DNA Structures

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Effect of Pressure on the Stability of G-Quadruplex DNA: Thermodynamics under Crowding Conditions**

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Noncanonical nucleic acid structures, such as G-quadruplexes, can regulate gene expression in cells.[1–3] It is not sufficient to evaluate the stabilities of these structures in dilute solution, because cells contain high concentrations of macromolecules and small-molecule effectors and are continually exposed to perturbations that may impact the structures of nucleic acids. To investigate the thermodynamics of nucleic acid structures under conditions that mimic those in cells, cosolutes have been used as so-called molecular crowding agents.[4–6] The thermal stabilities of various RNA and DNA structures under molecular crowding conditions differ from those in the absence of the cosolute.[7–11] For example, we have reported that crowding reagents like poly(ethylene glycol) (PEG) stabilize G-quadruplexes owing to changes in water activity and DNA hydration.[9–11] Furthermore, several kinds of the cosolutes may directly interact with nucleic acids and control their folding.[9–12]

Another informative approach to examine the properties of DNA in a living cell is through application of hydrostatic pressure. From a physicochemical point of view, pressure effects are mainly due to volumetric aspects resulting from the compressibility of the system. Several studies have revealed that the effects of high pressure on nucleic acids include changes in hydration,[13] protonation,[14] and hydrogen bonding[15,16] and that pressure may induce structural transitions.[17] Thermal stabilities of various nucleic acid structures have also been evaluated as a function of pressure.[18,19] It has been suggested that changes in hydration accompany the transition from coil to quadruplex.[20] It is possible that the volumetric characteristics of G-quadruplex DNA are also affected by molecular crowding agents. From the volumetric changes in the transition of G-quadruplex DNA, the molecular interaction of crowding reagents with DNA may be evaluated.

In this study, we investigated the effect of high pressure on the stability of G-quadruplex DNA in the presence of molecular crowding agents. We studied the thrombin binding aptamer (TBA; 5′-GGTTGGTGTGGTTGG-3′),[21] which folds into an intramolecular, antiparallel G-quadruplex structure in the presence of various monovalent and divalent cations and cosolutes.[22] Unlike other G-rich sequences such as the human telomeric sequence, TBA does not show structural polymorphism under different solution conditions.[10,23] Thus, TBA is an appropriate model for the investigation of the thermodynamics of G-quadruplex formation in crowding conditions under various pressures. UV melting experiments were performed under pressures from 0.1 MPa (ambient pressure) to 400 MPa in solution containing tris(hydroxymethyl)aminomethane (Tris-HCl; 30 mM, pH 7.0) and KCl (100 mM, see the Supporting information). Figure 1a shows typical UV melting curves of TBA assessed

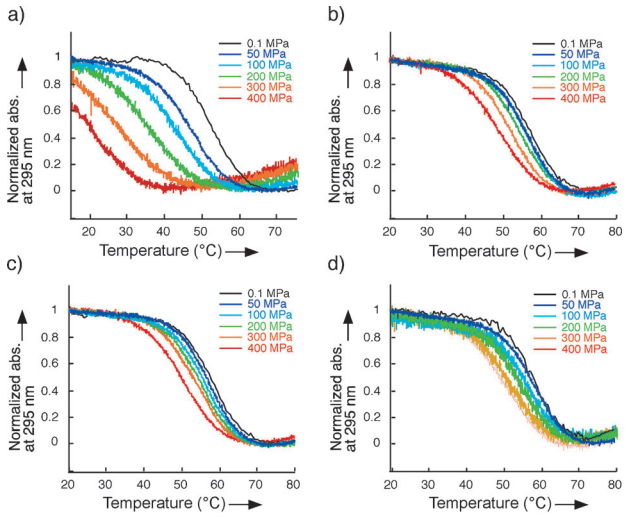

Figure 1. Effect of cosolute on the transition of TBA (40 μM) from a quadruplex to a coil form under various pressures. UV melting curves were obtained a) in the absence of cosolute or in the presence of b) 40 wt% EG, c) 40 wt% PEG200, and d) 40 wt% PEG4000. The changes of absorbance at 295 nm were analyzed under atmospheric pressure (0.1 MPa), 50, 100, 200, 300, and 400 MPa. Each solution was buffered with Tris-HCl (30 mM, pH 7.0) and KCl (100 mM).

