Electronic Supplementary Information (ESI)

Stimuli-responsive DNAzyme displaying Boolean logic-gate responses

Ayaka Banno,1 Sayuri Higashi,2 Aya Shibata,1 and Masato Ikeda*,1,2,3

1Department of Life Science and Chemistry, Graduate School of Natural Science and Technology, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
E-mail: m_ikeda@gifu-u.ac.jp, Tel: +81-58-293-2639, Fax: +81-58-293-2794
2United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University
3Center for Highly Advanced Integration of Nano and Life Sciences (G-CHAIN), Gifu University

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1. Experimental generals. Unless stated otherwise, all commercial reagents were used as received. Human O6-methylguanine-DNA-methyltransferase (MGMT) protein (His tag, Recombinant) was purchased from Antibodies-online GmbH (ABIN805634) through Funakoshi (Japan). MGMT was used without further purifications and its concentration was evaluated on the basis of molar extinction coefficient calculated from the sequence. The unmodified 8-17 DNAzyme (8-17Dz), the substrate with a single ribonucleotide at the cleavage site bearing fluorescein (6-FAM) at 5’ end, and the other oligonucleotides were ordered from Fasmac Corp. (Kanagawa, Japan). The concentration of the oligonucleotides was determined by absorbance at 260 nm. All water used in the experiments refers to ultra-pure water obtained from a Millipore system having a specific resistance of 18 MΩ•cm. IP RP HPLC was conducted with a Shimadzu Prominence instrument LC-20AD and SPD-20A equipped with a GL Science Inertsil ODS-3 column (150 mm × 4.6 mm I. D., 5 μm) for analysis and GL Science Inertsil ODS-3 column (150 mm × 10 mm I. D., 5 μm) for purifications in a column oven CTO-20A. Matrix-assisted laser desorption/ionization (MALDI) coupled with time-of-flight (TOF) mass spectra were recorded using a Shimadzu AXIMA-CFR plus mass spectrometer. UV-vis spectra were obtained using a Jasco V-630 or a Shimadzu UV-2450 spectrophotometer.

2. Solid-phase oligonucleotide synthesis. Oligonucleotide synthesis of 8-17DzNB was carried out by using an NTS H-6 DNA/RNA synthesizer (Nihon Techno service) using phosphoramidite method (commercially available β-cyano phosphoramidites with synthesized dGNB phosphoramidite[1]) at 1 μmol scale. After cleavage from the solid support, deprotection of bases and phosphates was performed in aqueous ammonia (1.0 mL, 28%) at 55 °C for 12 h. The purification of the “trityl-on” oligonucleotide was carried out on IP RP HPLC {GL Science Inertsil ODS-3 column (150 mm × 10 mm I. D.)}, linear gradient from A:B = 100:0 (A: 0.1 M TEAA containing 5% acetonitrile, B: acetonitrile) to 50:50 over 30 min was used with a flow rate of 3.0 mL/min, detection wavelength = 260 nm}. The purified “trityl-on” oligonucleotide was treated with 80% CH3COOH for 15 min at 37 °C to remove the dimethoxytrityl residues. The detritylated oligomer was purified by IP RP HPLC {GL Science Inertsil ODS-3 column (150 mm × 10 mm I. D.)}, linear gradient from A:B = 100:0 (A: 0.1 M TEAA containing 5% acetonitrile, B: acetonitrile) to 50:50 over 30 min was used with a flow rate of 3.0 mL/min, detection wavelength = 260 nm}. IP RP HPLC charts were shown in Figure S1. The identity of the oligonucleotides 8-17DzNB has been established by MALDI-TOF MS {matrix: 3-hydroxypicolinic acid (3-HPA), negative, Figures S2}, and the observed molecular weights were in good agreement with their structures: 8-17DzNB, Caled.

For [M – H]: 10234.7 Found: 10220.4. The oligonucleotide concentrations were determined by UV spectroscopy. The molar extinction coefficient (at 260 nm) ratio of dGNB/dG (≈ 1.15) was used.
Table S1. Sequences of oligonucleotides used in this study.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Sequences (from 5’ to 3’)</th>
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<tbody>
<tr>
<td>8-17Dzn</td>
<td>CATCTCTCTCGAGCCGG TCGAAATAAGTGATG: 33 nt</td>
</tr>
<tr>
<td>8-17DznB</td>
<td>CATCTCTCTCGAGCCGCTCGAAATAAGTGATG: 33 nt</td>
</tr>
<tr>
<td>F-Substrate</td>
<td>FAM–ACTCACTATGGAAGAGATG: 20 nt</td>
</tr>
<tr>
<td>Product</td>
<td>FAM–ACTCACTATG: 10 nt</td>
</tr>
</tbody>
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*Catalytic core is highlighted in bold (stem part is shown in blue color). Small capital in the sequence of substrate represents ribonucleotide. Cleavage site of substrate is shown by an arrow.

3. Characterization of oligonucleotides

Figure S1. HPLC charts before (a: after aqueous ammonia (28%) treatment, b: after aqueous 80% CH₃COOH treatment) and after purification (c) of 8-17DznB.
Figure S2. MALDI-TOF MS spectra (3-HPA as matrix, negative mode) of (a) 8-17Dz^{NB} and (b) 8-17Dz. The asterisk in chart (a) denotes a peak most probably due to the removal of the 4-nitrobenzyl (NB) group during UV laser-induced ionization.[1]
4. **In vitro substrate cleavage assay.** The cleavage reactions were performed under single-turnover conditions. The reactions were carried out under the conditions (Reaction mixture: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz or 8-17Dz^{NB}] = 25 nM, [substrate] = 25 nM, [Zn(NO_3)_2] = 50 µM, [Na_2S_2O_4] = 2.0 mM, 37 °C). Typically, reaction samples were incubated at 37 °C and quenched upon the addition of an urea loading buffer (8 M urea, 500 mM EDTA•2Na 10% glycerol). Samples were loaded onto 8 M urea 20% PAGE gels (6 cm × 6 cm compact gels; ATTO Inc., Tokyo, Japan), which were run at 24 W for 30 minutes at room temperature in TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA•2Na, pH 8.0). The gels were imaged using by ImageQuant LAS 4000 (GE Healthcare) equipped with a blue epi light (460 nm) and Y515 filter (Epi-B set). The cleaved product and uncleaved substrate were quantified and analyzed by Image J software (freely available at https://imagej.nih.gov/ij/index.html).

