The structural transition and compaction of human telomeric G-quadruplex induced by excluded volume effect under cation-deficient conditions

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A B S T R A C T

The structure polymorphism of human telomeric G-quadruplex (ht-quadruplex) is currently an important topic but remains controversy. Here, we present study of the ht-quadruplex under the cation-deficient but molecular crowding conditions by circular dichroism (CD), microchip electrophoresis (MCE) and UV-melting experiments. Our results show that with concentration increasing of poly(ethylene glycol) (PEG), the structural transition of ht-quadruplex occurs accompanied by structural compaction and enhanced stabilization, which may be caused by excluded volume effect. This work also demonstrates that ht-quadruplex can be well assembled without cations and the structure of ht-quadruplex is actually very complex in vivo.

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1. Introduction

Guanine-rich oligonucleotides being able to self-associate to form four-stranded G-quadruplex was were discovered accidentally in the 1960s [1]. Since then, especially in recent twenty years, G-quadruplexes have drawn lots of attention in the areas such as biology, medicinal chemistry, supramolecular chemistry and nanotechnology [2–4]. G-quadruplex displays a great conformational diversity because of strand direction difference, loop polymorphism and glycosidic torsion angle variation [5,6]. Human telomeric G-quadruplex (ht-quadruplex) receives the extensive attention because it is regarded as a potential therapeutic target for cancer [4,7,8], furthermore, its highly polymorphic structure also makes it the focus of the research. Up to now, the structure of ht-quadruplex remains to be a controversial topic, and was extensively discussed in the first international meeting on quadruplex DNA [9,10]. A recent excellent review introduced the details of the controversy [11].

We should note that physiological milieu, for example in Escherichia coli, is crowded with, except for cations like Na⁺ and K⁺, various biomolecules such as proteins, nucleic acids and polysaccharides that occupies 30–40% of the internal cellular volume and reaches 300–400 g/L [12–14]. Recently, Tan reported that ht-quadruplex adopts the parallel structure under molecular crowding condition containing 150 mM K⁺ [15], and Chaires reported that crowding alters the structure of ht-quadruplex in K⁺ solution but fails to alter the Na⁺ form [16]. Moreover, molecular crowding can induce the formation of G-quadruplex in cation-deficient conditions [17–19]. Therefore, molecular crowding may be an important factor and should be taken into account when obtaining the accurate information on ht-quadruplex.

Here, we reported the structural transition, by CD spectra, from antiparallel to hybrid type, then to parallel structure of ht-quadruplex induced by increasing the concentration of PEGs under cation-deficient conditions. UV-melting results showed that ht-quadruplex is stabilized with concentration increasing of PEGs. Subsequently, microchip electrophoresis (MCE) experiments confirmed that ht-quadruplex forms compact structure under high concentration of PEG. We proposed that a nonspecific force, excluded volume, might be a major factor inducing ht-quadruplex compaction and structural transition.

2. Materials and methods

2.1. Materials

DNA oligomer G₃(T₂AG₃)₃ was purchased from Sangon (Shanghai, China). The fluorophore-labeled oligonucleotide (FAM-G₃(T₂AG₃)₃-TAMRA) (HPLC purified) was obtained from TaKaRa (Dalian, China). Single-strand concentration of the oligomer was determined by
measuring the absorbance at 260 nm. PEGs were purchased from Alfa-Aesar (Tianjin, China). The samples were heated to 90 °C for 5 min, cooled slowly to room temperature, and then stored at 4 °C overnight before use except for UV-melting experiments. All experiments were carried out in 10 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA and various concentrations of PEGs unless otherwise stated. Water used was distilled and deionized using a Milli-Q A10 water purification system.

2.2. Circular dichroism

CD spectra were obtained on a Jasco J-810 spectropolarimeter (JASCO, Japan). Samples containing 10 μM oligomer were prepared and treated as described above before collecting CD spectra. The CD spectra were obtained in a 0.1 cm path length cuvette by taking the average of three scans recorded from 220 to 320 nm at a scanning rate of 50 nm/min at room temperature. A background CD spectrum of corresponding PEG buffer solution was subtracted from the average scan for each sample.

2.3. Microchip electrophoresis

MCE was carried out on home-made microfluidic device including laser-induced fluorescence detection system at room temperature [20]. The details of the experiments were shown in the supporting information (Fig. S1). Samples containing 100 nM fluorophore-labeled oligomer were treated as described above before use. To make results credible, electrophoresis was made in the same channel and was repeated at least three times. The electrophoresis was run in TBE buffer (100 mM Tris, 100 mM boric acid and 1 mM EDTA, pH 7.4) while exciting at 473 nm. The migration time of electrophoretic mobility shift assay was analyzed with Origin 6.0 software.

