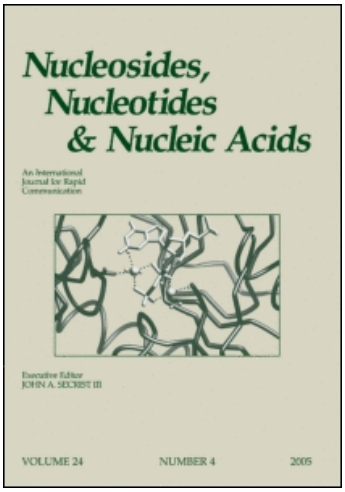

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Sugimoto, Naoki , Ohmichi, Tatsuo and Sasaki, Muneo(1996) 'The Stability of DNA and RNA G-Quartets', Nucleosides, Nucleotides and Nucleic Acids, 15: 1, 559 — 567
To link to this Article: DOI: 10.1080/07328319608002405
URL: http://dx.doi.org/10.1080/07328319608002405

NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 559-567 (1996)

THE STABILITY OF DNA AND RNA G-QUARTETS †

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ABSTRACT: We have investigated the thermodynamic stability of DNA and RNA G-quartet by circular dichroism spectroscopy, gel electrophoresis, and melting analysis. The free energy ($\Delta G°_{37}$) for G-quartet formation of $d(TTGGGG)_4$ and $r(UUGGGG)_4$ at 100 mM NaCl and 37°C were 1.4 kcal mol^{-1} and -3.0 kcal mol^{-1}, respectively. On the other hand, at 100 mM KCl, $\Delta G°_{37}$ of the DNA and RNA were -10.0 kcal mol^{-1} and -8.2 kcal mol^{-1}. This result indicates that the dependence of DNA G-quartet stability on these ions is larger than that of RNA.

INTRODUCTION

Non-Watson-Crick-paired nucleotides are important roles for three-dimensional structure of nucleic acids. Especially, guanine has the most interesting property among four bases: It was known that G-rich sequences in DNA like telomere[1] can form G-quartet, which is a cyclic hydrogen-bonded array of four guanine nucleotides as shown in Fig. 1A. It was also reported that the G-rich sequence was found in HIV (human immunodeficiency virus) genomic RNAs[5,6] and these RNAs can dimerize *in vitro* via G-quartet formation.[6] Thus, not only DNA but RNA G-rich sequences are able to form G-quartets. However, little is known about thermodynamic properties of the G-quartets. In this work, we have studied thermodynamic effects of Na^+ and K^+ ions on the stability of G-quartets, $r(UUGGGG)_4$ and $d(TTGGGG)_4$, which is the same telomeric sequence of *Tetrahymena* DNA[7] and can form the intramolecular G-quartet structure (Fig.1B).

MATERIALS AND METHODS

DNA/RNA synthesis and purification

Oligonucleotides, $r(UUGGGG)_4$ and $d(TTGGGG)_4$, were synthesized on solid support with a phosphoramidite method on an Applied Biosystems model 391 DNA/RNA

†This paper is dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday.

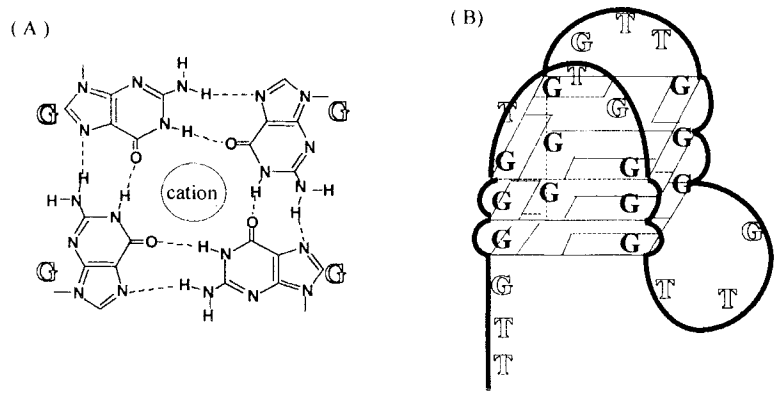

FIG.1. Schematic illustrations of (A) guanine tetrad and (B) intramolecular G-quartet structure of *Tetrahymena* DNA.

