Rhodamine B Hydrazide Revisited: Chemodosimetric Hg^{2+}-selective Signaling Behavior in Aqueous Environments

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The well-known Cu^{2+}-selective chemodosimetric behavior of rhodamine B hydrazide was successfully switched to selectivity for Hg^{2+}. The fluorescence signaling is remarkably selective toward Hg^{2+} ions compared to other common biologically and environmentally important metal ions, including Cu^{2+} ions. The detection limit was 0.2 μM in an acetate-buffered aqueous 10% methanol solution at pH 5. The OFF-ON type of signaling is due to the selective Hg^{2+}-induced hydrolysis of the lactam ring of the hydrazide as has been reported for the standard Cu^{2+}-signaling process of the same compound. A simple change in medium resulted in clear switching of selective signaling from Cu^{2+} to Hg^{2+}, which extends the applicability of the easily accessible hydrazide derivative.

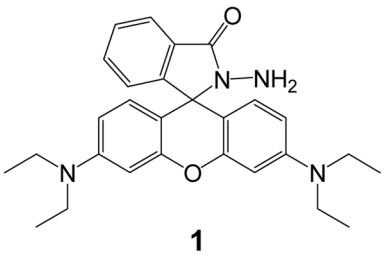
Key Words : Chemodosimeter, Hg^{2+}-selectivity, Rhodamine, Fluorescence

Introduction

Recently, a number of elegantly designed fluorescent signaling systems for the determination of important chemical species have been developed.[1,2] Among the widely used signaling approaches, chemodosimeters, in particular, have attracted a lot of interest because of advantages such as high selectivity, usually achieved by a specifically designed reaction, and a characteristic accumulative effect for the determination of analytes.[3]

Many unique signaling systems have been developed based upon the hydrazide derivative of rhodamine B, which is a classical example of Cu^{2+}-selective fluorescent chemodosimeter (Scheme 1).[4] For example, rhodamine B hydrazide **1** has been investigated for the analysis of chromium (VI)[5] in water and for sensitive determination of hydrogen peroxide and glucose.[6] Rhodamine 6G derivative, which has a carbohydrazone binding unit, exhibits selective Hg^{2+} ion signaling by ring opening of the lactam moiety.[7] Chemodosimetric determination of Hg^{2+} ions in aqueous media has been accomplished using an irreversible desulfurization reaction of a thiosemicarbazide derivative of fluorescein to its corresponding oxadiazole, with efficient chromogenic and fluorogenic signaling.[8] More recently, Zheng *et al.* reported an interesting finding that the Cu^{2+}-selective signaling behavior of hydrazide **1** could be effectively switched to Hg^{2+} selectivity by replacing one oxygen atom of the spirolactam with a sulfur atom to yield a thiohydrazide.[9] Other related derivatives of salicylaldehyde rhodamine B hydrazone[10] and fluorescein hydrazide[11] exhibited Cu^{2+}-selective chromogenic and fluorogenic signaling behavior *via* selective metal ion-induced hydrolytic cleavage of the amide bond.

During the course of developing new signaling systems by the preparation of fluorescein or rhodamine hydrazide derivatives, we have found that the well-known Cu^{2+}-selective chemodosimeter **1** exhibits a pronounced Hg^{2+} selectivity under other optimized conditions. In this paper, we report the prominent Hg^{2+}-selective fluorescence signaling behavior of the simple, well-known rhodamine B hydrazide **1** in acetate buffer at pH 5. Development of selective and efficient Hg^{2+} chemosensor is very important in chemical, biological, and environmental sciences as has been emphasized constantly.[12]



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Scheme 1. Czarnik's Cu^{2+}-selective chemodosimeter **1**.

