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Hg^{2+} Selective Fluorescent and Colorimetric Sensor: Its Crystal Structure and Application to Bioimaging

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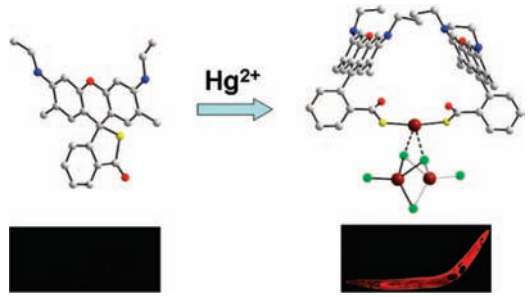
ABSTRACT



A Hg^{2+}-selective rhodamine 6G derivative bearing thiolactone moiety was synthesized, and its crystal structure with Hg^{2+} is presented to explain the binding mode. In addition, highly selective "off−on"-type fluorescent change upon the addition of Hg^{2+} was also applied to bioimaging.

Sensors based on the metal ion induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of the fluorescence.[1] In this regard, metal-selective fluorescent chemosensors serve as useful tools for detection of metal ions and thus have been widely exploited to detect biologically or environmentally relevant metal ions.[1] Mercury contamination occurs through oceanic and volcanic emission,[2] gold mining,[3] solid waste incineration, etc. Due to the high toxicity of mercury, considerable attention has been devoted to the development of new fluorescent chemosensors[1,4] for the detection of mercury and mercuric salts with sufficient selectivity.

Recently, rhodamine derivatives have received increasing attention in the design of chemosensors for metal ions.[5] Rhodamine derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color. Inspired by this strategy, spirolactam (nonfluorescent) to ring opened amide (fluorescent) process was utilized for the detection of metal ions.[6] Rhodamine derivatives have also been utilized as fluorescent chemosensors for Hg^{2+}.[6,7]

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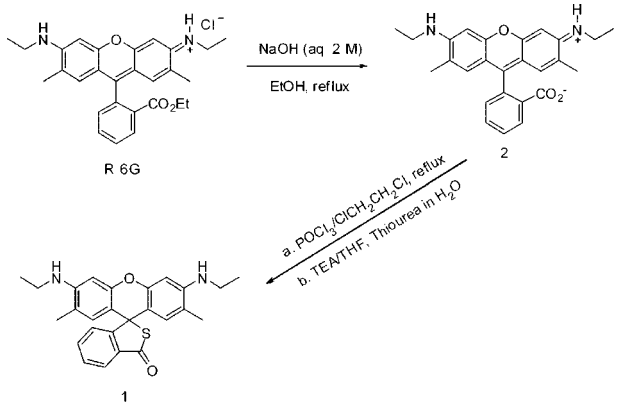
Herein, we introduced rhodamine 6G thiolactone derivative **1** as a selective fluorescent and colorimetric sensor for Hg^{2+} at pH 7.4. "Off−on" type fluorescent and colorimetric changes were observed only for Hg^{2+}. X-ray structure of **1**−Hg^{2+} was presented to explain the binding mode of sensor **1** with Hg^{2+}. Furthermore, our sensor was applied for in vivo imaging of Hg^{2+} using *C. elegans*. During the preparation our manuscript, similar approaches have been published by Xu et al. and Ma et al.[8] However, in our work, we could get the crystal structures of sensor as well as **1**−Hg^{2+} complex, which are very important and direct evidence of Hg^{2+} binding with the sensor. In addition, we demonstrated the nanomolar detection limit of our sensor, which is better than those reported. Finally, the biological application using *Caenorhabditis elegans* is reported as in vivo imaging.

As shown in Scheme 1, rhodamine 6G was first hydrolyzed to **2** in 97% yield, which was then reacted with phosphorus

Scheme 1. Synthesis of Chemosensor **1**



oxychloride and thiourea to give sensor **1** in 53% yield after the column chromatography using methylene chloride as an eluent. The detailed experimental procedures and ^1H NMR, ^{13}C NMR spectra are explained in the Supporting Information (S-Figure 1−S-Figure 4). Sensor **1** was further confirmed by X-ray analysis (Figure 1). A single crystal of **1** was grown in acetonitrile, and a unique thiospirolactone structure was observed.

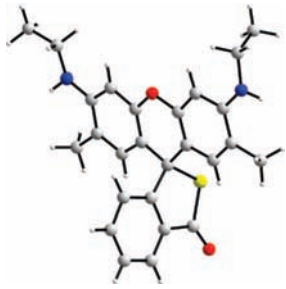


Figure 1. X-ray crystal structure of chemsensor **1**.

