

Effects of Polyethylene Glycol on DNA Duplex Stability at Different NaCl Concentrations

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Although the thermodynamic stability of ordered DNA is known to be affected by interactions with cations and water molecules, there is scarce quantitative information about the behavior of DNA under conditions of molecular crowding as found inside the cell. In this study, we measured the thermal stability of DNA base pairs under conditions of molecular crowding caused by polyethylene glycol (PEG) and at different concentrations of NaCl. Even though PEG 200 and 8000 (average molecular weights of 200 and 8000, respectively) decreased the stability of DNA base pairs at high NaCl concentrations, PEG 8000 substantially increased the stability at low NaCl concentrations. Quantitative analyses of the thermodynamic parameters made it possible to determine the numbers of water molecules ($-\Delta n_{\mathrm{w}}$) and sodium ions ($-\Delta n_{\mathrm{Na^+}}$) bound to the hairpin DNA. These measurements showed that PEG decreased the $-\Delta n_{\mathrm{Na^+}}$ value without changing the $-\Delta n_{\mathrm{w}}$ value, suggesting that PEG buffers sodium ion binding but may not affect the hydration status of the nucleotide or sodium ion. These results should help clarify the effects of molecular crowding on DNA stability and function and provide quantitative information about the effects of molecular crowding in cells and on chips and nanoparticles.

Hydrogen bonding and base stacking play major roles in the thermodynamic stability of Watson–Crick base pairs.[1] The solution conditions, such as cation and cosolute concentrations, also influence the stability. Metal ions primarily bind to nucleotide phosphates and stabilize the ordered DNA and RNA structures by reducing the repulsive forces between the phosphate groups.[2] The interaction between metal ions and the nucleotide phosphates is primarily through electrostatic "outer-sphere binding," wherein the hydration status of metal ions and the nucleotide is unchanged by the binding.[3] Because outer sphere-bound metal ions exchange rapidly with ions in the bulk solution, outer-sphere binding is relatively weak and is thought to be the predominant mode in which metal ions are bound by nucleotide duplexes with Watson–Crick base pairs.[3] Metal ions may also be bound by specific sites of nucleotides, as seen with G-quadruplexes, wherein the coordination of metal ions by guanine quartet centers is accompanied by the release of hydrating water molecules. This is referred to as "inner-sphere binding,"[4] and it is also observed for tertiary folded nucleotides.[5]

There has been increasing interest in the effect of cosolutes on the stability of ordered nucleotide structures, because they affect nucleotide hydration and reduce the water activity of a solution.[6–8] Studies using polyols and polysaccharides as cosolutes have shown that they decrease the thermal stability of calf thymus DNA duplex.[9] Also, alcohols, such as ethanol and propanol, decrease the stability of duplexes of DNA oligomers.[10]

Cationic molecules are abundant in living cells, and they play important roles in regulating the biological functions of nucleotides by stabilizing their ordered structures.[11,12] A variety of biomolecules, including nucleic acids, proteins, polysaccharides, and other soluble and insoluble low-molecular weight components, are also present in living cells.[13] These biomolecules occupy a significant fraction of the cellular volume (up to 40%) and result in a crowded intracellular environment, which is referred to as "molecular crowding."[14,15] Molecular crowding is thought to occur not only in cells but also on sensor chips and nanoparticles.[16]

To study the nucleotide properties in molecular crowding conditions, aqueous solutions containing high amounts of polyethylene glycol (PEG) are often employed, because PEG is neutral and does not interact with nucleotides and because various molecular weights of PEGs are available.[17] Chaires et al. and Serwer et al. have reported that the thermal stabilities of polymer duplexes of poly(dA)·poly(dT) and λ DNA are increased by a volume exclusion effect, when large PEGs with an average molecular weight above 1000 are used.[6,17] On the other hand, lower molecular weight PEGs, which exclude less volume, do not influence or slightly destabilize the polymer duplexes. In a previous study, we have found that the stability of a short DNA duplex (8-mer) in 1 M NaCl was decreases upon the addition of PEGs with molecular weights between 200 and 8000.[8] Measurement of the thermodynamic parameters of the 8-mer duplex indicate that the duplex stability is mostly influenced by the water activity of the solution. Chaires

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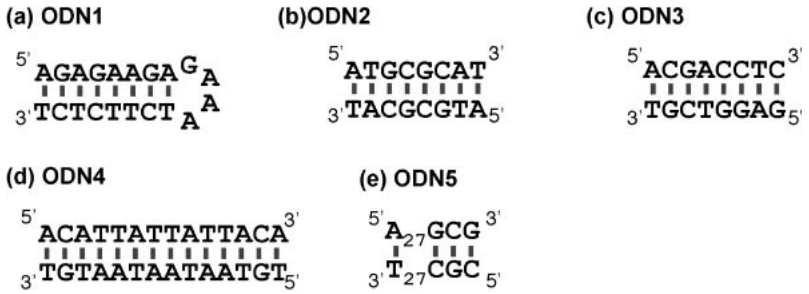

Fig. 1. Sequences of the DNA duplexes used in this study.

and colleagues[6] and Minsky and colleagues[7] have also reported the importance of the water activity on the stability of DNA triplexes. In addition, we have reported that a DNA G-quadruplex is stabilized by PEG with an average molecular weight of 200. In this case, metal ion coordination by the guanine O6 carbonyl groups is accompanied by release of hydrating water.[18] Accordingly, both the water molecules and the cations participating in the formation of DNA structures are responsible for controlling the stability of ordered DNA structures in solutions containing PEG. In these preceding reports, however, the roles of cations and water molecules in DNA structure formation have been investigated separately.

