Direct ^{23}Na NMR observation of mixed cations residing inside a G-quadruplex channel†

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We report direct ^{23}Na NMR observation for the presence of mixed cations (Na^+/K^+, Na^+/Rb^+, Na^+/Sr^{2+}) inside the G-quadruplex channel formed by the self-association of guanosine 5′-monophosphate at pH 8.

Alkali metal cations such as Na^+ and K^+ are known to play an important role in stabilizing G-quadruplex structures.[1] Although solid-state techniques such as X-ray crystallography and solid-state NMR are quite useful for localizing alkali metal cations in G-quadruplex DNA,[2,3] it is highly desirable to have biophysical techniques that can detect these cations in solution. To this end, NMR methodologies based on spin-1/2 probes such as $^{15}NH_4^+$ and $^{205}Tl^+$ have been developed and successfully applied to G-quadruplex DNA.[4,5] Recently, we demonstrated that solution metal (^{23}Na, ^{39}K, ^{87}Rb) NMR can be used for direct detection of alkali metal cations in G-quadruplex DNA.[6] Here we report that high-resolution ^{23}Na NMR spectra allow direct detection of mixed cations residing inside a G-quadruplex channel.

Fig. 1 shows a ^{23}Na NMR spectrum for Na_2(5′-GMP) at pH 8.‡ This spectrum exhibits two peaks, one at $\delta(^{23}Na)$ 0 ppm having a bi-Lorentzian line shape and the other at $\delta(^{23}Na)$ −17 ppm. As we have shown recently,[6] the former signal is due to free Na^+ ions and the latter is assigned to Na^+ ions residing inside the G-quadruplex channel. At pH 8, Na_2(5′-GMP) is known to self-associate into molecular cylinders of 10–30 nm in length, depending on the actual 5′-GMP concentration.[7] Under such a circumstance, the Na^+ ions residing inside the G-quadruplex channel can be seen as tightly bound to a macromolecule with an effective molecular weight of 30–100 kDa. An additional benefit of Na^+ binding to this large molecular species is that the line width for the slow decaying component becomes narrow again when the spin-3/2 system is far from the so-called extreme narrowing condition.[8] In the present case, the full width at the half height (FWHH) of the channel ^{23}Na NMR signal is only 218 Hz (<1.4 ppm at 14.1 T) at 5 °C. With such a high spectral resolution, it may be possible that the ^{23}Na chemical shift can be used as a sensitive reporter for the nature of cations occupying the neighboring site inside a G-quadruplex channel. Indeed, the existence of G-quadruplexes containing mixed cations has been observed recently in ^{1}H/^{15}N NMR and X-ray crystallographic studies.[9]

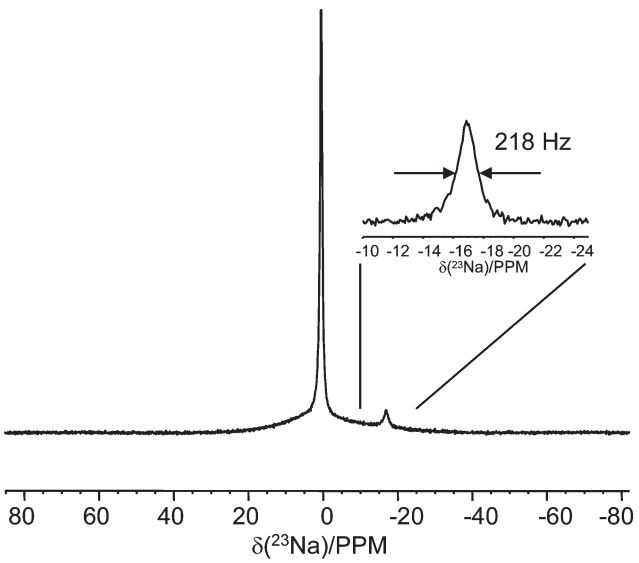

Fig. 1 ^{23}Na NMR spectrum for 1.0 M Na_2(5′-GMP) at 5 °C.

