Hydration Is an Important Factor to Regulate Thermodynamic Stability of a DNA Duplex under Molecular Crowding Conditions

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By using a DNA duplex, (5′-GTTACTATATGA-3′/5′-TCATATAGTAAC-3′) under molecular crowding conditions, it was revealed that hydration plays a significant role in the stability of the duplex, in contrast to viscosity and dielectric constant which have little effect.

Since cells contain various biomolecules such as proteins, nucleic acids, saccharides, vitamins, and metabolites, their total concentration can reach as high as 400 g/L, occupying most of the cellular volume.[1] These crowded conditions (molecular crowding) are very different from dilute solutions that are normally studied in vitro, and therefore it has been recognized that molecular crowding has profound effects on the stability, conformation, and activity of cellular biomolecules. Minton, Zimmerman, and co-workers studied the effects of molecular crowding on proteins, both theoretically and experimentally, and found that molecular crowding has a substantial impact on protein stability, activity, and folding properties.[2] However, there have been few studies of molecular crowding on DNA so far.[3,4] We and other groups have reported that molecular crowding destabilizes DNA duplexes,[3b,4a,4f] whereas it stabilizes DNA triplexes[4b–4e] and quadruplexes.[3a,3b] Furthermore, molecular crowding alters a DNA quadruplex from an antiparallel to a parallel structure.[3c] According to subsequent quantitative studies,[4c,5] water molecules involved in structure formation, which are related to the hydration factor of DNA under molecular crowding conditions, are taken up by DNA duplexes whereas DNA triplexes and quadruplexes release them. Given preliminary results, hydration seems to be the most important factor regulating the stability of DNAs under molecular crowding conditions. However, it is still difficult to conclude that hydration is involved in the stabilization or destabilization of DNA because highly different hydration environments among DNA duplexes, triplexes, and quadruplexes can have different estimated numbers of water molecules, which are not only directly solvating the DNA, but are also indirectly bound to metal ions and cosolutes surrounding the DNA. Furthermore, other factors such as viscosity and dielectric constant can possibly affect the stability of DNAs. To conclude that hydration is involved, we should use a DNA that is both stabilized and destabilized by changing molecular crowding conditions. Here, we have found that a DNA duplex (DNA1: 5′-GTTACTATATGA-3′/5′-TCATATAGTAAC-3′) can be both stabilized and destabilized by changing the molecular weight of poly(ethylene glycol)s (PEGs) that mimic cellular crowding environments. Using the DNA and PEGs, we have quantitatively evaluated what kinds of factors, including hydration, viscosity, and dielectric constant, are playing important roles in the stability of DNA duplexes under molecular crowding conditions.

PEGs with different molecular weights (PEG200, PEG3000, and PEG6000) were used as cosolutes to change the crowding environment. UV melting curves at 260 nm of DNA1 were obtained in 10 mM phosphate buffer (pH 7.0) containing 0.2 M NaCl, 0.1 mM EDTA, and 5–30 wt % PEG200, PEG3000, or PEG6000.

Figure 1 shows plots of the melting temperatures (T_m) of DNA1 as a function of PEG concentration. Changing the molecular weight of PEG induces stabilization or destabilization of DNA1 as the PEG concentration rises. PEG200 destabilizes the duplex with an increase in the PEG concentration, whereas PEG6000 stabilizes it. Interestingly, PEG3000, with a molecular weight in between PEG200 and PEG6000, scarcely changes (slightly stabilizes) the duplex even when the concentration rises up to 30 wt %.

To obtain further insights into the effect of molecular crowding on the stability of DNA1 quantitatively, we estimated the thermodynamic parameters using T_m^{-1} vs. $\log C_T$ plots.[6] To clarify the involvement of hydration, viscosity, and dielectric constant in the stability of DNA1, the stability parameters ($\ln K_{obs}$), which are calculated from ΔG°_{25} values,[5] were plotted as functions of parameters related to (a) water activity, (b) viscosity (η), and (c) dielectric constant (ε), respectively (Figure 2).[8]

Figure 2a shows the plots of $\ln K_{obs}$ vs. $\ln a_w$. According to previous studies,[5] when the plots obey a linear relation, the stability is ascribed to the hydration factor. As shown in Figure 2a, all the plots for PEG200, PEG3000, and PEG6000 obey linear relations, indicating that the stability difference induced by changes in the molecular weight of PEG results from the hydration factor. Furthermore, according to the reported

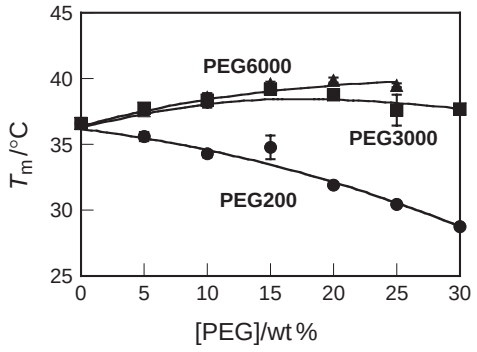

Figure 1. Plots of T_m as a function of PEG concentration for PEG200 (closed circle), PEG3000 (closed square), and PEG6000 (closed triangle).

