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A reversible B-A transition of DNA duplexes induced by synthetic cationic copolymers

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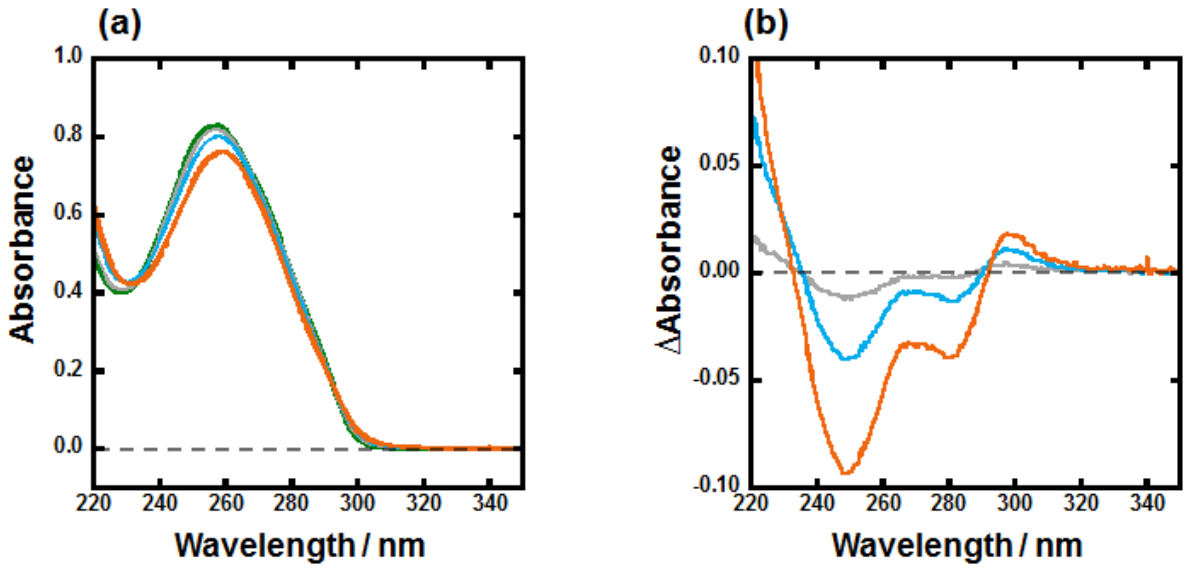

Figure S1. (a) UV spectra of 5 μM GC8 in the presence of PAA-*g*-Dex of various concentrations [N/P = 0 (green), 0.3 (gray), 1.0 (blue), 3.0 (yellow)] in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0), and 1 mM Na_2EDTA at 25ºC. (b) UV difference spectra of 5 μM GC8, obtained by subtraction of the spectrum in the absence of PAA-*g*-Dex from the spectra in the presence of PAA-*g*-Dex [N/P = 0.3 (gray), 1.0 (blue), 3.0 (yellow)].

