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Selective Binding of Monovalent Cations to the Stacking G-Quartet Structure Formed by Guanosine 5′-Monophosphate: A Solid-State NMR Study

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Abstract: We report a solid-state multinuclear (^{23}Na, ^{15}N, ^{13}C, and ^{31}P) NMR study on the relative affinity of monovalent cations for a stacking G-quartet structure formed by guanosine 5′-monophosphate (5′-GMP) self-association at pH 8. Two major types of cations are bound to the 5′-GMP structure: one at the surface and the other within the channel cavity between two G-quartets. The channel cation is coordinated to eight carbonyl oxygen atoms from the guanine bases, whereas the surface cation is close to the phosphate group and likely to be only partially hydrated. On the basis of solid-state ^{23}Na NMR results from a series of ion titration experiments, we have obtained quantitative thermodynamic parameters concerning the relative cation binding affinity for each of the two major binding sites. For the channel cavity site, the values of the free energy difference ($\Delta G°$ at 25 °C) for ion competition between M^+ and Na^+ ions are K^+ (−1.9 kcal mol^{-1}), NH_4^+ (−1.8 kcal mol^{-1}), Rb^+ (−0.3 kcal mol^{-1}), and Cs^+ (1.8 kcal mol^{-1}). For the surface site, the values $\Delta G°$ are K^+ (2.5 kcal mol^{-1}), NH_4^+ (−1.3 kcal mol^{-1}), Rb^+ (1.1 kcal mol^{-1}), and Cs^+ (0.9 kcal mol^{-1}). Solid-state NMR data suggest that the affinity of monovalent cations for the 5′-GMP structure follows the order $NH_4^+ > Na^+ > Cs^+ > Rb^+ > K^+$ at the surface site and $K^+ > NH_4^+ > Rb^+ > Na^+ > Cs^+ > Li^+$ at the channel cavity site. We have found that the cation-induced stability of a 5′-GMP structure is determined only by the affinity of monovalent cations for the channel site and that the binding of monovalent cations to phosphate groups plays no role in 5′-GMP self-ordered structure. We have demonstrated that solid-state ^{23}Na and ^{15}N NMR can be used simultaneously to provide mutually complementary information about competitive binding between Na^+ and NH_4^+ ions.

Introduction

Guanosine 5′-monophosphate (5′-GMP) is known to undergo spontaneous formation of a highly ordered structure in water in the presence of certain alkali metal cations.[1] The basic structural motif of 5′-GMP self-assembly at neutral pH is a tetrameric unit known as the G-quartet. In a G-quartet, four guanine base molecules are held together by a total of eight Hoogsteen-type hydrogen bonds forming a planar square, as illustrated in Figure 1. The G-quartet model was first proposed by Gellert et al.[2] on the basis of X-ray diffraction data for GMP fibers. Subsequent X-ray diffraction studies of Na_2(5′-GMP) single crystals confirmed that the molecular aggregates form a right-handed quadruple helix with G-quartets stacking along the helix axis.[3,4] In such a stacking G-quartet structure, two adjacent G-quartets are twisted by 30° and separated by 3.3 Å. The latest X-ray diffraction study suggested that the asymmetric unit cell contains 12 GMP molecules, i.e., three G-quartets.[4] However, because of the extreme difficulties in preparing good-quality single crystals of 5′-GMP in the quadruplex form, detailed information about atomic positions is still unavailable to date.

One of the most interesting properties of 5′-GMP self-association is that the formation of molecular aggregates at neutral pH depends critically on the presence of alkali metal cations. The pioneering work of Pinnavaia et al.[5,6] has established the following order of cation affinity for 5′-GMP structure: $K^+ > Na^+$, $Rb^+ \gg Li^+$, Cs^+. This sequence is similar to that reported earlier by Chantot and Guschlbauer[7] on the basis of the melting temperatures of Br_8-guanosine gels in various salt solutions, $K^+ \gg Rb^+ > NH_4^+ > Na^+ > Cs^+ > Li^+$. Laszlo and co-workers[8−12] have carried out extensive multinuclear

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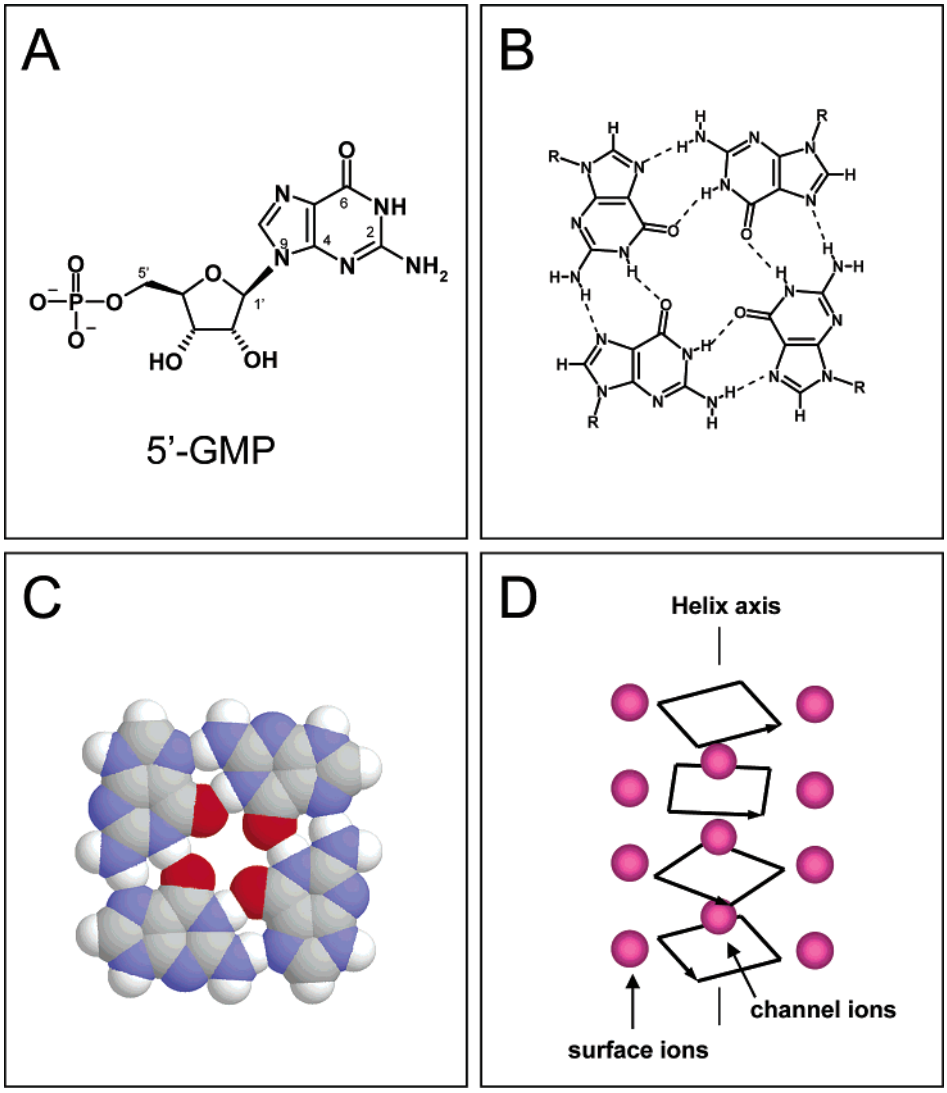

Figure 1. (A) Atomic numbering for 5′-GMP. (B) G-quartet model. (C) Space-filling model of the G-quartet. (D) Diagram showing the two types of cation environments in 5′-GMP.