In this study, we quantitatively measured the interaction between water and cation binding to nucleotides under conditions of molecular crowding generated by PEGs. This information was obtained by measuring the effects of PEGs with molecular weights of 200 (PEG 200) or 8000 (PEG 8000) on the thermal stability of oligomeric DNA duplexes at various NaCl concentrations. We found that PEG 8000 increased the thermal stability of the DNA duplexes at lower NaCl concentrations, whereas it decreased the stability at higher NaCl concentrations. The difference in the duplex stabilities in the absence and presence of PEG 8000 increased as the NaCl concentration fell below the physiological salt concentration. We also found that PEG 8000 and PEG 200 decreased the number of sodium ions bound ($-\Delta n_{Na^+}$), but had less of an effect on the number of water molecules bound upon duplex formation ($-\Delta n_w$). The results suggest that PEG reduces the number of sodium ions but not the number of water molecules required for Watson–Crick base pair formation, which is consistent with the outer-sphere model of cation binding. The results are useful for understanding DNA stability and hydration under conditions of molecular crowding, which occurs in cells, on chips, and on nanoparticles.[14,15]

Experimental

Materials. All oligodeoxynucleotides used in this study (ODN1-5; Fig. 1) were high-performance liquid chromatography grade and were purchased from Hokkaido System Science (Sapporo, Japan). Single-strand concentrations of DNA oligonucleotides were determined by measuring the absorbance at 260 nm at 90 °C and using single-strand extinction coefficients calculated from the mononucleotide and dinucleotide data and according to the nearest-neighbor approximation model.[19] The absorbance at 260 nm was measured using a Shimadzu 1700 spectrophotometer (Shimadzu, Kyoto, Japan) connected to a thermoprogrammer. PEGs were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan) and used without further purification.

Circular Dichroism (CD) Measurements. CD measurements were made on a JASCO J-820 spectropolarimeter (JASCO, Hachioji, Japan) at 20 μM total DNA strand concentration in buffers containing 10 mM Na_2HPO_4 (pH 7.0), 1 mM Na_2EDTA, and different concentrations of NaCl supplemented with PEG 200 or PEG 8000. The spectra at 4 °C were obtained by taking at least three scans from 200 to 350 nm in a cuvette with a path-length of 0.1-cm. The temperature of the cell holder was regulated by a JASCO PTC-348 temperature controller, and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid condensation of water on the cuvette exterior. Before the measurement, the sample was heated to 80 °C, cooled at a rate of $-2\,°C\,min^{-1}$, and incubated at 4 °C for 30 min.

Water Activity Measurements. The water activity was determined by the osmotic stressing method via vapor-phase osmometry using a model 5520XR pressure osmometer (Wescor, Logan, UT, U.S.A.) at 25 °C and under the assumption that PEG does not directly interact with DNA.[7]

Thermodynamic Analysis. The UV absorbance was measured on a Shimadzu 1700 spectrophotometer equipped with a temperature controller. The melting curves at 260 nm were measured in buffers containing 10 mM Na_2HPO_4 (pH 7.0), 1 mM Na_2EDTA, and different concentrations of NaCl in the absence and presence of PEG. A heating rate of $0.5\,°C\,min^{-1}$ was applied, because the melting curve was unaffected by heating rates between 0.1 °C and $0.5\,°C\,min^{-1}$ (data not shown). The thermodynamic parameters shown in Table 2 were obtained from three independently measured melting curves and were calculated by curve-fitting analysis using the previously reported theoretical equation.[18,20] Before the measurements, the DNA sample was heated to 80 °C, cooled to 0 °C at a rate of $-2\,°C\,min^{-1}$, and incubated at 0 °C for 30 min.

Determination of Δn_w, Δn_{cs}, and Δn_{Na^+}. Formation of an intramolecular DNA hairpin structure in solution containing cosolute (e.g., PEG 200 or PEG 8000) and cation (e.g., Na^+) can be represented as follows:

$$A_{ss} \rightleftharpoons A_h + \Delta n_w H_2O + \Delta n_{CS} CS + \Delta n_{Na^+} Na^+, \quad (1)$$

where A_{ss} and A_h indicate the single-strand and the hairpin, respectively, CS represents the cosolute, Na^+ represents sodium ion, and Δn_w, Δn_{cs}, and Δn_{Na^+} represent the numbers of water molecules, cosolute molecules, and sodium ions released upon hairpin formation, respectively. The value is negative when the molecule is associated during hairpin formation. The observed equilibrium constant (K_{obs}) for the hairpin 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 first derivative of $\ln K_{obs}$ by $\ln a_w$ or $\ln a_{Na^+}$ is represented by Eqs. 3 and 4, respectively, which contains the terms for the number of bound molecules.[8,18]

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

$$\frac{d\ln K_{obs}}{d\ln a_{Na^+}} = -\left[\Delta n_w\left(\frac{d\ln a_w}{d\ln a_{Na^+}}\right) + \Delta n_{cs}\left(\frac{d\ln a_{cs}}{d\ln a_{Na^+}}\right) + \Delta n_{Na^+}\right]. \quad (4)$$

When the activity coefficients are close to 1, Eq. 4 can be rewritten as follows:

$$\frac{d\ln K_{obs}}{d\ln [Na^+]} = -\left[\Delta n_w\left(\frac{d\ln [H_2O]}{d\ln [Na^+]}\right) + \Delta n_{cs}\left(\frac{d\ln [CS]}{d\ln [Na^+]}\right) + \Delta n_{Na^+}\right], \quad (5)$$

where $[H_2O]$, $[CS]$, and $[Na^+]$ are the molar concentrations of water, cosolute, and Na^+, respectively.