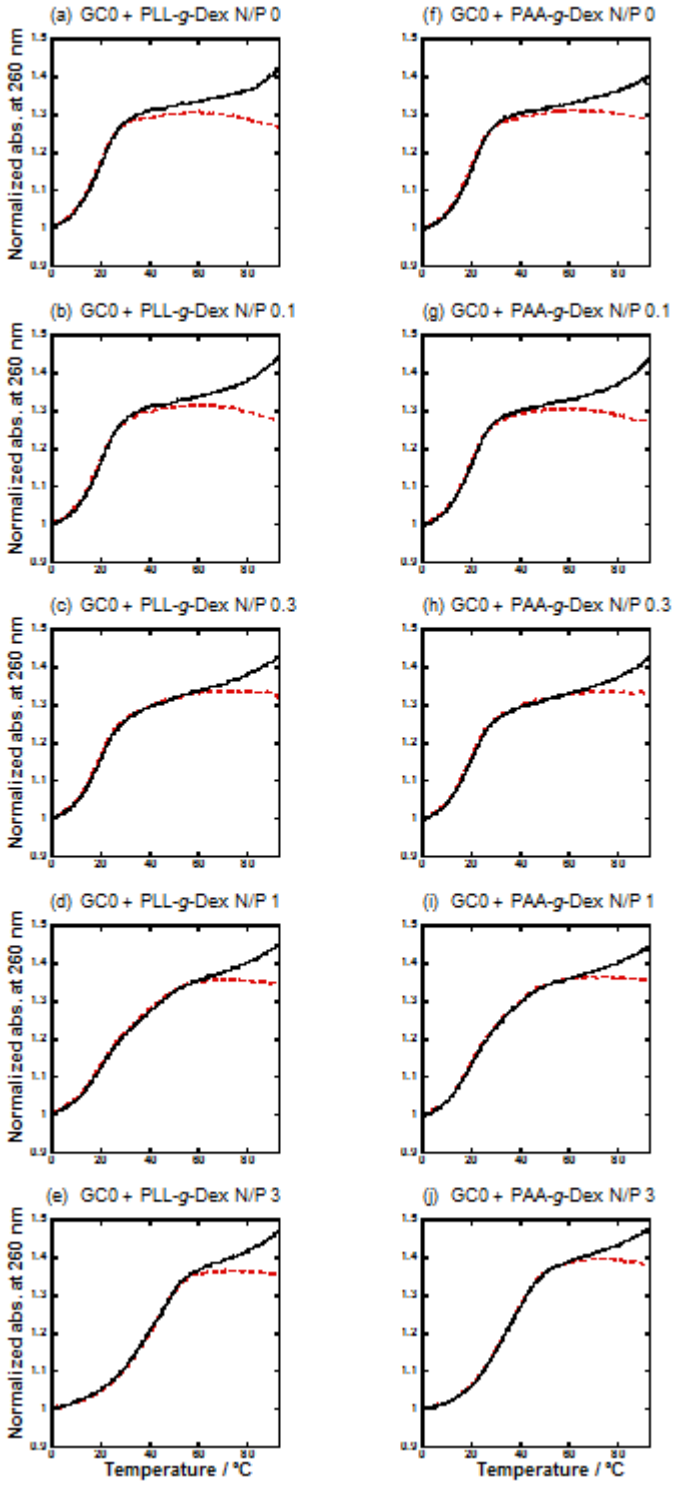

Figure S2. Melting and annealing curves of 20 μM GC0 in the presence of PLL-g-Dex (a: N/P = 0), (b: N/P = 0.1), (c: N/P = 0.3), (d: N/P = 1.0), (e: N/P = 3.0) or in the presence of PAA-*g*-Dex (f: N/P = 0), (g: N/P = 0.1), (h: N/P = 0.3), (i: N/P = 1.0), (j: N/P = 3.0) in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0), and 1 mM Na_2EDTA.