solution NMR studies to examine the role of cations in self-ordered 5′-GMP structure. Most interestingly, they discovered an interesting synergism between K^+ and NH_4^+ ions in the formation of 5′-GMP aggregates in neutral aqueous solution.[11] They also demonstrated for the first time that different cations may bind selectively to a 5′-GMP structure. As illustrated in Figure 1, the stacking G-quartets in the 5′-GMP structure form a central channel along the helix axis. The distance between the diagonal oxygen atoms in a G-quartet is approximately 5 Å. The central cavity between two G-quartets has a volume of ca. 40 $Å^3$, representing an ideal site for cation binding. The proposal that an alkali metal cation such as Na^+ and K^+ can reside at the central cavity between two G-quartets was first brought about by Pinnavaia et al.[6] The work of Detellier and Laszlo[11] strongly supported this model. However, direct crystallographic demonstration for the existence of such ion binding sites in G-quartet structures was not achieved until much later. The first case where a K^+ ion was detected between two G-quartets was reported by Rich and co-workers[13] in a study of the crystal structure of *Oxytricha nova* telomeric DNA repeat, $d(G_4T_4G_4)$ (Oxy-1.5). The high-resolution crystal structure of $[d(TG_4T)]_4$ was another remarkable example that illustrates the existence of an array of Na^+ ions inside the central channel formed by stacking G-quartets.[14] A number of recent crystallographic studies have provided further examples demonstrating that both monovalent (K^+, Na^+) and divalent metal cations (Ba^{2+}, Pb^{2+}, Sr^{2+}) can be sandwiched between two G-quartets.[15−21] Only two solution NMR studies have been reported so far in examining cation location in G-rich DNA sequences. Feigon and co-workers[22] identified the location of NH_4^+ ions in $[d(G_4T_4G_4)]_2$ by observing $^1H-^1H$ cross-peaks between imino

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protons on the guanine base and NH_4^+ ions in ROESY spectra. Strobel and co-workers[23] observed distinct ^{205}Tl NMR signals for Tl^+ ions bound to a parallel-stranded quadruplex formed by $d(T_2G_4T_2)$. These crystallographic and NMR studies have established unequivocally that the cavity site between two G-quartets is a preferred site for cation binding. It is, however, also important to point out that, in addition to the cavity site, the anionic phosphate group in 5′-GMP (or the phosphodiester group in oligonucleotides) is another site for cation binding. For this reason, when studying cation binding phenomena in G-quadruplexes, one must take into consideration the fact that both binding sites are operative.

Recent discoveries of the existence of the G-quartet motif in many biologically important systems such as telomeres, promoters of many genes, and sequences related to various human diseases have triggered tremendous research interest in this unusual type of nucleic acid structure.[24] In G-quadruplex systems, cations play an important role not only in structural stability but also in structural polymorphism. For example, Williamson et al.[25] found that the G-quadruplex structures of $d(T_4G_4)_4$ (Oxy-4) were stabilized by K^+, Na^+, or Cs^+ but not by Li^+. Sen and Gilbert[26] described a phenomenon termed as a sodium−potassium switch where the formation of either linear or folded quadruplex structures can be controlled by changes in Na^+ or K^+ concentrations. Hardin et al.[27] observed that cations stabilized the G-quadruplex structure of $d(CGCG_3CG)$ in the order $K^+ > Ca^{2+} > Na^+ > Mg^{2+} > Li^+$ and $K^+ > Rb^+ > Cs^+$. These affinity sequences are similar to that observed for 5′-GMP mentioned above. Marathias and Bolton[28] showed that G-quadruplex structures can be classified into distinct categories according to their dependence on Na^+ and K^+. Extensive thermodynamic data on G-quadruplex stability are also available in the literature.[29,30] However, it appears that no systematic evaluation has been performed to study the affinity of a G-quadruplex structure for group Ia cations from Li^+ to Cs^+. In addition, none of the previous studies has been able to provide information about cation affinity for a G-quadruplex in a *site-specific* manner.

In the case of 5′-GMP, neither solution NMR nor X-ray diffraction studies have been able to yield definite information about precise cation location and stoichiometry. The most direct evidence for the cation location in 5′-GMP gels has come from our recent solid-state ^{23}Na NMR study.[31] In particular, we obtained unambiguous NMR signatures for the two major types of Na^+ ions bound to 5′-GMP self-ordered structure. The Na^+ ions bound to the peripheral phosphate groups (referred to as surface Na^+ ions) give rise to a solid-state ^{23}Na NMR signal at approximately $\delta(^{23}Na)$ −4 ppm, whereas the Na^+ ions residing inside the G-quartet channel (referred to as channel Na^+ ions) exhibit a signal at $\delta(^{23}Na)$ −19 ppm. This spectral assignment was further confirmed by examining solid-state ^{23}Na NMR spectra for the stacking G-quartet structure formed by a lipophilic guanosine nucleoside.[32] Very recently, we also demonstrated that solid-state ^{39}K NMR at high magnetic fields can be used for direct detection of K^+ ions bound to G-quadruplexes.[33] As we predicted several years ago,[34] the ability of solid-state NMR methodology to detect alkali metal cations with different chemical environments has made it a unique technique for studying cation binding phenomena in biomolecular systems. The relevance of the G-quartet motif in 5′-GMP gels to the structures of telomeric DNA and other G-rich sequences warrants an in-depth solid-state NMR investigation. Indeed, solid-state ^{23}Na NMR spectra for 5′-GMP gels[31] are very similar to those observed for a DNA quadruplex, $[d(TG_4T)]_4$, reported by Rovnyak et al.[35]

The primary focus of the present report is on the relative affinity of monovalent cations for the stacking G-quartet structure formed by 5′-GMP self-association at pH 8. Here we apply solid-state NMR techniques including ^{23}Na magic-angle spinning (MAS) and two-dimensional multiple-quantum (MQ) MAS[36] to characterize various types of 5′-GMP gels. We have designed a titration experiment to monitor the competitive equilibria between Na^+ and M^+ (M = K, Rb, NH_4, and Cs) ions. From these solid-state NMR experiments, it is possible to obtain quantitative thermodynamic data on relative cation affinity for the 5′-GMP structure. The most important advantage of our solid-state NMR approach is that we can simultaneously determine cation affinity for *different* binding sites. This type of information is difficult to obtain by other analytical techniques. Another objective of the present study is to use 5′-GMP as an example to demonstrate the utility of solid-state NMR as a general approach for direct detection of cations in biomolecular systems. The novel feature of our method is that solid-state ^{23}Na NMR spectra are used as "snapshots" of the cation distribution present in solution. In many aspects, solid-state NMR is advantageous over traditional solution NMR in obtaining precise information about the location of alkali metal cations in a molecular system. In general, rapid cation exchange occurring in solution renders it difficult to obtain *site-specific* information by alkali metal NMR.[37−39] To date, the most effective solution NMR method for studying alkali metal cations bound to G-quadruplexes relies on the use of surrogate spin-$^1/_2$ NMR probes such as ^{15}N and ^{205}Tl.

Experimental Section

Sample Preparation. Hydrated disodium salt of guanosine 5′-monophosphate, NaCl, KCl, RbCl, CsCl, and tetramethylammonium

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(TMA) chloride were purchased from Sigma−Aldrich (Ontario, Canada). Ammonium chloride was obtained from Fisher Scientific (Canada). NH_4Cl (99% ^{15}N atom) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). All 5′-GMP gel samples were prepared in doubly distilled deionized water. Two different methods were used to prepare 5′-GMP gel samples. In the first method, each of the 5′-GMP gel samples with mixed cations (denoted as M/Na gels) was prepared by mixing a known amount of MCl salt and 25 mg of Na_2(5′-GMP)·$7H_2O$ in water. The solution (at pH = 8.0) was then stirred for 10−15 min under gentle heating to ensure that all substances were dissolved. Gelation occurred upon cooling of the warm solution to room temperature. The white gel-like materials were then further dried under a stream of N_2 gas. Formation of a gel-like material is a reliable indication for the presence of highly ordered molecular aggregates. The 5′-GMP sample containing mixed Li^+ and Na^+ ions does not form a gel, indicating the absence of ordered aggregates. Because the gel formation in this procedure occurred in a homogeneous environment, we refer to this method as homogeneous preparation. In the second procedure for gel preparation, each sample was prepared by adding a known amount of 2.46 M MCl(aq) to a vial containing Na 5′-GMP gel, which was generated from 25 mg of Na_2(5′-GMP)·$7H_2O$ in a way described above. Because the amount of MCl(aq) added to the gel was small (less than 1 mL), the gel did not dissolve, resulting in a heterogeneous (two-phase) mix. The vial was then sealed with Parafilm and left at room temperature for 55 h to equilibrate. The sample was finally lyophilized and used for solid-state NMR measurement. We refer to this method as heterogeneous preparation. As will be discussed in detail later, 5′-GMP samples prepared by either homogeneous or heterogeneous approaches exhibit essentially the same characteristics in terms of cation distribution among different binding sites.

