J Fluoresc (2008) 18:1187–1192
DOI 10.1007/s10895-008-0365-7

SHORT COMMUNICATION

A Novel Hg^{2+} Selective Ratiometric Fluorescent Chemodosimeter Based on an Intramolecular FRET Mechanism

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Received: 10 January 2008 / Accepted: 14 March 2008 / Published online: 11 May 2008

Abstract A irreversible Hg^{2+} selective ratiometric fluorescence probe **FR**, a fluorescein fluorophore linked to a rhodamine B hydrazide by a thiourea spacer, was designed and synthesized. The developed probe **FR** exhibited great ratiometric fluorescence enhancement and remarkable yellow-magenta color change toward Hg^{2+} with excellent selectivity in aqueous acetone solution, and the ratiometric fluorescence response to Hg^{2+} was not interfered by other metal cations including Fe^{3+}, Co^{2+}, Ni^{2+}, Cr^{3+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+} and Mn^{2+}. The linear range and the detection limit of this supposed ratiometric fluorescence method for Hg^{2+} were $0.0–10.0\times10^{-6}$ and 5×10^{-8} M, respectively.

Keywords Fluorescence · Chemosensor · Ratiometric · FRET · Hg^{2+}

Introduction

Design and synthesis of fluorescence chemosensors for Hg^{2+} with desirable properties have been of interest to chemists for many years because of the lethal effects of Hg^{2+} on the environment and living organisms [1–4], and a number of chemosensors for Hg^{2+} have been reported [5–19]. Among these chemosensors, most are direct fluorescence quenching [6–9] or fluorescence enhancement models [10–19]. However, in most practical applications, changes in fluorescence intensity only can also be caused by many other poorly quantified or variable factors such as photobleaching, concentration of probe molecule, the microenvironment around the probe molecule, or the stability of light source. Therefore, there is still an urgent need for feasible chemosensors for the determination of Hg^{2+}. Ratiometric method measuring the ratio of fluorescence intensities at two wavelengths provides an alternative approach, which can overcome the drawbacks of intensity-based measurements due to a built-in correction for environmental effects and increase the selectivity, sensitivity and dynamic range of the method [20–25]. However, up to now, only a few ratiometric fluorescence probes for Hg^{2+} [26–28] have been reported in literature. Moreover, the reported sensors were mainly based on the mechanism of intramolecule charge transfer [26–27] or excimer-monomer transfer [28]. To our knowledge, ratiometric fluorescence probe for Hg^{2+} based on fluorescence resonance energy transfer (FRET) has not been reported in literature.

FRET is an interaction between a fluorophore at the electronic excited state (energy donor) and a fluorophore at the ground state (energy acceptor), which leads to the transfer of excitation energy from the donor to the acceptor. Although the efficiency of energy transfer is affected by the distance between the donor and the acceptor and the relative orientation of transition dipoles of both the donor and acceptor, it is also mainly determined by the extent of the spectral overlap between the donor emission and acceptor absorption [29]. Therefore, we thought that it would be possible to fabricate a probe based on the FRET mechanism if a molecule could dramatically generate a suitable fluorescent energy acceptor by the interaction with

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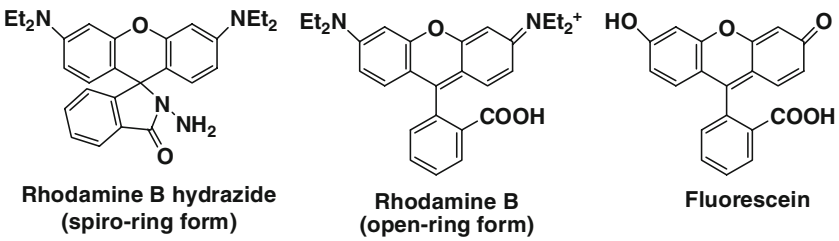


Fig. 1 The structures of *rhodamine B hydrazide*, *rhodamine B* and *fluorescein*

target analyte. It was anticipated that a rhodamine B spirolactam derivative would be appropriate candidate because it has two conformations (spirolactam form and ring opened amide form) with distinctly different absorption and fluorescence properties [30]. The ring-opening amide form of rhodamine B derivative has strong absorption at around 560 nm and emits strong fluorescence, whereas the spirolactam form displays absorption only in the ultraviolet region and no fluorescence. Obviously, if a fluorescein derivative is chosen as the energy donor, there is a significant spectral overlap between the emission of fluorescein derivative and the absorption of ring-opening amide form of rhodamine B derivatives (see Figs. 1 and 2).

