

## Solid-state $^{23}\text{Na}$ NMR determination of the number and coordination of sodium cations bound to *Oxytricha nova* telomere repeat $d(\text{G}_4\text{T}_4\text{G}_4)$

Gang Wu\*, Alan Wong

Department of Chemistry, Queen's University, Kingston, Ont., Canada K7L 3N6

Received 29 July 2004

Available online 17 September 2004

### Abstract

We report a solid-state  $^{23}\text{Na}$  NMR study of the bound sodium cations in a G-quadruplex formed by *Oxytricha nova* telomere DNA repeat,  $d(\text{G}_4\text{T}_4\text{G}_4)$  (Oxy-1.5). Using a 2D multiple-quantum magic-angle spinning  $^{23}\text{Na}$  NMR method, we observed three sodium cations residing inside the quadruplex channel of the  $\text{Na}^+$  form of Oxy-1.5. Each of these sodium cations is sandwiched between two G-quartets. We found no evidence for sodium cations in the  $\text{T}_4$  loop region. For comparison, solid-state  $^{15}\text{N}$  MAS NMR spectra were also obtained for the  $^{15}\text{NH}_4^+$  form of Oxy-1.5. The insufficient resolution in the  $^{15}\text{N}$  MAS NMR spectra did not permit determination of the number of  $\text{NH}_4^+$  ions inside the quadruplex channel. The solid-state  $^{23}\text{Na}$  and  $^{15}\text{N}$  NMR spectra for Oxy-1.5 were also compared with those obtained for guanosine 5'-monophosphate.

© 2004 Elsevier Inc. All rights reserved.

**Keywords:** Alkali metal; Cation binding; G-quartet; Solid-state NMR; Telomeric DNA

The importance of alkali metal cations in formation, stability, and function of G-quadruplex structures has been well documented in the literature [1–8]. Structural details regarding the mode of cation binding in G-quadruplexes have been reported in recent years [9–17]. In addition to the crystallographic approach, two NMR studies have demonstrated that  $^{15}\text{NH}_4^+$  (spin  $-1/2$ ) and  $^{205}\text{Tl}^+$  (spin  $-1/2$ ) ions can be used as surrogate probes for studying alkali metal cation binding to G-quadruplex in solution [18,19]. While direct studying of alkali metal cations by conventional liquid-state NMR is hampered by the lack of site-specific information on spectra, solid-state NMR has emerged as a new technique complementary to crystallography for direct detection of alkali metal cations [20]. Rovnyak et al. [21] reported the first solid-state  $^{23}\text{Na}$  (spin  $-3/2$ ) NMR study for G-rich oligonucleotides. We have subsequently established the solid-state  $^{23}\text{Na}$  and  $^{39}\text{K}$  (spin  $-3/2$ ) NMR

signatures for  $\text{Na}^+$  and  $\text{K}^+$  ions bound to a G-quadruplex [22–25]. Here, we use this novel solid-state  $^{23}\text{Na}$  NMR approach to determine the exact number and coordination geometry of bound  $\text{Na}^+$  ions in a G-quadruplex formed by *Oxytricha nova* telomere DNA repeat,  $d(\text{G}_4\text{T}_4\text{G}_4)$  (Oxy-1.5).

Oxy-1.5 is perhaps one of the most studied telomeric oligonucleotides. In solution, the  $\text{Na}^+$  form of Oxy-1.5 adopts a symmetric foldback quadruplex structure consisting of four stacked G-quartets and two diagonal thymine loops [26]. The crystal structure of the  $\text{K}^+$  form of Oxy-1.5 showed that the overall structure of Oxy-1.5 in the solid state is identical to that found in solution [12]. Feigon and co-workers also examined the structural details between various forms of Oxy-1.5 in solution and concluded that the nature of monovalent cations present in the solution ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{NH}_4^+$ ) does not affect the overall fold of Oxy-1.5 [27]. However, there is strong evidence suggesting that the details of cation binding in Oxy-1.5 may be different, depending on the type of cations present. For example, five  $\text{K}^+$  ions were found in the  $\text{K}^+$  form of Oxy-1.5 [12], whereas only three  $\text{NH}_4^+$

\* Corresponding author. Fax: +1 613 533 6669.

E-mail address: [gangwu@chem.queensu.ca](mailto:gangwu@chem.queensu.ca) (G. Wu).

ions were observed in the  $\text{NH}_4^+$  form [18]. Because the crystal structure for the  $\text{Na}^+$  form of Oxy-1.5 has not been reported, the question regarding the mode of  $\text{Na}^+$  binding in Oxy-1.5 has remained unanswered until now.

