Switching the Recognition Preference of Rhodamine B Spirolactam by Replacing One Atom: Design of Rhodamine B Thiohydrazide for Recognition of Hg(II) in Aqueous Solution

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ABSTRACT

A new chemosensor based on rhodamine B thiohydrazide is described. Chemosensor B was found to show a reversible dual chromo- and fluorogenic response toward Hg^{2+} in aqueous solution in a highly selective and sensitive manner. This was suggested to result from the coordination of Hg^{2+} at the N, S binding sites in B to open its spiro ring.

Rhodamine-based dyes, known by their excellent spectroscopic properties of large molar extinction coefficient and high fluorescence quantum yield, have found applications in the study of complex biological systems as molecular probes,[1] but few as chemosensors.[2]

Recently, based on the protocol of metal coordination inducing spiro-ring opening of a sensing molecule,[3] rhodamine B spirolactam as a molecular scaffold was employed to design chemosensors for selective recognition of Cu^{2+} and Pb^{2+}.[4,5] In detrimental transition and post transition metal ions, mercury is considered a highly dangerous element because both elemental and ionic mercury can be converted by bacteria in the environment to methyl mercury, which subsequently bioaccumulates through the food chain.[6] Moni-

toring of mercury in the environment and in industrial waste streams is hence in high demand. Since chromo- or fluoro-oionophores are highly effective for the determination in terms of handling easy and equipment simplicity, much effort has been paid to the development of optical chemosensors that selectively respond to the mercuric ion,[7] especially reversible ones since they can be reused with proper treatment.

Designing chemosensors based on rhodamine spirolactams has several advantages: they display not only great absorbance and fluorescence intensity enhancement toward some

(1) Haugland R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes, Inc.: Eugene, OR 97402, and examples therein.

(2) Chemosensors based on hybrids of fluoresceins and rhodamines, namely rhodafluors, see: (a) Clark, M. A.; Duffy, K.; Lippard, S. J. *Org. Lett.* **2003**, *5*, 2051–2054. (b) Burdette, S. C.; Lippard, S. J. *Inorg. Chem.* **2002**, *41*, 6816–6823.

(3) Selective examples of opening the spiro ring of the spiropyran scaffold, see: (a) Winkler, J. D.; Bowen, C. M.; Michelet, V. *J. Am. Chem. Soc.* **1998**, *120*, 3237–3242. (b) Chibisov, A. K.; Gorner, H. *Chem. Phys.* **1998**, *237*, 425–442. (c) Gorner, H.; Chibisov, A. K. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2557–2564. (d) Tanaka, M.; Kamada, K.; Ando, H.; Kitagaki, T.; Shibutani, Y.; Kimura, K. *J. Org. Chem.* **2000**, *65*, 4342–4347. (e) Tanaka, M.; Nakamura, M.; Salhin, M. A. A.; Ikeda, T.; Kamada, K.; Ando, H.; Shibutani, Y.; Kimura, K. *J. Org. Chem.* **2001**, *66*, 1533–1537. (f) Suzuki, T.; Kato, T.; Shinozaki, H. *Chem. Commun.* **2004**, 2036–2037.

(4) Dujols V.; Ford F.; Czarnik A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387.

specific metal ions, but also a strong color development against the colorless blank during the sensing event, a feature that would facilitate "naked-eye" detection, and very recently, an excellent example of opening the spiro ring of rhodamine spirolactam-based chemodosimeter was reported via the Hg^{2+}-induced chemical reaction.[8] Bearing this in mind, we envisaged that, by incorporating proper binding sites, it would be possible to achieve a rhodamine spirolactam based chemosensor highly selective for Hg^{2+} via color/fluorescence changes. For the selective recognition of such a soft heavy metal ion, a sulfur-based functional group was considered and introduced, and the N, S binding sites might be a choice to be parts of a selective receptor.[7a,d,g] Obviously, a suitably designed rhodamine B spirolactam might be a good candidate. We report here in this regard compound **B**, namely rhodamine B thiohydrazide, containing an S atom and a $-NH_2$ group attached to the N-bearing spiro ring (Figure 1).
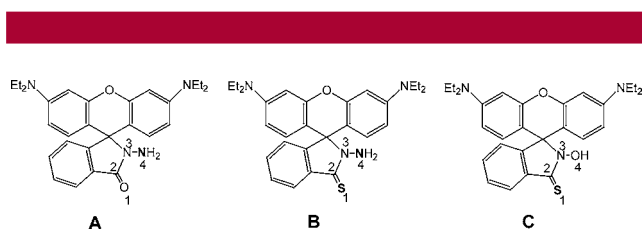
Figure 1. Chemical structures of **A**, **B**, and **C**.