Based on the above thinking, a ratiometric fluorescence probe **FR** for Hg^{2+} was fabricated, which was composed of fluorescein fluorophore linked to a rhodamine B hydrazide by a thiourea spacer and could function as a dual colorimetric and ratiometric fluorescent reporter for Hg^{2+}. Further experiments showed that **FR** made a feature of good selectivity for Hg^{2+} in water and a red region emission excited at visible wavelength with a ratiometric mode.

Experimental

Apparatus

A Hitachi F-4500 spectrofluorometer (Tokyo, Japan) equipped with a plotter unit and a 1.0 cm quartz cell was used for recording fluorescence spectra and making fluorescence measurements. The absorption spectra were made on a Beckmamn DU7400 absorption spectrophotometer (America).

Reagents

All the reagents were used as received from Shanghai Chemicals Group Company except for FITC from Acros Organics. The inorganic salts were of the highest purity available and existed in their nitrates or chlorides. Twice deionized water was further distilled in the presence of $KMnO_4$.

Buffer solution (pH5.0) prepared by mixing 0.2 M 3, 3-dimethylglutaric acid solution and 0.2 M sodium hydroxide solution was used.

The synthesized dye **FR** dissolved in acetone solution to make a 1.0×10^{-3} M stock solution. A 1.0×10^{-3} M standard solution of mercuric chloride was prepared by dissolving 13.6 mg of the reagent in water and diluting up to 50 mL.

The probe **FR** was synthesized as follows (Scheme 1): Rhodamine B hydrazide was synthesized according to the literature [31]. Fluorescein isothiocyanate (FICT, 0.50 g, 1.3 mmol) and rhodamine B hydrazide (0.59 g, 1.3 mmol) were dissolved in 4.0 mL dry dimethylfuran, and the reaction mixture was stirred at room temperature under N_2 atmosphere for 48 h. After removal of the solvent, the residue was purified by flash chromatography with $CHCl_3$/acetone as the eluent to afford **FR** (0.98 g, yield: 89%). The product was further confirmed by the results of ^1H NMR (CD_3OD, 500 MHz), ^{13}C NMR (DMSO-d_6, 400 MHz) and electrospray ionization (ESI) mass spectrometry (MS).

