A highly sensitive and selective detection of Hg(II) in 100% aqueous solution with fluorescent labeled dimerized Cys residues†

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A simple design of a ratiometric fluorescent sensor for detecting Hg(II) ion in 100% aqueous solution was demonstrated, based on the structure of dimerized Cys residues with two dansyl fluorophores. The sensor highly sensitively and selectively detected mercury ion ($K_d = 41$ nM) in 100% aqueous solution *via* a turn-on and ratiometric response. The sensor showed no interferences of other metal ions and satisfied for monitoring the maximum allowable level (2 ppb) of mercury ion in drinking water demanded by EPA *via* a turn-on response.

Introduction

Among heavy and transition metal ions, mercury is considered as a highly toxic metal ion and its environmental contamination caused serious problems for human health and ecology.[1] A major source of human exposure was mainly due to the contamination of natural waters and long or heavy exposure to mercury will damage the digestive organs, kidneys, central nervous system and endocrine system.[1] In order to detect Hg(II) in the environmental or biological samples, considerable efforts have been made to develop colorimetric or fluorescent Hg(II) sensors.[2–4] Fluorescence has been regarded as a most powerful optical technique for detecting low concentration of mercury ion in water. Some degree of successful accomplishment has been achieved in the development of fluorescent Hg(II) sensors. However, fluorescent sensors that provide turn on response to Hg(II) are still particularly valuable because mercury ion like many other heavy metal ions, causes quenching of fluorescence *via* the well defined mechanism, electron transfer to the metal ions. Thus, ideal fluorescent Hg(II) sensors are required for turn on response with reversible Hg(II)-dependent chemical reaction. Furthermore, the concentration of the common heavy metal ions are generally much higher than the concentration of Hg(II) in drinking water that is allowed less than 10 nM (2 ppb) by the EPA.[5] Therefore, new fluorescent mercury sensors require water compatibility as well as a extremely high sensitivity for Hg(II) in 100% aqueous solution. In addition, ratiometric sensing is highly recommended because this property makes it possible to measure the analytes more accurately with minimization of background signal. However, ratiometric fluorescent sensors for the detection of Hg(II) in aqueous solution were rarely reported. Recently, Nolan and Lippard reviewed the sensors for optical detection of Hg(II).[2a] Only a few chemical sensors are known showed ratiometric fluorescent response to Hg(II) in aqueous buffer solution.[2a,4d] However, these sensors did not satisfy for monitoring the maximum allowable level (2 ppb, 10 nM) of Hg(II) in drinking water demanded by the Environmental Protection Agency (EPA) or suffered from interference either by Cu^{2+}, Zn^{2+}, or Cd^{2+}. Therefore, it is highly challenging for the synthesis of new simple fluorescent sensors that satisfy for monitoring Hg(II) below 10 nM in 100% aqueous solution *via* turn on and ratiometric response.

The soft ligands such as thiol and thioether are frequently found in the Hg(II) sensors.[2] The high affinity of Cys residues for mercury is well known and methylmercury, a highly toxic organic compound of mercury is mostly found complexed with free cysteine and with proteins and peptides containing cysteines.[6] In nature, proteins such as MerP and MerR family that shared cysteine rich metal binding motif play an important role in the detoxification and transport systems of Hg(II).[7] We and other researchers have previously reported fluorescent peptide sensors containing cysteine rich metal binding motif that exhibit sensitive response to heavy metal ions including Hg(II) in aqueous solution.[8] In comparison to the properties of chemical sensors, it is generally accepted that amino acids and peptides have good solubilities in a neutral buffer solution and show potent binding affinities for specific metal ions in 100% aqueous solution. The straightforward way to guarantee a ratiometric sensing is to use an internal charge transfer fluorophore with strong electron donor and acceptors within the designed sensors. Thus, we design a new mercury sensor based on L-Cys residue with a dansyl [3-dimethylamino-1-naphtalenesulfonamido] fluorophore in this research. The dansyl fluorophore has been extensively used in a wide variety of fluorescent chemical sensors because the sulfonamide of the dansyl group has been used to detect metal ions by chelation enhanced fluorescence (CHEF) effect and the dansyl fluorophore is sensitive to the polarity of its microenvironment because of the charge-transfer character of the transition.[4b,4e,4i,8–11]

As shown in Scheme 1, we synthesized a L-Cys residue with a dansyl fluorophore (**Mcys**) and dimerized L-Cys residues (**Dcys**) with two dansyl fluorophores. **Mcys** containing a free thiol group showed response to several heavy metal ions including Hg(II) in 100% aqueous solution, whereas **Dcys** showed only response to Hg(II) among heavy transition metal ions. Furthermore, **Dcys** shows distinct advantages for sensing Hg(II) such as a fast, reversible, turn-on, and ratiometric response in a neutral buffer

