Sensors and Actuators B 224 (2016) 661–667

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A novel solvent-dependently bifunctional NIR absorptive and fluorescent ratiometric probe for detecting Fe^{3+}/Cu^{2+} and its application in bioimaging

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ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form 17 October 2015
Accepted 24 October 2015
Available online 27 October 2015

Keywords:
NIR
Fluorescent probe
Fe^{3+}
Cu^{2+}
Cyanine
Rhodamine

ABSTRACT

A novel rhodamine-cyanine based probe **LS1** was designed and synthesized, which can act as a bifunctional NIR ratiometric colorimetric and fluorescent probe for detecting Fe^{3+} and Cu^{2+} in solvent of MeOH/H_2O and MeCN/H_2O respectively. As expected, it exhibited high selectivity and sensitivity for detecting Fe^{3+} and Cu^{2+} over other commonly coexistent metal ions in their respective systems with a broad pH span. The detection limit was measured to be 0.737 μM for Fe^{3+} and 1.019 μM for Cu^{2+}. Furthermore, fluorescence imaging experiments of Cu^{2+} ions in living SH-SY5Y109 cells demonstrated its value of practical application in biological systems.

1. Introduction

Recently, the design and development of chemosensors for sensing and recognition of environmentally and biologically important heavy- and transition-metal ions has attracted wide-spread interests of biologists, chemists, clinical biochemists and environmentalists [1–5]. Among transition metal ions, iron is the most abundant essential trace element for both plants and animals. Fe^{3+} plays an important role in enzyme catalysis, cellular metabolism, and as an oxygen carrier in hemoglobin and a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain [6]. Both of its deficiency and excess can result in serious disorders such as Huntington [7], Parkinson's disease [8]. And copper, rank only to iron and zinc, is the third most abundant essential trace element in the human body, and performs some important roles in many fundamental physiological processes in organisms [9], but excessive levels of cooper have malign influences [10–12]. Therefore, many excellent works of Fe^{3+} and Cu^{2+} sensing by synthesized colorimetric/fluorescent probes have been reported and investigated [13–19].

Most of these chemosensors have the absorption and emission in the ultraviolet-visible (UV/Vis) light rage, which renders them difficult to be employed for sensing and imaging targets of interest in living lives, as the absorption and auto-fluorescence of biomolecules in UV/Vis region are high [20]. Recently, cyanine dyes, as a long wavelength (NIR region at around 650–900 nm) analyte-responsive fluorescent probes, have become the focus of analytical and biological. As it has minimum photo-damage to biological samples, deep tissue penetration, and minimum interference form background auto-fluorescence by biomolecules in the living systems, cyanine dyes have been well used in vivo fluorescence imaging [21–26]. And as the heptamethine cyanine has a rigid chlorocyclohexenyl ring in the polymethine chain that could increase its photostability, enhance the fluorescence quantum yield, and provide an ideal site for further modification with amino or phenol substitutions, we choose heptamethine cyanine as a fluorophore along with other cyanine analogues. According to our knowledge, only few NIR analyte-responsive probes based on heptamethine cyanine for sensing metal ions have been reported [27–36].

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http://dx.doi.org/10.1016/j.snb.2015.10.086
0925-4005/

However, in the detecting of most probes, the absorbance and fluorescence intensity measurements may be influenced by the variations in the assay sample environment (pH, concentration, temperature, and so forth), due to the only optical signal change. To eliminate those effects, a ratiometric colorimetric/fluorescent measurement is desirable [37–39]. Because ratiometric chemosensors can provide a built-in correction for environmental effects by simultaneous measurement of two fluorescence signals at different wavelengths followed by calculation of their intensity ratio [40,41]. Although some ratiometric chemosensors have been constructed recently, there barely are reports on the ratiometric sensing of metal ions in the near-infrared (NIR) region. Thus, there is still an intense demand for new ratiometric optical probes for Fe^{3+}/Cu^{2+} with favorable photophysical proprieties in the NIR region.

Tuning metal ion selectivity by altering the solvent system is in keep with the newly emerged chemosensor design concept of "single sensor for multiple analytes", namely, analysis of more than one analyte by one receptor using a single or an array of detection method [42–45]. In this paper, we reported a novel probe **LS1**, based on cyanine-coupled rhodamine B, that could detect Fe^{3+} and Cu^{2+} ions in two kinds of solvent systems separately with high selectivity and sensitivity.

2. Experimental

2.1. Apparatus

Absorption spectra were measured on a on a UV-2102 double-beam UV/VIS spectrometer, Perkin Elmer precisely. Fluorescence spectra were recorded on the F-4500 FL Spectrophotometer, and the excitation and emission wavelength band passes were both set at 10.0 nm. The pH was measured with a Model pHs-3C meter (Shanghai, China). ^1H and ^{13}C NMR spectra were recorded using a Bruker DTX-400 spectrometer. Samples were dissolved in $CDCl_3$ and placed in 5 mm NMR tubes. TMS was used as internal reference. ESI mass spectra were carried out on an HPLC Q-Exactive HR-MS spectrometer (Thermo, USA) by using methanol as mobile phase. Fluorescence images experiments were carried out with a Nikon-80i inverted fluorescence microscope.

