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A fluorescent "turn-on" probe for the dual-channel detection of Hg(II) and Mg(II) and its application of imaging in living cells

Yan Zhao[a], Baozhan Zheng[a], Juan Du[a,*], Dan Xiao[a,b,**], Li Yang[a]

[a] College of Chemistry, Sichuan University, Chengdu 610064, PR China
[b] College of Chemical Engineering, Sichuan University, Chengdu 610065, PR China

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ABSTRACT

A novel rhodamine-based fluorescent chemosensor (RND) was synthesized that contains two independent fluorophores and acts as a very sensitive, selective and reversible off–on probe for Hg(II). Importantly, this newly developed sensing system exhibited different fluorescent responses toward Hg(II) and Mg(II) at 589 nm and 523 nm, respectively. RND also displayed detectable color change upon treatment with Hg(II). Results from confocal laser scanning microscopy experiments demonstrated that this chemosensor is cell permeable and can be used as a fluorescent probe for monitoring Hg(II) or Mg(II) in living cells. This probe can also indirectly detect glutathione (GSH) and cysteine (Cys) with good linear relationships.

1. Introduction

Mercury is a major environmental and health concern as a result of its toxicity in living systems [1,2], and considerable efforts have been devoted to its detection. Despite a reduction in its industrial use as a result of stricter regulations, high concentrations of mercury are still present in many environmental areas. Therefore, the effective and selective detection of mercury is of great significance for biochemistry, environmental science and medicine [3,4]. Of the many modes of detection available, fluorescence-based methods have attracted much attention because of their simplicity, selectivity, high sensitivity, adaptability and online imaging capabilities [5–7]. Hg(II) is a heavy metal ion that is recognized as a fluorescence quencher due to the enhancement of spin–orbit coupling commonly associated with the heavy atom effect [8,9]. To date, many reported fluorescence chemosensors based on the complexation-induced fluorescence quenching mechanism [10–12] have led to the nonspecific identification of Hg(II) against a background of competing metal ions and a lower detection limit compared to the fluorescence enhancing mechanism. Therefore, it would be advantageous to design fluorescence sensors that can turn on and provide a specific response following Hg(II) recognition [13–15]. Another cation, Mg(II), is the most abundant divalent cation in cells, and it plays a critical role as an enzyme cofactor in DNA synthesis [16,17] and protein phosphorylation [18]. Recent findings confirmed that Mg(II) is a crucial modulator of cell function and that knockdown of genes encoding for magnesium transporters leads to cell death [19,20]. Mg(II) has long been regarded as a chronic regulator of cell function, but the development of Mg(II)-specific probes has not attracted much attention. However, the development of fluorescence imaging techniques can also be considered for use in Mg(II) research.

Biothiols, such as glutathione (GSH), homocysteine (Hcy), and cysteine (Cys), are critical physiological components and are extensively present in animal tissues and fluids [21,22]. They play extremely significant roles in metabolism and homeostasis. Thus, the rapid, sensitive and selective detection of biothiols is of considerable importance and interest.

Because of the outstanding spectroscopic properties [23] and binding-promoted fluorescence-enhancing process of rhodamine-based dyes [24–26], a new rhodamine derivative (RND) (as shown in Scheme 1) was designed and synthesized as a fluorescent turn-on probe for Hg(II) in two steps. The original purpose of introducing 2-hydroxy-1-naphthaldehyde into rhodamine B was to provide a suitable binding site, therefore improving the ability of complexation. Based on experimental results, it was reasonably hypothesized that the 2-hydroxy-1-naphthaldehyde group itself might also act as a Mg(II) detector through a fluorescent "turn-on" mechanism.

Rhodamine-based chemosensors have been widely used for detecting intracellular analytes [27,28]; therefore, we speculated that RND may be applicable for imaging Hg(II) in living cells. However, the strong thiophilic nature of Hg(II) [29–31] may cause

* Corresponding author. Tel.: +86 028 85415029; fax: +86 028 85416029.
** Corresponding author at: College of Chemistry, Sichuan University, Chengdu 610064, PR China. Tel.: +86 028 85415029; fax: +86 028 85416029.
E-mail addresses: lxdj@vip.sina.com (J. Du), xiaodan@scu.edu.cn (D. Xiao).

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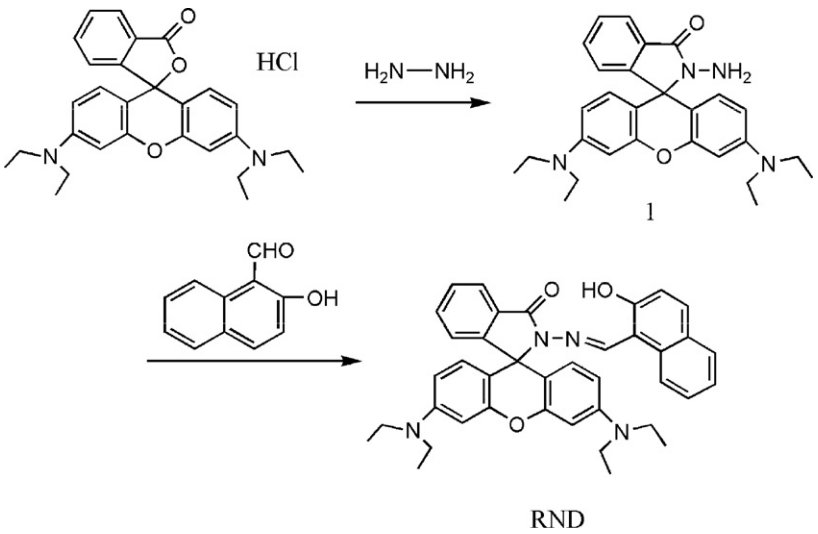