## Materials and methods

**Sample preparation.** Hydrated sodium salt of guanosine 5'-monophosphate (5'-GMP), sodium chloride, and cesium chloride were purchased from Sigma–Aldrich (Ont., Canada).  $^{15}\text{NH}_4\text{Cl}$  (99%  $^{15}\text{N}$  atom) was purchased from Cambridge Isotope Laboratories (Andover, MA). DNA oligonucleotide d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) (Oxy-1.5) was synthesized by Cortec DNA Service Laboratories (Kingston, Ont., Canada). The oligonucleotide was synthesized on a 12-channel oligonucleotide synthesizer (Northwest Engineering) using standard CE phosphoramidite chemistry. The oligonucleotide was cleaved from the CPG support and protecting groups were removed using a 50/50 mixture of ammonium hydroxide and methylamine (AMA) for 5 h at room temperature. Prime pure oligonucleotide purification cartridges were used to purify the oligonucleotide according to the manufacturer's protocol.

A sample of the  $\text{Na}^+$  form of Oxy-1.5 was prepared by dissolving 5.638 mg d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in 1 mL of deionized water and was extensively dialyzed against NaCl(aq) in the following sequence of concentrations, 500, 250, 20, and 1 mM. The solution was then dialyzed against a mixture of NaCl(aq) (1 mM) and CsCl(aq) (40 mM) prior to drying in order to suppress the amount of free  $\text{Na}^+$  cations. The DNA oligonucleotide was then lyophilized. A sample of Oxy-1.5 in the  $^{15}\text{NH}_4^+$  form was prepared by dialyzing d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) against a series of  $^{15}\text{NH}_4\text{Cl}$ (aq) solutions, 100, 50, 25, and 15 mM, and finally against a mixture of  $^{15}\text{NH}_4\text{Cl}$ (aq) (3.7 mM) and CsCl(aq) (37 mM). Cellulose dialysis membranes with molecular weight cut-off of 1000 kDa (Sigma–Aldrich) were used in all dialysis experiments.

Two 5'-GMP samples were also prepared for solid-state NMR experiments. The first sample was prepared by dissolving 320 mg  $\text{Na}_2(5'\text{-GMP}) \cdot 7\text{H}_2\text{O}$  with 1.7 mL of 4.0 M NaCl(aq). The solution (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 gently washed with 2-methylpentane-2,4-diol aqueous solution (MPD) (40% v/v) to reduce the amount of free salts. This sample was denoted as the  $\text{Na}^+$  form of 5'-GMP. The second 5'-GMP gel samples were prepared in a similar fashion, except that  $^{15}\text{NH}_4\text{Cl}$ (aq) was used rather than NaCl(aq). This second sample was denoted as the  $\text{NH}_4^+$  form of 5'-GMP.

**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, and 500.13 MHz for  $^{23}\text{Na}$ ,  $^{15}\text{N}$ , and  $^1\text{H}$  nuclei, respectively. The radio-frequency (RF) field strength at the  $^{23}\text{Na}$  frequency was 96 kHz. Sodium-23 chemical shifts were referenced to NaCl(aq) by setting the  $^{23}\text{Na}$  NMR signal of a solid NaCl sample to  $\delta(^{23}\text{Na}) = 7.21$  ppm. Solid-state  $^{15}\text{N}$  NMR spectra were obtained under the cross-polarization (CP) MAS condition with high power proton decoupling. Nitrogen-15 chemical shifts were referenced to liquid  $\text{NH}_3$ ,  $\delta(^{15}\text{N}) = 0$  ppm, by setting the  $^{15}\text{N}$  NMR signal of a solid  $^{15}\text{NH}_4\text{Cl}$  sample to  $\delta(^{15}\text{N}) = 41$  ppm. For 2D  $^{23}\text{Na}$  MQMAS experiments [28], the  $z$ -filter MQMAS pulse sequence [29] was used: P1( $\phi_1$ )-t1-P2( $\phi_2$ )- $\tau$ -P3( $\phi_3$ )-Acq(t<sub>2</sub>, $\phi_4$ ), where  $\phi_1 = (0^\circ)$ ;  $\phi_2 = (0, 0, 60, 60, 120, 120, 180, 180, 240, 240, 300, \text{ and } 300^\circ)$ ;  $\phi_3 = (0, 180^\circ)$ ;  $\phi_4 = (0, 180, 180, \text{ and } 0^\circ)$ , and  $\tau = 20$   $\mu\text{s}$ . The optimized excitation (P1) and conversion (P2) pulse widths were 4.5 and 2.0  $\mu\text{s}$ , respectively. The pulse width for the selective  $^{23}\text{Na}$  90° pulse (P3) was 18  $\mu\text{s}$ . The hypercomplex data method was used for obtaining pure-phase 2D spectra. The 2D time-domain data were treated with a shear Fourier transformation (FT).