synthesizer. The synthesized oligomers were removed from solid support, and base blocking groups were removed by treatment with concentrated ammonia at 50°C overnight.[8,9] The oligonucleotides were purified by high-performance liquid chromatography (HPLC) on C18 column (TOSOH) with a gradient of 0-50 % methanol/H_2O containing 0.1 M triethylammonium acetate (TEAA) (pH 7.0) at 60 °C, because at the lower temperature the peak on HPLC was broadened because of forming the intramolecular G-quartet or an aggregated complex (Fig. 2). After purification on HPLC, the oligomers were desalted with a C18 Sep-Pak cartridge. Final purities of the oligomers were rechecked by HPLC and were greater than at least 98 %.

Nondenaturing gel electrophoresis

Electrophoresis experiments were conducted in gels containing 20% polyacrylamide (29% acrylamide/1% bisacrylamide) with a Atto AEP-500 apparatus cooled with circulating water to provide a running temperature of 5.0±0.5 °C. Both oligonucleotides were labeled with [γ-^{32}P] ATP at the 5' end with T4 polynucleotide kinase, and precipitated with ethanol. The samples were diluted into 5 µl buffer containing 10 mM Tris•HCl, 0.1 mM Na_2EDTA, and 100 mM NaCl, pH 7.0. The solutions were heated to 90 °C for 2 min, cooled slowly, incubated at 5.0 °C for 24 h, and run at 5.0 °C in 1xTBE buffer (0.09 M Tris, 0.09 M boric acid, and 0.2 mM EDTA, pH 8.3) for 3 h. The radioactivity of oligonucleotides were analyzed by Fuji BAS 2000 bio-imaging analyzer.

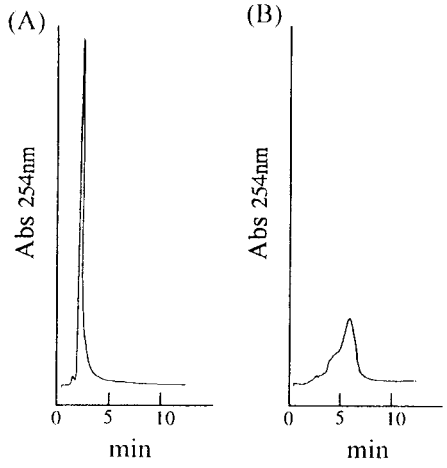

FIG. 2. HPLC elution profiles of r(UUGGGG)$_4$ at 60°C (A) and at 25°C (B). A linear gradient from 30% to 50% of methanol / 0.1 M triethylamine acetate (pH7.0) for 20 min was used, and wavelength of detection is 254 nm.

Circular dichroism

CD spectra were obtained on a JASCO J-600 spectropolarimeter equipped with the temperature controller and interfaced to a NEC PC-9801 computer. The experimental temperature was regulated by temperature controller. The cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid water condensation on the cuvette exterior. All CD spectra were measured from 350 nm to 200 nm in 0.1 cm path length cuvettes. The oligonucleotide concentrations (C_t) as strand concentration were calculated from the high-temperature absorbance.[10] Single-strand extinction coefficients were calculated from extinction coefficients of dinucleotide monophosphates and nucleotides.[11] A NaCl buffer contained 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA, pH7.0, while a buffer of KCl contained 100 m M KCl, 10 mM K_2HPO_4, and 1 mM Na_2EDTA, pH7.0.

Date analysis

Assuming a two-state process, the helix-coil transition of a G-quartet is represented by the equilibrium;[12) random coil $\rightleftarrows$ G-quartet

The equilibrium constant, K, for this transition is

$$K = f/(1-f) = \exp(-\Delta H^\circ/RT + \Delta S^\circ/R)$$

$$\Delta G^\circ = -RT \ln K = \Delta H^\circ - T\Delta S^\circ$$

where ΔH° and ΔS° are enthalpy and entropy changes, respectively, and f is the fraction of G-quartet. When f=0.5, $T_m = \Delta H^\circ/\Delta S^\circ$. The fraction f is related to the ellipticity at any temperature, $\varepsilon(T)$, by

$$f(T) = [\varepsilon_r(T) - \varepsilon(T)] / [\varepsilon_r(T) - \varepsilon_q(T)]$$

where $\varepsilon_r(T)$ and $\varepsilon_q(T)$ are the ellipticity of the random coil and G-quartet, respectively, at temperature T and are approximated by assuming linear upper and lower base lines:

$$\varepsilon_q(T) = m_q T + b_q, \quad \varepsilon_r(T) = m_r T + b_r$$

Obtained melting curves were fitted to six adjustable parameters ΔH°, ΔS°, m_q, b_q, m_r, and b_r using the nonlinear least-squares method.[13]

