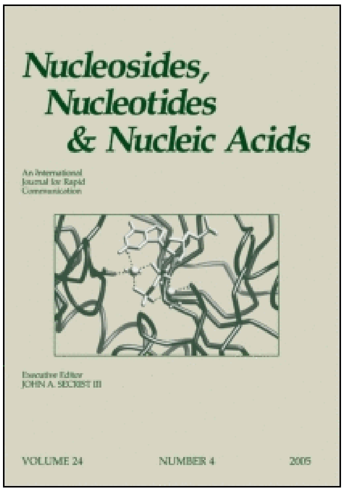

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HYDRATION REGULATES THE THERMODYNAMIC STABILITY OF DNA STRUCTURES UNDER MOLECULAR CROWDING CONDITIONS

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□ *Although water is an integral part of DNA structures, the effects of water molecules on various DNA structures which are formed by not only Watson-Crick but also Hoogsteen base pairs are still unclear. Here, we studied quantitatively the effects of molecular crowding on the thermodynamics of a parallel G-quadruplex formation of* $[d(TG_4)_2]_4$ *with Hoogsteen base pairs. It was demonstrated that molecular crowding conditions stabilized the parallel G-quadruplex. Moreover, the plot of stability of the parallel G-quadruplex structure versus water activity suggested that water molecules were released through the G-quadruplex formation. The stabilization of the DNA structures consisting of Hoogsteen base pairs under cell-like conditions may lead to a structural polymorphism of various DNA sequences regulated by water molecules.*

Keywords DNA G-quadruplex; molecular crowding; hydration; thermodynaimics

INTRODUCTION

The inside of a living cell is occupied by biomolecules. The total concentration of the biomolecules can reach 400 g L^{-1}, leading to a crowded intracellular environment.[1] However, biochemical studies of the biomolecules

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are generally examined in dilute conditions. Thus, the study of biochemical reactions and processes in the crowded condition has attracted considerable attention in recent years.[2] We reported that the length of DNA as well as the size of the cosolute was critical for the hybridization energy of the DNA duplex in molecular crowding conditions and that the number of water molecules affecting base pair formation may differ depending on the cosolute.[3] It was further investigated that the molecular crowding effects the thermodynamics of an antiparallel G-quadruplex formation with Hoogsteen base pairs among four guanines in cyclic and coplanar arrangement (G-quartet),[4] and that of an intramolecular hairpin duplex formation with Watson-Crick base pairs. The quantitative analysis showed that molecular crowding conditions stabilized the G-quadruplex and destabilized the duplex, because water molecules were released and taken up through the antiparallel G-quadruplex and the duplex formations, respectively.[5] These previous results revealed that water is an integral part of DNA structures. However, the effect of water molecules on the thermodynamic stability of the G-quadruplex remains unclear, because orientation of the four strands connected by the G-quartets is either antiparallel or parallel, which can be altered by the sequence and surrounding condition. Therefore, systematic and quantitative studies on the effect of molecular crowding not only on the antiparallel but also the parallel G-quadruplexes are required to reveal the structure and stability of DNA oligonucleotides under cell-like conditions.

Here we studied the effect of molecular crowding on the thermodynamics of an intermolecular parallel G-quadruplex formation of $[d(TG_4)_2]_4$ (Figure 1). It was demonstrated that molecular crowding conditions stabilized the parallel G-quadruplex. Moreover, the plots of stability ($\ln K_{obs}$) of the parallel G-quadruplex versus water activity ($\ln a_w$) demonstrated that water molecules were released through the parallel G-quadruplex formation. It is noteworthy release that the number of water molecules released through the parallel G-quadruplex formation was more than that released through the antiparallel G-quadruplex formation. The results showed quantitatively that water molecules were a critical participant in the formation of the parallel G-quadruplex as well as the antiparallel G-quadruplex.

