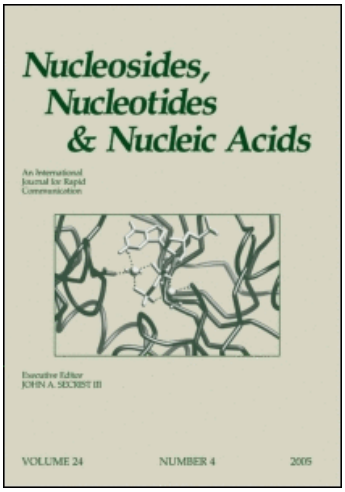

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NMR STUDY OF AMMONIUM ION BINDING TO $d[G_3T_4G_4]_2$ AND $d[G_4(T_4G_4)_3]$ G-QUADRUPLEXES

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□ *Quantitative NMR study has shown a significant difference in affinity of $^{15}NH_4^+$ ions for cation binding sites within G-quadruplexes adopted by $d[G_3T_4G_4]_2$ and $d[G_4(T_4G_4)_3]$.*

Keywords G-Quadruplex; ammonium ion; NMR; binding constants

INTRODUCTION

G-rich DNA regions are abundant throughout the genome of most organisms.[1] Oligonucleotides with repeating G-tracts have been found to fold into G-quadruplex structures.[2,3] Their basic structural element is a G-quartet, which is held together by eight hydrogen bonds. Electrostatic repulsions of four carbonyl oxygen atoms are shielded by cations (Figure 1a). Recently, considerable efforts have been made to identify cation binding sites inside G-quadruplexes by NMR in solution.[4–8]

We herein focus on determination of binding constants of $^{15}NH_4^+$ ions to two G-quadruplexes. The assignments of NMR resonances of bimolecular $d[G_3T_4G_4]_2$ and unimolecular $d[G_4(T_4G_4)_3]$ G-quadruplexes and their respective 3D structures have been already described (Figures 1b and 1c).[9–12] We have shown earlier that $d[G_3T_4G_4]_2$ quadruplex binds two $^{15}NH_4^+$ ions which are localized between two adjacent G-quartets.[13] In this work we extend our previous studies by analyzing preferences of $^{15}NH_4^+$ ions for different binding sites within bimolecular $d[G_3T_4G_4]_2$ and unimolecular $d[G_4(T_4G_4)_3]$ G-quadruplexes in a quantitative way.

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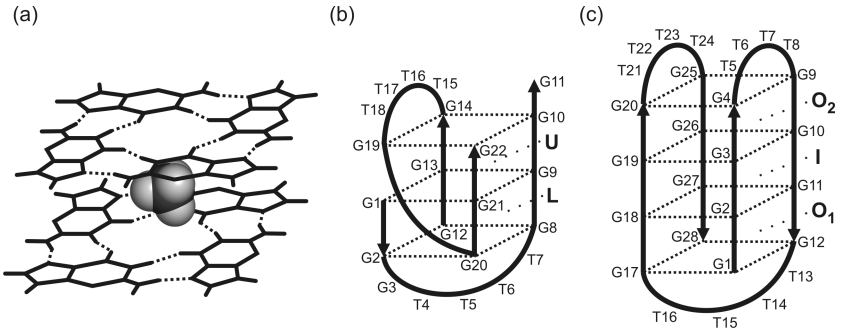

FIGURE 1 a) Schematic presentation of two stacked G-quartets with location of $^{15}NH_4^+$ ion in the center; b) topologies of $d[G_3T_4G_4]_2$[9,10]; and c) $d[G_4(T_4G_4)_3]$[11,12] with designation of individual cation binding sites.

RESULTS AND DISCUSSION

The concentration of ^{15}N-labeled ammonium ions in NMR samples has been gradually increased by titrating aqueous solution of its chloride salt into solutions of $d[G_3T_4G_4]_2$ and $d[G_4(T_4G_4)_3]$. 2D ^1H-^{15}N HSQC spectra displayed three cross-peaks in the case of $d[G_3T_4G_4]_2$ and four for $d[G_4(T_4G_4)_3]$ (Figure 2). The cross-peaks corresponding to $^{15}NH_4^+$ ions which are localized at the binding sites within both G-quadruplexes have been assigned using NOESY correlations and kinetics of $^{15}NH_4^+$ ion movement.[13] The most intense cross-peak (**B**) in both spectra corresponds to free ammonium ions in bulk solution. Cross-peaks labeled as **L** and **U** in Figure 2a correspond to $^{15}NH_4^+$ ions at two distinct binding sites within $d[G_3T_4G_4]_2$ (Figure 1b). Cross-peaks $\mathbf{O}_1$, **I** and $\mathbf{O}_2$ in Figure 2b correspond to $^{15}NH_4^+$ ions at three binding sites within $d[G_4(T_4G_4)_3]$ (Figure 1c).

