Rhodamine Derivative Bearing Histidine Binding Site as a Fluorescent Chemosensor for Hg^{2+}

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Sensors based on the ion-induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of the fluorescence.[1] Especially, a few rhodamine B derivatives have also been used as fluorescent chemosensors for metal ions, in which the spirolactam (non-fluorescent) to ring opened amide (fluorescent) process was utilized.[2-6] Czarnik *et al.* reported a pioneering work utilizing this unique process, in which a rhodamine B hydrazide was utilized as a fluorescent chemodosimeter for Cu^{2+}.[2] Recently, we also reported a new fluorescent sensor based on rhodamine B for Pb^{2+}.[3] Structure of chemosensor and ring-opening process was confirmed by X-ray crystallography in addition to NMR, IR and ESI mass data. On the other hand, Tae *et al.* reported an intelligent and highly selective chemodosimeter system, which utilized an irreversible Hg^{2+}-promoted oxadiazole forming reaction of rhodamine derivative.[4] Recent few years, enormous efforts have been devoted to the development of new rhodamine based sensors for mercury ions[5] and other metal ions.[6] The "Off-On" type fluorescence enhancement along with colorimetric changes can be the important merits of rhodamine based sensors.

Mercury contamination occurs through oceanic and volcanic emission,[7] gold mining,[8] solid waste incineration, and etc. Due to the high toxicity of mercury, considerable attention has been devoted to the development of new fluorescent chemosensors for the detection of mercury and mercuric salts with sufficient selectivity.[9]

Herein, we synthesized a new rhodamine derivative bearing histidine unit (**1**). Among the various metal ions, chemosensor **1** displayed a highly selective 'Off-On" type fluorescent change with Hg^{2+}.

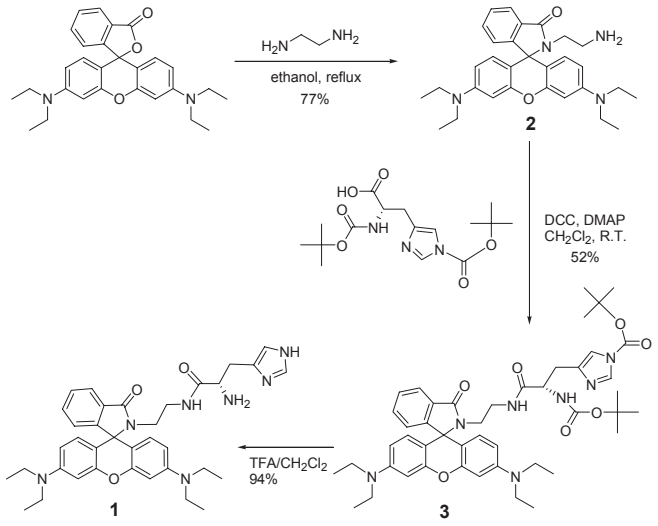
The intermediate **2**[5e] was efficiently synthesized from rhodamine B in a relatively good yield. Boc-protected histidine intermediate **3** was obtained in 52 % yield from the treatment of **2** with Boc protected histidine, DCC (N,N′-dicyclohexyl carbodiimide) and DMAP (4-dimethylamino pyridine) after the column chromatography using ETOAC-Hexane as eluent (1:1, v/v). Compound **1** was then obtained after the Boc deprotection using trifluoroacetic acid in 94% yield (Scheme 1). The ^1H and ^{13}C NMR spectra can be found in the supporting information.

The perchlorate salts of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} ions were used to evaluate the metal ion binding properties of compound **1** in 0.02M pH 7.4 HEPES: EtOH (1:9, v/v). The fluorescence spectra were obtained by excitation of the rhodamine fluorophore at 510 nm. Both the excitation and emission slits were either 5 nm. Among these metal ions (100 eq.), compound **1** showed an extremely selective fluorescence enhancement only with large with Hg^{2+} (Figure 1). An overall emission change of over 100-fold was observed for Hg^{2+}.

From the fluorescence titrations (Figure 2), the association constant of **1** with Hg^{2+} was observed to be 2.0×10^3 (errors < 15%).[10]

The proposed mechanism for these fluorescent changes can be attributed to the spiro-lactam ring opening, which was induced by the complexation of Hg^{2+}.[6a] Two carbonyl oxygens as well as imidazole nitrogen can provide a nice binding pocket for Hg^{2+}. Because the fluorescent enhancement disappeared upon the addition of excess 1,10-diaza-4,7, 14,17-tetrathiacyclooctadecane, it is believed that this process is reversible.