Fig. 2 shows portions of the ^{23}Na NMR spectra for 5′-GMP containing mixed cations Na^+/M^{n+} (M^{n+} = K^+, Rb^+, Sr^{2+}). It is

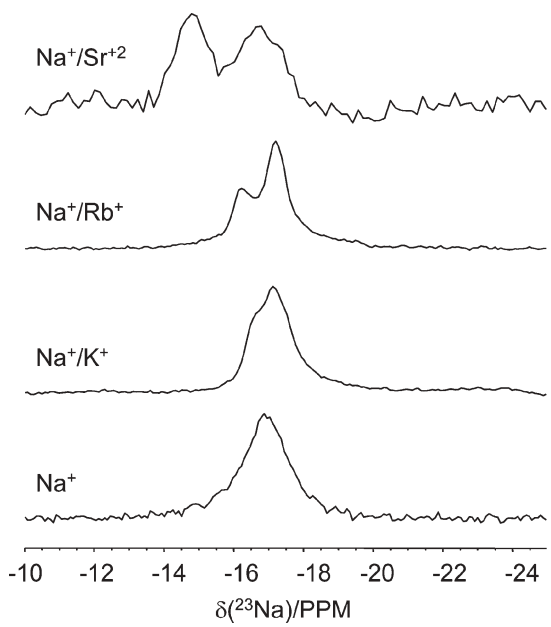

Fig. 2 Portions of the ^{23}Na NMR spectra of 1.0 M Na_2(5′-GMP) containing mixed cations at 5 °C. The concentrations of the added cations are: K^+, 100 mM; Rb^+, 100 mM; Sr^{2+}, 20 mM.

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† Electronic supplementary information (ESI) available: Plot showing the NMR results from Na/Rb titration experiment and atomic coordinates of the G-quadruplex model used in quantum chemical calculations. See DOI: 10.1039/b613105k

well known that K^+, Rb^+ and Sr^{2+} are all capable of entering a G-quadruplex channel.[1] As seen in Fig. 2, when M^{n+} is added to Na_2(5′-GMP) solution, a new signal appears in the ^{23}Na NMR spectra. In the cases studied here, each of the new signals exhibits a less negative ^{23}Na chemical shift (corresponding to a less shielded environment) than that of the original ^{23}Na signal. We assign these new signals to the Na^+ ions with one of the two neighboring sites being occupied by M^{n+}, *i.e.* G_4-M-G_4-Na-G_4-Na-G_4. Because the concentration of the added cations employed in our study is always much smaller than that of Na^+, we can safely neglect the population of the Na^+ ions with both neighboring sites being occupied by M^{n+} ions, *i.e.* G_4-M-G_4-Na-G_4-M-G_4. We also performed a Na/Rb titration experiment in which various amounts of Rb^+ ions were added to the Na_2(5′-GMP) solution. As expected, as the concentration of Rb^+ ions increases, the relative intensity of the ^{23}Na signal at $\delta(^{23}Na)$ −16.2 ppm also increases, indicating that more Rb^+ ions have entered the channel (see ESI†).

Another important point worth emphasizing is that the observed ^{23}Na NMR spectra will provide clues to the rate of cation movement through the channel. The fact that a separate ^{23}Na NMR signal is actually observed for the channel Na^+ ions with M^{n+} as an immediate neighbor immediately suggests that the movement of the added M^{n+} ions (K^+, Rb^+ and Sr^{2+}) in and out of the G-quadruplex channel must be slow on the ^{23}Na NMR time scale employed in this study. In particular, the smallest signal separation observed for the different channel Na^+ ions is 0.6 ppm (corresponding to 95 Hz at 14.1 T) as observed in the Na/K case. This indicates that the averaged residence time of K^+ ions inside the channel (not necessarily the residence time at a particular site) must be much longer than $(2\pi \times 95\ \text{Hz})^{-1} \approx 2$ ms. Otherwise, the two ^{23}Na NMR signals would be averaged into one. This is consistent with recent findings that the residence time of NH_4^+ ions in a G-quadruplex channel containing mixed Na^+/NH_4^+ ions is 36 ms[8] and that the residence time of Tl^+ ions in d($G_4T_4G_4$) is approximately 100 ms.[5]

