Responsive europium emission for paralytic shellfish saxitoxin detection in water

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ABSTRACT

A water-soluble europium complex (EuL1), with the chromophore conjugated with both the Eu³⁺ ion and the aza-18-crown-6 ether moiety (receptor), were synthesized and its photophysical properties were studied. Saxitoxin (STX) selectively binds to EuL1 and induced a 4-fold enhancement in europium emission in the presence of a variety of metal ions. A mechanistic study indicated that the luminescence enhancement could be triggered by a two-interaction binding mechanism, in which the initial interaction between STX and the aza-18-crown-6 ether moiety of EuL1 induces the secondary interactions between STX and the Eu³⁺ ion, which resulted in displacement of the coordinated water molecule on Eu³⁺ ion.

1. Introduction

Paralytic shellfish poisons (PSP) are a group of marine neurotoxins produced by a variety of marine dinoflagellates and natural water cyanophytes (blue-green algae) [1]. Bioaccumulation of PSP in marine organisms has become an increasing wide public concern on local marine environment, aquaculture industry and human health [2]. The most representative PSPs are saxitoxin (STX) and its analogues, such as neosaxitoxin and decarbamoylsaxitoxin (Fig. 1). The toxicity of STX is a result of binding towards the voltage gated sodium channel, blocking the passage of nerve impulses and leading to death via respiratory paralysis [3]. It is undoubtedly important and necessary to develop accurate and reliable tools for PSP toxins detection. There are several common methods for detection of STX, such as cells and mouse biosays [4–6], immunoassays [7–19] and high-performance liquid chromatography (HPLC) coupled with chemical derivatization or mass spectrometric (MS) detection [20–24]. Although immune analysis technology is highly sensitive, convenient and rapid, it is not easy to obtain pure antibodies, cross-reaction of the toxin is low, and does not fully reflect the toxicity of the source of the sample. The post column fluorescent derivatization HPLC method is the most widely adopted analytical protocol for PSP determination. However, the current analytical process is tedious, and the results obtained are not always reproducible.

Compared to the existing analysis methods, molecular chemo-sensors could be a promising alternative for detection of PSP toxins. This strategy usually involves a reversible binding of the analyte with an appropriate receptor, follows by a cascade transduction of the binding event into the generation of physically measurable signals. Recently, the group of Gawley reported a number of organic chemo-sensors containing a diaza-18-crown-6 moiety for STX detection [25–27]. Our group is particularly interested in developing lanthanide-based luminescence sensors for biologically important molecules [28–31] due to their long-lived luminescence lifetimes and fingerprint emission bands [32,33]. We have previously reported a europium-based fluorescent indicator for paralytic shellfish saxitoxin with the europium-crown-chromophore motif [28], in which the energy transfer efficiency of the chromophore to the Eu³⁺ ion was weakened since they were separated by the crown ether moiety (STX receptor). To improve the antenna effect, we herein report a new europium-based fluorescent STX sensor (EuL1) with the chromophore is conjugated with the both Eu³⁺ ion and the crown ether moiety (Fig. 1). This molecular architecture could provide a good environment for the chromophore-to-europium energy transfer efficiency and the fluorescence signal change upon binding with STX. Moreover, the positive charge of EuL1 should improve the solubility of the complex for the detection of STX in water/food samples.
2. Experimental section

2.1. Materials and methods

All air and water sensitive reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. All the chemicals were purchased commercially and used without further purification. NMR spectra were recorded on either a 400 (1H: 400 MHz, 13C: 100 MHz), or 500 (1H: 500 MHz, 13C: 125 MHz). High-resolution mass spectra were obtained from a MALDITOF Mass Spectrometer. UV–Visible absorption spectra in the spectral range 200–1100 nm were recorded by an HP UV-8453 spectrophotometer. Single-photon luminescence spectra were recorded using an Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and

![Fig. 1. (a) Structures of paralytic shellfish poisons (PSP), (b) Europium complex receptor EuL1 and (c) control compounds EuL2 and EuL3.](image)

Steady state spectrophotometer that was equipped with a red sensitive single photon counting photomultiplier by Peltier Cooled Housing. The spectra were corrected for detector response and stray background light phosphorescence. Values of the number of coordinated water molecules, \( q \), (±20%), were determined according to the literature procedures [34].

