Organic &
Biomolecular
Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 2350

www.rsc.org/obc

PAPER

A methionine-based turn-on chemical sensor for selectively monitoring Hg^{2+} ions in 100% aqueous solution†

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Received 25th September 2010, Accepted 13th December 2010
DOI: 10.1039/c0ob00780c

Dansyl-labeled methionine is synthesized by solid-phase synthesis, and found to be a highly sensitive and selective sensor for Hg^{2+}. The sensor sensitively detects Hg^{2+} ions in aqueous solution by a turn-on response; however, the sensor detects Hg^{2+} ions by a turn-off response in organic and mixed aqueous–organic solutions. We investigated the binding stoichiometry, binding constant, and binding mode of the sensor under various solvent conditions. In 100% aqueous solution, 2 : 1 complexation of the sensor with Hg^{2+} ions is more favorable than 1 : 1 complexation, whereas the sensor preferentially forms a 1 : 1 complex in 100% CH_3CN or in 50% CH_3CN–aqueous solutions. Results reveal that the stoichiometry of the sensor–Hg^{2+} complex plays an important role in the type of response to Hg^{2+} ions, and that 2 : 1 complexation is required for a turn-on response to Hg^{2+} ions in aqueous solution.

Introduction

Hg^{2+} is one of the most toxic metal ions, and its presence in the environment has caused serious problems for human health and ecology.[1] Therefore, the development of selective and sensitive methods for the detection of mercury ions has received much attention. In particular, fluorescence has been regarded as the most powerful optical technique for detecting low concentrations of metal ions, and considerable efforts have been devoted to the development of fluorescent chemical sensors that detect mercury ions.[2–4] Since mercury ions cause quenching of fluorescence emission intensity,[5] fluorescent chemical sensors that detect Hg^{2+} ions by a turn-on response are much in demand. Furthermore, as human exposure to mercury is mainly due to water contamination,[6] the ideal Hg^{2+} sensor will show a sensitive response to Hg^{2+} ions in 100% aqueous solution. Even though various types of chemical sensors for Hg^{2+} ions have been reported, most of them have some shortcomings in terms of sensitivity, selectivity, interference from other metal ions, a turn-off response, and insufficiently high solubility in aqueous solutions for environmental applications.[2–4] Therefore, a current challenge is the development of fluorescent chemical sensors that show a selective and sensitive turn-on response to Hg^{2+} ions in aqueous solution.

If chemical sensors for mercury ions are to be used in the environment, they clearly need to have environmental compatibility. As amino acids are water-soluble and biologically and environmentally compatible, new chemical sensors based on amino acids are a promising route of development. However, as none of the natural amino acids except Trp and Tyr have fluorescent properties, conjugation of a fluorophore into the amino acid is a critical step for the synthesis of fluorescent chemical sensors based on amino acids. Several research groups, including ourselves, have synthesized fluorescent chemical sensors based on peptides or amino acids.[5–6] The dansyl fluorophore has been frequently used in chemical sensors for metal ions because it contains a sulfonamide group, and thus plays a critical role in the recognition of specific metal ions.[7] In addition, internal charge transfer (ICT) involving the dimethylamino and sulfonamide groups makes the dansyl group sensitive to its microenvironment and specific metal ions.[8]

In this paper, we chose methionine (Met) as a receptor for chemical sensors, because we considered that its thioether group may act as a ligand for soft Hg^{2+} ions. We therefore synthesized a dansyl-labeled L-Met residue (**Dansyl-Met**) by solid-phase synthesis, and investigated the response of this compound to several metal ions. As we expected, the compound shows a highly sensitive and selective response to mercury ions among various heavy and transition metal ions in aqueous solution, as well as in aqueous–organic mixed solvents. Interestingly, the sensor shows a solvent-dependent response to Hg^{2+} ions. In 100% aqueous solution, the sensor detects Hg^{2+} ions by a turn-on response, while the sensor exhibits a turn-off response to Hg^{2+} ions in 50% CH_3CN–aqueous solutions or in 100% CH_3CN. We investigated the binding stoichiometry, binding affinity, binding mode, and pH-dependent fluorescence enhancement of the sensor for Hg^{2+} ions. Results revealed that the binding stoichiometry of the sensor might play an important role in the type of response to Hg^{2+} ions, depending on the solvent. In 100% aqueous solution, the sensor forms a 2 : 1 complex with Hg^{2+} ions and shows a turn-on response, whereas the sensor forms a 1 : 1 complex with Hg^{2+} ions in 50%

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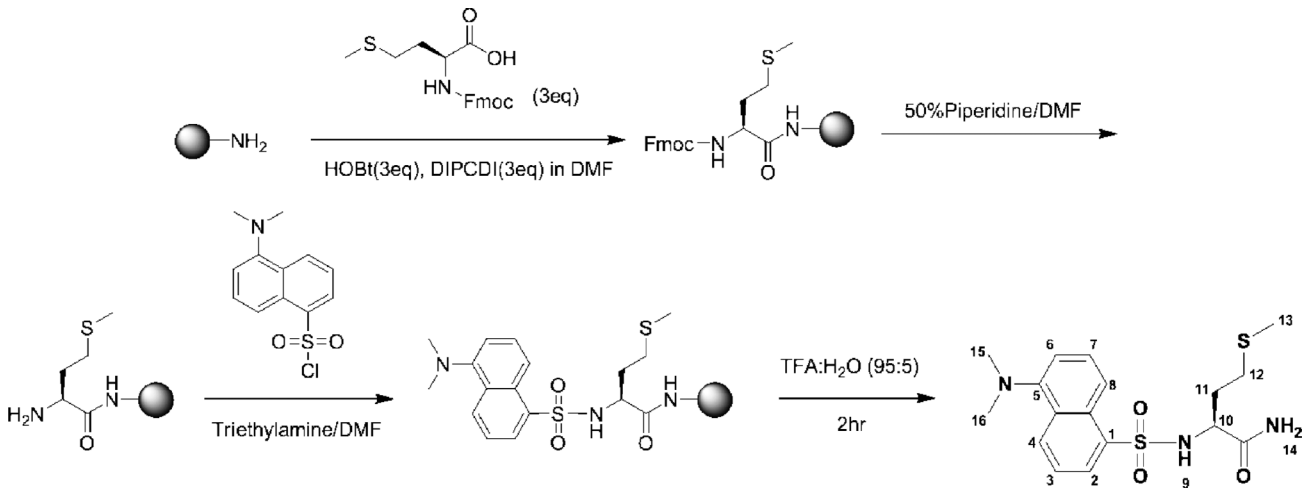
† Electronic supplementary information (ESI) available: UV absorbance, HPLC chromatogram, ESI mass spectra, and ^1H NMR and ^{13}C NMR spectra of **Dansyl-Met**. ITC titration curves of **Dansyl-Met** with Hg^{2+}, titration curve with Hg^{2+}) and non-linear least-squares fitting.



