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A naked-eye visible and turn-on fluorescence probe for Fe^{3+} and its bioimaging application in living cells

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ABSTRACT

A novel turn-on fluorescence probe based on rhodamine B for Fe^{3+} has been synthesized and characterized by various analytical techniques. Compared with other commonly coexisting metal ions, the probe showed 100-fold fluorescence enhancement for detecting Fe^{3+} with a 1:1 stoichiometry in MeOH/H_2O (1:1, v/v) solution. After adding Fe^{3+}, the rapid enhancement of fluorescence intensity provided a rapid method for detection of Fe^{3+}. The color change was visible, so it could be used for naked-eye detection. The association constant between the probe and Fe^{3+} was detected to be $0.67 \times 10^4\ M^{-1}$ and the corresponding detection limit were calculated to be 5.7×10^{-8} M according to fluorescence titration analysis. The reversible binding of probe L to Fe^{3+} was confirmed by reacting with $Na_4P_2O_7$, which captured the Fe^{3+} from the L-Fe^{3+}complex and turned off the L-Fe^{3+}complex. Fluorescence imaging experiments demonstrated that this probe was cell permeable and suitable for monitoring intracellular Fe^{3+} in living cells by confocal microscopy. What's more, MTT assay indicated that this probe had no significant cytotoxicity to living cells.

1. Introduction

Transition-metal ions have received increasing attention due to their importance in many biological and environmental processes [1–4]. Iron, one of the most abundant and versatile transition metal, plays various and important roles in many biochemical process, such as oxidation reaction [5,6], electron transfer [7,8] and oxygen transport [9]. There is increasing evidence that neither deficiencies nor excesses of iron can induce biology disorders in the human body such as Huntington, Parkinson's [10] and Alzheimer's disease [11]. These health problems of Fe^{3+} have prompted researchers to develop efficient methods for selective and sensitive assay of the metal to understand its distribution and potential hazard. Toward this goal, conventional methods have been developed in the past decades [12–16]. However, these conventional methods require sophisticated equipment, tedious sample preparation procedures and highly skilled individuals. Recently, considerable efforts have been undertaken to develop fluorescence probe because of their high selectivity, sensitivity and simplicity. Researchers have developed a series of probes such as Hg^{2+} [17–20], Cu^{2+} [21,22], Zn^{2+} [23–25], Pb^{2+} [26,27] and so on, while fluorescence probe for Fe^{3+} are relatively scare. Most early probes showed a fluorescence quenching (turn-off) response due to paramagnetic of the Fe^{3+} which are not as sensitive as required for desired analytical purposes [28,29]. Turn-on type signal is more superior than the turn-off type due to their better overall signal processing in biosystems, so it is necessary to develop probes which exhibit fluorescent enhancement with Fe^{3+}. The rhodamine moiety to construct turn-on fluorescent probe is a desirable platform due to their structure change from non-fluorescent spirolactam to highly fluorescent ring-opened amide induced by analytes at room temperature [30–34].

To date, some Fe^{3+}-selective probes based on rhodamine have been synthesized and assessed in bioimaging. For example, Ozay synthesized a novel on/off fluorescent rhodamine-based hexapodal Fe^{3+} probe containing a cyclotriphosphazene core [35]. Meng reported a water-soluble fluorescent probe for Fe^{3+} based on rhodamine B, which shows high selectivity in water containing less than 1% organic cosolvent [36]. Bao synthesized a rhodamine B-based sensor consisting of a rhodamine B moiety linked to the amine end of a 1, 4, 7, 10-tetraoxa-13-azacyclopentadecane which

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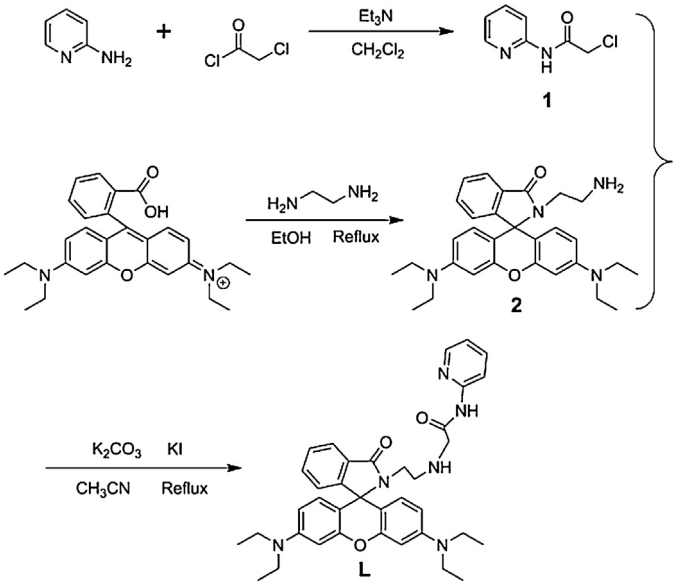

Scheme 1. Schematic of probe L synthesis.

exhibits a high selectivity for Fe^{3+} in the presence of many other metal cations [37]. Although these rhodamine-based fluorescent probes for Fe^{3+} show potential application and unique properties. Many Fe^{3+}-selective probes based on rhodamine still have some shortcomings, such as long response time, unsatisfactory detection limit and high cytotoxicity. These shortcomings restrict the development of Fe^{3+}-selective probes. Therefore, it is worthwhile to develop Fe^{3+}-selective probes with short response time, low detection limit and low cytotoxicity.