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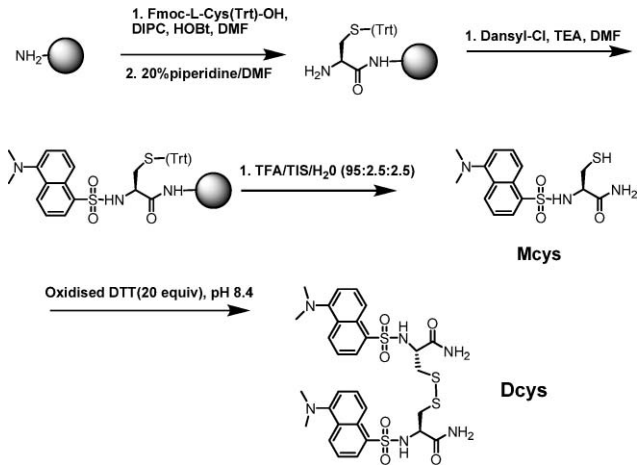
† Electronic supplementary information (ESI) available: UV-Vis absorption spectra, HPLC chromatogram, ESI mass spectra, and ^{1}H NMR and ^{13}C NMR of **Mcys** and **Dcys**. Emission spectra of **Dcys** at different pH. See DOI: 10.1039/b925744f



Scheme 1 Synthesis of **Dcys** and **Mcys**.

solution. **Dcys** showed exclusive selectivity for Hg(II) because the monitoring ability for Hg(II) was not interfered with the presence of other heavy metal ions such as Cd(II), Pb(II), and Cu(II). The sensor that has a simple structure satisfies for monitoring the maximum allowable level (2 ppb, 10 nM) of Hg(II) in drinking water demanded by EPA *via* a turn-on and ratiometric response in a neutral buffer solution.

Results and discussion

Solid phase synthesis of Amino acid probes

The probes were synthesized by Fmoc-chemistry in solid phase synthesis (Scheme 1).[12] After cleavage of the product from resin, the dansyl labeled Cys residue was purified from crude product by semi-preparative HPLC with a C_{18} column. The successful synthesis and purity of **Mcys** (>95%) were confirmed by analytical HPLC with a C_{18} column and ESI mass spectrometer. **Dcys** was synthesized by oxidation of **Mcys** in the presence of 20 equiv. of oxidized dithiothreitol (DTT). The synthesis and purity of **Dcys** (>95%) were confirmed by analytical HPLC with a C_{18} column and ESI mass spectrometer. Details on the synthesis and characterization of **Mcys** and **Dcys** are described in experimental section.

As **Dcys** and **Mcys** have good solubility in water, the stock solutions of the compounds were prepared in 100% distilled water and all of the photochemical experiments were carried out in 10 mM HEPES buffer solution. Fig. 1 shows fluorescence response of **Dcys** and **Mcys** in the presence of each metal ions (Ca^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Ag^{+}, Mg^{2+}, Mn^{2+}, Ni^{2+},Hg^{2+}, Zn^{2+} as perchlorate anion and Na^{+}, Al^{3+}, K^{+}, as chloride anion) by excitation with 330 nm. **Dcys** exhibits an outstanding selectivity to Hg(II) and turn-on response with an obvious blue shift (34 nm) of the maximum emission intensity. **Dcys** shows no fluorescent response to the other metal ions except Hg(II), whereas **Mcys** containing free thiol group exhibits response to several heavy and transition metal ions except alkali and alkaline earth metal ions. **Mcys** showed turn on response to Zn(II), Cd(II), and Ag(I) but turn off response to Cu(II), Hg(II), and Pb(II).

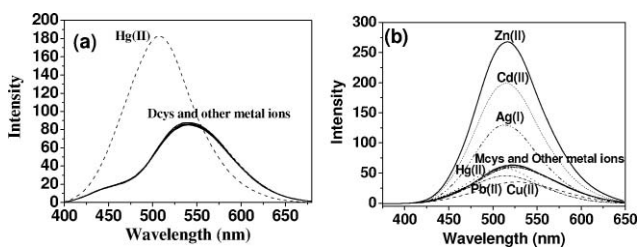


Fig. 1 Fluorescence spectra of (a) **Dcys** (5 μM) and (b) **Mcys** (5 μM) in 10 mM HEPES buffer solution (pH = 7.4) in the presence of various metal ions (2 equiv.) except Mg(II), Ca(II), Na(I) and K(I) which were used 1000 equiv. (λ_{ex} = 330 nm).

