Selective and sensitive turn on detection of Hg$^{2+}$ in aqueous solution using a thioether-appended dipeptide

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A fluorescent chemosensor (4) was synthesized by coupling two dansyl labeled amino acids with the thioether bond. Compound 4 showed exclusively selective and sensitive response toward Hg$^{2+}$ among 14 tested metal ions in aqueous solution. Compound 4 exhibited 11-fold enhanced emission intensity in the presence of Hg$^{2+}$ (1 equiv). The detection limit of 4 for Hg$^{2+}$ in aqueous solution is 14.2 nM (2.8 ppb) and 4 showed a sensitive linear response to Hg$^{2+}$ in the concentration range (0–5 × 10$^{-7}$ M). The sensitive detection of Hg$^{2+}$ using 4 was not interfered by other competitive metal ions such as Cd$^{2+}$, Cu$^{2+}$, and Ag$^{+}$.

Mercury among the heavy metal ions has been regarded as the most toxic metal ions. As Hg$^{2+}$, the oxidized form of mercury accumulates in biological systems such as microorganisms, fish, and crops through the contaminated water, there is a high demand for a sensitive detection of Hg$^{2+}$ ions in aqueous solutions. Current several sensitive techniques for the determination of mercury ions including atomic absorption/emission spectrometry, stripping voltammetry, and inductively coupled plasma mass spectrometry generally require expensive instrumentation and laborious sample preparation. The fluorescence method for detecting mercury ions has been regarded as an alternative way due to its high sensitivity, simple and inexpensive instrumentation, and quick and easy sample preparation. Thus, various types of fluorescent chemosensors for Hg$^{2+}$ ions have been reported.

Fluorescent chemosensors can be classified into reversible chemosensors and chemodosimeters (reactive probes). Many different types of reversible fluorescent chemosensors were reported to show a sensitive response to Hg$^{2+}$. However, most of them displayed one or more drawbacks such as interference from other metal ions (e.g., Ag$^{+}$, Pb$^{2+}$, Cu$^{2+}$, and Cd$^{2+}$), synthetic difficulty, low aqueous solubility, fluorescence quenching, and low sensitivity in aqueous media. In special, turn off response by quenching mechanism is not favorable in practical applications because quenching process is non-selective and the decrease of fluorescence also can be induced by other factors such as precipitation of the chemosensor, absorbance of impurities, and pH change.

Thus, there is still a challenge to design water-soluble fluorescent chemosensors that detect Hg$^{2+}$ ions sensitively and selectively over competing metal ion contaminants by turn on response. A fluorescent chemosensor consists of a receptor part and a fluorophore part. The receptor part plays a critical role in the recognition of specific target molecules, whereas the fluorophore part converts the recognition events of target analytes into fluorescent signals. As the ligands containing soft donor element sulfur have a potent binding affinity for Hg$^{2+}$, several fluorescent chemosensors for Hg$^{2+}$ were designed to contain thioether or thiourea groups in a receptor part. Fluorescent chemosensors for Hg$^{2+}$ have been synthesized on the basis of various fluorophores such as dansyl, pyrene, anthracene, BODIPY, and so on. Among the various fluorophores, the dansyl fluorophore has been widely used for monitoring metal ions because this fluorophore is sensitive to micro-environmental change induced by metal binding via an internal charge-transfer (ICT) mechanism.

In recent years, several research groups including us have reported fluorescent chemosensors for heavy metal ions based on the scaffold of amino acids and peptides, because these biomolecules with biological compatibility and high water solubility had potent binding affinities to some metal ions in aqueous solutions. Previously, we synthesized dansyl labeled Cys and its oxidized form and investigated their responses to metal ions in aqueous solutions. Interestingly, the oxidized form of dansyl labeled Cys showed sensitive and selective response to Hg$^{2+}$ in aqueous solution. However the disulfide bond (–S–S) of the sensor can be easily reduced by mild reducing reagents or in reducing circumstances, which limits the practical application of this sensor for environmental field.
Considering the application of the chemosensor for environmental field, we extended our previous work to design a new fluorescent chemosensor (4), as shown in Scheme 1 and investigated the sensing ability of 4 for metal ions in aqueous solution. Compound 4 was synthesized by coupling two amino acids by the thioether bond as a linker as well as a binding site for Hg$^{2+}$ ion. Dansyl labeled Cys (1) was synthesized in solid phase synthesis following the published procedure. Compound 3 was prepared by treating L-serine methyl ester hydrochloride with dansyl chloride and then bromination. Compound 4 was easily synthesized in a high yield (70%) by coupling reaction of 1 and 3 in the presence of triethylamine (Scheme 1). The successful synthesis and high purity of 4 (>98%) were confirmed by analytical HPLC with a C18 column, ESI mass spectrometer, and $^1$H NMR (Figs. S1–S3).