RESULTS

Nondenaturing gel electrophoresis of d(TTGGGG)$_4$ and r(UUGGGG)$_4$

Figure 3 shows the electrophoretic mobilities of d(TTGGGG)$_4$ and r(UUGGGG)$_4$ in nondenaturing gel. The native gel was run in 100 mM NaCl (pH 7.0) at 5.0 °C. In the NaCl buffer, r(UUGGGG)$_4$ consisting of 24 nucleotides showed the slightly faster migrating band than dT$_8$ in Fig. 3. In the KCl buffer, each oligonucleotide, d(TTGGGG)$_4$ or r(UUGGGG)$_4$, also showed only one band like the band in the above case. These faster migrating forms indicate the intramolecular G-quartet structures, because previous polyacrylamide gel electrophoresis (PAGE) experiment showed that the intramolecular G-quartet structure of d(TTTTGGGG)$_4$, which was the sequence of *Oxytricha* telomeric DNA, had the faster migrating form than dT$_{12}$ in the presence of Na^+.[7,14] On the other hand, in the NaCl buffer, d(TTGGGG)$_4$ showed not only the slightly faster migrating band than dT$_8$ but also a slower migrating band than dT$_{12}$ in Fig. 3. These results indicate that r(UUGGGG)$_4$ forms only the G-quartet conformation in both NaCl and KCl buffers, while d(TTGGGG)$_4$ forms not only the G-quartet structure but also the other structure in the NaCl buffer.

CD spectra of d(TTGGGG)$_4$ and r(UUGGGG)$_4$ in solution containing Na^+ or K^+ ion

Since the CD spectroscopy is as good method as gel electrophoresis to research for the overall structure of nucleic acids,[15] we measured the CD spectra of d(TTGGGG)$_4$ and r(UUGGGG)$_4$ in 100 mM NaCl or KCl buffer (pH 7.0) at 5.0 °C in order to monitor the G-quartet. These spectra are shown in Fig. 4. The CD spectrum of d(TTGGGG)$_4$ in

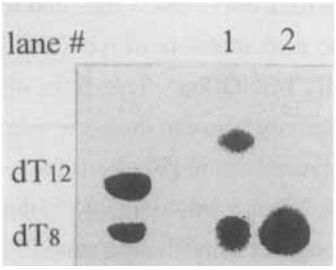

FIG.3. Electrophoresis of $d(TTGGGG)_4$ and $r(UUGGGG)_4$ at 1.0 X10 $^{-5}$M in 20% nondenaturing gels. Lanes 1 and 2 show the mobilities in the 100 mM NaCl buffer of $d(TTGGGG)_4$ and $r(UUGGGG)_4$. The gel was run at 5.0 °C in 1xTBE buffer for 3 h.

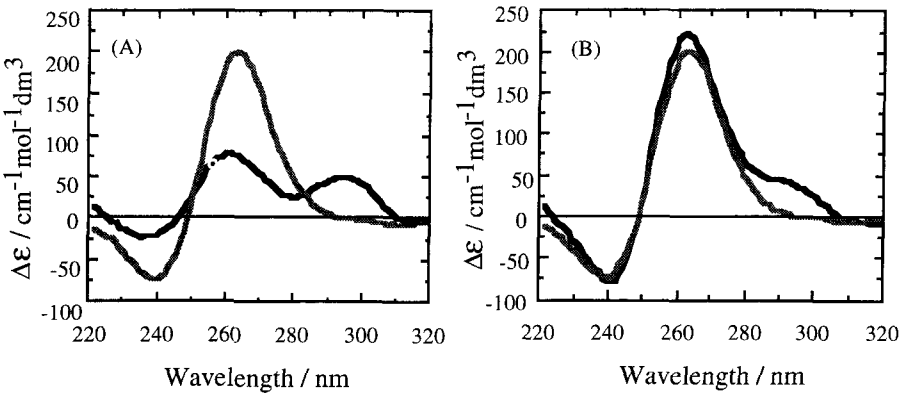

FIG. 4. (A) CD spectra for $d(TTGGGG)_4$ (—) and $r(UUGGGG)_4$ (—) at 5°C in 100 mM NaCl - phosphate buffer (pH 7.0). (B) CD spectra for $d(TTGGGG)_4$ (—) and $r(UUGGGG)_4$ (—) at 5°C in 100 mM KCl -phosphate buffer (pH 7.0).

