Properties of long human telomeric DNAs under cell-mimicking conditions

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ABSTRACT

We investigated the stability and structure of long telomeric DNAs derived from human, (TTAGGG)ₙ (n =4–12) in the presence of 100 mM K⁺ at 0 wt% or 20 wt% poly(ethylene glycol) 200 (PEG200) utilizing circular dichroism and UV melting analysis. The results showed that the values of enthalpy and entropy changes for the G-quadruplex formation of the telomeric DNAs whose repeat number was multiple of four, such as n=4, n=8, and n=12, increased gradually under the dilute condition (100 mM K⁺), demonstrating no interaction existed between the individual G-quadruplex units composing of four repeats. Therefore, the reasonable arrangement of the intramolecular G-quadruplexes formed by long telomeric DNAs (n≥8) was proposed to be a head-string structure in which the G-quadruplex units were connected each other by one TTA linker. Furthermore, the results of melting experiments demonstrated that thermodynamic stabilities of G-quadruplex structures of the long telomeric DNAs (n=5–12) are mostly independent of sequence length, although telomeric DNA including four repeats (n=4) is more stable than the longer ones. Moreover, the melting temperatures of the G-quadruplexes under the crowding condition (100 mM K⁺ and 20 wt% PEG200) are higher than those under the dilute condition, indicating the crowding condition can increase the stability of G-quadruplex. These information are useful for researches of the telomere biology and a better development of therapeutic agents targeting telomeric DNAs.

INTRODUCTION

Telomeric DNAs composing of tandemly-repeated guanine-rich (G-rich) sequences set on the ends of eukaryotic chromosomes in many species including vertebrates, Saccharomyces cerevisiae, Tetrahymena, and Oxytricha nova.¹ In human somatic cells, telomere is typically 5–8 kb long with a single-strand 3’ overhang of 200±75 bases.² Since a strong association between telomere length and cellular aging was demonstrated,³,⁴ telomere researches have been in focus for issues on cellular senescence and immortalization. Especially, numerous studies have been done for the structures, stabilities, and thermodynamic or kinetic properties of G-quadruplex, which is a possible structure of telomeric DNA.⁵,⁷ However, most of these studies for structural, biochemical and biophysical properties of G-quadruplexes are only for short telomeric DNAs whose number of the repeats is not more than four. More importantly, the difference between the living cell condition in which the macromolecules occupy 20–40% of the total volume and the dilute condition in vitro has been found.⁸ Some distinct changes of the structure and thermodynamic stability of short telomeric DNAs under cell-mimicking condition were observed.⁵,⁹ However, there is no report about the structure and thermodynamic properties of long telomeric DNAs under a cell-mimicking condition.

In this study, we show the evidence of different properties of stability and structure of G-quadruplex under cell-mimicking conditions. The properties of G-quadruplexes under molecular crowding conditions provide important information for exploring telomere science and developing the drugs and ligands targeting telomeric DNAs and telomerase.

MATERIALS AND METHODS

CD (circular dichroism) spectra of the oligonucleotides were obtained by using a Jasco J-820 spectropolarimeter. The CD spectra were measured in a buffer containing 100 mM KCl, 50 mM Tris-HCl (pH 7.0), in the presence of 0 wt% or 20 wt% PEG200 at 4 °C. Antiparallel G-quadruplex has positive and negative CD peaks around 295 nm and 260 nm, respectively. On the other hand, parallel G-quadruplex has positive and negative CD peaks around 260 nm and 240 nm, respectively.¹¹ Before the CD measurements, the samples were incubated at 4 °C after annealing from 90 °C to 0 °C by the rate of 1 °C min⁻¹. The UV melting curves for DNA G-quadruplexes were recorded at 295 nm using a Shimadzu UV1700 instrument. The measurements were carried out in the buffer containing 100 mM KCl, 50 mM Tris-HCl (pH 7.0), in the presence of 0 wt% or 20 wt% PEG 200. The heating and cooling rate was 0.5 °C min⁻¹. The melting curves were analyzed using a curve fitting procedure with the theoretical equation for an intramolecular association to calculate thermodynamic parameters.
RESULTS AND DISCUSSION