Results and Discussion

Effect of PEG on the Structure and the Stability of a Hairpin DNA. To investigate the effect of PEG on the structure and the stability of DNA base pairings, we examined the formation of an intramolecular hairpin structure by ODN1 (Fig. 1a). We also examined the influences of PEG 200 and PEG 8000 on the hairpin structure of ODN1 formed at 4 °C in 600 or 10 mM NaCl by CD spectroscopy. The CD spectra in the presence of 20 wt % PEG 200 or PEG 8000 and at 600 or 10 mM NaCl showed positive peaks around 270 nm and negative peaks around 250 nm, indicating a B-form structure[1,21] as observed in PEG-free solution (Fig. 2). This suggests that the base stacking and the sugar–phosphate backbone conformation of the hairpin structure are not substantially affected by PEG, consistent with the idea that PEG does not bind to nucleotides. We have also previously observed this phenomenon for the 8-mer DNA duplex (ODN2).[8] Additionally, the NaCl concentration had little affect on the hairpin structure in the absence or presence of PEG.

To investigate the stability of the hairpin stability in the solutions of ODN1, we measured the melting temperature (T_m). Figure 3 shows the UV melting curves at 600 or 10 mM NaCl in the presence and absence of 20 wt % PEG 200 or PEG 8000. All of the melting curves showed a two-state transition from a hairpin to a single strand. Also, changing the oligomer concentration from 5 to 50 μM did not affect the T_m (data not shown), which is consistent with the melting behavior of an intramolecular hairpin structure.[22] Comparison of the T_m data of at 600 mM NaCl indicated that T_m decreased by 7.5 °C (from 70.8 to 63.3 °C) upon addition of PEG 200 and by 1.9 °C (from 70.8 to 68.9 °C) upon addition of PEG 8000 (Fig. 3a). More importantly, when the NaCl concentration was changed from 600 to 10 mM, T_m decreased by 17.3 °C (from 70.8 to 53.5 °C) in the absence of PEG, 13.6 °C (from 63.3 to 49.7 °C) in the presence of 20 wt % PEG 200, and

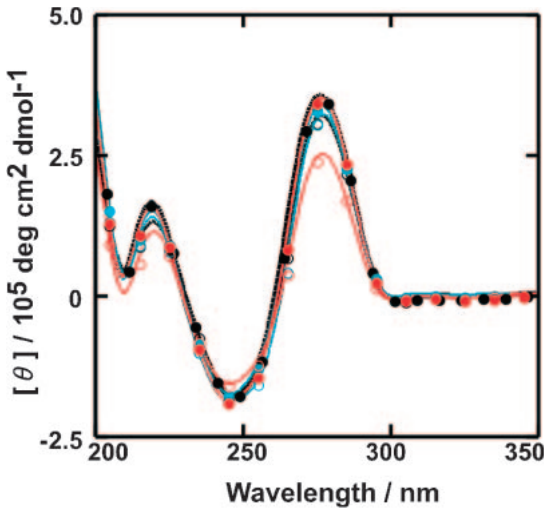

Fig. 2. CD spectra of 20 μM ODN1 at 4 °C measured in solutions containing 10 mM (closed circles) or 600 mM NaCl (open circles) and in the absence (black) and presence of 20 wt % PEG 200 (cyan) or 20 wt % PEG 8000 (red).