## Results and discussion

Fig. 1 shows 1D solid-state  $^{23}\text{Na}$  NMR spectra for the  $\text{Na}^+$  forms of Oxy-1.5 and 5'-GMP. The 5'-GMP sample was used as a model system, because the spectral assignment for this system has been unambiguously established [22,23]. As seen from Fig. 1, the  $^{23}\text{Na}$  MAS spectra for Oxy-1.5 and 5'-GMP exhibit very similar features. In particular, three groups of  $\text{Na}^+$  ions can be identified in each of the spectra. The  $^{23}\text{Na}$  NMR signal at 7 ppm is due to excessive free NaCl salt. The signal centered at about  $-5$  ppm arises from the  $\text{Na}^+$  ions bound to the phosphodiester group in Oxy-1.5 and the phosphate group in 5'-GMP, respectively. These  $\text{Na}^+$  ions are referred to as surface ions. The signal centered at approximately  $-20$  ppm is characteristic of the  $\text{Na}^+$  ions residing inside a G-quadruplex channel (referred to as channel  $\text{Na}^+$  ions). Although the  $^{23}\text{Na}$  MAS spectrum of Oxy-1.5 shows spectral separation for  $\text{Na}^+$  ions from different locations, the resolution is insufficient to allow an accurate determination of the exact number of channel  $\text{Na}^+$  ions.

Fig. 2 shows 2D  $^{23}\text{Na}$  multiple-quantum magic-angle spinning (MQMAS) spectra obtained for Oxy-1.5 and 5'-GMP at 11.75 T. The most important feature in the 2D MQMAS spectrum for Oxy-1.5 is the fine spectral feature associated with the channel  $\text{Na}^+$  ions (shown as an expansion in Fig. 2). In particular, the channel  $\text{Na}^+$  ions give rise to two distinct NMR signals with a peak volume ratio of approximately 2:1. In the dimeric structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>), there are four G-quartets stacking on the top of one another, giving rise to three pockets inside the quadruplex channel. The simplest

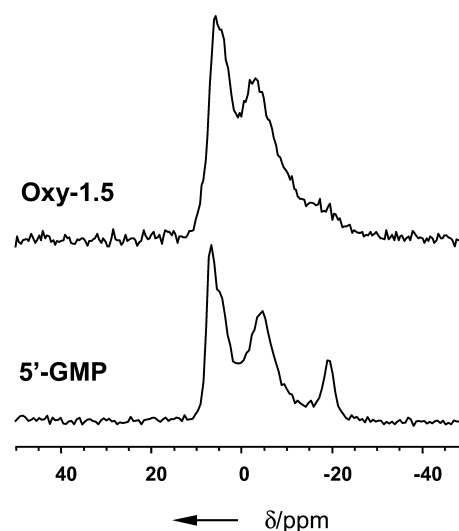


Fig. 1. Solid-state  $^{23}\text{Na}$  MAS spectra of the  $\text{Na}^+$  forms of Oxy-1.5 (upper trace) and 5'-GMP (lower trace). Detailed NMR experimental parameters are as follows. 5'-GMP: 72 transients; recycle time, 5 s, and sample spinning frequency, 8000 Hz. Oxy-1.5: 325 transients; recycle time, 2 s, and sample spinning frequency, 8000 Hz.