Results and Discussion

The signaling behavior of **1** towards representative alkali, alkaline earth, and transition metal ions was investigated by absorption and fluorescence spectroscopy. First, the UV-Vis characteristics of **1** were measured in an aqueous 10% methanol solution (H_2O:CH_3OH = 90:10, v/v) buffered at pH 5.0 (acetate buffer, 10 mM). As can be seen from Figure 1, compound **1** showed almost no absorption above 450 nm due to the existence of the spiro ring in lactam form. Upon interaction with various metal ions (100 equiv), an intense absorption band centered at 556 nm (λ_{max}) appeared exclusively with Hg^{2+} ions. This increase in absorbance at 556 nm was prominent and A/A_o, which is the ratio of the absorbance of **1** in the presence and absence of metal ions, is 70.5 for Hg^{2+} ions. On the other hand, other metal ions induced almost no changes (A/A_o = 1.1-2.0) in the absorption spectra of **1**. Specifically, Cu^{2+} ions, which are the main target for chemodosimetric signaling by hydrazide **1** in

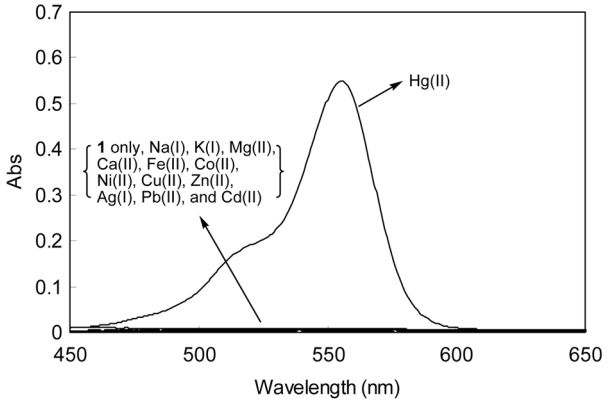


Figure 1. UV-Vis spectra of **1** in the presence of various metal ions in an aqueous methanol solution at pH 5. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, in H_2O-CH_3OH (90:10, v/v) at pH 5.0 (acetate buffer, 10 mM). The indicated pH value denotes the pH of the H_2O portion before adding methanol.

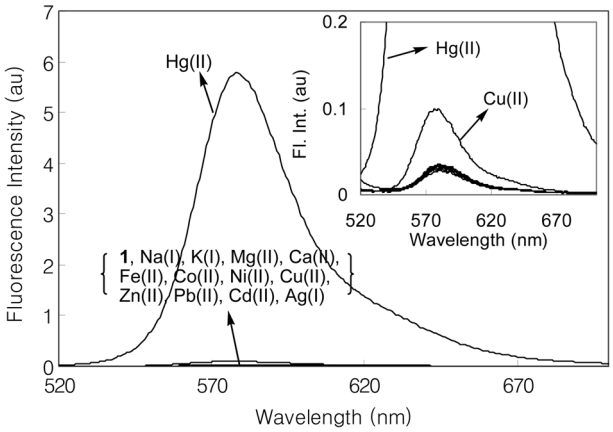
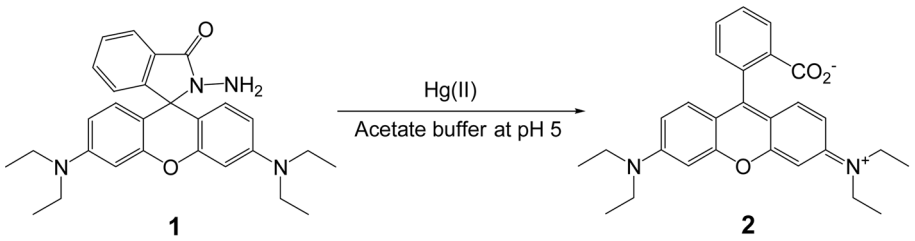


Figure 2. Fluorescence spectra of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, in buffered H_2O-CH_3OH (90:10, v/v) at pH 5.0. λ_{ex} = 500 nm.