2.4. UV spectroscopy

UV-melting experiments were carried out on a Shimadzu 2450 spectrophotometer (Shimadzu, Japan) equipped with a Peltier temperature control accessory. All UV/Vis spectra were measured in a sealed quartz cell with a path length of 1.0 cm. Solutions containing 4 μM oligomer and variable amounts of PEGs were prepared. Samples were first heated to 95 °C for 5 min and then cooled to 18 °C with the temperature gradient of 0.5 °C/min and held at this temperature for 20 min without data collection. Samples were then heated to 95 °C using the same temperature gradient during which absorbance data at 295 nm were recorded [21]. The thermodynamic parameters were calculated by analyzing their melting profiles according to the method described previously [21]. Experiments were done in triplicate, the values tabulated reflect the averages of those measurements.
3. Results

3.1. Structural transition of ht-quadruplex

CD spectroscopy is a sensitive and useful technique for probing the structures of G-quadruplexes [6]. Fig. 1a shows the CD spectra of 10 μM G3(T2AG3)3 in the presence of various concentrations of PEG 200. The CD spectra in both 0 and 0.1 M PEG 200 have weak positive and negative peaks around 295 and 265 nm, respectively, indicating that a small quantity antiparallel ht-quadruplex is formed under these conditions [22]. The spectrum of G3(T2AG3)3 in the pure water is consistent with the result reported previously [23]. When the concentration of PEG 200 is increased from 0.25 M to 1.25 M, the positive shoulder peak around 265 nm appears gradually, which shows that G3(T2AG3)3 adopts a hybrid-type G-quadruplex [24]. The CD spectra recorded in 1.5 and 2 M PEG 200 have distinct positive and negative peaks around 265 and 240 nm, respectively, and the peak around 290 nm diminishes dramatically, indicating that the parallel structure is formed [25].

As described above, the increasing concentration of PEG 200 induces the structural transition of G3(T2AG3)3 from antiparallel to hybrid type, then to parallel G-quadruplexes. Furthermore, we studied the structural transition of G3(T2AG3)3 induced by different concentrations of PEG 400. In Fig. 1b, we observed the similar results in various concentrations of PEG 400 to those in PEG 200. We also found that the structural transition induced by PEG 400 changes more quickly than that by PEG 200. For example, CD spectra indicated that G-quadruplex is a parallel structure in solution containing 1 M PEG 400 whereas it is a hybrid type in the presence of 1 M PEG 200. PEG 200 and 400 have the same composition, however, with different average molecular weight. For ht-quadruplex, volume excluded by PEG augments with crowding agents occupy solution volume increasingly. Therefore, we proposed that the nonspecific force of excluded volume drives the G-quadruplex forming the compact structure, and leads to its structural transition.

3.2. Structural compaction of ht-quadruplex

MCE, which can analyze a small amount of sample in a short time, was used to characterize the compaction of ht-quadruplex. As shown in Fig. 2, the migration time of ht-quadruplex decreases from about 44.1 s (±0.8 s) to 36.1 s (±0.30 s) gradually as the concentrations of PEG increasing from 0 M to 2 M, which indicates that ht-quadruplex forms compact structure at high concentration of PEG, because the mobility rate of the sample is inversely proportional to its volume. It is known that G-quadruplex is extremely stable and dissociates very slowly once it is formed, we also found that when the ht-quadruplex was diluted four times using 10 mM Tris–HCl buffer, its intensity at 515 nm is almost invariable in 2 min (Fig. S2), suggesting that the structure of G-quadruplex is stable during electrophoresis.

3.3. Stability of ht-quadruplex

To test the thermal stability and measure the thermodynamic parameters of ht-quadruplex under molecular crowding conditions, UV-melting method was used. Fig. 3 shows UV-melting curves for ht-quadruplexes in various concentrations of PEG 200 and cation-deficient conditions. The melting temperatures (Tm) of 4 μM ht-quadruplexes are increased from 33.7 to 53.8 °C with the concentrations of PEG 200 increased from 0.1 M to 1.5 M. At the concentration of 2 M PEG 200, the spectrum does not exhibit a two-state model under our detection condition, therefore, we could not obtain its Tm and thermodynamic parameters. The Tm values and thermodynamic parameters of ht-quadruplexes under various amounts of PEG 200 and PEG 400 were summarized in Table 1. Notably, we could not examine samples at PEG 400 concentration higher than 0.75 M in our experiments due to their high viscosity. As shown in Table 1, in similar content of PEG, the stability of G-quadruplex in PEG 400 is greater than that in PEG 200. The volume excluded by PEG 400 is greater than that in PEG 200 because the molecular weight of PEG 200 is less than that of PEG 400, therefore, the nonspecific force of excluded volume may be attributed to the stabilization of ht-quadruplex. The free energy change at 25 °C for ht-quadruplex formation decreased from ~5.92 to ~11.43 kJ/mol when the concentration of PEG 200 was increased from 0 M to 1 M, which showed that the ht-quadruplex is stabilized under these conditions. When the concentration of PEG 200 increased to 1.25 M and 1.5 M, however, both the free energy change and Tm are increased, these this difference may be caused by the structural transition of ht-quadruplex (Fig. 2). For PEG 400, the free energy is changed from ~6.17 to ~8.25 kJ/mol when the concentration increased from 0 M to 0.75 M, which is in accord with the results observed in PEG 200.