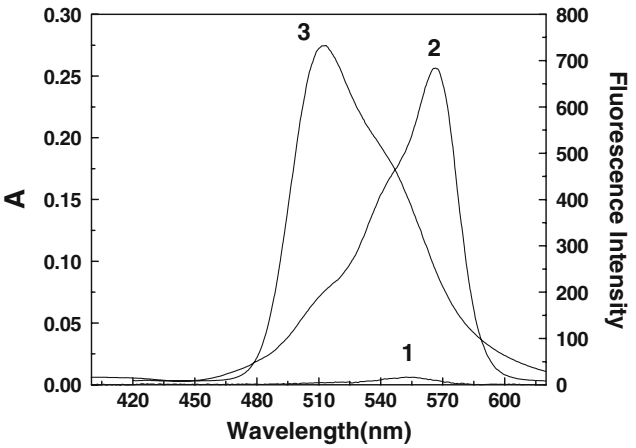
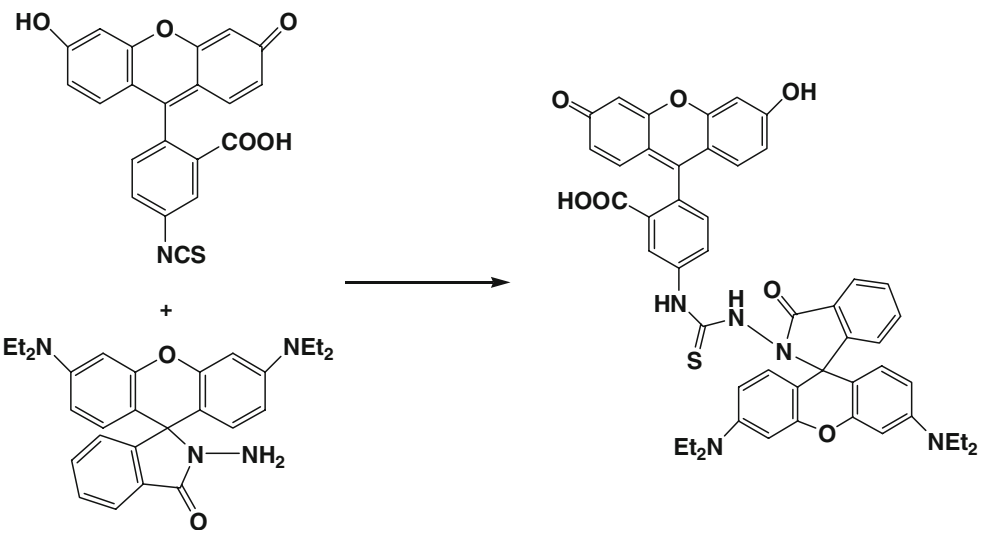


Fig. 2 The absorption spectra of rhodamine B hydrazide and rhodamine B (*1* and *2*, respectively) and the fluorescence emission spectrum of fluorescein (λ_{ex}=490 nm)

Scheme 1 Synthesis of FR



^{1}H NMR (CD_3OD, 500 MHz) δ 1.098 (t, *J*=8.0 Hz, 12 H, 4×NCH_2CH_3), 3.298–3.345 (m, 12 H, including 4×NCH_2CH_3; and 4 active H: 1× OH, 1× COOH, 2 H in thiourea group, exchangeable with MeOD), 6.328 (d, *J*= 10.0 Hz, 2 H), 6.480 (S, 1 H), 6.484 (S, 2 H), 6.502 (d, *J*= 3.0 Hz, 2 H), 6.554 (S, 2 H), 6.576 (S, 1 H), 6.654 (d, *J*=3.0, 2 H), 6.954 (d, *J*=10.0 Hz, 1 H), 7.265(d, *J*=9.5 Hz, 1 H), 7.368 (dd, *J*=10.0, 2.0 Hz, 1 H), 7.666 (S, 1 H), 7.638 (d, *J*=9.5 Hz, 1 H), 7.720 (dt, *J*=9.5, 1.0 Hz, 1 H), 7.996 (d, *J*=9.5 Hz, 1 H)

^{13}C NMR (DMSO-d6, 100 MHz) δ 12.902, 19.019, 30.064, 32.561, 40.900, 44.121, 56.294, 56.500, 63.268, 66.749, 68.979, 83.336, 97.557, 102.763, 104.929, 110.071, 112.975, 123.414, 123.513, 124.729, 126.158, 129.157, 130.107, 134.129, 140.947, 148.949, 152.285, 153.995, 159.939, 168.717, 181.700

ESI mass spectrometry, *m/z*: 846.2 $(M)^+$

General procedure

Transfer appropriate amounts of $HgCl_2$ solutions into a series of 10.0 mL volumetric flasks, then add 1.0 mL 3,3-dimethylglutaric acid–NaOH buffer (pH 5.4) and 4.0 mL acetone. Dilute the solution with doubly-distilled water to the mark, then add 100 μL 1.0×10^{-3} M **FR** stock solution with a micropipette and mix thoroughly. After the mixture was incubated at room temperature for 20 min, measure the