Solid-State NMR. Solid-state NMR spectra were recorded at 11.75 T on a Bruker Avance-500 spectrometer operating at 132.26, 50.57, 125.77, 202.91, and 500.13 MHz for ^{23}Na, ^{15}N, ^{13}C, ^{31}P, and ^{1}H nuclei, respectively. The radio frequency (RF) field strength at the ^{23}Na frequency was 96 kHz. Sodium-23 chemical shifts were referenced to NaCl (aq) by setting the ^{23}Na NMR signal of a solid NaCl sample to $\delta(^{23}Na) = 7.21$ ppm.[40] Nitrogen-15 chemical shifts were referenced to liquid NH_3, $\delta(^{15}N) = 0$ ppm, by setting the ^{15}N NMR signal of a solid $^{15}NH_4Cl$ sample to $\delta(^{15}N) = 41$ ppm.[41] One-dimensional ^{23}Na MAS spectra were recorded with a sample spinning frequency of 8000 ± 2 Hz. Single-pulse excitation with a pulse width of 1.0 μs was used, and 128 transients were collected with a recycle delay of 2 s. A recycle delay of 10 s was also tested to ensure that both the surface and channel signals recover fully from spin−lattice relaxation with a recycle delay of 2 s. Solid-state ^{15}N MAS spectra were recorded with a sample spinning frequency of 5000 ± 2 Hz. Single 90° pulse (6.5 μs) excitation with a recycle delay of 10 s was used to obtain the MAS spectra. Solid-state ^{13}C and ^{31}P NMR spectra were obtained under the cross polarization (CP) MAS condition. The sample spinning frequency was 8000 Hz. Carbon-13 chemical shifts were referenced to TMS, $\delta(^{13}C) = 0$ ppm, by use of a solid sample of adamantane as a secondary referencing sample. Phosphorus-31 chemical shifts were referenced to 85% H_3PO_4, $\delta(^{31}P) = 0$ ppm, by use of a solid sample of $NH_4H_2PO_4$ as a secondary referencing sample. All 1D NMR spectra were processed and analyzed on a personal computer using WinNuts.[42] A careful multicomponent line shape deconvolution was performed to obtain the peak areas under different signals in ^{23}Na MAS spectra. For two-dimensional (2D) ^{23}Na MQMAS experiments, the sample spinning frequency was controlled at 8500 ± 2 Hz. The z-filter MQMAS pulse sequence[43] was used: $P1(\phi1)-t_1-P2(\phi2)-\tau-P3(\phi3)-ACQ(t_2, \phi4)$,

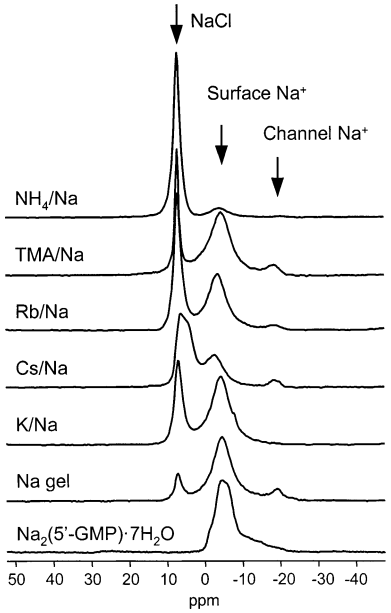

Figure 2. Experimental ^{23}Na MAS NMR spectra for 5′-GMP gels containing mixed M^+ and Na^+ ions. The $[M^+]/[Na^+]$ ratio was 2.0 in all cases, except for the Na gel. The samples were prepared by the homogeneous method. All ^{23}Na NMR spectra were obtained under identical conditions: 128 transients, 2 s recycle delay, and 8000 Hz sample spinning.

where $\phi1 = (0°)$, $\phi2 = (0°, 0°, 60°, 60°, 120°, 120°, 180°, 180°, 240°, 240°, 300°, 300°)$, $\phi3 = (0°, 180°)$, $\phi4 = (0°, 180°, 180°, 0°)$, and $\tau = 20$ μs. The optimized excitation ($P1$) and conversion ($P2$) pulse widths were 5.0 and 2.0 μs, respectively. The pulse width for the selective ^{23}Na 90° pulse ($P3$) was 18 μs. Typically, 480∼600 transients were collected for each of the 42∼52 t_1 increments with a recycle delay of 2 s. The hypercomplex data method[44] was used for obtaining pure-phase 2D spectra. The 2D time-domain data were treated with a shear Fourier transformation (FT).

Results and Discussion

Solid-State ^{23}Na NMR. Figure 2 shows the 1D ^{23}Na MAS spectra for several 5′-GMP gel samples containing different monovalent cations. For comparison, the ^{23}Na MAS spectrum for crystalline Na_2(5′-GMP)·$7H_2O$ (orthorhombic) is also shown in Figure 2. The spectral assignment for the 5′-GMP gel samples has been unambiguously established in our recent studies.[31,32] That is, the ^{23}Na NMR signals at approximately 7, −4, and −19 ppm are associated with free NaCl, surface Na^+ ions, and channel Na^+ ions, respectively. For the crystalline sample of Na_2(5′-GMP)·$7H_2O$, a line shape is observed between −7 and −20 ppm, due to an overlap of four crystallographically distinct Na sites.[31] As seen from Figure 2, whether a 5′-GMP sample is a gel or a crystalline sample can be readily distinguished from their ^{23}Na MAS spectra. Among the gel samples, the relative signal intensities between the surface and channel Na^+ ions show a strong dependence on the type of cations present in the sample. For example, the signal associated with the surface Na^+ ions, $\delta(^{23}Na) \approx -4$ ppm, is significantly smaller in the NH_4/Na sample than in the Na gel sample. This suggests that the NH_4^+ ions have partially replaced the Na^+ ions that are originally on the surface of the stacking G-quartet structure. In contrast to

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the case of NH_4^+, no replacement of the surface Na^+ ions by K^+ ions occurs in the K/Na sample. For the Rb/Na and Cs/Na samples, Rb^+ and Cs^+ ions also replace partially the surface Na^+ ions.

Similar to the above discussion, any change in the ^{23}Na NMR signal associated with the channel Na^+ ions, $\delta(^{23}Na) \approx -19$ ppm, would reflect the competition between Na^+ and M^+ ions for the cavity site of the 5′-GMP structure. For example, as seen in Figure 2, the signal at -19 ppm disappears in the ^{23}Na NMR spectrum for the K/Na gel sample, immediately suggesting that the channel Na^+ ions are completely replaced by the K^+ ions. In comparison, the signal intensity for the channel Na^+ ions in the Cs/Na gel is essentially the same as that in the Na gel. This indicates that Cs^+ ions do not enter the channel. On the basis of these observations, we can conclude that both K^+ and NH_4^+ ions are strongly favored over Na^+ by the channel cavity and that the surface prefers NH_4^+ and Cs^+ ions. We found no interaction between tetramethylammonium (TMA) cation and the 5′-GMP structure. These observations can be understood qualitatively on the basis of ionic size and electrostatic interaction.

Cation Titration Experiment. Since we have achieved spectral separation for Na^+ ions in different locations, it is now possible to quantitatively examine the affinity of various monovalent cations for the 5′-GMP structure in a site-specific manner. The approach used in this study is a titration experiment. In particular, we prepared a series of 5′-GMP gel samples, each containing mixed Na^+ and M^+ ions. By varying the amount of M^+ added to a Na gel sample and following the corresponding change in ^{23}Na NMR signal intensities, we can obtain a thermodynamic equilibrium constant (K_{eq}) for the binding competition between Na^+ and M^+ ions. Because there are two major types of ion binding sites in the 5′-GMP system (channel and surface sites), two equilibrium constants can be determined for the two exchange processes. The two-site model used in this study involves the following two ion competition equilibria:

$$Na^+\cdot P + M^+(aq) \overset{K_1}{\rightleftharpoons} M^+\cdot P + Na^+(aq) \qquad (1)$$

$$Na^+\cdot C + M^+(aq) \overset{K_2}{\rightleftharpoons} M^+\cdot C + Na^+(aq) \qquad (2)$$

where P (phosphate) and C (carbonyl) denote the surface and channel sites of the 5′-GMP structure, respectively. In eqs 1 and 2, M^+ represents the monovalent cation added in the ion titration experiment.