HEPES buffer, did not induce any discernible change with A/A_o = 1.8. Concomitantly, the solution changed from being colorless to pink in color, allowing for detection of Hg^{2+} ions by the naked eye. This dramatic OFF-ON type of signaling is due to the selective Hg^{2+}-induced hydrolysis of the lactam ring of the hydrazide (Scheme 2) as has been reported for the standard Cu^{2+}-signaling process of the same compound.[4]

The Hg^{2+}-induced selective transformation of **1** into rhodamine B **2** was analyzed by UV-Vis, fluorescence, NMR, and mass spectroscopy measurements. The UV-Vis and fluorescence spectra of **1** (5.0×10^{-6} M) treated with 10 equiv of Hg^{2+} ions were almost identical with those obtained for a solution of rhodamine B **2** in the presence of 10 equiv of Hg^{2+} ions at pH 5. The structural transformation of hydrazide **1** was also confirmed by NMR. After treatment of **1** with $Hg(OAc)_2$ in CD_3OD-D_2O (4:1, buffered at pH 5 with deuterated acetate buffer), the ^1H NMR spectrum of the resulting solution was similar to the spectrum for rhodamine B itself. The characteristic signals of the xanthene moiety of hydrazide **1** at 6.4-6.5 ppm were completely disappeared and shifted to around 7.0-7.2 ppm. The signals of lactam containing phenyl ring were also experienced significant downfield shift from 7.0-7.9 ppm to 7.4-8.3 ppm. A significant downfield shift was also observed for the ethyl protons of diethylamine function from 1.17 and 3.40 ppm to 1.33 and 3.71 ppm, respectively. Although there exist minor differences, the appearance of the spectrum of the hydrazide derivative **1** obtained after treatment with 2 equiv of $Hg(OAc)_2$ was almost identical with that of the rhodamine B **2** in the presence of 2 equiv of $Hg(OAc)_2$ under identical condition. In ^{13}C NMR spectra, the carbon signal at 64.7 ppm, which is characteristic of the spirolactam ring carbon of **1**, disappeared, while another peak at 85.0 ppm, corresponding to the same carbon atom in rhodamine B, appeared. The mass measurement (EI, DIP) also provided additional evidence with an intense peak at *m/z* = 443.23 for rhodamine B $[M+H]^+$ and concomitant disappearance of the hydrazide peak at *m/z* = 456.25 $[M]^+$ of **1**.

Subsequently, the fluorescent signaling behavior of hydrazide **1** was investigated in the same aqueous 10% methanol solution at pH 5.0 ([**1**] = 5.0×10^{-6} M). As can be seen in Figure 2, the fluorescence spectra of **1** did not exhibit any considerable emissions above 520 nm. However, upon treatment with 100 equiv of various metal ions, an intense emission band around 578 nm was developed exclusively with Hg^{2+} ions. The enhancement factor of the fluorescence intensity, I/I_o, which is the ratio of the fluorescence intensity in the presence and the absence of metal ions, measured at 578 nm was 166.2. The solution also changed from colorless to fluorescent pink when illuminated with a UV lamp. The I/I_o values for the other metal ions ranged from 0.77 (Ca^{2+}) to 2.81 (Cu^{2+}). Of particular interest is the relative lack of response to the Cu^{2+} ions, which are possible sources of interference as inferred from the well-known Cu^{2+} selectivity of **1**. Compound **1** also exhibited Hg^{2+} selective signaling behavior in Tris or HEPES buffered aqueous methanol solution as well as other aqueous solutions of dioxane or



Scheme 2. Hg^{2+}-selective signaling of hydrazide **1**.

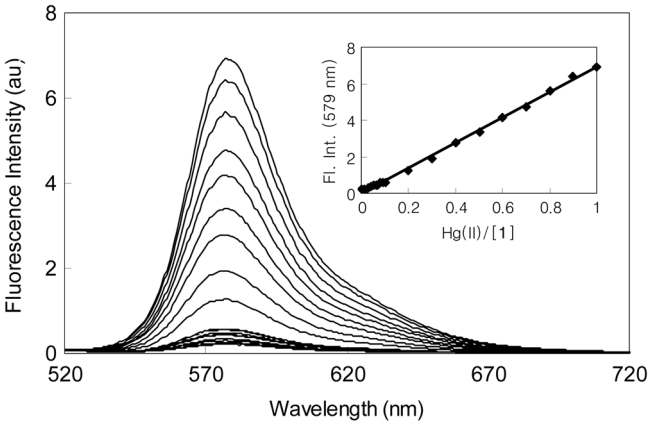


Figure 3. Fluorescence spectra of **1** as a function of concentration of Hg^{2+} ions. [**1**] = 2.0×10^{-5} M in H_2O-CH_3OH (90:10, v/v) at pH 5.0. λ_{ex} = 500 nm.

