Pressure-dependent formation of i-motif and G-quadruplex DNA structures under high pressure

*Shuntaro Takahashi and Naoki Sugimoto**

Frontier Institute for Biomolecular Engineering Research (FIBER) and Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University,

7-1-20 Minatojima-minamimachi, Kobe 650-0047, Japan

Fax: (+) 81-78-303-1495

E-mail: sugimoto@konan-u.ac.jp

Supporting Information

Materials and Methods

Oligonucleotides and reagents

All oligonucleotides were purchased from Japan Bio Service (Saitama, Japan). DNA oligonucleotides 5'-dCGG$(CCT)_{10}$CGG-3', 5'-dC$_3$T$(A_2C_3T)_3$-3', and 5'-d$(C_4A_4)_3C_4$-3' form intramolecular i-motif structures. 5'-d$(AG_3T_2)_3AG_3$-3' and d$(G_4T_4)_3G_4$ forms an intramolecular G4 structure. For the UV melting analysis, 20 μM of DNA was dissolved in 10 mM Na_2HPO_4, 1 mM Na_2EDTA. pH was adjusted by adding HCl at atmospheric pressure. The 5'-[Alexa Fluor 488]-modified C strand and the 3'-[Dabcyl]-modified G strand used for the fluorescence experiments were purchased from Japan Bio Service. All DNA oligonucleotides were purified by HPLC by the manufacturer, and the concentration of the DNA was determined by measuring the absorbance at 260 nm at 90 ^{o}C using a Shimadzu 1700 spectrophotometer connected to a thermoprogrammer. Before each experiment, DNA samples were heated at 95 °C for 5 min and gradually cooled at 1 °C/min. Ethylene glycol (monomer, EG) and other chemicals were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and were used without further purification.

Apparatus for analysis of UV spectroscopy under high pressure

The system for generating high pressure (maximum 400 MPa = 4 kbar) connected to an optical unit was

built by Syn Corporation (Kyoto, Japan). This system was installed in a Shimadzu UV-1700 spectrometer. The temperature was controlled using an F25-ME refrigerator and heater circulator from Julabo (Seelbach, Germany). To maintain constant pressure, a PV-400 pressure reservoir (Syn Corporation) was used during UV melting experiments.

UV melting analyses

For the melting temperature analyses, the absorbance at 295 nm was recorded every 6 seconds using PC probe software (Shimadzu). Melting was monitored at 100, 200, 300, and 400 MPa. DNAs were dissolved at 20 μM strand concentration in 10 mM Na_2HPO_4 (pH 6.0 or 7.0) and 1 mM Na_2EDTA. The temperature of the high pressure cell was recorded and regulated using the Julabo Easy TEMP professional software. The temperature was increased from 20 °C to 80 °C at a rate of 0.5 °C min^{-1}. The UV melting curves were normalized and analyzed by curve fitting to determine thermodynamic parameters using KaleidaGraph (Synergy Software). For melting analyses as a function of pressure, the absorbance of 20 μM $(CCT)_{10}$ DNA at 295 nm was recorded at 25 °C and 50 °C every 50 MPa from ambient pressure to 400 MPa. The sample was incubated 10 min after each pressure change prior to recording of the absorbance.

Structural transitions in solutions of duplex, i-motif and G4 as a function of pressure

Solutions containing 100 nM Alexa Fluor 488 modified C-strand (C_4A_4 or cHT DNA) Dabcyl modified G-strand (G_4T_4 or HT DNA) in 10 mM Na_2HPO_4 (pH 5.5) and 1 mM Na_2EDTA were prepared. As for C_4A_4 and G_4T_4 case, we also analyzed in 10 mM K_2HPO_4 (pH 5.5) and 1 mM K_2EDTA. The same volumes of each solution were mixed and heated at 90 °C for 5 min at each pressure. After measuring fluorescence, solutions were cooled to 37 °C at a rate of 0.5 °C min^{-1} while recording fluorescence intensities at 508 nm (excitation at 480 nm). To normalize the results, the intensities were divided by intensity measured at 90 °C in each pressure condition. The normalized value of "0" means complete duplex formation, whereas "1" means there is no duplex. To obtain the percentages of the population of G4 and i-motif in each temperature, each normalized UV melting curve was multiplied by the normalized fluorescence data at each temperature. Fluorescence measurements were carried out on FP-6500 spectrofluorometer (JASCO). For the analysis of the effect of cosolute, 40 wt% EG was added to each oligonucleotide solution prior to mixing of the strands, and other procedures were the same as the analysis without EG. Annealing analyses were carried out using the procedure described for melting of $(CCT)_{10}$ DNA. The percentages of each structured species were estimated from each melting curve at 37 °C.

Figures and Tables

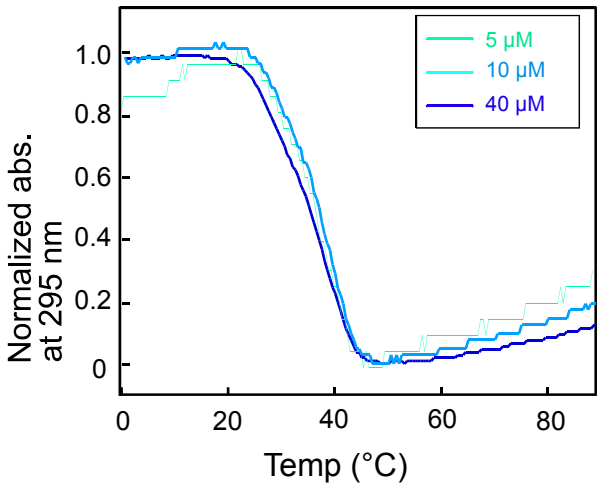

Figure S1. UV melting curves of 5, 10, and 40 μM $(CCT)_{10}$ DNA at atmospheric pressure in a solution of 10 mM NaH_2PO_4 (pH 6.0) and 1 mM Na_2EDTA.

