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Characterization of a highly Cu^{2+}-selective fluorescent probe derived from rhodamine B

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ABSTRACT

A rhodamine B derivative was synthesized and characterized as a highly selective and sensitive probe for Cu^{2+} in ethanol–water solution (2:3, v:v, pH7.4, 50 mM HEPES). A prominent fluorescence enhancement at 575 nm was observed in the presence of Cu^{2+}, accompanied by the change in the absorption spectrum. Under the optimal conditions, a good linear range of 0.5–1.5 μM with a detection limit of 1.6×10^{-7} M were obtained. Furthermore, confocal laser scanning microscopy experiments have proven that this probe is cell-permeable and can respond to changes in intracellular Cu^{2+} in living cells.

1. Introduction

Fluorescence spectroscopy offers a powerful tool for sensing and imaging trace amounts of species by virtue of its simplicity, high sensitivity and selectivity, and instantaneous response [1–3]. In particular, selective heavy metal ions (HTM) recognition by fluorescence probes has attracted increasing interest in biological and environmental chemistry. Among the HTM, Cu^{2+} plays an important role in living systems and has an extremely ecotoxicological impact on the human health result from its catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome *c* oxidase and tyrosinase [4]. However, Cu^{2+} exhibits toxicity under overloading conditions in that it causes neurodegenerative diseases, probably by its involvement in the production of reactive oxygen species [4]. Owing to the biological significance of Cu^{2+}, a considerable effort has been devoted to the development of the efficient methods to detect Cu^{2+}, and many studies focus on the design of fluorescent probes for the analysis of Cu^{2+} have been reported [5–12]. Whereas most of the reported Cu^{2+} fluorescent probes show "on-off" signal upon the binding of Cu^{2+} due to its paramagnetic nature [5–8], which is not as sensitive as a fluorescence enhancement response [9–12]. Therefore, the development of highly sensitive and selective "off–on" fluorescent probes for Cu^{2+} is still significant.

Based on our previous research [9–14], it is necessary to choose an efficient fluorophore in the design of fluorescent probes. Rhodamine derivative is one of the most useful fluorophores for the construction of artificial fluorescent probes owing to its excellent photophysical properties [15]. In the light of the equilibrium between the spirolactam (non-fluorescence) and the ring-opened amide (fluorescence) of rhodamine derivatives, rhodamine-based probes are ideal modes for in vitro detection and in vivo imaging [16–21]. In addition, the receptor should be preliminarily considered because it is responsible for the selectivity and binding efficiency of the whole probes. According to the Soft-Hard Acid-Base theory, the probes attached the recognition moiety with N and O atoms could show good affinity to Cu^{2+}. Keeping this in mind, a new probe **L** was designed and synthesized as a novel cell membrance-permeable, Cu^{2+}-selective probe in aqueous media and living cells. (Scheme 1).

2. Experimental

2.1. Reagents and instruments

All chemicals were used are of analytical grade or of the highest purity available. Rhodamine B and Hydrazine hydrate (100%) were purchased from Sigma–Aldrich. Glyoxal and other reagents were

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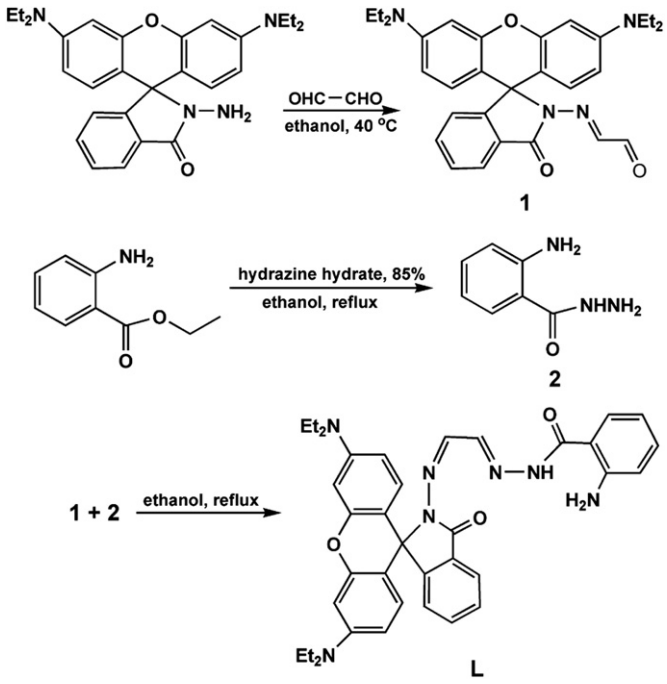

Scheme 1. The synthesis route of probe **L**.

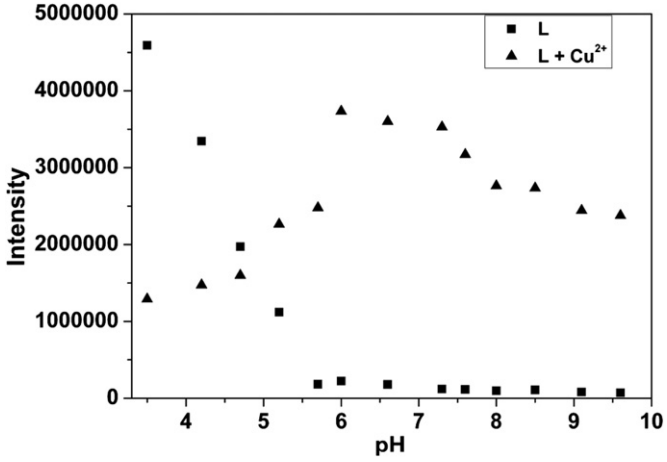

Fig. 1. Influences of pH on the fluorescence spetra of **L** (2 μM) (■) and **L** (2 μM) plus Cu^{2+} (50 μM) (▲) in ethanol–water solution (2:3, v:v). The pH was modulated by adding 1 M HCl or 1 M NaOH in HEPES buffers.

obtained from Shanghai Reagent Company. All solutions were prepared with double–distilled water.