Before we proceed further with data analysis, we should examine whether the equilibria described by eqs 1 and 2 indeed exist. To this end, we performed a test to see whether the cation distribution in a sample prepared by the homogeneous method would be identical to that of a sample with the same chemical composition but prepared by the heterogeneous method. Details of the heterogeneous method of gel preparation are given in the Experimental Section. Briefly, in the heterogeneous preparation, a pure Na 5′-GMP gel forms first and is then soaked in a solution containing M^+ ions. Under such a circumstance, any replacement of Na^+ by M^+ would be an indication of the existence of a competitive equilibrium. As can be seen from Figure 3, both surface and channel Na^+ ions bound to the 5′-GMP structure can be replaced by M^+ ions. Furthermore, the

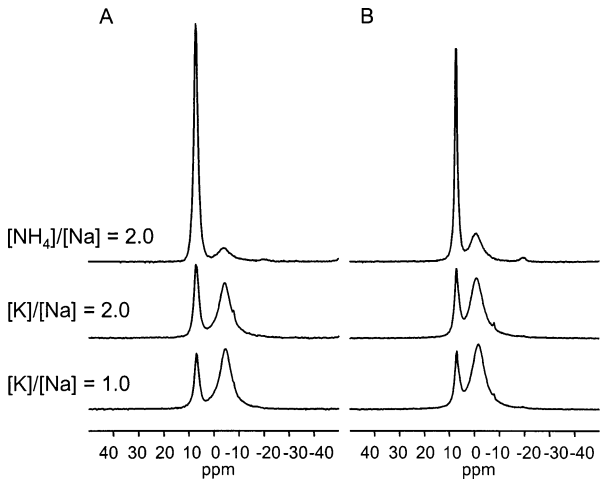

Figure 3. Experimental ^{23}Na MAS NMR spectra for 5′-GMP gels prepared by (A) homogeneous and (B) heterogeneous methods.

samples from the two different preparation methods exhibit essentially the same characteristics regarding the cation distribution among different binding sites. This provides strong evidence that the equilibria given in eqs 1 and 2 exist in both homogeneous and heterogeneous environments. Equations 1 and 2 also imply that the formation of the stacking G-quartet structure in 5′-GMP is independent of the cation exchange process. This is a reasonable assumption because the dissociation rates of G-quadruplex structures are known to be much slower than the movement of cations. For example, the lifetimes of Na^+ and NH_4^+ ions bound to the channel cavity site of $d(G_4T_4G_4)$ (Oxy-1.5) are 180 μs and 250 ms, respectively.[22,39] In contrast, the lifetimes of G-quadruplex structures could be on the order of days, weeks, or even months.[45−47]

In the two-site model described by eqs 1 and 2, the two equilibrium constants, K_1 and K_2, are related to the various concentrations at equilibrium in the following fashion:

$$K_1 = \frac{x(x+y)}{([Na^+\cdot P]_0 - x)([M^+]_0 - x - y)} \qquad (3)$$

$$K_2 = \frac{y(x+y)}{([Na^+\cdot C]_0 - y)([M^+]_0 - x - y)} \qquad (4)$$

where x and y are the individual contributions due to eqs 1 and 2, respectively, to the total free Na^+ concentration, $[Na^+(aq)] = x + y$. $[Na^+\cdot P]_0$ and $[Na^+\cdot C]_0$ are the initial concentrations of Na^+ ions bound to the surface and channel sites, respectively. $[M^+]_0$ is the total concentration of added MCl salt. In the case of 5′-GMP, since x is usually much greater than y, eqs 3 and 4 can be further simplified, for which the following solutions can be readily obtained:

$$x = \frac{([Na^+\cdot P]_0 + [M^+]_0)K_1 - \sqrt{([Na^+\cdot P]_0 - [M^+]_0)^2 K_1^{\,2} + 4K_1[Na^+\cdot P]_0[M^+]_0}}{2(K_1 - 1)} \qquad (5)$$

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(46) Smith, F. W.; Feigon, J. *Nature (London)* **1992**, *356*, 164−168.
(47) Wang, Y.; Patel, D. J. *Biochemistry* **1992**, *31*, 8112−8119.

$$y = \frac{K_2[\mathrm{Na}^+\cdot\mathrm{C}]_0([\mathrm{M}^+]_0 - x)}{x + K_2([\mathrm{M}^+]_0 - x)} \quad (6)$$

In the ion titration experiment, $[M^+]_0$ was varied while the total amount of Na^+ ions was kept constant. The concentrations of $[Na^+\cdot P]$ and $[Na^+\cdot C]$ at equilibrium, which can be directly monitored by ^{23}Na MAS NMR, are related to K_1 and K_2 in the following fashion:

$$[\mathrm{Na}^+\cdot\mathrm{P}] = [\mathrm{Na}^+\cdot\mathrm{P}]_0 - x \quad (7)$$

$$[\mathrm{Na}^+\cdot\mathrm{C}] = [\mathrm{Na}^+\cdot\mathrm{C}]_0 - y \quad (8)$$

By measuring $[Na^+\cdot P]$ and $[Na^+\cdot C]$ as a function of $[M^+]_0$, we can determine the values of K_1 and K_2. Once a thermodynamic equilibrium constant is determined, the Gibbs free energy difference ($\Delta G°$) for the aforementioned cation competition process can be readily calculated from

$$K_{\mathrm{eq}} = \exp(-\Delta G°/RT) \quad (9)$$

The ^{23}Na MAS NMR spectra from the titration experiment for four types of 5′-GMP gel samples with mixed M/Na cations are presented in Figure 4. The corresponding experimental data and theoretical fits, using eqs 7 and 8, are shown in Figure 5. As seen in Figure 4, a small but nonnegligible amount of free Na^+ ions are present in the pure Na 5′-GMP gel sample before any M^+ is added. This factor was also taken into account in the data analysis. The thermodynamic parameters obtained from the analyses of the data shown in Figure 5 are reported in Table 1. Because the two binding sites of the 5′-GMP structure exhibit very different selectivity behaviors, we examine them separately in the following sections.

Cation Affinity for the Channel Cavity. As seen from Table 1, the channel cavity site of the 5′-GMP structure significantly prefers K^+ and NH_4^+ over Na^+, whereas Cs^+ is much less favored than Na^+. On the basis of the thermodynamic parameters shown in Table 1 and the fact that Li^+ does not promote 5′-GMP gel formation, we obtain the following cation affinity sequence for the channel cavity site: $K^+ > NH_4^+ > Rb^+ > Na^+ > Cs^+ > Li^+$. This cation affinity sequence is in agreement with the qualitative ranking first reported for 5′-GMP by Pinnavaia et al.,[5,6] $K^+ > Rb^+$, $Na^+ \gg Li^+, Cs^+$. By studying the melting temperatures of Br_8-guanosine gels in various salt solutions, Chantot and Guschlbauer[7] also observed a stability sequence: $K^+ \gg Rb^+ > NH_4^+ > Na^+ > Cs^+ > Li^+$. Except for the position of NH_4^+, the stability sequence for Br_8-guanosine gels is also similar to what we observed for 5′-GMP. As mentioned above, several telomeric DNA sequences show a similar trend in cation-induced stability, $K^+ > Na^+ > Cs^+$.[24] Our results indicate that the overall stability of the stacking G-quartet structure in 5′-GMP depends only on the affinity of cations for the cavity site.

In Figure 6, we plot the value of $\Delta G°$ against the reciprocal ionic radius. The shape of the curve suggests that the affinity of monovalent cations for the 5′-GMP channel cavity site follows the order of Eisenman sequence V.[48] Among the monovalent cations examined in this study, K^+ has the highest affinity for the cavity site and Cs^+ the least. It should be noted that although Cs^+ is too large to actually enter the channel cavity, our NMR data did show a slow replacement of the channel Na^+ ions by Cs^+ ions. The most plausible explanation for this observation is that the addition of Cs^+ ions to the 5′-GMP/Na system breaks the long columnar 5′-GMP aggregates into shorter segments. It is also possible that the Na^+ ions at the ends of the stacking G-quartet structure are replaced by Cs^+ ions that serve as capping ions, in a similar fashion as those observed in lipophilic guanosines.[19,32]

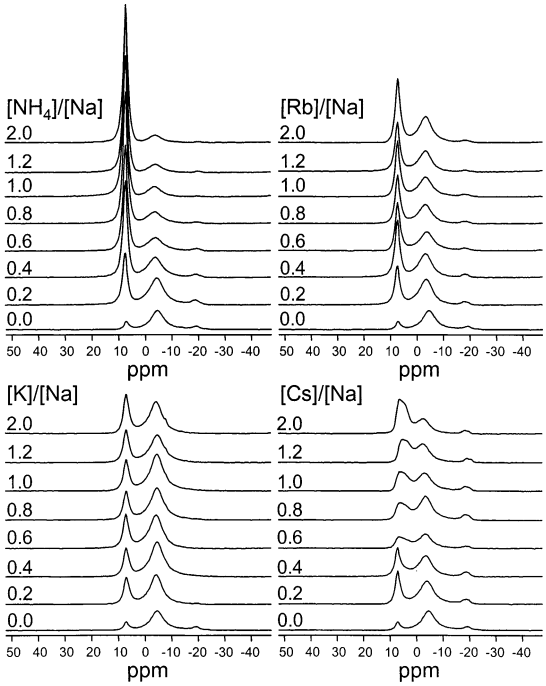

Figure 4. Experimental ^{23}Na MAS spectra for 5′-GMP samples as a function of the amount of M^+ and Na^+ ions. The samples were prepared by the homogeneous method. All ^{23}Na NMR spectra were obtained under identical conditions: 128 transients, 2 s recycle delay, and 8000 Hz sample spinning.