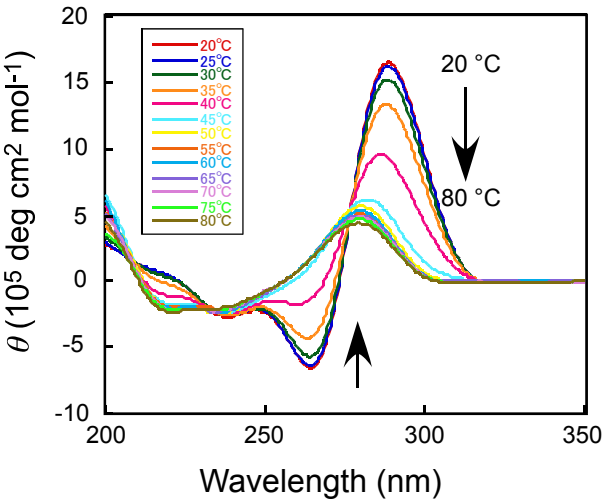

Figure S2. Dependence of CD profile of 20 μM of $(CCT)_{10}$ DNA on temperature at atmospheric pressure. The CD spectrum was measured every 5 °C between 20 °C and 80 °C.

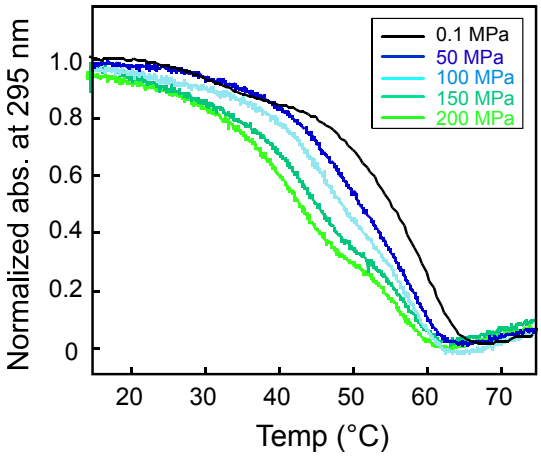

Figure S3. UV melting behavior of G4 forming sequence from G_4T_4 DNA under high pressure. The changes of absorbance at 295 nm of 20 μM G_4T_4 DNA under 0.1 MPa (black), 50 MPa (blue) 100 MPa (light blue), 150 MPa (light green), and 200 MPa (green).

