An easily prepared hypersensitive water-soluble fluorescent probe for mercury(II) ions†

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A hypersensitive water-soluble fluorescent probe, dansyl-L-tryptophan methyl ester (1), was easily prepared for the detection of Hg^{2+} with a significantly improved detection limit (5 nM *vs.* 500 nM) in buffered aqueous solution.

Mercury pollution, which mainly stems from mercury(II) ion (Hg^{2+}) contaminated natural water, has become a worldwide environmental problem since Hg^{2+} can easily pass through biological membranes, causing serious damage to the central nervous and endocrine systems.[1] To ensure human health, the upper limit for Hg^{2+} in drinking water has been decreased to 10 nM.[2] Therefore, new mercury detection methods that are effective, rapid, facile, and applicable to environmental and/or biological systems have become a significant and insistent goal.

As opposed to traditional instrumental techniques, small-molecule probes are well-suited for quick detection of Hg^{2+} in the field and for *in vivo* studies in biological systems.[1,3–7] However, most small-molecule probes require the use of organic[3] or mixed aqueous–organic solutions,[4] features that may be problematic for real-world samples. In addition, since the emission of many fluorophores is easily quenched by heavy-metal ions, probes for Hg^{2+} normally display a turn-off response,[5] although a turn-on response has proven to be more effective. Therefore, the search for new probes with the characteristics of high selectivity and affinity, fast and sensitive turn-on response to Hg^{2+}, as well as good water-solubility, pH-stability, and cell-permeability has been the focus of extensive investigation. Such probes that demonstrate practical application in aqueous solution[6] and/or live cells[7] have recently attracted much attention.

To improve the water-solubility of fluorescent probes, hydrophilic amino acids have been introduced into their design.[8] A fluorescent probe derived from pyrene and tryptophan showed a detection limit of 0.15 μM for lead ion and good solubility in aqueous solution.[8a] In a recent approach to Hg^{2+} detection, we have designed a protein-supported fluorimetric assay using dansyl-L-aspartic acid whose fluorescence emission spectrum underwent an obvious blue-shift and an enhancement induced by Hg^{2+} in aqueous solution.[8b] We proposed that the non-covalent complexation between the probe *via* its carboxyl group and the protein weakened the bond strength of the N–H in the sulfonamide moiety and further generated an affinity binding site for Hg^{2+}. Therefore, covalent modification of the carboxyl group by a methyl ester may favor the deprotonation of the amide group and strengthen the binding between the amino group and the metal ions. We extended our studies to the design and synthesis of dansyl-L-tryptophan methyl ester (**1**) (Fig. 1a), as a more efficient and selective fluorescent Hg^{2+} probe (Fig. S1, ESI†). This compound displayed a rapid and specific response to Hg^{2+} in buffered aqueous solution with a significant improvement of detection limit (5 nM *vs.* 500 nM), which was also effective in live cells.

Compound **1** was easily synthesized in 81.7% yield from the reaction of dansyl chloride and L-tryptophan methyl ester and its structure was confirmed by spectroscopic data (see ESI†). This fluorescent probe displayed remarkable advantages including: (i) simple and high-yielding synthesis; (ii) turn-on fluorescence properties upon Hg^{2+} addition; (iii) rapid and specific response to Hg^{2+}; (iv) high sensitivity at the nanomolar concentration range; and (v) suitable detection both in buffered aqueous solution and in live cells. In addition, the crystal structure of **1**–Hg^{2+} offers a suggestion for the Hg^{2+}-induced blue-shift and enhancement of the fluorescence emission intensity.

Compound **1** itself has a very weak emission band at 550 nm in aqueous solution (Fig. 1b). Upon addition of small amounts of Hg^{2+}, a significant enhancement of the emission band at 487 nm occurred, resulting in a 63 nm blue-shift and a large increase in the intensity (saturated at 2.5 μM Hg^{2+} with a 35-fold enhancement) of the fluorescence emission (Fig. S2, ESI†), and the fluorescent color of the solution turned from brown to green (inset in Fig. 1b). The effect can be compared

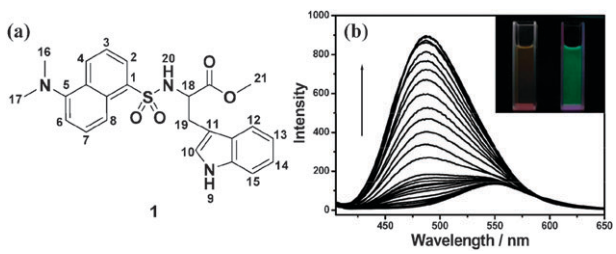

Fig. 1 (a) Chemical structure and atomic numbering of dansyl-L-tryptophan methyl ester (**1**). (b) Fluorescence titration spectra of **1** (5.0 μM) in 10.0 mM HEPES solution (pH 7.5) which is blue-shifted and enhanced upon gradual addition of Hg^{2+} ions from 5.0 nM to 7.0 μM (λ_{ex} = 355 nm).