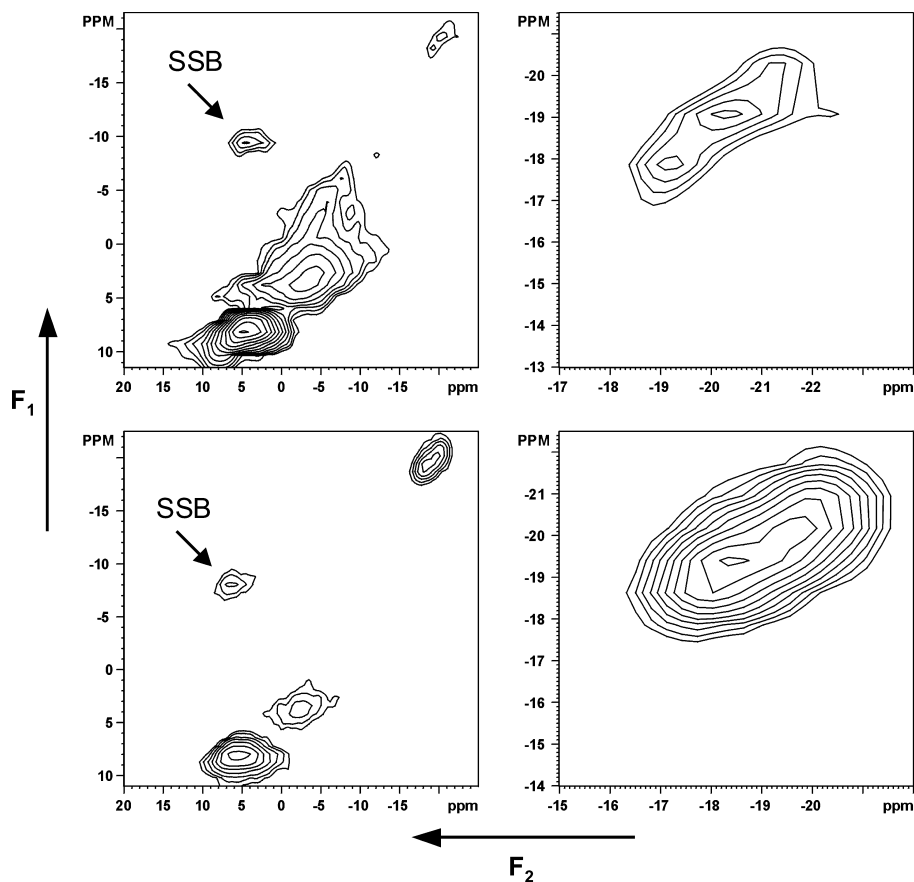


Fig. 2. Two-dimensional  $^{23}\text{Na}$  MQMAS spectra of the  $\text{Na}^+$  forms of Oxy-1.5 (upper) and 5'-GMP (lower). Spectral expansions are shown on the right. Detailed NMR experimental parameters are as follows. Oxy-1.5: 3000 transients for each  $t_1$ ; recycle time, 2 s; 26  $t_1$  increments; sample spinning frequency, 8500 Hz, and total experimental time, 43 h. 5'-GMP: 48 transients for each  $t_1$ ; recycle time, 2 s; 64  $t_1$  increments; sample spinning frequency, 8000 Hz, and total experimental time, 4.3 h. The spinning sidebands are marked as "SSB."

interpretation for the above  $^{23}\text{Na}$  NMR observation is that the signal with the twice intensity can be attributed to two  $\text{Na}^+$  ions in the symmetry-related outer pockets and the less intense signal to a  $\text{Na}^+$  ion in the central pocket. This situation is in analogy to the  $\text{NH}_4^+$  coordination in the  $\text{NH}_4^+$  form of Oxy-1.5 [18]. Analyses of the 1D and 2D spectra yield the following  $^{23}\text{Na}$  NMR parameters for the channel  $\text{Na}^+$  sites in Oxy-1.5: outer

site,  $C_Q = 1.2 \pm 0.2$  MHz,  $\eta_Q = 0.4 \pm 0.2$ , and  $\delta = -19.0 \pm 0.2$  ppm; central site,  $C_Q = 0.9 \pm 0.2$  MHz,  $\eta_Q = 1.0 \pm 0.2$ , and  $\delta = -17.5 \pm 0.2$  ppm. As seen from Table 1, these NMR parameters are comparable to those observed for the channel  $\text{Na}^+$  ions found in other G-quadruplexes. On the basis of the crystal structures for  $[\text{d}(\text{TG}_4\text{T})_4]$  [10] and  $[\text{G1}]_{16}$   $[\text{3Na/Cs Picrate}_4]$  [21], the channel  $\text{Na}^+$  ion is known to coordinate to eight car-

Table 1

A summary of solid-state  $^{23}\text{Na}$  NMR parameters for  $\text{Na}^+$  ions residing inside a G-quadruplex channel

System	$\delta_{\text{iso}}$ (ppm)	$C_Q$ (MHz)	$\eta_Q$	Ref.
$[\text{d}(\text{TG}_4\text{T})_4]$	-19	ND <sup>b</sup>	ND	[21]
5'-GMP	-18	1.1	1.0	[22]
$[\text{G1}]_{16}$ $[\text{3Na/Cs} \cdot \text{Picrate}_4]^{\text{a}}$				[23]
Site 1	-12.8	1.65	0.6	
Site 2	-16.5	1.35	0.8	
Site 3	-15.0	1.70	0.6	
$[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$				This work
Outer site	-19.0	1.2	0.4	
Inner site	-17.5	0.9	1.0	

<sup>a</sup> G1, 5'-*tert*-butyl-dimethylsilyl-2',3'-*O*-isopropylidene guanosine.

