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The Sodium Ions Inside a Lipophilic G-Quadruplex Channel as Probed by Solid-State ^{23}Na NMR

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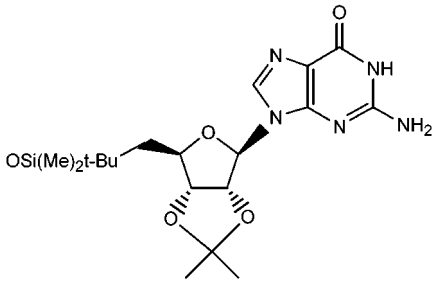
The biological implications of G-quadruplex structures have attracted considerable attention in recent years.[1,2] Monovalent cations such as K^+ and Na^+ stabilize the G-quadruplex structure; however, precise cation locations inside G-quadruplexes have only been determined by X-ray crystallography in $[d(G_4T_4G_4)]_2$[3] and $[d(TG_4T)]_4$.[4] In these systems, the K^+ and Na^+ ions, located between two adjacent G-quartets, have an octacoordinate geometry. In general, direct detection of Na^+ ions in biological structures is a challenge even with state-of-the-art X-ray diffraction techniques.[5] Although solution NMR has also been used for studying alkali metal ion binding to G-quadruplexes,[6] it is generally difficult to obtain site-specific information, with two notable exceptions where spin-$^1/_2$ nuclear probes of $^{205}Tl^+$ and $^{15}NH_4^+$ are used.[7,8] We recently proposed that solid-state ^{23}Na NMR can be used as a complementary technique to X-ray crystallography for detecting Na^+ ions in proteins and nucleic acids.[9] Rovnyak et al. successfully obtained solid-state ^{23}Na NMR spectra for a G-quadruplex, $[d(TG_4T)]_4$.[10] To gain further understanding about the interaction between Na^+ ions and G-quadruplexes, it is desirable to study nucleoside systems rather than nucleotides, because in the former systems no phosphate-bound Na^+ ions are present to complicate the ^{23}Na NMR spectra. Here we report solid-state ^{23}Na NMR and X-ray crystallographic results for a self-assembled G-quadruplex formed by a guanine nucleoside, 5′-*tert*-butyl-dimethylsilyl-2′, 3′-*O*-isopropylidene guanosine (G **1**).



We recently showed that G **1** self-assembles in the presence of K^+ and Cs^+ picrates to form a lipophilic G-quadruplex channel structure consisting of 16 equiv of G **1**.[11] A similar complex is formed when Na^+ and Cs^+ picrates are used.[12] As shown in Figure 1, the crystal structure[13] of $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic_4]$ reveals a G-quadruplex structure consisting of four G-quartets that are stacked on top of one another, forming a central ion channel. The channel is fully occupied by three collinear Na^+ ions and one Cs^+ ion along the central axis. As shown in Figure 2, each of the three Na^+ ions is sandwiched by two G-quartet planes. The separation between the two adjacent G-quartet planes around Na1 and Na3, 3.4 Å, is slightly larger than that around Na2, 3.3 Å. For Na1 and Na3, the twist between the two adjacent G-quartets is 30°. In contrast, the two G-quartets around Na2 are stacked with a 90° twist between the planes. Therefore, Na2 is at the center of a more regular cube than are Na1 and Na3. The Cs^+ ion, too large to enter the channel, is located on the top of the channel as a capping ion. Another unusual feature of $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic_4]$ is that all nucleoside molecules adopt a syn conformation. The two inner G-quartets (G_4 **2** and G_4 **3**) have an average glycosyl torsion angle of 65.5°, while G_4 **1** and G_4 **4** are in the "high syn" region with an average glycosyl torsion angle of 98.2°.

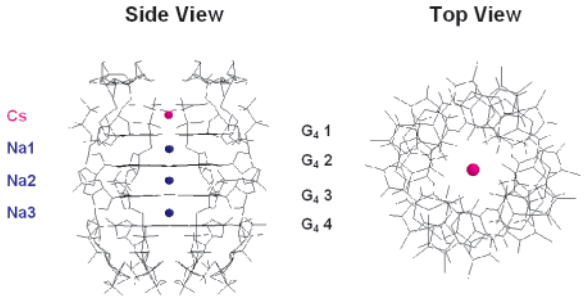


Figure 1. Crystal structure of $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic4]$. Picrate molecules and hydrogen atoms are omitted for clarity. Only G-quartets are shown in the top view diagram to illustrate the ion channel structure.

