

# Effect of divalent cations on antiparallel G-quartet structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>)

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**Abstract** The thermodynamic parameters of an antiparallel G-quartet formation of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) with 1 mM divalent cation (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, and Zn<sup>2+</sup>) were obtained. The thermodynamic parameters showed that the divalent cation destabilizes the antiparallel G-quartet of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in the following order: Zn<sup>2+</sup> > Co<sup>2+</sup> > Mn<sup>2+</sup> > Mg<sup>2+</sup> > Ca<sup>2+</sup>. In addition, a higher concentration of a divalent cation induced a transition from an antiparallel to a parallel G-quartet structure. These results indicate that these divalent cations are a good tool for regulating the G-quartet structures. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

**Key words:** G-quartet; Divalent cation; Structural transition; Nucleic acid; Telomere

## 1. Introduction

The guanine quartet (G-quartet) structure is a tertiary structure of nucleic acid formed by a cyclic Hoogsteen base pairing of four guanines in a coplanar arrangement [1]. Although direct evidence of G-quartet formation in vivo is still lacking, there are interesting G-quartet structures as structural motifs potentially adopted in many biological roles [2–5]. Because of their importance, G-quartets were investigated using X-ray [6], NMR [7], gel electrophoresis [8], and spectroscopic analyses [9] in the presence of monovalent cations such as Na<sup>+</sup> and K<sup>+</sup>. Kallenbach and coworkers estimated the thermodynamic parameters of G-quartet formation of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) [10,11]; however, these experiments were examined only in the presence of monovalent cations, and little was known about the divalent cation effect on the G-quartet formation. On the other hand, some nucleic acids discovered as functional molecules such as aptamers [12,13] and deoxyribozymes [14,15] form G-quartet structures, and their activities were shown to be strongly dependent on a coexistent divalent cation. Therefore, the divalent cation should play critical roles in regulating G-quartet structures and then the functions of the functional nucleic acids. The quantitative effect of the divalent cation on G-quartet structure, however, is not yet clear.

Analyses of G-quartet structures of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>), which are formed by *Oxytricha* telomere DNA, have been well investi-

gated [6,16]. These results showed that self-complementary d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) forms an antiparallel G-quartet with the thymines in a loop. Therefore, quantitative analysis of the G-quartet structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) is presumably able to lead to the assumption of a two-state transition model that is very useful in calculating thermodynamic parameters [11]. Here, we investigated quantitatively the divalent cation effect on the structures and stabilities of the d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) G-quartet in the presence of 100 mM and 7 mM Na<sup>+</sup>. This is the first quantitative report to elucidate the divalent cation effect on a G-quartet structure. The thermodynamic and conformational analyses of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) with the divalent cations (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, or Zn<sup>2+</sup>) revealed two major points. First, the antiparallel G-quartet of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) was destabilized with 1 mM divalent cation. Second, a continuous structural transition of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) from an antiparallel to a parallel G-quartet was also observed with Ca<sup>2+</sup> titration in the presence of 100 mM Na<sup>+</sup>. The structural transition was completed by adding 20 mM Ca<sup>2+</sup>. These results suggest that the divalent cations are able to regulate the stability and further the structure of the G-quartet. Sen and Gilbert proposed that the intracellular balance of cations might control the switching between the antiparallel and parallel G-quartets [17]. In this study, the structural switching between antiparallel and parallel G-quartets was regulated by divalent cations. These results indicate that the divalent cations used in this study are a good tool for regulating the G-quartet structures.

## 2. Materials and methods

### 2.1. Sample preparation

DNA oligonucleotide d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) was synthesized chemically on a solid support using the phosphoramidite method on an Applied Biosystems model 391 DNA synthesizer. The synthesized oligonucleotide was purified with reversed-phase high performance liquid chromatography (HPLC) after the deblocking operations. The oligonucleotide was desalted with a C-18 Sep-Pak cartridge column. The final purity of the oligonucleotide was confirmed to be greater than 98% by HPLC. Single-strand concentration of the oligonucleotide was determined from the absorbance at 260 nm with single-strand extinction coefficients calculated from mononucleotide and dinucleotide data using a nearest-neighbor approximation [18].