MATERIALS AND METHODS

Material Preparations

Oligodeoxynucleotides used in this study were high performance liquid chromatography (HPLC) grade and were purchased from Hokkaido System Science (Sapporo, Japan). Single-strand concentrations of the DNA oligonucleotide were determined as described previously.[5]

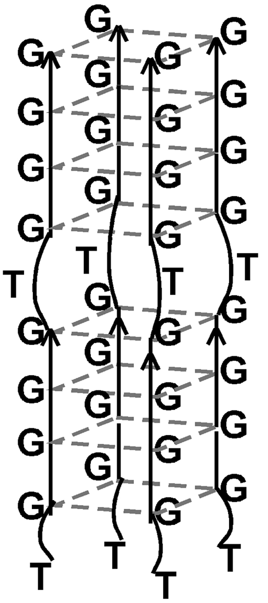

FIGURE 1 Schematic structure of $[d(TG_4)_2]_4$. Arrows indicate the directions of strands.

Water Activity Measurements

The water activity was determined by the osmotic stressing method via vapor phase osmometry using a model 5520XR pressure osmometer (Wescor, Utah, USA).[3]

Circular Dichroism (CD)

CD experiments utilizing a JASCO J-820 spectropolarimeter (JASCO, Hachioji, Japan) were measured as described previously.[5] All measurements were carried out at 4°C in a 0.1-cm path length cuvette for 20 μM total strand concentration of DNA in buffers of 100 mM NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA containing various concentrations of poly(ethylene glycol) with an average molecular weight of 200 (PEG 200).

Thermodynamic Analysis

The UV absorbance was measured with a Shimadzu 1700 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a temperature controller. Melting curves of the G-quadruplex were obtained by measuring the UV absorbance at 295 nm, in buffers containing 100 mM NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA containing various concentrations of PEG 200. The thermodynamic stability (ln K_{obs}) were calculated as reported previously.[6]

RESULTS

Sequence Design and Structural Analysis of a Parallel DNA G-Quadruplex Under Dilute and Molecular Crowding Conditions

In order to perform quantitative analysis, we need a guanine-rich DNA that folds into a parallel G-quadruplex structure via a two-state transition under various conditions. We tested $[d(TG_4)_2]_4$ (Figure 1) instead of $[d(TG_4T)]_4$, because the folding and unfolding kinetics of $[d(TG_4T)]_4$, which is one of the most popular sequences forming a parallel G-quadruplex, is slow, resulting in a non-two-state transition behavior of structure association and dissociation of $[d(TG_4T)]_4$.[6] Figure 2 shows CD spectra of $[d(TG_4)_2]_4$ at 4°C in the presence of Na^+ with 0, 10, 20, or 30 wt % of PEG 200. Both CD spectra had positive and negative peaks around 260 and 240 nm, respectively. In addition, the CD intensities at the peaks are almost identical under the conditions. These CD spectra showed that $[d(TG_4)_2]_4$ folded into a parallel G-quadruplex structure under the dilute and molecular crowding conditions.[5,8]

We examined the thermal denaturation and renaturation of the parallel G-quadruplex in the presence of Na^+ with 30 wt% PEG 200 or without PEG 200. The denaturation and renaturation behaviors traced with UV absorbance at 295 nm were similar in the conditions (data not shown), indicating the two-state transition of $[d(TG_4)_2]_4$ between a single strand and the parallel G-quadruplex that allows us to estimate thermodynamic parameters of the structure with the assumption of the two-state transition model.

