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A New Fluorescent Chemodosimeter for Hg^{2+}: Selectivity, Sensitivity, and Resistance to Cys and GSH

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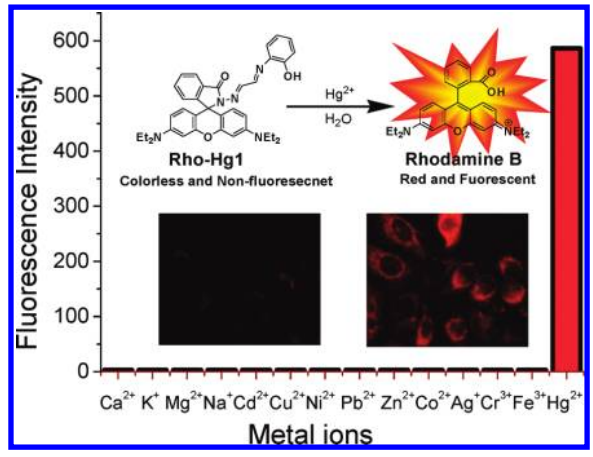
ABSTRACT



On the basis of the mechanism of Hg^{2+}-promoted hydrolysis, a new fluorescent chemodosimeter (Rho-Hg1) is reported for single-selective and parts per billion level-sensitive detection of Hg^{2+} in natural waters. Moreover, the fluorescence response of Rho-Hg1 to Hg^{2+} has little interference from sulfur compounds such as cysteine and glutathione and could be used in the Hg^{2+} imaging in living cells.

Up to the present, many good fluorescent probes for multifarious targets have been described.[1] Recently, as the demand of sensitivity and high selectivity is continuously increasing, some fascinating chemodosimeters have been designed to detect analyte utilizing chemical events. They have played significant roles in the detection of ion and small molecules, especially in monitoring heavy and transition metal ions.[2] Mercury is one of the most toxic and dangerous heavy metal elements because of its high affinity for thiol groups in proteins and enzymes, leading to the dysfunction of cells and consequently causing many health problems in the brain, kidney, and central nervous system.[3] Thus, concerns over toxic damage of mercury provide motivation to explore selective reactions to monitor Hg^{2+} in biological and environmental samples.

At present, most of the reported chemodosimeters for Hg^{2+} are based on thiophilic specialty.[4] However, mercury is abundant in the sulfur-rich environments.[5] Unfortunately, few literatures discuss how to avoid the potential interference from mercapto-chemicals in organism and sulfur-rich environments

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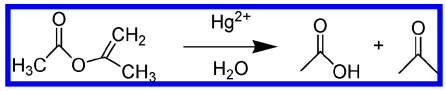
in the detection of Hg^{2+} until the recent report from Koide who developed a chemodosimeter based on Hg-catalyzed hydration of alkynes into ketones at 90 °C.[6] The high temperature, however, could not be used in thermosensitive systems such as living cells and other biosamples. Therefore, mild reactions for selective and sensitive Hg^{2+}-signaling without interference from organo-sulfide are very important.

It is known that Hg^{2+} promoted an irreversible hydrolysis of isopropenyl acetate in mild conditions[7] (Scheme 1), and

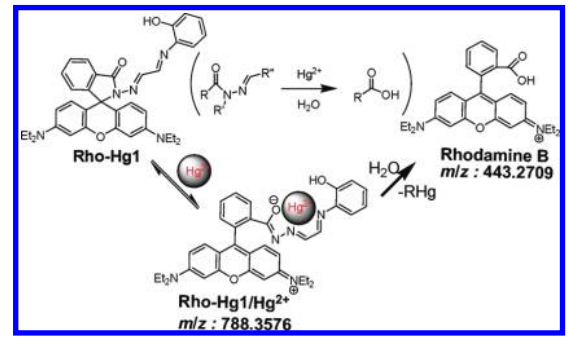
Scheme 1. Mercury-Promoted Hydrolysis of Isopropenyl Acetate



rhodamines undergo a great fluorescence enhancement via their structure change from spirocyclic (nonfluorescent and colorless) to ring-open (fluorescent and colorated) states induced by specific chemical environments at room temperature.[8] We envisioned that combining the spirolactam ring opening of rhodamine derivatives with the mild Hg^{2+}-promoted hydrolysis reaction of isopropenyl acetate would serve as the fundamental reactions for a novel chemodosimeter for Hg^{2+}.