The fluorescent response of **Dcys** to the amount of Hg(II) was measured in 10 mM HEPES buffer solution (pH = 7.4). Upon the addition of increasing concentration of Hg(II), about 3 fold enhancement of the maximum intensity and 34 nm blue shift from 541 to 507 nm of the maximum emission intensity were observed (Fig. 2). A significant increase of the emission intensity around 507 nm and decrease at 583 nm were observed with a single isosbestic point at 555 nm. The intensity ratio (I_{507}/I_{583}) at 507 and 583 nm increased with the concentration of Hg(II) (0–1 equiv.). In the titration curve of intensity ratio *versus* concentration, about 1 equiv. of Hg(II) was required for the saturation of the emission intensity of **Dcys** (5 μM), which suggested that **Dcys** has a great sensitivity to Hg(II) in 100% aqueous solution (Fig. 3). A Job's plot, which exhibits a maximum at 0.5 mole fraction indicates that the sensor forms 1 : 1 complex with Hg(II). Assuming 1 : 1 complex formation, the dissociation constant was calculated based on the titration curve with Hg(II) by non-linear least square fitting. The

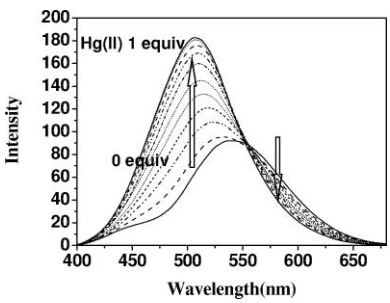


Fig. 2 Fluorescence emission spectra of **Dcys** (5 μM) in the presence of increasing concentration of Hg(II) (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 equiv.) in 10 mM HEPES buffer (pH 7.4), λ_{ex} = 330 nm.

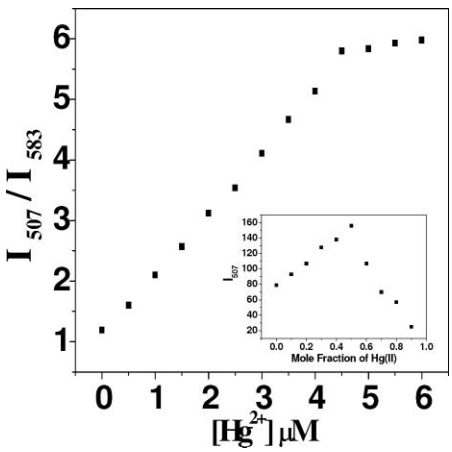


Fig. 3 Titration curve of **Dcys** (5 μM) with Hg(II) and a Job plot for **Dcys** in 10 mM HEPES buffer solution (pH 7.4).

dissociation constant, K_d (4.12×10^{-8}M, $R^2 = 0.9963$) indicated that **Dcys** has a potent binding affinity for Hg(II) in a neutral buffer solution.

The sensitivity of **Dcys** for Hg(II) was calculated on the basis of the linear relationships between the maximum emission intensity at 507 nm and the concentration of Hg(II). The intensity at 507 nm increased linearly with the concentration of Hg(II) and a linear measurement is possible until 0.6 equiv. with high accuracy (Fig. 4A). The inflection point of the intensity *vs.* concentration was calculated to be 7.9 μM. This sensor has a detection limit of 7.8 nM (1.6 ppb), based on $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity *versus* sample concentration. We also measured the sensitivity of the sensor for monitoring nanomolar concentration of Hg(II) ranged from 0 nM to 500 nM using 500 μM of **Dcys**. Interestingly, the sensor showed a good linear response to nanomolar concentration of Hg(II) in 100% aqueous solution, as shown in Fig. 4B. This suggests **Dcys** can be used to quantitatively detect the low level of Hg(II) in aqueous solution. The detection limit of **Dcys** for Hg(II) is lower than the EPA's drinking water maximum allowable level (10 nM). We expect that this detection limit of the sensor for Hg(II) can be optimized by further modification of C-terminal amide form of the sensor and can be improved by optimization techniques such as a more intense light source, a longer integration time, and slit size.

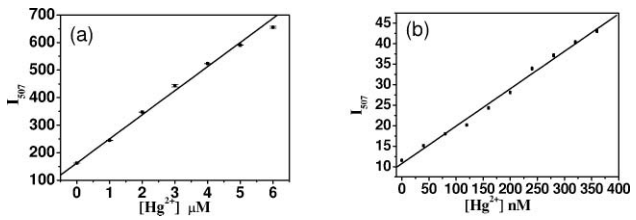


Fig. 4 Emission intensity ($I_{507\,nm}$) change of (a) **Dcys** (10 μM) and (b) **Dcys** (500 nM) with Hg(II) in 10 mM HEPES buffer solution (pH 7.4), $\lambda_{ex} = 330$ nm.