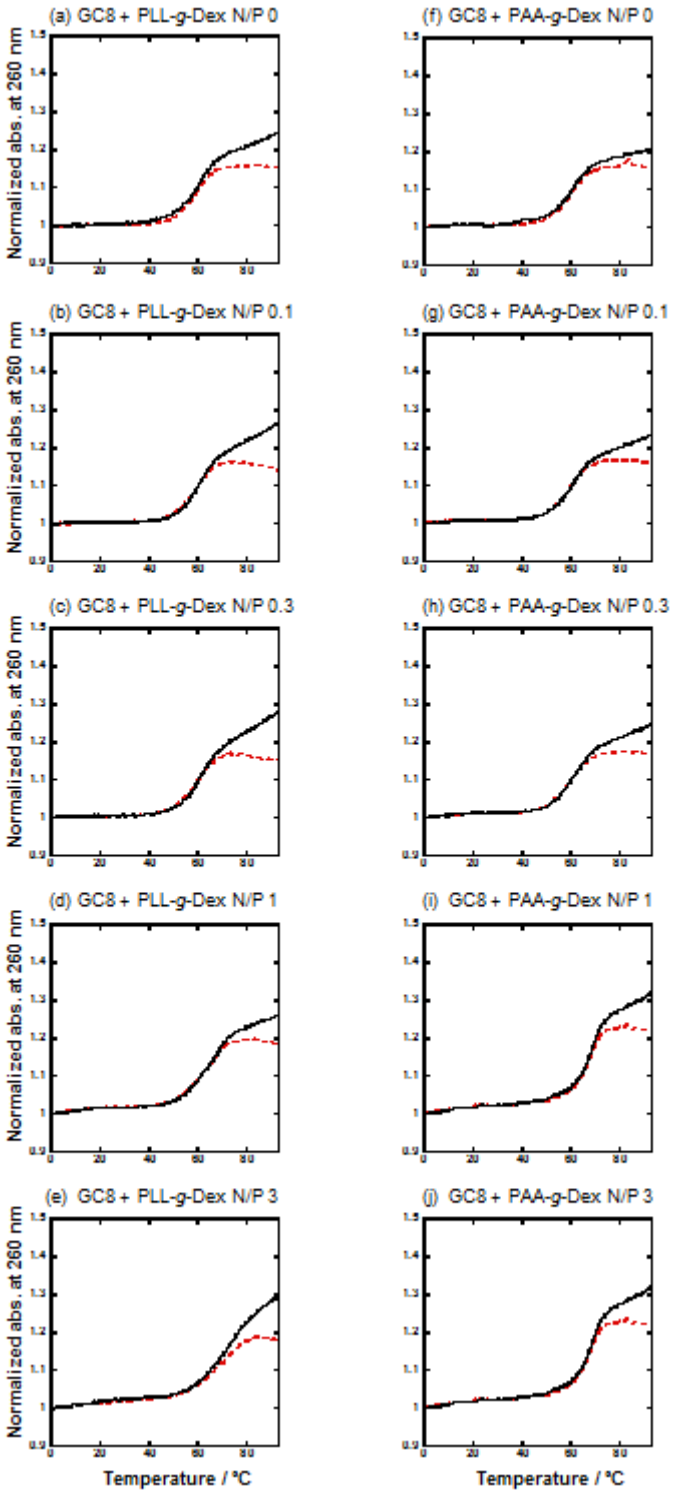

Figure S3. Melting and annealing curves of 20 μM GC8 in the presence of PLL-g-Dex (a: N/P = 0), (b: N/P = 0.1), (c: N/P = 0.3), (d: N/P = 1.0), (e: N/P = 3.0) or in the presence of PAA-*g*-Dex (f: N/P = 0), (g: N/P = 0.1), (h: N/P = 0.3), (i: N/P = 1.0), (j: N/P = 3.0) in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0), and 1 mM Na_2EDTA.