Herein, a new rhodamine-based fluorescent Hg^{2+} chemodosimeter **Rho-Hg1** is presented, which has a similar structure with isopropenyl acetate (Scheme 2). It was facilely

Scheme 2. Hg^{2+}-Promoted Ring Opening and Hydrolysis of **Rho-Hg1**



synthesized from Rhodamine B by a three-step procedure in a total yield of 45.6% (Scheme S1 in Supporting Information (SI)). We anticipated that it would undergo hydrolysis reaction when a similar but modified molecular moiety of isopropenyl acetate was liberated by Hg^{2+}-facilitated ring opening of the spirocycle group (Scheme 2). Here, Hg^{2+} acts not only as an analyte but also as the promoter for the hydrolytic reaction. Compared with other Hg^{2+} fluorescent probes, **Rho-Hg1** shows two advantages: first, **Rho-Hg1** has single selectivity and parts per billion (ppb) level sensitivity for Hg^{2+} detection in natural waters at room temperature; second, **Rho-Hg1** realizes the avoidance of potential interference from sulfide such as cysteine and glutathione in the detection of Hg^{2+} and then is successfully applied in the fluorescence imaging of Hg^{2+} in living cells.

To explore the mechanism, **Rho-Hg1**/Hg^{2+} complexes were detected by the ESI-TOF high resolving mass spectrum analysis. As shown in Scheme 2, a peak at *m/z* 788.3576 corresponding to [**Rho-Hg1** + Hg^{2+} − H^+]$^+$ was observed after the addition of $Hg(ClO_4)_2$ to the ethanol solution of **Rho-Hg1** (Figure S2, SI). At the same time, the complex exhibited obvious fluorescence emission at 580 nm and color of amaranth with absorption at 554 (Table S1 and Figure S1, SI). After addition of water (ethanol/water, 1/1, v/v), the peak at *m/z* 788.3576 disappeared, and then a new peak of *m/z* 443.2709 appeared which was confirmed as Rhodamin B (Figure S2, SI). In the absence of Hg^{2+}, **Rho-Hg1** is colorless and nonfluorescent due to the closed spirolactam ring. In the presence of Hg^{2+}, the complex of **Rho-Hg1** − Hg^{2+} forms in the first equilibrium resulting in ring opening, and then the complex further hydrolyzed to brilliant pink and strong fluorescent Rhodamine B in the presence of water.

An ethanol/water (1/1, v/v, pH 7.0) solution was selected as a testing system to investigate the chemical response of **Rho-Hg1** to Hg^{2+} at room temperature. A time course study revealed the recognizing event could complete in 8 min (Figure S3, SI).

One challenge for the Hg^{2+} probe is to obtain systems that are selective to Hg^{2+} over a wide range of potentially competing ions, such as alkali or alkaline-earth metals (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and heavy and transition metal ions (Co^{2+}, Cr^{3+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ag^+, Fe^{3+}, and Cu^{2+}).

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The **Rho-Hg1** exhibited no obvious fluorescence and absorption because it mostly exists in the spirocyclic form. Only after addition of Hg^{2+}, the intensity of fluorescence emission was significantly enhanced by over 370-fold at 579 nm with a quantum yield of 0.75 (Figure 1a,b), and also, an absorption