The volume intensities of HSQC cross-peaks corresponding to **U** and **L** binding sites reached a plateau at 8 mM concentration of $^{15}NH_4^+$ ions in the case of $d[G_3T_4G_4]_2$, whereas the volume intensities for $\mathbf{O}_1$, **I** and

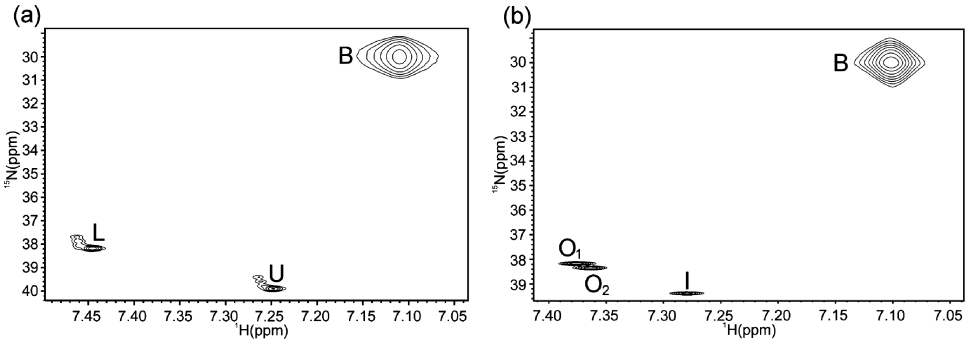

FIGURE 2 ^1H-^{15}N HSQC spectra of a) $d[G_3T_4G_4]_2$ at 60 mM $^{15}NH_4Cl$, and b) $d[G_4(T_4G_4)_3]$ at 120 mM $^{15}NH_4Cl$.

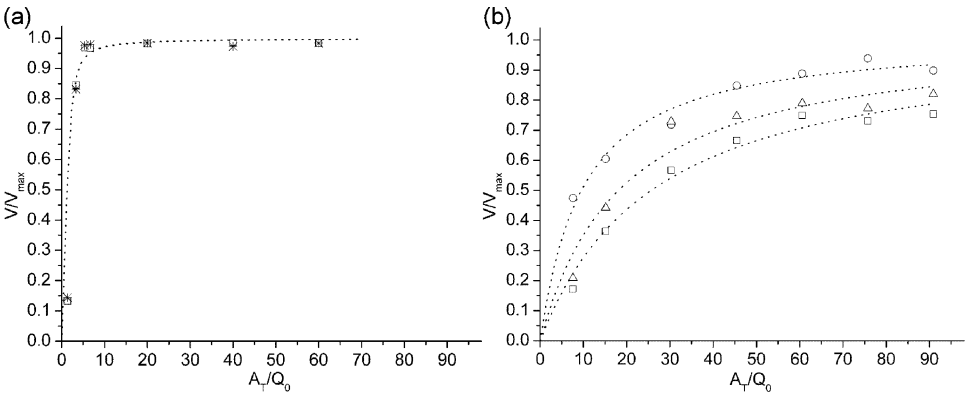

FIGURE 3 Increase of V/V_{max} as a function of ratios of total concentration of $^{15}NH_4^+$ ions and G-quadruplex. a) Experimental data for **L** (stars) and **U** (squares) binding sites within $d[G_3T_4G_4]_2$, and b) $\mathbf{O}_1$ (circles), $\mathbf{O}_2$ (triangles) and **I** (squares) within $d[G_4(T_4G_4)_3]$. Dotted curves represent the best fits to the experimental data.