Al^{3+}, Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cs^+, Cu^{2+}, Fe^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+} ions were used to evaluate the metal ion binding properties of **1** (10 μM) in CH_3CN−HEPES buffer (0.01 M, pH 7.4) (1:99, v/v). The fluorescence spectra were obtained by excitation of the rhodamine 6G fluorophore at 515 nm. Among these metal ions (10 equiv), sensor **1** showed large fluorescence enhancement only with Hg^{2+} among the various metal ions examined (Figure 2). As large as 200-fold "off−on" type

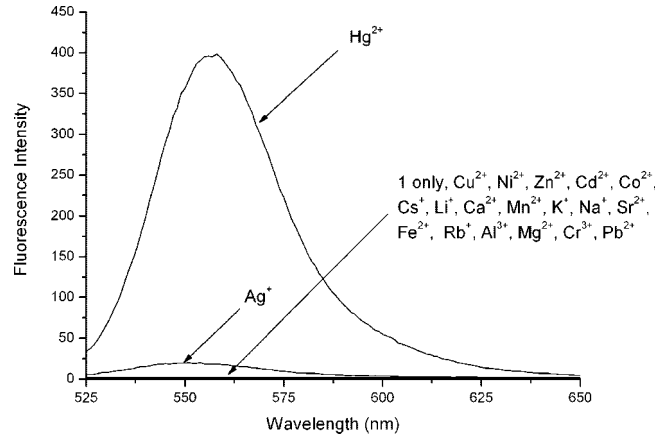


Figure 2. Fluorescence spectra of **1** (10 μM) with metal ions (100 μM) in CH_3CN−HEPES buffer (0.01 M, pH 7.4) (1:99, v/v) (λ_{ex} = 515 nm, slit: 1.5 nm/1.5 nm).

fluorescence enhancement was observed upon the addition of Hg^{2+}. The ring-opening process of spirolactone can enable the colorimetric change upon the addition of Hg^{2+}. A color change to dark pink from colorless was observed as naked eye detection of Hg^{2+} (S-Figure 5, Supporting Information).

The evidence of the binding mode comes from the ESI-MS spectra of the complex of probe **1** with Hg^{2+}. The peaks at *m*/*z* 531.5 and 1161.1 were clearly observed, corresponding to $[Hg(1)_2]^{2+}$ and $[Hg(1)_2ClO_4]^+$, respectively (S-Figure 6, Supporting Information). The 2:1 stoichiometry was also confirmed by the Job plot (S-Figure 7, Supporting Information). The X-ray crystal structure of probe **1** binding with Hg^{2+} is shown in Figure 3. The spirothiolactone ring-opened structure as well as the coordination of two sulfur atoms to Hg^{2+} was clearly confirmed. The large fluorescence enhancement as well as the colorimetric change can be attributed to this spirothiolactone ring opening, which was induced by

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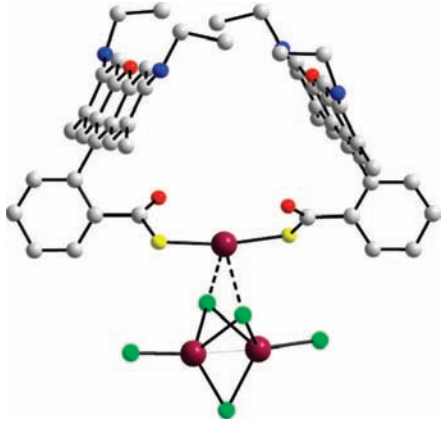

Figure 3. View of the X-ray crystal structure of complex **1**−Hg^{2+}.

the complexation of Hg^{2+}. Figure 4 explains the fluorescence spectra of **1** in the presence of different concentrations of Hg^{2+} in CH_3CN−HEPES buffer (0.01 M, pH 7.4) (1:99, v/v). As shown in Figure 5, sensor **1** can detect Hg^{2+} as low as nanomolor range. When 10 mM **1** was exposed to 1 equiv of Hg^{2+}, the pink color faded and fluorescence disappeared upon addition of 4 equiv of KI to Hg^{2+}, and when Hg^{2+} was added to the system again, the fluorescence could be reproduced. The results showed that the spectral sensing is reversible (S-Figure 8, Supporting Information).

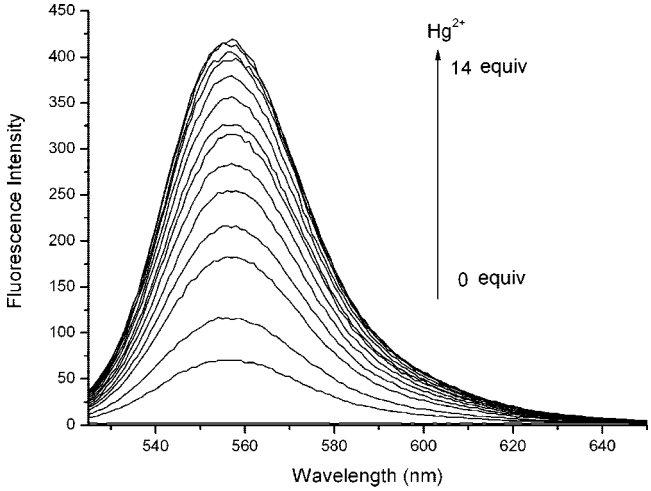

Figure 4. Fluorescence spectra of **1** (10 μM) in the presence of different concentrations of Hg^{2+} (0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 μM) in CH_3CN−HEPES buffer (0.01 M, pH 7.4) (1:99, v/v) (λ_{ex} = 515 nm, slit: 1.5 nm/1.5 nm).