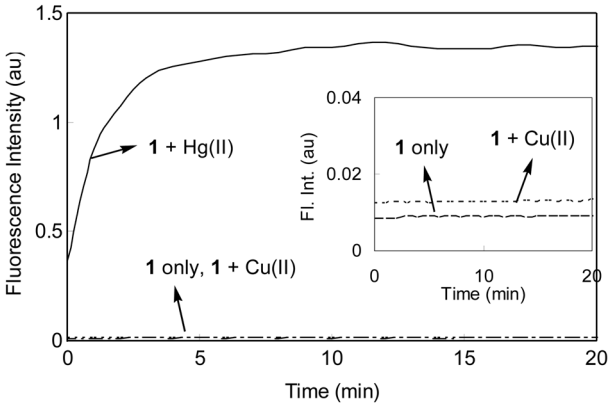


Figure 4. Fluorescence intensity of **1** at 578 nm as a function of time in the presence of Hg^{2+} and Cu^{2+} ions. [**1**] = 5.0×10^{-6} M, [M^{2+}] = 5.0×10^{-4} M, in H_2O-CH_3OH (90:10, v/v) at pH 5.0. λ_{ex} = 500 nm.

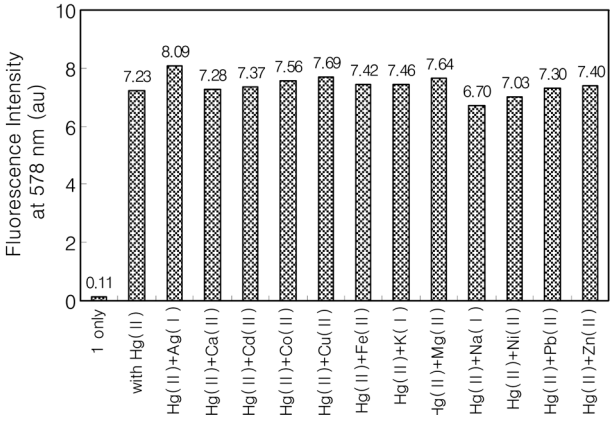


Figure 5. Selective signaling of Hg^{2+} ions by **1** in the presence of common interfering metal ions. [**1**] = 5.0×10^{-6} M, [Hg^{2+}] = 5.0×10^{-5} M, [M^{n+}] = 5.0×10^{-4} M, in buffered H_2O-CH_3OH (90:10, v/v) at pH 5.0. λ_{ex} = 500 nm.

acetonitrile in acetate buffered at pH 5. However, the Hg^{2+} selectivity observed is somewhat inferior to the acetate buffered aqueous 10% methanol solution due to the enhanced Cu^{2+} responses in these media. The trends in selectivity could be easily assessed by comparing the fluorescence intensity ratios of **1** in the presence of Hg^{2+} and Cu^{2+} ions (I_{Hg}/I_{Cu}) observed at 578 nm. For example, the I_{Hg}/I_{Cu} values for aqueous 10% methanol solution at pH 7 (HEPES) and pH 8 (Tris) were 11.4 and 13.3, respectively, which are much smaller than the value of 59.1 at pH 5. On the other hand, the I_{Hg}/I_{Cu} values for aqueous 10% acetonitrile and dioxane solutions were 4.4 and 17.5, respectively, which also are much smaller than the value of the aqueous 10% methanol solution at pH 5 (59.1).

The quantitative nature of the Hg^{2+}-dependent signaling was investigated by titration of **1** with $Hg(OAc)_2$. As can be seen in Figure 3, the fluorescence of **1** increased linearly as a function of [Hg^{2+}] up to 2.0×10^{-5} M. The detection limit, which was calculated as three times the standard deviation of the background noise from the calibration curve, for the determination of Hg^{2+} ions in the same medium was found to be 0.2 μM.[13] This observation suggests that the prepared compound may be utilized as a new chemodosimeter for the analysis of Hg^{2+} ions in the micromolar concentration range in aqueous environment.

