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The Effect of Molecular Crowding with Nucleotide Length and Cosolute Structure on DNA Duplex Stability

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The thermal stability of nucleic acid structures has been investigated to understand their structures and folding events.[1] Most attempts to obtain this stability have been performed in diluted aqueous solutions to avoid complexity of reaction. However, quantitative information in cell-like conditions is also essential for understanding nucleic acid structures and functions in living cells that contain macromolecules occupying 20−40% of the total volume.[2]

Poly(ethylene glycol) (PEG) is one of the most commonly used molecules as a cosolute in aqueous solution to mimic cellular environments because PEGs are inert with nucleotides and different molecular weight PEGs are available. We have previously found a structural transition of the G-quadruplex from an antiparallel to a parallel orientation induced by PEG.[3] There has also been a report that T_m (melting temperature) of a triplex but not a duplex is increased as the size of PEG in solution increased, while low molecular weight PEGs and ethylene glycol (EG) decrease the T_m of a duplex.[4] These observations suggest that the cosolutes alter the stability of nucleic acid structures, although they do not directly interact with nucleotides. In this study, we investigated the influence of the molecular weight of PEG on the thermal stability of DNA duplexes with a different nucleotide length and show that the effects of high concentration of the cosolutes on the duplex stability differ considerably, depending on the nucleotide length and cosolute structure as well as the size of the PEG.

The T_m of DNA duplexes of differing length—an 8-mer self-complementary duplex (5′-ATGCGCAT-3′), a 17-mer duplex (5′-CACAAACATGCACCTCA-3′/5′-TGAGGTGCATGTTTGTG-3′), and a 30-mer duplex (5′-A_{27}GCG-3′/5′-CGCT_{27}-3′), were measured in a solution containing 20 wt % EG (MW = 62), PEG 200,[5] PEG 1000, or PEG 8000.[6] That low molecular weight cosolutes destabilize the polymer duplexes of poly(dA)·poly(dT) and *Escherichia coli* DNA, while high molecular weight PEGs (MW > 1000) stabilize these duplexes, as assessed by the exclusion volume from the large cosolutes, has been reported.[4b,c] The T_m data in Table 1 indicate that PEG 8000 stabilizes the 30-mer duplex, as reported for the polymer duplexes, but EG, PEG 200, and PEG 1000 decrease the T_m of all the duplexes (15.9−1.6 °C). Intriguingly, the destabilization was greater for the shorter duplexes and by lower molecular weight PEG. It is therefore likely that the length of a DNA duplex as well as the size of PEG affects the duplex stability and the exclusion volume of the larger cosolutes may not be significant for the shorter duplex. To reveal destabilization of the short duplex, we further investigated the thermodynamic properties of the 8-mer duplex in the presence of various cosolutes.

Duplex formation by a self-complementary strand (A) in an aqueous solution containing cosolute (CS) and sodium ion (Na^+) can be represented by the following equilibrium

$$2A \rightleftharpoons A_2 + \Delta n_w H_2O + \Delta n_{cs} CS + \Delta n_{Na^+} Na^+ \quad (1)$$

where Δn_w, Δn_{cs}, and Δn_{Na^+} are the numbers of water, cosolute, and sodium ion released upon the duplex (A_2) formation, respectively.[4c,7] The observed equilibrium constant (K_{obs}) for the duplex formation is thus given as

$$K_0 = K_{obs}\, a_w^{\Delta n_w}\, a_{cs}^{\Delta n_{cs}}\, a_{Na^+}^{\Delta n_{Na^+}} \quad (2)$$

where K_0 is the true thermodynamic equilibrium constant, and a_w, a_{cs}, and a_{Na^+} are the activities of water, cosolute, and sodium ion, respectively. At a constant temperature and pressure, the derivative of ln K_{obs} by ln a_w is represented by eq 3 containing the terms for the number of bound molecules.

$$\frac{d \ln K_{obs}}{d \ln a_w} = -\left[\Delta n_w + \Delta n_{cs}\left(\frac{d \ln a_{cs}}{d \ln a_w}\right) + \Delta n_{Na^+}\left(\frac{d \ln a_{Na^+}}{d \ln a_w}\right)\right] \quad (3)$$

