

# A comparison of the association of spermine with duplex and quadruplex DNA by NMR

Max A. Keniry\*

*Research School of Chemistry, The Australian National University, Canberra, ACT 0200, Australia*

Received 7 March 2003; revised 7 March 2003; accepted 8 March 2003

First published online 17 April 2003

Edited by Hans Eklund

**Abstract** The association of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  ( $[1',1''\text{-}^{13}\text{C}_2]\text{-}N,N'\text{-bis(3-aminopropyl)-1,4-butanediamine}$ ) with duplex and quadruplex DNA has been studied by nuclear magnetic resonance spectroscopy. 1D NOESY experiments using two-way selective cross-polarization (ISI-SCP-NOESY) showed spermine intramolecular NOEs are either weakly positive or weakly negative when spermine is complexed to duplex B-DNA and linear four-stranded quadruplex DNA. In contrast, large negative intramolecular NOEs are observed when spermine is complexed to two distinct forms of folded quadruplex DNA suggesting greater immobilization of spermine on these folded DNA quadruplexes. No changes in the quadruplex stem structure are observed but there are minor changes to the loop structure of a two-stranded folded quadruplex on binding spermine.

© 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

**Key words:** DNA;  $^1\text{H}$  nuclear magnetic resonance; Nucleic acid; Spermine; Quadruplex

## 1. Introduction

The natural polyamines, spermine, spermidine and putrescine, are present in millimolar concentrations in all living cells [1]. They have essential roles in cell differentiation, proliferation (reviewed in [2]), and, surprisingly, in apoptosis [3]. Indeed, the absolute requirement for cell growth and evidence of enhanced polyamine concentrations in tumor cells [4] suggests polyamine metabolism and function may be a possible target for antineoplastic intervention [5].

The polyamines are highly charged, organic polycations at physiological pH. Hence, they are attracted to nucleic acids and anionic patches on proteins. That polyamines bind to DNA is not disputed, but there has been considerable debate over the specificity of the interaction with DNA and the influence of polyamines over the structure of DNA [6]. To date, no X-ray crystallographic or NMR study of the interaction of spermine with DNA has unequivocally located a specific interaction between spermine and B-DNA. There is abundant crystallographic evidence that spermine and its analogs bind to A- and Z-DNA in a variety of different locations [7–10].

Several nuclear magnetic resonance (NMR) studies confirm

the mobile nature of the interaction of spermine and DNA [11–14]. Initial work with the Dickerson–Drew dodecamer revealed spermine had motion that was effectively independent of the dynamics of the oligonucleotide [12]. Later work by Banville et al. [13] confirmed this finding for a small hexanucleotide, which also assumed the B-conformation, but more restricted motion was observed when spermine bound to a hexanucleotide with a strong tendency to assume the Z-conformation. No intramolecular nuclear Overhauser effects (NOEs) between spermine and either hexanucleotide were observed. These observations were confirmed by van Dam and Nordenskiöld [14].

The aim of this present work is to investigate and compare the interaction and dynamics of spermine with duplex and quadruplex DNA. Selectively  $^{13}\text{C}$ -labeled spermine was used to probe the mobility of spermine when bound to duplex and quadruplex DNA, compare the intramolecular NOEs between the bound spermine in complex with duplex and quadruplex DNA and search for intermolecular NOEs between spermine and DNA. We have studied complexes of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  ( $[1',1''\text{-}^{13}\text{C}_2]\text{-}N,N'\text{-bis(3-aminopropyl)-1,4-butanediamine}$ ) with a self-complementary hexamer, a complementary trisdecannucleotide, a linear four-stranded quadruplex, a two-stranded diagonally looped folded quadruplex and a single-stranded folded quadruplex. Intramolecular  $^1\text{H}$  NOEs confirm that the interaction of spermine with B-DNA is weak but detectable, whereas the interaction with folded DNA quadruplex is stronger.

