Electronic Supplementary Information (ESI)

Consecutive GC base pairs determine the energy barrier of a DNA duplex formation under molecular crowding conditions

Xiao-Bo Gu, Shu-ichi Nakano and Naoki Sugimoto

Materials

All the oligodeoxynucleotides used in this study were high performance liquid chromatography (HPLC) grade purchased from Hokkaido System Science (Sapporo, Japan). The single-strand concentrations of the DNA oligonucleotides were determined by measuring the absorbance at 260 nm and high temperature [1] using a Shimadzu 1700 spectrophotometer (Shimadzu, Kyoto, Japan) connected to a thermoprogrammer. The single-strand extinction coefficients were calculated from the mononucleotide and dinucleotide data using the nearest-neighbor approximation.[1]

All the PEGs [poly(ethylene glycol)] were purchased from Wako Pure Chemical Co., Ltd., (Japan), and used without further purification. PEG 200, PEG 600 and PEG 8000 indicate the PEG with an average molecular weight of 200, 600 and 8000, respectively.

Methods

Melting Curves. The melting curves (absorbance versus temperature curves) of the 10-mer DNA duplexes were measured at 260 nm by a Shimadzu UV1700 spectrophotometer in a buffer (1 M NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA at

pH 7.0) with and without the 20 wt% PEGs. The heating rate was 0.5 ^{o}C min^{-1}. The T_m was obtained from the UV melting curve (Fig. S1) as previously described.[8]

Temperature-jump Kinetics. The temperature-jump instrument was manufactured by Unisoku (Japan). The data collection and analysis were controlled by a Dell personal computer using the Unisoku Spectroscopy & Kinetics program. The light path length was 0.5 cm, and the sample volume was 250 μL. The range of the temperature jump discharge at 15 kV in each buffer was determined by calibrating a test sample using the phenolphthalein.[11] The relaxation curves (Fig. 2a) were measured eight times, and the relaxation times were evaluated from the eight relaxation curves.

For a reaction using the scheme (A + B $\underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}}$ AB), where A and B are the non-self complementary DNA single-strands and AB is the fully-matched DNA duplex, the plots (Fig. 2b) of τ^{-2} (τ, the relaxation time) versus C_t (the total DNA concentration) follow the equation:

$$\tau^{-2} = 2k_{+1}k_{-1}C_t + k_{-1}^2$$

Because the intercept of the plot was very low and produced a large error, we calculated the association rate constant (k_{+1}) and the dissociation rate constant (k_{-1}) from the slope (S) of the plot and the equilibrium constant (K) obtained from the melting curves (Figure S1) [4,12] using the following equations:

$$k_{+1} = (S \cdot K/2)^{1/2}$$

$$k_{-1} = k_{+1}/K$$

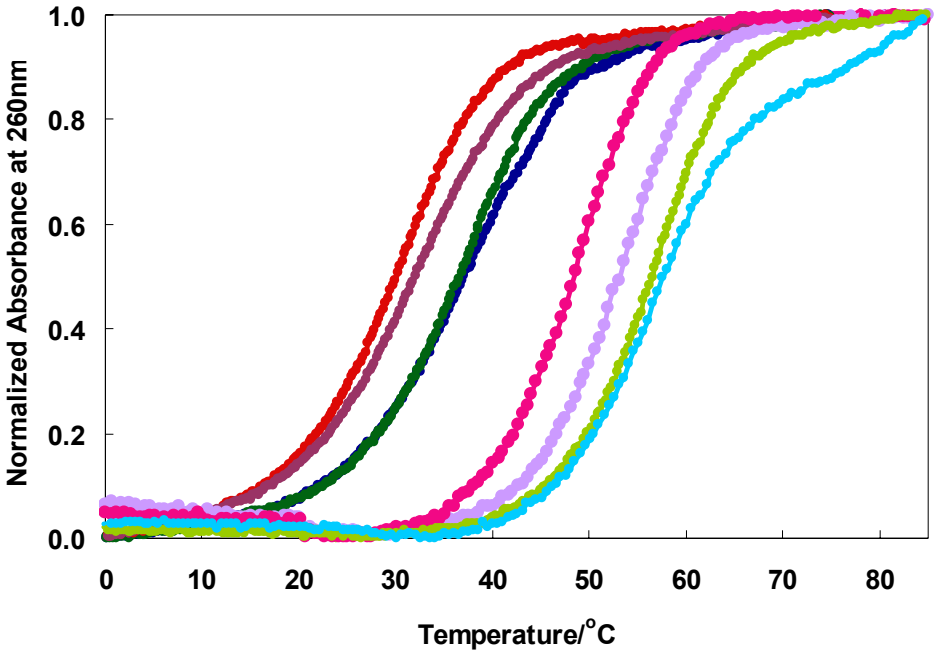

Fig. S1 The normalized melting curves of 50 μM 5'-TAGGTTATAA-3'/ 5'-TTATA ACCTA-3' (duplex **1**) in the absence of PEG (blue) and in the presence of 20 wt % PEG 200 (red), PEG 600 (purple) or PEG 8000 (green), and the normalized melting curves of 50 μM 5'-CAGGTCACAG-3'/5'-CTGTGACCTG-3' (duplex **2**) in the absence of PEG (light blue) and in the presence of 20 wt % PEG 200 (light red), PEG 600 (light purple) or PEG 8000 (light green).

