Effects of cosolutes on the thermodynamic stability of parallel DNA duplex and triplex

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ABSTRACT

We investigated molecular crowding effects on the thermodynamic stability of Hoogsteen and Watson-Crick base pairs in an intramolecular duplex and triplex. The melting temperature ($T_m$) of Hoogsteen base pair formations in the triplex and the duplex increased 3.7 °C and 3.2 °C, respectively, by adding 20 wt% PEG 200. On the other hand, the $T_m$ of Watson-Crick base pair formations in the triplex and the duplex decreased 5.7 °C and 5.2 °C, respectively. These results suggested that molecular crowding conditions generally stabilized and destabilized Hoogsteen and Watson-Crick base pairs, respectively, even in the different DNA structures.

INTRODUCTION

Most of biochemical studies are regularly conducted in highly dilute solutions, whereas the intracellular environment is extremely crowded; 20-40% of the total volume is physically occupied by macromolecules1. In fact, it has been reported that molecular crowding is a critical factor determining the structure, stability, and function of a variety of proteins2,3. To understand physiology and metabolism in vivo, elucidation of the effects of molecular crowding on the structure and stability of DNAs is also currently of great interest. In fact, DNA structures depend on not only type of base pair formation but also surrounding conditions. For example, we reported that an antiparallel G-quadruplex formation with Hoogsteen base pairs was stabilized under molecular crowding conditions but an antiparallel duplex formation with Watson-Crick base pairs was destabilized4. These results are consistent with the molecular crowding effect on triplex DNA reported by Spink and Chaires6. They demonstrated that DNA triplexes consisting of Hoogsteen base pairs were stabilized by molecular crowding. These quantitative reports suggested that noncanonical DNA structures consisting of Hoogsteen base pairs were more preferable than canonical DNA duplexes consisting of Watson-Crick base pairs under molecular crowding conditions. However, not only base pair types but also structures and sequences used in these studies were totally different from each other. Therefore, the effects of molecular crowding on Hoogsteen and Watson-Crick base pairs still remain unclear. In order to study directly and quantitatively the effects of molecular crowding on the Hoogsteen and Watson-Crick base pairs, here we investigated thermodynamic stability of Hoogsteen and Watson-Crick base pairs in the duplex and the triplex (Fig 1). As a result, DNA structures consisting of Hoogsteen base pairs stabilized under molecular crowding conditions, whereas that consisting of Watson-Crick base pairs destabilized.

MATERIALS AND METHODS

The melting curves of DNA structures were obtained by measuring the UV absorbance at 295 or 260 nm in buffers of 100 mM NaCl, 1 mM Na2EDTA, and 10 mM Na2HPO4 (pH 7.0) containing various concentrations of PEG 200. The $T_m$ values for 5 μM DNA structures were obtained from the UV melting curves as described previously4,5. The heating rate was 0.2 °C min⁻¹ or 0.5 °C min⁻¹ for the WC duplex. The thermodynamic parameters were calculated from the fit of the melting curves to a theoretical equation7. Before the measurement, the sample was heated to 80°C, gently cooled at a rate of 1°C min⁻¹, and incubated at 0°C for 1 h.
RESULTS AND DISCUSSION

We designed and synthesized the parallel and antiparallel stranded DNA duplexes (H duplex and WC duplex) consisting of Hoogsteen and Watson-Crick base pairs, respectively, as well as the intramolecular triplex. The thermal stability of DNA structures was measured by the UV melting analysis. The melting temperature ($T_m$) of the H duplex increased from 13.0°C to 16.7°C when the PEG 200 concentration increased from 0 to 20 wt% (Fig. 2a). On the other hand, the $T_m$ of the WC duplex decreased from 67.4°C to 62.2°C when the PEG 200 concentration increased from 0 to 20 wt% (Fig. 2b). In the case of the triplex, two melting transitions were observed (Fig 2c). The transition at the lower temperature corresponds to formation of Hoogsteen base pairs in the triplex, whereas the transition at the higher temperature corresponds to formation of Watson-Crick base pairs in the triplex DNA. The low melting temperature ($T_m$) increased from 33.9°C to 37.1°C, whereas the high $T_m$ decreased from 67.7°C to 61.8°C by adding 20 wt% PEG200. These results suggested that DNA structures consisting of Hoogsteen base pairs stabilized under molecular crowding conditions, whereas that of Watson-Crick base pairs destabilized.

Furthermore, we also investigated effects of PEG200 concentration on the thermodynamic stability of DNA structures. Figure 3 shows the plots of $T_m$ for DNA structures vs. concentration of PEG200. The plot indicates that the stability of the Hoogsteen base pairs in triplex and duplex linearly increased with increase in PEG200, whereas that of the Watson-Crick base pairs in triplex and duplex linearly decreased. These results are consistent with the previous reported demonstrating for molecular crowding destabilizes and stabilizes duplex and G-quadruplex, respectively.\(^4\)\(^5\). Compared with these previous studies, the results obtained in this study demonstrated that molecular crowding conditions generally stabilized and destabilized Hoogsteen and Watson-Crick base pairs, respectively, even in the different DNA structures. These results suggest that base pair conformation of DNA structures regulates the molecular crowding effects on the thermodynamic stability of DNA structures.

CONCLUSION

It was revealed that Hoogsteen and Watson-Crick base pairs are stabilized and destabilized, respectively, under molecular crowding conditions. These results indicate that DNA structures consisting of Hoogsteen base pairs are more favourable than the DNA duplex consisting of Watson-Crick base pairs under molecular crowding conditions.

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