## 2. Materials and methods

### 2.1. Oligonucleotide synthesis and purification

The oligonucleotides  $\text{d(G}_4\text{T}_4\text{G}_4)$ ,  $\text{d(ATGCAT)}$ ,  $\text{d(C}_2\text{TGTGGA-TA}_2\text{CA)}$  and  $\text{d(TGT}_2\text{ATCCACAG}_2)$  were synthesized by a cyanophosphoramidite method on an Applied Biosystems 394 DNA synthesizer.  $\text{d(TG}_4\text{T)}$  and  $\text{d(G}_2\text{T}_2\text{G}_2\text{TGTG}_2\text{T}_2\text{G}_2)$  were purchased from Geneworks (Adelaide, Australia). All oligonucleotides were purified by anion exchange high performance liquid chromatography and dialyzed against water over 24 h with one change of the dialysate. The solutions were lyophilized and redissolved in 10 mM phosphate, 50 mM NaCl, pH 7  $\text{D}_2\text{O}$  buffer or 10%  $\text{D}_2\text{O}/90\%$   $\text{H}_2\text{O}$  buffer except for the thrombin aptamer,  $\text{d(G}_2\text{T}_2\text{G}_2\text{TGTG}_2\text{T}_2\text{G}_2)$ , which was dissolved in 10 mM phosphate, 50 mM KCl, pH 7  $\text{D}_2\text{O}$  buffer.

### 2.2. $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$ synthesis

$[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  was synthesized from 1,4-diaminobutane (Fluka) and  $[3\text{-}^{13}\text{C}]\text{acrylonitrile}$  (Isotec) following the method of Israel et al. [15]. The molecular structure of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  and the labeling scheme are shown in Fig. 1A (the atom notation scheme of van Dam and Nordenskiöld [14] was adopted). The two symmetrical  $^{13}\text{C}$ -labeled sites are indicated with asterisks.

\*Fax: (61)-2-6125 0750.

E-mail address: max@rsc.anu.edu.au (M.A. Keniry).

**Abbreviations:** NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy

### 2.3. $[1',1''^{13}\text{C}_2]$ Spermine–DNA complex formation

It is desirable to investigate the NMR spectra of spermine–DNA complexes at concentrations where there is little or no aggregation of the complex and where most of the spermine is associated with the DNA. Accordingly, all the complexes were 1 mM or less in DNA and had a stoichiometry as near as possible to 1:1  $[1',1''^{13}\text{C}_2]$ spermine:DNA structure. All DNA duplexes and quadruplexes were preformed prior to adding  $[1',1''^{13}\text{C}_2]$ spermine as described previously [16,17] and the  $[1',1''^{13}\text{C}_2]$ spermine titrated from a 20 mg/ml stock solution.

### 2.4. Nuclear magnetic resonance

All NMR spectra were recorded on a Varian INOVA NMR spectrometer at a  $^1\text{H}$  frequency of 600 MHz and at 20°C unless otherwise indicated. One-dimensional selective  $^1\text{H}$  NOE experiments were acquired using a selective two-way cross-polarization NOE experiment (ISI-SCP-NOESY) [18]. The cross-polarization time was set to 7.7 ms ( $1/J_{\text{CH}}$ ) and the RF field was set to approximately 60 Hz and was optimized manually by varying the carbon field strength until maximum signal of the  $\alpha_{2''}$  protons was obtained. The NOE mixing time was either 80 ms, 500 ms or 1 s and the recycle time was 3.1 s.

## 3. Results

A number of NMR studies of complexes of unlabeled spermine and duplex DNA have investigated a specific binding site for spermine on DNA by probing intermolecular  $^1\text{H}$  NOEs between spermine and DNA [12–14]. This is possible in the case of duplex DNA because the resonance peaks of the  $\alpha$  protons of spermine appear in a window of the  $^1\text{H}$  NMR spectrum where there are no DNA resonances. Hence, it is possible, in principle, by either low power saturation transfer or 2D NOESY techniques, to identify, unequivocally, intermolecular  $^1\text{H}$  NOEs between the  $\alpha$  protons of spermine and DNA. There are two problems with this strategy. First, it limits the detection of  $^1\text{H}$  NOEs between spermine and DNA to only certain conformations of DNA, that have no  $^1\text{H}$  nucleotide resonances near  $\sim 3$  ppm. Secondly, because all three types of  $\alpha$  proton ( $\alpha_1$ ,  $\alpha_{2'}$  and  $\alpha_{2''}$ ) are excited or are poorly resolved, these NMR techniques reveal little about the

proximity of the individual  $\alpha$  protons to the  $\beta$  and  $\gamma$  protons of spermine and/or the relative mobilities of the  $\alpha$ ,  $\beta$  and  $\gamma$  protons. Fig. 1B shows a  $^1\text{H}$  spectrum of  $[1',1''^{13}\text{C}_2]$ spermine and the folded quadruplex,  $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$ , showing the position of the  $\alpha$ ,  $\beta$  and  $\gamma$  protons of  $[1',1''^{13}\text{C}_2]$ spermine. The asterisks indicate the location of the  $^{13}\text{C}$  satellites of the  $\alpha_{2''}$  resonances. Inspection of the region around 3 ppm illustrates the problems of spectral overlap that make strategies employing only homonuclear NMR techniques to study spermine–quadruplex interactions ineffective in the case of folded DNA quadruplexes.

