Electronic Supplementary Information

A label-free thrombin binding aptamer as probe for highly sensitive and selective detection of lead(II) ions by resonance Rayleigh scattering method

Xiao Bei Fu, Fei Qu, Nian Bing Li*and Hong Qun Luo*

Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

* Corresponding authors: linb@swu.edu.cn (N.B. Li); luohq@swu.edu.cn (H.Q. Luo)
1. Apparatus

A Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan) was used to record the RRS spectra and measure the scattering intensities. Rapid mixing device (Ronghua Instrument Plant, Jiangsu, China) was used to mix the reaction completely. A Chirascan-CD spectrometer (Photophysics Company, Britain) was used to analyze the structure of oligonucleotide. A UV-vis 2450 spectrophotometer (Shimadzu, Japan) was used to record the absorption spectra and measure absorbance. A pHS-3C pH meter (Shanghai Analytical Instrument Factory, Shanghai, China) was used to adjust pH values.

2. Reagents

A stock Pb(II) solution ($1.0 \times 10^{-3}$ M) was prepared by dissolving 0.0331 g of Pb(NO$_3$)$_2$ (Beibei Chemical Reagents Main Workshop, Chongqing, China) in water with nitric acid (15 μL, 6.0 M) and diluting to the mark in a 100 mL calibrated flask. Working standard solutions of Pb$^{2+}$ were prepared by appropriate dilution of the stock solution with water to 1 µM. 100 µM TBA probe stock solution was prepared in the 70 µL of Tris-acetate (10 mM pH 7.4) buffer solution. The working solution was further diluted with Tris-acetate buffer solution to 1 µM and stored in the dark at −20 °C. Other oligonucleotides solutions were prepared and stored as TBA. All the aptamers were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). Tris-acetate buffer solution (pH 7.4) contains 10 mM Tris was used to control the pH values of the interacting system. All the reagents used were of analytical reagent grade and doubly distilled water was used throughout.

3. Procedure

Aliquots (500 µL) of 10 mM Tris-acetate (pH 7.4) solution includes 80 µL of TBA solution (1 µM) and an appropriate amount of Pb(NO$_3$)$_2$ standard solution. The mixture was shaken at room temperature on the oscillator for 10 min and then equilibrated for 10 min. After reaction for 20 min, the RRS spectra were recorded with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ and the RRS intensity $I$ for binding product and $I_0$ for the reagent blank were measured at the maximum RRS wavelength, $\Delta I = I - I_0$. 
4. Effect of temperature

The effect of temperature on the RRS intensity of the system was investigated and the experimental results are shown in Fig. S1, it can be seen from Fig.S1 that the optimum temperature for the system is in the range of 15 – 25 °C. With the increase in temperature (> 25 °C) the RRS intensity will decrease gradually. This may be because the G-quartet structure split when the temperature increased. This conclusion consists with previous report that the DNA double helix is destabilized by Pb^{2+} on heating 1.

![Graph showing the effect of temperature on ΔI_{RRS}](image.png)

**Fig. S1** Effect of temperature on ΔI_{RRS}

[באSEN] = 0.1 μM; [TBA] = 0.1 μM; buffer: Tris-acetate 10 mM pH 7.4.

5. Effect of the TBA concentration

The dependence of ΔI_{RRS} on the TBA concentration for the TBA-Pb^{2+} system was studied. The experimental results are shown in Fig. S2. It can be seen that when the concentration of TBA was 0.16 μM, ΔI_{RRS} reached the maximum value. The ΔI_{RRS} value decreased when the TBA concentration was too low, resulting in incomplete reaction. However, when the TBA concentration was too high, the ΔI_{RRS} value also decreased due to the increase in scattering intensity of the reagent blank.
Fig. S2 Effect of TBA concentration on $\Delta I_{RRS}$

$[\text{Pb}^{2+}] = 0.1 \ \mu\text{M}$, buffer: Tris-acetate 10mM pH 7.4

6. Reaction speed and the stability

The stability of the system was studied by detecting the RRS intensity once every 5 min. The results showed that the reaction between TBA and Pb$^{2+}$ would be completed within 20 min at room temperature (15–25 °C). The intensities of RRS for the TBA-Pb$^{2+}$ system can be stable for over 45 min.

7. Effect of the instrument parameters

The influence of instrument parameters, such as scan speed, PMT voltage and slits on the RRS intensity of the reaction system was tested. To identify optimal instrument settings, we evaluated the $\Delta I_{RRS}$ values, the peak shape and spectral noise levels. Experimental results showed that when the scan speed, the slits (EX/EM), and the PMT voltage were 2400 nm/min, 5.0/5.0 nm and 700 V, respectively, the maximum $\Delta I_{RRS}$ value can be obtained.
8. Comparison of methods

A comparison of this method for the determination of lead (II) ion with other methods based on lead ions inducing conformational change of DNA from the random coil structure to a G-quartet structure is given in Table S1.

Table S1. Comparison of this method for the determination of lead (II) ion with other methods

<table>
<thead>
<tr>
<th>Method</th>
<th>System</th>
<th>Linear range (nM)</th>
<th>LOD (nM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td>AGRO100 / hemin/ H2O2 / AUR</td>
<td>0 – 1000</td>
<td>0.4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>T30695 / Znic PPIX / DOTA</td>
<td>20 – 1000</td>
<td>5.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>FAM / TBA- DABCYL</td>
<td>0.5 – 30</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Polyguanine / Tb</td>
<td>3.0 – 50</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>PW17 / hemin/ H2O2 / ABTS</td>
<td>50 – 1200</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Chemiluminescence</td>
<td>PS2.M / hemin/ H2O2 / luminol</td>
<td>1.0 – 316</td>
<td>1.0</td>
<td>7</td>
</tr>
<tr>
<td>Electrochemistry</td>
<td>G-rich DNA / [Fe(CN)6]4-/3-</td>
<td>0.5 – 50000</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>T30695/ crystal violet</td>
<td>1.0 – 1000</td>
<td>0.4</td>
<td>9</td>
</tr>
<tr>
<td>RRS</td>
<td>Label-free TBA</td>
<td>1.0 – 120</td>
<td>0.9</td>
<td>This study</td>
</tr>
</tbody>
</table>


9. Analytical application

The sample was collected (from our laboratory on the campus of Southwest University) after discharging tap water for about 20 min. Subsequently, the test sample was filtered through a 0.2 µm membrane and the filtrates were boiled for 5 min to remove chlorine. An appropriate
volume of the sample solution was added, and the following procedure is the same as the general procedure. The standard addition method was used to determine five parallel samples for each concentration of Pb$^{2+}$ and the results are listed in Table S2.

Table S2. Results for the determination of trace lead (II) ions in tap water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Found (nM)</th>
<th>Added (nM)</th>
<th>Total found (n = 5, nM)</th>
<th>RSD (n = 5, %)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water 1</td>
<td>ND a</td>
<td>9.9</td>
<td>10.2</td>
<td>5.51</td>
<td>103.0</td>
</tr>
<tr>
<td>Tap water 2</td>
<td>ND a</td>
<td>72</td>
<td>72.6</td>
<td>1.17</td>
<td>100.8</td>
</tr>
</tbody>
</table>

a ND: Not detected

Supplementary references: