the 5'-GMP channel [13], even at a Na⁺:K⁺ ratio of 10:1, a significant proportion of the K⁺ cations should enter the channel. Indeed, two ^{39}K NMR signals are clearly observed at 278 K. The signal at 0 ppm is due to surface/free K⁺ cations and the signal at

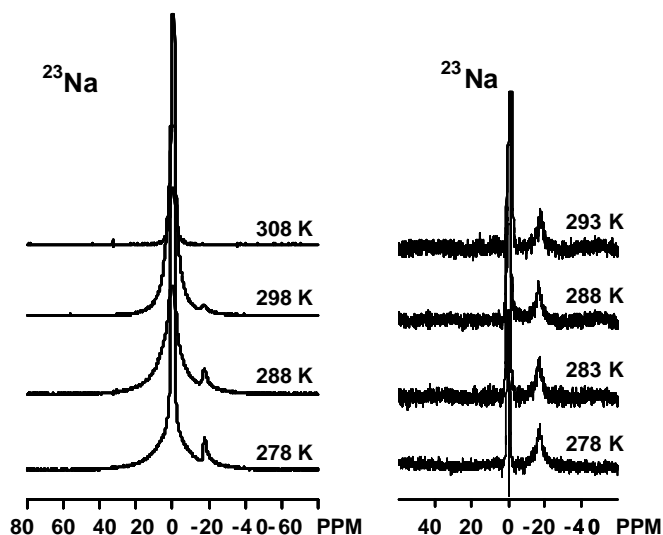


Fig. 1. Left: variable-temperature ^{23}Na NMR spectra of 0.80 M Na₂(5'-GMP) obtained on a Bruker Avance-500 spectrometer ($B_0 = 11.75 \text{ T}$) operating at 132.26 MHz for ^{23}Na nuclei. Right: ^{23}Na NMR spectra of d(TG₄T) obtained on a Bruker Avance-600 spectrometer ($B_0 = 14.1 \text{ T}$). An inversion-recovery sequence was used to partially suppress the large signal from the free Na⁺ ions. A recovery delay of 13 ms (close to the null point) was used. Typically, 250,000 scans were accumulated with a recycle time of 50 ms. The DNA sample was prepared at 8 mM strand concentration in 10 mM sodium phosphate buffer (pH 7.1) and 100 mM NaCl. All ^{23}Na chemical shifts were referenced to Na⁺(aq), $\delta = 0 \text{ ppm}$.

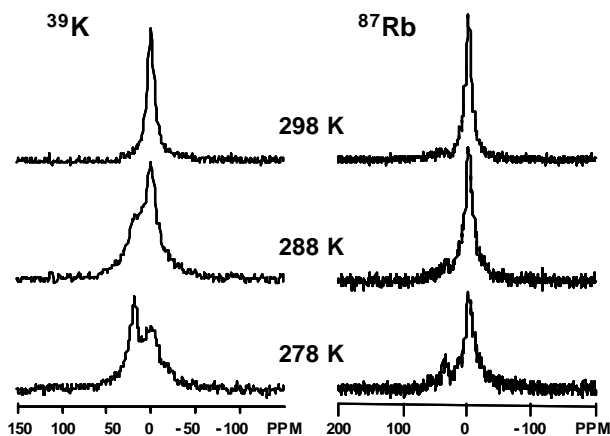


Fig. 2. Left: ^{39}K NMR spectra of 0.53 M $\text{Na}_2(5'\text{-GMP})$ in the presence of 0.10 M K^+ . All ^{39}K NMR spectra were obtained on a Bruker Avance-500 spectrometer ($B_0 = 11.75$ T) operating at 23.33 MHz for ^{39}K nuclei. A probe equipped with a solenoid coil (5 mm) was used to reduce probe ring-down. Typically, 500,000 scans were accumulated with a recycle time of 10 ms. Reported ^{39}K chemical shifts were referenced to $\text{K}^+(\text{aq})$, $\delta = 0$ ppm. Right: ^{87}Rb NMR spectra of 1.07 M $\text{Na}_2(5'\text{-GMP})$ in the presence of 0.10 M Rb^+ . All ^{87}Rb NMR spectra were obtained on a Bruker Avance-600 spectrometer ($B_0 = 14.1$ T) operating at 196.38 MHz for ^{87}Rb nuclei. Typically, 20,000 scans were accumulated with a recycle time of 50 ms. Reported ^{87}Rb chemical shifts were referenced to $\text{Rb}^+(\text{aq})$, $\delta = 0$ ppm.

18 ppm can be assigned to the channel K^+ cations. The observed ^{39}K chemical shift for channel K^+ cations is in agreement with the solid-state ^{39}K NMR data [12]. Fig. 2 also shows ^{87}Rb NMR spectra for 1.07 M $\text{Na}_2(5'\text{-GMP})$ in the presence of 0.10 M Rb^+ . Similar to the situations discussed earlier, the signal intensity for the channel Rb^+ cations (centered at 38 ppm at 14.1 T) shows a strong temperature dependence. This once again is related to the degree of 5'-GMP aggregation occurring at different temperatures.