### 2.2. Conformational analysis

Recent conformational analyses of G-quartet structures revealed that the CD spectra of an antiparallel G-quartet structure had positive and negative peaks near 295 and 265 nm, respectively [19], while a parallel G-quartet structure had positive and negative peaks near 260 and 240 nm, respectively [20]. With this information, a structural type of G-quartet can be determined by CD measurement. CD spectra were obtained on a JASCO J-820 spectropolarimeter equipped with a JASCO PTC-348 temperature controller and interfaced to a Dell

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OptiPlex GXi computer. The cuvette-holding chamber was flushed with a constant stream of dry  $N_2$  gas to avoid water condensation on the cuvette exterior. All the CD spectra were measured for a 50  $\mu$ M total strand concentration of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in a 0.1 cm path length cuvette with a buffer containing 50 mM MES (pH 6.1) and 1 mM MCl<sub>2</sub> (M = Mg, Ca, Mn, Co, or Zn) in the presence of 100 or 7 mM NaCl at 5°C. The CD spectrum was the average of at least three scans made at 0.1 nm intervals from 200 to 350 nm. Before the CD measurement, the DNA sample was heated to 90°C, gently cooled at a rate of 3°C min<sup>-1</sup>, and incubated at 5°C for several hours. We confirmed that the CD spectra were independent of long time incubation (for 3 days), repetition of the heating and cooling procedure (five times), and cooling rate (from 3°C min<sup>-1</sup> to 0.5°C min<sup>-1</sup>).

### 2.3. Thermodynamic analysis

All CD melting curves of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) were recorded at 295.6 nm in the buffer containing 50 mM MES (pH 6.1) and 1 mM MCl<sub>2</sub> (M = Mg, Ca, Mn, Co, or Zn) in the presence of 100 or 7 mM NaCl. Samples were heated from 0°C to 90°C at a rate of 0.5°C min<sup>-1</sup>. Before the CD measurement, all the samples were thermally treated as described above, and we confirmed that the melting behavior was not dependent on the treatments. Because the antiparallel G-quartet structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) is formed with two single strands [6,7,11,16], the helix-coil transition of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) is represented by the following equation:  $2S = Q$ , where  $S$  and  $Q$  are single-strand DNA and antiparallel G-quartet, respectively. Therefore, the CD melting curves of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) can fit the self-complementary two-state approximation of a helix-coil transition to obtain the thermodynamic parameters ( $\Delta H^\circ$ ,  $\Delta S^\circ$ , and  $\Delta G_{25}^\circ$ ) as described elsewhere [21] with non-linear least-squares fitting.

### 2.4. Titration experiment

The relationship between divalent cation concentration and the structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) was investigated with a CaCl<sub>2</sub> titration experiment. The structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) at each titration point was measured using CD after the thermal treatment as described above. 50  $\mu$ M d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in a buffer containing 100 mM NaCl, 100 mM CaCl<sub>2</sub> and 50 mM MES (pH 6.1) was added to 50  $\mu$ M d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in 100 mM NaCl and 50 mM MES (pH 6.1). Therefore, all CD spectra were measured for 50  $\mu$ M of total strand concentration of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in a 0.1 cm path length cuvette in a buffer containing 50 mM MES (pH 6.1), 100 mM NaCl and appropriate CaCl<sub>2</sub> concentration (from 0 to 100 mM) at 5°C. The transition from an antiparallel to a parallel G-quartet structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) by adding only a solution including an appropriate concentration of CaCl<sub>2</sub>, 100 mM NaCl, and 50 mM MES was also observed (data not shown). However, the concentration of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) was changed by adding the solution. Because antiparallel and parallel G-quartets are formed by two and four strands, respectively, there is a possibility that the effect of the concentration of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) on antiparallel and parallel G-quartets is different. Therefore, in this study, a solution including 50  $\mu$ M d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in 100 mM NaCl, 100 mM CaCl<sub>2</sub> and 50 mM MES (pH 6.1) was added to 50  $\mu$ M d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in 100 mM NaCl and 50 mM MES (pH 6.1).

### 2.5. Gel electrophoresis

DNA solutions were prepared in a buffer containing 50 mM NaCl and 50 mM MES (pH 6.1) with or without 50 mM CaCl<sub>2</sub>. DNA samples with a total strand concentration of 100  $\mu$ M were heated to 90°C, gently cooled at a rate of 3°C min<sup>-1</sup>, incubated at 5°C for several hours, and run on 20% non-denaturing polyacrylamide gels in 1×TBE buffer at 5°C for 24 h at 75 V (ca. 5 V cm<sup>-1</sup>). The electrophoresis plates were cooled in a refrigerator. The gel was stained in a 0.01% Stain-All formamide solution (9:11 formamide:H<sub>2</sub>O ratio) [11].