To evaluate the feasibility for the application of the mercury sensor **1** to in vivo imaging, the bacteriovorous nematode *Caenorhabditis elegans* was used. Since *C. elegans* inhabits the interstitial water between soil particles and its hermaphroditic nature minimizes the genetic variation in nematode population, it has been considered as an ideal

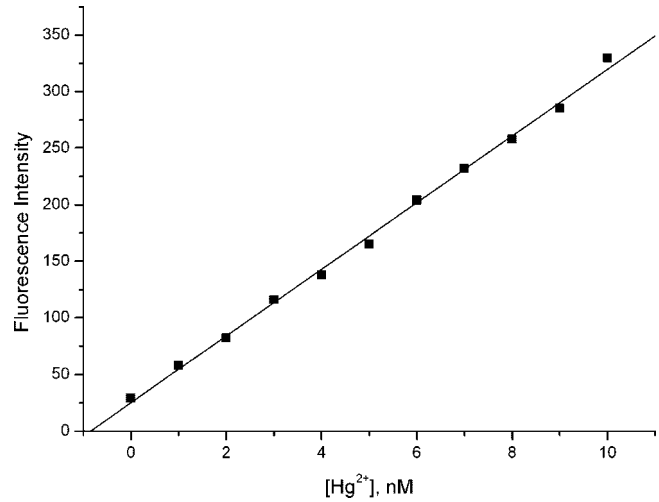

Figure 5. Plot of fluorescence intensity change of **1** (10 μM) against varied concentrations of Hg^{2+} from 1 to 10 nM (λ_{ex} = 515 nm, λ_{em} = 545 nm, slit: 5 nm/5 nm).

organism for testing the toxicity of aquatic media such as municipal and industrial wastewater.[8] Previously, the mortality of the nematode after exposure to metals was utilized to establish dose−response relationships.[9] In this study, young adult nematodes were incubated on a NGM (Nematode Growth Medium) agar plate containing $HgCl_2$ for 6 h at 25 °C.[10] Then, the nematodes were thoroughly washed with NGM buffer and then incubated with the mercury chemosensor (final concentration 200 μM) in NGM buffer for 20 min. Fluorescent images obtained from the nematodes are shown in Figure 6. When nematodes were incubated with the

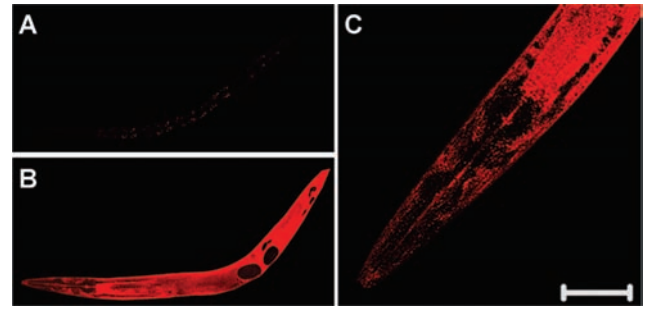

Figure 6. Imaging of *C. elegans* previously exposed to $HgCl_2$ with the mercury chemosensor. (A) A nematode previously not exposed to $HgCl_2$ showed only weak fluorescence in the intestine in the presence of the chemosensor. (B) A nematode previously exposed to $HgCl_2$ showed strong fluorescence in the presence of the chemosensor. (C) An enlarged image of the head region from the image (B). The scale bar represents 50 μM. To expose nematodes to $HgCl_2$, 250 μL of 50 μM $HgCl_2$ in NGM buffer were dropped onto NGM agar plates previously seeded with *E. coli* OP50 and dried at 25 °C before transferring nematodes on the plates. After 6 h of exposure, the nematodes were collected, washed three times with NGM buffer, and finally incubated with the mercury chemosensor (final conc. 200 μM) in NGM buffer for 20 min before the imaging.

mercury chemosensor alone, weak fluorescence was observed only in the intestine of the nematodes (Figure 6A).[11] When nematodes were first exposed to $HgCl_2$ and later incubated with the chemosensor, strong fluorescence was observed all over their body (parts B and C of Figure 6), indicating that mercury ions(II) might have penetrated into the nematodes through its cuticular layer. These results suggest that the chemosensor was highly suitable for sensing mercury ion(II) in vivo.

In conclusion, rhodamine 6G thiolactone derivative **1** was synthesized as a selective fluorescent and colorimetric sensor for Hg^{2+} in neutral aqueous solution. "Off-on"-type fluorescent and colorimetric changes were observed only for Hg^{2+}, which can be attributed to a spirothiolactone ring-opening process. Since sensor **1** can detect Hg^{2+} in the nanomolar range, this sensor has great potential for biological imaging and environmental purposes. The X-ray structure of **1**−Hg^{2+} supports and explains the 2:1 binding mode of sensor **1** with Hg^{2+}. Furthermore, our sensor was applied for in vivo imaging of Hg^{2+} using *C. elegans*.

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Supporting Information Available: Experimental procedures and characterization data of sensor **1**, fluorescent data, and job plot. This material is available free of charge via the Internet at http://pubs.acs.org.

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