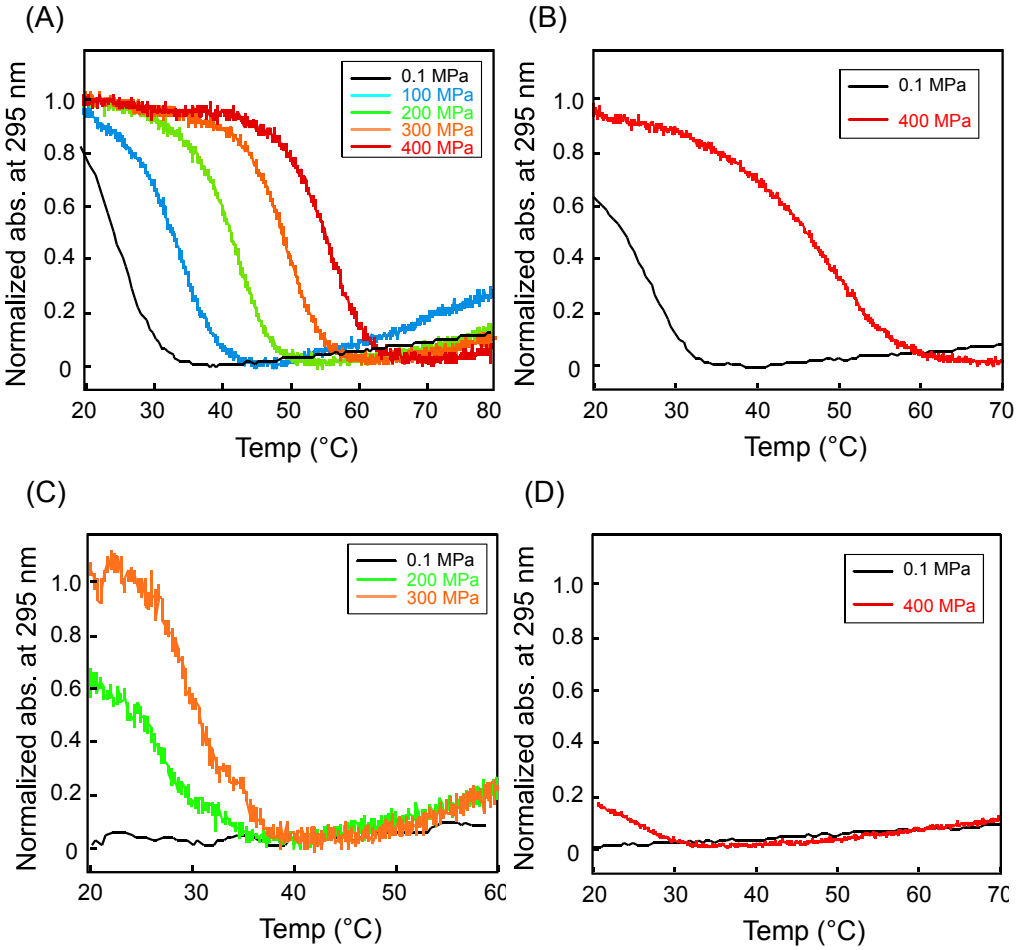

Figure S4. UV melting behavior of i-motif forming sequence under high pressure. (A) The changes of absorbance at 295 nm of C_4A_4 DNA buffered with 10 mM Na_2HPO_4 and 1 mM Na_2EDTA, pH 6.0 (adjusted at 0.1 MPa). (B) The changes of absorbance at 295 nm of cHT DNA buffered with 10 mM Na_2HPO_4 and 1 mM Na_2EDTA, pH 6.0 (adjusted at 0.1 MPa). (C) The changes of absorbance at 295 nm of $(CCT)_{10}$ DNA buffered with 10 mM Na_2HPO_4 and 1 mM Na_2EDTA, pH 7.0 (adjusted at 0.1 MPa). (D) The changes of absorbance at 295 nm of cHT DNA buffered with 10 mM Na_2HPO_4 and 1 mM Na_2EDTA, pH 7.0 (adjusted at 0.1 MPa). Melting was analyzed under 0.1 MPa (black), 100 MPa (light blue), 200 MPa (green), 300 MPa (orange), and 400 MPa (red).

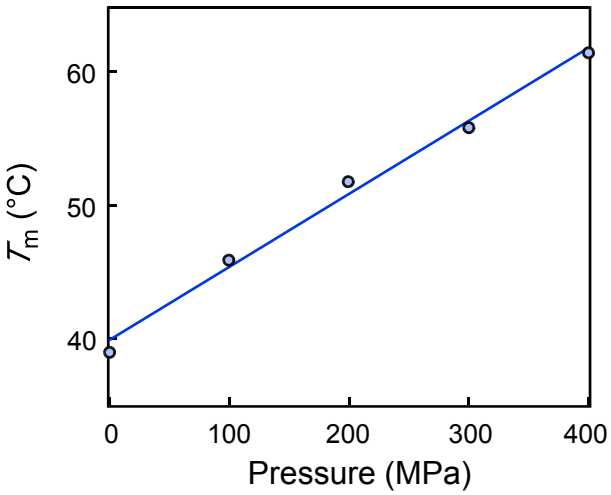

Figure S5. Dependence of T_m of $(CCT)_{10}$ on pressure.

