Electronic Supplementary Information

Riboadenosine-substituted DNA probes for self-illuminating real-time monitoring exonuclease III activity and exonuclease III-assisted target recycling

Qinfeng Xu and Chun-yang Zhang*

Single-molecule Detection and Imaging Laboratory, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, 518055, China

EXPERIMENTAL SECTION

Materials. Exonuclease III (Exo III), exonuclease I, exonuclease T, T7 exonuclease, lambda exonuclease, DNase I and NEBuffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9) were obtained from New England Biolabs (Ipswich, MA, USA). Phospho(enol)pyruvic acid monosodium salt hydrate (PEP), pyruvate kinase from rabbit muscle (PK), and adenylate kinase (AK) from chicken muscle were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The dCTP and riboadenosine (rA)-substituted oligonucleotide (Table 1) were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). Other oligonucleotides (Table S1) were synthesized and HPLC purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The ATP determination kit and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA, USA), and the 96-well white microplate was purchased from Fisher Scientific (Pittsburgh, PA, USA).

Investigation of the rA-substituted DNA substrate. To obtain the rA-substituted dsDNA, the rA-substituted ssDNA was mixed with its complementary ssDNA at a molar ratio of 1:1. Both the mixture and the rA-substituted ssDNA (both in 1× NEBuffer 4 with a final concentration of 1.0 μM) were heated at 95°C for 5 min, followed by gradual cooling to 25°C for 1 h. To investigate the effects of rA substitution upon the Exo III activity, the rA-substituted DNA and the DNA without substitution (10.0 μL, 1.0 μM) was mixed with Exo III (1.0 μL, 1U/μL) and incubated at room temperature (25°C) for 15 min, respectively. The reaction products (11.0 μL) were prestained by SYBR Green I (Invitrogen, USA) and analyzed by 10% native polyacrylamide gel electrophoresis. The electrophoresis images were screened with the Image Station 4000MM (Rochester, NY). To further investigate the influence of rA substitution upon the rate of DNA hydrolysis (Figure S1), 1.0 μM DNA (5.0 μL) was mixed with 1U/μL Exo III (0.5 μL) in 50 μL 1× NEBuffer 4 and incubated at 25°C for different time. After immediately terminating the hydrolysis reaction by the addition of excessive EDTA (10.0 μL, 100 mM), the reaction solution was mixed with SYBR Green I (100×, 1.0 μL) and the fluorescence intensity was measured at excitation/emission wavelength of 490/520 nm by the spectrofluorometer (F-4600, Hitachi).

Bioluminescence Monitoring of Exo III Activity. The bioluminescence monitoring of Exo III catalyzed hydrolysis was performed in the presence of various DNA substrates (6.0 μL, 1.0 μM), Exo III (1.0 μL, 1U/μL), 4.0 μL of the AMP-to-ATP conversion buffer (1U/μL AK (1.0 μL), 1U/μL PK (1.0 μL), 10 mM dCTP (1.0 μL), and 4.8 mM PEP (1.0 μL)), and 0.5 μL of ATP detection buffer (5 mM D-luciferin, 12.5 μg/mL firefly luciferase, 25 mM Tricine buffer (pH 7.8), 5 mM $MgSO_4$, 100 μM EDTA, and 10 mM DTT). After the above reagents were mixed in 60 μL of 1× NEBuffer 4, the bioluminescence signals were recorded with a Glomax 96-well

luminometer (Promega, Madison, WI, USA) at room temperature. For the Exo III activity assay, various concentrations of Exo III were mixed with 1.0 μM rA-substituted dsDNA (6.0 μL), the AMP-to-ATP conversion buffer (4.0 μL) and the ATP detection buffer (0.5 μL) in 60 μL of 1× NEBuffer 4. The bioluminescence signals were measured in a real time using the 96-well luminometer at room temperature.

Detection of DNA Target. Various concentrations of DNA target were mixed with 1.0 μM rA-substituted ssDNA probe (6.0 μL), 1U/μL Exo III (1.0 μL), 4.0 μL of the AMP-to-ATP conversion buffer and 0.5 μL of ATP detection buffer in 60 μL of 1× NEBuffer 4. The bioluminescence signals were measured in a real time with the 96-well luminometer at room temperature. For the bioluminescence imaging, the experimental conditions are same to those of bioluminescence assay except for the addition of 5.0 μL of ATP detection buffer. The bioluminescence images were recorded on the Image Station 4000MM with the excitation light source being turned off and the exposure time of 30 min.