Scheme 1. Synthesis of RND.

formation of Hg–S bonds in the presence of biothiols, such as GSH and Cys. As a result, the fluorescence of RND–Hg(II) could be quenched following the addition of biothiols. Thus, the double detection of Hg(II) and biothiols based on the same fluorescent probe could be realized.

A modified previously reported procedure [32,33] was employed for the synthesis of RND. RND was synthesized from the reaction of rhodamine B and hydrazine, followed by reaction with 2-hydroxy-1-naphthaldehyde for a relatively high yield. The detailed experimental procedures are described in Section 2. The product was dissolved in 1:1 H_2O/CH_3CN (v/v), and the structure of the RND was characterized by ^1H NMR and MALDI-TOF MS (Figs. S1 and S2).

2. Experimental

2.1. Apparatus

^1H NMR measurements were performed with a Bruker AV II-600 MHz spectrometer. Fluorescence spectra were measured on a Hitachi F-4500 spectrophotometer equipped with a 1 cm quartz cell. UV–visible spectra were acquired on a Techcomp UV1100 spectrophotometer (Shanghai, China). Mass spectra were obtained with a Bruker Autoflex MALDI-TOF MS at Hong Kong Baptist University.

2.2. Chemicals

Rhodamine B was purchased from Beijing Chemical Co. Glutathione (GSH) and L-cysteine (Cys) were purchased from Aldrich Chemical Co. Hydrazine hydrate and 2-hydroxy-1-naphthaldehyde were purchased from Alfa Aesar. All solvents used for synthesis and measurements were redistilled before use. All other chemicals were of analytical-reagent grade and were used without further purification.

2.3. Cell cultures and cell labeling

Hela cells were provided by the School of Life Science at Sichuan University (Sichuan, China). Confocal fluorescence imaging was performed with a Leica TCS SP5 laser scanning confocal microscope (excitation wavelengths 543 and 403 nm). Before the experiments, the Hela cells were exposed to 100 μmol L^{-1} RND for 30 min at room temperature to allow the probe to permeate into the cells. The cells were then centrifuged to remove excess sensor, and the treated cells were then incubated with 100 μmol L^{-1} $Hg(NO_3)_2$ or 100 μmol L^{-1} $Mg(NO_3)_2$ for another 30 min. Following incubation, the cells were imaged.

2.4. Synthesis of intermediates and probes

2.4.1. Synthesis of compound *1*

For compound **1**, 2.0 mL of 98% hydrazine hydrate was added drop wise to 30 mL of vigorously stirred 2.5 mmol L^{-1} rhodamine B in ethanol at room temperature. The solution was then refluxed for 3 h. The reaction mixture was subsequently cooled, and the solvent was removed under reduced pressure with a rotary evaporator. Next, 1 mol L^{-1} HCl was added until the solution became clear. Then, 1 mol L^{-1} NaOH was slowly added with stirring until the solution pH reached 9–10. The precipitate was filtered, washed with water and dried under reduced pressure to afford compound **1** (0.83 g, yield: 70%) as a light pink powder.

2.4.2. Synthesis of RND

Compound **1** (0.46 g) was dissolved in absolute ethanol to a concentration of 1 mmol L^{-1}. An excess of 2-hydroxy-1-naphthaldehyde (0.26 g, 1.5 mmol L^{-1}) was then added, Next, the mixture was refluxed for approximately 8 h, and TLC demonstrated that the reaction was complete. The solution was then concentrated and allowed to stand at room temperature overnight. The precipitate was filtered the following day, washed several times with cold ethanol, recrystallized from absolute ethanol and dried under reduced pressure to afford RND (0.55 g, yield: 90%) as a light yellow solid.

Using ^1H NMR (600 MHz, D_6-DMSO, ppm), the following chemical shifts were recorded: δ 1.05(12H, t), 3.30(8H, q), 6.37(2H, dd), 6.49(4H, d), 7.10(1H, d), 7.18(1H, d), 7.35(1H, t), 7.46(1H, t), 7.62(1H, t), 7.66(1H, t), 7.84(3H, m), 7.97(1H, d), 9.53(1H, s), and 11.92(1H, s). MALDI-TOF mass spectrometry showed a peak with m/z 611.3 ($M+H^+$).

3. Results and discussion

To examine the binding properties of RND with metal ions, the colorimetric and fluorescent responses of RND to various metal ions of interest, including Mg(II), Ba(II), Cd(II), Hg(II), Pb(II), Zn(II), Ni(II), Mn(II) and Cu(II), were investigated.