by monitoring absorbance at 295 nm as a function of temperature under ambient pressure and 50, 100, 200, 300, and 400 MPa. At ambient pressure, the UV absorbance decreased with increasing temperature as reported.[10,24] We also confirmed the validity for the two-state approximation analysis by CD melting (Figure S1 in the Supporting Information). As

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pressure was increased, the melting curves shifted significantly toward lower temperatures. The transition from a quadruplex to a coil occurred at less than 20 °C under 400 MPa. Thus, applying high pressure to G-quadruplex DNA induced the unfolding of the quadruplex. This result is in agreement with data from a previous study of the effect of high pressure on the structural transition of human telomeric oligonucleotide in the presence of Na^+ ions.[20] In contrast, the UV melting curve of the hairpin DNA under 400 MPa was not significantly different from that under ambient pressure (Figure S2 in the Supporting Information) as reported.[25]

Next we evaluated the effect of crowding reagents on the denaturation of a G-quadruplex under high pressure. Solutions containing ethylene glycol (EG), PEG200 (PEG with an average molecular weight [M_w] of 200), and PEG4000 (PEG with M_w of 4000) were employed. EG is a monomer unit of PEG, and these three reagents are nonionic. Each destabilizes Watson–Crick base pairs but stabilizes Hoogsteen base pairs owing to changes in water activity and DNA hydration.[9–12] Figure 1b shows normalized UV melting curves for TBA (40 μM) in the presence of EG (40 wt %) under indicated pressures. Interestingly, in the presence of EG, the melting curves did not dramatically shift toward lower temperature with elevating pressure as was observed in the absence of the cosolute. With PEG200 or PEG4000 as cosolute, the UV melting curves shifted slightly toward lower temperature as pressure was increased (Figure 1c, d). The effect of cosolute on the melting process under high pressure was similar in the case of human telomeric DNA (Figure S3 in the Supporting Information). The thermodynamic parameters for the formation of G-quadruplex were obtained from the thermal melting curves, and results are summarized in Tables 1 and 2 and Table S1 in the Supporting Information. The melting temperature (T_m) of TBA at 400 MPa was 18.3 °C, which was 34.3 °C lower than that at ambient pressure. In the presence of EG (40 wt %), the T_m under ambient pressure was only 5.5 °C higher than that in the absence of cosolute, but at 400 MPa, the T_m was 32.8 °C higher than that in the absence of cosolute, and only 7.0 °C lower than the value under ambient pressure. In the presence of 40 wt % PEG200 or PEG4000, T_m values decreased from 59.3 to 51.4 °C and from 60.6 to 52.9 °C, respectively, when ambient pressure and 400 MPa were compared. When the pressure in the absence of the cosolute increased from 0.1 to 400 MPa, the values of $\Delta H°$, $T\Delta S°$, and $\Delta G°_{25}$ of TBA DNA increased (Table 2 and Table S1 in the Supporting Information). These changes indicate that inhibition of G-quadruplex formation at high pressure is dominated by an enthalpic contribution more than entropic contribution. In EG, the values of $\Delta H°$, $T\Delta S°$, and $\Delta G°_{25}$ increased with pressure, although these increments were smaller than those in the absence of cosolute. These results indicated that high pressure perturbed stacking interactions or hydrogen bonding of DNA,[15] and the crowding conditions countered the effect of elevated pressure.

The T_m values of G-quadruplex DNA in the absence of the cosolute decreased linearly with increasing pressure (Figure 2). By employing the Clapeyron equation to the correlation between T_m and pressure,[22,25] we calculated the volume change during the transition, ΔV_{tr}, from the single strand to the folded quadruplex structure (see the Supporting Information). The value of ΔV_{tr} for TBA in the absence of cosolute was determined to be $(54.6 \pm 4.2)\ cm^3 mol^{-1}$ (Table 3). The positive value suggests that the formation of a quadruplex from a coiled form is accompanied by dehydration. This value was much larger than those determined for formation of the duplex form of calf thymus DNA, which is in

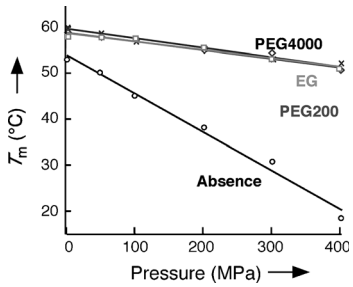

Figure 2. Dependencies of T_m for G-quadruplex DNA on pressure in the presence of EG (□), PEG200 (◇), PEG4000 (x), and in the absence of cosolute (○).