To test reversibility, EDTA was added to the **Dcys**-Hg(II) complex that exhibited a strong emission intensity at 507 nm. The addition of EDTA instantly resulted in the change of the emission intensity (Fig. 5). Addition of about 200 equiv. of EDTA to the **Dcys**-Hg(II) complex results in an immediate return of the original metal free spectrum, which demonstrates the reversibility of **Dcys** and potent binding affinity for mercury ion.

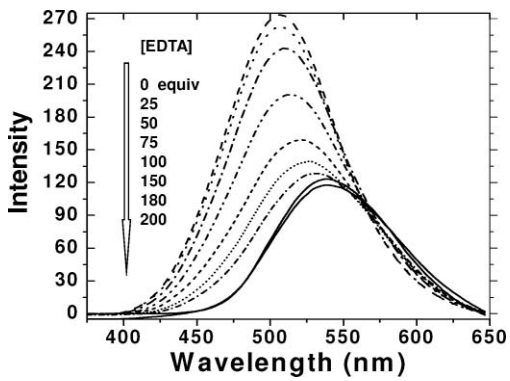


Fig. 5 Emission spectra of **Dcys**-Hg^{2+} complex in the presence of EDTA (0, 25, 50, 75, 100, 150, 180 and 200 equiv.) in 10 mM Hepes buffer, pH 7.4, $\lambda_{ex} = 330$ nm.

The pH influence on the fluorescence intensity of **Dcys** was investigated in the presence and absence of Hg(II) (Fig. 6). At lower pH than 4.5, the sensor exhibited very weak emission intensity, regardless of the presence and absence of Hg(II). Previously reported chemical and peptide sensors containing dansyl fluorophore also showed very weak emission intensity in acidic condition.[11] This weak emission intensity of **Dcys** at acidic condition was due to the protonated dimethylamino group ($pK_a \cong 4$) of dansyl fluorophore, which might prevent the charge transfer between dimethylamino group and naphthyl moiety.[11,13] The great intensity difference between **Dcys** and **Dcys**-Hg(II) was observed in a neutral and weak acidic pH (pH > 4.5). When the pH was higher than 4.5, the emission intensity of **Dcys**-Hg(II) increased.

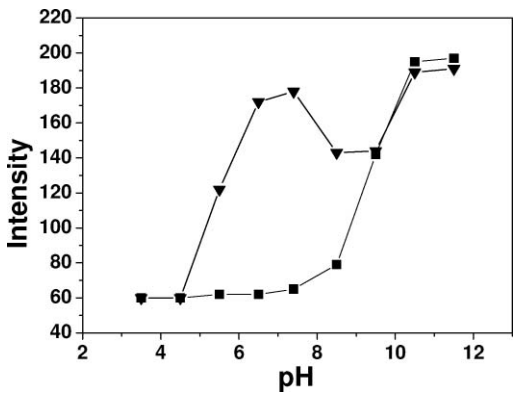


Fig. 6 Emission intensity of **Dcys** in the presence (▼) and absence of (■) Hg^{2+} (1 equiv.) at different pH ($\lambda_{ex} = 330$ nm).

At pH > 9, the intensity of free **Dcys** increased with increasing pH. This might be due to the deprotonation of NH of the sulfonamide group ($pK_a \cong 10$). Specially, the emission spectrum of free **Dcys** measured at pH = 11 was similar to that of the **Dcys**-Hg(II) complex (Fig S11†). This result suggests the mechanism of the emission spectrum change of **Dcys** in the presence of Hg(II) at a neutral pH. The similar blue shift of the emission spectra and an enhancement of the emission intensity were observed as results of the deprotonation of the sulfonamide group of dansyl fluorophore of chemical sensors.[11,12] This deprotonation process induced by Hg(II) was also confirmed by ESI mass spectrum (Fig. 7). Peak *m/z* 705.20 and 727.20 values corresponded to [**Dcys** + H^+]$^+$ and [**Dcys** + Na^+]$^+$, respectively. When 1.0 equiv. of Hg(II) was added, the peak of [**Dcys** +Na^+]$^+$ disappeared and the new peak at 905.00 corresponding to [**Dcys** + Hg^{2+}-H^+]$^+$ was observed, indicating that the deprotonation process proceeded in the ground state. Thus, we can conclude that Hg(II) chelates **Dcys** through a deprotonation process of the NH of the sulfonamide group and this process leads to a blue shift of the emission spectrum, which can be rationalized by a reduction of the acceptor character of sulfonamide.