## 3. Results

### 3.1. Effect of divalent cation on the structure and stability of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>)

Fig. 1A shows the CD spectra of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in 100 mM NaCl and 50 mM MES (pH 6.1) at various temperatures. These spectra showed that d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) formed a typical antiparallel G-quartet structure under this condition [19]. Because these CD spectra of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) had an isodichroic point near 250 nm, it was concluded that the d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) structure under this condition underwent a two-state transition between a single-strand and an antiparallel G-quartet. The CD spectra of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in the buffer containing 50 mM MES (pH 6.1) and 1 mM MCl<sub>2</sub> (M = Mg, Ca, Mn, Co, or Zn) in the presence of 100 mM NaCl also had positive and negative peaks near 295 and 265 nm, respectively, and an isodichroic point (data not shown). Therefore, the two-state model also can be applied to the antiparallel G-quartet structures with 1 mM divalent cations. Fig. 1B shows the melting behavior of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) at 295.6 nm versus temperature with the divalent cations in the presence of 100 mM Na<sup>+</sup>. Based on this melting behavior, the thermodynamic parameters and melting temperature were calculated. Table 1 shows the thermodynamic parameters of the G-quartet in the absence (only 100 mM Na<sup>+</sup> present) and presence of 1 mM divalent cations. In the absence of divalent cations, the  $\Delta G_{25}^\circ$  value of the antiparallel G-quartet formation of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) with 100 mM Na<sup>+</sup> was  $-24.3$  kcal mol<sup>-1</sup>. The values in the presence of 1 mM divalent cations were from  $-19.5$  kcal mol<sup>-1</sup> to  $-16.4$  kcal mol<sup>-1</sup>. Free energy changes,  $\Delta\Delta G_{25}^\circ$  ( $\Delta G_{25}^\circ$ (with a divalent cation)  $-\Delta G_{25}^\circ$ (without a divalent cation)), show that all divalent cations destabilized the antiparallel G-quartet structure in the presence of 100 mM Na<sup>+</sup> in the following order: Zn<sup>2+</sup> > Co<sup>2+</sup> > Mn<sup>2+</sup> > Mg<sup>2+</sup> > Ca<sup>2+</sup>. These results indicate that transition

Table 1  
Thermodynamic parameters of the antiparallel G-quartet structure of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in the presence of divalent cation at 25°C<sup>a</sup>

	In the presence of 100 mM NaCl				
	$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$-T\Delta S^\circ$ (kcal mol <sup>-1</sup> )	$\Delta G_{25}^\circ$ (kcal mol <sup>-1</sup> )	$T_m^b$ (°C)	$\Delta\Delta G_{25}^\circ$ (kcal mol <sup>-1</sup> )
Na <sup>+</sup>	$-201 \pm 12$	$176 \pm 10$	$-24.3 \pm 1.3$	59.6	–
Ca <sup>2+</sup>	$-144 \pm 12$	$125 \pm 11$	$-19.5 \pm 0.9$	56.8	4.8
Mg <sup>2+</sup>	$-136 \pm 4$	$118 \pm 4$	$-18.9 \pm 0.5$	55.6	5.4
Mn <sup>2+</sup>	$-163 \pm 17$	$145 \pm 16$	$-18.1 \pm 1.5$	51.1	6.2
Co <sup>2+</sup>	$-154 \pm 8$	$137 \pm 7$	$-17.1 \pm 1.0$	49.0	7.2
Zn <sup>2+</sup>	$-100 \pm 9$	$83 \pm 7.4$	$-16.4 \pm 1.3$	53.2	7.9
	In the presence of 7 mM NaCl				
Na <sup>+</sup>	$-189 \pm 5$	$169 \pm 7$	$-19.4 \pm 0.7$	54.1	–
Ca <sup>2+</sup>	$-142 \pm 6$	$126 \pm 6$	$-15.9 \pm 0.6$	49.0	3.5
Mg <sup>2+</sup>	$-152 \pm 6$	$137 \pm 6$	$-15.1 \pm 0.6$	48.3	4.3
Mn <sup>2+</sup>	$-141 \pm 11$	$129 \pm 10$	$-12.5 \pm 1.0$	40.5	6.9

<sup>a</sup>All experiments were conducted in a buffer containing 100 or 7 mM NaCl, 50 mM MES (pH 6.1), and 1 mM divalent cation chloride.

<sup>b</sup> $T_m$  was calculated for 100  $\mu$ M total strand concentration.

<sup>c</sup>Relative free energy:  $\Delta\Delta G_{25}^\circ = \Delta G_{25}^\circ$ (with a divalent cation)  $-\Delta G_{25}^\circ$ (without a divalent cation).