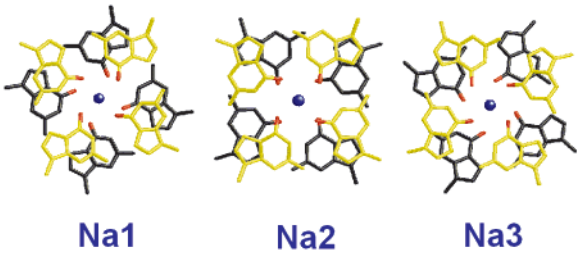


Figure 2. Sodium coordination geometry in $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic_4]$. Twisting between two consecutive G-quartets (deg): G_41-G_42, 30; G_42-G_43, 90; G_43-G_44, 30. Mean Na−O separations (Å): Na1−G_41(O), 2.77; Na1−G_42(O), 2.82; Na2−G_42(O), 2.87; Na2−G_43(O), 2.80; Na3−G_43(O), 2.88; Na3−G_44(O), 2.72.

Figure 3A shows the 1D ^{23}Na magic-angle spinning (MAS) NMR spectrum of $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic_4]$. The spectrum exhibits only a broad peak centered at −18 ppm. This immediately suggests that the previous assignment of a signal at 6.8 ppm to the Na^+ ions inside the G-quadruplex of $[d(TG_4T)]_4$ was erroneous.[10] Because only a featureless peak was observed in the 1D ^{23}Na MAS spectrum of $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic_4]$, it is not possible to distinguish the three crystallographically distinct Na sites. To this end, we have obtained a 2D multiple-quantum magic-angle spinning (MQMAS)[14] spectrum for $[G\ \mathbf{1}]_{16}\cdot[3Na/CsPic_4]$ (Figure 3B). The 2D MQMAS spectrum

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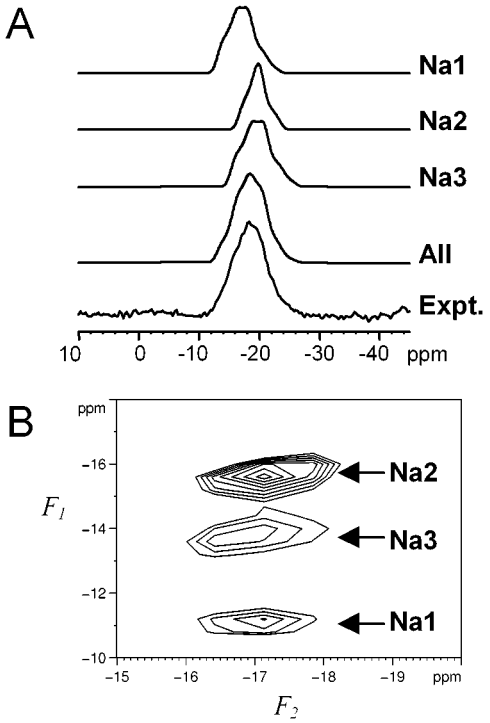

Figure 3. (A) Experimental and simulated 1D ^{23}Na MAS spectra of [G **1**]$_{16}$·[3Na/CsPic$_4$]. The sample spinning frequency was 8500 ± 2 Hz. A total of 1353 transients was collected with a recycle delay of 1 s. (B) Two-dimensional ^{23}Na MQMAS spectrum of [G **1**]$_{16}$·[3Na/CsPic$_4$]. The sample spinning frequency was 12000 ± 2 Hz. The pulse sequence with a *z*-filter[15] was used in obtaining the ^{23}Na MQMAS spectrum. The excitation and conversion pulses were 4.5 and 2.0 μs, respectively. A total of 2400 transients were collected for each of the 32 t_1 increments with a recycle delay of 2 s. All solid-state NMR experiments were performed on a Bruker Avance-500 spectrometer operating at 132.29 MHz for ^{23}Na nuclei. Reported ^{23}Na chemical shifts were referenced to NaCl(aq), $\delta = 0$ ppm.