Scheme 1 Synthesis of **Dansyl-Met**.

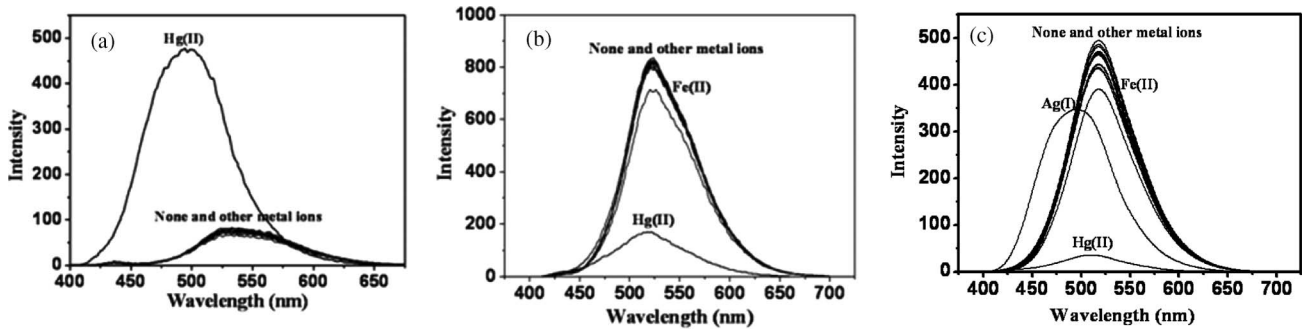


Fig. 1 Fluorescence spectra of **Dansyl-Met** (30 μM) in (a) 10 mM HEPES buffer solution (pH 7.4), (b) 50% CH_3CN–HEPES buffer solution, and (c) 100% CH_3CN. Fluorescence spectra of **Dansyl-Met** were measured in the presence of various metal ions ((a) 3 equiv, slit 10/6 nm, (b) 5 equiv, slit 10/6 nm, (c) 5 equiv, slit 10/4 nm) (λ_{ex} = 380 nm).

CH_3CN–aqueous solution, or in 100% CH_3CN, and shows a turn-off response.

Results and discussion

Solid-phase synthesis of the amino acid sensor

The sensor was synthesized by solid-phase synthesis using Fmoc-chemistry (Scheme 1).[9] After cleavage of the product from resin, the dansyl-labeled Met residue was purified from the crude product by semi-preparative HPLC with a C_{18} column. The successful synthesis and purity of **Dansyl-Met** (>98%) were confirmed by analytical HPLC with a C_{18} column and ESI mass spectrometry (Fig. 1S and 2S†).

Fluorescence properties in aqueous and non-aqueous solvents

As **Dansyl-Met** is water-soluble, the stock solution of the compound was prepared in 100% distilled water and the photochemical experiments were carried out in 100% aqueous solution without cosolvent. The molar extinction coefficient of **Dansyl-Met** ($\varepsilon = 4.3 \times 10^3$ M^{-1} cm^{-1} at 330 nm) was measured in 10 mM HEPES buffer solution (pH 7.4). The fluorescence quantum yield was determined by using anthracene in ethanol as a standard (λ_{ex} = 330 nm, Φ = 0.15).

Fig. 1 shows the fluorescence response of **Dansyl-Met** in the presence of metal ions (Ca^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Ag^{+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+} and Zn^{2+} as their perchlorate salts, and Na^{+}, Al^{3+} and K^{+} as their chloride salts) by excitation with 380 nm radiation. The sensor shows exclusive selectivity for Hg^{2+} in aqueous solution, and no fluorescent response to the other metal ions. **Dansyl-Met** detects Hg^{2+} in 100% aqueous solution by a turn-on response with a 23-fold enhancement of the emission intensity at 490 nm.

We also investigated the fluorescent response of **Dansyl-Met** to metal ions in organic and aqueous–organic mixed solvents. **Dansyl-Met** exhibits a high selectivity to Hg^{2+} in 100% CH_3CN and 50% CH_3CN–HEPES buffer solutions. However, interestingly, the sensor shows a turn-off response to Hg^{2+} ions in these solvents.