Herein, we report a naked-eye, reversible fluorescence turn-on probe L based on rhodamine B for Fe^{3+} with high sensitivity and selectivity. This probe L has wide pH range of 4–8, which makes it possible to apply in physiological pH window. According to the obtained results, we propose a plausible mechanism. Finally, probe L shows low cytotoxicity and excellent membrane permeability toward living cells, which can be successfully applied to monitor intracellular Fe^{3+} effectively by confocal fluorescence imaging.

2. Experiment

2.1. Materials and instruments

All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. Mass spectra (ESI) were performed on Bruker MicroTOF ESI-TOF Mass Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on JNM-ECS-400 MHz and referenced to the solvent signals. Absorption spectra were recorded on Cary-5000 UV–vis-NIR spectrophotometer. Fluorescence spectra were performed by using a Hitachi F-7000 spectrofluorophotometer. All pH measurements were made with a pH-10C digital pH meter.

2.2. Synthesis of probe L

The general procedure of synthesis is shown in Scheme 1. Intermediate compounds 1 and 2 were synthesized as reported method [38–41]. The probe L was synthesized by the reaction of compound 1 and 2 in acetonitrile. Compound 1 (0.342 g 2 mmol), compound 2 (0.968 g 2 mmol), K_2CO_3 (0.276 g 2 mmol) and KI (0.166 g 0.1 mmol) were dissolved in 30 mL acetonitrile and reflux for 8 h. After reaction finished, the flask was naturally cooled to room temperature. The filtrate obtained by filter was evaporated under reduced pressure. The residue was purified by silica gel column using a mixture of dichloromethane and methanol (20:1, v/v). Yield: 56.3% ^{1}H NMR (400 MHz,) δ 9.69 (s, 1H), 8.25 (d, J = 4.8 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.91 (dd, J = 5.7, 3.1 Hz, 1H), 7.70–7.61 (m, 1H), 7.46 (dd, J = 5.7, 3.0 Hz, 2H), 7.13–7.06 (m, 1H), 6.99 (dd, J = 7.2, 4.9 Hz, 1H), 6.45 (d, J = 8.9 Hz, 2H), 6.37 (d, J = 2.2 Hz, 2H), 6.25 (dd, J = 8.9, 2.4 Hz, 2H), 5.30 (s, 1H), 3.30 (dd, J = 14.1, 7.0 Hz, 10H), 3.19 (s, 2H), 2.49 (t, J = 6.0 Hz, 2H), 1.13 (t, J = 7.1 Hz, 12H). ^{13}C NMR (100 MHz,) δ 170.97, 168.92, 153.48, 153.39, 151.14, 148.93, 147.97, 138.18, 132.63, 131.20, 128.75, 128.21, 123.94, 122.91, 119.65, 113.79, 108.17, 105.35, 97.64, 65.18, 52.75, 48.96, 44.43, 40.12, 12.66, 12.66. ESI-MS m/z: Calcd for $C_{37}H_{42}N_6O_3$ 618.3318, Found: 619.3718 $[M+H]^+$.

2.3. General spectroscopic procedures

Stock solutions (1×10^{-2} M) of perchlorate salts (Na^+, K^+, Li^+ Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Hg^{2+}, Ag^+, Cd^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, and Fe^{3+}) and $FeSO_4$ were prepared. The probe L stock solution (1×10^{-2} M) was also prepared in dimethyl sulfoxide (DMSO). Test solutions were prepared by placing 2 μL of probe L stock solution into a test tube with 2.0 mL MeOH/H_2O (1:1, v/v) solution, and adding an appropriate aliquot (0–20 μL) of each metal stock solution. To make the metal ions chelate with the probe sufficiently, solutions were shaken for 10 s and waited for 15 min before determination. Fluorescence measurements were carried out with excitation and emission slit widths of 5.0 and 5.0 nm and excitation wavelength was 520 nm.

2.4. Cell culture and confocal fluorescence imaging

Vitro experiments were performed by using A549 cells. A549 cells were cultured in RPMI-1640 medium supplemented with 10% FBS (Fetal bovine serum), 100 units per mL penicillin, and 100 μg mL^{-1} streptomycin at 37 °C in a humid atmosphere containing 5% CO_2. Cells (5×10^8 L^{-1}) were plated on 18 mm glass coverslips and allowed to adhere for 24 h. Then they were treated with L (20 μM) and incubated for 30 min. Subsequently, the cells were treated with 100 μM Fe $(ClO_4)_3 \cdot 6H_2O$. Cells were incubated for 30 min and washed with PBS three times to remove free compound and ions before analysis. Cells only incubated with 20 μM L for 30 min acted as a control. Confocal luminescence images of cells were carried out on an Olympus FV1000 laser scanning confocal microscope and a 100× oil-immersion objective lens. Emission was collected at 530–630 nm for the A549 cells.

2.5. Cell cytotoxicity

The cytotoxic effect of probe L was determined by MTT (3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) assays. A549 cells were placed in 96-well microassay culture plates (1×10^4 cells per well) and grown for 24 h at 37 °C in a 5% CO_2 incubator. Probe L was then added to the wells to achieve final concentrations of 12.5, 25, 50 and 100 μM. Control wells were prepared by addition of culture medium (100 μM). The plates were incubated at 37 °C in a 5% CO_2 incubator for another 24 h. Upon completion of the incubation, stock MTT dye solution (20 μL, 5 mg/ mL) was added to each well. After 4 h, dimethyl sulfoxide (150 μL) was added to solubilize the MTT formazan. An enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad, Model 550) was used to measure the OD570 (absorbance value) of each well referenced at 655 nm. Each experiment was repeated at least three times to obtain the mean values.