The equilibrium constant of ATGCGCAT was measured in the absence of and in the presence of various amounts (10, 20, 30, 40, and 50 wt %) of PEG 200.[8] As the amount of PEG 200 increased, the duplex stability decreased. As has been examined for the duplex destabilization by low molecular weight cosolutes,[4b,c,f,7] the relationship of the stability with the water activity was investigated. Figure 1 shows the plots of ln K_{obs} at 25 °C versus ln a_w obtained by the osmotic pressure measurements at 25 °C.[9] The plot indicates that the duplex stability (ln K_{obs}) is linearly decreased with the decrease in ln a_w,[10] as already reported.[4b,c,f,7] Although the slope of the plot includes the two variable terms responsible for the cosolute and cation binding, the linear plot in Figure 1 suggests that these variable terms are insignificant, and the slope approximately equals the constant term $-\Delta n_w$.[11]

To further confirm the destabilization relevant to the change in the water activity, PEG 1000 (40 and 20 wt %), PEG 2000 (40

Table 1. T_m (°C) of the DNA Oligomer Duplexes in the Presence of 20 wt % Ethylene Glycol (EG) or Poly(ethylene glycol) (PEG)[a]

duplex	none	EG	PEG 200	PEG 1000	PEG 8000
8 mer	47.1	37.1 (−10.0)	31.2 (−15.9)	41.1 (−6.0)	42.0 (−5.1)
17 mer	69.1	64.1 (−5.0)	60.1 (−9.0)	66.4 (−2.7)	68.3 (−0.8)
30 mer	71.3	64.1 (−7.2)	62.6 (−8.7)	69.7 (−1.6)	72.1 (0.8)[b]

[a] All experiments were carried out with 5 μM DNA in a buffer containing 1 M NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA in the absence of and in the presence of 20 wt % EG or PEG. Values in parentheses are the difference of the T_m values in the absence of and in the presence of the cosolute. [b] The PEG 8000 data for the 30-mer DNA duplex was measured with 10 wt % PEG because of a low solubility of the longer nucleotides.

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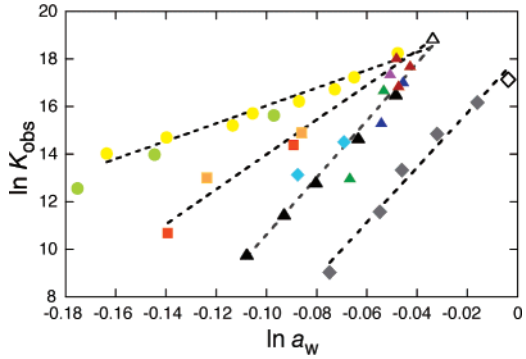

Figure 1. ln K_{obs} vs ln a_w plots for ATGCGCAT in the absence of (open symbols) and in the presence of PEG (triangles) [PEG 200 (black), PEG 1000 (dark green), PEG 2000 (dark blue), PEG 6000 (purple), PEG 8000 (dark red)], glycerol (yellow circles), ethylene glycol (green circles), 1,3-propanediol (orange squares), 2-methoxyethanol (red squares), or 1,2-dimethoxyethane (blue diamonds) in 1 M NaCl, and in the presence of PEG 200 in 100 mM NaCl (gray diamonds).

and 20 wt %), PEG 6000 (20 wt %), and PEG 8000 (20, 15, and 10 wt %) were also examined.[12] As indicated in Figure 1, all the data for the various sizes of PEGs were located on the same straight line as those for PEG 200, suggesting that the decrease in the DNA duplex stability with the addition of PEG is mostly caused by the decrease in the water activity.

The slope of the linear plot in Figure 1 provides a negative Δn_w, leading to the uptake of 15.0 ± 0.6 water molecules per base-pair formation[13] in 1 M NaCl in the presence of PEG 200. The linear plot for the 100 mM NaCl data (Figure 1) also gives a water uptake of 14.4 ± 0.5, leading to the conclusion that Δn_w is unaffected by the change in the NaCl concentration from 1 M to 100 mM. On the other hand, the linear plot in the presence of glycerol (MW = 92) leads to the uptake of 4.7 ± 0.3 water molecules which is close to the values reported for EG or glycerol as a cosolute (2−4 water molecules).[4c,7] It is therefore likely that solvation of the nucleotides in the presence of PEG differs considerably from that of glycerol as a cosolute.

