Electronic Supplementary Information

Specific Recognition of DNA Depurination by a Luminescent Terbium(III) Complex

Xiaohui Wang, Xiaoyong Wang,* Shanshan Cui, Yan Wang, Guangju Chen and Zijian Guo*

1 Experimental

1.1 Materials and Methods

Common reagents used in the experiments were all of analytical grade. TbL and TbL′ were prepared as reported previously. 1 MMS was purchased from Alfa Aesar. Tris(hydroxymethyl)aminomethane (Tris), EB, CT-DNA, GMP, AMP, CMP, and TMP were purchased from Sigma. Oligonucleotides ploy(G), ploy(A), ploy(C), ploy(T), ON1, ON2, and ON3 were purchased from GenScript Co. UV-vis spectra were determined on a Perkin-Elmer Lambda 35 UV-vis spectrometer. Time-resolved luminescence spectra were recorded on a Perkin-Elmer LS 55 luminescence spectrometer with the following settings: delay time, 100 μs; gate time, 2.00 ms; and cycle time, 20 ms. The excitation and emission slit widths were 12 nm for TbL and 5 nm for TbL′, respectively. The photomultiplier voltage was 900 V. CD spectra were recorded on a Jasco J-810 spectropolarimeter. The concentrations of oligonucleotides were measured by Thermo Scientific NanoDrop 1000. The concentration of CT-DNA was determined by measuring the UV absorption at 260 nm using the known molar absorption coefficient of 6600 M⁻¹ L cm⁻¹. The ratio of the UV absorption at 260 and 280 nm in Tris-HCl buffer is around 1.83, indicating that DNA was sufficiently free of protein. 2

1.2 Reactions of TbL with nucleotides, oligonucleotides or CT-DNA

All the reactions were carried out in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 37 °C for 10 min. The time-resolved luminescence spectra of TbL (100 μM) upon reacting with GMP, AMP, CMP, TMP, ploy(G), ploy(A), ploy(C), ploy(T), ON1, ON2, ON3, and CT-DNA, respectively, were measured at 25 °C after excitation at 260 nm. The time-resolved luminescence spectra of TbL′ upon reacting with CT-DNA were measured similarly.

1.3 Reactions of TbL with CT-DNA in the presence of MMS

CT-DNA (90 μM) was incubated with MMS (0.9, 1.35, 1.8, 3.6, 7.2 mM, respectively) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 37 °C for 24 h. TbL (100 μM) was added to the solution and incubated at 37 °C for 10 min. The time-resolved luminescence spectra were measured at 25 °C after excitation at 260 nm.

1.4 Reactions of TbL with CT-DNA in acidic conditions

The depurination of DNA induced by acid was prepared according to the literature. 3 CT-DNA (0.9 mM) in Tris-HCl buffer (pH 7.4) was adjusted with HCl (0.1 M) to obtain a final pH of 5 or 4.5 at 25 °C. Depurination of DNA was achieved by heating the samples for different times at 70 °C. Depurianted DNA was added to the buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) of TbL (100 μM) at 37 °C for 10 min (DNA final concentration: 45 μM). The time-resolved
luminescence spectra were measured at 25 °C after excitation at 265 nm.

1.5 Response of TbL to CT-DNA in the presence of denaturants

CT-DNA was incubated with common DNA denaturants formamide and urea (2 mM) at 90 °C for 5 min. The solution was then cooled with ice and added to the buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) of TbL (100 μM) at 37 °C and reacted for 10 min ([DNA] final = 90 μM). The time-resolved luminescence spectra were measured at 25 °C after excitation at 265 nm.

1.6 Minimum detection limit and linear range of TbL for CT-DNA

The time-resolved luminescence spectra of free TbL (0.1 mM, λex = 265 nm) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) was collected for 20 times to determine the background noise σ. The time-resolved luminescence spectra of TbL in the presence of CT-DNA at various concentration was measured. A linear regression curve was fitted according to the emission intensity at 545 nm in the range of 0.08 – 10 μM, and the slope of curve was obtained using Origin 8.0.

1.7 DNA binding mode of TbL

CT-DNA (100 μM) was incubated with TbL (10, 20, 40, 60, 80, and 100 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 37 °C for 12 h. CD spectra were measured at 25 °C in the wavelength range of 220–320 nm.