shows three well-resolved spectral regions. By combining the 1D MAS and 2D MQMAS spectra,[16] we were able to accurately determine the values of the isotropic chemical shift (δ_{iso}), the quadrupole coupling constant (C_Q) and the asymmetry parameter (η_Q) for each of the Na sites: Na1, $\delta_{iso} = -12.8 \pm 0.2$ ppm, $C_Q = 1.65 \pm 0.05$ MHz, $\eta_Q = 0.60 \pm 0.05$; Na2, $\delta_{iso} = -16.5 \pm 0.2$ ppm, $C_Q = 1.35 \pm 0.05$ MHz, $\eta_Q = 0.80 \pm 0.05$; Na3, $\delta_{iso} = -15.0 \pm 0.2$ ppm, $C_Q = 1.70 \pm 0.05$ MHz, $\eta_Q = 0.60 \pm 0.05$. It is important to note that the simulations of the 1D ^{23}Na MAS spectrum shown in Figure 3A would not be possible without the MQMAS results. The Na signal associated with the smallest C_Q is assigned to Na2, because Na2 is in the most symmetrical environment. Careful examination of the crystal structure indicates that Na1 and Na3 have very similar coordination environments. Therefore, the above assignment for Na1 and Na3 signals is tentative. As also seen from Figure 3B, each of the three spectral regions in the 2D spectrum is parallel to the F_2 axis, consistent with the crystalline nature of the [G **1**]$_{16}$·[3Na/CsPic$_4$] sample.

In summary, we have presented an unambiguous solid-state ^{23}Na NMR characterization for the Na^+ ions inside a 9-kDa G-quadruplex channel. The crystalline G-quadruplex formed from G **1** and various salts is an excellent model for DNA G-quadruplex systems. The study demonstrates the utility of 2D MQMAS NMR in obtaining accurate site-specific information about ion binding in a self-assembled system. Our results strongly suggest that solid-state ^{23}Na NMR can be useful in the direct detection of Na^+ ions in biological structures.

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Supporting Information Available: Crystallographic tables, atomic coordinates and thermal parameters, selected bond lengths and angles (PDF). X-ray crystallographic files (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) 5′-*tert*-Butyl-dimethylsilyl-2′,3′-*O*-isopropylidene guanosine (G **1**) was prepared as previously described.[11] A solution of 39 mg of G **1** dissolved in 4.0 mL of chloroform was shaken with 4.0 mL of 75 mM NaPic (aq) and 25 mM of CsPic (aq) for salt extraction. The chloroform layer was separated and dried under a stream of N_2(g) to give [G **1**]$_{16}$·[3Na/CsPic$_4$] as a yellow powder. Single crystals of [G **1**]$_{16}$·[3Na/CsPic$_4$] were obtained from recrystallization from acetonitrile. ^{1}H NMR (400 MHz, CD_3CN at 298 K) δ (ppm): 11.69 (NH_A), 11.52 (NH_B), 9.97 (NH_{2A}), 9.43 (NH_{2B}), 8.78 (picrate), 7.57 ($H8_A$), 6.92 ($H8_B$), 6.27 ($H1'_A$), 6.05 ($H2'_A$), 5.68 ($H2'_B$), 5.62 ($H1'_B$), 4.79 ($H3'_A$), 4.44 ($H4'_B$), 4.14 ($H3'_B$, $H4'_A$), 3.60 ($H5'_B$), 3.26 ($H5'_A$), 1.60 and 1.509 (CH_3), 1.40 and 1.29 (CH_3), 0.88 and 0.44 (t-Bu), 0.19 and 0.16 ($Si(CH_3)$), −0.39 and −0.45 ($Si(CH_3)$).
(13) Crystal data for [G**1**]$_{16}$·[3Na/CsPic$_4$]·(CH_3CN)$_4$: $C_{357}H_{546}Cs_{0.92}N_{106.50}Na_{3.08}O_{108}Si_{16}$; $M_r = 8700.53$, tetragonal, space group *I*4, $a = b = 30.622(7)$ Å, $c = 25.748(8)$ Å, $V = 24$, 144(11) Å^3, $Z = 2$, $D_x = 1.197$ mg/mm^3, μ(Mo Kα) = 0.283 mm^{-1}. Data were collected on a Bruker SMART 1000 CCD diffractometer at 153(2) K. The structure was determined by direct methods using the program XS.[17] Refinement was performed using the program XL.[18] The final *R* factors for 21 262 unique reflections were $R(F) = 6.20\%$ and $wR(F^2) = 16.66\%$.
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