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Original article

A fluorescent probe for Hg^{2+} sensing in solutions and living cells with a wide working pH range

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ABSTRACT

In this paper, 2-carboxybenzaldehyde rhodamine B thiohydrazine (**1**) was synthesized and developed as a fluorescent probe to recognize Hg^{2+} in DMF/H_2O (1:9, v/v) solution with high selectivity. The probe can be applied to the quantification of Hg^{2+} with a linear concentration range covering from 1.0×10^{-7} mol/L to 1.0×10^{-5} mol/L ($R^2 = 0.9985$) and a detection limit of 4.2×10^{-8} mol/L. The experiment results show that the response of probe **1** to Hg^{2+} is pH-independent in a wide range from 4.0 to 9.0. Moreover, the probe **1** exhibits excellent selectivity toward Hg^{2+} over other common metal cations. Most importantly, the probe can be employed to monitor Hg^{2+} in living cells using fluorescent imaging technique with satisfied results.

1. Introduction

Design and synthesis of artificial fluorescent probes for selective and sensitive quantification of metal ions has attracted widespread interest from chemists, biologists, clinical biochemists and environmentalists in recent years [1]. Among heavy metals, mercury has been considered as the most toxic and dangerous element due to its high affinity for the thio group in proteins and enzymes, leading to the dysfunction of cells and consequently causing many health problems [2]. Thus, searching for efficient analytical methods for monitoring Hg^{2+} with high selectivity and rapid response in a wide pH range is of great importance for the environment and human health.

Compared with various traditional analytical techniques for Hg^{2+} quantification, fluorescent methods are preferred due to their high sensitivity, fast analysis with spatial resolution, and non sample/cell-destructive nature [3]. Many efforts in the past decade have been made to develop novel fluorescent probes for Hg^{2+} by utilizing various fluorophores as signal reporters [4]. Among them, rhodamine is an ideal fluorophore to construct high signal-noise ratio fluorescent probes. This is because its spirolactam derivative is an excellent platform on which to construct a fluorescent-enhancement probe that is almost colorless, has almost zero background fluorescent signal, and can be converted to a ring-opening form with pink color and strong fluorescence upon addition of analytes. Inspired by this unique feature, considerable attention has been focused on rhodamine spirolactam-based fluorescent probes for various metal ions (including Hg^{2+}) and other biologically important species in recent years [5]. Kim *et al.* developed a novel rhodamine-based tris(2-aminoethyl)-amine with tosyl groups Hg^{2+} probe. The optimized conditions of Hg^{2+} determination were 90% CH_3CN aqueous solution with a pH spanning 3–6 [6]. Lin and co-workers have employed a rhodamine–thioamide–alkyne scaffold as a highly selective and sensitive fluorescent probe for Hg^{2+} imaging in live cells, but the probe can detect Hg^{2+} only in the pH 6.0–7.4 range [7]. Zheng and co-workers utilized rhodamine B thiohydrazide for highly selective and sensitive colorimetric and fluorescent detection of Hg^{2+} in pH 3.4 [8]. For practical applications of Hg^{2+} determination, though, probes must be highly effective in a wider pH range and under physiological conditions. To the best of our knowledge, there are only few rhodamine-based probes which can respond to Hg^{2+} in wide pH range [9].

To improve effective pH range, chelating ability and water solubility, a novel rhodamine-based Schiff base, 2-carboxybenzaldehyde rhodamine B thiohydrazine (**1**), was designed, synthesized,

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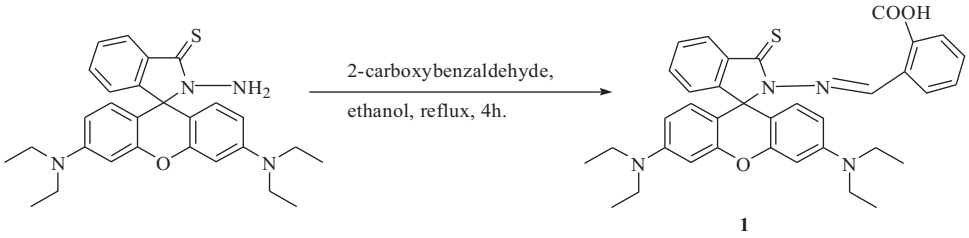

Scheme 1. Synthetic pathway of probe **1**.

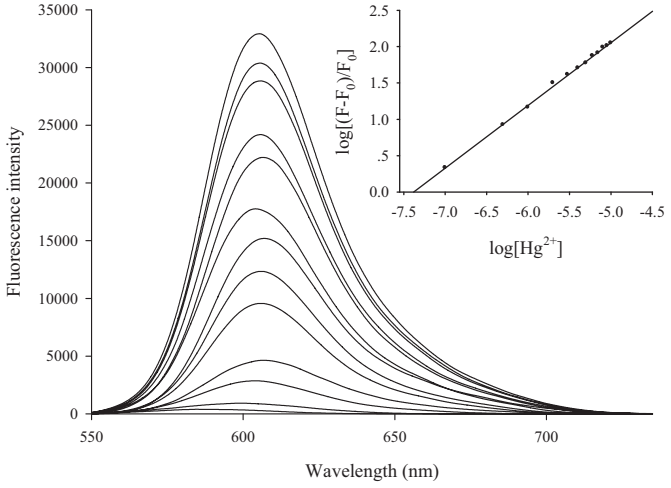

Fig. 1. Fluorescence spectra of probe **1** (10 μmol/L) upon titration of Hg^{2+} (from bottom to top: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μmol/L) in the test solutions. Inset: plot of $\log[(F - F_0)/F_0]$ as function of $\log[Hg^{2+}]$, F is the fluorescence intensity at 606 nm.