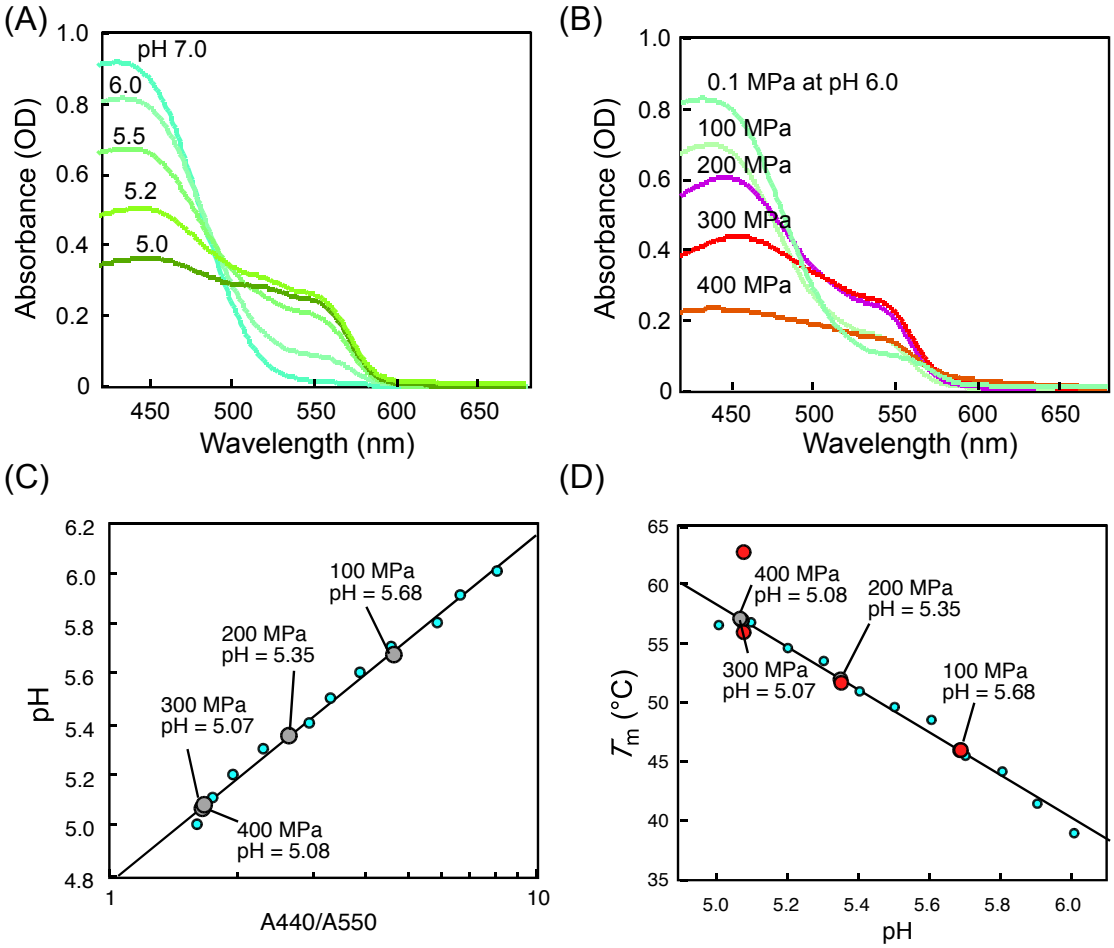

Figure S6. Ratio of absorbance at 440 nm to that at 550 nm of methyl red in a solution containing 10 mM Na_2HPO_4 and 1 mM Na_2EDTA (A) at pH 5.0, 5.2, 5.5, 6.0 and 7.0 at 0.1 MPa and (B) at pH 6.0 under atmospheric pressure, 100, 200, 300, and 400 MPa. (C) Dependence of the pH on the ratio of absorbance (A_{440}/A_{550}) of methyl red. Gray plot indicates the predicted pH value under each pressure. (D) Dependence of the T_m on the value of pH. Gray plot indicates the predicted pH value under each pressure. The measured values were overlaid as red plots.

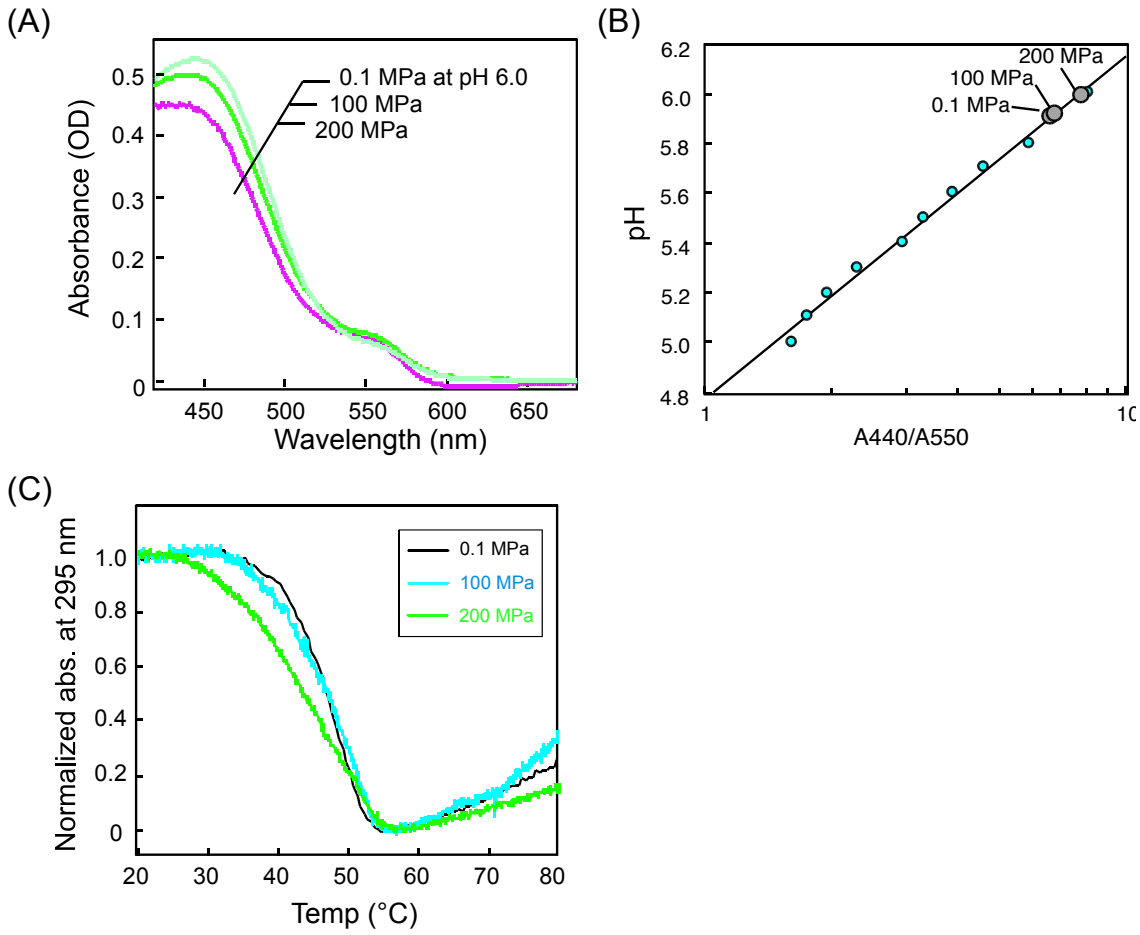

Figure S7. (A) Ratio of absorbance at 440 nm to that at 550 nm of methyl red in a solution containing 10 mM MES and 1 mM Na_2EDTA pH 6.0 (adjusted at 0.1 MPa) under 0.1, 100, and 200 MPa. (B) Dependence of the pH on the ratio of absorbance (A_{440}/A_{550}) of methyl red. Gray plot indicates the point from each A_{440}/A_{550} value in (B) corresponding to the liner regression line in Fig. S6C. (C) Typical UV melting curves of $(CCT)_{10}$ DNA in 10 mM MES and 1 mM Na_2EDTA pH 6.0 (adjusted at 0.1 MPa) under 0.1, 100, and 200 MPa.

