Single-molecule analysis of thymine dimer-containing G-quadruplexes formed from the human telomere sequence

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Experimental procedures

Oligodeoxynucleotide preparation

Oligodeoxynucleotides (ODNs) containing the thymine dimer (T=T) were synthesized at the DNA/Peptide Core Facility at the University of Utah. The ODNs were deprotected using previously established techniques,[1] avoiding any light exposure to ensure that the T=T did not degrade back to TT. Next, they were purified using an ion-exchange HPLC on a Dionex DNA Pac PA-100 column with a method that consisted of a linear gradient from 5% to 40% B over 30 min while monitoring the absorbance at 260 nm, and the mobile phases were: A: 10% MeCN and 90% ddH₂O B: 1 M LiCl, 25 mM Tris, 10% MeCN, pH 8. Dialysis was used to remove excess salts from the purification. Annealing was done by first heating the ODN samples at a 50 μM concentration to 90 °C in the buffers listed below. Second, the samples were allowed to cool to room temperature (~4 h) and left at 4 °C for 48 h. The different buffers used were 1) hybrid fold: 950 mM LiCl, 50 mM KCl, 25 mM Tris, 1 mM EDTA, pH 7.9; 2) basket fold: 1 M NaCl, 25 mM Tris, 1 mM EDTA, pH 7.9; 3) propeller fold: 5 M LiCl, 20 mM KCl, 25 mM Tris, 1 mM EDTA, pH 7.9. The ODNs were handled with caution to prevent any light exposure through all experimental procedures.

Thermal denaturation, CD and EMSA measurements

The thermal denaturation studies were conducted at 3 μM concentration of ODN and in three different buffers as listed above. In the Tₘ studies, the wavelength monitored for basket and hybrid folds was 295 nm, and 260 nm was monitored for the propeller fold. The temperature was increased from 20 °C to 90 °C followed by cooling to 20 °C. The CD spectra were obtained at 50 μM ODN concentration and the wavelengths monitored were between 220 nm to 320 nm at 20 °C. The electrophoretic mobility shift assays were performed at lower salt concentrations for the basket and hybrid folds. The salt used for casting the gels was 0.1 M KOAc, with TBE buffer pH 7.9 for the hybrid folds, and 0.1 M NaOAc, TBE buffer pH 7.9 for the basket fold. The ODNs were 5’-end labeled using T4-polynucleotide kinase, [γ-³²P] ATP and G-25 spin X column (GE Healthcare) following manufacturer’s protocol. The native PAGE (20 %) experiments were run for 8 h, at room temperature.
Nanopore experiments

Three different electrolyte solutions were prepared for the experiments (the compositions mentioned above). Ultrapure water (> 18 MΩ·cm) was used to prevent any contamination, and electrolyte solutions were filtered using a sterile 0.22 μm Millipore vacuum filter. The surface of the glass nanopore membrane (GNM) was modified with 3-cyanopropyltrimethylchlorosilane before the measurements. The phospholipid used to form the lipid bilayer was 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC). The nanopore setup was as follows: (1) a Ag/AgCl electrode was positioned inside the GNM (filled with the electrolyte solution), which was attached to a pressure gauge and a 10-mL gas-tight syringe. (2) Another Ag/AgCl electrode was placed in the nanopore holder chamber that was then filled with electrolyte solution. Once the lipid bilayer was painted across the GNM the protein channel was inserted into the lipid bilayer by applying a pressure to the GNM, resulting in the protein insertion associated with an increase of the resistance. The data was collected using a 100 kHz filter and 500 kHz data acquisition rate; however, for the purposes of the presentation it was then re-filtered to 10 kHz. The data was extracted and analyzed using QUB 2.0.0.20. OriginPro 9.1 and Event States Analysis (EBS software) was used for statistical analysis of the events.
Figure S1. HPLC traces of the T=T containing ODNs.

5’ TAGGGTTAGGGTTAGGGTTAGGGTT
Figure S2. Representative $T_m$ curves (hybrid) and EMSA for the hybrid and basket folds.

Study conducted at 3 μM G-quadruplex concentration in 950 mM LiCl, 50 mM KCl, 25 mM Tris, 1 mM EDTA, pH 7.9.
Figure S3. Types and percent distribution of the current-time ($i$-$t$) traces (damaged ODNs and standard) for the nanopore experiments in KCl.

| Event Types | Std [\%]* | 5’[\%]* | M[\%]* | 3’[\%]* |
|-------------|-----------|-----------|---------|---------|
| Type 1 (a + b) | 74 ± 9 | 70 ± 8 | 61 ± 7 | 69 ± 8 |
| Type 2 | 21 ± 3 | 21 ± 2 | 26 ± 4 | 25 ± 3 |
| Type 3 | 5 ± 2 | 9 ± 1 | 12 ± 1 | 6 ± 1 |

*The percent distribution was corrected according to the entry rate ratios previously established by our laboratory. [$^{[4]}$]
Figure S4. Examples of the 1a and 1b traces in the native and T=T-containing ODNs as hybrid folds (K+).

For the purposes of the presentation only shorter traces were shown (some traces go up to seconds).
Figure S5. Color plots and statistical analysis of the event durations in 5 M LiCl during the nanopore experiments of the T=T-containing human telomere sequence.

The data were plotted using a single exponential decay model. As shown in the color plots there are two populations that show voltage dependence.
Figure S6. $T_m$ and CD spectra of the human telomere folds and triplex folds in a 5M LiCl solution.

The comparison between a propeller fold (black), triplex folds (5’-purple and 3’-red) and T=T-containing ODNs (5’-blue and 3’-green).

$T_m$ comparison between triplex folds and T=T-containing folds.

|            | $T_m$ (°C) |
|------------|------------|
| 3’ T=T     | 49.4 +/- 0.4|
| 3’ triplex | 47.2 +/- 0.8|
| 5’ T=T     | 51.0 +/- 0.6|
| 5’ triplex | 50.8 +/- 0.5|
Figure S7. CD and $T_m$ data for the physiological salt concentrations of G-quadruplexes containing the T=T.

|                | Hybrid fold$^a$ | Basket fold$^b$ |
|----------------|-----------------|-----------------|
|                | $T_m$ ($^o$C)   | $T_m$ ($^o$C)   |
| Natural sequence | 65.4 +/- 0.7    | 56.3 +/- 0.8    |
| 5' T=T          | 60.6 +/- 1.0    | 52.6 +/- 0.6    |
| 3' T=T          | 62.0 +/- 0.7    | 52.4 +/- 0.7    |
| M T=T           | 51.6 +/- 0.6    | 52.3 +/- 0.4    |

$^a$120 mM KCl, 20 mM PBS, pH 7.4.

$^b$120 mM NaCl, 20 mM PBS, pH 7.4.
References

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