To further look into the nature of the interactions between **Dcys** and Hg(II), ^{1}H NMR experiments were carried out in DMSO-d_6 (Fig. 8). For free **Dcys**, the chemical shift of NH of the dansyl sulfonamide group is 7.11 ppm, whereas the complexation of **Dcys** by Hg(II) results in the disappearance of NH peak of the sulfonamide group and downshifts (0.1–0.3 ppm) of the aromatic protons of the dansyl moiety. ^{1}H NMR spectra support that

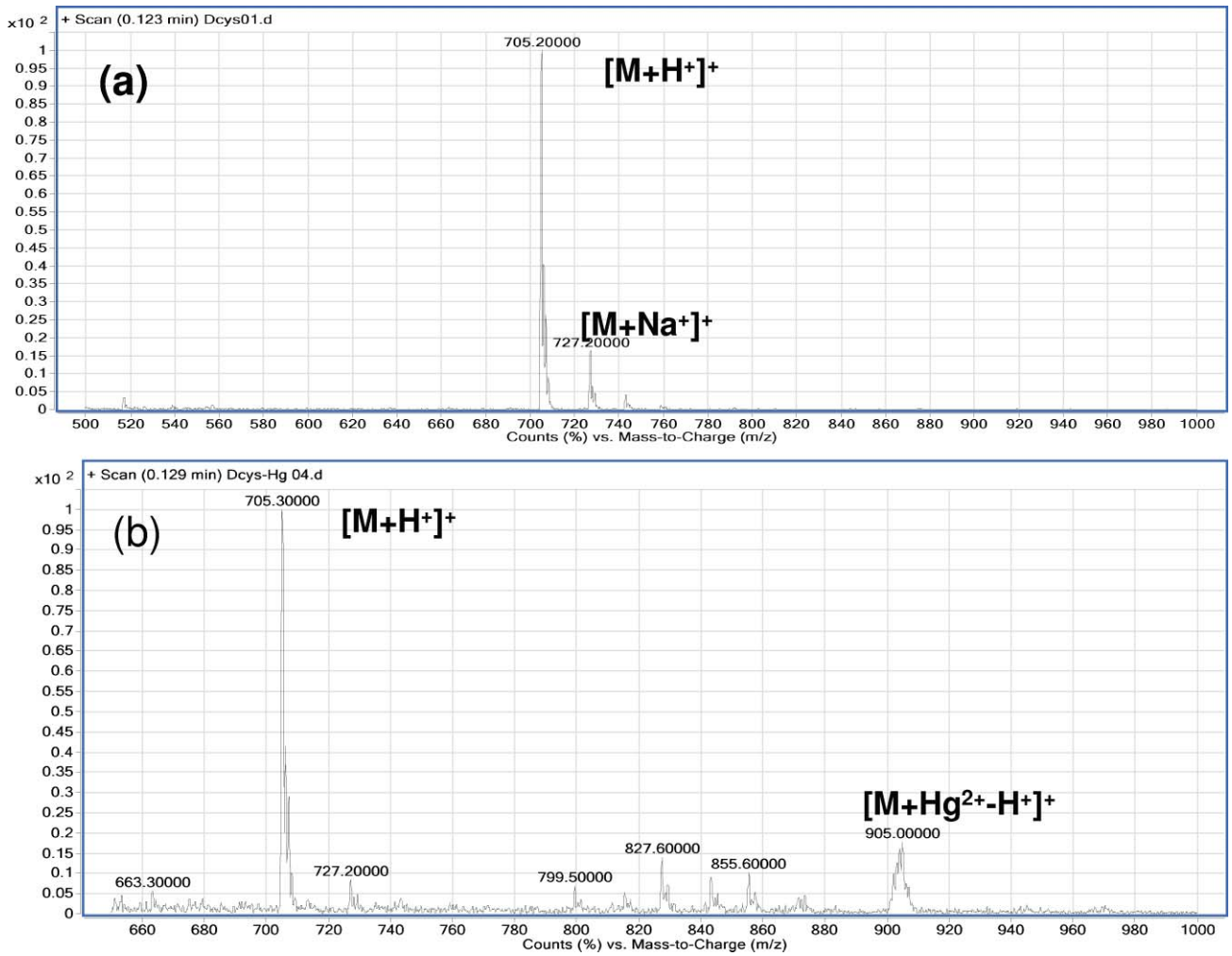


Fig. 7 ESI mass spectrum of **Dcys** in the absence (a) and presence (b) of 1 equiv. of $Hg(ClO_4)_2$.