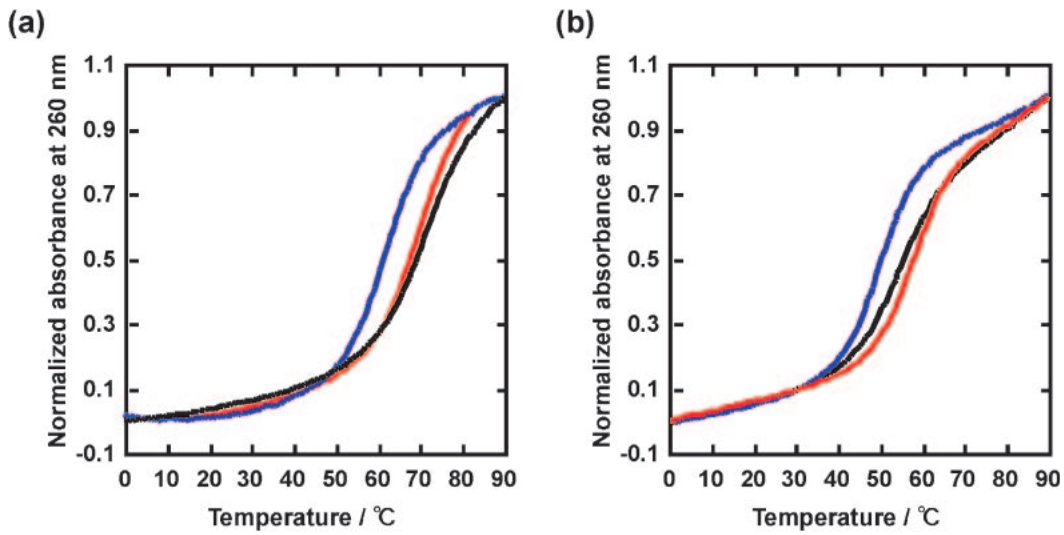

Fig. 3. Normalized melting curves for ODN1 at 5 μM in solutions containing (a) 600 mM or (b) 10 mM NaCl and in the absence (black) or presence of 20 wt % PEG 200 (cyan) or 20 wt % PEG 8000 (red).

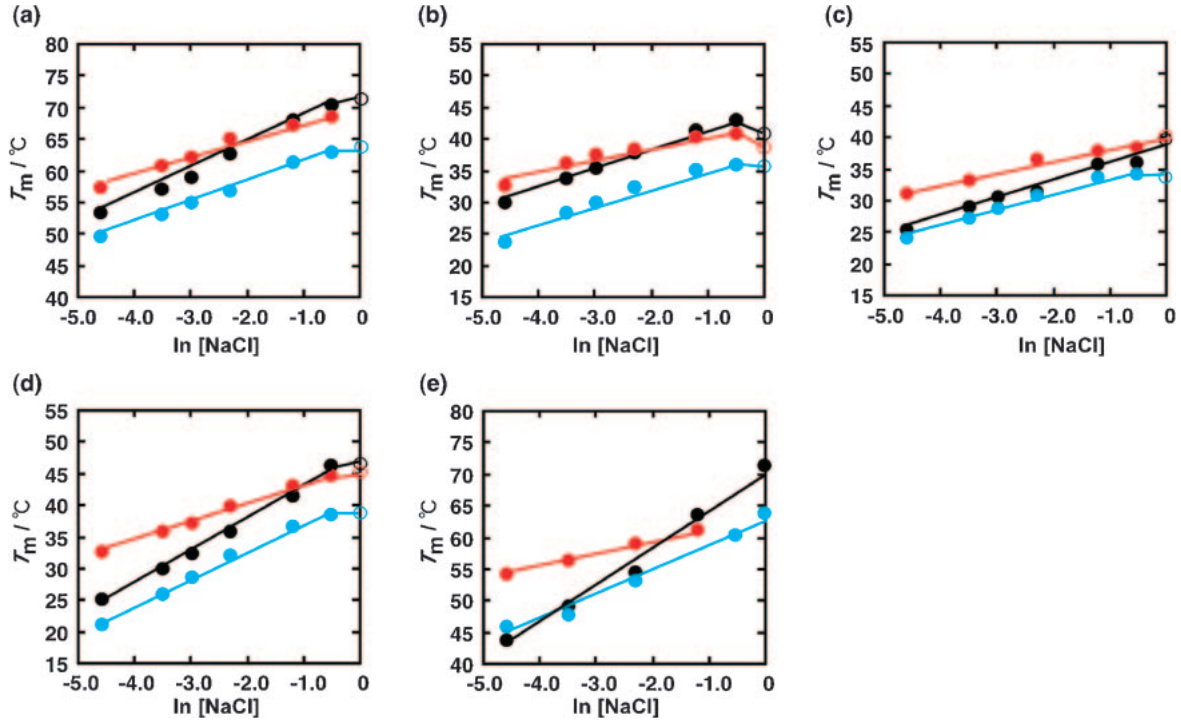

Fig. 4. Dependencies of the T_m for (a) ODN1, (b) ODN2, (c) ODN3, (d) ODN4, and (e) ODN5 on the NaCl concentration. The T_m values were measured for 5 μM DNA in solutions without (black) or with 20 wt % PEG 200 (cyan) or 20 wt % PEG 8000 (red). The data points that deviated from a linear plot are indicated with open symbols.

11.3 °C (from 68.9 to 57.6 °C) in the presence of 20 wt % PEG 8000 (Fig. 3b). Thus, T_m increased by 4.1 °C (from 53.5 to 57.6 °C) when 20 wt % PEG 8000 was added in the presence of 10 mM NaCl.

DNA Duplex Stabilities at Various NaCl Concentrations in the Presence of PEG. To examine the effect of PEG on Watson–Crick base pairing in greater detail, we also measured the T_m for the 8-mer self-complementary duplex (ODN2), the 8-mer non-self-complementary duplex (ODN3), the 14-mer duplex (ODN4), and the 30-mer duplex (ODN5) (Fig. 1). Figure 4 shows the T_m values of the DNA duplexes obtained at different NaCl concentrations in the absence and presence of 20 wt % PEG. The stabilities of all of the duplexes including ODN1 decreased as the NaCl concentration was decreased, and there was a linear correlation between the T_m and the natural logarithm of the NaCl concentration, although, as previously reported,[12] the linear correlation was lost at higher NaCl concentrations (>600 mM). The slopes of the plot of T_m vs. ln [NaCl] ($dT_m/d\ln$ [NaCl]), which is related to the number of sodium ions bound to nucleotides upon duplex formation, are listed in Table 1. Comparison of the fully matched duplexes of ODN2, ODN3, ODN4, and ODN5 revealed that the longer DNA duplexes had steeper slopes in the absence of PEG. This supports the idea that sodium ions mostly bind to phosphate groups, because the number of phosphates increases with the duplex length; however, $dT_m/d\ln$ [NaCl] for forming a hairpin structure was higher for ODN1 than for ODN2 and ODN3, even though they have the same number of base-pairs. This is probably due to the presence of a loop nucleotide in ODN1.