The fluorescence of the EB-DNA system was determined in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 25 °C. CT-DNA (12 μL, 0.9 mM) was added to EB buffer (1.3 μM, 3 mL). Aliquots of TbL (3 μL, 1 mM) were then added to the EB-DNA system at 25 °C in 1 min and the fluorescence spectra were measured on the Perkin-Elmer LS 55 (λex = 495 nm, λem = 610 nm). The apparent binding constant (Kapp) was determinated by the following equation

\[ K_{EB}[EB] = K_{app}[complex] \]

where \( K_{EB} = 1.0 \times 10^7 \text{ M}^{-1} \), [EB] = 1.3 μM, and [complex] was the final concentration at which a 50% reduction of the fluorescence had occurred.6

1.8 MD simulation

The initial coordinate of the DNA sequence was generated by using the HyperChem program. The structure of TbL was optimized by Gaussian09 program at the density functional theory (DFT) level. All molecular dynamics (MD) simulations were carried out using the AMBER9 package with the AMBER force fields of ff03 and gaff. The equilibration time for each simulation was 200 ps (NPT) followed by 20 ns of data collection for analysis under the conditions of constant volume with a periodic boundary at 300 K. The ptraj program from AMBER9 simulation software was used to analyze the trajectory.

2 Supplementary Figures
**Fig. S1** The time-resolved luminescence intensity of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) at 545 nm in the absence or presence of oligonucleotides or nucleotides (40 $\mu$M) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

**Fig. S2** Plot of luminescence intensity of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 545 nm as a function of CT-DNA concentration in the range of 0.08 – 10 $\mu$M.

**Fig. S3** The time-resolved luminescence spectra of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) in the presence and absence of CT-DNA (45 $\mu$M) determined in H$_2$O and D$_2$O.
**Fig. S4** The time-resolved luminescence spectra of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) in the absence or presence of DNA after incubation at 70 °C for 0 or 30 h at different pH values.

**Fig. S5** The effect of MMS (0.9 mM, pH 7.4) or acid (pH 5.0) on the time-resolved luminescence spectra of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) in the absence of DNA in buffer (5 mM Tris-HCl, 50 mM NaCl).

**Fig. S6** The time-resolved luminescence spectra of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) in the absence or presence of CT-DNA (90 µM) with or without denaturants formamide (2 mM) and urea (2 mM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).
Fig. S7 The structure of TbL′ and its time-resolved luminescence spectra (0.1 mM, \( \lambda_{ex} = 265 \) nm) upon addition of increasing amount of CT-DNA in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) after incubation at 37 °C for 10 min. Inset shows the emission intensity ratio \( (I/I_0) \) at 545 nm versus the ratio of [DNA] to [TbL'].

Fig. S8 RMSD values of all backbone atoms in the simulation of TbL-DNA model with respect to the starting structure.
The time-resolved luminescence spectra of TbL (0.1 mM, $\lambda_{ex} = 265$ nm) in response to CT-DNA (3.6 µM) in the absence or presence of EB (1.3 µM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

Table S1 Parameters for the $\pi$-$\pi$ interactions between metronidazole plane of TbL and purine bases of DNA derived from the MD simulations.

<table>
<thead>
<tr>
<th>$\pi$-$\pi$ interaction</th>
<th>Distance</th>
<th>Dihedral angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-binding mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole – G6</td>
<td>3.878 Å</td>
<td>9.645°</td>
</tr>
<tr>
<td>Double-binding mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole – G6</td>
<td>3.556 Å</td>
<td>11.988°</td>
</tr>
<tr>
<td>Metronidazole – A16</td>
<td>3.773 Å</td>
<td>32.258°</td>
</tr>
</tbody>
</table>

Table S2 Occupancies of hydrogen bonds between oxygen atoms in nitro groups of TbL and N-H groups of DNA bases.

<table>
<thead>
<tr>
<th>H-bonds</th>
<th>Distance</th>
<th>Occupancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-binding mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A16/N6 – H61 ... O86</td>
<td>3.0408 Å</td>
<td>83.33%</td>
</tr>
<tr>
<td>A16/N6 – H61 ... O87</td>
<td>3.9651 Å</td>
<td>8.54%</td>
</tr>
<tr>
<td>Double-binding mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5/N3 – H3 ... O86</td>
<td>2.9336 Å</td>
<td>47.82%</td>
</tr>
<tr>
<td>T5/N3 – H3 ... O87</td>
<td>4.0885 Å</td>
<td>39.26%</td>
</tr>
</tbody>
</table>

References

7 HyperChem Inc., ON, Canada, *Molecular Simulations*, Inc. San Diego, CA, USA.
8 M. J. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V.

9 D. A. Case, T. A. Darden, T. E. Cheatham, III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo,
Roitberg, G. Seabra, K. F. Wong, F. Paesani, X. Wu, S. Brozell, V. Tsui, H. Gohlke, L. Yang, C.