To investigate the influence of the cosolute structure on the number of water molecules taken up, other low molecular weight cosolutes, EG, 1,3-propanediol (MW = 76), 2-methoxyethanol (MW = 76), and 1,2-dimethoxyethane (MW = 90) were also examined. As shown in Figure 1, the data of EG are close to those of glycerol, whereas those of 1,2-dimethoxyethane are close to those of the PEGs, and those of 1,3-propanediol and 2-methoxyethanol appear intermediate between the linear plots of the PEGs and glycerol. These observations suggest that the number of water molecules taken up per base-pair formation, which influences the duplex stability, differ depending on the cosolute structure. It is possible that the solvation of nucleotides by glycerol and EG may eliminate the apparent number of water molecules taken up because they have hydroxyl groups at the vicinal position suitable for hydrogen bond formation with nucleotides, in contrast with those of PEGs which are separated by an ethylene glycol chain and hydroxyl groups are absent in the vicinal position in 1,2-dimethoxyethane. The absence of the hydroxyl groups at the vicinal position in 1,3-propanediol and 2-methoxyethanol may partially participate in the solvation of the nucleotides, although other effects such as the change in dielectric constant, ability of the cavity formation, and cosolute cluster formation also still deserve consideration.

Hybridization of short nucleotide duplexes is fundamental to antisense, RNAi, and DNA chip technologies. Since these techniques are aimed at in-cell or solid surface use, quantitative information regarding duplex stability in cell-like or cell-surface-like conditions is essential. Our results demonstrate that the length of DNA as well as the size of the cosolute is critical for the hybridization energy in molecular crowding conditions and the number of water molecules taken up per base-pair formation may differ, depending on the cosolute structure. These findings might be useful not only for understanding nucleic acid structures and functions in cells but also for the design of oligonucleotides applicable for cells such as antisense nucleic acids, RNAi, and DNA chips.

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Supporting Information Available: Thermodynamic parameters for the 8-mer duplex formations, osmolarity and ln a_w, typical melting curves, the plots of $\Delta H°$ vs cosolute wt %, and CD spectra in various solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) PEG 200 indicates poly(ethylene glycol) with a molecular weight (MW) of 200, on average. All cosolutes were purchased from Wako Pure Chemical Co. Ltd. (Japan), and used without further purification.

(6) T_m was obtained from the UV melting curve (Figure S1) as described previously [e.g. Sugimoto, N.; Nakano, S.; Katoh, M.; Matsumura, A.; Nakamuta, H.; Ohmichi, T.; Yoneyama, M.; Sasaki, M. *Biochemistry* **1995**, *34*, 11211−11216; Nakano, S.; Kanzaki, T.; Sugimoto N. *J. Am. Chem. Soc.* **2004**, *126*, 1088−1095]. The examined heating rate was 0.5 °C min^{-1} because it was confirmed that the shape of the melting curve and T_m were unaffected by the heating rate in the range from 0.01 to 0.5 °C min^{-1}.

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(8) Thermodynamic parameters (Table S1) were determined from T_m^{-1} vs log C_t plot and curve fittings as reported previously.[6]

(9) The water activity (Table S2) was determined by the osmotic stressing method[4f] via vapor phase osmometry (a Wescor pressure osmometer, model 5520XR) or freezing point depression osmometry (a Halbmikro osmometer, Typ Dig.L), with the assumption that the cosolutes do not directly interact with DNAs which could lead to underestimating the Δn_w.[4c,7]

(10) The $\Delta H°$ and $\Delta S°$ values in Table S1 suggest that these parameters differ, depending on the size of PEG and the concentration (Figure S2). Accordingly, the Δn_w value derived from the linearity of the plot of ln K_{obs} vs ln a_w is more accurate than that obtained from the T_m^{-1} vs ln a_w plot, which requires the assumption that $\Delta H°$ is constant over the experimental conditions.[4f,7] The CD spectra indicated that the conformation of the 8-mer duplex was unaffected by the cosolutes (Figure S3).

(11) The upper and lower limits of the derivative term for the cosolute binding under the conditions examined here were 89−20, suggesting that Δn_{cs} is nearly zero, consistent with no interaction of nucleotide with PEG. Additionally, the plots of ln K_{obs} vs ln a_{cs} for PEG 200 were nonlinear (data not shown), consistent with the idea that the decrease of water activity is the primary factor for destabilization of the 8-mer duplex by PEG 200. Thus, the use of a cosolute is useful for investigating the DNA hydration.

(12) Solubility limited measurements with higher concentrations of large PEGs.

(13) In B-DNA, ~20 water molecules per base pair are found in the primary hydration shell [e.g. Feig, M.; Pettitt, B. M. *Biopolymers* **2000**, *48*, 199−209; Auffinger, P.; Westhof, E. *J. Mol. Biol.* **2001**, *305*, 1057−1072].

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