### 3.1. Intramolecular $^1\text{H}$ – $^1\text{H}$ NOE measurements of

#### $[1',1''^{13}\text{C}_2]$ spermine bound to duplex and quadruplex DNA

Fig. 2 shows the 1D NOESY spectra of complexes between  $[1',1''^{13}\text{C}_2]$ spermine and different forms of duplex and quadruplex DNA. In each spectrum, the selectively excited  $\alpha_{2''}$  peak (3.1 ppm) is scaled to the same relative intensity for each spectrum (four times the size displayed in Fig. 2) so that the relative NOE intensities are comparable. Fig. 2A shows the expected positive NOE between the  $\alpha_{2''}$  protons and the  $\gamma$  protons (2.1 ppm) of  $[1',1''^{13}\text{C}_2]$ spermine (a positive NOE has the opposite sign of the selectively excited resonance and a negative NOE has the same sign). As expected, the NOE to the  $\gamma$  protons is larger than the NOE to the  $\beta$  protons (1.8 ppm) because the  $\gamma$  methylene protons are closer to the  $\alpha_{2''}$  methylene protons than are the  $\beta$  methylene protons in the low energy all-*trans* conformation. This emphasizes an advantage of this work over previous studies in which all of the  $\alpha$  protons were irradiated at the same time and hence no meaningful information regarding the relative dynamics and distances between the  $\alpha$ ,  $\beta$  and  $\gamma$  protons of spermine could be deduced.

A positive NOE is also observed between the  $\alpha_{2''}$  and  $\gamma$  methylene protons when spermine is mixed with the small self-complementary duplex,  $\text{d}(\text{ATGCAT})_2$  (Fig. 2B), confirming the results of previous studies of spermine with small,

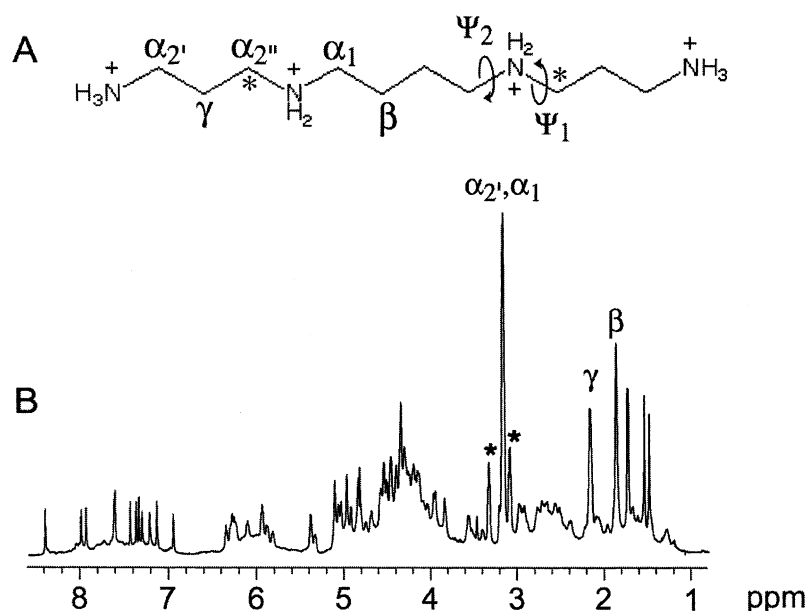


Fig. 1. A: Diagrammatic representation of  $[1',1''^{13}\text{C}_2]$ spermine depicting the location of the  $\alpha$ ,  $\beta$  and  $\gamma$  carbons and the  $^{13}\text{C}$  label (indicated by an asterisk). B:  $^1\text{H}$  NMR spectrum of a 1:1 complex of  $[1',1''^{13}\text{C}_2]$ spermine: $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$ .