Table 1: The effect of pressure on the melting temperature of G-quadruplex DNA in the absence and presence of cosolute.

Pressure [MPa]	T_m [°C]			
	Absence of cosolute	EG	PEG200	PEG4000
0.1	52.6 ± 3.4	58.1 ± 1.4	59.3 ± 2.3	60.6 ± 3.2
50	50.4 ± 5.1	58.0 ± 3.0	58.4 ± 1.3	59.5 ± 2.1
100	45.1 ± 2.6	57.7 ± 2.0	58.1 ± 1.9	57.7 ± 2.5
200	38.2 ± 2.0	56.1 ± 3.0	56.1 ± 1.2	56.0 ± 3.3
300	30.8 ± 1.2	53.6 ± 3.6	54.9 ± 1.8	53.3 ± 4.4
400	18.3 ± 1.9	51.1 ± 3.9	51.4 ± 4.3	52.9 ± 2.5

Table 2: Thermodynamic parameters for G-quadruplex formation.

Pressure [MPa]	Cosolute	$\Delta H°$ [kcal mol^{-1}]	$T\Delta S°$ [kcal mol^{-1}]	$\Delta G°_{25}$ [kcal mol^{-1}]
0.1	(absence)	−50.4 ± 2.2	−46.1 ± 2.1	−4.2 ± 0.1
0.1	EG	−54.5 ± 0.9	−49.0 ± 0.6	−5.2 ± 0.2
0.1	PEG200	−55.1 ± 1.6	−49.4 ± 1.4	−5.6 ± 0.1
0.1	PEG4000	−52.4 ± 2.0	−46.8 ± 1.8	−5.6 ± 0.1
400	(absence)	−20.1 ± 1.7	−21.4 ± 1.7	0.4 ± 0.1
400	EG	−39.0 ± 2.1	−35.9 ± 1.9	−3.1 ± 0.2
400	PEG200	−37.0 ± 2.2	−34.0 ± 2.0	−3.0 ± 0.1
400	PEG4000	−44.7 ± 1.5	−40.9 ± 1.3	−3.8 ± 0.1

Table 3: The value of $\Delta T_m/\Delta P$ and the molar volume change ΔV_{tr} of the transition for G-quadruplex DNA in the presence and absence of cosolute.

Cosolute	$\Delta T_m/\Delta P$ [10^{-2} K MPa^{-1}]	ΔV_{tr} [cm^3 mol^{-1}]
absence	−8.4 ± 0.5	54.6 ± 4.2
EG	−1.8 ± 0.2	12.5 ± 0.8
PEG200	−1.9 ± 0.2	12.9 ± 0.9
PEG4000	−2.0 ± 0.2	13.1 ± 1.0

the range of less than 5 $cm^3 mol^{-1}$,[26] but comparable to the 56 $cm^3 mol^{-1}$ determined for formation of the human telomeric oligonucleotide G-quadruplex structure in the presence of NaCl (100 mM).[21] In the presence of EG (40 wt%), the slope of $\Delta T_m/\Delta P$ was smaller than that in the absence of cosolute, T_m values linearly decreased with increasing pressure, and ΔV_{tr} was calculated as 12.5 $cm^3 mol^{-1}$. Therefore, the transition volume of TBA in the presence of EG was about one fourth as large as that in the absence of cosolute. With PEG200 as the cosolute, the value of ΔV_{tr} was calculated to be 12.9 $cm^3 mol^{-1}$, which was comparable to that obtained in the presence of EG. A similar value (13.1 $cm^3 mol^{-1}$) was obtained when PEG4000 was the cosolute. The transition volume ΔV_{tr} can be calculated as follows:[16,17]

$$\Delta V_{tr} = \Delta V_M + \Delta V_T + \Delta V_I + \Delta V_{K^+} \quad (1)$$

