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. 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(G1T1G2T2)]$^5$ and [d(TG1T2)]$^4$. 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.

Although solution NMR has also been used for studying alkali metal ions in proteins and nucleic acids, 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, S′- tert-butyl-dimethylsilyl-2′, 3′-O-isopropylidene guanosine (G1).

We recently showed that G1 self-assembles in the presence of K$^+$ and Cs$^+$ picrates to form a lipophilic G-quadruplex channel structure consisting of 16 equiv of G1. A similar complex is formed when Na$^+$ and Cs$^+$ picrates are used. As shown in Figure 1, the crystal structure of [G1]_4[3Na/CsPic4] 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

1/2 nuclear probes of $^{205}$Tl have been determined by X-ray crystallography in [d(G4T4G4)]$^7$ and [d(TG1T2)]$^4$. 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. Although solution NMR has also been used for studying alkali metal ions in proteins and nucleic acids, 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, S′- tert-butyl-dimethylsilyl-2′, 3′-O-isopropylidene guanosine (G1).

Figure 1. Crystal structure of [G1]_4[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 [G1]_4[3Na/CsPic4]. Twisting between two consecutive G-quartets (deg): G1–G2, 30; G2–G3, 30; G3–G4, 30. Mean Na–O separations (Å): Na1–G1(O), 2.77; Na1–G2(O), 2.82; Na2–G2(O), 2.87; Na2–G3(O), 2.80; Na3–G3(O), 2.88; Na3–G4(O), 2.72.

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 [G1]_4[3Na/CsPic4] is that all nucleoside molecules adopt a syn conformation. The two inner G-quartets (G1 2 and G2 3) have an average glycosyl torsion angle of 65.5°, while G1 1 and G3 4 are in the “high syn” region with an average glycosyl torsion angle of 98.2°.

Figure 3A shows the 1D $^{23}$Na magic-angle spinning (MAS) NMR spectrum of [G1]_4[3Na/CsPic4]. 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(TG1T2)]$^4$ was erroneous. Because only a featureless peak was observed in the 1D $^{23}$Na MAS spectrum of [G1]_4[3Na/CsPic4], 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 [G1]_4[3Na/CsPic4] (Figure 3B). The 2D MQMAS spectrum...
Na NMR characterization for the Na\textsuperscript{23}MAS and 2D MQMAS spectra, we were able to accurately show three well-resolved spectral regions. By combining the 1D\textsuperscript{23}Na chemical shifts were referenced to NaCl(aq), was used in obtaining the 23\textsuperscript{Na} MQMAS spectrum. The excitation and dimension\textsuperscript{23}Na MQMAS spectrum of [G\textsuperscript{16}]. The crystalline G-quadruplex formed from the crystalline nature of the [G\textsuperscript{16}]\textsuperscript{+}. 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 \textsuperscript{23}Na NMR can be useful in the direct detection of Na\textsuperscript{+} 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.

**References**


(11) Crystal data for [G\textsuperscript{16}]\textsuperscript{+} were obtained from recrystallization from acetone. H NMR (400 MHz, CD\textsubscript{3}CN at 298 K) (ppm): 11.69 (NH\textsubscript{A}), 11.52 (NH\textsubscript{B}), 9.97 (NH\textsubscript{2A}), 9.43 (NH\textsubscript{2B}), 8.87 (picate), 7.57 (H\textsubscript{8A}), 6.92 (H\textsubscript{8B}), 6.27 (H\textsubscript{1A}), 6.05 (H\textsubscript{1B}), 5.68 (H\textsubscript{2A}), 5.62 (H\textsubscript{2B}), 4.44 (H\textsubscript{4A}), 4.14 (H\textsubscript{4B}), 3.60 (H\textsubscript{3A}), 3.26 (H\textsubscript{3B}), 1.60 and 1.50 (CH\textsubscript{3}), 0.88 and 0.44 (t-Bu), 0.19 and 0.16 (Si(C\textsubscript{2}H\textsubscript{5})), 0.39 and 0.45 (Si(C\textsubscript{2}H\textsubscript{5})).

(12) S-tert-Butyl-dimethylsilyl-2,3-\textsuperscript{23}O-isopropylidene guanosine (G\textsubscript{1}) was prepared as previously described. A solution of 39 mg of G\textsubscript{1} dissolved in 4.0 mL of chloroform was shaken with 4.0 mL of 75 mM NaPic (aq) and 25 mL of NaPic (aq) for salt extraction. The chloroform was separated and dried under a stream of N\textsubscript{2} to give [G\textsuperscript{16}]\textsuperscript{[3Na/CsPic]}. The data collected for a Bruker SMART 1000 CCD diffractometer at 153(2) K. The structure was performed using the program XL. The final R factors for 216 unique reflections were R\textsubscript{c}(F) = 0.20 and wR\textsubscript{c}(F) = 16.66%.

(13) Crystal data for [G\textsuperscript{16}]\textsuperscript{[3Na/CsPic]} (CH\textsubscript{3}CN): C\textsubscript{357}H\textsubscript{546}Cs\textsubscript{0.92}N\textsubscript{106.50}Na\textsubscript{3.08}O\textsubscript{108}Si\textsubscript{16}; M = 8700.53, tetragonal, space group I\textsubscript{4}, a = b = 30.62(2) Å, c = 25.748(8) Å, V = 24, 144(11) Å\textsuperscript{3}, Z = 2, D\textsubscript{c} = 1.197 g/cm\textsuperscript{3}, µ(Mo K\textalpha) = 0.283 mm\textsuperscript{-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. Refinement was performed using the program X\textsuperscript{\textregistered}. The final R factors for 216 unique reflections were R\textsubscript{c}(F) = 0.20 and wR\textsubscript{c}(F) = 16.66%.


