Supplementary Material (ESI) for Molecular BioSystems

Human telomeric DNA has a peroxidase apoenzyme activity

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Supplementary Information

Experimental Section

Oligonucleotides. All oligonucleotides were purchased from Integrated DNA Technologies (IDT), Inc. and purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE). The DNA sequences were listed below:

1) 24 nt human telomere DNA: 5'-$(TTA\ GGG)_4$-3';
2) 72 nt human telomere DNA: 5'-$(TTA\ GGG)_{12}$-3';
3) original DNAzyme, PS2.M: 5'- GTG GGT AGG GCG GGT TGG-3';
4) unrelated dsDNA: 5'-GAT GAC GCT AGT TCT GAT CGT TCA CAC TGG ATC TAC ATA CTG C-3' / 5'-GCA GTA TGT AGA TCC AGT GTG AAC GAT CAG AAC TAG CGT CAT C-3';
5) unrelated ssDNA: 5'-AGT GCT GAT TCG GAC ATG AGT GAC-3';
6) c-Myc promoter: 5'-TGG GGA GGG TGG GGA GGG TGG GGA AGG-3'.

Other materials. Both hemin (fom Frontier Scientific, Inc.) and ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ammonium salt)] (from TCI America) were used without further purification. A hemin stock solution (5 mM) was prepared by dissolving hemin in Dimethylsulfoxide (DMSO). A diluted stock solution (100 μM) was made by further diluting the 5 mM hemin solution in DMSO and used in the following experiments. Both stock solutions were stored in dark at -20°C. An ABTS stock solution (18.6 mM) was prepared by dissolving ABTS solid in H_2O and stored in the dark at 4°C. H_2O_2 30 wt.% was purchased from Sigma and freshly diluted into appropriate concentration immediately before use.

Preparation of hemin/DNA complex. DNA oligomers with appropriate concentrations were mixed with 2.5 μM hemin (hemin concentration was kept at 500 nM in the final redox reaction solution) in 20 μL phosphate-citrate buffer (pH=5.0) which consisted of 200 mM NaCl, 20 mM KCl, 1% DMSO, and 0.05% Triton X-100. The mixture was incubated at 22°C for 3 hours to allow hemin to complex with DNA.

Preparation of redox reaction solution. The reaction solution contained three components: 20 μL hemin/DNA complex solution described above, 70 μL ABTS solution, and 10 μL H_2O_2 solution. Each component contained the same buffer (phosphate-citrate buffer (pH = 5.0), together with 200 mM NaCl, 20 mM KCl, 1% DMSO and 0.05% Triton X-100). The reaction was initiated by adding 10 μL H_2O_2 solution into a mixture of DNA-hemin complex (20 μL) and ABTS solution (70 μL). In the overall 100 μL reaction solution, the initial concentrations of ABTS and H_2O_2 were kept at 1.17 mM and 3.54 mM, respectively; the DNA concentrations were 100 nM or otherwise indicated; the hemin concentration was kept at 500 nM, which was in excess.

Kinetic measurements. Kinetic data were obtained by monitoring the absorbance of $ABTS^{\bullet+}$ at 405 nm on a Beckman Du®520 general purpose UV/Vis spectrophotometer at 22°C. The molar absorbance at this wavelength is 31600 $M^{-1}\cdot cm^{-1}$. The data were recorded every 5 seconds during the first 5 minutes of the reaction. The initial reaction rates were calculated from the slope of the initial linear portion of the increase in absorbance. Every measurement was repeated five times and the averages and error bars were put on the final results.