<sup>b</sup> Not determined.

bonyl oxygen atoms ( $O_6$ ) from two adjacent G-quartets, having a square anti-prism coordination geometry with an averaged Na–O distance of 2.81 Å.

As also seen from Fig. 2, each of the two signals for the channel  $Na^+$  ions exhibits a line shape parallel to the  $F_2$  axis, indicating that the coordination environment for the channel  $Na^+$  ions is well-defined. In contrast, the  $^{23}Na$  NMR signal for the channel  $Na^+$  ions in 5'-GMP exhibits a diffuse shape along the slope of unity. This latter spectral feature suggests that the channel  $Na^+$  ions in 5'-GMP occupy a distribution of  $Na^+$  sites that have essentially the same value of  $C_Q$  but slightly different chemical shifts. Similarly, the signal from the surface  $Na^+$  ions in Oxy-1.5 exhibits a diffuse line shape, indicating that the chemical environment for the surface  $Na^+$  ions is not homogeneous. It is also noted that the signal for the surface  $Na^+$  ions in Oxy-1.5 shows more diffused features than that for 5'-GMP. This may be due to the fact that Oxy-1.5 has two different types of phosphodiester groups (stacking G-quartets and  $T_4$  loops), whereas 5'-GMP has a mononucleotide.

Another important region where  $Na^+$  ions may potentially occupy is at the mouth of the quadruplex channel inside the  $T_4$  loop region. In the  $K^+$  form of Oxy-1.5, two  $K^+$  ions are located in this region, each coordinating to eight oxygen atoms (four from the  $O_6$  atoms of the end G-quartet, two from the  $O_2$  atoms of the loop thymine residues, and two from water molecules). For the  $Na^+$  form, the only model available is that proposed by Feigon and co-workers [27] where

each of the two outer  $Na^+$  ions is located within the end G-quartet plane also coordinating to the  $O_2$  atom from  $T_7$  in a square-pyramidal fashion. Because we have accumulated some  $^{23}Na$  NMR data on pentacoordinate  $Na^+$  ions in mononucleotides [30], we are confident that in-plane  $Na^+$  ions are not present in Oxy-1.5. Although our solid-state  $^{23}Na$  NMR data does not support Feigon's model, we cannot completely discount the possibility that there may be  $Na^+$  ions residing inside the  $T_4$  loop whose NMR signals are overlapped with those for the surface  $Na^+$  ions. At the present time, the cation binding in the  $Na^+$  form of Oxy-1.5 appears to be identical to that in the  $NH_4^+$  form, despite the fact that the loop conformations for these two forms are known to be different in solution [27]. A similar observation is that, although the  $K^+$  and  $NH_4^+$  forms of Oxy-1.5 have the same topology and loop conformation, they have different cation binding in the loop region [12,27]. It might be possible that factors other than cation binding may be responsible for the formation of different loop structures in different forms of Oxy-1.5. Since different  $T_4$  loop structures are quite common between the  $Na^+$  and  $K^+$  forms of G-quadruplexes [31,32], further investigations are necessary to fully understand this phenomenon.

At this point, it is useful to summarize the currently available information about the mode of cation binding in various forms of Oxy-1.5. As illustrated in Fig. 3, there are two types of  $K^+$  forms of Oxy-1.5 that have been determined by X-ray crystallography. One is a pure  $K^+$  form where five  $K^+$  ions are found to coordinate to the G-quadruplex structure: three inside the channel and two in the  $T_4$  loop regions [12]. Another  $K^+$  form is a Oxy-1.5/drug complex where one of the loop  $K^+$  ions is replaced by a drug molecule [33]. The  $NH_4^+$  form studied by liquid-state  $^{15}N$  NMR [27] and the  $Na^+$  form by solid-state  $^{23}Na$  NMR in the present work have the same mode of cation binding. A very different  $Na^+$  form of Oxy-1.5 was observed in a DNA–protein complex [11]. The crystal structure of the Oxy-1.5/protein complex indicates that the general folding of dimeric Oxy-1.5 structure is similar to those for other forms of Oxy-1.5. However, a significantly different feature found in the Oxy-1.5/protein complex is that the stack of four G-quartets is slightly tilted, as depicted in Fig. 3. This tilting makes it difficult for a  $Na^+$  ion to interact simultaneously with eight  $O_6$  atoms from the adjacent G-quartets. Consequently, all  $Na^+$  ions are located essentially in the G-quartet planes. In addition to the four  $O_6$  atoms from the same G-quartet, each of the two outer  $Na^+$  ions is also coordinated to two  $O_2$  atoms from the loop thymine residues and each of the two inner  $Na^+$  ions to another  $O_6$  atom from the neighboring G-quartet in a square-pyramidal arrangement. On the basis of this new mode of cation binding, we anticipate that this DNA–protein complex would exhibit a