Probe **B** was designed on the basis of the consideration that compound **A** (Figure 1), the rhodamine B hydrazide, was an irreversible chemodosimeter for Cu^{2+} by opening the spiro ring in **A** via metal ion binding at the N, O atoms at positions 1 and 4. [4] We expected that such a mode of opening the spiro ring might also be realized by the mercury ion if the O atom in **A** at position 1 was replaced by an S atom affording N, S atoms at positions 1 and 4 as the Hg^{2+} binding

sites. In particular, the lack of additional binding sites except for these two atoms would make the probe show no or little coordination to any other metal ions but allow only the most preferred mercuric ion to bind to the chelating unit.

Treatment of rhodamine B hydrazide (compound **A**) with Lawesson's reagent in refluxing benzene gave, after flash column chromatography on silica, the corresponding rhodamine B thiohydrazide (compound **B**) in a yield of ca. 19%.

A 1,4-dioxane/water solution (1:99, v/v) at pH 3.40 of 0.02 M 3,3-dimethylglutaric acid−NaOH buffer solution was seleced for the spectral investigation (Figure S1, Supporting Information). The absorption spectra of **B** with varying Hg^{2+} concentration were recorded, as shown in Figure 2. The
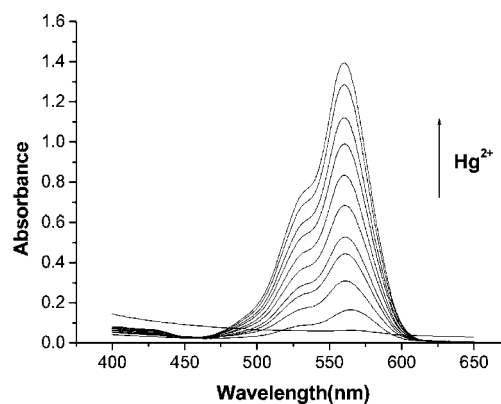
Figure 2. The absorption spectra of **B** in the presence of Hg^{2+} in an aqueous solution of pH 3.40. [**B**] = 20.0 μM. The Hg^{2+} concentration is 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μM, respectively.

colorless free **B** solution exhibited almost no absorption peak in the visible wavelength range (>400 nm) in aqueous solution. Upon addition of Hg^{2+}, **B** showed a new maximum absorption wavelength at 561 nm ($\epsilon = 1.25 \times 10^5$ M^{-1} cm^{-1}), which can be ascribed to the delocalized xanthene moiety of rhodamines. Accordingly, it showed a strong magenta color, indicating that chemosensor **B** can indeed serve as a highly sensitive "naked-eye" indicator for Hg^{2+} in water. The absorbance of **B** at 561 nm increased linearly with Hg^{2+} concentration, and Hg^{2+} could be detected at least down to 1.0×10^{-7} M, a concentration in the ppb range, when **B** was employed at 1.0×10^{-5} M.

The selective coordination studies of **B** were then extended to related heavy, transition, and main group metal ions by UV−vis spectroscopy. Figure 3 shows the representative chromogenic behavior of **B** toward metal ions in aqueous solution. As shown, 10.0 μM **B** exhibits very little absorbance at 561 nm upon addition of 1.0 equiv of metal ions such as Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Cd^{2+}, Ag^+, Fe^{3+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, and Pb^{2+}; however, only the addition of Hg^{2+} resulted in a prominent enhancement of absorbance at 561 nm, which indicated the high spectro-photometric selectivity of **B** to Hg^{2+}.