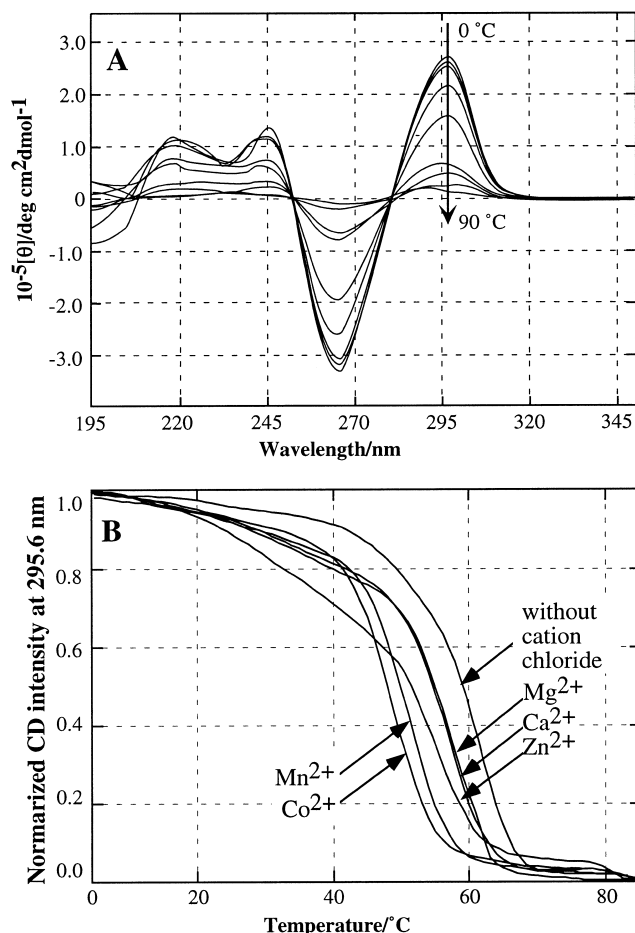


Fig. 1. A: CD spectra of 50  $\mu\text{M}$  d( $\text{G}_4\text{T}_4\text{G}_4$ ) in the buffer containing 50 mM MES (pH 6.1) and 100 mM NaCl at various temperatures from 0°C to 90°C (from the lower to the upper spectrum at 295.6 nm). B: Normalized CD melting curves of 50  $\mu\text{M}$  d( $\text{G}_4\text{T}_4\text{G}_4$ ) in the buffer containing 50 mM MES (pH 6.1), 100 mM NaCl and 1 mM  $\text{MCl}_2$  (M = Mg, Ca, Mn, Zn, or Co).

metal ions destabilize the antiparallel G-quartet more strongly than alkaline earth ions in the presence of 100 mM  $\text{Na}^+$ .

The structures and stabilities of d( $\text{G}_4\text{T}_4\text{G}_4$ ) with the divalent cations in the presence of a low concentration of  $\text{Na}^+$  (7 mM) were also investigated. Although d( $\text{G}_4\text{T}_4\text{G}_4$ ) forms an antiparallel G-quartet with all divalent cations in the presence of 100 mM  $\text{Na}^+$ , the structures with the divalent cations in the presence of 7 mM  $\text{Na}^+$  depend on the coexistent divalent cations. Fig. 2A shows that d( $\text{G}_4\text{T}_4\text{G}_4$ ) forms an antiparallel G-quartet with 1 mM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , or  $\text{Mn}^{2+}$ , and without the divalent cation in the presence of 7 mM  $\text{Na}^+$ . On the other hand, the CD spectra of d( $\text{G}_4\text{T}_4\text{G}_4$ ) with 1 mM  $\text{Co}^{2+}$  or  $\text{Zn}^{2+}$  had two positive peaks near 290 and 260 nm, and two negative peaks near 270 and 240 nm, respectively, as shown in Fig. 2B. These CD spectra suggest that d( $\text{G}_4\text{T}_4\text{G}_4$ ) forms antiparallel and partly parallel G-quartet structures with 1 mM  $\text{Co}^{2+}$  or  $\text{Zn}^{2+}$  in the presence of 7 mM  $\text{Na}^+$ . These results suggest that the coexistent divalent cation can regulate the G-quartet structures of d( $\text{G}_4\text{T}_4\text{G}_4$ ), and that 1 mM of a transition metal ion may induce the parallel G-quartet structure.