All photochemical experiments of 4 were carried out in 10 mM HEPES buffer solution containing 1% CH$_3$CN at pH 7.4. Figure 1a shows the fluorescence response of 4 to various metal ions (Hg$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Pb$^{2+}$, Ag$^{+}$, Mg$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$) as perchlorate anion, and Na$^+$, Al$^{3+}$, and K$^+$, as chloride anion) by excitation with 380 nm. Among the tested metal ions, 4 showed sensitive turn on response only to Hg$^{2+}$ (Fig. 1a). As shown in Figure 1b, the fluorescence emission intensity increased with increasing the concentration of Hg$^{2+}$ and the intensity reached to plateau region at about 0.7 equiv of Hg$^{2+}$. Upon the addition of Hg$^{2+}$ (1 equiv), about 11-fold enhancement of the emission intensity at 500 nm was observed. This suggests that 4 selectively and sensitively detects Hg$^{2+}$ in aqueous solution by turn on response.

Compound 4 showed a sensitive linear response to Hg$^{2+}$ in the concentration range (0–500 nM) (Fig. 2). The detection limit of 4 was calculated as 14.2 nM (2.8 ppb) based on 3$\sigma$/m, where $\sigma$ is the standard deviation of the blank measurements, and m is the slope of the intensity versus sample concentration plot (Fig. 2). This confirms that 4 can detect qualitatively low levels of Hg$^{2+}$ in aqueous solutions.

Although the detection limit of 4 is slightly higher than the maximum allowable level (10 nM, 2.0 ppb) of Hg$^{2+}$ in drinking water demanded by the EPA, the low detection limit of 4 is much better than those of the other turn on chemosensors for Hg$^{2+}$ previously reported.$^{6e,7d,16}$ In general, the detection limit can be further optimized because the detection limit depends on the light source, detector, and integration time, and so on.

Figure 2 presents a visible emission change of 4 (10 μM) in the presence of various metal ions (1 equiv) under UV light ($\lambda$ = 365 nm) of UV lamp for thin layer chromatography. Compound 4 displayed a yellow color in the presence or absence of the other tested metal ions except Hg$^{2+}$, whereas 4 displayed a brighter color in the presence of Hg$^{2+}$. Compound 4 provides a simple and easy detection way for Hg$^{2+}$ using UV lamp.

We investigated the binding affinity and the binding stoichiometry of 4 in aqueous solutions. As shown in Figure 4a, Job’s plot exhibits a maximum at 0.34 mol fraction. This indicates that 4 may form a 2:1 complex with Hg$^{2+}$ in aqueous solution. The titration experiment indicates that 0.7 equiv of Hg$^{2+}$ was required for the complete change of the emission intensity (Fig. 1b), which also confirms that 4 may form a 2:1 complex with Hg$^{2+}$ and 4 has a tight binding affinity to Hg$^{2+}$ ions. Assuming a 2:1 complex formation, the association constant of 4 for Hg$^{2+}$ was calculated as 1.09 × 10$^{10}$ M$^{-2}$ ($R^2 = 0.966$) (ESI, Fig. S4). We investigated the binding mode of 4 with Hg$^{2+}$ by ESI mass spectrometry (ESI, Fig. S5). When Hg$^{2+}$ was added to the solution (50% CH$_3$CN/H$_2$O) of 4, a new peak appeared at 885.87 (m/z), which corresponds to [4+Hg$^{2+}$−H$^+$]. ESI mass spectra indicate that 4 directly interacted with Hg$^{2+}$-H$^+$.
with Hg^{2+} and formed a 1:1 complex in 50% CH$_3$CN/H$_2$O. This result suggests that the binding stoichiometry of 4 with Hg^{2+} may depend on the solvent. Thus, we investigated the binding stoichiometry and fluorescence response of 4 to Hg^{2+} in 50% CH$_3$CN–HEPES buffer solution. Interestingly, a job's plot with a maximum at 0.5 mol fraction indicates that 4 may form a 1:1 complex with Hg^{2+} in 50% CH$_3$CN–HEPES buffer solution. In addition, 4 showed a turn off response to Hg^{2+} ions in 50% CH$_3$CN–HEPES buffer solution (ESI, Fig. S6a). The titration result indicates that about 1.3 equiv of Hg^{2+} was required for the complete change of the emission intensity. The intensity change as a function of Hg^{2+} was well fitted using 1:1 complex model equation. The dissociation constant of 4 for Hg^{2+} in 50% CH$_3$CN–HEPES buffer solution was calculated as 2.19 \times 10^{-7} \text{ M} (R^2 = 0.97) by nonlinear regression analysis (ESI, Fig. S6b). Overall results suggest that even though 4 is tightly bound to Hg^{2+} in aqueous solution and mixed organic-aqueous solution, the binding stoichiometry and the binding mode might depend on the solvent.

