Analysis of unbinding force between telomestatin derivatives and human telomeric G-quadruplex by atomic force microscopy

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Supplementary Materials and Methods

Immobilization of human telomeric G-quadruplexes on a gold chip

The 5'-biotinylated oligonucleotides consisting of a human telomeric repeat sequence, 5’-biotin-(TTAGGG)4 (telo24), and 5’-biotin-(TTAGGG)16 (telo96), and a nontelomeric sequence, 5’-biotin-(T)20 (polyT), were immobilized on gold surfaces, respectively, as follows. 1 µM biotinylated oligonucleotide in PBS (10 mM phosphate, 145 mM Na+, 5 mM K+, pH 7.4) was heated at 95°C for 2 min and then cooled to 25°C gradually to form a G-quadruplex. The DNA solution was dropped on Sensor Chip SA (GE Healthcare), which is a streptavidin-immobilized gold chip, followed by incubation for more than 10 h at 4°C. The gold chip was washed with PBS before use.

Immobilization of 6OTDs on a silicon cantilever

6OTD monomer and dimer were immobilized on a silicon cantilever (ATEC-CONT, Nanoworld), respectively, as follows. A silicon cantilever with a spring constant of 0.05-0.08 N/m was selected and washed with sulfuric peroxide mixtures (SPM) for 15 min at 85°C and then with ammonium peroxide mixtures (APM) for 10 min at room temperature, followed by washing with ultrapure water. The cantilever was immersed in 2% 3-mercaptopropyltrimethoxysilane (MPTMS, Sigma) in ethanol at room temperature for 1 h and then washed with ethanol. The cantilever was immersed in 10 mM N-(ε-maleimidocaproyloxy)sulfo succinimide (EMCS, Pierce) in dimethylformamide at room temperature for 1 h and then washed with dimethylformamide. The cantilever was immersed in 4 mM 6OTD monomer/dimer in 10% DMSO solution at 4°C for more than 10 h. The cantilever was washed with 10% DMSO solution and then with PBS before use.

Force analysis

Force measurements between oligonucleotides on gold chips and 6OTD monomer/dimer on cantilevers were performed by AFM (Nanowizard II, JPK Instrument) at room temperature in PBS with a loading rate of 200 nN/s. In the analysis of force curves, “unbinding force” and “detachment force” were defined as “the force measured in the final unbinding event” and “the maximum force measured when the cantilever was retracted from the surface”. The unbinding forces were measured at different positions on the gold chips and represented as histograms. The histograms were analyzed by Gaussian fitting. The detachment forces were measured 100 times consecutively at a single site on the gold chips. The forces obtained by 10 consecutive measurements were averaged and plotted.
**Supplementary Figures**

![Chemical Structures](image)

**Fig. S1** Structures of synthesized 6OTD monomer (A) and dimer (B).

![Force Curves](image)

**Fig. S2** Force curves obtained when a 6OTD monomer-immobilized cantilever was detached from oligonucleotide-immobilized gold chips. PolyT (A), telo24 (B) and telo96 (C) were immobilized on the gold chips, respectively. Only retraction curves were shown.

![Possible Binding Modes](image)

**Fig. S3** Possible binding modes of the 6OTD dimer to G-quadruplexes. The dimer binds to two adjacent G-quadruplexes in parallel (A) or a single G-quadruplex in a sandwiching manner (B).
Fig. S4 Force curves obtained when a 6OTD dimer-immobilized cantilever was detached from oligonucleotide-immobilized gold chips. PolyT (A), telo24 (B) and telo96 (C) were immobilized on the gold chips, respectively. Only retraction curves were shown.

Fig. S5 Average values of the detachment forces obtained by consecutive force measurements at a single site of the telo96-immobilized chip with the 6OTD monomer-immobilized cantilever. Two sets of 100 consecutive measurements were performed with a 5 min interval. The cantilever with the spring constant of 0.35 N/m was used. One force measurement required 0.27 sec. The detachment forces obtained by 10 consecutive measurements were averaged and plotted. The decreased averaged forces in the first 100 measurements were partially recovered after the 5 min interval and then decreased again in the second 100 measurements.