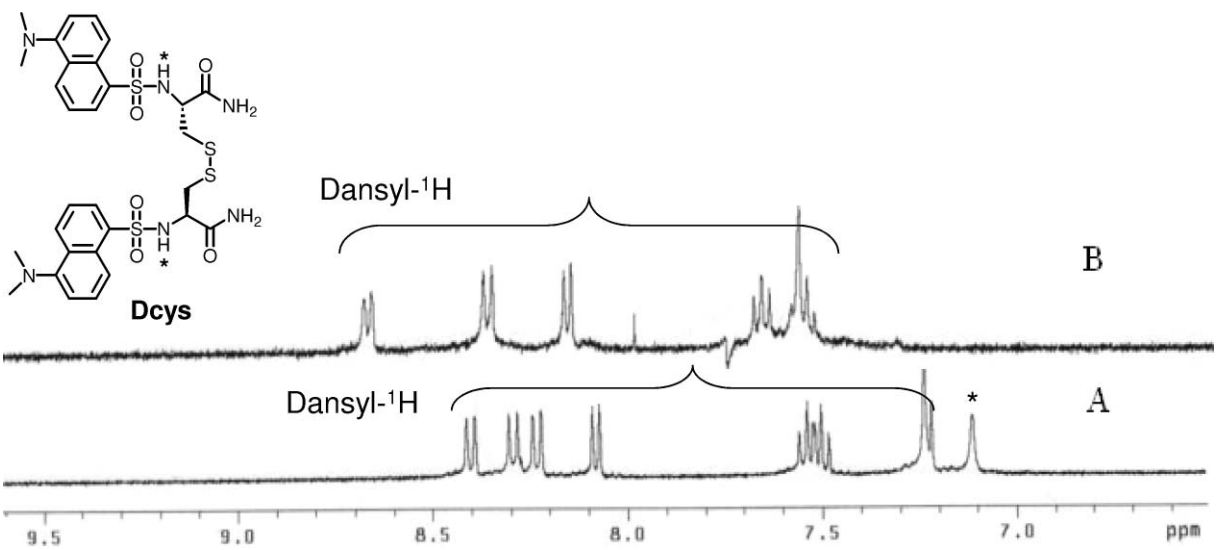


Fig. 8 Partial ^1H NMR (400 MHz) of **Dcys** (5.7 mM) in DMSO-d_6 at 25 °C in the absence (a) and presence (b) of 2 equiv. of $Hg(ClO_4)_2$.

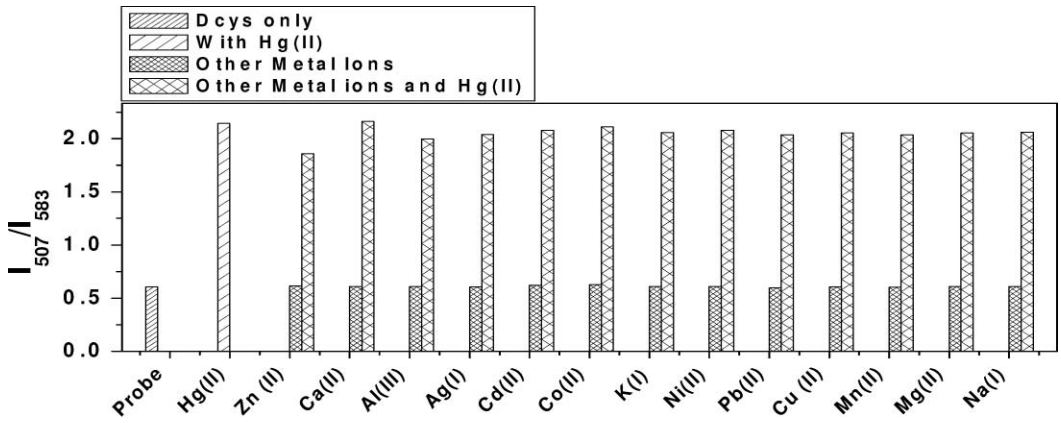


Fig. 9 Fluorescence response of **Dcys** (5 μM) in the presence of Hg(II) (2 equiv.) and additional various metal ions in 10 mM HEPES buffer (pH 7.4). All metal ions are evaluated at one equivalent to Hg(II) except Na(I), K(I), Ca(II), and Mg(II), which are used at 1000 equiv. (λ_{ex} = 330 nm).

chelation of Hg(II) with the nitrogen atom of the sulfonamide groups increased electron withdrawing effect of the sulfonamide groups on the napthalene moiety. On the basis of NMR and ESI mass spectra, we confirmed that the blue shift and enhancement of the emission intensity of **Dcys** in the presence of Hg(II) may be attributed to the strong binding between the Hg(II) and the sulfonamide group of the dansyl moiety, which induced the disruption of internal charge transfer between the sulfonamide group and the napthalene moiety of the dansyl fluorophore.