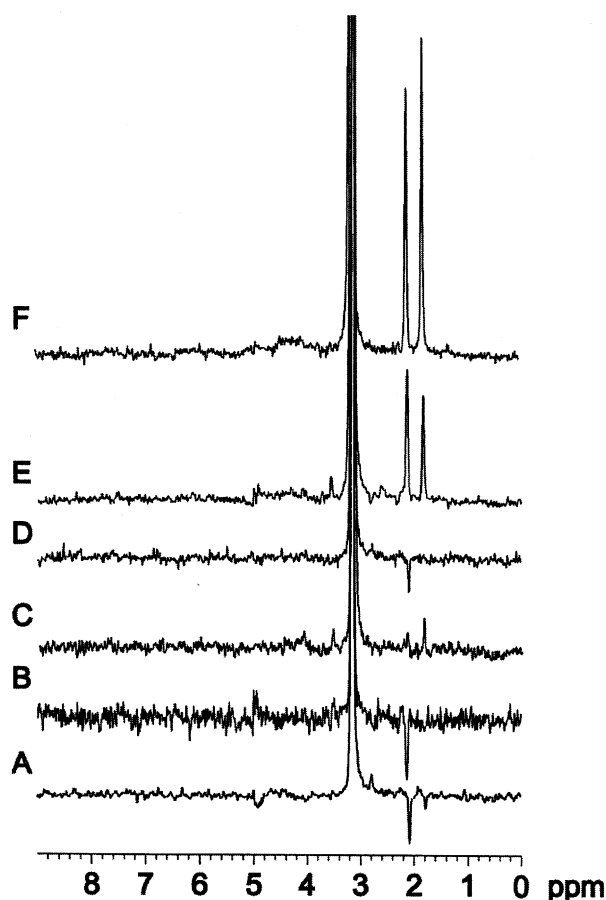


Fig. 2. ISI-SCP-NOESY experiments at 600 MHz of (A) 4 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$ , (B) 0.8 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine-d(ATGCAT)}_2$ , (C) 1 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine-d(C}_2\text{TGTG}_2\text{ATA}_2\text{CA)-d(TGT}_2\text{ATC}_2\text{ACAG}_2\text{)}$ , (D) 1 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine-[d(TG}_4\text{T)}_4]$ , (E) 1 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine-d(G}_2\text{T}_2\text{G}_2\text{TGTG}_2\text{T}_2\text{G}_2\text{)}$ , (F) 1 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine-[d(G}_4\text{T}_4\text{G}_4)]_2$ . The selectively excited peak has been cut and is actually four times the size shown in the figure. The mixing time was 0.5 s, the recycle time 3.5 s and the data collection time 16–20 h for each experiment.

B-form duplex DNA [12]. The interaction of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  with a much larger complementary B-form duplex (henceforth referred to as the 13mer), encompassing the initiation site of the dnaA protein [19], displayed small negative NOEs to the  $\beta$  and  $\gamma$  protons (Fig. 2C). This also confirms earlier work with larger duplex DNA [14] and shows that spermine does interact weakly with B-DNA and that the size and sign of the NOE is dependent on the size of the interacting duplex as expected of a complex of two species in fast exchange. The 1D NOESY spectrum of the complex of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  and a small linear, four-stranded quadruplex,  $[\text{d(TG}_4\text{T)}_4]$  [20] (Fig. 2D), is similar to that of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  and the hexamer (Fig. 2B) displaying a positive NOE between the  $\alpha$  and  $\gamma$  protons indicative of a weak interaction between spermine and this form of quadruplex.

The most unexpected result in this work comes from the interaction of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  with folded DNA quadruplexes that contain thymine loop structures. In the prototypical folded quadruplex,  $[\text{d(G}_4\text{T}_4\text{G}_4)]_2$ , there are two identical diagonal  $\text{T}_4$  loops [21], whereas in the thrombin aptamer,  $\text{d(G}_2\text{T}_2\text{G}_2\text{TGTG}_2\text{T}_2\text{G}_2\text{)}$ , there are two lateral  $\text{T}_2$  loops and a central diagonal TGT loop [22,23]. In both cases,  $[1',1''\text{-}$

$^{13}\text{C}_2]\text{spermine}$  displays strong negative intramolecular NOEs between the  $\alpha_{2'}$  protons and the  $\beta$  and  $\gamma$  protons (Fig. 2E,F). The magnitudes of these interactions are comparable, indicative of a change in the time-averaged relative distance between these protons and/or an immobilization of the  $\beta$  protons relative to the  $\alpha$  and  $\gamma$  protons. The relative intensities of the  $\beta$  and  $\gamma$  peaks are little changed at an 80 ms mixing time indicating that spin diffusion is not significant at the longer mixing time.

### 3.2. Intermolecular NOEs between $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$ and duplex and quadruplex DNA

Despite several NOE studies of the interaction of spermine with duplex DNA, no clear evidence of intermolecular NOEs between spermine and DNA has ever been presented. In accord with these observations, no evidence of any specific intermolecular NOEs was observed in spectra 2B–F. We further investigated the complex of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  and  $[\text{d(G}_4\text{T}_4\text{G}_4)]_2$  by recording a 1D NOE spectrum at longer mixing time (1 s) and lower temperature ( $4^\circ\text{C}$ ). Under these conditions, there is definite broad component in the 4', 5' and 5'' region of the spectrum (Fig. 3B) but no evidence of NOEs to protons connected to the nucleotide bases such as the thymine methyl protons. A spectrum of  $[\text{d(G}_4\text{T}_4\text{G}_4)]_2$  is shown in Fig. 3A for comparison.