Melting points were determined using a Shanghai Melting points WRS–1B apparatus. NMR spectra were measured with a Brucker WM–500 spectrometer, using TMS as an internal standard. The pH measurements were carried out on a PHS-3C meter. Mass spectra were performed on a Thermo TSQ Quantum Mass Spectrometer. Fluorescence emission spectra were conducted on a HORIBA Fluoromax-4 spectrofluometer. UV–Vis spectra were obtained on a Beckman DU–800 spectrophotometer. Fluorescence imaging was performed by confocal fluorescence microscopy on an Olympus FluoView Fv1000 laser scanning microscope.

2.2. Synthetic procedure

Compounds **1** was synthesized as reported method [21].

The synthesis of compound **2**: Compound **2** was synthesized as reported procedure with some modification [22]. Under N_2 atmosphere, ethyl 2-aminobenzoate (1.0 mmol, 0.165 g) and hydrazine hydrate (12.0 mmol, 0.6 mL) were mixed in 30 mL ethanol. The mixture was refluxed for 6 h. After the reaction was finished, the solvent was removed under reduced pressure, and then 50 mL petroleum ether was added to the oily residue, and the precipitate so produced was filtered and used directly. Yields: 87.2%. M.p.: 152.0–153.1 °C. MS: *m/z* 152.09 $[M + H]^+$.

The synthesis of fluorescent probe **L**: Compound **1** (0.496 g, 1.0 mmol) and compound **2** (0.181 g, 1.2 mmol) were mixed in 30 mL ethanol and refluxed for 4 h. After cooling to room temperature, the precipitate so obtained was washed with water and ethanol, and then dried in vacuum. The **L** was obtained by recrystallization with ethanol as pale yellow solid. Yields: 85.6%. ^1H NMR (d_6-DMSO, δ ppm): 11.64 (s, 1H), 7.99 (d, 1H, $J = 8.10$), 7.42 (d, 1H, $J = 7.45$), 7.82 (d, 1H, $J = 8.20$), 7.60 (t, 1H, $J = 7.47$), 7.54 (t, 1H, $J = 7.26$), 7.43 (d, 1H, $J = 7.85$), 7.18 (t, 1H, $J = 7.67$), 7.45 (d, 1H, $J = 7.55$), 6.02 (d, 1H, $J = 8.05$), 6.53 (t, 1H, $J = 7.87$), 6.45 (s, 2H), 6.41 (s, 2H), 6.36 (d, 2H, $J = 9.00$), 6.33 (b, 2H), 3.32 (m, 8H, $J = 6.95$), 1.09 (t, 12H, $J = 6.97$). ^{13}C NMR (d_6-DMSO, δ ppm): 165.74, 164.71 (C=O), 162.76, 153.51, 152.64, 152.54, 152.34, 150.62, 149.13, 148.60, 146.02, 144.08, 134.91, 133.07, 132.84, 130.10, 129.27, 128.80, 128.58, 128.16, 127.76, 127.50, 124.11 (ArC), 123.97, 123.73 (C=N), 116.86, 115.08, 113.25, 108.67, 108.28, 105.95, 104.83, 97.97, 97.92 (ArC), 65.23, 44.12, 12.89. MS (ESI) *m/z*: 630.4 $[M + H]^+$.

2.3. General spectroscopic methods

Metal ions, anions and fluorescent probe **L** were dissolved in deionized water and DMSO to obtain 1.0 mM stock solutions,

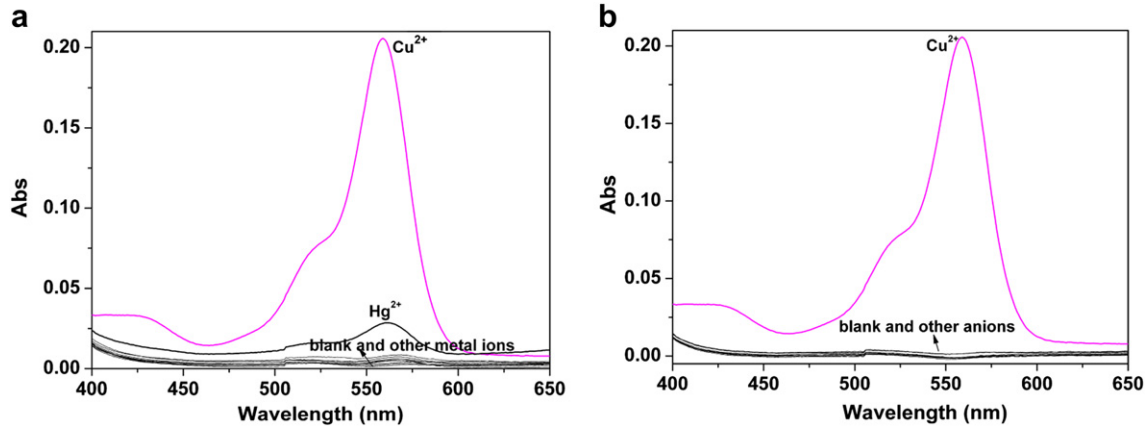

Fig. 2. (a) UV–vis spectra of **L** (2 μM) with different metal ions (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4). (b) UV–vis spectra of **L** (2 μM) with different anions (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4).