Another striking feature of the data presented in Figure 6 is that the affinity sequence observed for the 5′-GMP channel site is remarkably similar to that for K^+ ion channel proteins.[48] The similarity between G-quadruplex structures and ion channel proteins was first noted by Feigon and co-workers.[45] This concept was also utilized by Davis and co-workers in the design of artificial ion channels.[19] The structural basis for the observed similarity in cation selectivity becomes quite clear after MacKinnon and co-workers[49] published the first high-resolution crystal structure for a K^+ ion channel protein (KcsA) from the bacterium *Streptomyces lividans*. Four K^+ binding sites were identified inside the selectivity filter of KcsA channel, each coordinating to eight carbonyl oxygen atoms from four signature sequences, Thr^{75}-Val^{76}-Gly^{77}-Tyr^{78}. At each binding site, the K^+ ion resides near the center of a square antiprism with a mean K−O distance of 2.85 Å. This type of cation coordination environment is remarkably similar to those found in the 5′-GMP structure and in the G-quadruplex structures formed by telomeric DNA oligomers.[16,17] A detailed comparison of thermodynamic data between 5′-GMP and telomeric DNA sequences will be presented in a later section.

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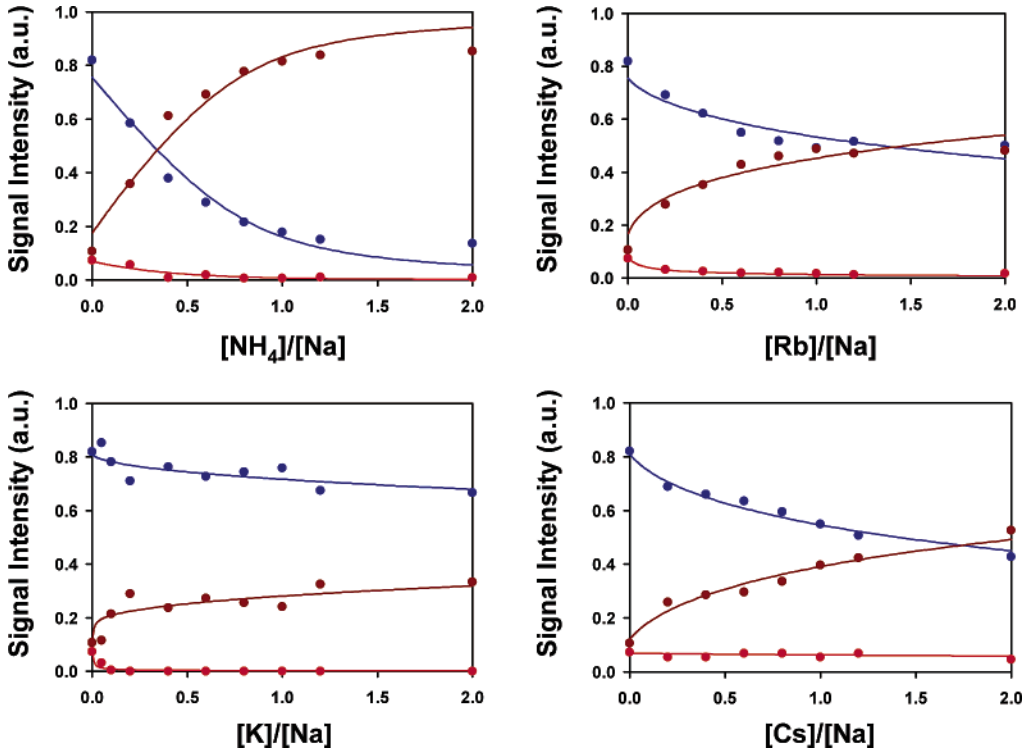

Figure 5. Experimental ^{23}Na NMR results (data points) and theoretical fits (solid lines) of the ion titration experiments for 5′-GMP samples containing mixed cations. The color codes used in the graph are as follows: surface Na^+ ions (blue), channel Na^+ ions (red), and free NaCl (brown).

Table 1. Thermodynamic Parameters Obtained for 5′-GMP Systems from Solid-State ^{23}Na NMR Titration Experiments at 298 K

cation	ionic radius (Å)	K_{eq}	$\Delta G°$ (kcal mol^{-1})	ΔG_{hyd} (kcal mol^{-1})	ΔG_{bind} (kcal mol^{-1})
		Channel			
Na^+	0.95	1	0	−98.2[a]	0
K^+	1.33	25 ± 5	−1.9 ± 0.4	−80.6[a]	15.7
NH_4^+	1.45	20 ± 2	−1.8 ± 0.2	−78.6[b]	17.8
Rb^+	1.48	1.8 ± 0.1	−0.3 ± 0.1	−75.5[a]	22.4
Cs^+	1.69	0.05 ± 0.05	1.8 ± 0.4	−67.8[a]	32.4
		Surface			
Na^+		1	0		
K^+		0.015 ± 0.010	2.5 ± 0.6		
NH_4^+		9 ± 2	−1.3 ± 0.2		
Rb^+		0.15 ± 0.05	1.1 ± 0.3		
Cs^+		0.20 ± 0.05	0.9 ± 0.4		

[a] From ref 57. [b] From ref 58.

As we noted previously,[34] the cation binding environment in G-quadruplexes is also similar to those found in the alkali metal salts of nonactin, a naturally occurring antibiotic ionophore. The ^{23}Na chemical shift for the nonactin/NaSCN complex in the solid state was found to be $\delta(^{23}Na) = -16$ ppm,[50] indeed very similar to the corresponding values observed for the channel Na^+ ions in G-quadruplexes.[31,32] As is the case for 5′-GMP, nonactin also shows a preferential affinity for K^+ over Na^+, $\Delta G°(Na^+ \rightarrow K^+) = -1.7$ kcal mol^{-1}.[48] For general monovalent cations, nonactin exhibits selectivity in the order $NH_4^+ > K^+$, $Rb^+ > Cs^+ > Na^+ > Li^+$.[51] This sequence is somewhat different from the one observed for 5′-GMP, especially in the placement of Cs^+. This is probably due to the overall structural difference between these two systems. For example, the G-quadruplex structure is quite rigid and Cs^+ is too large to fit into the channel cavity, whereas the nonactin molecule is flexible enough to wrap around a Cs^+ ion.[52]

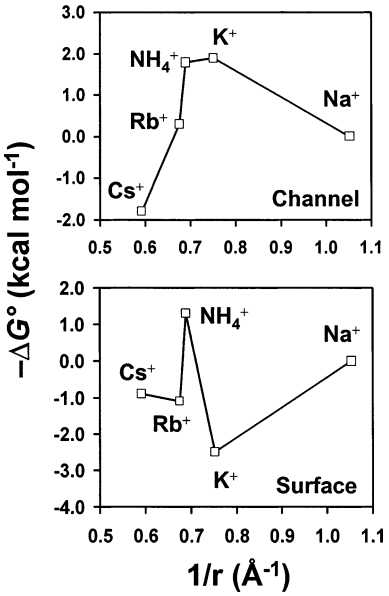

Figure 6. Diagrams of free energy difference versus reciprocal ionic radius for binding of monovalent cations to 5′-GMP structure.