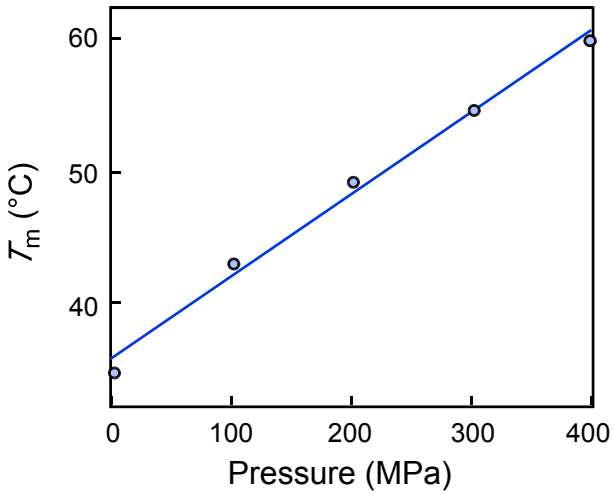

Figure S8. Dependence of the T_m of $(CCT)_{10}$ on pressure in the presence of 40 wt% EG.

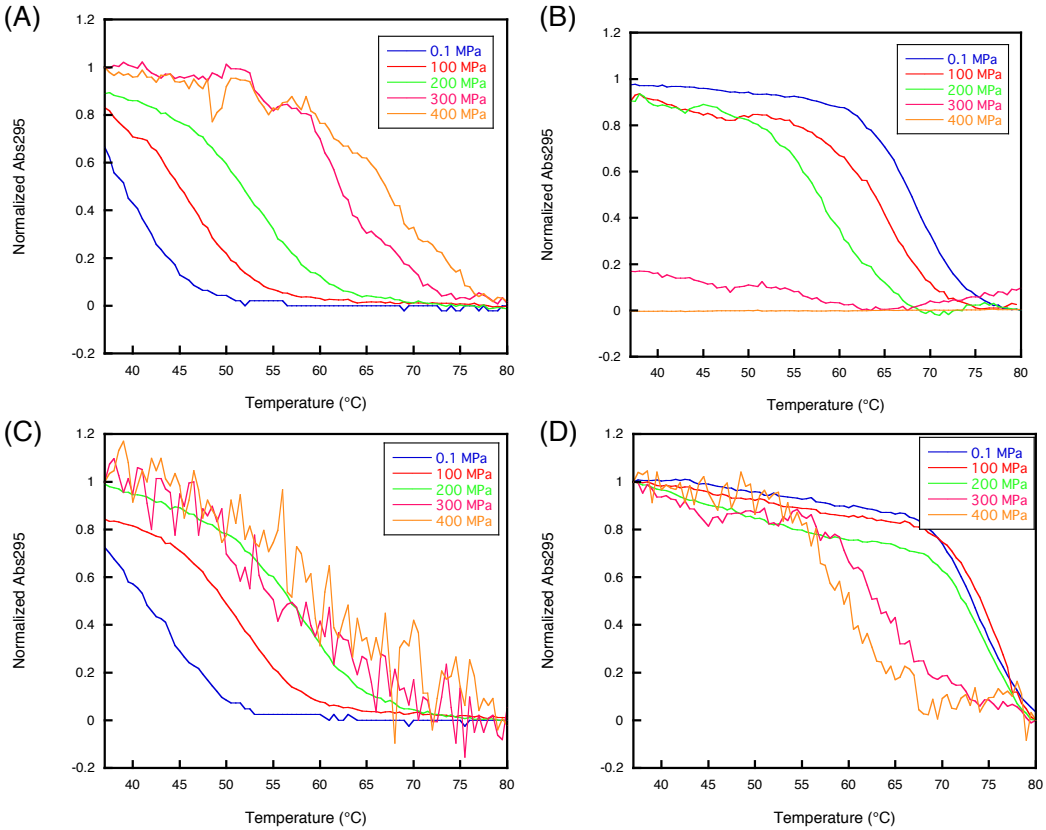

Figure S9. Normalized UV melting curves of (A) C_4A_4 in the absence of EG, (B) G_4T_4 in the absence of EG, (C) C_4A_4 in the presence of 40 wt% EG, and (D) G_4T_4 in the presence of 40 wt% EG. UV changes were recorded at 0.1 MPa (blue), 100 MPa (red), 200 MPa (green), 300 MPa (pink), and 400 MPa (orange). The buffer condition was 10 mM Na_2HPO_4 and 1 mM Na_2EDTA at pH 5.5.

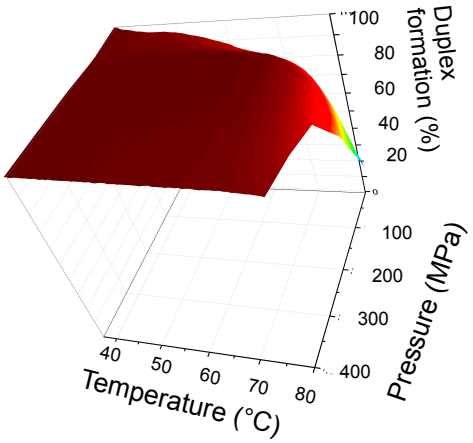

Figure S10. *P*-*T* stability diagrams of the normalized ratio of duplex formation by G_4T_4 strand, and C_4A_4 strand in 30 mM Tris-HCl pH 7.5 and 100 mM LiCl.