2.2. Synthesis and characterization

2.2.1. Synthesis of complex EuL1

2.2.1.1. Synthesis of ligand L1

To a degassed solution of \( \text{I} \) (50 mg, 0.08 mmol) and NaHCO\(_3\) (31 mg, 0.4 mmol) in MeCN (15 mL) was added DO2A(O\(_{\text{t}}\)Bu)\(_2\) (32 mg, 0.08 mmol) at 80 °C for 2 h. The resulting mixture was heated under refluxed for 4 h. After cooling to room temperature, the reaction was quenched by addition of water (20 mL). The aqueous solution was extracted with CH\(_2\)Cl\(_2\) (3 × 25 mL) and the combined organic layers were dried over Mg\(_2\)SO\(_4\), filtered, and concentrated. Silica gel flash column chromatography (CH\(_2\)Cl\(_2\)/MeOH; 25/1–10/1) of the residue gave a white solid (30 mg, 40%) as the product. \( \text{L1} \):

- \( R_f = 0.30 \) (silica gel, CH\(_2\)Cl\(_2\)/MeOH = 10 : 1);
- \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.57 (s, 1H), 7.48 (d, \( J = 9 \) Hz, 2H), 7.37 (s, 1H), 6.91 (d, \( J = 9 \) Hz, 2H), 4.00 (t, \( J = 7 \) Hz, 2H), 3.90 (s, 2H), 3.73–3.53 (m, 24H), 3.40 (s, 2H), 3.30 (s, 2H), 3.13–2.55 (m, 16H, cyclen), 1.88–1.80 (m, 2H), 1.44 (s, 18H), 1.06 (t, \( J = 7 \) Hz, 2H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \( \delta \) 170.9, 172.0, 168.9, 160.2, 155.5, 153.8, 133.5, 133.1, 126.0, 123.3, 114.8, 113.4, 95.9, 85.2, 81.9, 70.4 (10C, crown ether), 69.7, 58.1, 56.4, 50.0, 49.1, 48.1, 45.7, 28.0, 22.4, 18.4; HRMS (ESI) \( m/z \) calcd. for C\(_{50}\)H\(_{79}\)N\(_6\)O\(_{11}\) (M+H\(^+\)) 939.5807, found 938.5729.

2.2.1.2. Synthesis of complex EuL1.

\( \text{L1} \) (20 mg, 0.021 mmol) was dissolved in TFA (2 mL) and the mixture was stirred at room temperature under argon for 12 h. After removal of TFA under reduced pressure, the pale yellow residue was dissolved in CH\(_2\)Cl\(_2\) and concentrated to ensure the removal of TFA. Deprotection of the \( \text{t} \)Bu groups was confirmed by \(^1\)H NMR of the crude product. The residue was then dissolved in H\(_2\)O/MeOH (2:1 v/v, 3 mL) and treated with EuCl\(_3\)·6H\(_2\)O (0.021 mmol). After adjustment of the pH to 7.0 with an aqueous sodium hydroxide solution, the mixture was stirred at room temperature for 30 h. Concentration and recrystallization of the crude product from CH\(_3\)CN gave a pale white solid (12.5 mg, 66%) as the product. HRMS (ESI) \( m/z \) calcd. for C\(_{42}\)H\(_{60}\)EuN\(_6\)NaO\(_{11}\)Cl (M+H\(^+\)) 1012.3221, found 1012.3290.