The fluorescent response of **Dansyl-Met** to the amount of Hg^{2+} was measured, and is shown in Fig. 2. The emission intensity increased with the concentration of Hg^{2+} in 10 mM HEPES buffer solution (pH 7.4). Upon addition of increasing concentrations of Hg^{2+}, we observed an enhancement of the emission intensity of about 23-fold at 490 nm, and a 40 nm blue-shift of the maximum emission intensity from 535 to 495 nm. This result suggests that the formation of a complex between the compound and Hg^{2+} has an influence on the dimethylamino or sulfonamide groups of the dansyl fluorophore with regard to internal charge transfer. In the titration curve, about 2 equiv of Hg^{2+} was sufficient for the

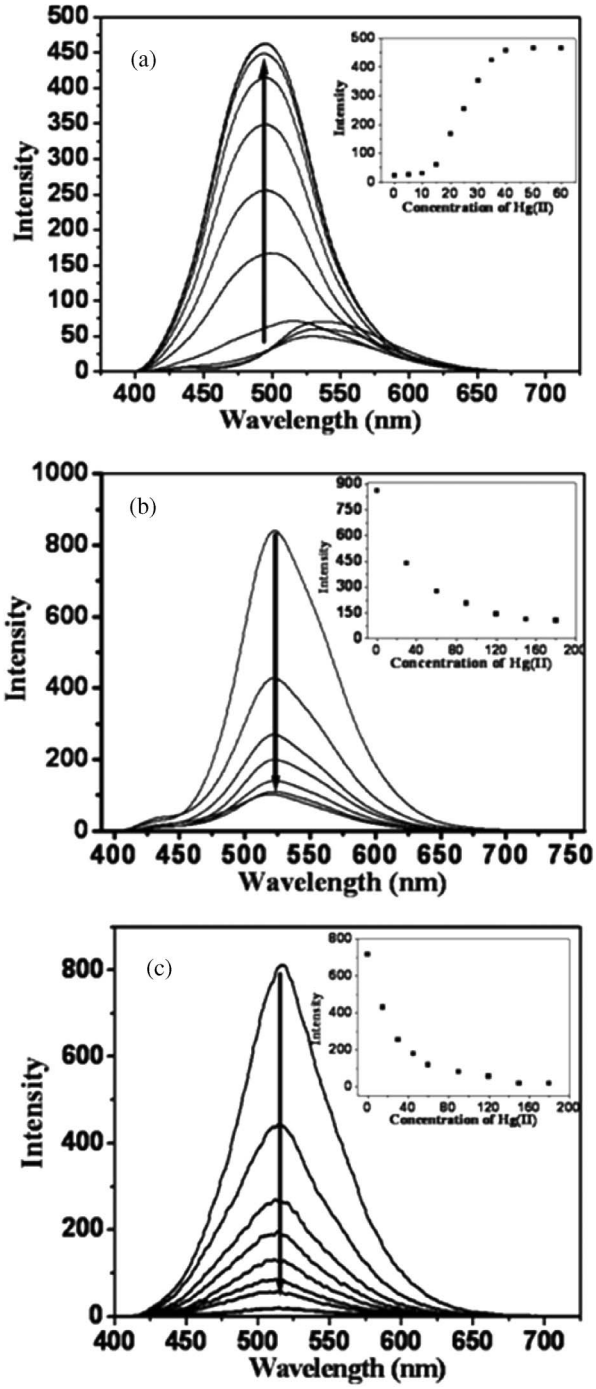


Fig. 2 Fluorescence emission spectra of **Dansyl-Met** (30 μM) in the presence of increasing concentrations of Hg^{2+} in (a) 10 mM HEPES buffer (pH 7.4); slit 10/6 nm (b) 50% CH_3CN–HEPES buffer solution; slit 10/6 nm and (c) 100% CH_3CN; slit 10/5 nm (λ_{ex} = 380 nm).

saturation of the emission intensity of the sensor (30 μM), which indicated that **Dansyl-Met** has hypersensitivity to Hg^{2+} in 100% aqueous solution.

Titration experiments were also performed in 100% CH_3CN and 50% CH_3CN–HEPES buffer solution. About 5 equiv of Hg^{2+} was sufficient for the saturation of the emission intensity change. A strong decrease of the emission intensity is observed upon mercury complexation with the compound in these conditions, which can be tentatively explained in terms of electron transfer from the excited dansyl fluorophore to the complexed mercury cation.[10] This kind of fluorescence quenching by Hg^{2+} is a well-documented phenomenon, a turn-off response being observed in fluorescent chemical sensors for Hg^{2+} ions.[11–13]

Fig. 3 shows the visible emission change of the **Dansyl-Met**–Hg^{2+} complex depending on solvent. In 100% aqueous solution, the solution containing the sensor and Hg^{2+} ions (2 equiv) shows a brighter color than those of the solution containing the sensor and the other metal ions. In 50% CH_3CN–HEPES buffer solution, the solution containing the sensor and Hg^{2+} ions (5 equiv) shows no color, while the solutions containing the sensor alone, and the sensor and other metal ions, show a yellow color.

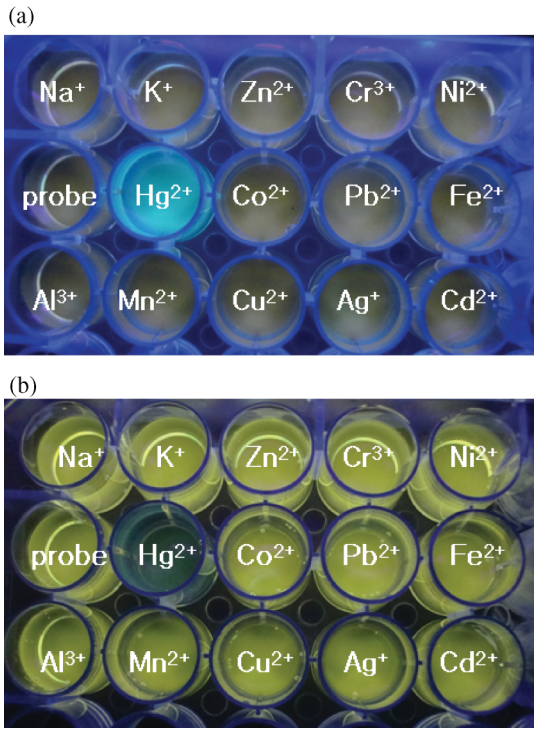


Fig. 3 Visible emission observed from samples of the sensor and various metal ions in (a) 10 mM HEPES buffer (pH 7.4), and (b) 50% CH_3CN–HEPES buffer solution.