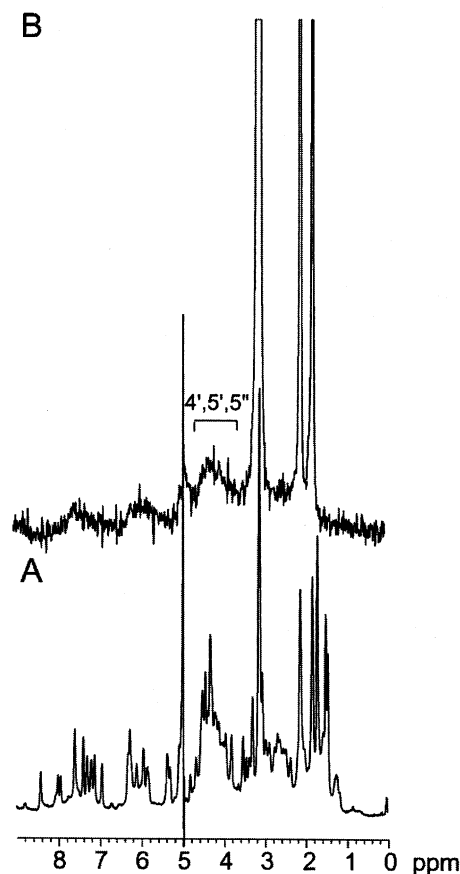


Fig. 3. A:  $^1\text{H}$  spectrum of  $[\text{d(G}_4\text{T}_4\text{G}_4)]_2$  in 10 mM phosphate, 50 mM NaCl, pH 7  $\text{D}_2\text{O}$  buffer. B: ISI-SCP-NOESY experiment of 1 mM  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine-[d(G}_4\text{T}_4\text{G}_4)]_2$  at  $4^\circ\text{C}$ . The mixing time was 1.0 s, the recycle time 5 s and the data collection time 48 h.

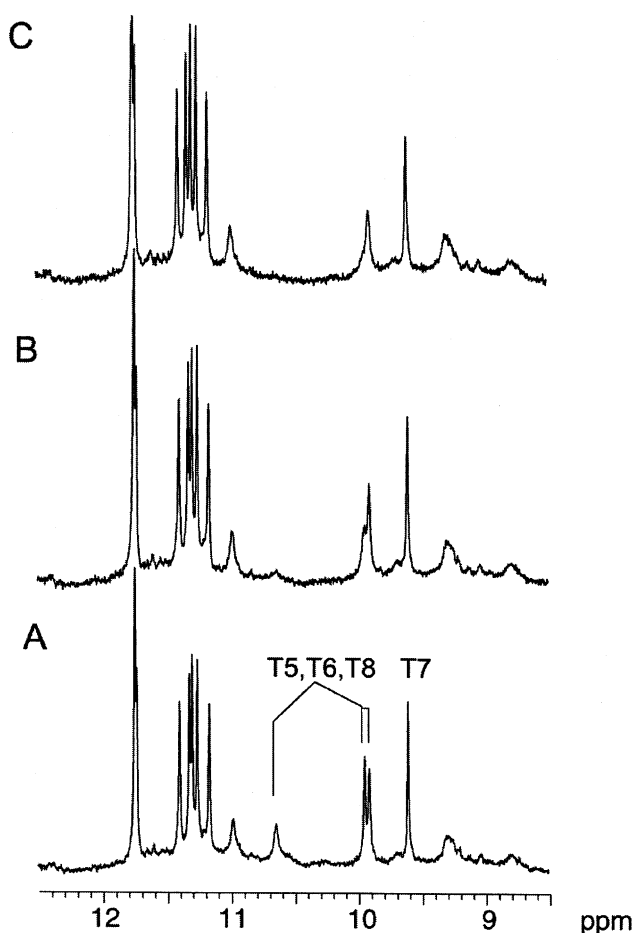


Fig. 4. Low field region of the 600 MHz  $^1\text{H}$  spectrum of 1 mM  $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$  (A) in the absence of spermine and (B,C) at molar ratios of 0.5 spermine: $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$  and 1.0 spermine: $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$  respectively.