Cation Affinity for the Surface. The second type of ion binding in 5′-GMP occurs between cations and doubly charged phosphate groups. As seen from Table 1 and Figure 6, the sequence of cation affinity for the 5′-GMP surface site is drastically different from that for the channel cavity site. For example, K^+ has the greatest affinity for the channel site ($\Delta G°$

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(52) Sakamaki, T.; Iitaka, Y.; Nawata, Y. *Acta Crystallogr., Sect. B* **1977**, *33*, 52−59.

$= -1.9$ kcal mol^{-1}), but the least affinity for the surface site ($\Delta G° = 2.5$ kcal mol^{-1}). Meanwhile, NH_4^+ exhibits equally strong affinity for both the channel and the surface sites. The surface site strongly prefers NH_4^+ over K^+ by a free energy difference of 3.8 kcal mol^{-1}. According to the observed $\Delta G°$ values, the order of cation affinity for the 5′-GMP surface site is $NH_4^+ > Na^+ > Cs^+ > Rb^+ > K^+$. This sequence is somewhat different from the affinity sequence of monovalent cations for the surface of double-stranded DNA, $NH_4^+ > Cs^+ > K^+ > Li^+ > Na^+$.[53] In addition, on the basis of careful ^{23}Na and ^{39}K NMR relaxation measurement, Xu et al.[38] found that Na^+ and K^+ ions have the same selectivity for atmospheric binding to a G-quadruplex structure formed by d(T_2G_4T). Presumably the doubly charged phosphate group in 5′-GMP behaves differently from the singly charged phosphodiester group in DNA sequences. Furthermore, structural topology may also be an important contributor to the observed cation affinity in 5′-GMP. Extension of the current solid-state NMR approach to double-stranded DNA structures is under way in this laboratory and the results will be published elsewhere. At this time it is sufficient to say that the presence of multiple binding sites in DNA systems may potentially complicate the analysis of solution NMR relaxation data, making it difficult to extract reliable site-specific information. However, the solid-state NMR approach presented in this study does not suffer from this problem.

Hydration Free Energy and Cation Binding Affinity. The traditional interpretation for the observed cation selectivity in G-quadruplex structures was based upon the "optimal fit" model.[6,11,25,54] In particular, K^+ was considered to have the optimal size to fit into the cavity between two G-quartets, compared with the smaller Na^+ and the larger Rb^+ ions. However, on the basis of extensive free energy perturbation/molecular dynamics (FEP-MD) calculations, Ross and Hardin[55] concluded that the "optimal-fit" model cannot fully explain the preference for K^+, because their calculations suggest that K^+ is too big for the G-quadruplex cavity. Although their calculations gave reasonable ion-quadruplex free energies, the FEP-MD calculations were apparently not accurate enough to predict the correct affinity order between K^+ and Na^+ ions. Later, Feigon and co-workers[45] proposed that the preferential binding of K^+ over Na^+ observed in DNA G-quadruplexes is dominated by relative free energies of hydration between the two cations. As mentioned above, the cation residing inside the G-quadruplex cavity is fully dehydrated; meanwhile, free cations in water are fully hydrated. A cation must undergo a dehydration process when entering the cavity from water. Therefore, any free energy difference between different cation-quadruplex complexes will be balanced by the free energy cost (or gain) during the dehydration process. The conclusion of Feigon and co-workers[45] was that although the Na^+-quadruplex complex has a lower free energy than the corresponding K^+ complex, it costs too much free energy to remove the water of hydration around a Na^+ cation. Consequently, the overall cation binding selectivity is $K^+ > Na^+$. This proposal was recently confirmed by ab initio calculations for G-quartet models.[56] Similar solvent effects have also been reported for crown ethers.[59,60]

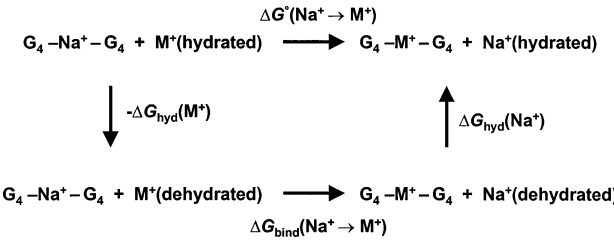

Figure 7. Diagram illustrating a thermodynamic cycle for ion competition process in 5′-GMP.

To illustrate the effect of hydration free energy, the thermodynamic cycle for the ion competitive equilibrium shown in eq 1 can be separated into three different steps as illustrated in Figure 7. The free energy difference for the ion exchange process, $\Delta G°$, can be formulated as a sum of two terms:

$$\Delta G° = \Delta G_{\text{solvation}} + \Delta G_{\text{bind}} \qquad (10)$$

where $\Delta G_{\text{solvation}} = \Delta G_{\text{hyd}}(Na^+) - \Delta G_{\text{hyd}}(M^+)$ describes the free energy cost (or gain) during the hydration of Na^+ and the dehydration of M^+ ions, and ΔG_{bind} describes the free energy cost (or gain) by mutating a Na^+ ion to an M^+ ion at a binding site in an aqueous environment.

Because we have determined $\Delta G°$ (see data in Table 1) and the ΔG_{hyd} values for monovalent cations have been well documented in the literature,[57,58] we are able to evaluate ΔG_{bind}. The values of ΔG_{hyd} and ΔG_{bind} are summarized in Table 1. The positive values of ΔG_{bind} indicate that the G-quadruplex structure prefers Na^+ over all the monovalent cations examined in this study. The sequence of ΔG_{bind} values is in the order $Na^+ < K^+ < NH_4^+ < Rb^+ < Cs^+$, consistent with the trend in ionic size or charge density for these cations. Although it is rather difficult to determine experimentally the values of ΔG_{bind}, our data may be useful as benchmarks to test the accuracy of quantum mechanical computations. For example, Gu and Leszczynski[56] reported that, at the HF/6-311G(d,p)//HP-6-31G(d,p) level, the free energy for G_4-Na^+-G_4 in water is lower than that of G_4-K^+-G_4 by 11.7∼13.2 kcal mol^{-1}, provided that the entropy difference between the two species can be ignored. Ross and Hardin[55] reported a similar value, 13 kcal mol^{-1}. These calculated values compare quite well with the one given in Table 1, $\Delta G_{\text{bind}}(Na^+ \rightarrow K^+) = 15.7$ kcal mol^{-1}. It would be of interest to see whether ab initio calculations can reproduce the ΔG_{bind} values reported in Table 1 for other cations. As seen from Table 1, the values of $\Delta G°$ between Na^+ and M^+ are on the order of ± 2 kcal mol^{-1}, which is much smaller than the corresponding $\Delta G_{\text{solvation}}$ value between the same pair of cations. Our solid-state ^{23}Na NMR data strongly suggest that the proposal of Feigon and co-workers[45] regarding the effect of hydration free energy on preferential binding between Na^+ and K^+ can be extended to include NH_4^+ and Rb^+ ions.

Solid-State 2D ^{23}Na MQMAS NMR. In the above discussion, we have focused only on analysis of 1D ^{23}Na MAS spectra. Potentially 2D ^{23}Na MQMAS may reveal more spectral features

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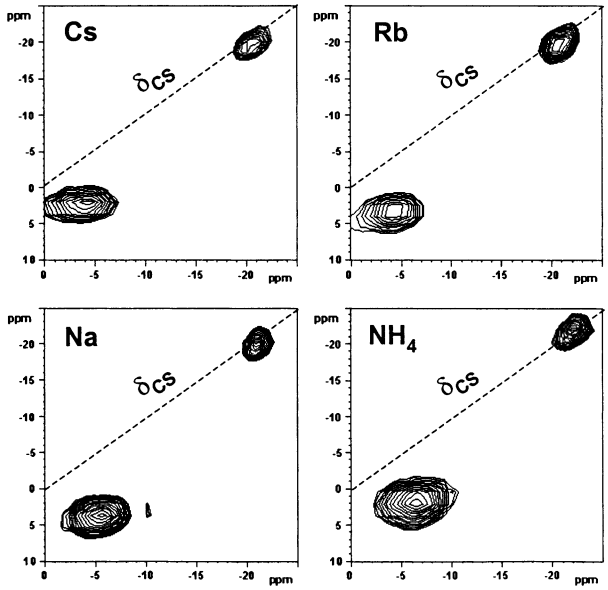