Fig. 2C shows the CD melting curves of d( $\text{G}_4\text{T}_4\text{G}_4$ ) with 1 mM  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , or  $\text{Mn}^{2+}$  in the presence of 7 mM  $\text{Na}^+$ .

The thermodynamic parameters of d( $\text{G}_4\text{T}_4\text{G}_4$ ) with 1 mM  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , or  $\text{Mn}^{2+}$  in the presence of 7 mM  $\text{Na}^+$  were calculated and are shown in Table 1. The thermodynamic parameters with  $\text{Co}^{2+}$ , or  $\text{Zn}^{2+}$  in the presence of 7 mM  $\text{Na}^+$  could not be calculated because the two-state assumption was not applied to these transitions. These thermodynamic parameters show that the destabilizing order of d( $\text{G}_4\text{T}_4\text{G}_4$ )

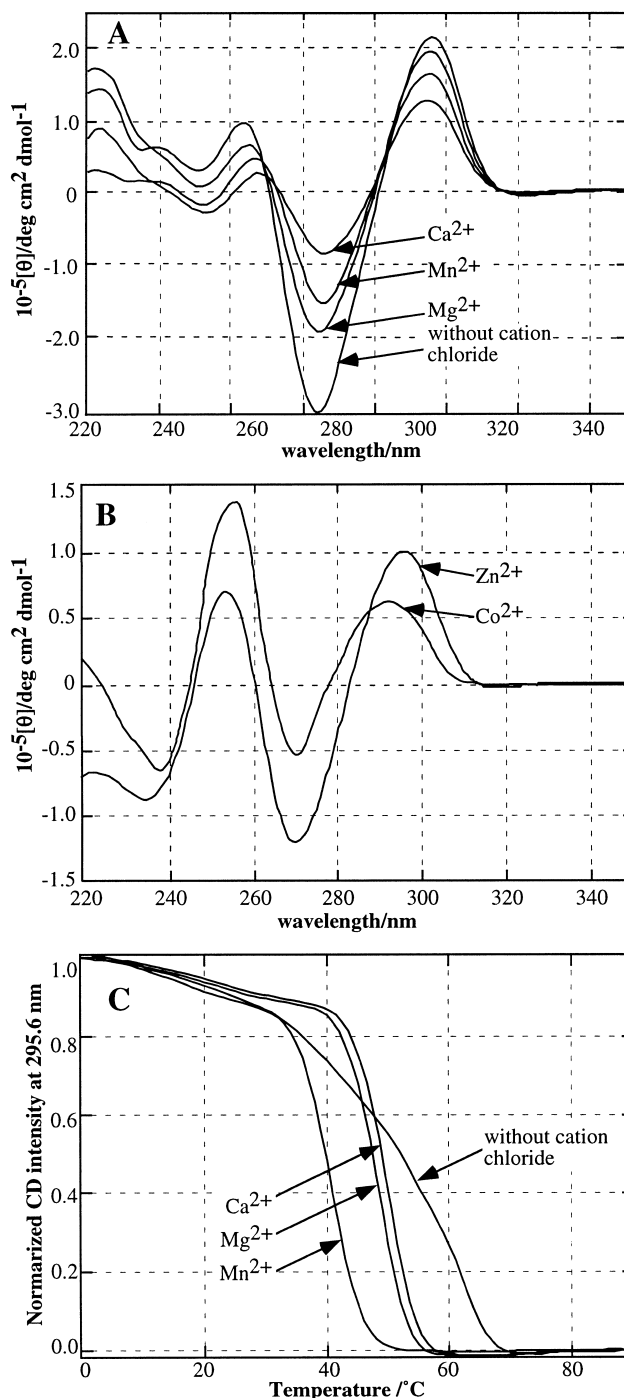


Fig. 2. CD spectra of 50  $\mu\text{M}$  d( $\text{G}_4\text{T}_4\text{G}_4$ ) in the buffer containing 50 mM MES (pH 6.1), 7 mM NaCl, and A: 1 mM  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , or  $\text{MnCl}_2$ , B: 1 mM  $\text{ZnCl}_2$  or  $\text{CoCl}_2$ . C: Normalized CD melting curves of 50  $\mu\text{M}$  d( $\text{G}_4\text{T}_4\text{G}_4$ ) in the buffer containing 50 mM MES (pH 6.1), 7 mM NaCl, and 1 mM divalent cation ( $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , or  $\text{MnCl}_2$ ).

by divalent cations ( $Mn^{2+} > Mg^{2+} > Ca^{2+}$ ) in the absence of  $Na^+$  has the same propensity in the presence of 100 mM  $Na^+$ . The transition metal ions, which strongly destabilize the antiparallel G-quartet in the presence of 100 mM  $Na^+$ , induce the parallel G-quartet in the presence of 7 mM  $Na^+$ .