To further verify our ^{23}Na NMR spectral assignment, we performed *ab initio* ^{23}Na chemical shielding calculations for Na^+ ions inside a G-quadruplex channel.§ Our model shown in Fig. 3 consists of four stacking G-quartets and three channel cations (a total of 259 atoms). Each cation is sandwiched between two adjacent G-quartets that are separated by 3.4 Å and twisted by 45°. To model the mixed cation cases, the top Na^+ ion is replaced by K^+, Rb^+ and Sr^{2+}, respectively, and the magnetic shielding at the central Na^+ ion is calculated. The computational results shown in Table 1 confirm that, when a K^+ ion (or Rb^+ and Sr^{2+}) occupies the neighboring channel site, the central Na^+ ion experiences a slightly less shielding environment. Furthermore, the observed trend on going from Na, K, Rb to Sr is well reproduced by quantum chemical calculations. Considering the approximations used in the model, the agreement between the experimental and calculated ^{23}Na chemical shifts is remarkable. To rule out the posibility of a Na^+ binding within the G-quartet plane, we performed further shielding calculations for a G_4-Na^+ model where the Na^+ ion is located at the center (in-plane) of the G-quartet. The calculations with different basis sets consistently predict that the ^{23}Na chemical shift for such a Na^+ ion should be approxiamtely +6 ppm. We have never observed any ^{23}Na signal at this chemical shift for our 5′-GMP samples. Another way to check the validity of our model is to calculate the chemical

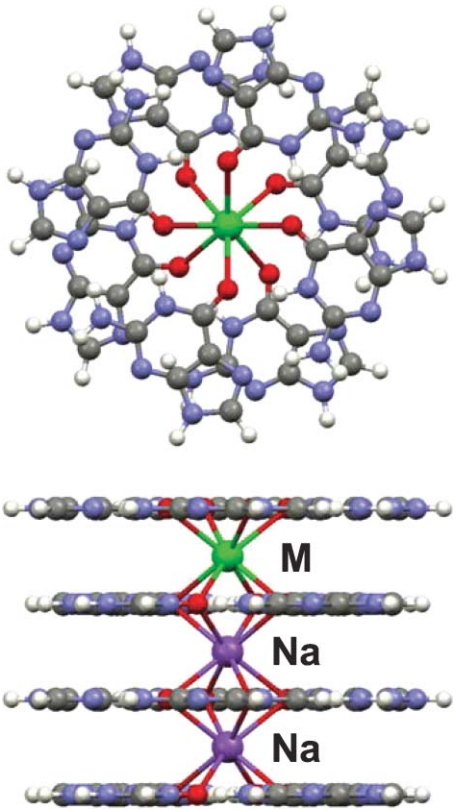

Fig. 3 Top (upper) and side (lower) views of the G-quadruplex model used for chemical shielding calculations where M = Na, K, Rb and Sr.

