Effects of various cations on the dynamic and thermodynamics of the dimerization of G-quadruplex DNA

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ABSTRACT

DNA fragment, d(TTAGGG), forms all-parallel G-quadruplex in the presence of K'. We found that G-quadruplex d(TTAGGG) dimerizes through end-to-end stacking of the 3'-terminal G-quartets. In this study, we report the effects of Mg2+ and Ca2+ on the dynamics and thermodynamics of the dimerization.

INTRODUCTION

The solution structure of a single repeat sequence of the human telomere, d(TTAGGG), has been shown to form a all-parallel G-quadruplex DNA in the presence of low K' concentrations ([K']) , which aggregates to form a higher order structure in the presence of high [K']. On the other hand, a parallel G-quadruplex DNA formed from the d(TTAGGGT) sequence does not aggregate to form a higher order structure even if [K'] increased, because the extra 3'-terminal thymine prevents the aggregation of the G-quadruplex DNA. Our previous study using 1H NMR and a size exclusion chromatography/multi angle laser light scattering demonstrated that a series of oligonucleotide sequences, d(TTAG)n, where n = 3–5, formed a dimer through end-to-end stacking of the 3'-terminal G-quartets of parallel G-quadruplexes formed from these sequences (Fig. 1). In addition, the dynamics and thermodynamics of the dimerization were also characterized. We report herein the effects of divalent cations such as Mg2+ and Ca2+ on the dynamics and thermodynamics of the dimerization of the G-quadruplex DNA, (d(TTAGGG))4.

RESULTS AND DISCUSSION

We first characterized the structure of G-quadruplex formed from d(TTAGGG) in the presence of various [Mg2+] , together with [K'] = 50 mM, using 1H NMR (Fig. 2). As shown in Fig. 2A, relative intensity of imino proton signals of dimer to monomer increased with increasing [Mg2+], demonstrating that the dimerization of G-quadruplex DNA is significantly promoted by Mg2+. Furthermore, in the base proton signal region, where proton signals due to single-stranded DNA were also observed, together with those of dimer and monomer G-quadruplex DNA, the relative signal intensities of single-stranded DNA and monomer to dimer decreased with increasing [Mg2+] (Fig. 2B). Those results indicated that Mg2+ promotes not only the dimerization of G-quadruplex DNA, but also the formation of G-quadruplex DNA.

Mg2+ has been thought to lower the stability of the G-quadruplex DNA because of its relatively high reactivity to N7 nitrogen atom of guanine base. In fact, base proton signal intensities of G-quadruplex DNA formed from (d(TTAGGGT))4, which does not form dimer because of the prevention of the end-to-end stacking of the 3'-terminal G-quartets by the presence of the 3'-terminal thymine, decreased relative to those of single-stranded DNA, with increasing [Mg2+] (Fig. 3). These results indicated that the dimerization plays a crucial role in the stabilization of G-quadruplex DNA by Mg2+. The molecular surface of G-quadruplex DNA is highly negatively charged and hence the electrostatic repulsion between molecules hampers the dimerization of G-quadruplex DNA. Mg2+ is likely to contribute to partial neutralization of the negative charges of the molecule, which in turn promotes the dimerization. DNA backbone phosphorus NMR signals exhibited an

![Fig. 1 Schematic representation of the dimerization of G-quadruplex DNA, (d(TTAGGG))4.](image)

![Fig. 2 Imino (A) and base (B) proton 1H NMR signals of G-quadruplex (d(TTAGGG))4 in the presence of the indicated [Mg2+] with [K'] = 50 mM, in 90%H2O/10%D2O, 50 mM Tris-HCl buffer, pH 7.0, at 25 °C. The assignments of proton signals are indicated with the spectra.](image)
upfield shift change of ~0.1 ppm by the addition of Mg$^{2+}$. Similar Mg$^{2+}$-induced shift changes were also observed for $^3$P NMR signals of ATP and cyclic AMP. Thus electrostatic interaction between Mg$^{2+}$ and DNA backbone phosphate ions were manifested in the $^3$P NMR data. In addition, similarly to the case of Mg$^{2+}$, Ca$^{2+}$ was also found to promote both the formation and dimerization of G-quadruplex DNA, due to the suppression of the electrostatic repulsion between monomers.

Secondly, we determined melting temperatures ($T_m$) of G-quadruplex DNA by monitoring the absorbance at 304 nm in order to determine the effects of Mg$^{2+}$ and Ca$^{2+}$ on the stability of G-quadruplex DNA. In the case of (d(TTAGGG))$_4$, the $T_m$ increased with increasing [Mg$^{2+}$] and [Ca$^{2+}$] up to 20 and 10 mM, respectively. In those conditions, the dimer was stabilized by the suppression of the electrostatic repulsion due to the partial neutralization of backbone phosphate ions by the divalent cations. Further increase of the cation concentrations decreased the $T_m$ value, as a result of their reactivity to N7 nitrogen atom of guanine base, which tends to break up G-quartet structure. The particularly large destabilization effect of Ca$^{2+}$ can be attributed to its high reactivity to the N7 nitrogen atom. On the other hand, the $T_m$ value of (d(TTAGGG))$_4$ did not increase, but simply decreased with increasing [Mg$^{2+}$] and [Ca$^{2+}$]. These results also supported that the dimerization is crucial in the stabilization of G-quadruplex DNA by Mg$^{2+}$ and Ca$^{2+}$.

Finally, the equilibrium constant ($K_{eq}$) can be obtained from the analysis of NMR signal intensities, and then the temperature dependence of the $K_{eq}$ values allows the determination of thermodynamic parameters. The entropy and enthalpy changes of dimerization ($\Delta S$ and $\Delta H$, respectively) at [Mg$^{2+}$] ~ 8 mM were $-47 \pm 5$ kJ/mol and $-68 \pm 10$ J/Kmol, respectively. $\Delta S$ and $\Delta H$ in the absence of Mg$^{2+}$ were $-47 \pm 5$ kJ/mol and $-91 \pm 10$ J/Kmol, respectively. Consequently, the promotion of the dimerization by Mg$^{2+}$ is attributed to $\Delta S$ in origin. It is likely that the reorientation of water molecule around the DNA, which could be one of factors responsible for $\Delta S$, is affected by Mg$^{2+}$.

**CONCLUSION**

The dimer of G-quadruplex DNA formed from d(TTAGGG) has been shown to be stabilized by Mg$^{2+}$ and Ca$^{2+}$, although Mg$^{2+}$ and Ca$^{2+}$ have been shown to prevent the G-quadruplex formation. This is due to the suppression of the electrostatic repulsion of the interface between the 3'-terminal G-quartets by these cations. This finding is useful for the molecular design of “heme-DNA complex”.

**REFERENCES**