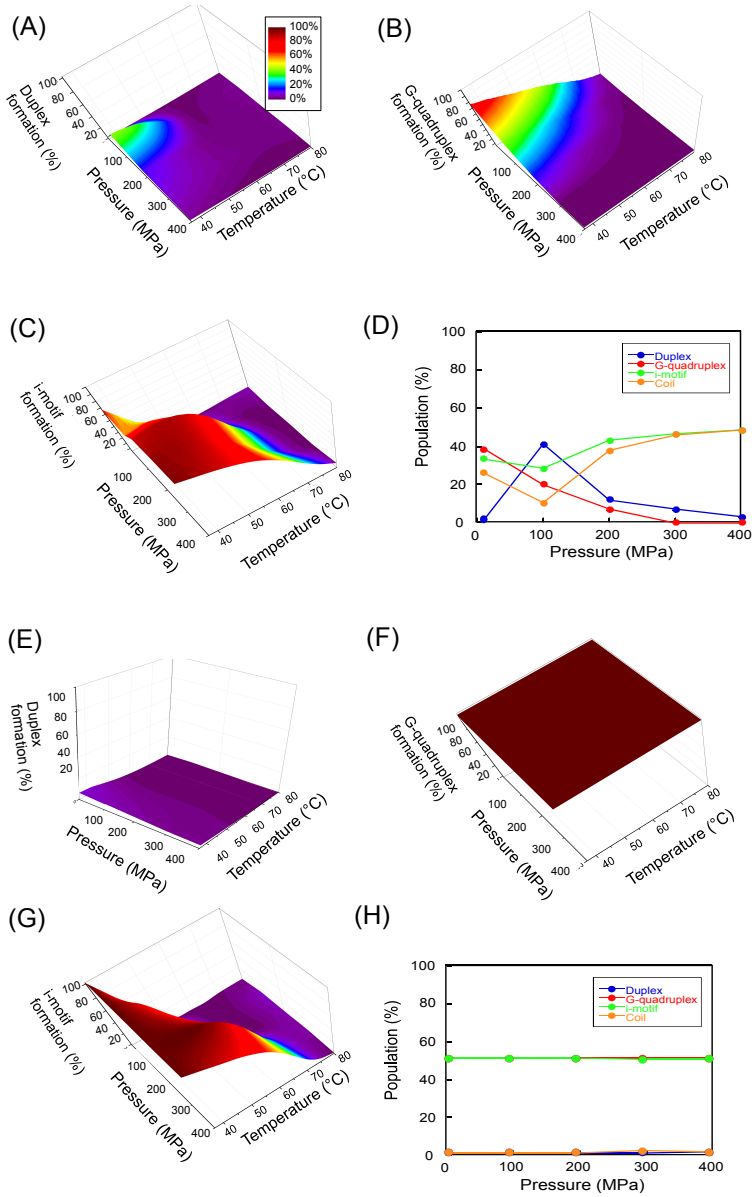

Figure S11. Transitions of DNA structures driven by pressure and temperature. (A-C) *P-T* stability diagrams of the normalized ratio of (A) duplex formation, (B) G-quadruplex formation by the G_4T_4 strand, and (C) i-motif formation by C_4A_4 strand in 10 mM K_2HPO_4 and 1 mM K_2EDTA at pH 5.5. (D) Percent of the population in duplex (blue), G-quadruplex (red), i-motif (green), and coil (orange) as a function of pressure at 37 °C in 10 mM Na_2HPO_4 and 1 mM Na_2EDTA at pH 5.5. (E-G) *P-T* stability diagrams of the normalized ratio of (E) duplex formation, (F) G-quadruplex formation, and (G) i-motif formation in 10 mM K_2HPO_4 and 1 mM K_2EDTA at pH 5.5 in 40 wt% EG. (H) Populations of duplex (blue), G-quadruplex (red), i-motif (green), and coil (orange) as a function of pressure at 37 °C in the presence of 40 wt% EG. In *P-T* diagrams, the color gradient indicates the percent of the population that adopts the structure.