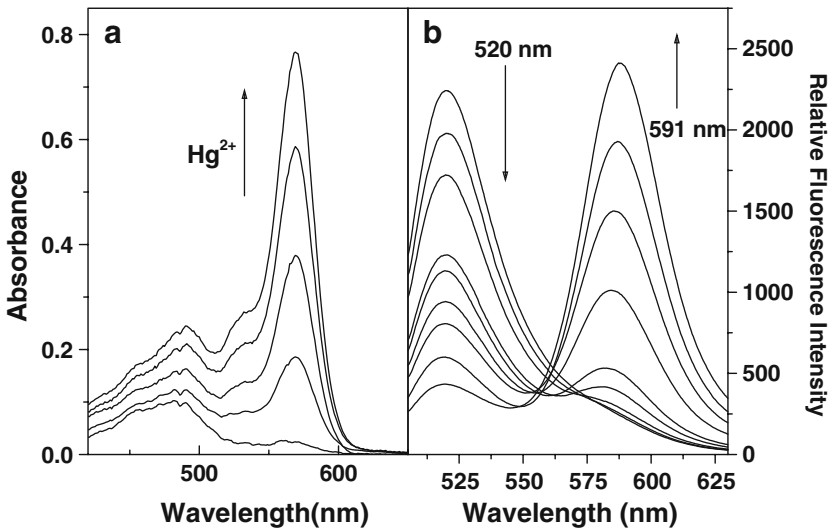
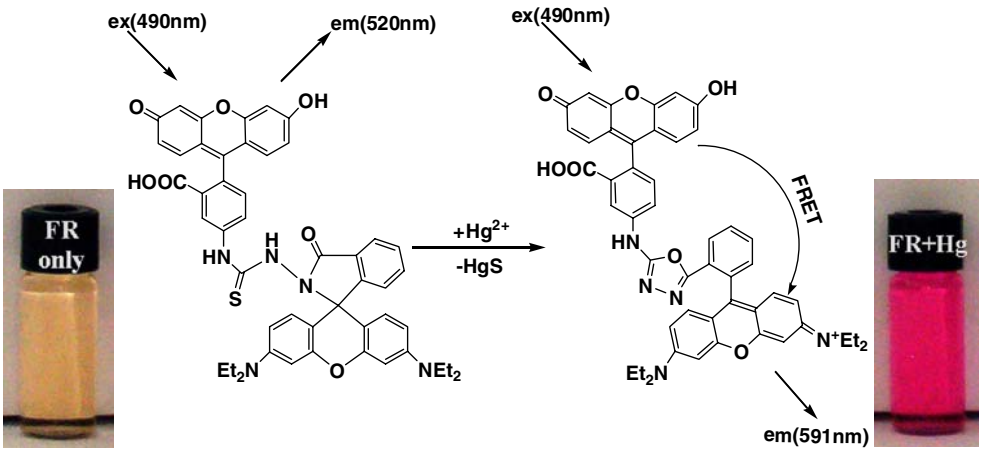


Fig. 3 The absorption spectra (**a**) and fluorescence spectra (**b**) of FR in the presence of Hg^{2+}. **a** [FR]=10.0 μM; [Hg^{2+}]=0, 8.0, 10.0, 20.0, 30.0 μM, respectively. **b** [FR]=10.0 μM; [Hg^{2+}]=0, 1.0, 2.0, 3.0, 5.0, 6.0, 7.0, 10.0 μM, respectively. Medium: acetone-water solution (40:60, *v*/*v*); pH 5.40

Scheme 2 FRET-based detection mechanism of Hg^{2+}

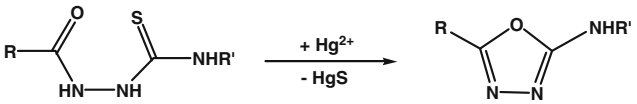


fluorescence ratio of I_{591}/I_{520} with the excitation and emission wavelengths at 490 and 591/520 nm, respectively.