the presence of 100 mM NaCl has two positive peaks at 260 and 295 nm, while the spectrum of r(UUGGGG)$_4$ in the same buffer has only one positive peak at 260 nm. The number of peaks for the CD spectra corresponds to that of the bands in the polyacrylamide gel electrophoresis experiment described above. The $\Delta\varepsilon$ value of d(TTGGGG)$_4$ at 260 nm in the NaCl buffer is 82.2 cm^{-1} mol^{-1} dm^3 and that of r(UUGGGG)$_4$ is 202.6 cm^{-1} mol^{-1} dm^3. That is, the peak intensity of r(UUGGGG)$_4$ at 260 nm is about three times larger than that of d(TTGGGG)$_4$. This ratio of the CD peak intensity is in agreement with that of radioactivity between the faster migrating forms of d(TTGGGG)$_4$ and r(UUGGGG)$_4$ in the polyacrylamide gel electrophoresis. These results lead to the consideration that the peak at 260 nm would be due to the G-quartet formation because there is no peak at this wavelength at a high temperature.

The CD spectrum of d(TTGGGG)$_4$ in the KCl buffer is very different from that in the NaCl buffer as shown in Fig. 4, although the CD spectra of r(UUGGGG)$_4$ are very similar in both buffers. The behavior was also found in the polyacrylamide gel electrophoresis as described in the above section. The peak intensity of d(TTGGGG)$_4$ at 260 nm increases from the NaCl buffer to the KCl one so that has almost the same ellipticities as r(UUGGGG)$_4$ in the KCl buffer. The result suggests that G-quartet formation of d(TTGGGG)$_4$ is easier in the presence of K^+ than Na^+. The peak at 295 nm would reflect other structure except for G-quartet.[16,17]

Thermodynamic stabilities of the DNA and RNA G-quartets

In many cases, thermodynamic parameters for double-helix formation of nucleic acids were obtained by UV absorbance melting curves.[18] In this case, UV absorbance of d(TTGGGG)$_4$ may be due to not only the G-quartet but also unknown other structure. So, in this study, melting curves were obtained by measuring the thermal changes of CD spectra at 260nm to investigate the effect of Na^+ and K^+ on the stability of the G-quartet. Melting curves of d(TTGGGG)$_4$ and r(UUGGGG)$_4$ at the same concentration are shown in Fig. 5. The melting curves were analyzed by fitting to a two-state model with sloping base line using a nonlinear least-squares program.[13] Obtained thermodynamic parameters are listed in Table 1. The free energy changes of the G-quartet formation ($\Delta G°_{37}$) for d(TTGGGG)$_4$ and r(UUGGGG)$_4$ in the KCl buffer were more favorable than those in the NaCl buffer. This result suggests that the intramolecular G-quartet in the presence of K^+ is more stable than that in the presence of Na^+.

DISCUSSION

As shown in Table 1, the thermodynamic parameters for formation of the intramolecular G-quartet structure depend on buffer solutions. This result provides that

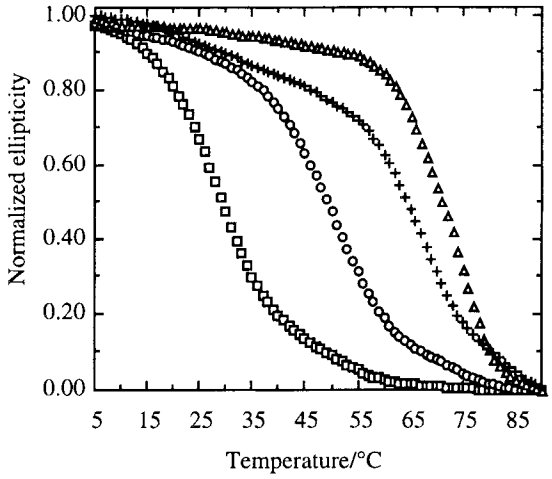

FIG. 5. Melting curves of 25 μM d(TTGGGG)$_4$ at the NaCl (□) and KCl buffers (Δ), and 25 μM r(UUGGGG)$_4$ at the NaCl (○) and KCl buffers (+).