where ΔV_M is the intrinsic volume change of the DNA, ΔV_T is the thermal volume change indicating the change of the void space of the DNA, ΔV_I is the interaction volume change (i.e., hydration volume change), and ΔV_{K^+} is the partial molar volume of one potassium ion. We previously found that the structure of the G-quadruplex of TBA was not altered by the presence of PEG200.[10] We hypothesize that the crowding reagent does not affect the structure-dependent volume of TBA DNA and that ΔV_M, ΔV_T, and ΔV_{K^+} are the same in the absence or presence of crowding reagents because these parameters can be calculated from a given structure.[20] ΔV_I reflects water expansion around polar and charged groups of DNA during transition and is presented as:

$$\Delta V_I = n_w(V_h - V_0) + n_{cs}(V_{cs} - V_{cs0}) \quad (2)$$

where n_w is the number of waters of hydration, V_h is the change in partial molar volume of water of hydration, and V_0 is the change in bulk water.[20] And, n_{cs} is the number of cosolute molecules bound on DNA, V_{cs} is the partial molar volume of cosolute of solvation, and V_{cs0} is the partial molar volume of cosolute in bulk. In the presence of EG (40 wt%), the partial molar volume of water should be slightly smaller (by less than approximately 3%) than that in the absence of the cosolute.[27] Thus, to explain the smaller ΔV_{tr} in the crowding condition, the number of waters of hydration and/or V_h should be much smaller than in the absence of crowding agent. The number of waters of hydration are likely smaller owing to EG binding to DNA because we have reported the cosolutes with hydroxy groups displace water from DNA.[9–11] Therefore, EG reduces the number of hydrating waters or the changes of partial molar volume of hydrating waters on DNA, which mitigates the effect of high pressure on the unfolding of G-quadruplex DNA. The decrease of the V_{cs} value may affect the ΔV_I value, but it is difficult to estimate the contribution of the reduction of V_{cs} because of the lack of information about the V_{cs} value on DNA.

Solutions containing PEG show a larger activity of water than solutions of EG, and we have previously concluded that PEG derivatives hardly bind to DNA.[9–11] The decrease of bulk water volume V_0 in the presence of PEG is very small as it was for EG.[28] Therefore, it was possible that the hydration term affects only the ΔV_I value in the presence of PEG. We hypothesize that the presence of PEG decreases V_h. PEG derivatives exhibit a larger excluded volume for DNA than EG.[29] This volume effect could cause the isolation of hydrating water from water possibly related with the second or third layer of hydration, which may induce a shorter radius of hydrating water because of strong binding to DNA. Another possibility is that PEG interacts with DNA in a different manner from EG under high pressure conditions.[30] This change might induce the binding of PEG to DNA and a decrease in the number of hydrating water molecules (n_w).

The structural transitions from duplexes to quadruplexes are important in not only the mechanism of biological reactions[31–33] but also DNA nanomaterials.[34,35] In order to use pressure to control DNA conformation, we tested whether the structural transition between G-quadruplex and duplex is switchable by high pressure (Figure 3a,b). For the

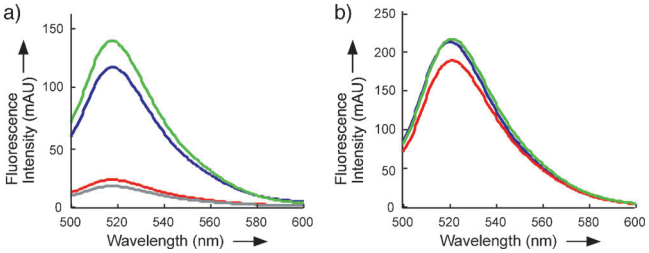

Figure 3. Regulation of the transition from quadruplex to duplex by pressure. a) Fluorescence intensities of the solution with G strand and C strand annealed under ambient pressure (blue), annealed under 400 MPa followed by release of pressure (red), re-annealed under ambient pressure (green), or annealed under the ambient pressure in the absence of KCl (gray). b) Fluorescence intensities of the solution with G strand and C strand in the presence of PEG4000 (40 wt%) annealed under ambient pressure (blue), annealed under 400 MPa followed by release of pressure (red), and re-annealed under ambient pressure (green). Each solution was buffered with Tris-HCl (30 mM, pH 7.0) and KCl (10 mM).