We also found that PEG 200 and PEG 8000 reduced the degree of duplex destabilization caused by reducing the NaCl concentration (Fig. 4 and Table 1); the values of $dT_m/d\ln$ [NaCl] for each DNA duplex were 5–35% at 20 wt % PEG 200 and 40–67% at 20 wt % PEG 8000, which are lower than the values obtained in the absence of PEG. Therefore, these PEGs can buffer the destabilization of Watson–Crick base pairs caused by decreasing the NaCl concentration. Interestingly, at lower NaCl concentrations, the T_m of each DNA in 20 wt % PEG 8000 was substantially higher than without PEG. As a result, the $dT_m/d\ln$ [NaCl] value was smaller in the presence of 20 wt % PEG 8000 than in the absence of PEG. The difference in the T_m values in the absence and presence of PEG 8000 increased as the NaCl concentration decreased below the physiological salt concentration.

Table 1. $dT_m/d\ln$ [NaCl] Values (°C) of the DNA Duplexes

Sequence	Without PEG	20 wt % PEG 200	20 wt % PEG 8000
ODN1	4.4 ± 0.2	3.4 ± 0.1	2.8 ± 0.2
ODN2	3.2 ± 0.1	3.0 ± 0.3	1.9 ± 0.2
ODN3	2.7 ± 0.2	2.6 ± 0.1	1.9 ± 0.2
ODN4	5.2 ± 0.2	4.4 ± 0.2	3.0 ± 0.1
ODN5	6.0 ± 0.4	4.0 ± 0.3	2.0 ± 0.1

Thermodynamic Parameters of DNA Hairpin Formation. To quantify the destabilization of the duplex in the PEG-containing buffers at different NaCl concentrations, we calculated the thermodynamic parameters for ODN1, because formation of a hairpin structure is an intramolecular reaction and it is expected to be barely affected by the excluded volume effect of PEG. In addition, as indicated in Fig. 4a, ODN1 was

stable enough to obtain accurate thermodynamic parameters even in the presence of PEGs. Table 2 shows the values of the thermodynamic parameters ΔH°, $T\Delta S^\circ$, and ΔG° at 25 °C (ΔG°_{25}) for hairpin formation in each solution. When the NaCl concentration was reduced from 600 to 10 mM, the hairpin was destabilized by a ΔG°_{25} of 2.73 kcal mol^{-1} (without PEG), 2.25 kcal mol^{-1} (20 wt % PEG 200), and 1.50 kcal mol^{-1} (20 wt % PEG 8000). This supports the idea that PEG decreases the degree of the duplex destabilization caused by decreasing the NaCl concentration. When the NaCl concentration was reduced from 600 to 10 mM, the $-\Delta H^\circ$ value was decreased by 4.7 kcal mol^{-1} without PEG, 3.0 kcal mol^{-1} with 20 wt % PEG 200, and 1.2 kcal mol^{-1} with 20 wt % PEG 8000, and the $-T\Delta S^\circ$ values were decreased by 1.9 kcal mol^{-1} without PEG and 0.8 kcal mol^{-1} with 20 wt % PEG 200 and increased by 0.3 kcal mol^{-1} with 20 wt % PEG 8000. These results suggest that PEGs reduce the decrement in the $-\Delta H^\circ$ value for hairpin formation when the NaCl concentration is decreased from 600 to 10 mM. This decrease in the $-\Delta H^\circ$ value was unfavorable for formation of a hairpin structure, indicating that PEGs buffered the destabilization of the hairpin caused by reducing the NaCl concentration.

Table 2. Thermodynamic Parameters for Hairpin Formation by ODN1 at 600 and 10 mM NaCl in the Absence and Presence of 20 wt % PEG 200 or PEG 8000

NaCl concentration /mM	$-\Delta H^\circ$ /kcal mol^{-1}	$-T\Delta S^\circ$ /kcal mol^{-1}	$-\Delta G^\circ_{25}$ /kcal mol^{-1}	T_m[a] /°C
Without PEG				
600	49.9 ± 0.7	43.2 ± 0.6	6.67 ± 0.24	70.8
10	45.2 ± 0.4	41.3 ± 0.4	3.94 ± 0.18	53.5
20 wt % PEG 200				
600	53.1 ± 0.7	47.0 ± 0.6	6.14 ± 0.26	63.3
10	50.1 ± 0.5	46.2 ± 0.4	3.89 ± 0.21	49.7
20 wt % PEG 8000				
600	52.2 ± 0.2	45.6 ± 0.3	6.58 ± 0.13	68.9
10	51.0 ± 0.6	45.9 ± 0.6	5.08 ± 0.23	57.6

a) The melting temperature was calculated at 5 μM ODN1.