For the application of sensor **1** to cell-imaging, human cervical cancer cell line, HeLa (Korean cell line bank) were cultured in culture medium (MEM supplemented with 2 mM L-glutamine, 100 units/mL penicillin, 100 mg/mL streptomycin, and 10% heat-inactivated fetal bovine serum) at 37 °C in a



Scheme 1. Synthesis of compound **1**.

[a]contributed equally to this work

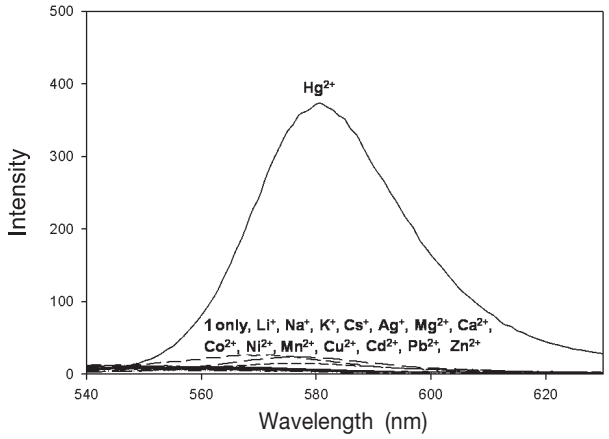


Figure 1. Fluorescent emission changes of **1** (6 μM) upon addition of various metal ions (100 eq.) in EtOH : HEPES = 9:1(v/v) (excitation at 510 nm) (excitation and emission slit: 5).

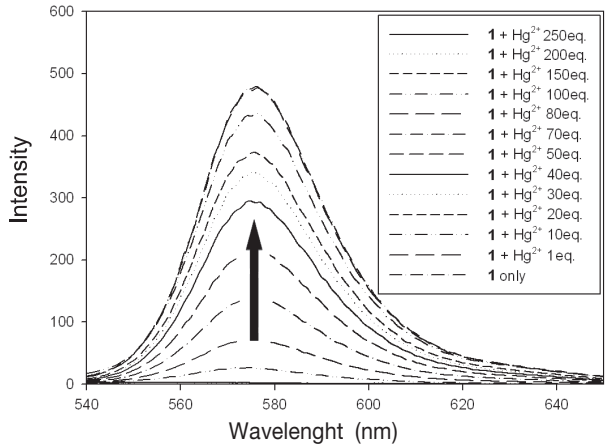


Figure 2. Fluorescent titrations of **1** (6 μM) with Hg^{2+} in EtOH: HEPES = 9:1 (v/v) (excitation at 510 nm) (excitation and emission slit: 5).

humidified incubator. Cells were seeded in a 6-well size confocal dish (coverglass bottom dish) at a density of 10^4 cells per well in a culture media overnight. HeLa cells were treated with 50 μM of compound **1** for 1 hour and washed with 3 times with PBS. Then cells were incubated with 50 μM $HgCl_2$ for 20 min and the cell cultures were washed with PBS to remove the remaining mercury ions. The images of the HeLa cell were obtained by a using confocal laser scanning microscope. Figure 3 explains the images of HeLa cells with sensor **1** (Figure 3a) and after the treatment of Hg^{2+} (Figure 3b).

In conclusion, a new rhodamine derivative bearing histidine group has been synthesized for the detection of Hg^{2+}. Sensor **1** displayed a highly selective fluorescent enhancement upon the addition of Hg^{2+}, in which the spirolactam (nonfluorescent) to ring opened amide (fluorescent) process was utilized. Furthermore, sensor **1** was successfully applied for sensing Hg^{2+} in the cell.

