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A visible-light-excited europium(III) complex-based luminescent probe for visualizing copper ions and hydrogen sulfide in living cells

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ABSTRACT

Development of visible-light-excited lanthanide (III) complex-based luminescent probes is highly appealing due to their superiority of less damage to the living biosystems over the conventional UV-light-excited ones. In this work, a visible-light-excited europium (III) complex-based luminescent probe, BPED-BHHCT-Eu^{3+}-BPT, has been designed and synthesized by conjugating the Cu^{2+}-binding N,N-bis(2-pyridylmethyl)ethanediamine (BPED) to a tetradentate β-diketone ligand 4,4′-bis(1″,1″,1″,2″,2″,3″,3″-heptafluoro-4″,6″-hexanedione-6″-yl)chlorosulfo-*o*-terphenyl (BHHCT) and coordinating with a coligand 2-(N,N-diethylanilin-4-yl)-4,6-bis(pyrazol-1-yl)-1,3,5-triazine) (BPT) for the time-gated luminescence detection of Cu^{2+} ions and hydrogen sulfide (H_2S) in living cells. BPED-BHHCT-Eu^{3+}-BPT exhibited a sharp excitation peak at 407 nm and a wide excitation window extending to beyond 460 nm. Upon its reaction with Cu^{2+} ions, the luminescence of BPED-BHHCT-Eu^{3+}-BPT was efficiently quenched, which could be reversibly restored by the addition of H_2S due to the strong affinity between Cu^{2+} ions and H_2S. The "on-off-on" type luminescence behavior of BPED-BHHCT-Eu^{3+}-BPT towards Cu^{2+} ions and H_2S enabled the sensing of the two species with high sensitivity and selectivity. The performances of BPED-BHHCT-Eu^{3+}-BPT for visualizing intracellular Cu^{2+} ions and H_2S were investigated, and the results have demonstrated the practical applicability of the probe for molecular imaging of cells.

1. Introduction

Trace elements (e.g., Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, I) and macroelements (e.g., Na, Mg, K, Ca, P, Cl, S) are essential to support various biochemical processes for human life [1]. As an example of trace elements, copper ion (Cu^{2+}) plays an important role in various physiological processes of organism, such as haemopoiesis, iron absorption, and enzyme-catalyzed and redox reactions [2]. As a typical macroelement, sulfur is mainly contained in amino acid (cysteine, methionine, etc.) and vitamin (biotin, thiamine, etc.). Currently, hydrogen sulfide (H_2S), generated from cysteine or homocysteine in biosystems [3,4], has been considered as the third endogenous signaling gasotransmitter along with nitric oxide (NO) and carbon monoxide (CO) [5]. H_2S also plays critical roles in diverse physiological processes including apoptosis, blood pressure regulation, neuromodulation, and inhibition of insulin signaling [6,7]. Increasing evidences have shown that the abnormal amounts of Cu^{2+} ions and H_2S will bring about health problems [8]. For example, excess Cu^{2+} ions can disrupt protein activity and induce tissue necrosis [9], which is related to Parkinson's disease [10] and Wilson's disease [11]. The abnormal production of H_2S is also associated with many diseases such as diabetes [12], chronic kidney disease [13], hypertension [14], and Alzheimer's disease [3]. Therefore, methods for the detection of Cu^{2+} ions and H_2S in biosystems, especially in living cells, are highly demanded for their physiological and pathological research [15].

To date, several traditional analytical methods have been adopted to detect Cu^{2+} ions and H_2S in various samples, including colorimetry [16], UV–vis spectroscopy [17] and fluorescence spectrometry [18] for Cu^{2+} ions, and electrochemistry [19], chemiluminescence [20], gas chromatography [21] and sulfide precipitation [22] for H_2S. These methods usually require post-mortem and destruction of samples, which are not suitable for the real-time monitoring of Cu^{2+} ions and H_2S in living biosystems. In addition, the low concentration of Cu^{2+} ions and high activity of H_2S also limit the usability of these methods. Recently, the optical