5. **Reduction responsive property**

5.1 **Monitoring reduction responsive structural change by IP RP HPLC.** The reductant (Na_2S_2O_4) was added to adjust 2.0 mM as the final concentrations in an aqueous buffer containing 8-17Dz^{NB} (25 nM) with substrate (25 nM). After designated incubation time, the reaction mixture was immediately desalted to remove of Na_2S_2O_4 by a Sep-pak cartridge (Waters) and the eluent was concentrate by approximately 20 folds, which was subjected to IP RP HPLC analysis (GL Science Inertsil ODS-3 column (150 mm × 4.6 mm I. D., 5 µm), linear gradient from A:B = 100:0 (A: 0.1 M TEAA containing 5% acetonitrile, B: acetonitrile) to 50:50 over 30 min was used with a flow rate of 1.0 mL/min, detection wavelength = 260 nm). The identity of the starting oligonucleotides (8-17Dz^{NB}) and the desired reduction product (8-17Dz) were established by comparing the retention time of authentic standard. Experiment was repeated in at least duplicates.
Figure S3. IP RP-HPLC traces (detection wavelength = 260 nm) of 8-17Dz\textsuperscript{NB} ((a) 0.5 min, (b) 5 min, and (c) 30 min after the addition of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} in the presence of substrate, (d) 8-17Dz, (e) 8-17Dz\textsuperscript{NB}, and (f) substrate. Conditions: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz or 8-17Dz\textsuperscript{NB}] = 25 nM, [substrate] = 25 nM, [Zn(NO\textsubscript{3})\textsubscript{2}] = 50 µM, [Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4}] = 2.0 mM, 37 °C.
6. Na$_2$S$_2$O$_4$ dependent cleavage activity of 8-17Dz$^{NB}$

**Figure S4.** PAGE (20%, denatured) analysis to evaluate substrate cleavage by DNAzymes 8-17Dz and 8-17Dz$^{NB}$ with or without the addition of Na$_2$S$_2$O$_4$. *Hydrolysis conditions:* 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz or 8-17Dz$^{NB}$] = 25 nM, [substrate] = 25 nM, [Zn(NO$_3$)$_2$] = 50 µM, [Na$_2$S$_2$O$_4$] = 2.0 mM, 37 °C.

**Figure S5.** (A) PAGE (20%, denatured) analysis (gel image) to evaluate Na$_2$S$_2$O$_4$ dependent cleavage of substrate by 8-17Dz$^{NB}$. (B) Na$_2$S$_2$O$_4$ dependent cleavage activity of 8-17Dz$^{NB}$ (6 h after the addition of Na$_2$S$_2$O$_4$). *Hydrolysis conditions:* 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz or 8-17Dz$^{NB}$] = 25 nM, [substrate] = 25 nM, [Zn(NO$_3$)$_2$] = 50 µM, 37 °C.
7. Substrate cleavage activity of 8-17Dz

![Image](8-17Dz)

**Figure S6.** PAGE (20%, denatured) analysis for substrate hydrolysis (single-turnover conditions) by 8-17Dz. See also Fig. 3. *Hydrolysis conditions:* 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz] = 25 nM, [substrate] = 25 nM, [Zn(NO₃)₂] = 50 µM, [Na₂S₂O₄] = 2.0 mM, 37 °C.

8. MGMT responsive property and OR logic-gate response

![Image](8-17DzNB)

**Figure S7.** PAGE (20%, denatured) analysis (gel image) to evaluate the cleavage of substrate by the DNAzymes (8-17Dz and 8-17DzNB) upon the addition of Na₂S₂O₄ or MGMT. *Hydrolysis conditions:* 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz] or [8-17DzNB] = 25 nM, [substrate] = 25 nM, [Zn(NO₃)₂] = 50 µM, [Na₂S₂O₄] = 2.0 mM, [MGMT] = 100 nM, 37 °C.

![Image](A) 8-17DzNB + MGMT

**Figure S8.** (A) PAGE (20%, denatured) analysis (gel images) and (B) kinetic plots for hydrolyzing substrate upon the addition of MGMT in the presence (a) or absence (b) of 8-17DzNB. *Hydrolysis conditions:*
50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz^{NB}] = 25 nM, [substrate] = 25 nM, [Zn(NO$_3$)$_2$] = 50 µM, [MGMT] = 100 nM, 37 °C.

9. Zn$^{2+}$ responsive property and AND logic-gate response

![Zn$^{2+}$ responsive property and AND logic-gate response](image)

**Figure S9.** PAGE (20%, denatured) analysis (gel image) to evaluate the cleavage of substrate by the 8-17Dz$^{NB}$ upon the addition of Zn$^{2+}$ and Na$_2$S$_2$O$_4$. **Hydrolysis conditions:** 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [8-17Dz$^{NB}$] = 25 nM, [substrate] = 25 nM, [Zn(NO$_3$)$_2$] = 50 µM, [Na$_2$S$_2$O$_4$] = 2.0 mM, 37 °C.

**References**