Experimental

Compound 3. To a 100 mL flask, 0.5 g (1.4 mmol) Boc protected histidine, 0.58 g (2.8 mmol) DCCL(N,N'-dicyclohexyl carbodiimide) and DMAP (catalytic amount) were dissolved in 10 mL distilled dichloromethane at room temperature. Add rhodamine B (0.68 g, 1.4 mmol) dropwise and the mixture was stirred for 12 hours in room temperature. Then the mixture was cooled and filtered. After Evaporation of the solvent, the crude product was purified by silica column chromatography (ETOAC: Hexane = 1:1, v/v) to give the redish yellow solid protected rhodamine derivative **3** in 52% yield (0.62 g): mp 103-105 °C; ^{1}H NMR (CD_3OD) δ 7.79 (m, 1H), 7.52 (s, 1H), 7.34 (t, 1H, J = 3.11 Hz), 7.06 (s, 1H), 6.96 (t, 3H, J = 2.86 Hz), 6.3 (m, 2H), 5.96 (d, 2H, J = 7.76 Hz), 4.3 (d, 2H, J = 6.16 Hz), 4.03 (q, 8H, J = 3.11 Hz), 3.25 (m, 2H), 2.88 (m,2H), 1.94 (m, 9H), 1.45 (m, 9H), 1.15 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 171.3, 169.6, 155.8, 154.1, 153.4, 149.1, 147.1, 139.8, 136.7, 132.8, 130.7, 128.6, 128.2, 124.0, 123.1, 114.6, 108.5, 105.1, 98.0, 85.4, 79.7, 77.7, 77.4, 77.1, 65.5, 60.5, 54.3, 44.5, 40.4, 39.7, 30.6, 28.6, 28.0, 21.2, 14.4, 12.8; HRMS (FAB) m/z = 822.4555 $(M+H)^+$, calc. for $C_{46}H_{59}N_7O_7$ = 822.4554.

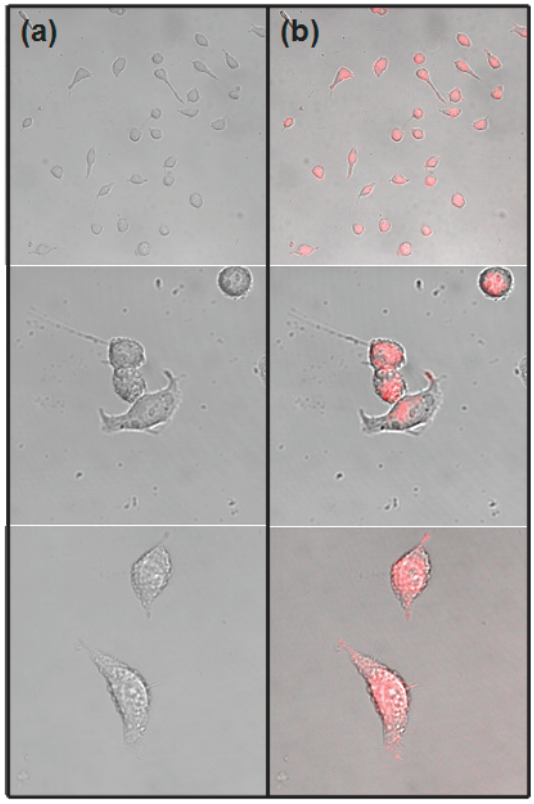


Figure 3. Images of HeLa cells treated with 50 μM of $HgCl_2$ after incubated with 50 μM of **1**. (a) fluorescent images of **1** only, (b) fluorescent images of HeLa cells treated with 50 μM of $HgCl_2$ after incubated with 50 μM of **1**. Images in a second low are enlarged by 20 times and third low images are enlarged by 60 times. (excitation = 556 nm, emission = 560-580 nm)

Compound 1. To rhodamine derivative **3** (0.62 g), add mixture of dichloromethane and trifluoroacetic acid (3:1, v/v), and stirred for 3 hours at room temperature. Compound **1** is afforded 0.58 g (94% yield) after removal of solvent under reduced pressure as a dark pink oily solid: ^{1}H NMR (CD_3OD) δ 8.87 (d, 2H, J = 1.1 Hz), 7.91 (m, 1H), 7.60 (m, 1H), 7.43 (s, 1H), 7.11 (dd, 2H, J = 4.5 Hz & 4.0 Hz), 6.83 (d, 3H, J = 7.0 Hz), 6.67 (m, 4H), 4.11 (t, 1H, J = 6.4 Hz), 3.53-3.22 (m, 11H),

2.87 (s, 2H), 1.13 (m, 12H); ^{13}C NMR (CD_3OD) δ 167.8, 165.6, 151.8, 133.1, 131.8, 129.9, 128.8, 127.4, 127.2, 125.5, 122.3, 121.1, 116.8, 64.1, 50.4, 46.7, 46.5, 46.4, 46.2, 46.0, 45.9, 45.7, 44.6, 44.0, 37.7, 37.0, 24.5, 10.0, 9.5; HRMS (FAB) m/z = 622.3503 $(M+H)^+$, calc. for $C_{36}H_{43}N_7O_3$ = 622.3506.

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