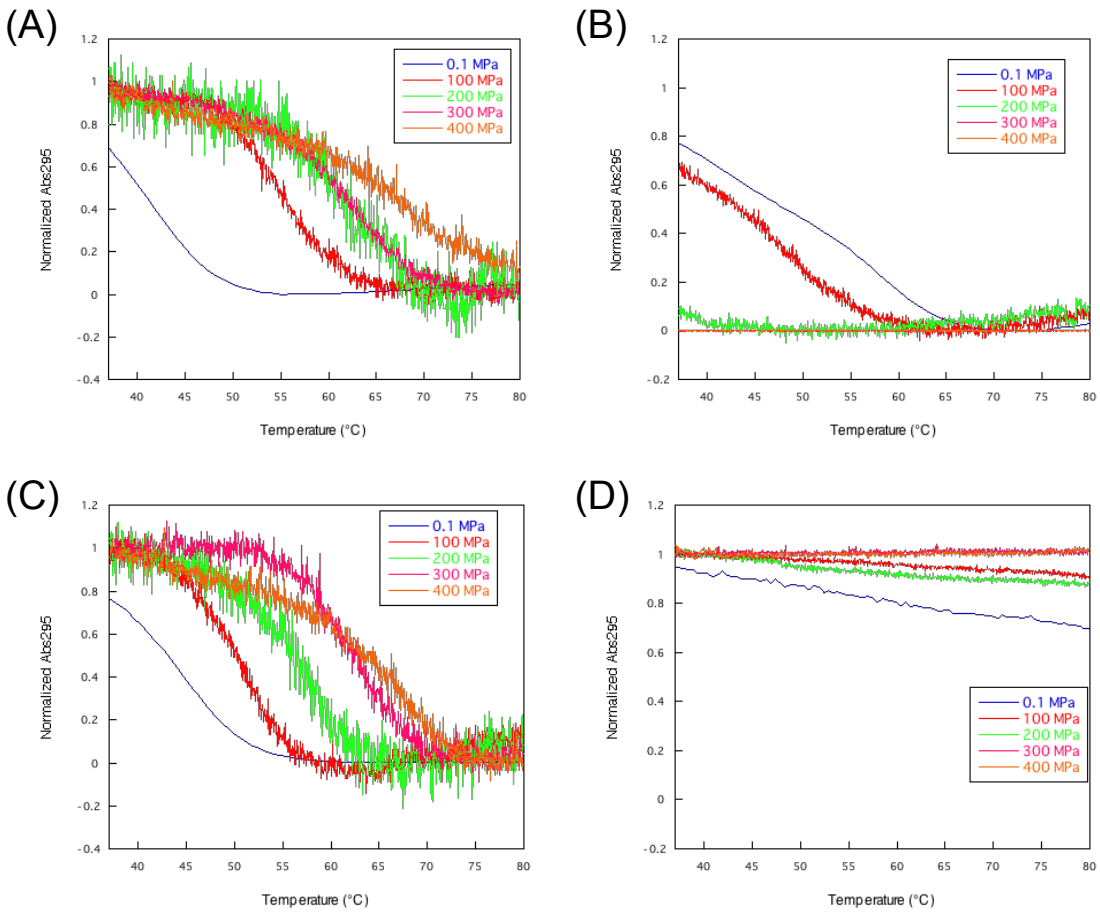

Figure S12. Normalized UV melting curves of (A) C_4A_4 in the absence of EG, (B) G_4T_4 in the absence of EG, (C) C_4A_4 in the presence of 40 wt% EG, and (D) G_4T_4 in the presence of 40 wt% EG. UV changes were recorded at 0.1 MPa (blue), 100 MPa (red), 200 MPa (green), 300 MPa (pink), and 400 MPa (orange). The buffer condition was 10 mM K_2HPO_4 and 1 mM K_2EDTA at pH 5.5.

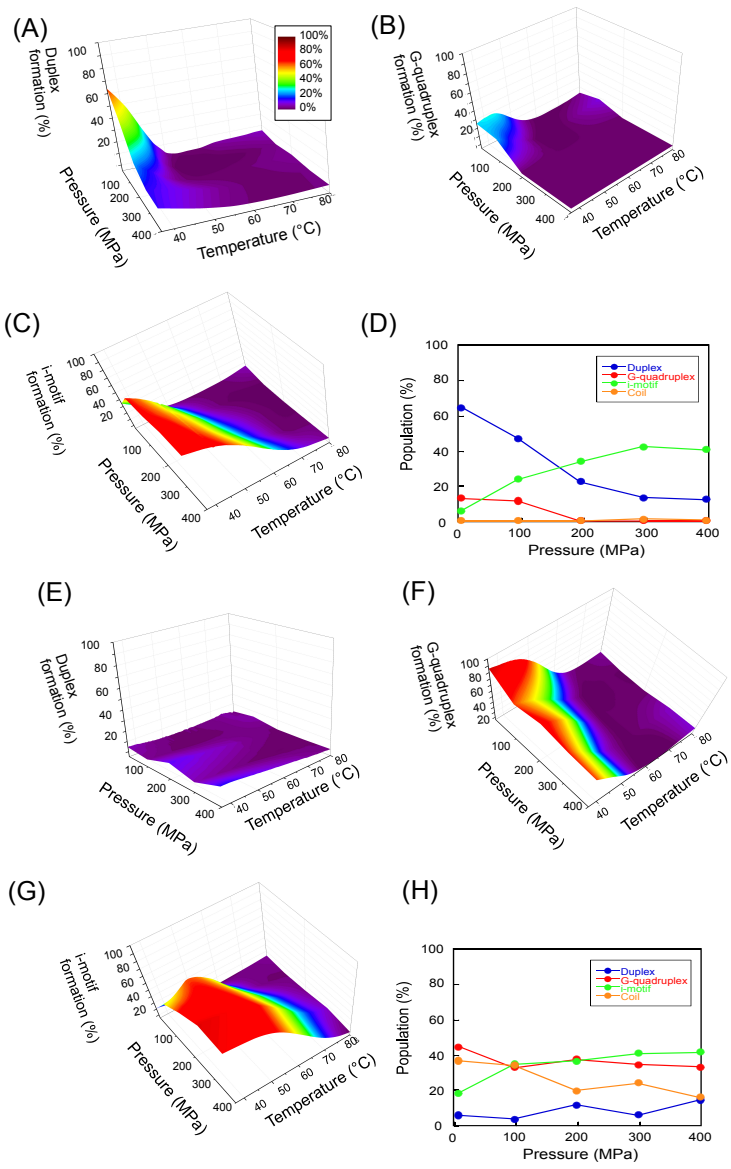

Figure S13. Transitions of DNA structures driven by pressure and temperature. (A-C) *P-T* stability diagrams of the normalized ratio of (A) duplex formation, (B) G-quadruplex formation by the HT strand, and (C) i-motif formation by cHT strand in 10 mM K_2HPO_4 and 1 mM K_2EDTA at pH 5.5. (D) Percent of the population in duplex (blue), G-quadruplex (red), i-motif (green), and coil (orange) as a function of pressure at 37 °C in 10 mM Na_2HPO_4 and 1 mM Na_2EDTA at pH 5.5. (E-G) *P-T* stability diagrams of the normalized ratio of (E) duplex formation, (F) G-quadruplex formation, and (G) i-motif formation in 10 mM K_2HPO_4 and 1 mM K_2EDTA at pH 5.5 in 40 wt% EG. (H) Populations of duplex (blue), G-quadruplex (red), i-motif (green), and coil (orange) as a function of pressure at 37 °C in the presence of 40 wt% EG. In *P-T* diagrams, the color gradient indicates the percent of the population that adopts the structure.