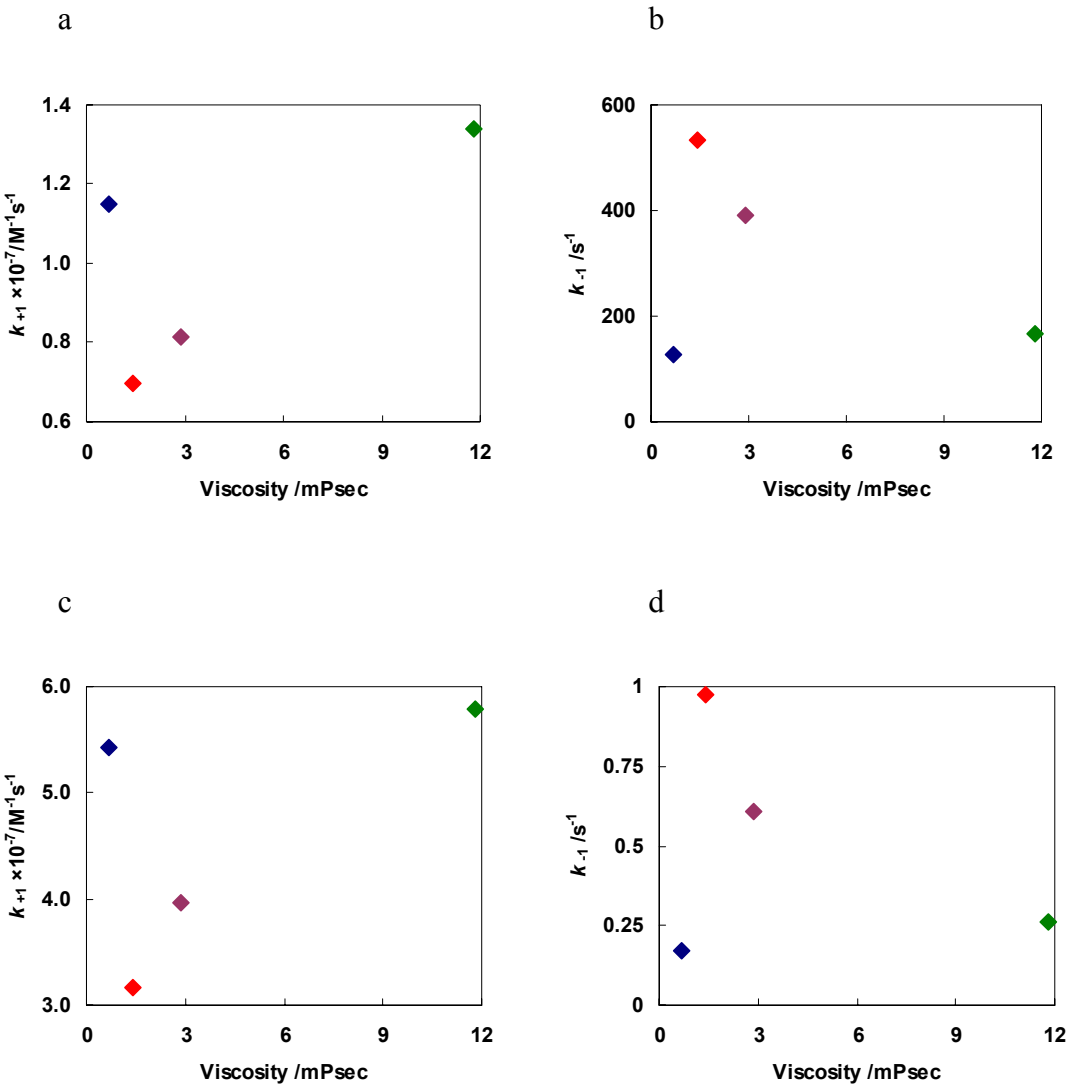

Fig. S2 The plots of k_{+1} and k_{-1} vs. the solution viscosity of duplex **1** (5'-TAGGTTAT AA-3'/5'-TTATAACCTA-3') (a and b, respectively) and duplex **2** (5'-CAGGTCACA G-3' /5'-CTGTGACCTG-3') (c and d, respectively) in the absence of PEG (blue) and in the presence of 20 wt% PEG 200 (red), PEG 600 (purple) or PEG 8000 (green).