Results and discussions

It can be seen from the curve in Fig. 3a that the free FR ($[Hg^{2+}]$=0.0 mol/L) showed a maximum absorption wavelength at 490 nm, which exhibited slightly yellow color dominating by the fluorescein chromophore, and no intramolecular FRET phenomenon can be observed in free **FR** because the rhodamine B hydrazide group in **FR** shows only a very small absorption in the wavelength region of the fluorescein emission and this group is nonfluorescent. Therefore, only green fluorescence (520 nm) of fluorescein itself was observed when free **FR** was excited at 490 nm (Fig. 3b, the curve of $[Hg^{2+}]$=0.0 mol/L).

As reported [32–33], Hg^{2+} promoted the desulfurization reaction of thiosemicarbazide to form 1,3,4-oxadiazole:



thiosemicarbazide 1,3,4-oxadiazole

Similarly, a thiosemicarbazide group exists in **FR**, therefore, the reaction of Hg^{2+} at the thiosemicarbazide group will force **FR** to form a 1,3,4-oxadiazole group as a new spacer and lead to the release of fluorescent rhodamine B moiety, which triggers a intramolecular FRET. Scheme 2 outlines the reaction mechanism of Hg^{2+} with **FR** based on this design.

The proposed mechanism was further confirmed by the succedent experimental results. Besides the absorption peak

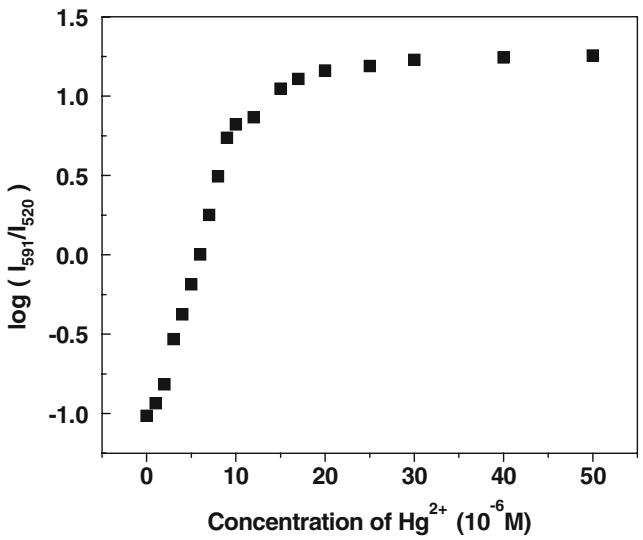
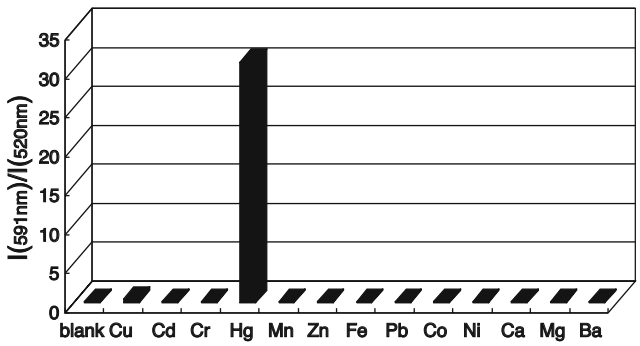


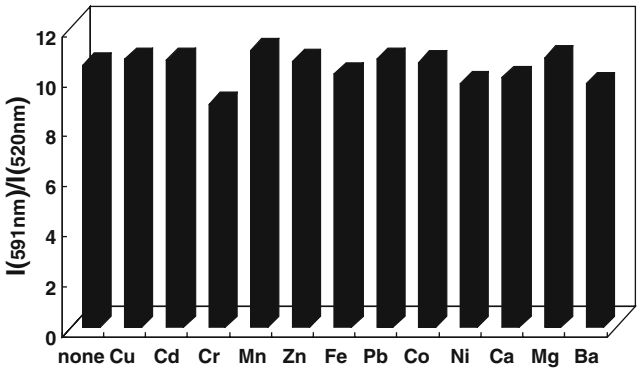
Fig. 4 The titration curves of fluorescent ratioof FR vs. Hg^{2+} ionic concentration. [FR]=10 μM; $\lambda_{ex}/\lambda_{em}$=490/520, 591 nm