The binding mode of 4 with Hg$^2+$ was characterized using UV absorbance spectroscopy, mass spectroscopy, and $^1$H NMR spectroscopy. The absorption spectrum of free 4 exhibited a maximum intensity at 216, 250, and 330 nm, respectively (ESI, Fig. S7). Upon the addition of Hg$^{2+}$, a gradual red shift at 330 nm was observed and the absorbance at 380 nm increased. This result suggests that the binding of Hg$^{2+}$ to 4 resulted in the increase of the electron density of the naphthyl moiety. The ESI mass spectra (ESI, Fig. S5) reveal that the deprotonated process may occur in the complex formation between 4 and Hg$^{2+}$ because the mass corresponding to [4+Hg$^{2+}$]$^-$ was not observed but the mass corresponding to [4+Hg$^{2+}$+H$^+$] was observed. The binding mode of 4 with Hg$^{2+}$ was further investigated by $^1$H NMR spectroscopy. $^1$H NMR experiments were carried out in CD$_3$CN (ESI, Fig. S8). 4 showed turn off response to Hg$^2+$ in 100% CH$_3$CN. When Hg$^2+$ was added, the disappearance of H(2) and H(7) corresponding to NH of the sulfonamide group was observed. The disappearance of these protons may be attributed to the coordination between Hg$^{2+}$ and NH of the sulfonamide group. The downfield shifts in H(13) and H(19) corresponding to the aromatic protons of the dansyl moiety suggest that Hg$^{2+}$ may induce the increase of electron density of the naphthyl moiety or directly interact with these protons of the dansyl moiety. The broadening of H(4) and H(5) was observed during the titration experiment, which suggests that Hg$^{2+}$ ion may coordinate with the thioether group. UV absorbance of NMR sample in the presence of Hg$^{2+}$ (0.5 equiv) confirmed the good solubility of 4 in NMR titration experiment (Data not shown). Overall results suggest that the sulfonamide groups of 4 may chelate with Hg$^{2+}$ and then the pK$_a$ value of the sulfonamide group decreased. The deprotonation of the NH group by chelation with Hg$^{2+}$ may induce the red shift by increase of the electron density of the naphthyl moiety. At the same time, the thioether group of 4 may chelate with Hg$^{2+}$. The binding mode of the previously reported fluorescent chemosensors containing dansyl fluorophore and/or the thioether group also suggests that the sulfonamide moiety and the thioether group of the chemosensors play an important role in the binding of target metal ions.

As an important feature of the chemosensor for practical application is a selective detection of target metal ion in the presence of other metal ions, we investigated whether 4 sensitively detects Hg$^{2+}$ in aqueous solution in the presence of other metal ions. Figure 5 shows that the turn on response of 4 to Hg$^{2+}$ was not significantly changed in the presence of the other metal ions (1 equiv) such as Ag$^+$, Cu$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$. Considering the cross sensitivity of the other Hg$^{2+}$ sensors toward Cu$^{2+}$, Ag$^+$, and Pb$^{2+}$, 4 has exclusive selectivity for Hg$^{2+}$ in aqueous solutions, which provides an advantage for practical application.

To test the reversibility, EDTA was added to solutions of 4-Hg$^{2+}$ that exhibited enhanced emission intensity (ESI, Fig. S9).

Upon the addition of EDTA to the solution, fluorescence emission spectrum changed immediately and returned to the Hg$^{2+}$ free spectrum, indicating that 4 detects Hg$^{2+}$ ions reversibly in aqueous solutions.

We measured the fluorescent spectrum of 4 in the presence and absence of Hg$^{2+}$ in different pH (Fig. 6). The maximum emission intensity of 4 in the absence of Hg$^{2+}$ was not sensitive to pH so the maximum emission intensity was not significantly changed in different pH (pH 4.5–11.5). However, the maximum emission intensity of 4 in the presence of Hg$^{2+}$ showed a dependence on pH. At pH 4.5, 4 showed no response to Hg$^{2+}$ due to the protonation of the dimethyl amino group (pK$_a$ ≈ 4) of dansyl fluorophore, which prevented charge transfer from the dimethylamino group to the naphthyl moiety. This indicates the enhancement of emission intensity of 4 in the presence of Hg$^{2+}$ is due to the electron donating ability of the dimethyl amino group to the naphthyl moiety. 4 exhibited a turn on response to Hg$^{2+}$ in pH 5.5–10.5. The largest enhancement of the emission intensity was observed at pH 7.4, showing that 4 is suitable for monitoring Hg$^{2+}$ ions in aqueous solution at physiological pH. At pH higher than 8.5, the emission intensity of 4 in the presence of Hg$^{2+}$ decreased as pH increased.