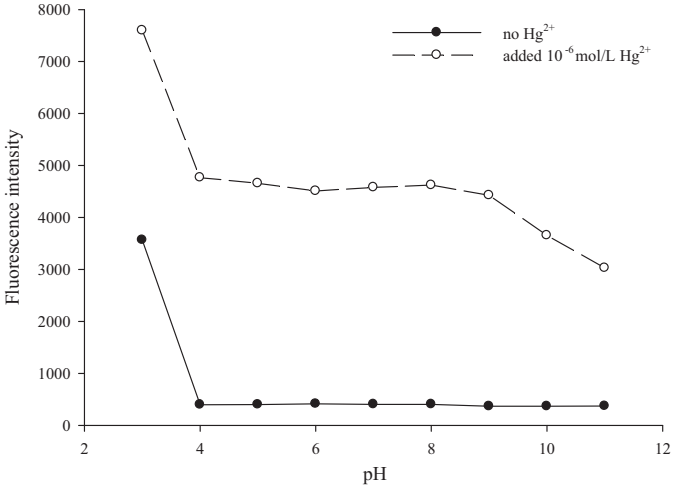

Fig. 3. Effect of pH on the fluorescence intensity of 10 μmol/L probe **1** in the absence (solid line) and presence (dashed line) of 1 μmol/L Hg^{2+}.

and employed as a colorimetric and fluorescent Hg^{2+} probe in aqueous solutions and living cells.

2. Experimental

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 spectrometer operating at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal reference. All chemical shifts are reported in the standard δ notation of parts per million. LC–MS and HRMS analyses were performed using an LXQ Spectrometer (Thermo Scientific, USA) and an LCQ Deca XP MAX spectrometer (Thermo Scientific, USA) operating on ESI, respectively. UV–vis absorption spectra were recorded with a Shimadzu UV-2400 spectrophotometer. All fluorescence measurements were conducted on a Thermo Scientific Lumina fluorescence spectrometer with excitation slit set at 5.0 nm and emission at 5.0 nm. Fluorescence images of HepG2 cells were carried out with an inverted fluorescence microscope (Carl Zeiss, Axio Observer

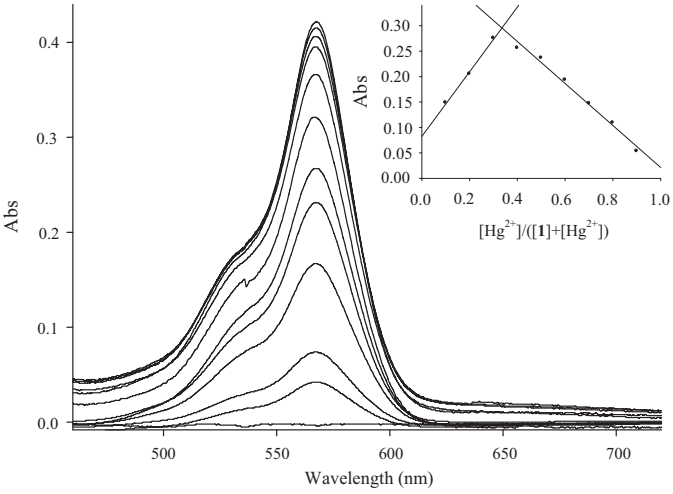

Fig. 2. Absorption spectra of probe **1** (10 μmol/L) upon titration of Hg^{2+} (from bottom to top: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μmol/L) in the test solutions. Inset: Job's plot for probe **1** in the test systems. The total concentration of **1** and Hg^{2+} was 20 μmol/L.

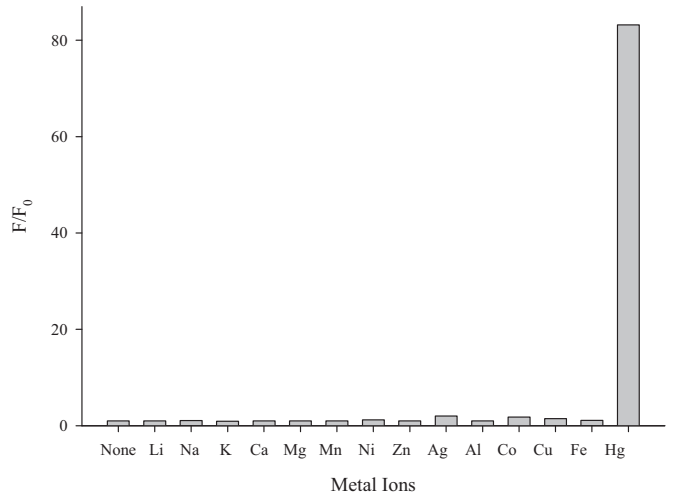

Fig. 4. Fluorescence responses of 10 μmol/L probe **1** to 10 μmol/L selected metal ions in the test systems. Bars represent the emission intensity ratios after (F) and before (F_0) addition of each metal ions.