Table 1 Fluorescence ratio of FR upon additon of 50.0 μM of various cations



From left to right: no cation (blank), Cu^{2+}, Cd^{2+}, Cr^{3+}, Hg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{3+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}. [FR]=10.0 μM; Reaction medium: acetone-water solution (40:60, *v*/*v*) of pH 5.40

Table 2 Fluorescence intensity of FR (1.0×10^{-5} mol/L) in the presence of mercury ion(1.0×10^{-5} mol/L) and competing ions(5.0×10^{-5} mol/L)



at 490 nm, **FR** showed a new strong absorption peak at 565 nm (Fig. 3a) when the presence of Hg^{2+}, which was attributed to the absorption peak of ring-opening rhodamine B moiety. We have validated the fluorescent product of **FR** after reaction with Hg^{2+} by ESI-MS. The intense peak of *m*/*z* 814.1 $(M)^+$ supported the proposed reaction product shown in Scheme 2. Accordingly, this spectral change resulted in the color change from yellow to magenta (Scheme 2), indicating that **FR** can also serve as a highly sensitive "naked-eye" indicator for Hg^{2+} in water.

Meanwhile, great changes in the fluorescence spectrum of **FR** in the presence of Hg^{2+} were also observed (Fig. 3b). The free **FR** displayed a single emission band centered at 520 nm when excited at 490 nm, which was attributed to the emission of fluorescein, whereas, the reaction system of **FR** with Hg^{2+} exhibited dual fluorescence peaks located at 520 and 591 nm, respectively, the latter agreed with the emission of ring-opening rhodamine B moiety. Furthermore, the fluorescence intensity at 520 nm was decreased and the fluorescence intensity at 591 nm was increased at the same time with the increase of Hg^{2+} concentration. Hence, the determination of Hg^{2+} can be performed by measuring the ratio of fluorescence intensities at 591 and 520 nm, respectively. This fact obviously indicates that an intramolecular FRET really exists between the fluorescein moiety and the rhodamine B moiety produced by the action of Hg^{2+} in PR. Figure 4 depicted the plot of the ratiometric fluorescence response of **FR** with the increasing amounts of Hg^{2+} and the presence of 1.0 equiv. of Hg^{2+} gave a ca. 65-fold enhancement in ratiometric value of I_{591}/I_{520} with respect to the metal-free solution.

Table 1 shows the fluorescence responses of **FR** to various background metal ions including some related heavy, transition and main group metal ions. As shown in Table 1, free **FR** (1.0×10^{-5} M) exhibits a rather low value of fluorescence ratio (signed as the blank), upon addition of 5.0 equiv. of tested background metal ions, the fluorescence ratio is nearly not affected, while the addition of Hg^{2+} results in a large ratio value, indicating the high fluorimetric selectivity for Hg^{2+}.

Furthermore, interferences from various coexistent metal cations for the determination of 10.0 μM Hg^{2+} ion were also investigated. The fluorescence ratio value of **FR** in the presence of 10.0 μM Hg^{2+} ion was almost unaffected (relative error≤±10%) by the addition of 5.0 equiv of competing metal ions except for Cr^{3+} caused an approximately −15% relative error (Table 2).

Conclusion

In summary, we have developed a new fluorescence probe, **FR**, for Hg^{2+} based on an intramolecular FRET with a high selectivity. The color of this probe changes from yellow to magenta when reacted with Hg^{2+}, which makes it available to detect Hg^{2+} either by ratiometric fluorimetry or by rapid "naked eye" detection. Moreover, the ratiometric fluorescence detection for Hg^{2+} provides a built-in correction for environmental effects, which is in favor of serving as a practical probe for rapid and accurate determination of mercuric ion in environmental systems. Furthermore, the successful fabrication of the proposed probe provides an alternative concept to design ratiometric fluorescence probes by utilizing two fluorophores satisfying the requirements of intramolecular FRET.

Acknowledgment This work was supported by the Science Research Foundation of Xiamen University (No.E43011) and the National Natural Science Foundation of China (No.20675067).

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