Table 1 shows values of the enthalpy change ($\Delta H$) and entropy change ($\Delta S$) of the G-quadruplex formed by (TTAGGG)$_n$ in the presence of 100 mM K$^+$. The $\Delta H$ value of (TTAGGG)$_4$ (-45.4 kcal mol$^{-1}$) is lower than that of (TTAGGG)$_7$ (-43.0 kcal mol$^{-1}$). Similarly, $\Delta S$ value of (TTAGGG)$_4$ (-136 cal mol$^{-1}$ K$^{-1}$) is also lower than that of (TTAGGG)$_7$ (-129 cal mol$^{-1}$ K$^{-1}$). The similar tendency was observed in the $\Delta H$ and $\Delta S$ values of (TTAGGG)$_2$ and (TTAGGG)$_1$. These phenomena are considered as the follows: For (TTAGGG)$_n$ and (TTAGGG)$_2$, two G-quadruplex units (the unit means each G-quadruplex structure composing of the four repeats in the whole structure of the telomeric DNA’s) can be folded by (TTAGGG)$_n$ while only one G-quadruplex unit can exist in (TTAGGG)$_2$. Thus, it is reasonable that the $\Delta H$ and $\Delta S$ of (TTAGGG)$_n$ were lower than those of (TTAGGG)$_2$ due to two G-quadruplex units of (TTAGGG)$_n$ with more stacking and hydrogen interactions as well as more cation coordinations to G-quadruplex. Same reason leads to the similar tendency that the values of $\Delta H$ and $\Delta S$ of (TTAGGG)$_2$ with three G-quadruplex units were lower than those of (TTAGGG)$_1$. Most importantly, it should be noted that the $\Delta H$ and $\Delta S$ values of (TTAGGG)$_n$ generally increase with growing the sequence length. These parameters suggest that there is not an interaction between the two or three G-quadruplex units since interactions such as stacking interaction and hydrogen bonding should decrease the $\Delta H$ and $\Delta S$ values. Therefore, it can be concluded that long telomeric DNAs are inclined to arrange the G-quadruplex units one by one with TTA linker, resembling a string of beads, but not to arrange the G-quadruplexes by stacking interactions.

Table 1 $\Delta H$ and $\Delta S$ values of G-quadruplexes of (TTAGGG)$_{n-4}$ in the presence of K$^+$.

<table>
<thead>
<tr>
<th>n</th>
<th>$\Delta H$ (kcal mol$^{-1}$)</th>
<th>$\Delta S$ (cal mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-47.9±3.83</td>
<td>-141±11.4</td>
</tr>
<tr>
<td>5</td>
<td>-39.0±1.96</td>
<td>-117±5.88</td>
</tr>
<tr>
<td>6</td>
<td>-40.1±2.05</td>
<td>-120±6.13</td>
</tr>
<tr>
<td>7</td>
<td>-43.0±2.58</td>
<td>-129±7.74</td>
</tr>
<tr>
<td>8</td>
<td>-45.4±2.92</td>
<td>-136±8.75</td>
</tr>
<tr>
<td>9</td>
<td>-38.5±2.70</td>
<td>-115±8.06</td>
</tr>
<tr>
<td>10</td>
<td>-41.6±2.91</td>
<td>-125±8.74</td>
</tr>
<tr>
<td>11</td>
<td>-43.2±3.89</td>
<td>-129±11.6</td>
</tr>
<tr>
<td>12</td>
<td>-44.3±3.98</td>
<td>-132±11.9</td>
</tr>
</tbody>
</table>

We further evaluated the thermal stabilities of the long human telomeric DNAs, (TTAGGG)$_{n-4}$, under the dilute and crowding conditions by analyzing the UV melting curves recorded at 295 nm. All sequences used here was found to increase the $T_m$ value of G-quadruplex structures in the presence of 20 wt% PEG200 compared with that in the absence of PEG200. These data indicate the molecular crowding condition can increase the stability of G-quadruplex of long telomeric DNAs, which are consistent with previous reports that PEG can stabilize the G-quadruplexes$^{12}$.

CONCLUSION

The results reported here indicate that the possible structure of long telomeric DNA is head-string structure, which is important for research the telomere biology and a better development of therapeutic agents targeting telomeric DNAs. Moreover, the molecular crowding condition increases the stability of G-quadruplex, indicating the molecular crowding is one of the most important environmental factors for structure and stability of G-quadruplexes of the long telomeric DNAs.

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REFERENCES