Similar to the earlier discussion on Na^+ transport rate, we can also estimate the rate at which K^+ and Rb^+ cations move through the 5'-GMP channel. At 11.7 T, the observed NMR frequency separation between channel and free cations is 420 and 5500 Hz for K^+ and Rb^+ , respectively. Because K^+ and Rb^+ have similar binding affinity to the channel site, it is safe to use 420 Hz as an upper limit for the transport rate for both K^+ and Rb^+ cations. Compared to Na^+ , K^+ and Rb^+ appear to move much slowly through the 5'-GMP channel. It is possible that K^+ and Rb^+ ions may have a similar residence time inside the G-quadruplex channel as that of NH_4^+ , 250 ms [16].

We have also found an interesting spectral phenomenon in the ^{87}Rb spectra of 5'-GMP. In particular, we found that the two ^{87}Rb NMR signals exhibit very different pulse-angle dependence. As seen from Fig. 3A, the signal for the channel Rb^+ cations reaches its maximum at approximately one-half of the pulse length observed for the surface/free Rb^+ signal. This observation suggests that the channel Rb^+ signal corresponds only to the central transition (CT) [25,26]. Because of the existence of dynamic shift for the CT, a well-known phenomenon for half-integer quadrupolar nuclei far outside the

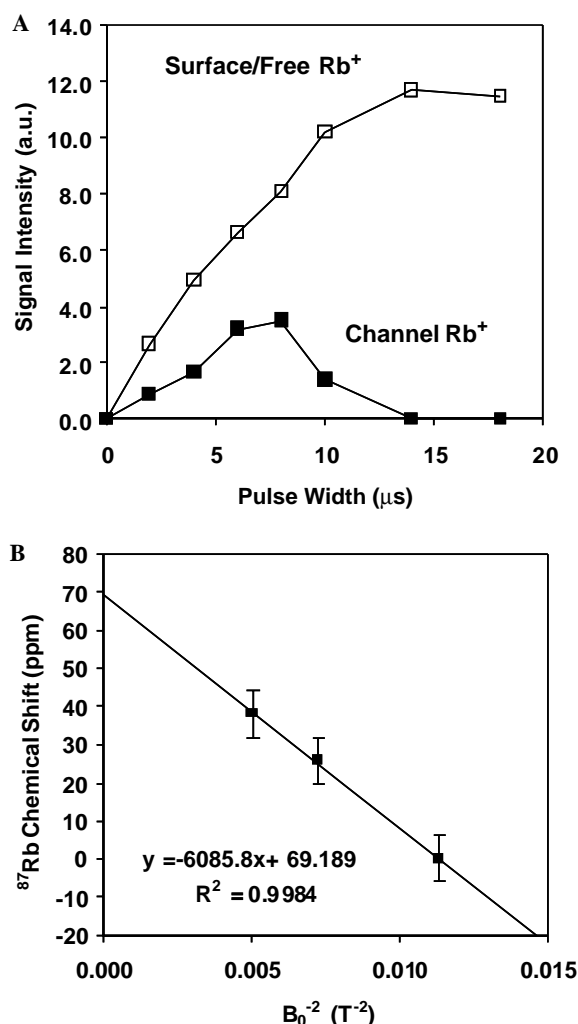


Fig. 3. (A) Pulse-width dependence for channel and surface/free Rb^+ cations. (B) Apparent ^{87}Rb NMR central-transition peak positions for the channel Rb^+ cations at three magnetic fields, 9.4, 11.7, and 14.1 T.

extremely narrowing regime (i.e., $\omega_0\tau_C \gg 1$) [27,28], the apparent peak position for the CT does not correspond to the true chemical shift. To obtain the true ^{87}Rb chemical shift, we recorded ^{87}Rb NMR spectra for the 5'-GMP sample at three different magnetic fields. As shown in Fig. 3B, the extrapolated ^{87}Rb chemical shift for the channel Rb^+ cations is 69 ± 5 ppm. This ^{87}Rb chemical shift is consistent with the solid-state ^{87}Rb NMR signature observed for channel Rb^+ cations [15]. Further, a dynamic shift of approximately 34 ppm at 14.1 T is also in qualitative agreement with the calculation based on the ^{87}Rb quadrupole parameters reported for the channel Rb^+ cations [15] and the equation for CT dynamic shift [27].

In summary, we have shown that, contrary to the commonly accepted viewpoint, Na^+ , K^+ , and Rb^+ cations residing inside a G-quadruplex channel can be directly observed by solution NMR spectroscopy. Our results suggest that the Na^+ cation transport rate through a G-quadruplex channel is much slower than previously believed. Furthermore, we find that Rb^+ and K^+ cations move through

the G-quadruplex channel at a much slower rate than that for Na^+ . Our finding opens up many new possibilities in the study of cation binding and transport dynamics in G-quadruplex DNA. For example, it might now be possible not only to monitor cation transport through a G-quadruplex channel, but also to measure cation binding affinity for the channel site in a direct and site-specific manner. Research along these directions is under way in this laboratory.

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Appendix A. Supplementary data

Variable temperature ^1H and ^{23}Na NMR data for Na_2 (5'-GMP). Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbrc.2005.08.275](https://doi.org/10.1016/j.bbrc.2005.08.275).

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