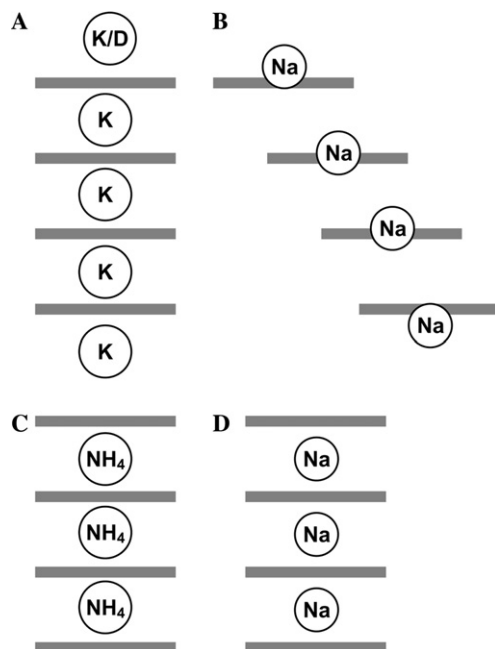


Fig. 3. Illustration of the mode of cation binding in various forms of Oxy-1.5. (A)  $K^+$  form. The top binding site can be occupied by either a  $K^+$  ion or a drug molecule. (B)  $Na^+$  form found in a DNA–protein complex. (C)  $NH_4^+$  form. (D)  $Na^+$  form. See text for discussion.

different cation affinity compared with other forms of Oxy-1.5. It is likely that, for this DNA–protein complex,  $\text{Na}^+$  ions are preferred over  $\text{K}^+$  ions.

Because the solid-state  $^{23}\text{Na}$  data have suggested that the cation binding in the  $\text{Na}^+$  form of Oxy-1.5 is identical to that in the  $\text{NH}_4^+$  form, we decided to further investigate the  $\text{NH}_4^+$  form of Oxy-1.5 using solid-state  $^{15}\text{N}$  NMR. Although  $^{15}\text{NH}_4^+$  has been used as a surrogate probe for studying alkali metal binding, it has not been possible to date to examine the same molecular system in the same physical state using  $^{15}\text{N}$  and alkali metal NMR methods simultaneously. Fig. 4 shows the solid-state  $^{15}\text{N}$  NMR spectra for Oxy-1.5 and 5'-GMP. Similar to the situation in  $^{23}\text{Na}$  NMR, three groups of  $^{15}\text{NH}_4^+$  ions can be identified in the  $^{15}\text{N}$  NMR spectra. The  $^{15}\text{N}$  chemical shift observed for the channel  $\text{NH}_4^+$  ions is in agreement with the solution  $^{15}\text{N}$  NMR data [18]. Unfortunately, the spectral resolution in the solid-state  $^{15}\text{N}$  spectra is insufficient to allow a determination of the number of  $\text{NH}_4^+$  ions inside the quadruplex channel. Here, Oxy-1.5 represents an unusual example where the NMR spectral resolution is actually higher in  $^{23}\text{Na}$  spectra than in  $^{15}\text{N}$  spectra for the same molecular system.

In summary, we have used a solid-state  $^{23}\text{Na}$  NMR approach to determine the exact number and coordination geometry of the  $\text{Na}^+$  ions bound to Oxy-1.5. Our results suggest that three  $\text{Na}^+$  ions are located inside the G-quadruplex channel and that no  $\text{Na}^+$  ions are found in the thymine loop regions. This situation is identical to that observed in the  $\text{NH}_4^+$  form of Oxy-1.5, but different from the  $\text{K}^+$  form. This study illustrates that the  $^{23}\text{Na}$  NMR signal from a single  $\text{Na}^+$  ion in a G-quadruplex of ca. 8 kDa molecular weight can be detected with confidence at a moderate magnetic field strength, 11.75 T. The very high spectral resolution

(less than 1 ppm) observed in the  $^{23}\text{Na}$  MQMAS spectra of Oxy-1.5 strongly suggests that solid-state  $^{23}\text{Na}$  NMR holds great promise to become a practical technique for obtaining detailed information about  $\text{Na}^+$  coordination sites. We are currently applying solid-state  $^{23}\text{Na}$  NMR to even larger biomolecular systems.

## Acknowledgments

This work was supported by research grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada, Queen's University, and the Province of Ontario.