### 3.2. $Ca^{2+}$ titration of $d(G_4T_4G_4)$

Fig. 3A shows CD spectra in the buffer containing 100 mM NaCl, 50 mM MES (pH 6.1) and 0–100 mM  $CaCl_2$  (from the lower to the upper spectrum at 260 nm). These CD spectra show that  $d(G_4T_4G_4)$  formed an antiparallel G-quartet in 100 mM  $Na^+$ , while  $d(G_4T_4G_4)$  formed a parallel G-quartet in 100 mM  $Ca^{2+}$  and 100 mM  $Na^+$ . A typical CD spectrum of an antiparallel G-quartet has a large positive peak near 295 nm, and this is useful for detecting an antiparallel G-quartet formation. On the other hand, the CD intensity at 260 nm is useful in detecting the structural transition between an antiparallel and a parallel G-quartet because the typical CD spectra of the parallel and antiparallel G-quartets show large positive and negative peaks near 260 nm, respectively. Fig. 3B

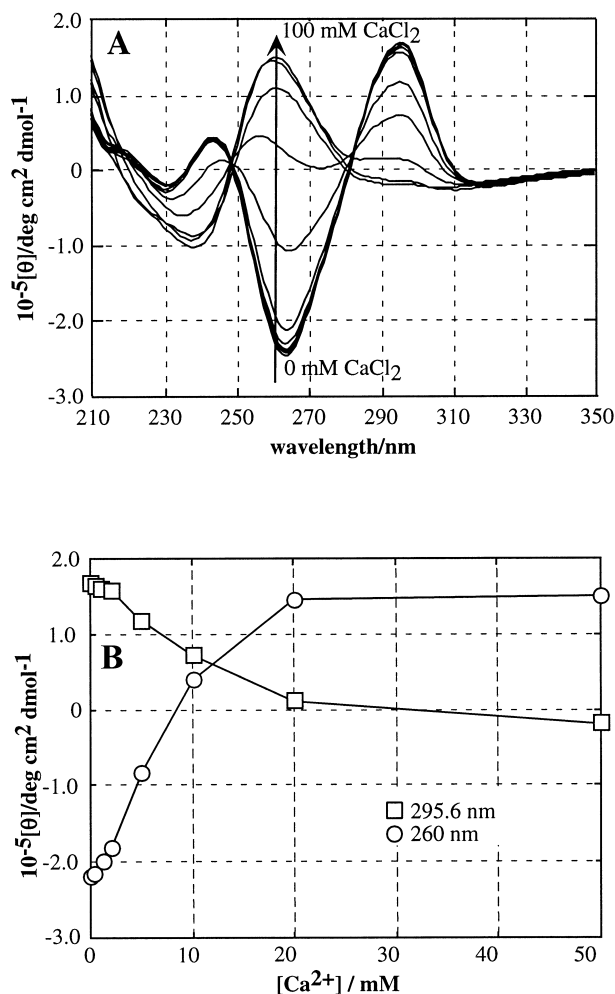


Fig. 3. A: CD spectra of 50  $\mu$ M  $d(G_4T_4G_4)$  in the buffer containing 50 mM MES (pH 6.1), 100 mM  $Na^+$  and 0–100 mM  $CaCl_2$  (from the upper to the lower spectrum at 295.6 nm) at 5°C. B: CD intensities of 50  $\mu$ M  $d(G_4T_4G_4)$  in the buffer containing 50 mM MES (pH 6.1), 100 mM NaCl, and various  $CaCl_2$  concentrations at 5°C. The square and circle indicate CD intensities at 295.6 and 260 nm, respectively.