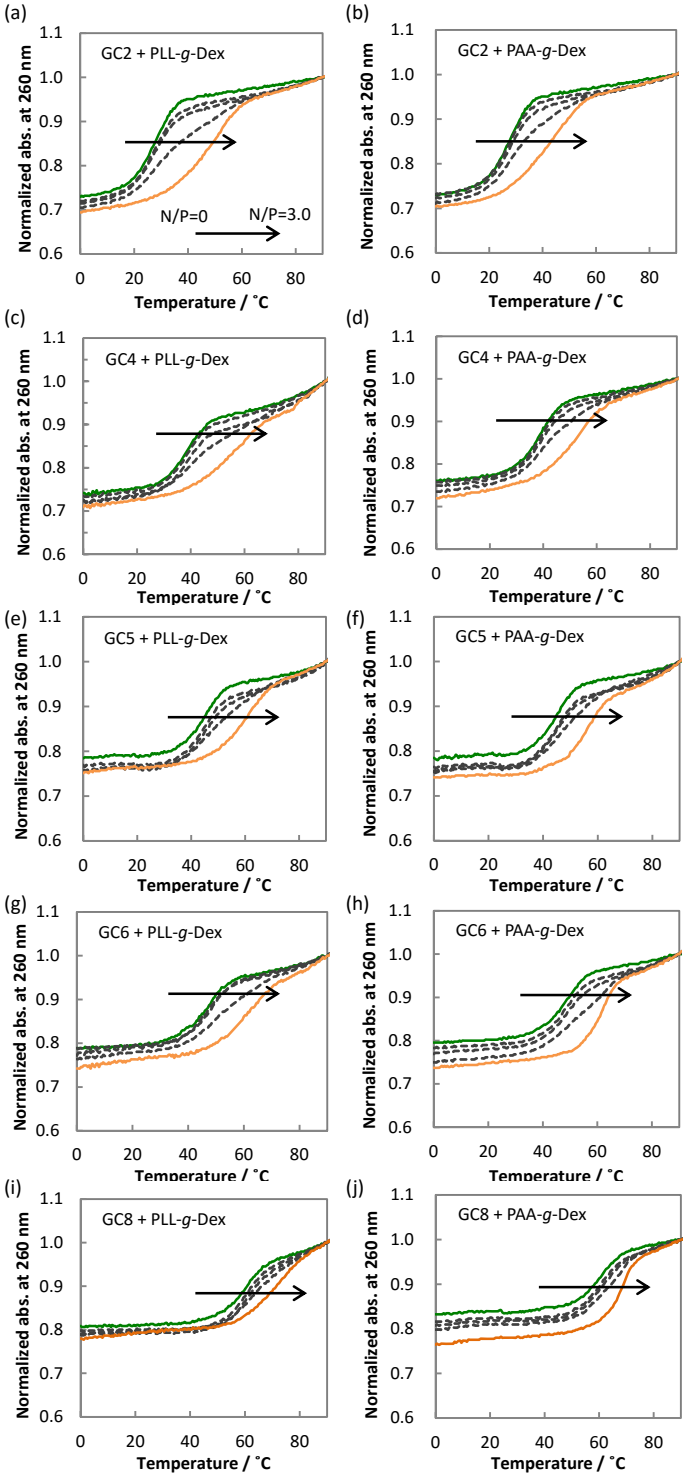

Figure S4. Normalized melting curves of 20 μM DNA duplexes in the presence of PLL-*g*-Dex (left column) or PAA-*g*-Dex (right column) of various concentrations [N/P = 0 (green), 0.1, 0.3, 1.0, 3.0 (yellow)] in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0) and 1 mM Na_2EDTA. (a)(b) GC2, (c)(d) GC4, (e)(f) GC5, (g)(h) GC6, (i)(j) GC8.

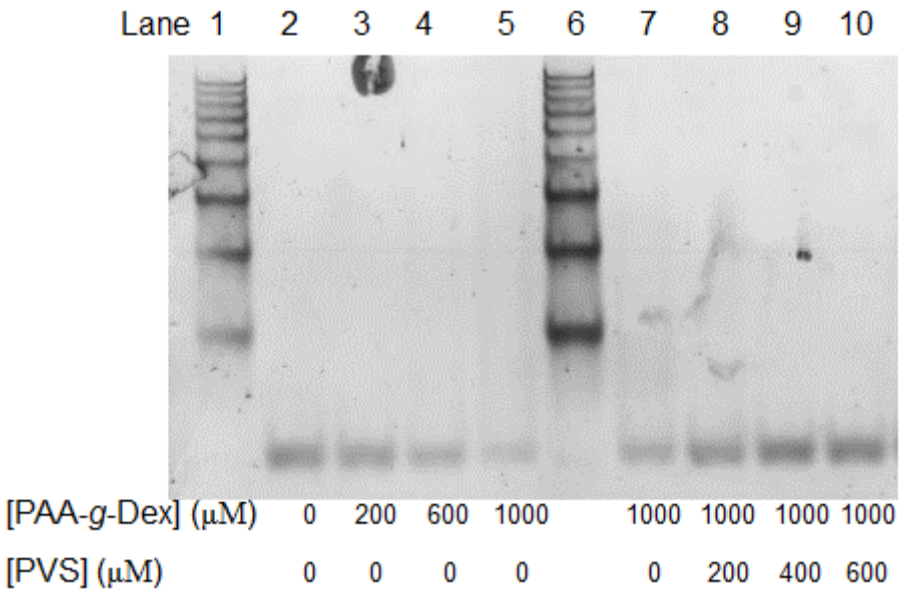

Figure S5. Non-denaturing 10% polyacrylamide gel electrophoresis of 10 μM GC8 in the presence of PAA-*g*-Dex and PVS. All experiments were carried out in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0), and 1 mM Na_2EDTA at 4ºC. Lanes 1 and 6, DNA ladder including 100, 90, 80, 70, 60, 50, 40, 30, 20 base pairs duplexes; Lane 2, [PAA-*g*-Dex] = 0 μM [PVS] = 0 μM; Lane 3, [PAA-*g*-Dex] = 200 μM [PVS] = 0 μM; Lane 4, [PAA-*g*-Dex] = 600 μM [PVS] = 0 μM; Lane 5, [PAA-*g*-Dex] = 1000 μM [PVS] = 0 μM; Lane 7, [PAA-*g*-Dex] = 1000 μM [PVS] = 0 μM; Lane 8, [PAA-*g*-Dex] = 1000 μM [PVS] = 200 μM; Lane 5, [PAA-*g*-Dex] = 1000 μM [PVS] = 400 μM; Lane 5, [PAA-*g*-Dex] = 1000 μM [PVS] = 600 μM.