We contemplate the reason why **Dcys** selectively detects Hg(II) ions. Both ^{1}H NMR and ESI mass spectrum strongly support that the two sulfonamide groups of the dansyl moieties play a critical role in stabilizing Hg(II)-**Dcys** complex in aqueous solution. As dansyl that has a strong acidic sulfonamide group (pK_a ≅ 10) produces a strong binding ability with cations, dansyl has been extensively used as a fluorophore in a variety of fluorescent chemical sensors for metal ions.[4b,4e,4i,8–11] Considering the structures of the previously reported Hg(II) sensors, interestingly, two sulfonamide groups were required for selective detection of Hg(II) in two dansyl appended calixarene sensor and a rhodamine fluorophore containing two tosyl group.[11b,13] Recently, Lixin Wu *et al.* reported that a water-soluble fluorescent probe, dansyl-L-tryptophan methylester showed a high selective detection of Hg(II) in buffered aqueous solution.[4e] Their X-ray crystal structure of the complex indicated that the two dansyl amino acids were required for the binding of one Hg(II). Their detailed crystallographic data revealed that the deprotonated NH of the sulfonamide group resulted in a strong interaction of the nitrogen atom and Hg(II) and that the two sulfur and four oxygen atoms in sulfonamide group stabilized the complex through weak interactions. On the basis of the binding mode of dansyl labelled L-Trp for Hg(II) and the spectroscopic data of **Dcys**, we can briefly explain the binding mode of **Dcys** for Hg(II). The two deprotonated NH of the sulfonamide groups of **Dcys** strongly interacts with Hg(II), resulting in high stability of **Dcys**-Hg(II) complex in aqueous solution just like the complex between dansyl labelled Trp and Hg(II). Li *et al.* investigated the binding interactions of Hg^{2+} ions towards human serum albumin (HSA).[14] Their spectroscopic data revealed that sulfur atoms of disulfide bonds of HSA are responsible for the binding sites to coordinate to Hg(II) ions with a certain geometry conformations. The disulfide bond (-S–S-) of **Dcys** may stabilize a certain conformation to provide a proper proximity and orientation of the two sulfonamide groups for interactions with Hg(II). In addition, we assumed that the disulfide bond may stabilize the complex through weak interactions because the slight downfield shift of methylene proton (-CH_2–S-) was observed in the presence of Hg(II) in NMR spectra.

To investigate the interference effect of other metal ions on the detection ability of **Dcys** for Hg(II), the fluorescence response of **Dcys** to Hg(II) in the presence of other metal ions was measured. Fig. 9 shows the fluorescence emission change of **Dcys** upon the addition of each metal ion (Ca^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Ag^{+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Na^{+}, Al^{3+}, K^{+}). The Hg(II)-dependent fluorescence change of **Dcys** was not affected by the presence of 5 mM Group I and Group II metal ions such as Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}. Specially, the fluorescence spectrum of **Dcys**-Hg(II) was not changed by other heavy and transition metal ions including Cu(II) and Cd(II). Most reported mercury sensors that work in aqueous solution displayed cross-sensitivities toward other heavy metal ions.[2–4] In addition, almost all chemical and peptide sensors containing dansyl fluorophore suffer from interference of Cu(II),[8–11] whereas even though **Dcys** also contained a dansyl fluorophore, **Dcys** showed no interference of Cu(II) that is relatively higher concentration than other heavy and transition metal ions in water.

Conclusions

We report a new small fluorescent sensor, which satisfies for monitoring the maximum allowable level of Hg(II) in drinking water demanded by EPA. The sensor has a simple and unique symmetric structure based on Cys dimer bearing two dansyl groups. This sensor exhibited a high selectivity and sensitivity toward Hg(II) ion over a wide range of metal ions in 100% aqueous solution *via* a turn-on and ratiometric response in 100% aqueous solution. Moreover, the presence of other metal ions did not interfere with the detection of Hg(II) ion. **Dcys** could be potential in practical applications for monitoring low level contamination of Hg(II) in environmental samples.

Experimental

General

Fmoc-Cys(Trt)-OH,*N*,*N*'-diisopropylcarbodiimide, 1-hydroxybenzotriazole, and Rink Amide MBHA resin were from advanced chem tech. Other reagents for solid phase synthesis including trifluoroacetic acid (TFA), triisopropylsilane (TIS), dansyl chloride, triethylamine, diethyl ether, *N*,*N*-dimethylformamide (DMF), and piperidine were purchased from Aldrich.