switching materials, two sequences found in *Oxytricha* telomeres, $d(G_4T_4)_3G_4$ (G strand) and $d(C_4A_4)_3C_4$ (C strand), were employed. The G strand folds into an antiparallel G-quadruplex, the structure of which is similar to that adopted by TBA. The two strands can work as a DNA logic gate based on the polymorphism responding to chemical signals.[34] To allow monitoring of the conformations of the strands, the 5′ terminus of the C strand was modified with Alexa Fluor 488, whereas the 3′ terminus of the G strand was modified with the quencher Dabcyl. Strong fluorescence should be observed when the G strand forms a G-quadruplex, whereas duplex formation of a duplex between the G strand and the C strand should result in quenching of the fluorescence (Figure S5 in the Supporting Information). Application of 400 MPa to the G strand in the presence of KCl (10 mM) inhibited G-quadruplex formation as indicated by a decrease in UV absorbance relative to ambient pressure (Figure S4a,b in the Supporting Information). In the same buffer, strong fluorescence of the mixture of both strands was observed at

518 nm after annealing under ambient pressure, which indicated that the G-quadruplex was preferentially formed despite the presence of C strand (Figure 3a, blue line). The mixture of strands was heated to 90 °C and cooled to room temperature at 400 MPa. After releasing the pressure, the fluorescence of the mixture was quenched, similar to the fluorescence of the mixture annealed in the absence of KCl (Figure 3a, red and gray lines). This result suggested that a duplex was formed during the annealing process under 400 MPa and fixed at room temperature even at ambient pressure. At 400 MPa, the quadruplex should be destabilized more than the duplex. After the pressure was released, the duplex form was stable for at least 4 h (Figure S6 in the Supporting Information), which indicated that the duplex was stable under the ambient conditions. The fluorescence was recovered completely after re-annealing under ambient pressure, thus indicating reformation of the G-quadruplex (Figure 3a, green line).

In the presence of PEG4000 as a cosolute, the fluorescence intensity did not change when the mixture of strands was subjected to high pressure. This indicated that the pressure effect was decreased by PEG4000 (Figure S4c, d) and the presence of cosolute inhibited the formation of duplex under high pressure (Figure 3b). These results suggest that the generation of high pressure within a living cell would favor G-quadruplex formation in G-rich genomic DNA strands. Therefore, the biological events based on the duplex to G-quadruplex transition such as transcription, recombination, and telomerase reactions can be controlled by pressure. Since the crowding conditions vary during the cell cycle,[4] the stabilization of G-quadruplex structures will depend on both cellular conditions and pressure.

In conclusion, our analyses demonstrated that G-quadruplex DNA can be unfolded by elevating pressure, but the effect of pressure is considerably diminished in the presence of crowding agents. This result indicated that coordinating water around DNA or the structure of bulk water affects unfolding of the G-quadruplex. These findings indicate that structural transitions can regulate gene expression. For example, DNA structures in a cell may be perturbed by local fluctuations of pressure owing to cell movement and division, although these pressure changes occurring in living cells seem to be smaller than those observed in this work.[36] However, the human stress sensor protein Ras showed a relatively small magnitude of transition volume of its reaction for the stress-signaling.[37] This fact indicates that a relatively high pressure as observed in this work is needed to facilitate the Ras reaction. If Ras works well as a stress sensor even at atmospheric pressure, G-quadruplexes should play a role against pressure stress occurring in a relatively mild environment. Enzyme-catalyzed reactions such as replication and transcription may occur in the highly structured region of G-rich sequences with the help of relatively low pressure, as observed in the reaction by some enzymes, which disrupt the proteins bound on DNA while translocating along DNA.[38] Therefore, the effect of pressure on quadruplex DNA in living cells may happen even at relatively low pressure stress, at most 100 MPa, which is an acceptable pressure for living cells on earth. In crowded conditions, G-quadruplexes are resistant to pressure-induced unfolding. However, changes in the crowding of the local environments caused by events such as cell cycle transitions may result in pressure-induced structural changes of genomic DNA structures. The ability to selectively perturb G-quadruplex stability by pressure will also provide a novel strategy for the control of DNA sensors and nanostructures.

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