### 3.3. The effect of $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$ on the quadruplex $^1\text{H}$ NMR spectrum

We further investigated the interaction of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  with the folded quadruplex,  $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$ , by detailed analysis of the 1D spectrum and 2D NOESY spectrum of the non-exchangeable protons of  $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$ . No substantial change was observed in the chemical shift of any of the non-exchangeable protons on adding  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  (data not shown). Detailed analysis of the base proton  $\text{H}1'$  fingerprint region of the 2D NOESY spectrum of the  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$ – $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$  complex showed that the structure of the  $\text{G}_4$  stem region of the quadruplex is unchanged. Because the concentration of the samples was only 1 mM in quadruplex, it was difficult to determine whether there were any changes to the chemical shift, linewidth or NOE pattern at the thymine resonances that might be indicative of a change in the loop structure.

We further investigated the possibility of detecting some spermine-induced changes in the nucleotide spectra by recording the 1D spectrum of the  $[\text{d}(\text{G}_4\text{T}_4\text{G}_4)]_2$  quadruplex in 90%  $\text{H}_2\text{O}/10\% \text{D}_2\text{O}$ . The sharp resonances in the region 11.1–11.9 ppm (Fig. 4A) are from the guanine imino protons in the quadruplex stem and the broad resonances upfield of 11.1 ppm are from slowly exchanging thymine imino protons in the loop and guanine amino protons from the stem [24]. On

titration of  $[1',1''\text{-}^{13}\text{C}_2]\text{spermine}$  into the quadruplex solution, all the exchangeable proton resonances are broadened but two of the thymine imino proton resonances are broadened to a much greater extent than the other resonances (10.7 and 10.0 ppm in Fig. 4B,C). Only one of the thymine imino protons has been unequivocally assigned [24]; the other three, two of which are broadened, were not previously assigned. Since T7 is hydrogen-bonded to T5 [17], the two broadened resonances are likely to be T6 and T8, which are located at the apex of the loop and which have been observed to have an altered conformation in quadruplexes formed around  $\text{Na}^+$  and  $\text{K}^+$  ions, respectively [25,26].

## 4. Discussion

Spermine is regularly used as an agent to assist in the crystallization of oligonucleotides. The precise location of spermine and its analogs in these crystal structures has only been located in a handful of cases, nevertheless, they appear to occupy a diverse range of different sites from the floor of the major groove [10], to minor groove binding sites [9] to linkers of complementary or neighboring strands [7]. This combination of irregular binding sites and substantial mobility makes spermine difficult to precisely locate in many X-ray crystal structures. The best-defined spermine or spermine analogs are found in the crystal structures of Z-DNA [7–9]. The most favored locations place the charged amino groups of spermine near the nucleotide phosphate groups frequently making intermolecular contacts between DNA duplexes [7,27].

There have been a number of NMR studies of complexes of spermine and DNA over the years and none have identified a specific binding site for spermine on any structural form of DNA [12–14]. The degree of association, in the thermodynamic sense, does vary depending on the DNA structure. In all investigations of spermine bound to B-DNA, spermine was found to be only loosely associated with the DNA with an overall correlation time less than 0.3 ns, much faster than the correlation time of the DNA, which was of the order 2–5 ns. Similar results are seen in this work for the hexamer, 13mer and the linear quadruplex. Greater immobilization of spermine was observed on binding to oligonucleotides capable of assuming the Z-conformation [13,14]. Not only does spermine encourage the B- to Z-transition but spermine is sufficiently immobilized to exhibit negative intramolecular NOEs between the  $\alpha$  protons and the  $\beta$  and  $\gamma$  protons. We observe similar large intramolecular negative NOEs between the spermine methylene protons in complexes with the two folded DNA quadruplexes. The effective correlation time of spermine bound to the folded quadruplexes is much longer than 0.3 ns, suggesting a longer residence time at the spermine binding sites on the folded quadruplexes compared to the corresponding sites on duplex DNA and the linear quadruplex. There is, however, no evidence that spermine substantially changes the conformation of the folded quadruplexes. It is tempting to assume that spermine might be associated with the thymine loop structures, since spermine is only loosely associated with the linear quadruplex, which does not possess a thymine loop. Support for this view comes from an analysis of yeast DNA where a clear preference of spermine binding at thymine residues was observed [28]. Further analysis revealed that spermine binding to runs of thymine was influenced by the sec-



ondary structure of the DNA and not just a preference for the thymine base [28]. However, linear and folded quadruplexes are not only distinguished by the absence or presence of secondary structures made up of thymine residues but are also distinguished by the width of the grooves. The linear quadruplex, [d(TG<sub>4</sub>T)]<sub>4</sub>, has four grooves of identical width [20]. The folded quadruplexes have three distinct grooves, one wide, two of medium width and one narrow groove [29]. The latter groove is a preferred binding site for multivalent cations [30]. The degree of association of spermine with DNA is known to be sensitive to the width of the DNA groove and the close proximity of interstrand phosphate groups is suggested as the major reason for the relatively tight association of spermine with Z-DNA [14]. In the absence of any specific intermolecular NOEs, it is difficult to assign a preferred binding site for spermine on the folded quadruplexes except to say the hydrogen bonding networks in the loops are sensitive to spermine binding.