Table S1. Sequences of oligonucleotides used in this work[α]

name	DNA sequence
ssDNA	5’-CGC ATG TCT ATG CGT GAA CTG-3’
ssDNA(rA)	5’-CGC ATG TCT ATG CGT G**rA**A CTG-3’
dsDNA	5’-CGC ATG TCT ATG CGT GAA CTG-3’
	5’-CAG TTC ACG CAT A*G*A *C-3’
dsDNA(rA)	5’-CGC ATG TCT ATG CGT G**rA**A CTG-3’
	5’-CAG TTC ACG CAT A*G*A *C-3’
DNA target	5’-CAG TTC ACG CAT AGA CAG TCC GTG GTA GGG CAG GTT
	GGG GTG ACT-3’
DNA target with single-base	5’-CAG TGC ACG CAT AGA CAG TCC GTG GTA GGG CAG GTT
mismatched	GGG GTG ACT-3’

[α]The underlined letters of ssDNA are the complementary sequence. The bold rA indicates the riboadenosine substitution. The asterisk indicates the phosphorothioate modification.

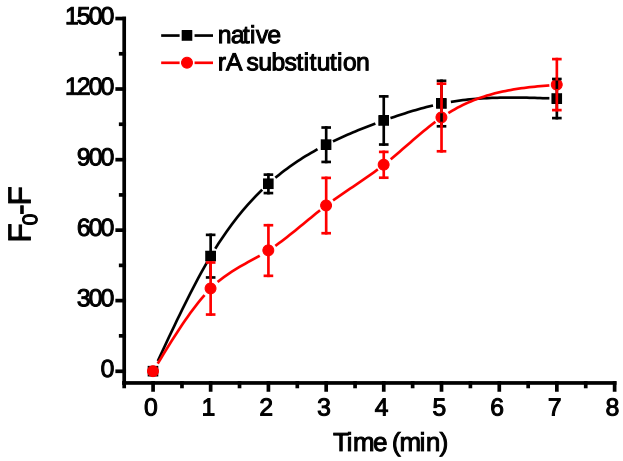

Figure S1. Study the influence of rA substitution upon the rate of DNA hydrolysis by fluorescence kinetics experiments. F_0 and F indicate the measured fluorescence intensity of native DNA or rA-substituted DNA in the absence (F_0) and in the presence (F) of Exo III, respectively.

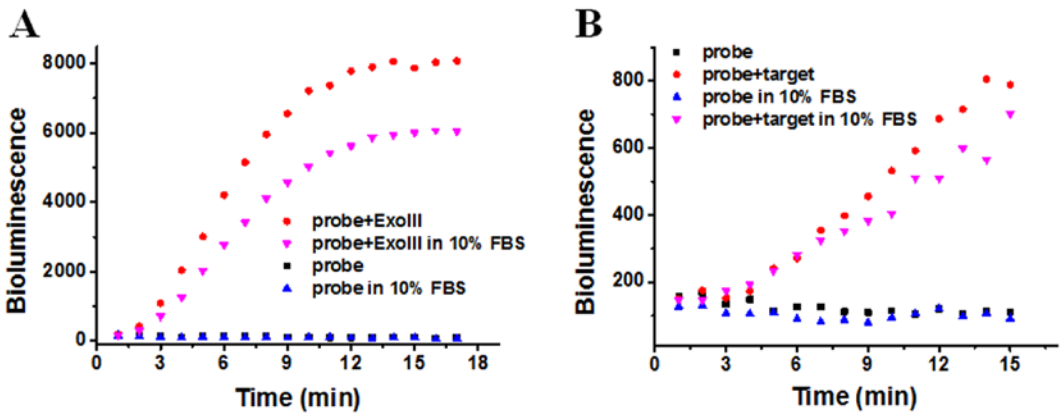

Figure S2. The rA-substituted DNA probe-based bioluminescent method can be used to detect Exo III (A) and DNA (B) in complex biological samples. The concentration of Exo III is 2 U/mL, and the concentration of target DNA is 0.5 nM.