The selective fluorimetric response of **B** to all the tested metal ions was also studied. The results showed that a large

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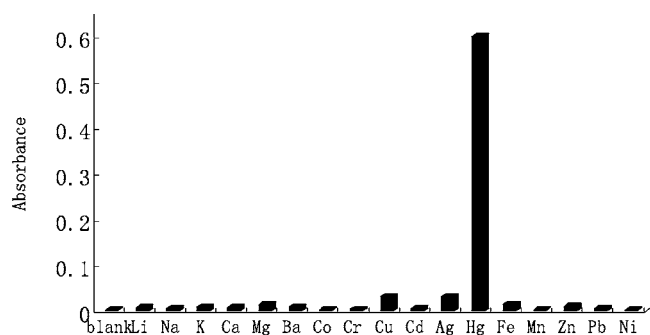(6) Nendza, M.; Herbst, T.; Kussatz, C.; Gies, A. *Chemosphere* **1997**, *35*, 1875−1885.

(7) Selective chemosensors based on photoinduced electron transfer (PET): (a) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270−14271. (b) Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272−2273. (c) Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769−6770. Intramolecular charge transfer (CT): (d) Descalzo, A. B.; Martinez-Màñez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418−3419. (e) Brümmer, O.; La Clair, J. J.; Janda, K. D. *Org. Lett.* **1999**, *1*, 415−418. (f) Choi, M. J.; Kim, M. Y.; Chang, S. K. *Chem. Commun.* **2001**, 1664−1665. (g) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968−969. (h) Sakamoto, H.; Ishikawa, J.; Nakao, S.; Wada, H. *Chem. Commun.* **2000**, 2395−2396. (i) Ros-Lis, J. V.; Martinez-Màñez, R.; Rurack K.; Sancenón, F.; Soto J.; Spieles M. *Inorg. Chem.* **2004**, *43*, 5183−5185. Chemical reaction (as chemodosimeter): (j) Sancenón, F.; Martinez-Màñez, R.; Soto, J. *Chem. Commun.* **2001**, 2262−2263. (k) Chae, M.-Y.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 9704−9705. (l) Ros-Lis, J. V.; Marcos M. D.; Martinez-Màñez, R.; Rurack K.; Soto J. *Angew. Chem., Int. Ed.* **2005**, *44*, 4405−4407. Redox process: (m) Hennrich, G.; Sonnenschein, H.; Resch-Genger, U. *J. Am. Chem. Soc.* **1999**, *121*, 5073−5074. Lipid bilayers: (n) Sasaki, D. Y.; Padilla, B. E. *Chem. Commun.* **1998**, 1581−1582. Oligo-nucleotide-based sensor: (o) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300−4302.

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Figure 3. Spectrophotometric response of **B** to 10.0 μM of various cations in an aqueous solution of pH 3.40. [**B**] = 10.0 μM. From left to right: no cation (blank), Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Cd^{2+}, Ag^+, Hg^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}.

enhancement of the fluorescence of **B** was observed only in the case of Hg^{2+} with the maximum excitation and emission wavelengths of 558 and 582 nm, respectively (Figure 4).
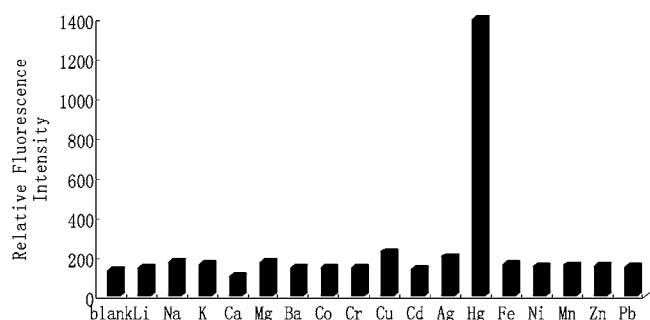
Figure 4. Fluorimetric response of **B** to 10.0 μM of various cations in aqueous solution of pH 3.40. [**B**] = 10.0 μM. From left to right: no cation (blank), Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Cd^{2+}, Ag^+, Hg^{2+}, Fe^{3+}, Ni^{2+} Mn^{2+}, Zn^{2+}, Pb^{2+}. Excitation/ emission was selected at 530/582 nm. Slit: excitation/emission = 5.0/10.0 nm.