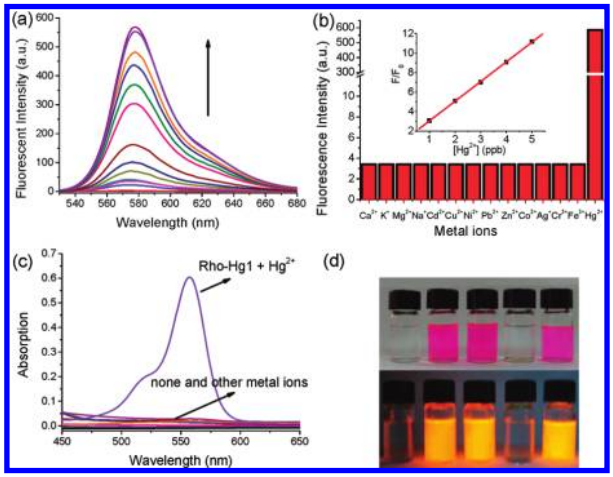

Figure 1. (a) Emission spectra of **Rho-Hg1** (5 μM) in the presence of increasing concentrations of Hg^{2+} (0, 15, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80 μM) in aqueous solution (ethanol/water = 1/1, v/v). (b) The fluorescence intensity at 580 nm of **Rho-Hg1** (5 μM each) in the presence of different metal ions (Hg^{2+} is 75 μM and other metal ions are 250 μM: Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Ag^{+}, Cr^{3+}, and Fe^{3+}). Inset: the changes of fluorescence intensity of **Rho-Hg1** (5 μM) upon addition of Hg^{2+} (1−5 ppb). (c) The absorption spectra of **Rho-Hg1** (5 μM) in the absence and presence of different metal ions (mentioned above). (d) Color (up) and fluorescence (down) change (from left to right: **Rho-Hg1** only; **Rho-Hg1** + Hg^{2+}; **Rho-Hg1** + Hg^{2+} + X; **Rho-Hg1** + X; Rhodamine B + Hg^{2+}; X denotes miscellaneous cations mentioned above and anions including NO_3^-, SO_4^-, ClO_4^-, CO_3^{2-}, Cl^-, $H_2PO_4^-$, SCN^-, and CH_3COO^-).

band centered at 554 nm appeared obviously (Figure 1c). The competition experiments revealed that the Hg^{2+}-induced fluorescence and absorption response were unaffected in the background of metal ions mentioned above and the anions including NO_3^-, SO_4^{2-}, ClO_4^-, CO_3^{2-}, Cl^-, $H_2PO_4^-$, SCN^-, and CH_3COO^- (Figure 1d).

The U.S. EPA (Environmental Protection Agency) standard for the maximum allowable level of inorganic Hg in drinking water is no more than 2 ppb.[9] When **Rho-Hg1** was added to a solution containing 2 ppb of Hg^{2+}, fluorescence enhanced nearly 400%, and the F/F_0 was proportional to the amount of Hg^{2+} added in ppb level with a detection limit of 0.91 ppb[10] (Figure 1b inset).

Normally, the spirolactam ring of the rhodamine derivative is open in acidic media and then shows the fluorescence of rhodamine. To apply **Rho-Hg1** in more complicated systems, like in organism and environment, the fluorescence responses

(9) Mercury Update: Impact on Fish Advisories. *EPA Fact Sheet EPA-823-F-01-011*; EPA, Office of Water: Washington, DC, 2001.

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of **Rho-Hg1** in the absence and presence of Hg^{2+} in different pH values were evaluated (Figure 2). The pK_a of **Rho-Hg1**

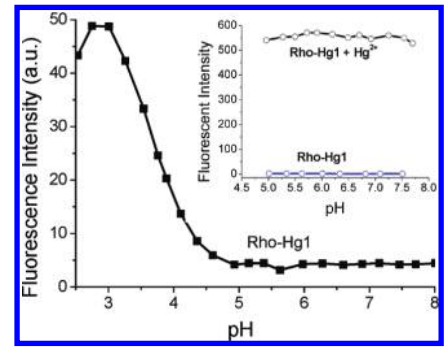

Figure 2. Effect of pH on the fluorescence intensity of **Rho-Hg1** (5 μM) and **Rho-Hg1**/Hg^{2+} (5 μM/75 μM) in the aqueous solution (ethanol/water = 1/1, v/v, pH 7.0). Excitation wavelength is 510 nm.

alone is 3.68 from the sigmoidal curve. In the pH range from 5.0 to 8.0, **Rho-Hg1** can respond to Hg^{2+} without any interference by protons (Figure 2 inset).

Generally, one of the most important and useful applications for a fluorescent probe is the detection of Hg^{2+} in natural water samples. Owing to its excellent spectroscopic properties, **Rho-Hg1** is sensitive enough to detect relevant concentrations of Hg^{2+} in natural water samples. We chose natural water samples from three different sources: seawater from Yellow Sea (Dalian), pool water, and tap water (in campus). The U.S. EPA standard for the limit of inorganic Hg in drinking water is 2 ppb. As shown in Figure 3 and

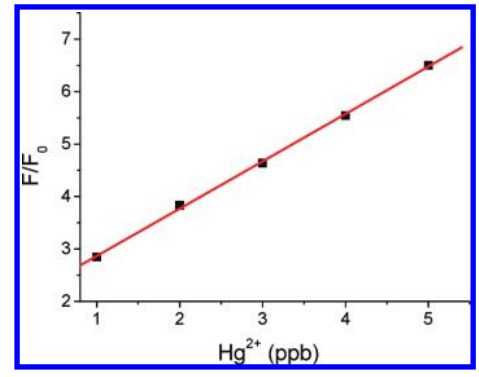

Figure 3. Linear fluorescence enhancement (F/F_0) of **Rho-Hg1** (1 μM) upon addition of Hg^{2+} (1−5 ppb) to seawater. The response (F) is normalized to the emission of the free **Rho-Hg1** (F_0). The samples were excited at 510 nm, and the emission intensities were recorded at 580 nm.