Effect of the Water Activity on Hairpin Stability. To investigate how PEG altered the thermodynamic stability of ODN1 at different NaCl concentrations, we measured the thermodynamic parameters. We focused on the water activity (a_w) of the solutions (Table S1). We measured the thermodynamic stability of ODN1 in 10–50 wt % PEG 200 and 10–20 wt % PEG 8000 at different NaCl concentrations. We were unable to examine the effects of higher concentrations of PEG 200 (>50 wt %) and PEG 8000 (>20 wt %), because of their limited solubility. Figure 5a shows the plots of $\ln K_{obs}$ for ODN1 at 25 °C vs. $\ln a_w$ at different NaCl concentrations. The plots indicated that the hairpin stability ($\ln K_{obs}$) increased linearly with $\ln a_w$ for all NaCl concentrations (closed symbols in Fig. 5a), although the data at high water activity (>−0.01) and low NaCl concentrations deviated from linearity (open symbols in Fig. 5a). These plots were linear regardless of the molecular weight of PEG. We have previously reported similar findings for ODN2 at NaCl concentration of 1 M and 100 mM.[8]

In principle, the thermodynamic stability of the hairpin structure is affected by the interactions of water molecules, cosolute, and cations with the nucleotide, which can be quantified by Eqs. 1–5 (see the Experimental section). According to Eq. 3, the slope of the $\ln K_{obs}$ vs. $\ln a_w$ plot includes the constant Δn_w, which represents the number of water molecules released by hairpin formation, and two variable terms corresponding to cosolute and sodium ion binding. However, the linear plots suggest that the two latter terms are insignificant and that the slope in Fig. 5a is approximately equal to

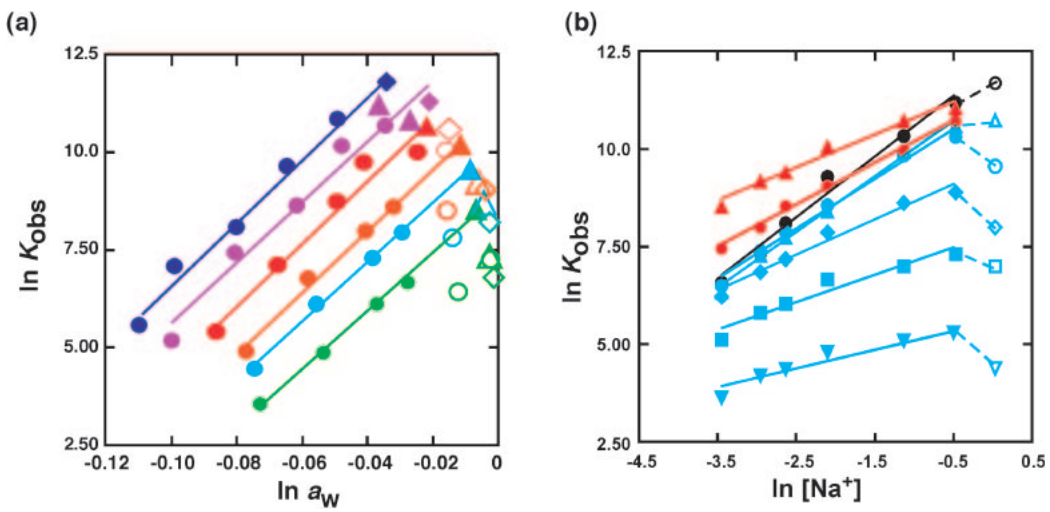

Fig. 5. (a) Plots of $\ln K_{obs}$ for ODN1 at 25 °C vs. $\ln a_w$ in the absence (diamonds) and presence of PEG 200 (circles) or PEG 8000 (triangles) in buffer containing 1 M (blue), 600 mM (pink), 300 mM (red), 100 mM (orange), 50 mM (cyan), or 10 mM NaCl (green). (b) Plots of $\ln K_{obs}$ for ODN1 at 25 °C vs. $\ln [Na^+]$. The $\ln K_{obs}$ values at 25 °C were measured in solutions without PEG (black), with PEG 200 (cyan) at 10 wt % (triangles), 20 wt % (circles), 30 wt % (diamonds), 40 wt % (squares), or 50 wt % (inverted triangles) or with PEG 8000 (red) at 10 wt % (triangles) or 20 wt % (circles). The data points that deviated from a linear plot are indicated with open symbols.

Table 3. Water Binding during Hairpin Formation by ODN1 as Determined from a Plot of $\ln K_{obs}$ at 25 °C vs. $\ln a_w$

NaCl concentration/mM	$-\Delta n_w$
10	73.6 ± 2.5
50	76.5 ± 5.2
100	78.7 ± 3.1
300	78.9 ± 6.9
600	79.3 ± 5.1
1000	80.2 ± 4.2