Figure 8. Experimental 2D ^{23}Na MQMAS spectra of 5′-GMP gels. Experimental parameters are given below. Na gel: 480 transients for each of 45 t_1 increments. Cs/Na gel: 480 transients for each of 42 t_1 increments. Rb/Na gel: 480 transients for each of 45 t_1 increments. NH_4/Na gel: 600 transients for each of 58 t_1 increments. The samples were prepared by the homogeneous method.

that are invisible in the 1D MAS spectra, as we recently demonstrated in a lipophilic G-quadruplex[32] and other Na−nucleotide systems.[61] To explore this possibility, we obtained 2D ^{23}Na MQMAS spectra for several 5′-GMP samples containing mixed cations. As shown in Figure 8, the 2D MQMAS spectra of these samples show similar spectral features. The most interesting feature is the different shapes between signals for surface and channel ions. In particular, the 2D signal for the surface ions is essentially parallel to the horizontal axis, whereas the 2D signals associated with the channel Na^+ ions exhibit a tilt along the axis of chemical shift distribution. This observation suggests that the surface binding sites in 5′-GMP show much less structural heterogeneity than do the channel sites. The 2D ^{23}Na MQMAS spectra shown in Figure 8 are also similar to those observed for $[d(TG_4T)]_4$.[35] Unlike the case for a crystalline G-quadruplex,[32] at 11.75 T we were unable to resolve different Na^+ sites present in the 5′-GMP samples. It is possible that solid-state ^{23}Na MQMAS spectra at higher magnetic fields may be able to provide definite information about the precise number of Na^+ ions inside the 5′-GMP channel. From a combined analysis of the 1D and 2D ^{23}Na NMR spectra, we have estimated the following parameters for the Na^+ ions in 5′-GMP gels: $\delta(^{23}Na) \approx 1$ ppm and $C_Q \approx 1.6$ MHz for the surface site; $\delta(^{23}Na) \approx -18$ ppm and $C_Q \approx 1.1$ MHz for the channel site. It should be noted that because small variations do exist among 5′-GMP gels containing different cations, the above NMR parameters are only qualitative. Unlike the situation for the channel Na^+ ions, the state of hydration for the surface Na^+ ions in 5′-GMP gels cannot be unambiguously determined. Comparing the NMR parameters observed for the surface Na^+ ion with our recent solid-state ^{23}Na NMR data for several Na−nucleotide systems,[61] we believe that the surface Na^+ ions in 5′-GMP are hexacoordinated and likely to be only partially hydrated. The remaining ligands coordinated to the surface Na^+ ion could be a phosphate oxygen atom and possibly a hydroxyl group from the ribose, similar to those seen in several Na−nucleotide systems.[61] It is entirely possible that the involvement of a hydroxyl group in the first coordination sphere may affect the cation affinity.

(61) Wong, A.; Wu, G. *J. Phys. Chem. A* **2003**, *107*, 579−586.

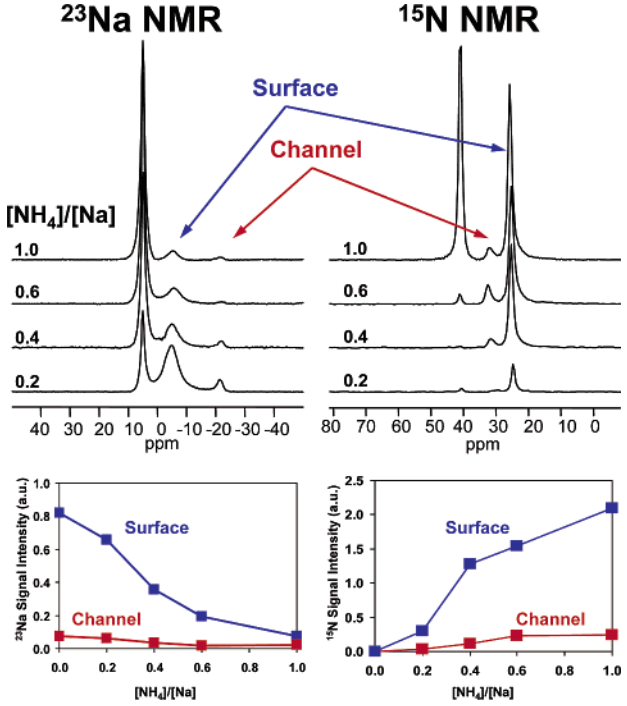

Figure 9. (Top) Solid-state ^{23}Na and ^{15}N MAS NMR spectra for 5′-GMP samples containing various amounts of $^{15}NH_4^+$ and Na^+ ions. The samples were prepared by the homogeneous method. Experimental parameters: for ^{23}Na NMR, 128 transients and 2 s recycle delay; for ^{15}N NMR, 1615 transients and 10 s recycle delay. (Bottom) NMR signal intensity as a function of the amount of added cations.

Solid-State ^{15}N MAS Spectra. So far we have demonstrated the use of solid-state ^{23}Na NMR to probe ion binding in 5′-GMP systems. In the case of 5′-GMP samples containing mixed NH_4^+ and Na^+ ions, it is also possible to use solid-state ^{15}N NMR to study the chemical environment around the NH_4^+ ion. Feigon and co-workers[22] recently showed that $^{15}NH_4^+$ can be used as a surrogate NMR probe for characterizing monovalent cation binding in oligonucleotides. Extension of this approach to solid-state ^{15}N NMR has not yet been demonstrated. Because we have obtained direct information about Na^+ binding from ^{23}Na NMR data, it would be ideal to see whether solid-state ^{15}N NMR can yield complementary information about NH_4^+ binding in a 5′-GMP system containing mixed NH_4^+ and Na^+ ions. To this end, we used 99% ^{15}N-enriched NH_4Cl salt to prepare four different NH_4/Na 5′-GMP gel samples with a $[NH_4]/[Na]$ ratio ranging from 0.2 to 1.0. Figure 9 shows the ^{23}Na and ^{15}N MAS spectra of these samples. In the ^{15}N MAS spectra, three well-resolved ^{15}N NMR signals were observed. Similar to the previous discussion about ^{23}Na NMR spectra, the ^{15}N NMR signals can be assigned in a straightforward manner to three different NH_4^+ binding sites. In particular, the signals at $\delta(^{15}N) = 41$, 32, and 25 ppm were attributed to free NH_4Cl, channel NH_4^+ ions, and phosphate-bound (surface) NH_4^+ ions, respectively. A chemical shift of $\delta(^{15}N)$ 32 ppm for the channel NH_4^+ ions in 5′-GMP is in agreement with the values reported by Feigon and co-workers, $\delta(^{15}N)$ 28 and 29 ppm, in $[d(G_4T_4G_4)]_2$.[22] In the solution ^{15}N NMR spectrum,

however, only one signal at $\delta(^{15}N)$ 20 ppm was observed for the so-called bulk NH_4^+ ions. Clearly, this signal is an average between the phosphate-bound and the free NH_4^+ (fully hydrated) ions as a result of rapid cation exchange occurring in solution. In contrast to the solution NMR spectrum, separate signals were observed for the phosphate-bound (surface) and the free NH_4^+ ions in the ^{15}N MAS spectrum. Similar to the case of surface Na^+ ions, the hydration state for the surface NH_4^+ ions cannot be determined with certainty from the 1D ^{15}N NMR experiment. However, we noted that the observed ^{15}N chemical shift for the surface NH_4^+ ions in the solid state, 25 ppm, is similar to the value observed for the bulk NH_4^+ ions in aqueous solution, 20 ppm. It is also possible that a rotational echo double resonance (REDOR) experiment[62] between ^{15}N and ^{31}P nuclei can establish the proximity of a surface $^{15}NH_4^+$ ion and a phosphate group.