It has been reported that PEG 200 induces a structural transition of Tetrathyamina telomere sequence, T3(G4T2)3G2, from the intramolecular quadruplex to multimolecular G-wire [26]. In this study, however, Tm of ht-quadruplex in the presence of 1 M PEG 200 is independent of the concentration of oligomer (Fig. S3), which demonstrates that ht-quadruplex forms a monomer intramolecular structure.

4. Discussion

A characteristic of physiological milieu is highly crowded with a diversity of molecules, and these crowding components occupy a significant fraction of the total volume [12–14], therefore, the molecular crowding condition used here is not able to mimic the real cell components completely. However, our results suggest that ht-quadruplex may display the structural diversity in vivo. It has been estimated that all macromolecules in the physiological fluid media collectively occupy a lower and upper limit of about 10% and 40% of total fluid volume, respectively [27], therefore, these structures observed here may occur permanently or temporarily and interconvert to each other in vivo. Moreover, such polymorphism of ht-quadruplex has not only biological significance, but also potential as nanotechnology materials, because it can be taken as the basis of the switchable molecular devices [2,3,26,28].

PEGs are neutral polymers and do not interact with G-quadruplex because the interaction is thermodynamically unfavorable [17]. The volume occupied by PEGs grows large as they are added increasingly, therefore, excluded volume might be a major factor inducing ht-quadruplex to form a compact structure, and further resulting in structural transition [29,30]. From the results of MCE, we observed that ht-quadruplex forms the compact structure with the concentrations increasing of PEG. Furthermore, the thermal stability of ht-quadruplex is enhanced with increasing the concentrations of PEG, which is in accordance with the results observed for G-quadruplex TBA that was stabilized by 0 to 40 wt.% PEG 200 [31].

Table 1

<table>
<thead>
<tr>
<th>PEG concentration (M)</th>
<th>Tm (°C)</th>
<th>ΔH° (kJ/mol)</th>
<th>ΔS (J/mol/K)</th>
<th>ΔG°25 (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>33.7</td>
<td>~209.37</td>
<td>~682.71</td>
<td>~5.92</td>
</tr>
<tr>
<td>0.25</td>
<td>37.9</td>
<td>~207.69</td>
<td>~667.99</td>
<td>~8.63</td>
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<tr>
<td>0.5</td>
<td>41.4</td>
<td>~190.12</td>
<td>~604.82</td>
<td>~9.88</td>
</tr>
<tr>
<td>0.75</td>
<td>44.5</td>
<td>~170.72</td>
<td>~537.65</td>
<td>~10.59</td>
</tr>
<tr>
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<td>48.6</td>
<td>~155.60</td>
<td>~483.80</td>
<td>~11.43</td>
</tr>
<tr>
<td>1.25</td>
<td>51.5</td>
<td>~122.96</td>
<td>~377.20</td>
<td>~10.17</td>
</tr>
<tr>
<td>1.5</td>
<td>53.5</td>
<td>~109.25</td>
<td>~334.65</td>
<td>~9.52</td>
</tr>
<tr>
<td>2</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Tm and thermodynamic parameters are not obtained under our detection condition.
of hq-quadruplex can be partially attributed to the effect of excluded volume as described about G-actin [32], though the hydration may be also an important factor to affect the thermal stability [31,33].

It has been reported that molecular crowding causes a structural transition from an antiparallel to a parallel G-quadruplex of Oxytricha nova telomeric sequences G₄T₄G₄ [17], while for Tetrahymena telomeric sequences T₂G₄T₂G₄, from antiparallel to a long and highly ordered parallel G-wires [26]. Therefore, the effect of molecular crowding is an important factor, which should not be neglected when developing an efficient drug against cancer based on the accurate structure of hq-quadruplex.

In summary, the results reported here suggest that the excluded volume should be a crucial factor governing the structural transition and enhancement of thermal stability of the hq-quadruplex. Our results also show that the structure of hq-quadruplex may be extraordinarily complex and can be well assembled without the assistant assistance of cations like Na⁺ and K⁺ under physiological environment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.bpc.2008.05.005.

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