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imaging technique with the use of luminescent probes provides an appropriate approach for in vivo detecting biomolecules due to its high sensitivity, high spatial resolution, and non-destructive detection [23,24]. A number of luminescent probes for Cu^{2+} ions have been designed utilizing its strong luminescence quenching ability [18,25,26]. Meanwhile, various luminescent probes for H_2S have also been developed based on its special properties such as strong reducing ability [27,28], quencher (e.g., Cu^{2+}, Hg^{2+}) removal [29,30] and nucleophilic reaction [31,32]. However, the major drawback of these organic fluorophore-based or ruthenium(II) complex-based probes is their susceptibility to photobleaching and strong biological autofluorescence.

As an improvement, lanthanide(III) complex-based luminescent probes (mainly Eu^{3+} and Tb^{3+} complexes) have attracted great interest due to their ideal luminescent properties including large Stokes shift, long luminescence lifetime, sharp emission profile and good photostability [33,34]. In particular, the super long-lived luminescence enables their usages in the time-gated detection technique, which can effectively eliminate the autofluorescence interference and contribute to improve the sensitivity and selectivity to the target species in complicated biosystems [35,36]. A few lanthanide(III) complex-based luminescent probes have been reported for the detection of Cu^{2+} ions [37,38] and H_2S [38,39]. However, a key problem of all these lanthanide(III) complex-based probes is that their excitation windows are limited to the UV region (<380 nm), which will cause excitation phototoxicity to some UV light-sensitive living biosamples and be unfavorable for the photostability of probes. Therefore, the development of visible-light-excited lanthanide complex(III)-based probes is urgently required for the pursuit of their wider bioassay applications.

Herein, we developed a unique visible-light-excited Eu^{3+} complex, BPED-BHHCT-Eu^{3+}-BPT, in which the ligand BPED-BHHCT was synthesized by conjugating a Cu^{2+}-binding moiety N,N-bis(2-pyridylmethyl)ethanediamine (BPED) to a tetradentate β-diketone 4,4′-bis(1″,1″,1″,2″,2″,3″,3″-heptafluoro-4″,6″-hexane- dione-6″-yl) chlorosulfo-*o*-terphenyl (BHHCT), and the coligand 2-(N,N-diethylanilin-4-yl)-4,6-bis(pyrazol-1-yl)-1,3,5-triazine (BPT) was introduced to achieve the visible-light excitation of the Eu^{3+} complex. BPED-BHHCT-Eu^{3+}-BPT exhibited a strong visible excitation peak at 407 nm, whose corresponding fluorescence emission at 611 nm could be selectively quenched by Cu^{2+} ions due to the coordination of Cu^{2+} with the BPED moiety and the resulting intramolecular photo-induced electron transfer (PET). The trapped BPED group could be released by the strong affinity between the coordinated Cu^{2+} and H_2S, which resulted in the formation of CuS precipitate and restored the luminescence of the quenched probe. The decrease of luminescence intensity at 611 nm was linearly correlated with the Cu^{2+} concentration, and the reverse luminescence enhancement also showed a good linearity against the H_2S concentration. Therefore, BPED-BHHCT-Eu^{3+}-BPT can be used as a luminescent probe for the time-gated luminescence detection of Cu^{2+} ions and H_2S in aqueous media. To examine the applicability of the new probe for bioimaging, the BPED-BHHCT-Eu^{3+}-BPT-loaded HepG2 cells were prepared, and the exogenous Cu^{2+} ions and H_2S in the cells were imaged on a true-color time-gated luminescence microscope. The results demonstrated the practical utility of the new probe for visualizing intracellular Cu^{2+} ions and H_2S. Scheme 1 shows the structure of BPED-BHHCT-Eu^{3+}-BPT and its luminescence response reaction with Cu^{2+} ion and H_2S.