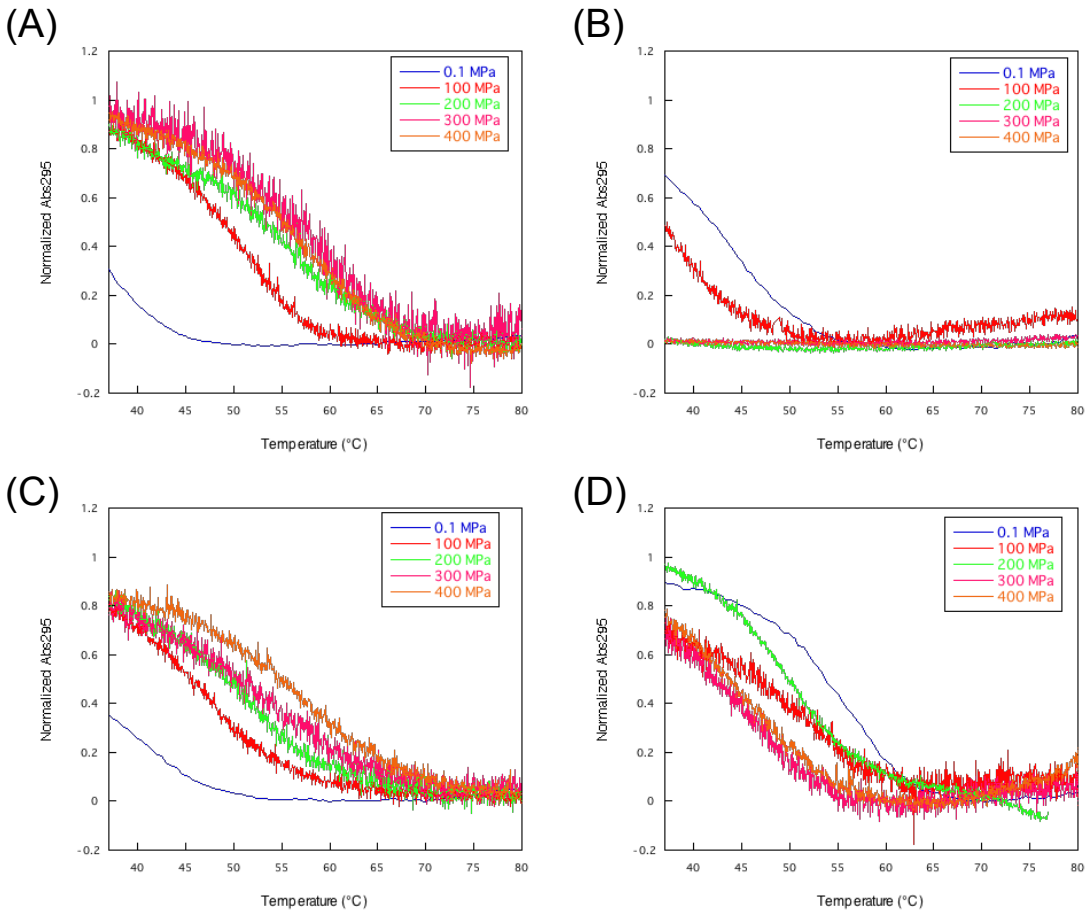

Figure S14. Normalized UV melting curves of (A) cHT in the absence of EG, (B) HT in the absence of EG, (C) cHT in the presence of 40 wt% EG, and (D) HT in the presence of 40 wt% EG. UV changes were recorded at 0.1 MPa (blue), 100 MPa (red), 200 MPa (green), 300 MPa (pink), and 400 MPa (orange). The buffer condition was 10 mM K_2HPO_4 and 1 mM K_2EDTA at pH 5.5.

Table S1. The ratio of A_{440}/A_{550} from the spectrum of methyl red

Pressure (MPa)	pH	A440/A550
0.1	6.0	8.20 ± 0.90
	5.9	6.66 ± 0.43
	5.8	5.86 ± 0.68
	5.7	4.62 ± 0.25
	5.6	3.87 ± 0.13
	5.5	3.30 ± 0.07
	5.4	2.95 ± 0.02
	5.3	2.30 ± 0.05
	5.2	1.96 ± 0.08
	5.1	1.74 ± 0.11
	5.0	1.60 ± 0.09
100	6.0 [a]	4.62 ± 0.07
200	6.0 [a]	2.64 ± 0.12
300	6.0 [a]	1.65 ± 0.04
400	6.0 [a]	1.67 ± 0.14

[a] pH was adjusted at 6.0 under atmospheric pressure (0.1 MPa).

Table S2. T_m values of $(CCT)_{10}$ DNA at various pHs under atmospheric pressure

pH	T_m (°C)
6.0	38.8 ± 0.9
5.9	41.5 ± 0.8
5.8	44.3 ± 1.0
5.7	45.6 ± 0.8
5.6	48.5 ± 1.1
5.5	49.8 ± 1.0
5.4	51.0 ± 0.7
5.3	53.7 ± 1.5
5.2	54.6 ± 0.3
5.1	56.8 ± 0.9
5.0	56.7 ± 1.1

Table S3. T_m values of $(CCT)_{10}$ DNA in the presence of 40 wt% EG

Pressure (MPa)	T_m (°C) [a]
0.1	35.0 ± 0.8
100	43.0 ± 0.3
200	49.3 ± 0.5
300	54.7 ± 0.5
400	60.0 ± 0.2

[a] pH was adjusted at 6.0 under atmospheric pressure (0.1 MPa).