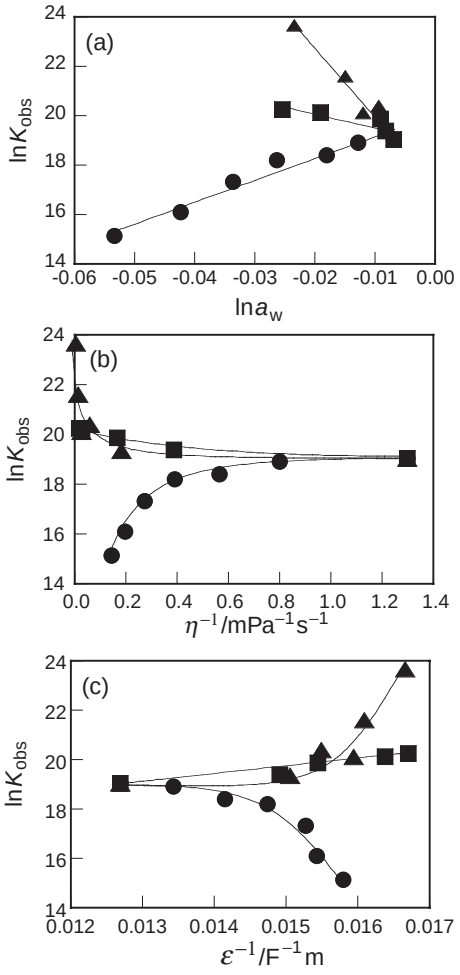

Figure 2. Plots of $\ln K_{obs}$ as functions of (a) $\ln a_w$, (b) η^{-1}, and (c) ε^{-1} for PEG200 (closed circle), PEG3000 (closed square), and PEG6000 (closed triangle).[8]

method, the estimated number of water molecules involved in the duplex formation[5] reveals that the take-up and release behavior of water molecules (4.0 water molecules were taken up for PEG200; 1.0 and 9.0 water molecules per nucleotide were released for PEG3000 and PEG6000, respectively) is consistent with stabilization and destabilization behavior for DNAs, indicating that hydration is the key factor in stabilizing and destabilizing the duplex.

Similar to the hydration factor, we evaluated the involvement of viscosity and dielectric constant in the stability of the duplex. Since high viscosity should affect intermolecular interactions due to a drastic decrement of the diffusion constant, $\ln K_{obs}$ values were plotted as a function of η^{-1} (Figure 2b) (diffusion constant is proportional to η^{-1}). The $\ln K_{obs}$ values for PEG3000 gradually increase as η^{-1} decreases, whereas those for PEG6000 drastically increase as η^{-1} decreases to less than 0.1. Furthermore, values for PEG200 non-linearly decrease as η^{-1} decreases. If the decrement of diffusion constant affects duplex formation, changes in $\ln K_{obs}$ for PEG200, PEG3000, and PEG6000 with high viscosity are expected to show similar behavior as η^{-1} decreases. However, these PEGs show little relation, indicating that viscosity is not the main factor affecting stability of the duplex.

Also, we considered the influence of dielectric constant (ε) on the stability of the DNA duplex (Figure 2c). A drastic decrement of ε should change the electrostatic interactions of DNAs.[4b,7] Therefore, $\ln K_{obs}$ values were plotted as a function of ε^{-1} (Figure 2c).[4b] Usually, when dielectric constants affect the stability of DNA duplexes under molecular crowding conditions, the plots give the same slope. However, as can be seen in Figure 2c, the plots not only show no linearity, but also show different behavior among PEG200, PEG3000, and PEG6000, suggesting that the stability of DNA duplex does not scale with the dielectric constant.

In conclusion, we examined the involvement of hydration, viscosity, and dielectric constant in terms of stability of a DNA duplex under molecular crowding conditions. Our quantitative analysis showed that hydration plays a significant role in the stability of the duplex under molecular crowding conditions, whereas there is little participation of viscosity and dielectric constant. Cellular hydration environments consistently vary during cell cycles and changes in the extracellular environment. Given such cellular environments, the present result implies that the stability of DNA duplexes might be regulated by cellular hydration factors, resulting in the regulation of cellular genomic events to alter the stability of genome duplexes.

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8 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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