Binding stoichiometry, binding affinity and binding mode

We investigated the binding stoichiometry of the sensor with Hg^{2+} ions in aqueous and non-aqueous solvents. To determine binding stoichiometry, we used a Job plot analysis (Fig. 4) and the model used for K_d calculation. As shown in Fig. 4, there is a maximum at 0.34 mole fraction in aqueous solution, indicating that the sensor forms a 2 : 1 complex with Hg^{2+} in aqueous solution. In contrast to this, the sensor forms a 1 : 1 complex with Hg^{2+} in 100% CH_3CN and 50% CH_3CN–HEPES buffer, on the basis of a Job plot that shows a maximum at 0.5 mole fraction.

Assuming 2 : 1 complex formation in aqueous solution, the titration curve was fitted by non-linear least-squares fitting. However, the titration curve could not be well fitted, perhaps because the sensor forms mixed complexes. We assume that the sensor forms 2 : 1 and 1 : 1 complexes in aqueous solution

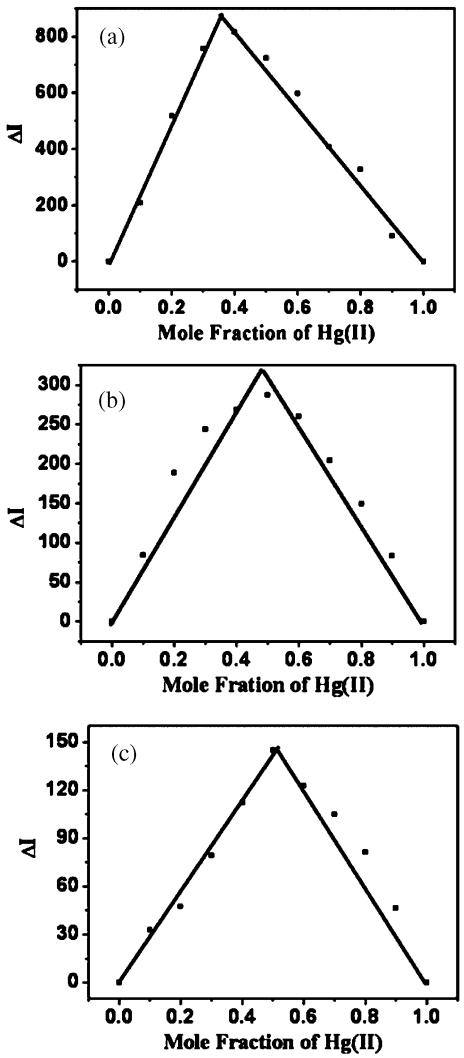


Fig. 4 A Job plot for **Dansyl-Met** in (a) 10 mM HEPES buffer (pH 7.4), (b) 50% CH_3CN–HEPES buffer solution, and (c) 100% CH_3CN.

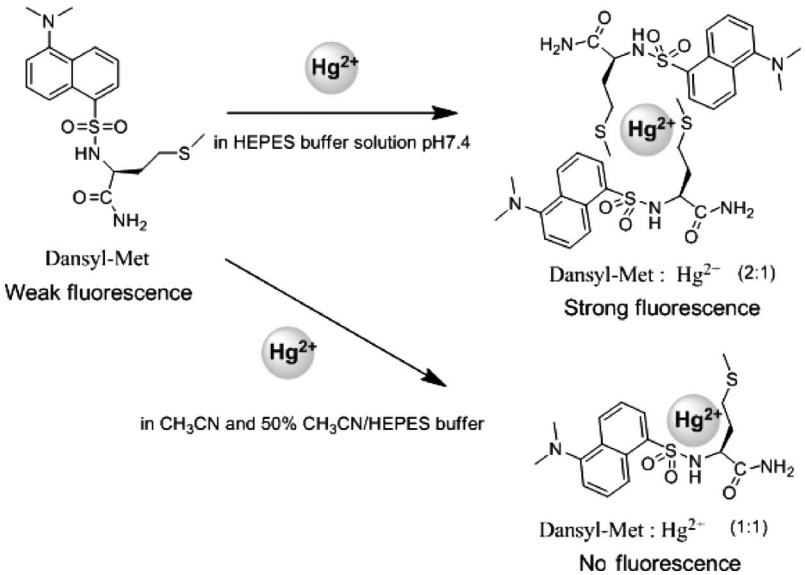
depending on the concentration of the sensor and Hg^{2+}. To confirm this, we investigated response type and binding stoichiometry at low concentrations of the sensor in aqueous solution. As shown in Fig. S3†, the sensor (5 μM) showed a turn-off response to a small amount of Hg^{2+} ions (1–2 equiv) and then a turn-on response to a relatively large amount of Hg^{2+} ions (3–5 equiv) in 100% aqueous solution. In addition, 5 equiv of Hg^{2+} was required for the saturation of the emission intensity change in this condition. A Job plot analysis at low concentrations of the sensor revealed a maximum intensity at around at 0.50 mole fraction in 100% aqueous solution (Fig. S4†). As the concentration of the sensor decreases, 1 : 1 complexation seems to be more favorable than 2 : 1 complexation because 2 : 1 complexation is dependent on the concentration of the sensor. This result indicates that the stoichiometry of the sensor–Hg^{2+} complex depends on the concentration of the sensor and Hg^{2+}, and that the stoichiometry may play an important role in the type of response for Hg^{2+} ions. Imperiali *et al.* reported that fluorescent peptide sensors for Zn(II) ions formed mixed complexes (1 : 1 and 2 : 1) depending on peptide concentration and Zn(II) concentration.[14] Here, we investigated the binding affinity and binding stoichiometry of the sensor in 100% aqueous solution using isothermal calorimetry titration (ICT). This indicated that the interaction involves a 2 : 1 binding of the sensor to Hg^{2+} ions with an association constant, K_a, of 1.38 × 10^5 M^{-2} in 100% aqueous solution (Fig. S5†).