In conclusion, we synthesized a new fluorescent chemosensor 4 based on amino acids. Compound 4 displayed a high selectivity and sensitivity for Hg$^{2+}$ in aqueous solution. Compound 4 exhibited an 11-fold enhancement of emission intensity in the presence of Hg$^{2+}$ in aqueous solution at neutral pH. Compound 4 with the detection limit of 14.2 nM (2.8 ppb) can detect low level of Hg$^{2+}$ ions without interference of the other competitive metal ions. The simple structure and high selectivity and sensitivity of 4 provide a valuable way for detecting Hg$^{2+}$ ions in aqueous solutions.
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Supplementary data

Supplementary data (experimental part, characterization data, fitting curve, mass of complex, titration with Hg²⁺, reversibility, ¹H NMR titration and the absorption spectra of compound 4) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.07.003.

References and notes


3. Compound 3. In a 25 mL round-bottom flask, l-serine methyl ester hydrochloride (100 mg, 0.64 mmol) was dissolved in 10 mL of DCM/DMF (3:1, v/v) solution and then dansyl chloride (205 mg, 0.76 mmol) and triethylalmine (268 µL, 1.92 mmol) in DCM/DMF were added into the solution. The resulting solution was stirred for 3 h at room temperature. The reaction solution was diluted with water and the product was extracted with DCM. DCM was removed by rotary evaporator and the resulting crude product was purified by column chromatography over silica gel to obtain 2. A mixture of 2 (50 mg, 0.142 mmol) and triphenylphosphine (74.5 mg, 0.284 mmol) were dissolved in DCM (5 mL) at 0 °C in nitrogen atmosphere and then carbon tetra-bromide (75.3 mg, 0.227 mmol) in 5 mL of DCM was added dropwise. The solution was stirred at 0 °C for 30 min and then stirred for 2 h at room temperature. The solution was diluted by adding 20 mL of water and the product was extracted with DCM. The organic solvent fraction was separated and DCM was removed by rotary evaporator. The crude solid product was re-dissolved in ethyl acetate and washed with water and then the organic layer was separated. And ethyl acetate was removed by rotary evaporation to get a solid crude product. The crude product was further purified by column chromatography on silica gel eluting with hexane/ethyl acetate (2:1, v/v) as eluent to give 78% of 3.

4. Compound 4 was synthesized by coupling reaction of 1 and 3 in presence of triethylaline. To a mixture of 1 (30 mg, 0.084 mmol) and 3 (34.77 mg, 0.084 mmol) in dry DCM, Et3N (70.29 µL, 0.504 mmol) was added. The reaction solution was stirred for 3 h at room temp. After 3 h, solvent was evaporated in vacuo and by finally purified by semi-preparative HPLC with a C18 column using a water (0.1% TFA)-acetonitrile (0.1% TFA) gradient to give 70% yield. The successful synthesis was confirmed by ESI mass spectrometry (Platform II, micromass, Manchester, UK) and its homogeneity (>95%) was confirmed by reverse phase analytical HPLC with a C18 column: mp 105–106 °C, H NMR (400 MHz, CDCl3) δ 8.67 (2H, d, J = 8.5 Hz), 8.52 (2H, d, J = 8.4 Hz), 8.34–8.30 (2H, m), 7.81–7.71 (4H, m), 7.60 (2H, d, J = 8.4 Hz), 6.58–6.52 (1H, m), 6.46–6.44 (1H, m), 6.41 (1H, br s), 5.88 (1H, br s), 3.97–3.92 (1H, m), 3.83–3.76 (1H, m), 3.31 (3H, s), 3.14 (12H, s), 2.54–2.39 (4H, m). ESI-mass [m/z]: [M+H]⁺ calcd for C55H37N10O12, 964–984; (b) Li, H.-Y.; Dang, Y.-Q.; Ma, L.-J.; Wu, Y.; Hoq, G.; Wu, L. Chem. Commun. 2009, 4453–4455; (c) Neupane, L. N.; Kim, J.-M.; Lee, K.-H. Org. Lett. 2013, 15, 254–257; (g) Yang, M.-H.; Thirupathi, P.; Lee, K.-H. Org. Lett. 2011, 13, 5028–5031; (b) Cheng, R. P.; Fisher, S. L.; Imperiali, B. J. Am. Chem. Soc. 1996, 118, 11340–11346; (i) Neupane, L. N.; Thirupathi, P.; Jang, S.; Jang, M. J.; Kim, J. H.; Lee, K.-H. Talanta 2011, 85, 1566–1574; (j) Zheng, Y.; Gattás-Afšura, K. M.; Konka, V.; Leblanc, R. M. Chem. Commun. 2002, 2350–2351.