Ammonium Ion as an NMR Probe for Monovalent Cation Coordination Sites of DNA Quadruplexes

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Cations play a central role in the stabilization of RNA and DNA structures at all levels.[1] While a number of divalent cation binding sites in crystal and solution structures of nucleic acids have been reported,[2] the localization of specific monovalent cation binding sites is less straightforward. In crystal structures monovalent cations, particularly Na^+, are difficult to distinguish from H_2O molecules.[3] In solution, direct detection of NMR active cations gives only indirect information on the nature of ion association with nucleic acids.[4] Among nucleic acid structural motifs the guanine quartet (G quartet) is a notable example of monovalent cation participation in structure stabilization.[5] A recent high-resolution nucleic acid crystal structure of d(TGGGGT), which forms dimers of four-stranded G quartet quadruplexes, provides the only case where monovalent cations have been localized on DNA, in this case within or between G quartets.[6] Prior analyses of coordination by G quartets in the solution state have relied upon indirect evidence.[7] Here we report the use of ammonium ions (NH_4^+) as a probe of monovalent ion binding to a DNA quadruplex using NMR spectroscopy. The detection of NOEs between the protons of NH_4^+ and guanine imino protons permits the localization of the nucleic acid monovalent cation binding sites. This provides the first direct experimental evidence in solution that one monovalent cation is coordinated between each stacked pair of G quartets in a DNA quadruplex and illustrates the utility of NH_4^+ as a probe for strong monovalent ion binding sites on macromolecules.

We have investigated the cation binding properties of the quadruplex formed by the oligonucleotide d($G_4T_4G_4$) (Oxy-1.5).[8] This oligonucleotide contains the G-rich repeat sequence found in *Oxytricha* telomeres.[9] We have previously shown that Oxy-1.5 forms a 2-fold symmetric bimolecular quadruplex which contains four stacked G quartets and two thymine loops (Figure 1).[8] In the related crystal structure of Oxy-1.5, electron density

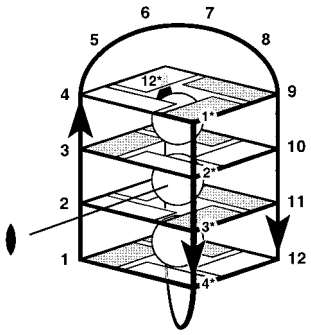

Figure 1. Schematic of the dimeric foldback quadruplex structure of d($G_4T_4G_4$) in solution (9). Numbers indicate the residues of one of the two strands of the 2-fold symmetric structure. Residues 1−4 and 12 of the symmetry-related strand are designated with an asterisk (*). Guanine bases are represented as rectangles, and thymine bases of the loops have been omitted. Shaded rectangles designate syn conformation. Spheres represent bound ammonium ions, the positions of which are determined by results presented in the text.

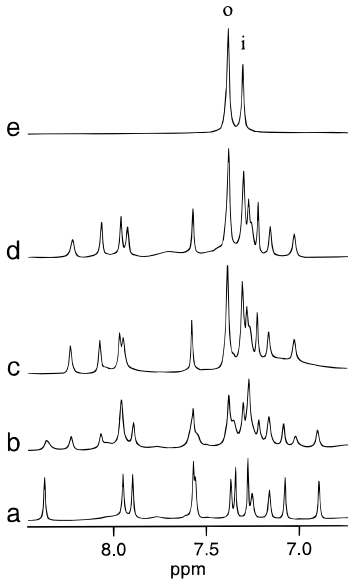

Figure 2. ^1H NMR spectra of 5.0 mM d($G_4T_4G_4$) at 10 °C and pH 7.0 in the presence of (a) 55 mM Na^+, (b) 55 mM Na^+, 20 mM $^{15}NH_4Cl$, (c) 55 mM Na^+, 80 mM $^{15}NH_4Cl$, (d) 55 mM $^{15}NH_4^+$. (e) One-dimensional $^1H-^{15}N$ HSQC spectrum of sample d. The ammonium resonances are designated "i" (inner) and "o" (outer). Spectra were collected at 500 MHz on a Bruker DRX 500 spectrometer. Water suppression was accomplished using the Watergate pulse sequence.[16] Spectra were acquired with a 10 kHz spectral width and 8K points; 128 FIDs were accumulated for each spectrum with ^{15}N decoupling.

is observed in the core of the quadruplex, which is interpreted as a single disordered K^+ located between the central two G quartets.[10]

One-dimensional ^1H NMR spectra from a titration of NH_4Cl into a solution of Oxy-1.5 containing Na^+ are shown in Figure 2a−c. As NH_4^+ is added to the sample, a second set of resonance lines appears which increase in relative intensity over the course of the titration. The changes observed in these spectra reveal that NH_4^+ competes with Na^+ for coordination by the G quartets.[11] Similar spectral changes have been observed for titrations of K^+

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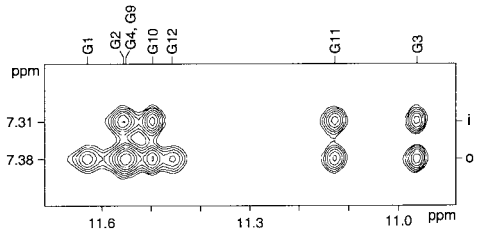