## References

- [1] W. Guschlbauer, J.-F. Chantot, D. Thiele, Four-stranded nucleic acid structures 25 years later: from guanosine gels to telomeric DNA, *J. Biomol. Struct. Dyn.* 8 (1990) 491–511.
- [2] G. Gottarelli, G.P. Spada, A. Garbesi, Self-assembled columnar mesophases based on guanine-related molecules, in: J.-P. Sauvage, M.W. Hosseini (Eds.), *Comprehensive Supramolecular Chemistry*, vol. 9, Elsevier, Rugby, UK, 1996, pp. 483–506.
- [3] D. Sen, W. Gilbert, Guanine quartet structures, *Methods Enzymol.* 211 (1992) 191–199.
- [4] J.R. Williamson, G-quartet structures in telomeric DNA, *Annu. Rev. Biophys. Biomol. Struct.* 23 (1994) 703–730.
- [5] D.E. Gilbert, J. Feigon, Multistranded DNA structures, *Curr. Opin. Struct. Biol.* 9 (1999) 305–314.
- [6] M.A. Keniry, Quadruplex structures in nucleic acids, *Biopolymers* 56 (2001) 123–146.
- [7] S. Neidle, G.N. Parkinson, The structure of telomeric DNA, *Curr. Opin. Struct. Biol.* 13 (2003) 275–283.
- [8] J.T. Davis, G-quartets 40 years later: from 5'-GMP to molecular biology and supramolecular chemistry, *Angew. Chem. Int. Ed.* 43 (2004) 668–698.
- [9] C. Kang, X. Zhang, R. Ratliff, R. Moyzis, A. Rich, Crystal structure of four-stranded *Oxytricha* telomeric DNA, *Nature* 356 (1992) 126–131.
- [10] G. Laughlan, A.I.H. Murchie, D.G. Norman, M.H. Moore, P.C.E. Moody, D.M.J. Lilley, B. Luisi, The high-resolution crystal structure of a parallel-stranded guanine tetraplex, *Science* 265 (1994) 520–524; K. Phillips, Z. Dauter, A.I.H. Murchie, D.M.J. Lilley, B. Luisi, The crystal structure of a parallel-stranded guanine tetraplex at 0.95 Å resolution, *J. Mol. Biol.* 273 (1997) 171–182.
- [11] M.P. Horvath, S.C. Schultz, DNA G-quartets in a 1.86 Å resolution structure of an *Oxytricha nova* telomeric protein–DNA complex, *J. Mol. Biol.* 310 (2001) 367–377.
- [12] S. Haider, G.N. Parkinson, S. Neidle, Crystal structure of the potassium form of an *Oxytricha nova* G-quadruplex, *J. Mol. Biol.* 320 (2002) 189–200.
- [13] G.N. Parkinson, M.P.H. Lee, S. Neidle, Crystal structure of parallel quadruplexes from human telomeric DNA, *Nature* 417 (2002) 876–880.
- [14] F.W. Kotch, J.C. Fettinger, J.T. Davis, A lead-filled G-quadruplex: insight into the G-quartet's selectivity for  $\text{Pb}^{2+}$  over  $\text{K}^+$ , *Org. Lett.* 2 (2000) 3277–3280.
- [15] S.L. Forman, J.C. Fettinger, S. Pieraccini, G. Gottarelli, J.T. Davis, Toward artificial ion channels: a lipophilic G-quadruplex, *J. Am. Chem. Soc.* 122 (2000) 4060–4067.

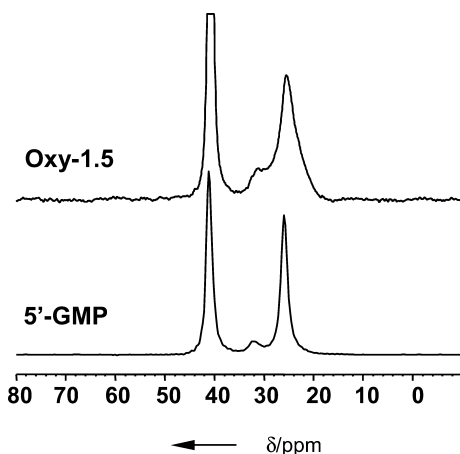


Fig. 4.  $^{15}\text{N}$  CP/MAS spectra of the  $\text{NH}_4^+$  forms of Oxy-1.5 (upper trace) and 5'-GMP (lower trace). Detailed NMR experimental parameters are as follows. Oxy-1.5: 918 transients; recycle time, 10 s; sample spinning frequency, 5000 Hz. 5'-GMP: 1615 transients; recycle time, 10 s; sample spinning frequency, 5000 Hz.