Similar to the treatment of the ^{23}Na NMR data, the intensities of the three ^{15}N signals were monitored as a function of the $[NH_4]/[Na]$ ratio in the sample. Figure 9 shows a comparison between the ^{23}Na and ^{15}N NMR data obtained for the same 5′-GMP sample. As the ratio of $[NH_4]/[Na]$ increases (i.e., when more NH_4^+ ions are added to the system), both the channel and surface Na^+ ions are gradually replaced by NH_4^+ ions. However, the rates of replacement are clearly different for the two binding sites. For example, at $[NH_4]/[Na] = 1.0$, more than 90% of the surface Na^+ ions are replaced by NH_4^+ ions, while only 70% of the channel Na^+ ions are replaced by NH_4^+ ions. When the ratio of $[NH_4]/[Na]$ reaches 10:1 (data not shown), essentially all of the Na^+ ions bound to 5′-GMP structure are replaced by NH_4^+ ions. The complementary nature of the two NMR probes is clearly illustrated by the data shown in Figure 9. This observation confirms the validity of the approach that $^{15}NH_4^+$ is used as a surrogate probe for studying alkali metal cations. However, it is also important to remember that the thermodynamic parameters for Na^+ and NH_4^+ binding are significantly different, as discussed in the previous sections. In principle, other NMR-active alkali metal isotopes such as ^{39}K (spin $^3/_2$) and $^{87/85}Rb$ (spin $^3/_2$) and ^{133}Cs (spin $^7/_2$) can be used in a similar manner to monitor the corresponding alkali metal cations. These metal isotopes have quadrupolar nuclei and, consequently, often give rise to broad NMR signals. At ultrahigh magnetic fields (21 T or higher), these quadrupolar nuclei should be accessible by solid-state NMR.

Solid-State ^{13}C and ^{31}P NMR Spectra. We have also obtained solid-state ^{13}C and ^{31}P CP/MAS NMR spectra for several 5′-GMP samples and for Na_2(5′-GMP)·$7H_2O$. The latter compound is a crystalline material and contains monomeric 5′-GMP molecules in the crystal lattice.[63] The primary objective here is to see whether additional information about cation binding to G-quartets can be obtained from these relatively easy NMR probes. Although ^{13}C and ^{31}P NMR studies have been shown to be useful in studying 5′-GMP self-association in solution,[64−66] solid-state ^{13}C and ^{31}P NMR spectra unfortunately did not yield useful information about cation binding in 5′-GMP. These spectra are included as Supporting Information.

Table 2. Comparison of Thermodynamic Parameters for Selective Binding of K^+ over Na^+ to G-Quadruplex Structures

system	method	$\Delta G°$ (kcal·mol^{-1})	ref
5′-GMP	solid-state ^{23}Na NMR	-1.9 ± 0.4	this work
$d(T_4G_4)_4$	folding	−0.8	67
$[d(G_3T_4G_3)]_2$	thermal melting	−2.1	68
$d(T_2AG_3)_4$	van't Hoff analysis	−1.3	69
$[d(G_3T_2AG_3)]_2$	van't Hoff analysis	−0.5	69
$[d(G_3T_4G_3)]_2$	solution ^1H NMR	−0.8	45
$d(T_2G_4)_4$	FEP/MD simulation	+4.3	55
G_4-M^+-G_4[a]	*ab initio* calculation	−5.9	56

[a] M = K, Na.

Comparison with Other G-Quadruplexes. Because of the relevance of the self-ordered 5′-GMP structure to G-rich DNA, it is important to compare our results with previously reported thermodynamic data for G-quadruplexes formed by oligonucleotides. Table 2 gives a summary of the thermodynamic parameters about selective K^+ binding in a number of related G-quadruplex systems. It should be noted that, in all the previous experimental studies, it has not been possible to associate the observed G-quadruplex stability with site-specific cation binding.

As we have mentioned in the above discussion, the stability of 5′-GMP structure depends only on the cation binding to the cavity site. From the solid-state ^{23}Na NMR titration experiment, we have determined that the free energy difference between K^+ and Na^+ binding to the cavity site of 5′-GMP is approximately −1.9 kcal mol^{-1}. Raghuraman and Cech[67] have analyzed the folding of $d(T_4G_4)_4$ (Oxy-4) in the presence of 50 mM NaCl and 50 mM KCl at 37 °C. Their estimated $\Delta G°$ value for selective binding of K^+ over Na^+ was −0.8 kcal mol^{-1}, assuming that three cations are sandwiched between the 4 G-quartets in folded Oxy-4 quadruplex. Scaria et al.[68] have also studied the thermal melting behavior of $d(G_3T_4G_3)$ in the presence of salt. They showed that at 25 °C the free energy of the quadruplex was 4.2 kcal mol^{-1} lower in 100 mM KCl than in 100 mM NaCl. Assuming that two cations are involved in $d(G_3T_4G_3)$, $\Delta G°(Na^+ \rightarrow K^+)$ was approximately −2.1 kcal mol^{-1}. Balagurumoorthy and Brahmachari[69] have determined thermodynamic data for cation binding stability in both intramolecular and hairpin dimer structures by performing van't Hoff analyses of CD melting profiles. For $d(T_2AG_3)_4$, they obtained a free energy difference of −2.7 kcal mol^{-1} between K^+ and Na^+ binding, which corresponds to a value of −1.3 kcal mol^{-1}, if two cations are assumed to reside between the three G-quartets. For the hairpin dimer formed by $d(G_3T_2AG_3)$, they determined a smaller value, −1.1 kcal mol^{-1}. Again, assuming two cations are bound to the three G-quartets, $\Delta G°(Na^+ \rightarrow K^+)$ is −0.5 kcal mol^{-1}.

There have also been several theoretical studies on the ion-induced stability of various G-quadruplexes. For example, Ross and Hardin[55] performed FEP/MD calculations on the relative affinity of the antiparallel $d(T_2G_4)_4$ quadruplex for K^+ and Na^+ ions. Using two sets of ionic Lennard-Jones 6−12 van der Waals

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parameters, they obtained a preferential binding of Na^+ over K^+ by 4.3 kcal mol^{-1} per cation. This is clearly inconsistent with the experimental results. Recently, Gu and Leszczynski[56] reported ab initio calculations for two G_4-M^+-G_4 models (M = K and Na). At the HF/6-311G(d,p)//HF/6-31G(d,p) level, their calculations predicted a free energy difference of −5.9 kcal mol^{-1} between K^+ and Na^+ binding, correctly reproducing the order of cation selectivity. Meyer et al.[70] have also examined the binding of metal ions to G-quartets. They reported that the interaction energy difference between Na^+-G_4 and K^+-G_4 is approximately 36 kcal mol^{-1}. After taking into consideration the hydration free energy difference between Na^+ and K^+, the study of Meyer et al.[70] predicted that Na^+-G_4 is about 18 kcal mol^{-1} lower in energy than K^+-G_4, indicating that Na^+ is preferred over K^+ by a G-quartet in aqueous solution. This prediction is clearly inconsistent with the experimental findings. It should be pointed out that, in the model of Meyer et al., the cation is assumed to be in the G-quartet plane. Although there is indeed evidence for this type of in-plane binding site in $[d(TG_4T)]_4$, it is safe to conclude that the in-plane binding to a G-quartet does not contribute significantly to the stability of the G-quadruplex structure. However, it is possible that in-plane binding at the termini of a G-quadruplex may contribute partially to structural polymorphism between sodium and potassium forms.

Conclusions

We have presented a comprehensive multinuclear solid-state NMR study of cation binding to a stacking G-quartet structure formed by 5′-GMP self-association at neutral pH. The most important finding of the study is the direct determination of thermodynamic parameters for competitive binding between Na^+ and M^+ (M = K, NH_4, Rb, and Cs) ions to two *different* sites of the 5′-GMP structure. Although extensive thermodynamic data for G-rich DNA oligomers are available in the literature, most of the previous studies are concerned with overall stability of the quadruplex structure rather than site-specific cation selectivity. Among the very few cases where cation selectivity in G-quadruplexes was examined, the consistency and accuracy between various methods are questionable. Here we introduce a new approach for direct determination of cation affinity. Using solid-state ^{23}Na NMR, we are able to directly monitor the Na^+ ions bound to a stacking G-quartet structure. The advantage of the solid-state NMR approach is that different cation binding sites can be studied separately. This type of information has been unavailable in the literature until now. We have found that the stability of the 5′-GMP structure is directly related to the relative affinity of monovalent cations for the channel cavity site and that the interaction between cations and phosphate groups plays no role in the formation of a stacking G-quartet structure. Using a combination of solid-state ^{23}Na and ^{15}N NMR techniques, we have shown that complementary information about competition between Na^+ and NH_4^+ ions can be obtained by simultaneously detecting both cations. Extension of the solid-state NMR methodology demonstrated in this study to other biomolecular systems should be straightforward, provided that sufficient spectral separation can be achieved for different cation binding sites. Further solid-state NMR studies can potentially provide information complementary to that obtainable from crystallographic experiments.

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Supporting Information Available: ^{13}C and ^{31}P CP/MAS NMR spectra for 5′-GMP samples and a table containing ^{13}C chemical shift data (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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