To further understand the Hg^{2+}-selective signaling behavior of **1**, the time course of the signaling process was investigated. Aliquots of stock solution of metal ions were added to a solution of **1** (using the optimized conditions of H_2O:CH_3OH = 90:10 v/v at pH 5.0), and the fluorescence intensity at 578 nm was followed at room temperature. As shown in Figure 4, the fluorescence intensity of **1** increased rapidly upon interaction with Hg^{2+} ions, reached a constant value within 10 min after preparation of the sample, and then remained at that plateau. However, with Cu^{2+} ions, the signal developed very slowly and only reached less than 1% of the **1**-Hg^{2+} system after 10 min and less than 3% even after 48 h of measurement, which is very inefficient compared to the case with Hg^{2+} ions. Other metal ions revealed almost no changes in the fluorescence spectra after 48 h of measurement.

The Hg^{2+}-selective signaling behavior of **1** was further investigated under competitive conditions. The fluorescence intensity of **1** (5.0×10^{-6} M) upon treatment with 10 equiv of Hg^{2+} ions was measured in the presence of 100 equiv of common coexistent metal ions (Figure 5). Although some fluctuations in the fluorescence intensity of **1** were observed, the signaling behavior of the **1**-Hg^{2+} system was not so significantly affected by coexistent metal ions. Therefore, compound **1** can effectively detect Hg^{2+} ions in the presence of other common interfering metal ions of representative alkali, alkaline earth, and transition metal ions.

Conclusions

We have found that the well-known Cu^{2+}-selective chemodosimetric behavior of rhodamine B hydrazide can be switched to a Hg^{2+}-selective fluorescence signaling behavior. The Hg^{2+}-signaling process of the hydrazide derivative is reminiscent of the signaling for Cu^{2+} ions and works well

in an aqueous solution. The signaling is selective and also sensitive toward Hg^{2+} ions over other common biologically and environmentally important interfering metal ions. This finding could become an extra applicability of the simple, but useful, rhodamine B hydrazide for the analysis of Hg^{2+} ions in micromolar concentration ranges in semi-aqueous media.

Experimental

General. All solvents were purchased from Aldrich Chemical Co. as 'anhydrous' or 'spectroscopic grade'. Rhodamine B hydrazide **1** was prepared following a reported procedure,[14] and an authentic sample was purchased from Aldrich Chemical Co. and used without further purification. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian Gemini-2000 spectrometer and referenced to the residual solvent signals. Mass spectra were obtained with a Micromass Autospec mass spectrometer using the direct insertion probe operated in electron impact ionization mode. UV-Vis spectra were recorded with a Jasco V-550 spectrophotometer equipped with a Peltier temperature controller. Fluorescence spectra were measured on an Aminco-Bowman Series 2 Spectrophotometer.

UV-Vis and Fluorescence Spectra Measurements. Stock solution of rhodamine B hydrazide **1** was prepared in methanol (1.0×10^{-3} M) and stock solutions (0.01 M) of metal ion (Na^+, K^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} Cd^{2+}, Pb^{2+}, and Hg^{2+} in perchlorate) were prepared in acetate buffered (pH 5.0) aqueous solution. To a stock solution of **1** (0.03 mL) was added a stock solution of metal ion (0.3 mL) and subsequently diluted with calculated amount of methanol and water to obtain aqueous 10% methanol solution. Final concentrations of **1**, metal ions, and buffer were 5.0×10^{-6} M, 5.0×10^{-4} M, and 10 mM, respectively. UV-Vis and fluorescence measurements were carried out after at least 10 min of sample preparation.

Time Course Measurements. Time course signaling experiments were carried out in aqueous 10% methanol solution buffered at pH 5.0 (10 mM acetate buffer). Measuring solution was prepared (1.0×10^{-6} M) in the cuvette by mixing freshly prepared stock solutions of **1** and $Hg(OAc)_2$ or $Cu(OAc)_2$ solution. Changes in absorbance at 556 nm or fluorescence intensity at 578 nm were followed at room temperature.

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