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Graphic Abstract

The Title:

A high selective and sensitive turn on fluorescent probe for detection of holmium ion and its bioimaging

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The statement:

The phenolphthalein aldehyde was synthesized and used to an turn-on fluorescent probe for the detection of holmium ion (Ho\(^{3+}\)), which is one of lanthanide ions in HEPES:DMF = 1:1 (V/V pH=7.4) solution with an excellent selectivity and sensitivity for Ho\(^{3+}\) over other metal ions.
A high selective and sensitive turn on fluorescent probe for detection of holmium ion and its bioimaging

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The phenolphthalein aldehyde was synthesized and used to a turn on fluorescent probe for the detection of holmium ion (Ho), which is one of lanthanide ions in HEPES:DMF = 1:1 (V/V pH=7.4) solution with an excellent selectivity and sensitivity for Ho over other metal ions.

Rare earth (RE) is an important non-renewable resources. With the development of science, it was made various demands for materials. Application of rare earth elements, from their use of homogeneous property, extends to the use of single rare earth specialty, and has gone deep into all areas of modern technology. Because rare earth element (REE) has the special properties to promote crop production and improve crop quality, the area of application of compound fertilizer of rare earth element is more and more widely. Generally, low concentrations of REEs is present in soil/plant, water, and atmosphere, but REEs can accumulate in such environments following anthropogenic inputs because of the low mobility of these elements. As the products containing rare earths continuously into the environment, long-term risks to plants, animals, the environment and human health has attracted widespread attention at home and abroad scientific community.

Holmium is one of lanthanide ions, however, it is 20 times more abundant than silver. There is a growing trend in the uses of holmium element, due to the fact that it is studied to produce catalysts, polish glass or use for biological applications. Lanthanides do not partake in any metabolic process. However, due to the similarity of lanthanides ionic radii to calcium ion, Lanthanides do not partake in any metabolic process. However, due to the similarity of lanthanides ionic radii to calcium ion, holmium ions in the body can be a threat to the liver. Nowadays, it is known that holmium causes damages to the cell membranes of the water animals with negative effects on the reproduction and the nervous systems. This makes the detections of Ho greatly important. Conventional methods of Ho ion analysis have included neutron activation analysis, ICP-MS, ICP-AES and isotope dilution mass spectrometry. In recent years, it was reported a number of opcode and membrane sensor for selective determination of Ho. However, the fluorescent probe to detect Ho has not been reported.

Herein, we report a novel fluorescent probe based on phenolphthalein aldehyde to selective detect Ho in medium of near neutral pH value. And the recognition ability of probe for Ho was studied. The result showed that this probe works well at physiological pH and has a high selectivity and sensitivity for Ho over other metal ions. These desirable attributes render the sensor suitable for detection of Ho. Furthermore, the system is used to bioimaging.

The synthesis of probe is summarized in Scheme 1. To a stirred TFA (10 mL) on ice was added phenolphthalein (636.64 mg, 2 mmol) and hexamethylenetetraamine (403.7 mg, 2.88 mmol). The solution was allowed to warm to room temperature, and then it was refluxed for 8h. The excess TFA was removed under vacuum. 30 mL water was then added to the remaining solution, and the mixture was warmed at 60°C whilst stirring for another 30 min. Upon cooling on ice, a pale yellow solid were precipitated out from the solution which were collected by filtration. Then the crude product was purified by chromatography on a silica gel column, and give the desired product as a white solid (57% yield). H NMR (300 MHz, 25°C, DMSO-d6): δ 11.04 (bs, 2H), 10.22 (bs, 2H), 7.94 (d, 1H, J = 7.2 Hz), 7.84 (m, 2H, J = 20.7 Hz), 7.68 (q, 1H, J = 14.4Hz), 7.51 (s, 2H), 7.43 (d, 2H, J = 8.7Hz), 7.02 (d, 2H, J = 8.7Hz); 13C NMR (75 MHz, DMSO-d6): δ 189.9, 168.1, 160.3, 150.5, 134.6, 134.2, 130.7, 129.4, 125.7, 125.1, 123.6, 121.1, 117.5, 89.3; ESI-MS [M-H] 373.25; Elemental analysis (caled %) for C23H10O6: C, 70.59; H, 3.77; Found: C, 70.38; H, 3.89. (Fig. S1, ESI†)