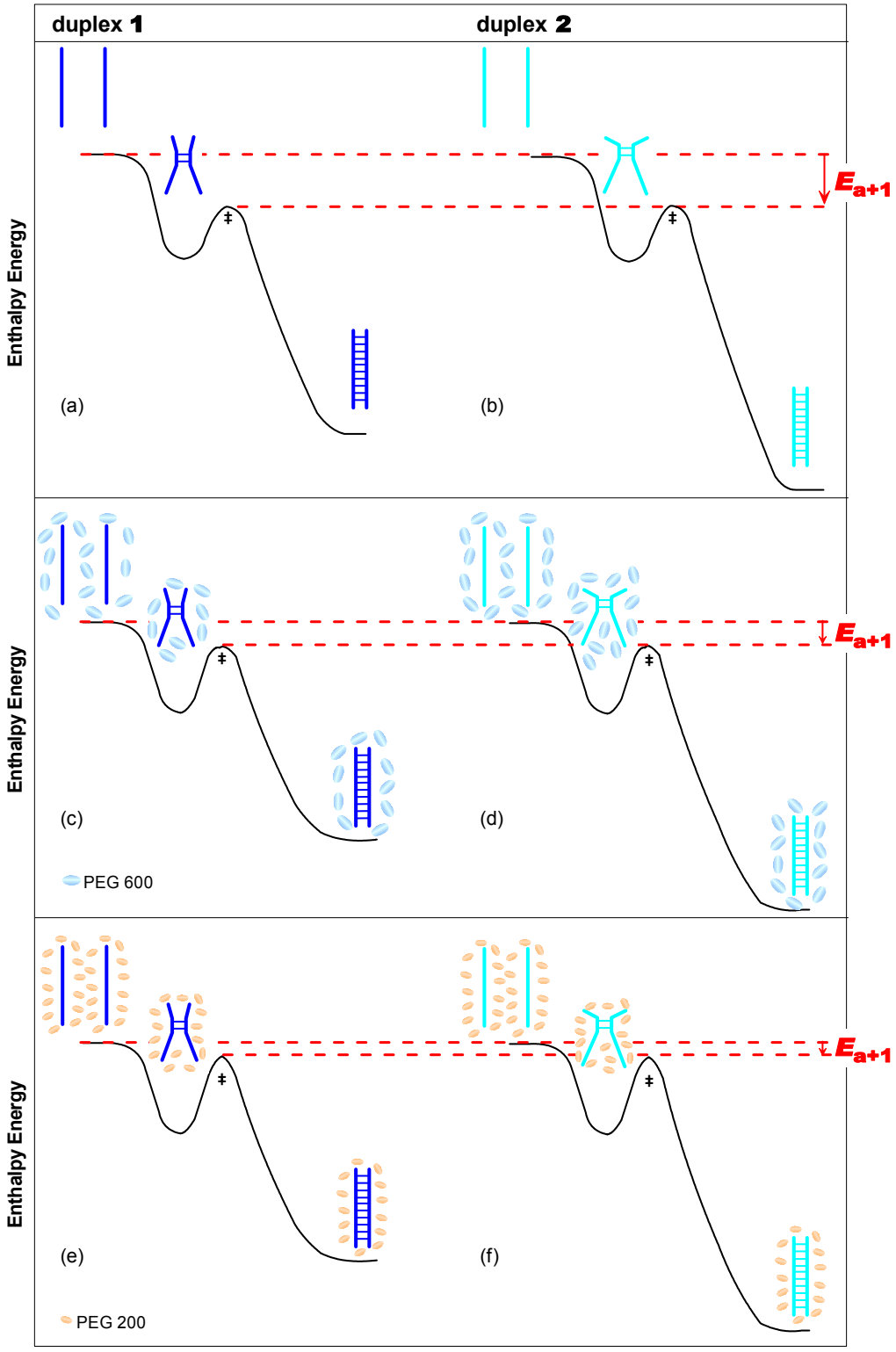

Fig. S3 Schematic view of the profiles of the enthalpy energy change for the duplexes **1** and **2** formations in the absence of the PEGs (a and b, respectively) and in the presence of PEG 600 (c and d, respectively) and PEG 200 (e and f, respectively). The profiles indicate that the E_{a+1} values of duplexes **1** and **2** are almost the same in the solutions whether with or without PEG and that the E_{a+1} values increase more in the presence of the lower mass PEGs.