In 50% CH_3CN–HEPES buffer solution, assuming 1 : 1 complex formation, the dissociation constant, K_d (8.80 × 10^{-6} M, R^2 = 0.989), was calculated based on the titration curve with Hg^{2+} by non-linear least-squares fitting (Fig. S6†). The value of the dissociation constant indicates that **Dansyl-Met** has a potent binding affinity for Hg^{2+} in 50% CH_3CN–HEPES buffer solution. In 100% CH_3CN, the dissociation constant was also successfully calculated by non-linear least-squares fitting with 1 : 1 complex model (Fig. S7†). The dissociation constant, K_d (7.11 × 10^{-6} M, R^2 = 0.996), indicates that **Dansyl-Met** also has a potent binding affinity for Hg^{2+} in 100% CH_3CN.

The dependence of the binding stoichiometry on solvent was also confirmed by ESI mass spectrometry, as shown Fig. S9†. In 50% CH_3CN–H_2O solution, a peak at *m*/*z* 381.84 corresponding to [**Dansyl-Met** + H^+]$^+$ was observed. When 5.0 equiv of Hg^{2+} was added, a new peak at 581.90 corresponding to [**Dansyl-Met** + Hg^{2+} – H^+]$^+$ appeared, which supports the fact that the sensor forms a 1 : 1 complex with Hg^{2+} in 50% CH_3CN–H_2O solution. However, when 3 equiv of Hg^{2+} was added to 10% CH_3CN–H_2O solution containing the sensor, new peaks at 963.02 and 1162.94 corresponding to [2**Dansyl-Met** + Hg^{2+} – H^+]$^+$ and [2**Dansyl-Met** + Hg^{2+}+ $2ClO_4^-$– H^+]$^+$ appeared. We then confirmed that the sensor shows a turn-on response to Hg^{2+} ion in 10% CH_3CN–H_2O solution (Fig. S8†). This result strongly supports the fact that the sensor forms a more favorable 2 : 1 complex in 100% aqueous solution at certain concentrations of the sensor and Hg^{2+}. Furthermore, when 0.1 equiv of Hg^{2+} was added to 10% CH_3CN–H_2O solution containing the sensor (Fig. S9.C†), we observed a large peak corresponding to [**Dansyl-Met** + Hg^{2+} – H^+]$^+$ and small peaks corresponding to [2**Dansyl-Met** + Hg^{2+} – H^+]$^+$ and [2**Dansyl-Met** + Hg^{2+} + $2ClO_4^-$ – H^+]$^+$. This supports the idea that the relative concentrations of the 1 : 1 and 2 : 1 complexes change depending on sensor concentration and Hg^{2+} concentration in aqueous solution. If the concentration of Hg^{2+} is lower than that of the sensor, the sensor may preferentially form a 1 : 1 complex with Hg^{2+} ions even in aqueous solution. ESI mass spectra revealed that deprotonation process of **Dansyl-Met** by chelation with Hg^{2+} occurred in aqueous solution and 50% CH_3CN–H_2O solution.

Overall, these results suggest that the stoichiometry of the complexes changes depending on the solvent and the concentrations of the sensor and Hg^{2+} ions, and that the stoichiometry may play an important role in the response type of the sensor to Hg^{2+} ions. When the sensor forms a 1 : 1 complex, the sensor shows turn-off response to Hg^{2+} ions, whereas it shows turn-on response to Hg^{2+} ions during 2 : 1 complex formation.

NMR studies provide information about the complexation of **Dansyl-Met** by Hg^{2+} ion. ^1H NMR experiments were carried out in 50% CD_3CN–D_2O solution (Fig. S12†). When 3 equiv of Hg^{2+} ions are added, large chemical shifts in H(11), H(12) and H(13) are observed, which indicates that Hg^{2+} coordinates the thioether of the sensor. Small chemical shifts of the aromatic protons of the dansyl moiety suggest that Hg^{2+} ion interacts with the dansyl moiety, while small shifts of H(15) and H(16) suggest that the Hg^{2+} ion directly coordinates the sulfonamide group of the dansyl fluorophore. As we mentioned in the Introduction, the



Scheme 2 Proposed binding modes of **Dansyl-Met** with Hg^{2+}.

sulfonamide group of the dansyl fluorophore is well known to interact with metal ions by a deprotonation process. As shown in Fig. S9, ESI mass spectra also confirm that deprotonation of the sulfonamide group of the dansyl fluorophore is induced by complexation of Hg^{2+} ions. Thus, we conclude that Hg^{2+} coordinates with the sulfonamide and the thioether of **Dansyl-Met**, as shown in Scheme 2.

Unfortunately, we could not investigate the binding mode of **Dansyl-Met** and Hg^{2+} in 100% aqueous solution because mM concentrations of **Dansyl-Met** did not dissolve in D_2O, even with a small amount of organic solvent. Consistent with our previous research in which a chemical sensor based on dimerized Cys residues with two dansyl fluorophores showed a turn-on response to Hg^{2+} ions in aqueous solution,[15] we are sure that interactions between two sulfonamide groups of the chemical sensor and Hg^{2+} ions are necessary for strong binding affinity to Hg^{2+} ions in 100% aqueous solution. NMR experiments and the X-ray crystal structure of the complex between dansyl-L-tryptophan methyl ester and Hg^{2+} ions reported by Ma *et al.* also suggest that the compound that showed turn-on response to Hg^{2+} ions in aqueous solution formed 2 : 1 complexes with Hg^{2+} ions, and that the two deprotonated sulfonamide groups of the sensors played a critical role in the strong interaction with Hg^{2+} ions in 100% aqueous solution.[7e] Therefore, we assume that the two sulfonamide groups and two thioether groups of two **Dansyl-Met** play an important role in the complexation with Hg^{2+} ions in aqueous solution, as shown in Scheme 2.