The correspondence of the distance between the charged groups on polyamines and the distance between the phosphate groups in DNA has long been recognized [31]. One of the earliest models suggested that each aminopropyl group is associated with the backbone on a single strand and the diaminobutyl group might be capable of spanning the minor groove [31]. The presence of a broad band of resonances in the 4',5'5'' region of the spectrum in the long mixing time NOE experiment at low temperatures is suggestive of a non-specific location near the phosphate groups for the  $\alpha_{2''}$  methylene of spermine. This is certainly consistent with a backbone location for the aminopropyl groups of spermine and certainly argues against a location either entirely in the grooves of the quadruplex stem or near any of the thymine bases in the loop. In the latter case, a NOE to the thymine methyl groups would be expected.

Selective enrichment at only one of the  $\alpha$  carbon sites permits a qualitative comparison of the relative orientation and/or mobility of the  $\beta$  and  $\gamma$  methylenes with respect to the  $\alpha_{2''}$  methylene. The most energetically stable conformation of spermine in isolation is the all-*trans* conformation. In this conformation, the distance between the center of mass of the  $\alpha_{2''}$  methylene protons and the center of mass of the  $\beta$  and  $\gamma$  methylene protons is 0.47 nm and 0.31 nm respectively. The NOE pattern of Fig. 2A is in qualitative agreement with the all-*trans* conformation being dominant for uncomplexed spermine. The conformation of spermine complexed to the two folded quadruplexes is substantially different. The NOE between the  $\alpha_{2''}$  methylene protons and the  $\beta$  and  $\gamma$  methylene protons is approximately the same. This means either one or both of the C–N bonds between the  $\alpha_{2''}$  and  $\beta$  methylenes (designated by angles of rotation  $\Psi_1$  and  $\Psi_2$  in Fig. 1A) are in the gauche conformational state as proposed by Liquori et al. [31] (6801) and/or the diaminobutyl group containing the  $\beta$  methylene is more immobile than the aminopropyl group containing the  $\alpha$  and  $\gamma$  methylenes. Unfortunately, the data cannot conclusively confirm whether spermine changes conformation so that the tetramethylene can span the narrow groove of the quadruplex or whether spermine is simply assuming a conformation to match the fold of the backbone of the quadruplex loop. It should be noted that the loop conformations in the two folded quadruplexes are substantially different and it is unlikely that spermine would assume similar conformations on binding to these loops.

This work further confirms that spermine does not interact indiscriminately with DNA, but rather, the binding is sensitive to the conformation of the DNA. This finding may have important implications for our understanding of gene expression particularly with regard to the recent evidence for a G-quadruplex in the promoter region of the *c-myc* oncogene [32]. *c-myc* is a transactivator of ornithine decarboxylase [33], which is a central enzyme in the biosynthetic pathway of polyamines. Both *c-myc* and ornithine decarboxylase also have important roles in cell proliferation and apoptosis [34] which are known to be sensitive to cellular polyamine levels.

**Acknowledgements:** This work benefited from the use of NMR facilities at the Australian National University. The author wishes to thank Rob Longmore for the synthesis of the [<sup>13</sup>C]spermine and gratefully acknowledges the assistance of Justin Coughlin in the early stages of this project and Prof. Rod Rickards for a critical reading of the manuscript.