2. Experimental

2.1. Materials and physical measurements

The tetradentate β-diketone BHHCT [40], BPT [41] and BPED [42] were synthesized according to the previously reported methods. Triton X-100 was purchased from Acros Organics. Dichloromethane (CH_2Cl_2) was used after appropriate distillation and purification. Cultured HepG2 cells were obtained from Dalian Medical University. The isotonic saline solution consisting of 140 mmol NaCl, 10 mmol glucose and 3.5 mmol KCl was prepared in our laboratory. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

^1H NMR spectrum was measured on a Bruker Avance spectrometer (400 MHz). Mass spectrum was recorded on a HP1100LC/MSD electrospray ionization mass spectrometer (ESI-MS). Elemental analysis was carried out on a Vario-EL analyser. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV–vis spectrometer. Time-gated luminescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the following conditions: delay time, 0.2 ms; gate time, 0.4 ms; cycle time, 20 ms; excitation slit, 10 nm; and emission slit, 10 nm. Luminescence lifetimes were measured on an FS5 spectrofluorometer of Edinburgh Instruments. Relative luminescence quantum yields of BPED-BHHCT-Eu^{3+}-BPT and its reaction product with Cu^{2+}, Cu^{2+}-BPED-BHHCT-Eu^{3+}-BPT, were measured with the Eu^{3+} complex, Eu^{3+}-(4′-phenyl-2,2′:6′,2″-terpyridine-6,6″-diyl) bis-(methylene-nitrilo)tetrakis(acetate), as a reference ($\Phi = 0.16$) [43]. All bright-field and luminescence imaging measurements were carried out on a laboratory-use true-color time-gated luminescence microscope [44].

2.2. Synthesis of BPED-BHHCT

BPED (35 mg, 0.12 mmol) was dissolved in dichloromethane CH_2Cl_2 (6 mL), and then BHHCT (100 mg, 0.12 mmol), triethylamine (0.15 mL) and dimethylaminopyridine (2.9 mg, 0.024 mmol) were added to the solution. The reaction mixture was stirred at room temperature in the dark for 5 days. After the solvent was evaporated, the residue was washed with diluted hydrochloric acid (1.0 M, 80 mL), and then dissolved in CH_2Cl_2 (200 mL). The CH_2Cl_2 solution was washed three times with 120 mL of water and dried with Na_2SO_4. After evaporation, the ligand BPED-BHHCT was obtained as yellow solid (120.5 mg, 89% yield). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.25 (s, 1H), 1.80 (m, 4H), 3.98–4.01 (d, 4H; $J = 12.0$ Hz), 6.56 (d, 2H; $J = 5.2$ Hz), 7.04 (m, 4H), 7.23–7.21 (d, 4H; $J = 8.0$ Hz), 7.77–7.86 (d, 8H; $J = 12.0$ Hz), 8.61 (m, 4H). ESI-MS (*m*/*z*): 1011.3 ($[M + H]^+$, 100%, calcd 1011.2). Elemental analysis calcd (%) for $C_{44}H_{33}F_{14}N_4O_{6.5}S$ (BPED-BHHCT·0.5H_2O): C, 51.82; H, 3.26; N, 5.49; found (%): C, 52.28; H, 3.19; N, 5.54.

2.3. Preparation of the stock solution of BPED-BHHCT-Eu^{3+}-BPT

The stock solution of the complex BPED-BHHCT-Eu^{3+}-BPT was prepared by in situ mixing equiv of BPED-BHHCT (0.5 mmol), BPT (1 mmol) and $EuCl_3 \cdot 6H_2O$ (0.5 mmol) in ethanol. The obtained stock solution was stored at 4 °C and properly diluted with 0.05 M Tris-HCl buffer of pH 7.5 containing 0.2% Triton X-100 before use.

2.4. Luminescence response of BPED-BHHCT-Eu^{3+}-BPT towards various metal ions

The reactions of BPED-BHHCT-Eu^{3+}-BPT with various metal ions were performed in 0.05 M Tris-HCl buffer of pH 7.5 containing 0.2% Triton X-100 with the same concentration of BPED-BHHCT-Eu^{3+}-BPT. The solutions were stirred at room temperature for 30 min, and then subjected to time-gated luminescence measurements.