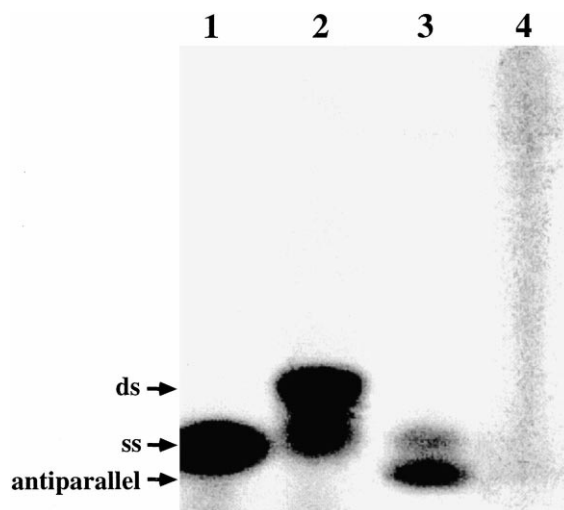


Fig. 4. Gel electrophoresis of oligonucleotides in 20% non-denaturing gel containing 50 mM NaCl. Lane 1,  $d(TTCCCTTCTT)$ ; lane 2,  $d(TTCCCTTCTT)/d(AAGAAAGGGAAA)$ ; lane 3,  $d(G_4T_4G_4)$  in 50 mM NaCl; lane 4,  $d(G_4T_4G_4)$  in 50 mM NaCl and 50 mM  $CaCl_2$ .

shows the CD intensities of  $d(G_4T_4G_4)$  at 295.6 and 260 nm during the  $Ca^{2+}$  titration. These results suggest that the structural transition from the antiparallel to the parallel G-quartet of  $d(G_4T_4G_4)$  occurred continuously and cooperatively with the  $Ca^{2+}$  titration. This is the first report stating that the structural transition between antiparallel and parallel G-quartets was induced by a divalent cation. The CD intensity changes at 260 and 295.6 nm were saturated at 20 mM  $Ca^{2+}$  concentration. To confirm that  $d(G_4T_4G_4)$  forms the parallel G-quartet structure in the presence of  $Ca^{2+}$ , the mobility of  $d(G_4T_4G_4)$  was examined on a non-denaturing gel. Fig. 4 shows the electrophoretic mobilities of  $d(G_4T_4G_4)$  in 50 mM NaCl (lane 3), and 50 mM NaCl and 50 mM  $CaCl_2$  (lane 4) on 20% non-denaturing gel containing 50 mM NaCl. The migration of  $d(G_4T_4G_4)$  in the absence of  $Ca^{2+}$  was faster than that of single- and double-stranded 12-mer oligonucleotides indicating that  $d(G_4T_4G_4)$  forms a compact structure, the antiparallel G-quartet structure [11]. On the other hand, smearing of the  $d(G_4T_4G_4)$  banding pattern occurred in 50 mM NaCl and 50 mM  $CaCl_2$ . The smearing band suggests that the parallel G-quartet structure of  $d(G_4T_4G_4)$  in the presence of 50 mM  $CaCl_2$  is not a homogeneous four-stranded parallel G-quartet but includes some highly ordered structures such as a G-wire [22]. The highly ordered structures can be formed only under conditions in which all strands are oriented in a parallel direction. These results of the gel electrophoresis of  $d(G_4T_4G_4)$  under different salt conditions are compatible with the results of CD measurements.

## 4. Discussion

### 4.1. Divalent cation effect on the antiparallel G-quartet structure

Although there are many ongoing studies on the divalent cation effect on G-quartet structures using  $T_m$  and the CD intensity change, our understanding of these features is qualitative but not quantitative. For example, Lee, Hardin et al., and Blume et al. showed that some divalent cations have an effect on poly[d(GGA)] [23],  $d(CGCG_3GCG)$  [24] and

d(CG<sub>4</sub>CG<sub>6</sub>AGC) [25], respectively. These DNAs form parallel G-quartet structures because their loop regions are too short to cross over the G-tetrad. The thermodynamic parameters obtained in this study (Table 1) are part of the ongoing results that show systematically and quantitatively the divalent cation effect on the antiparallel G-quartet structure. The parameters show clearly that the divalent cations destabilized the antiparallel G-quartet structure in the following order: Zn<sup>2+</sup> > Co<sup>2+</sup> > Mn<sup>2+</sup> > Mg<sup>2+</sup> > Ca<sup>2+</sup> (> Na<sup>+</sup>).