The recognition ability of the probe was investigated by the fluorescence spectra. The selectivity towards the metal ions is one of the most important criteria for probe design. The effect of a wide range of environmentally and physiologically active metal ions was investigated for probe using the fluorescence spectra of solutions containing probe and the metal ions (100 equiv.) in the HEPES:DMF = 1:1 (V/V pH=7.4) solution. As shown in Fig. 1, the fluorescence intensity, there is notable change when Ho is excited. The nice isolation between energy levels, which resulted from two way: one is low energy photon is easy to be quenched by high energy level structure inducing the bigger gap between the high metastable state. The second one is that the more abundant level structures, as well as some other rare earth element is excited, which results in the abundant level structures, as well as some other rare earth element.

In the present work, the fluorescence of Dy3+ and Eu3+ were excited by 365 nm, the emission peaks of Dy3+ and Eu3+ were 540 nm and 590 nm respectively. The probe could specifically recognize Ho3+ fluorescence to 520 nm, which is corresponding to the absorption peak of Ho3+ (Fig. S2, ESI†).

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The fluorescence of Dy3+ is easy to be quenched by high-energy...
vibration of O–H bond in water molecules.

A titration experiment of the probe for Ho$^{3+}$ was also carried out on fluorescence spectrophotometer. The probe displayed no emission at 480 nm. With addition and gradual increasing amount of Ho$^{3+}$ to the HEPES/DMF $= 1:1$ (V/V, pH=7.4) solution containing probe, a significant increase in fluorescence intensity at 480 nm was observed with an excitation wavelength 365 nm. When 260 µM Ho$^{3+}$ was added to the solution of the probe (20 µM), a more than 25-fold increase in fluorescence intensity at 526 nm was observed (Fig. 2) with a change of fluorescence quantum yield from $\Phi_{\text{probe}} = 0.001$ to $\Phi_{\text{probe-Ho}}^{3+} = 0.256$. In addition, with increasing Cu$^{2+}$ concentration, the absorption peaks at 330 gradually decreased and 380 nm increased. One isosbestic points was noted at 348 nm indicating the formation of a new species (Fig. S3, ESIf). According to linear Benesi Hildebrand expression, the measured intensity $[1/(A-A_0)]$ at 380 nm varied as a function of $[\text{Ho}^{3+}]$ in a linear relationship ($R = 0.99797$) (Fig. S4, ESIf), indicates formation of 1:1 stoichiometry between Ho$^{3+}$ and probe. The association constant of probe with Ho$^{3+}$ was calculated to be $1.08 \times 10^5$ M$^{-1}$.

The effect of pH on the fluorescence properties of the system was investigated for pH values of 2.0–13.0. The results are shown in Fig. 3. Over the pH 2.0–8.0, the fluorescence signal for the probe solution containing Ho$^{3+}$ increased sharply from pH 4.0 to 8.0, and then decreased from pH 8.0 to 11.0. The fluorescence intensity of the probe solution containing Ho$^{3+}$ has a little increases at pH 12.0 compared with at pH 11.0. Holmium exists in the form of Ho(OH)$_3$ precipitate due to the strong basicity of pH 13.0 which result in the fluorescence decrease of probe with addition of Ho$^{3+}$. Therefore, considering pH and fluorescence intensity, a pH of 7.4 was selected for the analytical system.

The effect of reaction time on the fluorescence emission of the system was also studied in the presence of 10 equiv. of Ho$^{3+}$ and the results are shown in Fig. 4. The fluorescence signal for the system increases gradually with increasing reaction time, and the addition of Ho$^{3+}$, under the selected reaction conditions, was rapid. Therefore, a 4 min reaction time was selected for the following experiments.

To investigate the detection limit of probe for Ho$^{3+}$, probe (20 µM) was treated with various concentrations of Ho$^{3+}$ (0–260 µM) and the relative emission intensities at 480 nm were plotted as a function of the Ho$^{3+}$ concentration (Fig. S5, ESIf). The fluorescence intensity of probe is linearly proportional to the Ho$^{3+}$ concentrations, and the detection limit, based on the definition by IUPAC (CDL = 3 Sbm/m), was found to be 0.055 µM indicating this probe is sensitive enough to monitor Ho$^{3+}$ compared with other method (Table 1).