Fluorescent sensing of Hg^{2+} in 100% aqueous solution

In order to assess the potential for environmental applications of the sensor, we next concentrated on the characterization of its photophysical properties in 100% aqueous solution. The influence of pH on the fluorescence of **Dansyl-Met** was examined in 100% aqueous solution (Fig. 5). The maximum emission intensity of free **Dansyl-Met** by excitation with 380 nm was not sensitive to pH, whereas the **Dansyl-Met**–Hg^{2+} complex shows emission

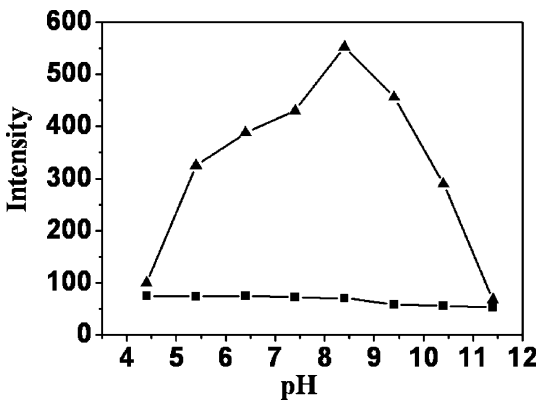


Fig. 5 Emission intensity of **Dansyl-Met** in the presence (▲) and absence of (■) Hg^{2+} (3 equiv) at different pH (λ_{ex} = 380 nm).

intensity dependent on pH. **Dansyl-Met** showed a sensitive turn-on response to Hg^{2+}, with at least a 10-fold enhancement in the pH range 5.5–10.5. The emission intensity of the complex at pH 4.5 was very weak because of the protonated dimethylamino group ($pK_a \approx 4$) of the dansyl fluorophore, which prevents charge transfer from the dimethylamino group to the naphthyl moiety.[16] The fluorescence of the **Dansyl-Met**–Hg^{2+} complex dramatically decreased from pH 8.5 to pH 11.5, due to the deprotonation process of sulfonamide ($pK_a \approx 10$) in this pH range.[16a,17] The fact that the largest enhancement of **Dansyl-Met** occurs at neutral pH suggests that the sensor is suitable for monitoring Hg^{2+} ion contamination in living cells.

To test reversibility, EDTA was added to the sensor–Hg^{2+} complex, which exhibited a strong emission intensity at 500 nm. The addition of EDTA instantly resulted in the change of the emission intensity (Fig. 6). Addition of about 3 equiv of EDTA to the sensor–Hg^{2+} complex results in an immediate return to the original metal-free spectrum, which demonstrates the reversibility of **Dansyl-Met**.

To investigate the interference effect of other metal ions on the ability of **Dansyl-Met** to detect Hg^{2+}, the response of

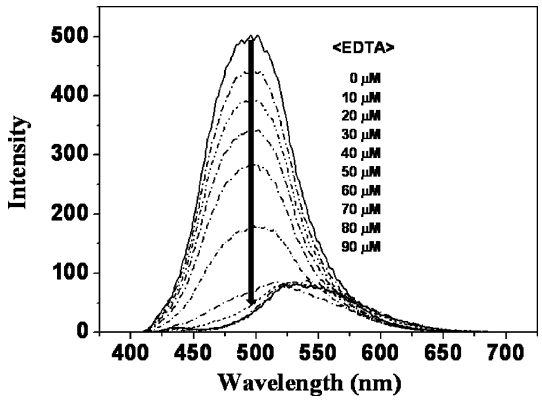


Fig. 6 Emission spectra of the **Dansyl-Met**–Hg^{2+} complex (30 μM) in the presence of EDTA in 10 mM HEPES buffer, pH 7.4 (λ_{ex} = 380 nm).

Dansyl-Met to Hg^{2+} in the presence of other metal ions was measured in HEPES buffer solution at pH 7.4. Fig. 7 shows the response of **Dansyl-Met** to Hg^{2+} ions in the presence of various metal ions (Ca^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Ag^{+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Na^{+}, Al^{3+} and K^{+}). The Hg^{2+}-dependent fluorescence response of **Dansyl-Met** was not affected by Group I and Group II metal ions, or other heavy/transition metal ions, including Cu(II) and Cd(II). Most of the reported mercury sensors that work in aqueous solution display cross-sensitivities toward other heavy metal ions.[2–4] However, **Dansyl-Met** could detect Hg^{2+} ions in 100% aqueous solution without interference from Cu(II) and Cd(II) ions.

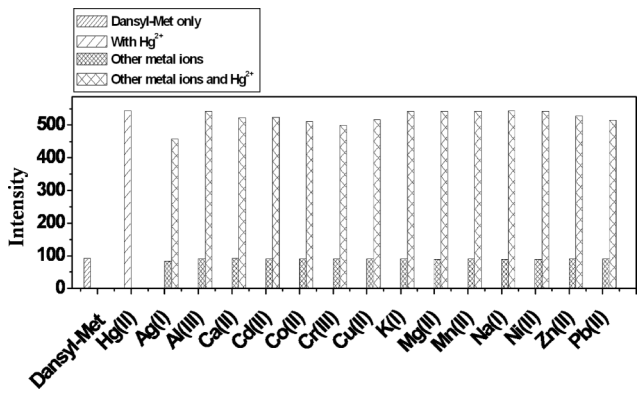


Fig. 7 Emission intensity response of **Dansyl-Met** (30 μM) in the presence of Hg^{2+} (3 equiv) and various other metal ions (3 equiv) in 10 mM HEPES buffer (pH 7.4).

