Rhodamine Derivative Bearing Histidine Binding Site as a Fluorescent Chemosensor for Hg²⁺

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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.²⁻⁵ Czarnik et al. reported a pioneering work utilizing this unique process, in which a rhodamine B hydrazide was utilized as a fluorescent chemodosimeter for Cu²⁺.² Recently, we also reported a new fluorescent sensor based on rhodamine B for Pb²⁺.³ Structure of chemodosimeter and ring-opening process was confirmed by X-ray crystallography in addition to NMR, IR and ESI mass data. On the other hand, Tao et al. reported an intelligent and highly selective chemodosimeter system, which utilized an irreversible Hg²⁺-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 ions² and other metal ions.⁵ 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, gold mining,⁶ solid waste incineration, and etc. Due to the high toxicity of mercury, considerable attention has been devoted to the development of new fluorescent chemo-sensors for the detection of mercury and mercury salts with sufficient selectivity.⁷

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 Hg²⁺.

The intermediate 2 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-dimethylaminopyridine) 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 ¹³C NMR spectra can be found in the supporting information.

The perchlorate salts of Ag⁺, Ca²⁺, Cd²⁺, Co²⁺, Cs⁺, Cu²⁺, Hg²⁺, K⁺, Li⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺ and Zn²⁺ 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 fluorescent 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 Hg²⁺ (Figure 1). An overall emission change of over 100-fold was observed for Hg²⁺.

From the fluorescence titrations (Figure 2), the association constant of 1 with Hg²⁺ was observed to be 2.0 × 10³ (errors < 15%)⁸

The proposed mechanism for these fluorescent changes can be attributed to the spiro-lactam ring opening, which was induced by the complication of Hg²⁺.⁹ Two carbonyl oxygens as well as imidazole nitrogen can provide a nice binding pocket for Hg²⁺. Because the fluorescent enhancement disappeared upon the addition of excess 1,10-diaza-4,7,14,17-tetraacyclooctadecane, 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 µg/mL streptomycin, and 10% heat-inactivated fetal bovine serum) at 37 °C in a

Scheme 1. Synthesis of compound 1.
humidified incubator. Cells were seeded in a 6-well size confocal dish (coverglass bottom dish) at a density of $10^3$ 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 HgCl₂ 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 using confocal laser scanning microscope. Figure 3 explains the images of HeLa cells with sensor 1 (Figure 3a) and after the treatment of Hg²⁺ (Figure 3b).

In conclusion, a new rhodamine derivative bearing histidine group has been synthesized for the detection of Hg²⁺. Sensor 1 displayed a highly selective fluorescent enhancement upon the addition of Hg²⁺, in which the spiro lactam (nonfluorescent) to ring opened amide (fluorescent) process was utilized. Furthermore, sensor 1 was successfully applied for sensing Hg²⁺ in the cell.

**Experimental**

**Compound 1.** 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 reddish yellow solid protected rhodamine derivative 3 in 52% yield (0.62 g): mp 103-105 °C; ¹H NMR (CDCl₃) 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), 6.30 (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); ¹³C NMR (CDCl₃) 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 C₂₉H₂₃N₂O₂ = 822.4554.

**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 reddish yellow solid protected rhodamine derivative 3 in 52% yield (0.62 g): mp 103-105 °C; ¹H NMR (CDCl₃) 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), 6.30 (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); ¹³C NMR (CDCl₃) 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 C₂₉H₂₃N₂O₂ = 822.4554.
2.87 (s, 2H). 1.13 (m, 12H); $^{13}$C NMR (CD$_3$OD) $\delta$ 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$_{33}$H$_{46}$N$_2$O$_4$ = 622.3506.

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