The reaction mechanism of the present system was also studied. We presumed that the fluorescence increase could be attributed to ring-open after the coordination of probe with Ho$^{3+}$. Namely, because of the probe with hydroxyl and aldehyde groups (their O atoms as hard bases), easily coordinating with Ln ions as hard acids and resulting in the ring open, increased the conjugate structure of intramolecular, which lead to the system became strong fluorescence. The identification of coordination product in the ESI-MS analysis made it possible to propose the signaling mechanism: a peak at m/z = 984.00, corresponding to [probe-Ho +4H$\text{O}$+H$^+$], is clearly observed (Fig. S6, ESIf). The proposed detection mechanism and the structures of the probes, both with and without the addition of Ho$^{3+}$, are shown in Scheme 2.

The ability of probe reacting with Ho$^{3+}$ within living cells was also evaluated by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope. The optical window at the yellow channel (450–550 nm) was chosen as a signal output. As shown in Fig. 5a, HepG2 cells incubated with 20 µM probe for 30 min at 37 °C and washed 3 times with PBS showed no fluorescence. In a further experiment it was found that HepG2 cells displayed green fluorescence when the cells were first incubated with 20 µM of probe for 30 min at 37 °C and washed 3 times with PBS, then incubated with 100 µM HoCl$_3$ and washed 3 times with PBS (Fig. 5b). These cell experiments show the good cell-membrane permeability of probe, and it can thus be used to mark Ho$^{3+}$ within living cells.

In summary, we have developed a fluorescent probe for the detection of Ho$^{3+}$ by turn-on fluorescence over other analytes in aqueous solution. This probe is based on a phenolphthalein derivative which has a high selectivity and sensitivity for Ho$^{3+}$ over other analytes in HEPES: DMF = 1:1 (V/V pH=7.4). Furthermore, the system is used to bioimaging.

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Inserting Graphics

Scheme 1 Synthesis of probe.
Ca^{2+}, Mg^{2+}, Sn^{2+}, Fe^{3+}, Dy^{3+}, Pr^{3+}, Tb^{3+}, Pm^{3+}, Lu^{3+}, Tm^{3+}, Nd^{3+} and Eu^{3+});
Inset: a color change photograph for Ho^{3+} and the other metal ions under illumination with a 365 nm UV lamp.

![Fluorescence spectral changes of probe (20 µM) in 10 mmol/L HEPES buffer/DMF (v/v=1:1, pH 7.4) (λex = 365 nm, slit: 5 nm/5 nm) upon addition of Ho^{3+}; Ho^{3+} was added gradually with [Ho^{3+}] = 0–260 µM; Inset: a color change photograph for Ho^{3+} under illumination with a 365 nm UV lamp.]

![Effect of pH on the fluorescence intensity of the probe and probe+Ho^{3+} (260 µM.)]

![Reaction time profiles of probe (20 µM) and Ho^{3+} (260 µM)]]

**Fig. 2.** Fluorescence spectral changes of probe (20 µM) in 10 mmol/L HEPES buffer/DMF (v/v=1:1, pH 7.4) (λex = 365 nm, slit: 5 nm/5 nm) upon addition of Ho^{3+}; Ho^{3+} was added gradually with [Ho^{3+}] = 0–260 µM; Inset: a color change photograph for Ho^{3+} under illumination with a 365 nm UV lamp.

**Fig. 3.** Effect of pH on the fluorescence intensity of the probe and probe+Ho^{3+} (260 µM.)

**Table 1.** A comparison table about the detection methods for Ho^{3+}.

<table>
<thead>
<tr>
<th>method</th>
<th>analyte</th>
<th>detection limit</th>
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<td>ref (18) membrane sensor</td>
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<td>this work Fluorescent probe</td>
<td>Ho^{3+}</td>
<td>0.055µM</td>
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</table>

**Scheme 2.** The mechanism of chemosensor.

![Scheme 2. The mechanism of chemosensor.]

**Fig. 4.** Reaction time profiles of probe (20 µM) and Ho^{3+} (260 µM)

**Notes and references**

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