2.2. Materials

All chemical reagents were used as received from commercial sources without further purification. Solvents for chemical synthesis and analysis were purified according to standard procedures. Deionized water was used throughout the experiment. Chloride salts of metal ions (Li^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Hg^{2+}, Al^{3+}) and the nitrate salts of Ag^+, Pb^{2+} and Fe^{3+} ions were prepared as 10.00 mM in water solution.

2.3. Synthesis

Compound **1** and **2** was synthesized by reported methods (Scheme 1) [46].

Compound **2** (0.61 g, 1 mM) was dissolved in anhydrous ethanol (20 mL) in a round bottom flask, then compound **1** (0.97 g, 2 mM) was added. The mixture was heated to reflux, and stirred under N_2 atmosphere for 8 h. Then the solvent of resulting mixture was evaporated in vacuum. The residue was purified by silica gel thin layer chromatography with EtOAc/MeOH (8:1, v/v) to obtain 0.46 g of dark blue solid in a yield of 43%.

^1H NMR (400 MHz, $CDCl_3$, ppm) δ: 1.14 (t, 12 H, J = 7.0 Hz), 1.30 (t, 2 H, J = 13.6 Hz), 1.52 (s, 12 H), 1.67 (t, 2 H, J = 6.0 Hz), 2.41 (bs, 4 H), 2.90 (t, 2 H, J = 14.0 Hz), 3.08 (s, 1 H), 3.31 (m, 8 H), 3.49 (s, 3 H), 5.19 (d, 2 H, J = 12.4 Hz), 6.18 (d, 1 H, J = 2.4 Hz), 6.20 (d, 1 H, J = 2.4 Hz), 6.35 (d, 2 H, J = 2.3 Hz), 6.43 (d, 2 H, J = 8.8), 6.57 (d, 2 H, J = 7.8 Hz), 6.80 (t, 2 H, J = 7.4 Hz), 7.10 (m, 6 H), 7.40 (t, 2 H, J = 3.5 Hz), 7.91 (t, 1 H, J = 4.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 12.65, 14.12, 21.41, 22.69, 26.33, 28.62, 29.69, 44.34, 44.41, 93.62, 97.87, 97.98, 104.77, 107.49, 108.00, 108.24, 121.63, 121.73, 122.73, 124.08, 127.51, 128.02, 128.28, 128.47, 128.82, 132.92, 139.60, 144.11, 148.88, 148.92, 153.41, 153.77; HR-MS *m/z*: Calcd for $C_{62}H_{71}N_6O_2^+$ $[M-I^-]^+$ 931.5633, found 931.5667.

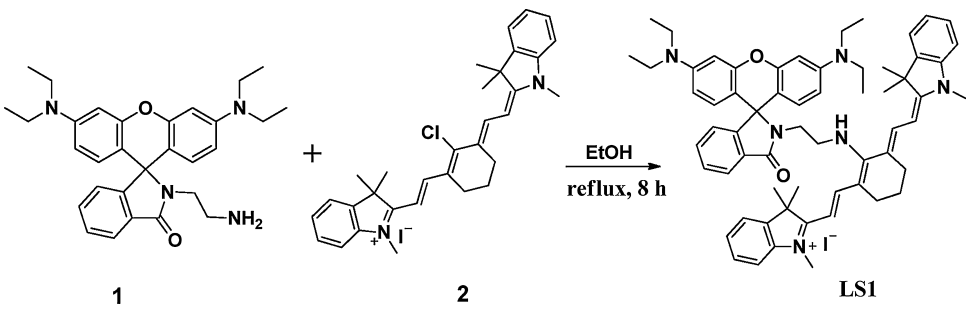
3. Results and analysis

Probe **LS1** was dissolved in MeCN or MeOH to make a 1 mM stock solution, respectively. Then the stock solution was further diluted to require concentration for measurement.

3.1. The detection of Fe^{3+} in H_2O-MeOH solution.

The UV–vis and Fluorescence characteristics of probe **LS1** in a solution of H_2O-MeOH (4:1, v/v) have been studied. As shown in Fig. 1 and Fig. S4, probe **LS1** (10 μM) exhibited obviously selective response to Fe^{3+} among 19 tested metal ions (Li^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Pb^{2+}, Cu^{2+}, Ag^+, Fe^{3+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Hg^{2+}, Al^{3+}). When there were no metal ions being added, the solution of probe **LS1** displayed a single strong absorption band at about 650 nm, which is attributed to the absorption of cyanine moiety. After all the 19 metal ions (100 μM) were added separately into the solution of probe **LS1** and placed for 30 min, only the solution containing Fe^{3+} changed its color from blue to red obviously which allowing naked-eye detection. In the meantime, a large range of hypochromatic shift (136 nm) in absorption spectrum could be observed as the peak decreased at 650 nm and increased at 514 nm, allowing colorimetric detection of Fe^{3+}.

The free probe **LS1** exhibited an emission band at about 777 nm attributed to cyanine moiety and another emission band at about 577 nm attributed to rhodamine moiety (Fig. S4). A prominent enhancement of characteristic fluorescence of rhodamine B emerged at 577 nm after 10 eq. of Fe^{3+} was added into the solution.



Scheme 1. Synthetic route of probe **LS1**.