Hg^{2+} could be detected at least down to 5.0×10^{-8} M by fluorimetric assay (Figure S3, Supporting Information).

The nice nonlinear fitting of the absorbance against metal concentration (Figure S2, Supporting Information) assuming a 1:2 binding ratio (Hg^{2+}/**B**) suggested a 1:2 binding stoichiometry. This (the overall binding constant is about 4.5×10^{10} M^{-2}) was supported by the results of continuous variations plot[9] (Figure S4, Supporting Information). Furthermore, this $Hg^{2+}\cdot\mathbf{B}_2$ complex formation can be reversibly switched by using KI and Hg^{2+} successively.[10,11]

To verify the binding sites in **B** for Hg^{2+}, two control molecules, **A** and **C** (Figure 1), were synthesized. The fact that the absorbance of **A** does not show any response toward Hg^{2+} under the same conditions indicates that the S atom in **B** plays a key role in the metal binding. On the other hand,

it was found that the absorbance of **C**, another control molecule for **B**, does not show any response toward Hg^{2+} either, indicating that in **B**, the N atom at position 4 takes part in the coordination. It is hence made clear that, in the **B**−Hg^{2+} system, both the S atom and the N atom at position 4 of **B** are involved in the binding of Hg^{2+}, forming a stable 5-membered cyclic complex that requires the opening of the spiro ring of **B** to establish the delocalized xanthene moiety that shows long wavelength absorption and fluorescence enhancement. On the basis of the aforementioned facts, a proposed binding mode of $Hg^{2+}\cdot\mathbf{B}_2$ is shown in Scheme 1.

Scheme 1. Proposed Hg^{2+} Binding Mode
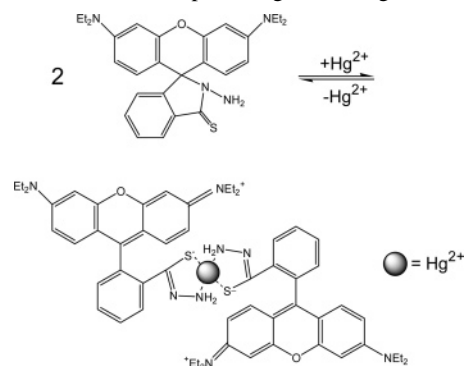
In conclusion, we showed a switching in the recognition preference in rhodamine B spirolactam by only replacing one atom of **A** at position 1, and thus only Hg^{2+} showed reversible high affinity toward N, S atoms at positions 1 and 4 to open the spiro ring of **B**, and the new dual chromo- and fluorogenic sensor displays merits not only in its excellent selectivity and sensitivity, but also in the obvious color change against the colorless blank during the sensing process. The design strategy that avoids introduction an S-bearing crown ether would simplify the synthesis steps, and it would help in the design of more chemosensors for some specific ions.

Supporting Information Available: Synthesis and characterization of compounds **B** and **C**, and absorption and fluorescence spectral responses of **B** for Hg^{2+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The procedure of preliminary investigation of the reversibility of the system: colorless 10.0 μM **B** was first exposed to a stoichiometric amount of Hg^{2+}; the resulting magenta solution was subsequently treated with KI (4 equiv of Hg^{2+}), which led to a nearly colorless solution. This can be ascribed to the formation of colorless HgI_4^{2-} and the decomplexation of $Hg^{2+}\cdot\mathbf{B}_2$ complex to release the colorless free **B**. This on/off chromogenic behavior can be reversed by introduction of more Hg^{2+}, restoring the magenta color due to the complexation of **B** to the newly added Hg^{2+} once again.