**Table 1**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Complexes</th>
<th>( \tau_{1200}/\text{ms}^{-1} )</th>
<th>( \tau_{1200}/\text{ms}^{-1} )</th>
<th>( q )</th>
</tr>
</thead>
<tbody>
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<td>EuL1</td>
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<td>0.65</td>
<td>1.03</td>
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<tr>
<td>2</td>
<td>EuL2</td>
<td>1.00</td>
<td>1.64</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>EuL3</td>
<td>0.21</td>
<td>0.28</td>
<td>1.13</td>
</tr>
<tr>
<td>4</td>
<td>EuL1 + STX</td>
<td>0.53</td>
<td>0.64</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>EuL2 + STX</td>
<td>0.10</td>
<td>1.63</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>EuL3 + STX</td>
<td>0.22</td>
<td>0.09</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Fig. 2. (a) The UV–vis spectra of \( \text{L1} \) (black solid line), \( \text{EuL1} \) (dot line) and the excitation spectrum of \( \text{EuL1} \) (grey solid line). (b) The emission spectra of \( \text{EuL1} \) and \( \text{EuL2} \) (10 μM in H\(_2\)O, \( \lambda_{\text{ex}} \) = 325 nm).

Fig. 3. The emission spectra of (a) \( \text{EuL1} \), (b) \( \text{EuL2} \) and (c) \( \text{EuL3} \) (10 μM in H\(_2\)O, \( \lambda_{\text{ex}} \) = 325 nm) with and without STX.

Fig. 4. Effects of various metal ions on the luminescence intensity of \( \text{EuL1} \). 1) Eu complex only; 2) STX; 3) K\(^+\); 4) Ca\(^2+\); 5) Ba\(^2+\); 6) Mn\(^2+\); 7) Co\(^2+\); 8) Zn\(^2+\); 9) Ni\(^2+\); 10) Fe\(^2+\); 11) Mn\(^2+\); 12) Ca\(^2+\); 13) Li\(^+\); 14) Na\(^+\); 15) all of the above metal ions.

2.2.2. Synthesis of complex EuL2
Complex EuL2 was prepared according to the literature procedures [31].

2.2.3. Synthesis of complex EuL3
2.2.3.1. Synthesis of compound 3. To a stirred solution of 2 (100 mg, 0.31 mmol) and Et3N (93 mg, 0.92 mmol) in CH2Cl2 (20 mL) at room temperature was added methanesulfonyl chloride (54 mg, 0.46 mmol) dropwise. After stirring at room temperature for 20 min, the reaction was quenched by addition of a saturated NaHCO3 solution (20 mL). The organic phase was separated, dried over MgSO4, filtered, and concentrated. Silica gel flash column chromatography (n-hexane/ethyl acetate; 15/1) of the residue gave a white solid (32 mg, 30%) as the product.

2.2.3.2. Synthesis of ligand L3.
With the compounds prepared, the photophysical properties of the ligand L1 and complex EuL1 were studied. In the UV–vis absorption spectrum (Fig. 2a), L1 showed strong absorption bands at 300 and 345 nm in methanol which were attributed to the π → π* transitions.
After complexation with Eu³⁺ ion, slight blue-shift of the absorption maxima (280 and 330 nm, fₐₘₐₓ = 8308 M⁻¹ cm⁻¹) were observed. The excitation spectrum of EuL₁ at 615 nm showed maxima at 280 and 345 nm, displaying an antenna effect due to energy transfer from the ligand to the Eu³⁺ ion. The emission spectrum of EuL₁ was composed of five regions corresponding to the D₉₋→F₇ transitions at 578 (J = 0), 585–603 (J = 1), 604–637 (J = 2), 646–658 (J = 3), and 673–712 nm (J = 4). Additionally, there are differences on the shapes of the emission spectra between the EuL₁ and EuL₂ (Fig. 2b). For example, the relative intensity of the electric-dipole allowed J = 2 transitions (around 620 nm) compared with the J = 1 magnetic-dipole allowed set (around 590 nm) of EuL₁ was a little smaller than that of EuL₂. Besides, the observed splitting of the J = 1 or J = 2 transition in several bands was not identical for EuL₁ and EuL₂. This phenomenon was determined both by symmetry of coordination environments and the polarizability of the capping axial donor [35,36].