Solid phase Synthesis of Mcys and Dcys

Mcys was synthesized by solid phase synthesis with Fmoc chemistry.[15] Fmoc protected L-Cys residue was assembled on Rink Amide MBHA resin, as shown in Scheme 1. After deprotection of Fmoc group, the coupling of dansyl chloride was performed by the following procedure. To the resin bound amino acid (65 mg. 0.05 mmol), dansyl chloride (40 mg, 0.15 mmol, 3 equiv.) in DMF (3 ml) and triethylamine (20 µl, 0.15 mmol, 3 equivalent) were added. Cleavage of the peptide from the resin was achieved by treatment with a mixture of 3 ml TFA: TIS: H_2O (95 : 2.5 : 2.5 v/v/v) at room temperature for 3 h. After filtration and washing of the resin by TFA, a gentle stream of nitrogen was used to remove the excess TFA. The crude **Mcys** was triturated with diethyl ether chilled at −20 °C and then centrifuged at 3,000 rpm for 10 min at −10 °C. The crude product was purified by prep-HPLC with a Vydac C_{18} column using a water (0.1% TFA)-acetonitrile (0.1% TFA) gradient to give 12 mg of **Mcys** (yield 78%). 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 C_{18} column: mp 189–191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, 1H, J = 4.4 Hz), 8.33 (d, 1H, J = 4.4 Hz), 8.25 (d, 1H, J = 4.2 Hz), 8.15 (d, 1H, J = 3.6 Hz), 7.62–7.57 (m, 2H), 7.27 (d, 2H, J = 3.8 Hz), 7.07 (s, 1H), 3.8 (m, 1H), 2.8 (s, 6H), 2.5 (br, 2H), 1.9 (m, 1H); ^{13}C NMR (DMSO-d6) δ 170.67, 150.62, 136.33, 129.37, 129.09, 128.76, 128.42, 127.76 123.56, 119.74, 115.25, 58.37, 45.15, 26.94; ESI-MS: calcd 353.09, obsd 354.1 $[M+H^+]^+$.

Dcys was synthesized by dimerization of **Mcys** in the presence of 20 equiv. of oxidized DTT. The oxidation reaction was monitored by HPLC and DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) assay by measuring the absorbance at 412 nm.[16] The resulting product was purified by prep-HPLC with a Vydac C_{18} column using a water (0.1% TFA)-acetonitrile (0.1% TFA) gradient to give 5 mg of **Dcys** (yield 95%). The successful synthesis of **Dcys** was confirmed by ESI mass spectrometry (Platform II, micromass, Manchester, UK) and its homogenity (>95%) was confirmed by reverse phase analytical HPLC with a C_{18} column: mp 124–126 °C; ^{1}H NMR (DMSO-D_6) δ 8.41 (d, 2H, J = 4 Hz), 8.31 (d, 2H, J = 4.4), 8.25 (d, 2H, J = 4.2), 8.09 (d, 2H, J = 3.6), 7.56–7.49 (m, 4H), 7.23 (br, 4H), 7.11 (s, 2H), 3.8 (m, 2H), 2.8 (s, 12H), 2.5 (b, 4H);

^{13}C NMR (DMSO-d6) δ 170.93, 150.78, 136.18, 129.39, 129.07, 128.82, 128.37, 127.64, 123.42, 119.64, 115.10, 55.17, 45.14, 38.88; ESI-MS: calcd 704.16, obsd 705.11 $[M+H^+]^+$.

General fluorescence measurements

Fluorescence emission spectrum of a probe in a 10 mm path length quartz cuvette was measured in 10 mM HEPES buffer solution (pH = 7.4) using a Perkin-Elmer luminescence spectrophotometer (model LS 55). Emission spectra of the probe (5 µM) in the presence of 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) were measured by excitation with 330 nm. The slit size for excitation and emission was 6 nm, respectively. The concentration of the probe was confirmed by UV absorbance at 330 nm for dansyl group.

Determination of dissociation constant and detection limit

The dissociation constant was calculated based on the titration curve of the probe with metal ion. The fluorescence signal, F, is related to the equilibrium concentration of the complex (HL) between Host (H) and metal ion (L) by the following expression:

$$F = F_o + \Delta F * [HL]$$

$$[HL] = 0.5 \times [K_D + L_T + H_T - \{(- K_D - L_T - H_T)^2 - 4 L_T H_T\}^{1/2}]$$

Where F_o is the fluorescence of the probe only and ΔF is the change in fluorescence due to the formation of HL. Association constants were determined by a nonlinear least squares fit of the data with the equation.[17]

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **Dcys** without any metal ions was measured by ten times and the standard deviation of blank measurements was determined. Three independent duplication measurements of emission intensity were performed in the presence of metal ions and each average value of the intensities was plotted as a concentration of metal ions for determining the slope. The detection limit is then calculated with the following equation.

$$\text{Detection limit} = 3\sigma_{bi}/m$$

Where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity *versus* sample concentration.

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