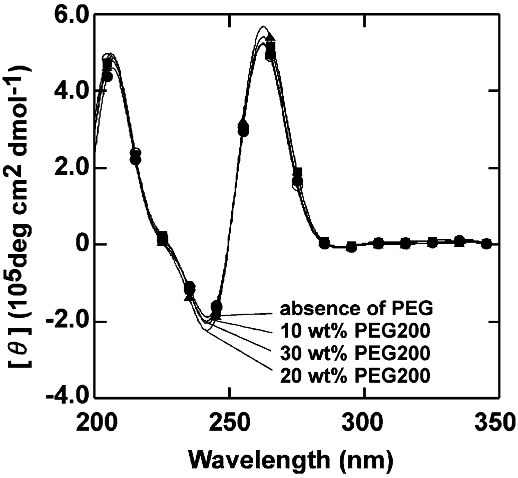

FIGURE 2 CD spectra of 20 μM $d(TG_4)_2$ in buffers consisting of 100 mM NaCl, 10 mM Na_2HPO_4 (pH 7.0), 1 mM Na_2EDTA in the absence (open circles) and presence of 10 wt% (closed circles), 20 wt% (closed triangles), or 30 wt% (closed squares) PEG 200 at 4°C.

Effect of Molecular Crowding on Thermodynamics of the Parallel G-Quadruplex Formation

We examined the thermal stability of the parallel G-quadruplex in the presence of various amounts of PEG 200. The melting temperature (T_m) of 5 μM [d(TG_4)$_2$]$_4$ increased from 30.5 to 44.0°C when the PEG 200 concentration increased from 0 to 30 wt% in the presence of Na^+ (Figure 3a). The T_m values showed that the molecular crowding condition with PEG 200 stabilized the parallel G-quadruplex as it was observed for the antiparallel G-quadruplex.[5] Although the molarity and strand orientation of the parallel G-quadruplexes are different from that of the antiparallel G-quadruplex, these results indicated that the molecular crowding stabilized both the parallel and antiparallel G-quadruplexes.

It was reported that a number of water molecules, Δn_w, released upon a structural formation can be estimated from the relationship between the equilibrium constant and water activity.[3] Thus, we investigated how water molecules affect the thermodynamic stability of the parallel G-quadruplex. At a constant temperature and pressure, the slope of the plot of the equilibrium constant for the structural formation (ln K_{obs}) against activity of water (ln a_w) approximately equals the number of water molecules released or taken up through the formation of the DNA structures, if the relationship between ln K_{obs} and ln a_w is linear.[3] Figure 3b shows a plot of stability (ln K_{obs}) of the parallel G-quadruplex structure at 25°C vs. water activity (ln a_w), demonstrating that the ln K_{obs} decreases linearly when the ln a_w increases.

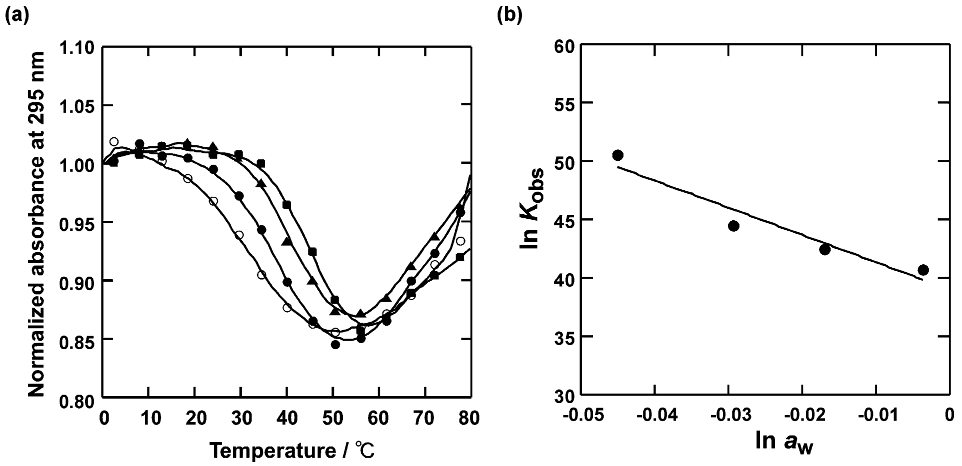

FIGURE 3 (a) Normalized UV melting curves at 295 nm for 5 μM d(TG_4)$_2$ in a buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH 7.0), 1 mM Na_2EDTA in the absence (open circles) and presence of 10 wt% (closed circles), 20 wt% (closed triangles), or 30 wt% (closed squares) PEG 200. (b) ln K_{obs} vs. ln a_w plot for the formation of the parallel G-quadruplex in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA containing 0, 10, 20, or 30 wt% PEG 200 at 25°C.