$\mathbf{O}_2$ approached a plateau at the highest concentration (120 mM) of $^{15}NH_4^+$ ions. The occupancies (V/V_{max}) of individual binding sites calculated from the volumes of cross-peaks in HSQC spectra are shown in Figure 3. The increase in volume integrals of cross-peaks corresponding to bound $^{15}NH_4^+$ ions can be explained by the following equation:

$$Q + A \overset{K_i}{\longleftrightarrow} QA_i,$$

where Q and A correspond to G-quadruplex and $^{15}NH_4^+$ ions, respectively. QA_i corresponds to G-quadruplex with its binding sites occupied by $^{15}NH_4^+$ ions and K_i is the equilibrium binding constant. The concentration of vacant binding sites was expressed as a difference in the total concentration of G-quadruplex (Q_0) and those occupied by cations (QA_i). The concentration of free ammonium ions was calculated from its total added concentration (A_T) and the amount of bound ammonium ions assuming that the binding constants for a specific G-quadruplex are the same for all binding sites. Individual equilibrium binding constants have been calculated using the least-squares fitting procedure and are reported in Table 1.

Data in Table 1 show a large difference in affinities of $^{15}NH_4^+$ ions for unimolecular and bimolecular G-quadruplexes. It is noteworthy that the curves

TABLE 1 Equilibrium binding constants for $^{15}NH_4^+$ ions to individual binding sites

	$d[G_3T_4G_4]_2$		$d[G_4(T_4G_4)_3]$		
Binding site	**L**	**U**	$\mathbf{O}_1$	**I**	$\mathbf{O}_2$
K_i (M^{-1})	2703 ± 742	2857 ± 585	93 ± 8	31 ± 2	46 ± 5

for sites **U** and **L** in $d[G_3T_4G_4]_2$ are practically identical resulting in similar binding constants. Therefore, the effect of different loop orientations on individual K_i values seems to be negligible. In contrast, the binding constants for $\mathbf{O}_1$, **I**, and $\mathbf{O}_2$ in $d[G_4(T_4G_4)_3]$ differ by up to three times. The two of T_4 loops span along the edges of the same outer G-quartet, while the third loop consisting of residues T13-T16 spans diagonally along the outer G-quartet on the opposite side of G-quadruplex. Binding site **I**, which is in the core of $d[G_4(T_4G_4)_3]$, has the lowest affinity for $^{15}NH_4^+$ ions. Site $\mathbf{O}_1$ exhibits three times higher affinity. It seems that the terminal 3′ and 5′ guanines within the outside G-quartet make $\mathbf{O}_1$ the preferred site for $^{15}NH_4^+$ ions.

In conclusion, the binding constants of $^{15}NH_4^+$ ions to bimolecular $d[G_3T_4G_4]_2$ are at least 30 times higher than to unimolecular $d[G_4(T_4G_4)_3]$ G-quadruplex. Binding constants are comparable for the two binding sites within $d[G_3T_4G_4]_2$. Three $^{15}NH_4^+$ ion binding sites within $d[G_4(T_4G_4)_3]$ differ in their equilibrium binding constants by two and three times, which could be attributed to different electrostatic properties influenced by the structure of T_4 loops. Apparently individual binding constant is also controlled with the number and relative positions of *syn* and *anti* guanine residues within two adjacent G-quartets.

EXPERIMENTAL SECTION

Oligonucleotides were synthesized on an Expedite 8909 DNA synthesizer, deprotected using aqueous ammonia, desalted and purified on a Sephadex G-15 column, which was followed by extensive dialysis (Spectrapor) against LiCl solution or centrifugal filtration (Centricon) using CsCl solution. Oligonucleotide concentrations were 3 mM per strand (1.5 mM in G-quadruplex) for $d[G_3T_4G_4]_2$ and 1.3 mM for $d[G_4(T_4G_4)_3]$. The $^{15}NH_4^+$ ion concentration was gradually raised by simple titration with aqueous $^{15}NH_4Cl$ to 2, 5, 8, 10, 30, 60, and 90 mM for $d[G_3T_4G_4]_2$ and to 10, 20, 40, 60, 80, 100, and 120 mM for $d[G_4(T_4G_4)_3]$. All NMR spectra were collected on a Varian Unity Inova 600 MHz NMR spectrometer at 298 K. Cross-peak volumes were integrated with VNMRJ 2.1A software, where the same chemical shift windows were used for all cross-peaks. Least-square fitting was done using Origin 7.5 software. Quality of the fits was monitored through parameter χ^2. Errors in Table 1 for binding constants are reported as given by the Origin program.

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