3.3. Luminescence change upon binding with saxitoxin

With the photophysical properties of EuL₁ evaluated, its binding properties towards STX were studied using fluorometric titration experiments. Upon addition of STX (1.25 equiv), EuL₁ (10 μM in water) showed a 4-fold enhancement in the signal intensity and changing in splitting of the europium emission peaks (Fig. 3a), indicating the binding of STX could alter the environment of the coordinated Eu³⁺ centre of EuL₁. In a comparison study, no significant europium emission change was observed upon addition of STX to the control compounds EuL₂ and EuL₃ (Fig. 3b-c). These results indicated that coordination environment around the Eu³⁺ ion centre and the azas-18-crown-6 ether moiety are important for the europium emission changes and the selective binding properties towards STX.

3.4. Competitive experiments

In a competitive study, the addition of a large excess of various metal ions, such as K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Co²⁺, Zn²⁺, Ni²⁺, Fe²⁺, Mn₂⁺, Ca²⁺, Li⁺ and Na⁺ ions, to EuL₁ resulted in only 1.05–1.20 fold enhancement of the europium emission (white columns in Fig. 4). Moreover, no significant change in europium emission was observed even in the presence of all the metal ions (entry 15 in Fig. 4). Subsequent addition of STX enhanced the europium emission by 4-fold in all cases (grey columns in Fig. 4). These results demonstrated the high selectivity of EuL₁ towards STX and the binding between the Eu complex and STX was not interfered by other metal ions.

3.5. Mechanistic study

To investigate the binding mechanism between EuL₁ and STX, the hydration states (q values) of Eu complexes EuL₁-EuL₃ before and after the addition of STX were estimated [34]. As shown in the Table 1, EuL₁ and EuL₃ contain one coordinated water molecule (entries 1 and 3) but EuL₂ contains none (entry 2) probably due to its more hindered environment around the Eu³⁺ ion centre. Upon addition of STX, the q value of EuL₁ dropped dramatically (entry 4), suggesting the dissociation of the coordinated water molecule. The luminescence enhancement was presumably due to the effective energy transfer from the antenna to the lanthanide ion without vibrational quenching from coordinated water through non-radiative energy dissipation. In contrary, the q values of EuL₂ and EuL₃ have no significant change after addition of STX (entries 5 and 6). These results suggested that the binding of Eu complex with STX and the dissociation of the coordinated water molecule are both important for the luminescence enhancement.

Based on the above results, we proposed that a two-interaction binding mechanism between EuL₁ and STX. As shown in Fig. 5a, STX first interacted with the azas-18-crown-6 ether moiety of EuL₁. This interaction brings STX close to the Eu³⁺ ion, allowing the secondary interaction between STX and the Eu³⁺ ion and resulting in displacement of the coordinated water molecule on the Eu³⁺ ion. Although the azas-18-crown-6 ether moiety of control compound EuL₂ could interact with STX, no luminescence change was observed due to the lack of a coordinated water molecule in EuL₂. Model compound EuL₃ does not contain the azas-18-crown-6 ether moiety. STX could not get close to the Eu³⁺ ion centre for displacement of the coordinated water molecule in EuL₃. (Fig. 5b)

4. Conclusion

In summary, we have developed a new water-soluble and emissive Eu complex EuL₁ with the chromophore conjugated with both the azas-18-crown-6 ether and the DO2A(Eu³⁺) moieties for detection of STX. EuL₁ binds to STX selectively and results in a 4-fold luminescence enhancement without interference by a variety of metal ions. Based on the results of the mechanistic and comparison studies, the luminescence enhancement was rationalized by a two-interaction binding mechanism between EuL₁ and STX, in which the initial interaction between STX and azas-18-crown-6 ether moiety induces the secondary interaction between STX and the Eu³⁺ ion and resulted in displacement of the coordinated water molecule. Due to its high selectivity and sensitivity towards STX, this new Eu-complex could find its applications for STX detection in water/food samples.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jlumin.2018.02.061.

References