The slope of ln K_{obs} vs. ln a_w for the parallel G-quadruplex in the presence of Na^+ was estimated to be -233.3 ± 46.1. This value corresponds to the release of -5.8 ± 1.2 per nucleotide water molecules per G-quartet upon formation of the parallel G-quadruplex. Previously, we reported that the number of water molecules per G-quartet released upon formations of the antiparallel G-quadruplex was -4.0 ± 0.1 per nucleotide in the presence of Na^+.[5] These results demonstrated that water molecules were released during the formation of the parallel G-quadruplex, and that the parallel G-quadruplex released more water molecules through the structure formation than the antiparallel G-quadruplex.

DISCUSSION

Thermodynamics of the parallel G-quadruplex reported here and that of the antiparallel G-quadruplex reported previously[5] revealed that molecular crowding stabilized both parallel and antiparallel G-quadruplexes composed of Hoogsteen base pairs, whereas it destabilized the duplex composing of Watson-Crick base pairs.[3] Moreover, the linear relationship between ln K_{obs} vs. ln a_w for the parallel G-quadruplex suggested that the origin of stabilization of the parallel G-quadruplex was the dehydration through the structural formation. The dehydration of the G-quadruplex leads to stabilization of the structure under molecular crowding conditions, because the activity of water molecules and the number of water molecules around the DNA strands decrease. In the case of the G-quadruplex, the water molecules are released from the nitrogen and oxygen atoms of a guanine which are occupied by the cyclic Hoogsteen base pairs. Moreover, water molecules should be released from the cations because their full dehydration is required for their binding to G-quadruplexes.

We reported previously that molecular crowding induced a structural transition of telomere DNAs from an antiparallel to a parallel G-quadruplex.[8,9] Although a reason for the difference in hydrations of the parallel and antiparallel G-quadruplexes is still unclear, the numbers of water molecules released through the parallel and antiparallel G-quadruplexes suggested that the structural transition was induced by releasing water molecules. These results demonstrate that the water molecules released or taken up during the formation of the DNA structures are key factors in the thermodynamics under molecular crowding conditions.

REFERENCES

1. Zimmerman, S.B.; Minton, A.P. Macromolecular crowding: Biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, 22, 27–65.
2. Ellis, R.J.; Minton, A.P. Cell biology: Join the crowd. *Nature* **2003**, 425, 27–28.

3. Nakano, S.; Karimata, H.; Ohmichi, T.; Kawakami, J.; Sugimoto, N. The effect of molecular crowding with nucleotide length and cosolute structure on DNA duplex stability. *J. Am. Chem. Soc.* **2004**, 126, 14330–14331.
4. Williamson, J.R. G-quartet structures in telomeric DNA. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, 23, 703–730.
5. Miyoshi, D.; Karimata, H.; Sugimoto, N. Hydration regulates thermodynamics of G-quadruplex formation under molecular crowding conditions. *J. Am. Chem. Soc.* **2006**, 128, 7957–7963.
6. Pugilisi, J.D.; Tinoco, I., Jr. Absorbance melting curve of RNA. *Methods Enzymol.* **1989**, 180, 304–325.
7. Mergny, J.L.; De Cian, A.; Ghelab, A.; Sacca, B.; Lacroix, L. Kinetics of tetramolecular quadruplexes. *Nucleic Acids Res.* **2005**, 33, 81–94.
8. Miyoshi, D.; Karimata, H.; Sugimoto, N. Drastic effect of a single base difference between human and tetrahymena telomere sequences on their structures under molecular crowding conditions. *Angew. Chem. Int. Ed.* **2005**, 44, 3740–3744.
9. Miyoshi, D.; Nakao, A.; Sugimoto, N. Molecular crowding regulates the structural switch of the DNA G-quadruplex. *Biochemistry* **2002**, 41, 15017–15024.