Conclusion

The sensor **Dansyl-Met** shows exclusive selectivity for Hg^{2+} ions in 100% aqueous solution, mixed aqueous–organic solution, and 100% CH_3CN. The addition of Hg^{2+} ions to aqueous solutions of **Dansyl-Met** (30 μM) induces a significant increase of emission intensity with the shift of emission maxima, whereas the addition of Hg^{2+} ions, both in mixed aqueous–organic solution and in 100% CH_3CN, induces complete quenching of the fluorescence spectra. Overall, these results reveal that the stoichiometry of the sensor–Hg^{2+} complex changes depending on the solvent and the concentration of the sensor, and that Hg^{2+} ions and the stoichiometry play an important role in the type of response to Hg^{2+} ions. When the sensor forms a 1 : 1 complex, the sensor shows a turn-off response to Hg^{2+} ions, whereas it shows a turn-on response to Hg^{2+} ions during 2 : 1 complex formation with Hg^{2+} ions. The selectivity of the sensor for Hg^{2+} over other metal ions is remarkably high in 100% aqueous solution, and the sensor is able to detect Hg^{2+} ions in 100% aqueous solution without interference from other metal ions.

Experimental

General

Fmoc-Met-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), dansyl chloride, triethylamine, *N*,*N*-dimethylformamide (DMF) and piperidine, were purchased from Aldrich.

Solid-phase synthesis of Dansyl-Met

Dansyl-Met was synthesized by solid-phase synthesis with Fmoc chemistry.[9] Fmoc-protected L-Met was assembled on Rink Amide MBHA resin, as shown in Scheme 1. After deprotection of Fmoc group, coupling of dansyl chloride was performed by the following procedure. To the resin-bound amino acid (100 mg, 0.05 mmol), dansyl chloride (40 mg, 0.15 mmol, 3 equiv) in DMF (3 ml) and triethylamine (40 μl, 0.30 mmol, 6 equiv) were added. Cleavage of the peptide from the resin was achieved by treatment with a mixture of 3 ml TFA–H_2O (95 : 5 v/v) at room temperature for 2 h. After filtration and washing of the resin with TFA, a gentle stream of nitrogen was used to remove the excess TFA. The crude **Dansyl-Met** was purified by prep-HPLC with a Vydac C_{18} column using a water (0.1% TFA)–acetonitrile (0.1% TFA) gradient to give 78 mg of **Dansyl-Met** (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 191–193 °C; ^{1}H NMR (400 MHz, 50% CD_3CN–D_2O) δ 8.39 (d, 1H, J = 8.8 Hz), 8.07 (d, 1H, J = 8.4 Hz), 8.0 (d, 1H, J = 7.4 Hz), 7.61 (d, 1H, J = 7.7 Hz), 7.52–7.45 (m, 2H), 3.40–3.36 (m, 1H), 3.0 (s, 6H), 1.79–1.75 (m, 1H), 1.55–1.50 (m, 1H), 1.38–1.34 (m, 2H), 1.27 (s, 3H); ^{13}C NMR (50% CD_3CN–D_2O) δ 175.1, 144.2, 136.2, 131.3, 129.8, 128.8, 128.3, 127.8, 126.4, 124.8, 118.7, 55.8, 46.8 (×2), 32.1, 29.9, 14.5; ESI-MS: calcd 382.12 $[M + H^+]^+$, obsd 381.83 $[M + H^+]^+$.

General fluorescence measurements

The fluorescence emission spectrum of the sensor 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). The emission spectra of the sensor 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 their perchlorate salts, and Na^{+}, Al^{3+} and K^{+} as their chloride salts) were measured by excitation with 380 nm radiation. The slit sizes for excitation and emission were 10 and 6 nm, respectively. The concentration of the sensor was confirmed by UV absorbance at 330 nm for the dansyl group.

Determination of dissociation constant

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

$$F = F_o + \Delta F\,[\mathrm{HL}]$$

where

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

where F_o is the fluorescence of the sensor only, and ΔF is the change in fluorescence due to the formation of HL, L_T and H_T is total concentration of metal ion (L), and host (H). Association constants were determined by a nonlinear least-squares fitting of the data with the equation.[18]

Acknowledgements

This work was supported by a grant (2009-0076572) from the Basic Research Program and a grant (2010-0018728) from the Atomic Research Program of the Korea Science & Engineering Foundation.

References

1 (*a*) T. C. Hutchinson and K. M. Meema, *Lead, mercury, cadmium and arsenic in the environment*, Wiley, New York, 1987; (*b*) G. H. Scoullos, M. J. Vonkeman, L. Thorton and Z. Makuch, in *Mercury, Cadmium and Lead: Handbook for Sustainable Heavy Metals Policy and Regulation* (Environment & Policy, V. 31), Kluwer Academic, Norwell, MA, 2001; (*c*) C. J. Coester, *Anal. Chem.*, 2005, **77**, 3737; (*d*) S. D. Richardson and T. A. Temes, *Anal. Chem.*, 2005, **77**, 3807; (*e*) *Mercury Update: Impact on Fish Advisories (EPA Fact Sheet EPA-823-F-01-011),* Office of Water, EPA, Washington, DC, 2001.

2 (*a*) E. M. Nolan and S. J. Lippard, *Chem. Rev.*, 2008, **108**, 3443; (*b*) A. Coskun, M. D. Yilmaz and E. U. Akkaya, *Org. Lett.*, 2007, **9**, 607; (*c*) B. Liu and H. Tian, *Chem. Commun.*, 2005, 3156; (*d*) H. Zheng, Z. H. Qian, L. Xu, F. F. Yuan, L. D. Lan and J. G. Xu, *Org. Lett.*, 2006, **8**, 859; (*e*) P. Chen and C. He, *J. Am. Chem. Soc.*, 2004, **126**, 728; (*f*) A. Ono and H. Togashi, *Angew. Chem., Int. Ed.*, 2004, **43**, 4300; (*g*) J. S. Wu, I. C. Hwang, K. S. Kim and J. S. Kim, *Org. Lett.*, 2007, **9**, 907.