- [16] X. Shi, J.C. Fettinger, J.T. Davis, Homochiral G-quadruplexes with  $\text{Ba}^{2+}$  but not with  $\text{K}^+$ : the cation programs enantiomeric self-recognition, *J. Am. Chem. Soc.* 123 (2001) 6738–6739.
- [17] J. Deng, Y. Xiong, M. Sundaralingam, X-ray analysis of an RNA tetraplex (UGGGGU)<sub>4</sub> with divalent  $\text{Sr}^{2+}$  ions at subatomic resolution (0.61 Å), *Proc. Natl. Acad. Sci. USA* 98 (2001) 13665–13670.
- [18] N.V. Hud, P. Schultze, V. Sklenář, J. Feigon, Binding sites and dynamics of ammonium ions in a telomere repeat DNA quadruplex, *J. Mol. Biol.* 285 (1999) 233–243.
- [19] S. Bazu, A.A. Szewczak, M. Cocco, S.A. Strobel, Direct detection of monovalent metal ion binding to a DNA G-quartet by <sup>205</sup>Tl NMR, *J. Am. Chem. Soc.* 122 (2000) 3240–3241.
- [20] G. Wu, Recent developments in solid state NMR of quadrupolar nuclei and applications to biological systems, *Biochem. Cell Biol.* 76 (1998) 429–442.
- [21] D. Rovnyak, M. Baldus, G. Wu, N.V. Hud, J. Feigon, R.G. Griffin, Localization of <sup>23</sup>Na<sup>+</sup> in a DNA quadruplex by high-field solid-state NMR, *J. Am. Chem. Soc.* 122 (2000) 11423–11429.
- [22] G. Wu, A. Wong, Direct detection of the bound sodium ions in self-assembled 5'-GMP gels: a solid-state <sup>23</sup>Na NMR approach, *Chem. Comm.* (2001) 2658–2659.
- [23] A. Wong, J.C. Fettinger, S.L. Forman, J.T. Davis, G. Wu, The sodium ions inside a lipophilic G-quadruplex channel as probed by solid-state <sup>23</sup>Na NMR, *J. Am. Chem. Soc.* 124 (2002) 742–743.
- [24] G. Wu, A. Wong, Z. Gan, J.T. Davis, Direct detection of 9potassium cations bound to G-quadruplex structures by solid-state <sup>39</sup>K NMR at 19.6 T, *J. Am. Chem. Soc.* 125 (2003) 7182–7183.
- [25] A. Wong, G. Wu, Selective binding of monovalent cations to the stacking G-quartet structure formed by guanosine 5'-monophosphate: a solid-state NMR study, *J. Am. Chem. Soc.* 125 (2003) 13895–13905.
- [26] F.W. Smith, J. Feigon, Quadruplex structure of *Oxytricha* telomeric DNA oligonucleotides, *Nature* 356 (1992) 164–168.
- [27] P. Schultze, N.V. Hud, F.W. Smith, J. Feigon, The effect of sodium, potassium and ammonium ions on the conformation of the dimeric quadruplex formed by the *Oxytricha nova* telomere repeat oligonucleotide d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>), *Nucleic Acids Res.* 27 (1999) 3018–3028.
- [28] L. Frydman, J.S. Harwood, Isotropic spectra of half-integer quadrupolar spins from bidimensional magic-angle spinning NMR, *J. Am. Chem. Soc.* 117 (1995) 5367–5368.
- [29] J.-P. Amoureux, C. Fernandez, S. Steuernagel, *J. Magn. Reson. Ser. A* 123 (1996) 116–118.
- [30] A. Wong, G. Wu, Characterization of the pentacoordinate sodium ions in hydrated nucleoside 5'-phosphates by solid-state <sup>23</sup>Na NMR and quantum mechanical calculations, *J. Phys. Chem. A* 107 (2003) 579–586.
- [31] M.A. Keniry, G.D. Strahan, E.A. Owen, R.H. Shafer, Solution structure of the Na<sup>+</sup> form of the dimeric guanine quadruplex [d(G<sub>3</sub>T<sub>4</sub>G<sub>3</sub>)]<sub>2</sub>, *Eur. J. Biochem.* 233 (1995) 631–643.
- [32] G.D. Strahan, M.A. Keniry, R.H. Shafer, NMR structure refinement and dynamics of the K<sup>+</sup>-[d(G<sub>3</sub>T<sub>4</sub>G<sub>3</sub>)]<sub>2</sub> quadruplex via particle mesh Ewald molecular dynamics simulations, *Biophys. J.* 75 (1998) 968–981.
- [33] S.M. Haider, G.N. Parkinson, S. Neidle, Structure of a G-quadruplex–ligand complex, *J. Mol. Biol.* 326 (2003) 117–125.