$-\Delta n_w$.[8,18] The plots were linear with a positive slope ($-\Delta n_w > 0$) due to water binding upon hairpin formation (Table 3). This observation is consistent with the fact that the formation of Watson–Crick base pairs is a water-binding reaction[23] and that the primary hydration shell for B-form DNA is composed of approximately 20 water molecules per base pair.[24] The $-\Delta n_w$ values at 10, 50, 100, 300, 600, and 1000 mM NaCl were 73.6 ± 2.5, 76.5 ± 5.2, 78.7 ± 3.1, 78.9 ± 6.9, 79.3 ± 5.1, and 80.2 ± 4.2, respectively. Thus, the NaCl concentration had little effect on the $-\Delta n_w$ value. Our previous study using ODN2, similar $-\Delta n_w$ values at 1 M NaCl (120 ± 4.8) and 100 mM NaCl (115 ± 4.0) have been obtained.[8]

We next calculated the number of sodium ions required for formation of a single Watson–Crick base pair, under the assumption that fewer sodium ions bind to loop nucleotides. The number of sodium ions required to form a single Watson–Crick base pair for ODN1 and ODN2 was 9.8 ± 0.4 and 14.4 ± 0.5, respectively, which are substantially different; however, the value for a 28-mer hairpin DNA of 5′-TCTTTCTCTTCTTTTTAGAAGAGAAAGA-3′ (loop nucleotides are underlined) has been reported to be 8.3 ± 0.8 in 100 mM NaCl,[18] which is similar to the value obtained for ODN1 forming a hairpin structure. These observations suggest that the loop nucleotides affect sodium ion binding or that the number of sodium ions bound to Watson–Crick base pair differs according to the base pair or the sequence.

Effect of the Sodium Ion Concentration on DNA Base Pair Stability. Using Eq. 5, we calculated the number of sodium ions bound (Δn_{Na^+}) upon ODN1 formation at 10, 20, 30, 40, and 50 wt% PEG 200 and 10 and 20 wt% PEG 8000. Figure 5b shows plots of $\ln K_{obs}$ of ODN1 at 25 °C vs. $\ln [Na^+]$ at different concentrations of PEG. The hairpin stability ($\ln K_{obs}$) increased linearly with the $\ln [Na^+]$ between 10 and 600 mM NaCl. This trend is similar to that in the T_m plots shown in Fig. 4. As described by Eq. 5, the slope of the plot includes a constant term, $-\Delta n_{Na^+}$, along with two variable terms for water and cosolute binding, although the linear plots suggest that the slope is approximately equal to $-\Delta n_{Na^+}$ as applied for the determination of $-\Delta n_w$.

The slope of the plot in the absence of PEG was positive ($-\Delta n_{Na^+} > 0$), indicating sodium ion binding during hairpin formation (Table 4). A $-\Delta n_{Na^+}$ value of 1.6 ± 0.1 indicates that 0.080 sodium ions bind per DNA phosphate, which agrees with previous reports that 0.020 to 0.075 sodium ions bind per phosphate to hairpin DNAs containing a six base-pair stem and six loop nucleotides.[20,25] The $-\Delta n_{Na^+}$ values in the presence of 10, 20, 30, 40, and 50 wt% PEG 200 were 1.4 ± 0.1, 1.2 ± 0.1, 0.91 ± 0.08, 0.70 ± 0.09, and 0.48 ± 0.09, respectively, and the values in the presence of 10 and 20 wt% PEG 8000 were 1.1 ± 0.1 and 0.84 ± 0.06, respectively, which were lower than the value in the absence of PEG. These results indicate that the number of sodium ions bound to the hairpin DNA decreases as the PEG concentration increases. Nordstrom et al. have recently reported that the addition of ethylene glycol reduces the number of sodium ions bound by calf thymus DNA due to a reduction of the dielectric constant of the solution.[26] It is also thought that the $-\Delta n_{Na^+}$ value is relevant not only for the real number of sodium ions bound to nucleotides but also for the strength of the electrostatic interactions between sodium ions and nucleotide phosphates, which is a weak interaction that allows the ions to exchange rapidly with those in the bulk solution.[3] According to the polyelectrolyte theory,[2] the $-\Delta n_{Na^+}$ value is related to changes in the charge density or the average axial spacing of nucleotide phosphate groups. When the charge density of nucleotide phosphates is increased, cations can bind more strongly. We found that the conformation of single-stranded DNA oligomers in the presence of PEG 200 or PEG 8000 differed substantially from that in a solution without PEG (our unpublished results), although PEGs did not change the CD spectra measured at 4 °C (Fig. 2). Therefore, it is possible that molecular crowding caused by PEGs enhances the affinity of cation–nucleotide binding by reducing the dielectric constant of the solution and conformational changes by single-stranded DNA.

Table 4. Sodium Ion Binding during Hairpin Formation by ODN1 as Determined from a Plot of $\ln K_{obs}$ at 25 °C vs. $\ln [Na^+]$

PEG	$-\Delta n_{Na^+}$
Without PEG	1.6 ± 0.1
10 wt% PEG 200	1.4 ± 0.1
20 wt% PEG 200	1.2 ± 0.1
30 wt% PEG 200	0.91 ± 0.08
40 wt% PEG 200	0.70 ± 0.09
50 wt% PEG 200	0.48 ± 0.09
10 wt% PEG 8000	1.1 ± 0.1
20 wt% PEG 8000	0.84 ± 0.06