TABLE.1. Thermodynamic parameters for the G-quartet formation

	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1}K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[a] (°C)
d(T$_2$G$_4$)$_4$ at 0.1M NaCl	-52.3	-173	1.4	28.4
r(U$_2$G$_4$)$_4$ at 0.1M NaCl	-76.6	-237	-3.0	49.8
d(T$_2$G$_4$)$_4$ at 0.1M KCl	-104	-302	-10.3	70.8
r(U$_2$G$_4$)$_4$ at 0.1M KCl	-98.8	-292	-8.2	64.2

a) Melting temperatures are calculated at the total oligomer concentration of 25 μM.

both DNA and RNA intramolecular G-quartets are salt-induced structures. The free energy change forming the G-quartet of d(TTGGGG)$_4$ in the K^+ buffer was 11.7 kcal mol^{-1} smaller than that in the Na^+ buffer. The free energy change of r(UUGGGG)$_4$ in the K^+ buffer was also smaller (5.2 kcal mol^{-1}) than in the Na^+ buffer.

This difference of the free energy change in buffer solutions reflects the contribution from the enthalpy ($\Delta H°$) and the entropy changes ($\Delta S°$). It would mean that the stacking interaction in the K^+ buffer are more stable than that in the Na^+ buffer, and so the entropy of G-quartet formation is more favorable. The favorable enthalpy would be due to the relation between the cavity of the G-quartet and the cation size. When the G-quartet form, four carbonyl oxygen atoms are juxtaposed in a square-planar geometry.[19] Further,

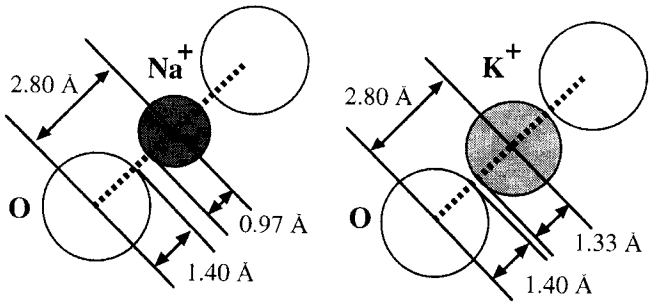

FIG. 6. Model for oxygen-ion interactions in two stacked guanine tetrads. Oxygen, sodium, and potassium atoms are represented as (○), (●), and (◎).

assuming two G-quartet planes were separated by the pitch of B-DNA (=3.4Å),[19] the distance between carbonyl oxygen and the center of cation is 2.80 Å. This distance is close to the expected K-O bond length (2.73Å) as calculated from the sum of the appropriate van der Waals radius of each atom (Fig. 6). On the other hand, the expected Na-O bond length (2.37Å) is not enough to just fit the cavity of the G-quartet plane (Fig. 6). Thus, Na^+ is too small to fill the cavity of the G-quartet plane, while K^+ just fits the cavity, so the stacking interaction between two G-quartet planes in K^+ buffer would be more stable than in Na^+ buffer.

The difference in free energy for the G-quartet formation at Na^+ and K^+ represents as $\Delta\Delta G^\circ$ (= ΔG°_{37} (in the Na^+ buffer)-ΔG°_{37} (in the K^+ buffer)). The $\Delta\Delta G^\circ$ value of $r(UUGGGG)_4$ is 6.5 kcal mol^{-1} smaller than that of $d(TTGGGG)_4$, indicating that the dependence of the RNA G-quartet for cation species is less than that of DNA. This result may be due to the difference in the flexibility between DNA and RNA. RNA has the flexible tertiary structures,[20,21] and may be able to make the cavity fitting not only K^+ ion but also Na^+ one. Thus, the thermodynamic data obtained in this study should be very useful to elucidate the potential role of the G-quartet in biological systems.

ACKNOWLEDGEMENT

This work was supported in part by grants from the Ministry of Education, Science and Culture, Japan, and Hirao Science Foundation to N.S.

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