Figure 3. Portion of a ^{1}H ROESY spectrum (τ_m = 50 ms) of 5.0 mM d($G_4T_4G_4$) at 10 °C and pH 7.0 in the presence of 55 mM $^{15}NH_4^+$ showing the region containing the NH_4^+ and guanine imino cross-peaks. Assignments of the imino and ammonium resonances are indicated on the spectrum. Spin lock field was 3750 Hz with maximum offset 2.5 ppm from the carrier frequency; 3K points were collected in t_2 and 1K blocks in t_1 using States-TPPI phase cycling.[17] The spectral width was 7K Hz in F1 and F2. Spectra were ^{15}N decoupled in both F1 and F2.

into Na^+ containing samples of DNA G quadruplexes.[8b,11,12] At an approximate [NH_4^+]:[Na^+] ratio of 3:2, the ^{1}H NMR spectrum of Oxy-1.5 is virtually identical to that of the pure NH_4^+ form (Figure 2d), indicating an essentially complete replacement of coordinated Na^+ by NH_4^+. Although the ^{1}H chemical shifts of the Na^+ and NH_4^+ forms of Oxy-1.5 are different, H−H distances determined from 2D NOESY spectra show that the two forms have the same global fold (unpublished data), as is the case for the Na^+ and K^+ forms of Oxy-1.5[8b] and d($G_3T_4G_3$).[11,12]

Examination of the spectra of the NH_4^+ form of Oxy-1.5 also reveals two additional resonances at 7.31 and 7.38 ppm. A one-dimensional ^{1}H−^{15}N HSQC spectrum[13] identifies these as resonances from the NH_4^+ (Figure 2e). The proton resonance intensity from the bulk NH_4^+ is broadened to baseline at this pH (7.0) and temperature (283 K), due to chemical exchange with H_2O. At lower pH and/or temperature, the ^{1}H resonance of free NH_4^+ is observed at 7.1 ppm (not shown). The unusual observation of the two downfield shifted NH_4^+ ^{1}H resonances indicates that there are two distinct binding sites for NH_4^+ on Oxy-1.5 which are protected from exchange with water. The integrated intensities of the two NH_4^+ ^{1}H resonances increase over the course of the NH_4Cl titration to a maximum of four and two times that of resonances from individual DNA protons. These observations indicate that there are a total of three bound NH_4^+ ions per Oxy-1.5 quadruplex, one of which gives rise to the resonance at 7.31 ppm and two which give rise to the resonance at 7.38 ppm.

A selected region from a 2D ROESY spectrum[14] of a NH_4^+-Oxy-1.5 sample is shown in Figure 3. Cross-peaks are observed between the two NH_4^+ and the eight G imino resonances. These G imino resonances can be divided into two sets: *outer G iminos*, those belonging to guanine residues of the G quartet composed of G1, G4*, G9*, and G12 (the asterisk (*) designates bases of the symmetry related strand); and *inner G iminos*, belonging to guanine residues G2, G3*, G10* and G11 (Figure 1). The pattern of NH_4^+ imino cross-peaks observed along the chemical shift of each G imino resonance is similar among the inner G iminos and among the outer G iminos, respectively (Figure 3). The NH_4^+ proton resonance at 7.31 ppm shows cross-peaks exclusively with the imino protons of the inner G iminos. This localizes the NH_4^+ resonance at 7.31 ppm to between the planes of the two inner G quartets of Oxy-1.5. This is consistent with the fact that Oxy-1.5 has 2-fold symmetry, and thus a unique NH_4^+ binding site must be located on the 2-fold rotation axis. The NH_4^+ resonance at 7.38 ppm exhibits cross-peaks with both the inner and the outer G iminos (Figure 3), which localizes this NH_4^+ between the inner and outer G quartets. Since the ROESY cross-peaks between this NH_4^+ and the G3 and G11 iminos are equal in volume to the cross-peaks between the NH_4^+ resonance at 7.31 ppm and the same G imino protons, this second NH_4^+ coordination site is centered exactly between the planes of the inner and outer G quartets. Given the 2-fold symmetry of the dimer, there are two such binding sites per quadruplex.

The cation coordination sites of Oxy-1.5 revealed here by the localization of bound NH_4^+ (ionic radius 1.45 Å) are consistent with an earlier proposal that the similar size K^+ (ionic radius 1.33 Å) would be preferentially coordinated between the planes of two successive quartets.[7b,15] Also consistent with the identified coordination sites is our previous study which demonstrated that the three G quartets of the closely related dimeric quadruplex [d($G_3T_4G_3$)]$_2$ contain two cation coordination sites which can be occupied by either Na^+ or K^+.[11] Thus, it appears that there is in general one K^+ or NH_4^+ cation binding site between each stacked pair of G quartets of DNA quadruplexes. The precise location of Na^+ coordination within these quadruplexes may, however, differ from that observed for NH_4^+. This is suggested by the X-ray crystal structure of d(TGGGGT) in which the smaller Na^+ (ionic radius 0.95 Å) is coordinated both intra- and interplanar by different G quartets within the same quadruplex.[6] A detailed analysis of NH_4^+ exchange between coordination sites of a DNA quadruplex and solution will be reported elsewhere.

We have shown here that NH_4^+ provides a unique solution state probe for monovalent cation binding to nucleic acids. The G quadruplex is perhaps a special case in which the tight ion binding allows direct observation of NOEs between the bound ion and the DNA. We are currently investigating the potential for NH_4^+ coordination as a probe of monovalent ion sites on other nucleic acid and protein structures.

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