Binding of Water and Sodium Ions during Hairpin Formation. The plots in Fig. 5a for the binding of water indicated that $-\Delta n_w$ values were in the range of 73.6–80.2 and values close to 80 at high NaCl concentrations. Moreover, the plots in Fig. 5b for sodium ion binding gave $-\Delta n_{Na^+}$ values of 1.4–0.48 depending on the concentration of added PEG and a value of 1.6 in the absence of PEG. Consequently, hairpin formation in ODN1 in the absence of PEG can be represented by Eq. 6:

$$A_{ss} \rightleftharpoons A_h + 80H_2O + 1.6Na^+. \quad (6)$$

The Δn_w and Δn_{Na^+} values can be obtained from the intercepts of the plots in Fig. 5. In Fig. 5a, the intercept of the plots reflect the $\ln K_{obs}$ values when $\ln a_w = 0$ ($\ln K_{obs,\ln a_w=0}$); thus, as determined according to Eq. 5, the intercept vs. $\ln [Na^+]$ plot gave a $-\Delta n_{Na^+}$ value of 1.6. Similarly, the intercept of the plots in Fig. 5b ($\ln K_{obs,\ln Na^+=0}$ vs. $\ln a_w$) gave a $-\Delta n_w$

value of 84.7 (Fig. S1). Because these values are close to those determined from the slopes of these plots, the linear approximations for the plots in Fig. 5 is valid, and Eq. 6 accurately represents the stoichiometry of water and sodium ion binding during hairpin formation by ODN1. Notably, more water molecules and sodium ions associate with the DNA duplex than determined here, because the numbers reflect only those required for the structured transition and because water and sodium ion also associate with single-stranded DNA.

In the presence of PEG, the $-\Delta n_{Na^+}$ value became smaller than 1.6. The observation that the addition of PEG altered the $-\Delta n_{Na^+}$ value but not the $-\Delta n_w$ value is consistent with the outer-sphere binding model, wherein the hydration of a cation and the nucleotide is unchanged by binding.[3] Although it is difficult to study outer-sphere binding of cations due to the relatively weak binding and rapid exchange with bulk ions, our approach was able to address outer-sphere binding.[27] Therefore, a thermodynamic approach is important for the study of outer-sphere binding of cations by nucleotides.

Possible Roles of Molecular Crowding in Regulating DNA Structure and Stability. Quantitative analyses of the effects of PEG on Watson–Crick base pairs at different NaCl concentrations indicated that PEG reduced the number of sodium ions required for DNA base pair formation. This observation provides a detailed understanding of how molecular crowding can offset perturbations in the salt concentration levels in living cells.[28,29] Buffering the effects of changes in the environmental salt concentration favors the maintenance of nucleotide structures and cellular metabolism. Moreover, the genomic DNA and proteins in the nucleus can continue to carry out their biological functions even under highly condensed conditions.[30] Berg has suggested that intracellular crowding may act as an evolutionary force that tends to bias conformational equilibria of a DNA toward compact conformations.[28] Buffering of metabolism may also be mediated by the thermodynamics of biomolecular reactions.[15] It is reported that molecular crowding by PEG increases the DNA binding affinity of DNA polymerases.[31] Also, the G-quadruplex structure of the thrombin-binding DNA aptamer can be induced by adding PEG 200 as well as by adding human thrombin.[32] These effects of PEG likely reflect biological effects of molecular crowding. The concentration of proteins inducing molecular crowding inside living cells is known to vary with the cell cycle.[33] This might buffer changes in DNA stability and structure under physiological conditions. Accordingly, quantitative analyses of the biomolecular reactions under molecular crowding conditions may provide significant insight into the reactions that occur in living cells.

Conclusion

We investigated the thermal stability of DNA Watson–Crick base pairs in solutions containing PEG at different NaCl concentrations. We found that PEG 200 and PEG 8000 buffer the destabilization of the base pairs when the concentration of NaCl is decreased; when the NaCl concentration was decreased from 600 to 10 mM, the $-\Delta G^\circ_{25}$ value for ODN1 decreased by 2.73 kcal mol^{-1} in the absence of PEG, 2.25 kcal mol^{-1} in the presence of 20 wt % PEG 200, and 1.50 kcal mol^{-1} in the presence of 20 wt % PEG 8000. Our results also indicated that PEG decreased the $-\Delta n_{Na^+}$ value without changing the $-\Delta n_w$ value, suggesting that PEG reduces the number of sodium ions required for base pair formation. These phenomena led to an enhancement in base pair stability at 10 mM NaCl by the addition of PEG 8000. The reduction in the number of sodium ions bound to nucleotides by the addition of PEG suggests that molecular crowding helps maintain DNA stability in the face of changing intracellular cation concentrations. Quantitative analyses of the effects of PEG and NaCl in the current study help clarify the binding of both sodium ions and water molecules by nucleotides. In addition, our results suggest that a change in the sodium ion concentration can be used to control DNA hybridization in technological applications. Finally, the present results should help clarify the structure and stability of DNA under conditions of molecular crowding, which are thought to be present not only in cells but also on sensor chips and nanoparticles.

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Supporting Information

Table S1 shows the values of water activity in the absence and presence of PEG in PDF format. Figure S1 shows the plots of $(\ln K_{obs,\ln a_w=0})$ vs. $\ln[Na^+]$ and $(\ln K_{obs,\ln Na^+=0})$ vs. $\ln a_w$ for ODN1 in PDF format. These materials are available free of charge on the web at: http://www.csj.jp/journals/bcsj/.

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