Previously, it was shown by ab initio calculation that the stabilization of divalent cations for a Watson–Crick base pair through coordination to the N7 of guanine was in the following order: Li<sup>+</sup> < Na<sup>+</sup> < Ca<sup>2+</sup> < Mg<sup>2+</sup> < Zn<sup>2+</sup> [26]. This propensity scale is almost opposite to that for the antiparallel G-quartet obtained in this study (Table 1). It was also reported that a divalent cation coordinates to the N7 of purines in a PyPuPu DNA triple helix and stabilizes it due to a Hoogsteen hydrogen bond enhancement [27]. In the triple and double helices of the previous studies, the guanine N7 is not involved in a hydrogen bond, although this is used for the cyclic Hoogsteen hydrogen bonds in the antiparallel G-quartet [1]. Therefore, the divalent cation coordination to the guanine N7 in the antiparallel G-quartet has to break the Hoogsteen hydrogen bonds and destabilize the antiparallel G-quartet structure. On the basis of these considerations, a novel mechanism for the antiparallel G-quartet destabilization by the divalent cations is now proposed: The divalent cation coordinates to the guanine N7 and decreases the Hoogsteen hydrogen bond; as a result, the divalent cation destabilizes the d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) G-quartet, although the mono- and divalent cation effects on the G-quartet were noted to involve enhancement of the G-quartet structures with their cation coordination with guanine O6. Moreover, on the basis of the enthalpy change ( $\Delta H^\circ$ ) and entropy change ( $T\Delta S^\circ$ ) of the antiparallel G-quartet formation of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) in the presence of each of the divalent cations, all of the  $\Delta\Delta H^\circ$  ( $\Delta H^\circ$ (with a divalent cation)– $\Delta H^\circ$ (without a divalent cation)) and  $-T\Delta\Delta S^\circ$  ( $-T\Delta S^\circ$ (with a divalent cation)– $-T\Delta S^\circ$ (without a divalent cation)) indicated positive and negative values, respectively (Table 1). The relationship among the free energy, enthalpy, and entropy changes for the G-quartet formation at 25°C is described by the following equation:  $\Delta G_{25}^\circ = \Delta H^\circ - 298.15\Delta S^\circ$ . Thus, the destabilization of the divalent cations for the antiparallel G-quartet structure formation is predominantly induced by the enthalpy increment. The enthalpy change generally occurs with hydrogen bonding and stacking interaction changes. Therefore, these thermodynamic parameters also support the presented mechanism that the divalent cations decrease the hydrogen bond with coordination to the guanine N7.

#### 4.2. Structural transition induced by divalent cations

Miura et al. showed a phase diagram of the structural transition between antiparallel and parallel G-quartet of d(T<sub>4</sub>G<sub>4</sub>)<sub>4</sub> depending on monovalent cation (Na<sup>+</sup> and K<sup>+</sup>) concentrations and their ratio [28]. In their study, 225 mM Na<sup>+</sup> or 65 mM K<sup>+</sup> was required for the transition from an antiparallel to a parallel G-quartet structure. However, in this study, only 1 mM transition metal cations may induce the parallel G-quartet structure in the presence of 7 mM Na<sup>+</sup>. These results indicate that not only ionic strength but also the specific divalent cation–G-quartet interaction is essential for the structural transition and that the specific divalent cation–G-quartet

interaction may be stronger than the Na<sup>+</sup> coordination to the guanine O6. Generally, a transition metal ion is prone to coordinate to a purine O6 more strongly than an alkaline earth ion [29]. Therefore, the structural transition induced by only 1 mM transition metal ion may be tentatively explained by the greater ability for coordination to the guanine O6 than the alkaline earth ions (Ca<sup>2+</sup> or Mg<sup>2+</sup>) have. Previously, Chen showed that 2 mM Sr<sup>2+</sup> facilitates the parallel G-quartet formation of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) [30]. In this study, although 1 mM Ca<sup>2+</sup> has little effect on the structural transition in the presence of Na<sup>+</sup>, over 20 mM Ca<sup>2+</sup> completes the transition. The results of gel electrophoresis show that the strand orientation of the d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) G-quartet in the presence of 50 mM Ca<sup>2+</sup> is in a parallel direction. From these results, a coexistent divalent cation generally can regulate the structural transition between an antiparallel and a parallel G-quartet. We revealed here several monovalent and divalent cation relationships that can possibly regulate the structural transition of the d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) G-quartet.

## 5. Conclusions

The divalent cations used in this study have the ability not only to decrease the antiparallel G-quartet stability of d(G<sub>4</sub>T<sub>4</sub>G<sub>4</sub>) but also to induce its structural transition to the parallel structure. Divalent cations, for example Ca<sup>2+</sup> and Mg<sup>2+</sup>, are often observed in the human body [31,32]; thus, the structural transition of the G-quartet can be controlled by the divalent cation and its balance. The divalent cation effect on the Hoogsteen base pair in the antiparallel G-quartet was also shown to be opposite to that on the Watson–Crick base pair in the double helix and the Hoogsteen base pair in the triple helix, due to whether the guanine N7 is occupied by a hydrogen bond. The G-quartet structural transition regulated with a divalent cation is very useful for the regulation of a functional molecule and the material development of a molecular switch.

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