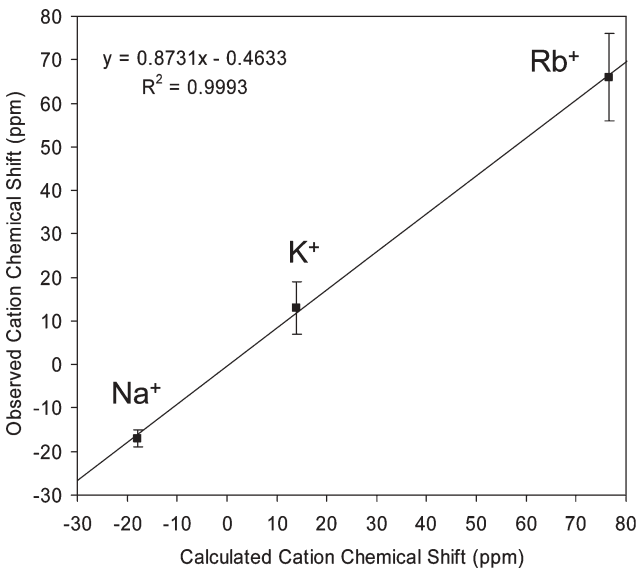

Fig. 4 Comparison between calculated and observed chemical shifts for Na^+, K^+, and Rb^+ ions residing inside the G-quadruplex channel.

Table 1 Observed and calculated ^{23}Na chemical shifts (in ppm) for Na^+ ions inside a G-quadruplex containing mixed cations[a]

	Na^+	K^+	Rb^+	Sr^{2+}
Observed	−17.0	−16.6	−16.2	−14.8
Calculated	−18.0	−17.5	−17.6	−14.8

[a] See footnote for computational details.

shielding values at the K^+ and Rb^+ sites using the same model, because ^{39}K and ^{87}Rb NMR signatures for these cations residing between two G-quartets have also been established.[6] As seen in Fig. 4, the calculated chemical shifts are in good agreement with the experimental values. This provides strong evedence that the G-quadruplex model used in our calculations is reasonable.

In summary, our ^{23}Na NMR results illustrate the remarkable resolution achievable in solution-state ^{23}Na NMR spectra for Na^+ ions tightly bound to a large molecular self-assembly. It is anticipated that similarly high resolution should be observed for Na^+ ions bound to other biological macromolecules. Possible extensions of this type of experiment to ^{39}K and ^{87}Rb NMR are under way in our laboratory.

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Notes and references

‡ The hydrated disodium salt of 5′-GMP (>99% purity) was purchased from Sigma-Aldrich. All ^{23}Na NMR spectra were obtained on a Bruker Avance-600 NMR spectrometer operating at 600.13 and 158.76 MHz for ^1H and ^{23}Na nuclei, respectively. All ^{23}Na chemical shifts are referenced to Na^+(aq.) at $\delta = 0$ ppm.

§ Quantum chemical calculations were performed using the Gaussian 03 suite of programs[10] on a SunFire 6800 symmetric multiprocessor system. Each of the four nodes is equipped with 24 × 1.05 GHz (8 MB E-Cache) UltraSPARC-III processor and 96 GB of RAM. For the central Na, a high-level correlation consistent basis set, cc-pV5Z, was used. For the two outer metal cations (Na, K, Rb and Sr), the all-electron pVTZ basis sets of Sadlej[11] were used. A 3-21G(d) basis set was used for all other non-metal atoms. Shielding calculations were performed at the Hartree–Fock (HF) level using the GIAO method as implemented in Gaussian 03. The computed absolute shielding (σ) was converted to the chemical shift (δ) scale using $\delta = \sigma_{ref} - \sigma$, where σ_{ref} is the absolute shielding constant for the reference sample, Na^+(aq.). We used σ_{ref} = 587.6 ppm, which is the value calculated for $[Na(H_2O)_6]^+$ (Na–O_W: 2.433 Å) at the HF/6-31G(d)/cc-pVQZ level.[12] For calculations of ^{39}K and ^{87}Rb chemical shifts, we used σ_{ref} = 1246 and 3218 ppm, respectively. These values were calculated at the HF/3-21G(d)/pVTZ levels for fully hydrated clusters, $[K(H_2O)_8]^+$ (K–O_W: 2.712 Å) and $[Rb(H_2O)_8]^+$ (Rb–O_W: 3.000 Å). All computations were performed at the High Performance Computing Virtual Laboratory (HPCVL) at Queen's University. Each calculation takes about 3–4 days of CPU time.

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