3 (*a*) J. Wang, X. Qian and J. Cui, *J. Org. Chem.*, 2006, **71**, 4308; (*b*) E. M. Nolan and S. J. Lippard, *J. Mater. Chem.*, 2005, **15**, 2778; (*c*) E. M. Nolan and S. J. Lippard, *J. Am. Chem. Soc.*, 2007, **129**, 5910.

4 (*a*) W. Guo, J. Yuan and E. Wang, *Chem. Commun.*, 2009, 3395; (*b*) R. Yang, J. Jin, L. Long, Y. Wang, H. Wang and W Tan, *Chem. Commun.*, 2009, 322; (*c*) M. Tian and H. Ihmels, *Chem. Commun.*, 2009, 3175; (*d*) A. Jana, J. S. Kim, H. S. Jung and P. K. Bharadwaj, *Chem. Commun.*, 2009, 4417; (*e*) S. Yoon, E. W. Miller, Q. He, P. H. Do and C. J. Chang, *Angew. Chem., Int. Ed.*, 2007, **46**, 6658; (*f*) Q. Zou and H. Tian, *Sens. Actuators, B*, 2010, **149**, 20.

5 (*a*) J. C. Koziar and D. O. Cowan, *Acc. Chem. Res.*, 1978, **11**, 334; (*b*) P. Svejda, A. H. Maki and R. R. Anderson, *J. Am. Chem. Soc.*, 1978, **100**, 7138.

6 D. W. Boening, *Chemosphere*, 2000, **40**, 1335.

7 (*a*) B. P. Joshi, J. W. Park and K. H. Lee, *Talanta*, 2009, **78**, 903; (*b*) B. R. White, H. M. Liljestrand and J. A. Holcombe, *Analyst*, 2008, **133**, 65; (*c*) Z. Wang, J. H. Lee and Y. Lu, *Chem. Commun.*, 2008, 6005; (*d*) B. P. Joshi, W. M. Cho, J. S. Kim, J. Y. Yoon and K. H. Lee, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 6425–6429; (*e*) B. P. Joshi and K. H. Lee, *Bioorg. Med. Chem.*, 2008, **16**, 8501–8509; (*f*) H. Li, Y. Li, Y. Dang, L. Ma, Y. Wu, G. Hou and L. Wu, *Chem. Commun.*, 2009, 4453; (*g*) L. Ma, Y. Li, L. Li, J. Sun, C. Tian and Y. Wu, *Chem. Commun.*, 2008, 6345.

8 (*a*) I. Leray, J. P. Lefevre, J. F. Delouis, J. Delaire and B. Valeur, *Chem. Eur. J.*, 2001, **7**, 4590; (*b*) V. Balzani, P. Ceroni, S. Gestermann, C. Kauffmann, M. Gorka and F. Vogtle, *Chem. Commun.*, 2000, 853; (*c*) F. Vogtle, S. Gestermann, C. Kauffmann, P. Ceroni, V. Vicinelli and V. Balzani, *J. Am. Chem. Soc.*, 2000, **122**, 10398; (*d*) S. Deo and H. A. Godwin, *J. Am. Chem. Soc.*, 2000, **122**, 174.

9 G. B. Fields and R. L. Nobel, *Int. J. Pept. Protein Res.*, 1990, **35**, 161.

10 (*a*) R. A. Nissell, A. P. de Silva, H. Q. N. Gunarantne, P. L. M. Lynch, G. E. M. Maguire, C. P. McCoy and K. R. A. S. Sandanayake, *Top. Curr. Chem.*, 1993, **168**, 223–264; (*b*) H. Masuhara, H. Shioyama, T. Saito, K. Hamada, S. Yasoshima and N. Mataga, *J. Phys. Chem.*, 1984, **88**, 5868.

11 X. Zhu, S. Fu, W. Wong, J. Guo and W. Wong, *Angew. Chem., Int. Ed.*, 2006, **45**, 3150.

12 Z. Wu, Y. Zhang, J. S. Ma and G. Yang, *Inorg. Chem.*, 2006, **45**, 3140.

13 (*a*) Z. Wu, Q. Chen, S. Xiong, B. Xin, Z. Zhao, L. Jiang and J. S. Ma, *Angew. Chem., Int. Ed.*, 2003, **42**, 3271; (*b*) R. Métivier, I. Leray and B. Valeur, *Chem. Eur. J.*, 2004, **10**, 4480; (*c*) S. H. Kim, K. C. Song, S. Ahn, Y. S. Kang and S. K. Chang, *Tetrahedron Lett.*, 2006, **47**, 497; (*d*) S. Y. Moon, N. J. Youn, S. M. Park and S. K. Chang, *J. Org. Chem.*, 2005, **70**, 2394.

14 Melissa D. Shults, Dierdre A. Pearce and B. Imperiali, *J. Am. Chem. Soc.*, 2003, **125**, 10591.

15 15 B. P. Joshi, C. R. Lohani, K. H. Lee, *Organic & Biomolecular Chemistry*, 2010, 8, 3220.

16 (*a*) Y. Zheng, K. M. Gattas-Asfura, V. Konak and R. M. Leblanc, *Chem. Commun.*, 2002, 2350; (*b*) R. M'etivier, I. Leray and B. Valeur, *Photochem. Photobiol. Sci.*, 2004, **3**, 374; (*c*) R. Metivier, I. Leray and B. Valeur, *Chem. Eur. J.*, 2004, **10**, 4480.

17 T. Koike, T. Watanabe, S. Aoki, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1996, **118**, 12696.

18 A. R. Reddi, T. R. Guzman, R. M. Breece, D. L. Tierney and B. R. Gibney, *J. Am. Chem. Soc.*, 2007, **129**, 12815.