## References

- [1] Tabor, C.W. and Tabor, H. (1984) *Annu. Rev. Biochem.* 53, 749–790.
- [2] Thomas, T. and Thomas, T.J. (2001) *Cell. Mol. Life Sci.* 58, 244–258.
- [3] Brüne, B., Hartzell, P., Nicotera, P. and Orrenius, S. (1991) *Exp. Cell Res.* 195, 323–329.
- [4] Russel, D.H. (1978) *Prog. Cancer Res. Ther.* 8, 157–165.
- [5] Marton, L.J. and Pegg, A.E. (1995) *Annu. Rev. Pharmacol. Toxicol.* 35, 55–91.
- [6] Korolev, N., Lyubartsev, A.P., Nordenskiöld, L. and Laaksonen, A. (2001) *J. Mol. Biol.* 308, 907–917.
- [7] Ohishi, H., Terasoma, N., Nakanishi, I., van der Marel, G., Van Boom, J.H., Rich, A., Wang, A.H.J., Hakoshima, T. and Tomita, K. (1996) *FEBS Lett.* 398, 291–296.
- [8] Ohishi, H., Suzuki, K., Ohtsuchi, M., Hakoshima, T. and Rich, A. (2002) *FEBS Lett.* 523, 1–6.
- [9] Bancroft, D.L., Williams, D., Rich, A. and Egli, M. (1994) *Biochemistry* 33, 1073–1086.
- [10] Jain, S., Zon, G. and Sundaralingam, M. (1989) *Biochemistry* 28, 2360–2364.
- [11] Andreasson, B., Nordenskiöld, L. and Braunlin, W.H. (1996) *Biopolymers* 38, 505–513.
- [12] Wemmer, D.E., Srivenugopal, K.S., Reid, B.R. and Morris, D.R. (1985) *J. Mol. Biol.* 185, 457–459.
- [13] Banville, D.L., Feuerstein, B.G. and Shafer, R.H. (1991) *J. Mol. Biol.* 219, 585–590.
- [14] Van Dam, L. and Nordenskiöld, L. (1999) *Biopolymers* 49, 41–53.
- [15] Israel, M., Rosenfeld, J.S. and Modest, E.J. (1964) *J. Mol. Biol.* 7, 710–716.
- [16] Strahan, G.D., Shafer, R.H. and Keniry, M.A. (1994) *Nucleic Acids Res.* 22, 5447–5455.
- [17] Keniry, M.A., Strahan, G.D., Owen, E.A. and Shafer, R.H. (1995) *Eur. J. Biochem.* 233, 631–643.
- [18] Chiarparin, E., Pelulessy, P., Cutting, B., Eykyn, T.R. and Bodenhausen, G. (1999) *J. Biomol. NMR* 13, 61–65.
- [19] Gray, B.N., Owen, E.A. and Keniry, M.A. (1994) *Eur. J. Biochem.* 226, 115–124.
- [20] Phillips, K., Dauter, Z., Murchie, A.I.H., Lilley, D.M.J. and Luisi, B. (1997) *J. Mol. Biol.* 273, 171–182.
- [21] Schultze, P., Smith, F.W. and Feigon, J. (1994) *Structure* 2, 221–233.
- [22] Schultze, P., Macaya, R.F. and Feigon, J. (1994) *J. Mol. Biol.* 235, 1532–1547.
- [23] Wang, K.Y., Krawczyk, S.H., Bischofberger, N., Swaminathan, S. and Bolton, P.H. (1993) *Biochemistry* 32, 11285–11292.
- [24] Smith, F.W. and Feigon, J. (1993) *Biochemistry* 32, 8682–8692.
- [25] Strahan, G.D., Keniry, M.A. and Shafer, R.H. (1998) *Biophys. J.* 75, 968–981.
- [26] Schultze, P., Hud, N.V., Smith, F.W. and Feigon, J. (1999) *Nucleic Acids Res.* 27, 3018–3028.

- [27] Wang, A.H.J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., Van Boom, J.H. and Rich, A. (1979) *Nature* 282, 680–686.
- [28] Xiao, L., Swank, R.A. and Matthews, H.R. (1991) *Nucleic Acids Res.* 19, 3701–3708.
- [29] Smith, F.W. and Feigon, J. (1992) *Nature* 356, 164–168.
- [30] Wang, K.Y., Gerena, L., Swaminathan, S. and Bolton, P.H. (1995) *Nucleic Acids Res.* 23, 844–848.
- [31] Liquori, A.M., Constantino, L., Crescenzi, V., Elia, V., Gigli, E., Puliti, R., De Santis Savino, M. and Vitagliano, V. (1967) *J. Mol. Biol.* 24, 113–122.
- [32] Siddiqui-Jain, A., Grand, C.L., Bearss, D.J. and Hurley, L.H. (2002) *Proc. Natl. Acad. Sci. USA* 99, 11593–11598.
- [33] Packham, G. and Cleveland, J.L. (1994) *Mol. Cell. Biol.* 14, 5741–5747.
- [34] Grassilli, E., Desiderio, M.A., Bellesia, E., Salomoni, P. and Franceschi, C. (1995) *Biochem. Biophys. Res. Commun.* 216, 708–714.