Figures S4 and 5 (SI), about 2.8-fold, 1.7-fold, and 1.5-fold enhancement of fluorescence intensity were displayed when 2 ppb of Hg^{2+} was added into those waters with **Rho-Hg1**. Furthermore, the F/F_0 is well proportional to the amount of Hg^{2+} (1−5 ppb). The result shows that **Rho-Hg1** is capable

of distinguishing between the safe and toxic levels of Hg^{2+} in more complicated natural water systems.

It is known that there are some important mercapto biomolecules in organisms, such as cysteine, homocysteine, and glutathione,[11] which could interact with Hg^{2+}. Therefore, for more practical application, we investigated the effect of cysteine and glutathione on the detection of Hg^{2+} in ethanol/HEPES buffer solutions (1/1, v/v, pH 7.0, 50 mM). As shown in Figure 4, the addition of cysteine (100 μM) could induce

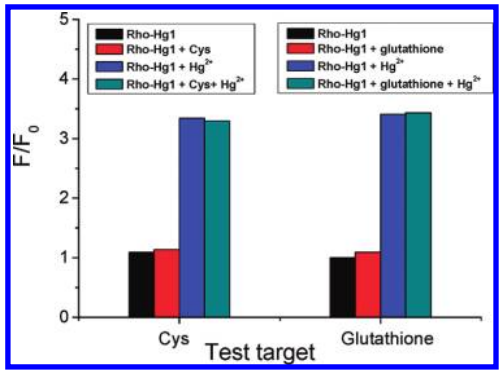

Figure 4. Hg^{2+} (2 μM) detection in the presence of L-cysteine and glutathione (100 μM) in ethanol/HEPES buffer (1/1, v/v, pH 7.0, 50 mM), [**Rho-Hg1**] = 0.3 μM. Excitation wavelength is 510 nm.

little fluorescence change (red bar) compared with **Rho-Hg1** (black bar); when Hg^{2+} (2 μM) is added into the mixed solution of **Rho-Hg1** (0.3 μM) and cysteine (100 μM), the obvious fluorescence enhancement (green bar) was observed, which is very close to the enhancement induced by Hg^{2+} only (blue bar). There is a similar result in the glutathione testing. Therefore, **Rho-Hg1** could detect Hg^{2+} in the sulfur-rich environment insusceptibly.

Cultured Hela cells were incubated with **Rho-Hg1** in culture medium for 30 min at 37 °C, and very weak fluorescence of **Rho-Hg1** inside the living Hela cells was observed (Figure 5a).

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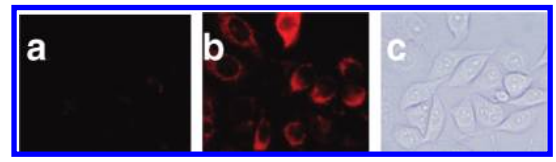

Figure 5. Fluorescence image of Hela cells incubated with 10 μM **Rho-Hg1** for 30 min (a) and then further incubated with 10 μM Hg^{2+} for 30 min (b). (c) Bright-field transmission image of cells in (b). The excited light is WB510-570 nm (Nikon eclipase TE 2000-5, 32 × objective lens).

After three times washing with PBS buffer, the cells were incubated with Hg^{2+} (10 μM) in the medium for another 30 min at 37 °C, and the fluorescence in living cells was much brighter (Figure 5b). A bright-field transmission image of cells treated with **Rho-Hg1** and Hg^{2+}confirmed that the cells were viable throughout the imaging experiments (Figure 5c). It is proved that **Rho-Hg1** is cell-permeable and primarily nontoxic to the cell culture.

In summary, a highly selective chemodosimer **Rho-Hg1** for Hg^{2+} based on hydrolysis was reported. It exhibits ultra sensitivity for Hg^{2+} detection in natural waters, even in the presence of cysteine and glutathione. Moreover, fluorescence imaging for Hg^{2+} in living cells shows **Rho-Hg1** has ideal chemical and spectroscopic properties that satisfy the criteria for further biological applications.

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Supporting Information Available: Experimental procedure, structural data, spectral property in ethanol, time course, TOF-MS data, and the testings for Hg^{2+} in pool and tap water are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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