Factors regulating thermodynamic stability of DNA structures under molecular crowding conditions

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ABSTRACT

The condition in a living cell is molecularly crowded with various biomolecules. The total concentration of the biomolecules inside Escherichia coli is in the range of 300–400 g/L. This is distinct from typical biomolecular concentrations of less than 1g/L, which is generally used for experiments in vitro. Here, we analyzed quantitatively the effects of molecular crowding on the thermodynamics of antiparallel G-quadruplex formation via Hoogsteen base pairs and of antiparallel hairpin-looped duplex (HP duplex) formation via Watson-Crick base pairs. The free energy changes for G-quadruplex and duplex formations decreased and increased when the concentration of poly(ethylene glycol) 200 was increased from 0 to 40 wt%, respectively. These results showed that the antiparallel G-quadruplex is stabilized under molecular crowding conditions but the HP duplex is destabilized.

INTRODUCTION

In a living cell, biomacromolecules such as a nucleic acid, protein, and polysaccharide together with other soluble and insoluble components occupy significant fraction (30–40%) of the cellular volume, and their total concentration can reach 400 g/L. Therefore, biochemical reactions in vitro progress under the crowding condition. Most studies of the biochemical reactions in vitro, however, have been performed in solutions containing low concentrations of biomacromolecules.

In order to understand physiology and metabolism in vivo, elucidation of the effect of molecular crowding on DNA structures and their stabilities is currently of great interest. Especially, understanding the effects of molecular crowding on not only duplexes and triplexes but also G-quadruplexes is important because of the potential roles of G-quadruplexes in biological systems. G-quadruplexes are formed by intermolecular or intramolecular association of guanine-rich oligonucleotides with four Hoogsteen-paired coplanar guanines (G-quartet). It was reported that that molecular crowding is one of the most important factors controlling the formation of G-quadruplex structures. However, the thermodynamics of G-quadruplex formation under molecular crowding conditions remain unclear. Moreover, it is not fully understood how molecular crowding affects the structure and stability of DNA oligonucleotides. Therefore, quantitative studies on the effect of molecular crowding on the DNA structures are required.

Here, we investigated quantitatively thermodynamics of DNA duplex and G-quadruplex under dilute and molecular crowding conditions.

MATERIALS AND METHODS

The UV melting curves for the antiparallel G-quadruplex and HP duplex were recorded at 295 and 260 nm, respectively, using a Shimadzu UV1700 instrument. The measurements were carried out in buffers containing 100 mM NaCl, 10 mM Na₂HPO₄ (pH 7.0), and Na₂EDTA in the presence and absence of poly(ethylene glycol) (PEG) 200. The heating rate was 0.5 °C min⁻¹. The thermodynamic parameters were calculated from the fit of the melting curves to a theoretical equation for an intramolecular association as described previously.

CD experiments utilizing a JASCO J-820 spectropolarimeter were measured at 4 °C in a 0.1-cm path length cuvette for 50 μM total strand concentration of DNA in buffers of 100 mM NaCl, 10 mM Na₂HPO₄ (pH 7.0), and 1 mM Na₂EDTA containing various concentrations of PEG200. Before the measurement, the sample was heated to 80 °C, gently cooled at a rate of 2 °C min⁻¹, and incubated at 4 °C for 1 h.

RESULTS AND DISCUSSION

We studied the systematically effect of molecular crowding induced by neutral cosolutes on the thermodynamics of thrombin binding aptamer forming intramolecular antiparallel G-quadruplex formation with Hoogsteen base pairs (Fig. 1a) and of intramolecular antiparallel hairpin-looped duplex (HP duplex) formation via Watson-Crick base pairs (Fig. 1b). Figure 2a shows the CD spectra of 50 μM of thrombin DNA aptamer d(G₂T₂G₂TGTG₃T₂G₂) at 4°C in the presence of Na⁺ and with or without 40 wt% of PEG 200. All CD spectra had positive and negative peaks around 295 and 265 nm, respectively, demonstrating an antiparallel G-quadruplex structure under the conditions. We also used a 28-mer DNA,
d(TCTTCTCTCTTTTAGAAGAGAAAGA) (loop region is underlined), that folds into an intramolecular HP duplex for comparison with the antiparallel G-quadruplex (see Fig. 2a for CD spectra of the HP duplex).

The thermodynamic parameters for the formation of antiparallel G-quadruplexes and HP duplexes were obtained from their thermal melting curves, which are measured by UV absorbance at 295 and 260 nm, respectively. Surprisingly, the melting temperature \( T_m \) for 5 \( \mu \)M of the antiparallel G-quadruplex increased from 24.1 °C to 36.6 °C. In contrast to the G-quadruplex, the \( T_m \) for 5 \( \mu \)M of the HP duplex in the presence of Na⁺ decreased from from 67.0 °C to 55.5 °C as the PEG 200 concentration was increased from 0 to 40 wt\% in the presence of Na⁺.

Next, we investigated how water molecules affect the thermodynamic stability of the antiparallel G-quadruplex and the HP duplex. Figure 2b shows the plots of \( \ln K_{obs} \) for antiparallel G-quadruplex formation at 25 °C vs. \( \ln a_w \) as determined by osmotic pressure measurements at 25 °C. The plots reveal that the stability of the antiparallel G-quadruplex (\( K_{obs} \)) decreases linearly with the increase of \( a_w \), whereas \( K_{obs} \) for the HP duplex at 25 °C increased linearly with the increase of \( a_w \). The slopes of \( K_{obs} \) vs. \( a_w \) for the G-quadruplex and the HP duplex were estimated to be -67.0 ± 5.2 and 99.1 ± 9.9, respectively, which are approximately equal to numbers of water released upon formation of the structure. These values correspond to release of 4.5 ± 0.4 and taking up of 3.5 ± 0.4 water molecules per nucleotide upon formation of the antiparallel G-quadruplex and the HP duplex, respectively. Notably, these results demonstrated dehydration and hydration during the formation of the antiparallel G-quadruplex and the HP duplex, respectively.

It was reported previously that a DNA triplex consisting of Hoogsteen base pairs is stabilized as the water activity decreases. Thus, it appears that molecular crowding conditions, wherein water activity decreases and hydration is unfavorable, stabilize DNA structures containing Hoogsteen base pairs and destabilize those containing Watson-Crick base pairs.

**CONCLUSION**

In this study, it was suggested that, under molecular crowding conditions, noncanonical DNA structures such as G-quadruplexes and triplexes consisting of Hoogsteen base pairs may be more favored than canonical DNA duplexes consisting of Watson-Crick base pairs. These results lead to conclusion that the structure of various DNA sequences can be regulated by the state of hydration.

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