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Communication

Single-molecule investigation of human telomeric G-quadruplex interactions with Thioflavin T

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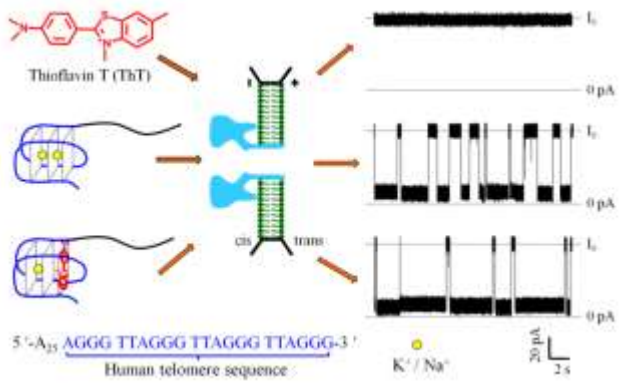
Graphical Abstract



The interactions between human telomere sequence and a typical highly selective G-quadruplex ligand ThT were studied at the single-molecule level through *α*-hemolysin protein nanopore.

ABSTRACT

G-quadruplex ligands have been accepted as potential therapeutic agents for anticancer treatment. Thioflavin T (ThT), a highly selective G-quadruplex ligand, can bind G-quadruplex with a fluorescent light-up signal change and high specificity against DNA duplex. However, there are still different opinions that ThT induces/stabilizes G-quadruplex foldings/topologies in human telomere sequence. Here, a sensitive single-molecule nanopore technology was utilized to analyze the interactions between humantelomeric DNA (Tel DNA)and ThT. Both translocation time and current blockade were measured to investigate the translocation behaviors. Furthermore, the effects of metal ion (K^+ and Na^+) and pH on the translocation behaviors were studied. Based on the single-molecule level analysis, there are some conclusions: (1) In the electrolyte solution containing 50 mmol/L KCl and 450 mmol/L NaCl, ThT can bind strongly with Tel DNA but nearly does not change the G-quadruplex form; (2) In the presence of high concentration K^+, the ThT binding induces the structural change of hybrid G-quadruplex into antiparallel topology withan enhanced structural stability; (3) In either alkaline or acidic buffer, G-quadruplex form will change in certain degree. All above conclusions are helpful to deeply understand the interaction behaviors between Tel DNA and ThT. This nanopore platform, investigating G-quadruplex ligands at the single-molecule level, has great potential for the design of new drugs and sensors.

Keywords:
α-Hemolysin nanopore
Interaction
Human telomere sequence
Thioflavin T
G-quadruplex ligand

Human telomeric G-quadruplex has been attracting intense research attention as drug targets to halt the function of telomerase and regulate gene expression because induction/stabilization of the G-quadruplex form directly affects the activity of telomerase [1,2]. Molecular ligands that selectively stabilize G-quadruplex foldings/topologies can be potential therapeutic agents for anticancer treatment [3,4]. Therefore, figuring out the interactions between G-quadruplex ligands and human telomere sequence are important for the design of new drugs and sensors.Thioflavin T (ThT) is a commercial G-quadruplex ligand that can bind G-quadruplex with a fluorescent light-up signal change and high specificity against DNA duplex [5]. Fluorescence spectra [6], circular dichroism (CD) spectroscopy [7], melting temperature (Tm) experiments [5] and electrospray ionization mass spectrometry (ESI-MS) [8] analyses have been carried out to characterize the binding behaviors.Thereported stoichiometric ratio of G-quadruplex to ThT was 1:2 [5].Shao et al. demonstrated that the ThT binding does not disturb native G-quadruplex structures preformed in Na^+ or K^+ solutions, but the binding model is strongly dependent on the G-quadruplex structures [9]. When binding to the G-quadruplex possessing hybrid structures, the excited state of ThT has a significant fluorescence enhancement because of the rotation restriction of benzothiazole (BZT) and dimethylaminobenzene