Table S1. Thermodynamic parameters for formation of DNA duplex of GC8 in the absence of polymer, in the presence of PAA-*g*-Dex (N/P = 3.0), and in the presence of PAA-*g*-Dex (N/P = 3.0) and PVS (1200 μM sulfonate) in the buffer of 100 mM NaCl 10 mM Na_2HPO_4 (pH7.0), and 1 mM Na_2EDTA.

	ΔG°_{25} (kcal mol^{-1})	ΔH° (kcal mol^{-1})	$T\Delta S^\circ$ (kcal mol^{-1})	T_m (°C)
Without	-14.7	-75.2	-60.5	57.8
+PAA-*g*-Dex	-21.9	-117	-95.0	67.6
+PAA-*g*-Dex + PVS	-15.4	-81.4	-66.0	58.2

Materials and Methods

Materials

The PLL-*g*-Dex copolymer (M_n of PLL = 14400, M_n of Dex = 5300, degree of substitution of Dex = 14.4%, weight fraction of Dex = 86%) and PAA-*g*-Dex (M_n of PAA = 15000, M_n of Dex = 5300, degree of substitution of Dex = 11.4 %, weight fraction of Dex = 92%) were prepared by a reductive amination reaction between poly(L-lysine) and dextran, as described in detail previously (1,2). PVS (poly(vinyl sulfonate) was purchased from Sigma-Aldrich.

DNA oligonucleotides were HPLC grade and were purchased from Hokkaido System Science (Sapporo, Japan) and and Sigma-Aldrich Japan K.K. (Tokyo, Japan). The single-strand concentrations of the DNA oligodeoxynucleotides were determined by measuring the absorbance at 260 nm and high temperature using a UV-1800 spectrometer (Shimadzu Co., Ltd., Kyoto, Japan) connected to a Shimadzu TMSPC-8 thermoprogrammer. Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data using the nearest-neighbor approximation (3).

All chemical reagents were of reagent grade from Wako Pure Chemical Co., Ltd. (Osaka, Japan) and Sigma-Aldrich Japan K.K. And the chemical reagents were used without further purification.

Circular dichroism measurements

Circular Dichroism (CD) spectra of DNA oligonucleotides were measured for 20 μM DNA total strand concentration using a J-820 spectropolarimeter (JASCO Co., Ltd., Hachioji, Japan) with a 0.1-cm path length quartz cell at 25°C. The CD spectrum was obtained by taking the average of three scans made at 0.2-nm intervals from 200 to 350 nm. Before measurement, the DNA samples were heated at 90°C for 3 min, gently cooled at 0.5°C min^{-1}, and incubated at 25°C. The temperature of the cell holder was regulated by a PTC-348 temperature controller (JASCO), and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid water condensation on the cuvette exterior. Before the measurement, the sample was heated to 90°C, gently cooled at a rate of 0.5 °C min^{-1}, and incubated at 25°C for 1 h.

Thermal and thermodynamic analysis

The UV melting curves of DNA oligonucleotides were measured by a Shimadzu 1800 spectrophotometer (Shimadzu) equipped with the Shimadzu TMSPC-8 temperature controller. The UV melting curves were measured at 260 nm where DNA duplexes show a hyperchromic transition (4). All experiments were carried out in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0) and 1 mM Na_2EDTA. The heating rate was 0.5°C min^{-1}. The thermodynamic parameters were calculated from the fit of the melting curves (with at least five different concentrations of DNA oligonucleotides) to a theoretical equation for an intramolecular association as described previously (5,6). Before the measurement, the sample was heated to 90°C, gently cooled at a rate of 0.5°C min^{-1} for annealing, and incubated at 0°C for 1 h.

Non-denaturing gel electrophoresis

Gel electrophoresis was performed on non-denaturing gels containing 10% polyacrylamide. Ice-cold loading buffer (980 μL) was mixed with 20 μL of 10 μM GC8, and a 5 μL aliquot of the mixed solution was loaded and analyzed by electrophoresis at 10 V cm^{-1} for 3 h at 4°C. All experiments were carried out in the buffer of 100 mM NaCl, 10 mM Na_2HPO_4 (pH7.0) and 1 mM Na_2EDTA at 4ºC. Gels were stained by using SYBR® Gold (Molecular Probes) and imaged with FLA-5100 (Fuji Film Co., Ltd., Tokyo, Japan). Before the measurement, the sample was heated to 90 °C, gently cooled at a rate of 0.5 °C min^{-1}, and incubated at 4 °C for 1 h.

References

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