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to the results previously obtained with dansyl-containing probes.[9] Coordinated with a metal ion, the amino group loses its ability to donate an electron to the dansyl moiety. Consequently, the involved internal charge transfer (ICT) between the amide group and the dansyl moiety is inhibited and the emission band shifts toward the blue region of the emission spectrum (Fig. 1b). More importantly, the fluorescence intensity of **1** corresponded to the concentrations of Hg^{2+} in a linear manner at low concentrations (<150 nM) (Fig. S3, ESI†). Thus, **1** can be used to quantitatively detect Hg^{2+} at low levels. A detection limit of 5.0 nM for Hg^{2+} was estimated for **1**, which is only 1% of that achieved in the previous research (500 nM)[8b] and is lower than the EPA mandate of 10 nM for Hg^{2+} in drinking water.[2] To the best of our knowledge, **1** displays the lowest detection limit of all the small-molecule fluorescent probes used for Hg^{2+} detection in pure water.

Over the pH range tested, compound **1** alone was not sensitive to pH as illustrated by fluorescence intensity at 487 nm (I_{487}) (Fig. S4, ESI†). In the presence of Hg^{2+}, however, the complex of **1**–Hg^{2+} had a strong pH-dependence which showed a stable and sensitive fluorescence response in the pH range of 7.5–10.0, affording the possibility for detection of Hg^{2+} by **1** under weakly basic conditions. Thus, in the current study, all the determinations of Hg^{2+} and other metal ions were carried out in the presence of 5.0 μM **1** in 10.0 mM HEPES buffer solution (pH 7.5).

We also examined the fluorescence response of **1** to 15 other metal ions under the same conditions used to test Hg^{2+} (Fig. 2). No obvious fluorescence changes were observed for **1** in the presence of these metal ions, even at concentrations as high as 20.0 μM. Perhaps incompatible ion size or binding affinity between these metal ions and **1** prevented such an interaction. To test the practical application of **1** as a Hg^{2+} probe, we conducted competitive experiments in mixing 2.0 μM Hg^{2+} with other metal ions at 10.0 μM. Little interference was observed by these metal ions in the detection of Hg^{2+} (Fig. S5, ESI†), confirming that **1** can be used for the practical detection of Hg^{2+} in aqueous solution.

In addition, the continuous variation (Job's plot, Fig. S6, ESI†) and metal-binding fluorescence titrations indicated that a 2 : 1 stoichiometric ratio of **1** : Hg^{2+} was the most stable species in solution with an overall K_a of 3.98×10^{13} L^2 mol^{-2} (Fig. S7, ESI†). Using NMR experiments, we examined the coordination mechanism of **1** and Hg^{2+} in solution.

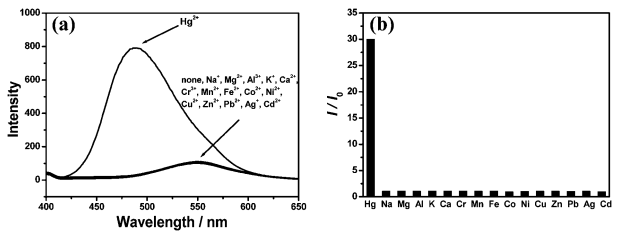

Fig. 2 (a) The fluorescence intensity of **1** (5.0 μM) in 10.0 mM HEPES solution (pH 7.5) in the presence of 2.0 μM of Hg^{2+} and 20.0 μM of the other indicated ions (λ_{ex} = 355 nm). (b) The intensity ratio of the fluorescence emission bands at 487 nm in the presence of each indicated ion.

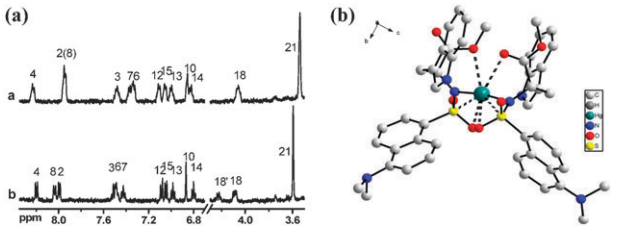

Fig. 3 (a) Region of the ^1H NMR spectra of (a) **1** (0.5 mM) and (b) **1**–Hg^{2+} (1 : 1) in CD_3OD : D_2O = 1 : 2. (b) View of the X-ray crystal structure of complex **1**–Hg^{2+} (H-atoms were omitted for clarity).

Characteristic structural changes can be observed upon interaction with Hg^{2+} (Fig. 3a, Table S1, ESI†). Chelation of Hg^{2+} with the nitrogen atoms of the sulfonamide groups reduced the charge transfer between it and the dansyl moiety and resulted in large chemical shifts of protons in the naphthalene ring. In addition, coordination between Hg^{2+} and oxygen in the carboxylic ester induced a large chemical shift in H(21) ($\Delta\delta_{H21}$ = 0.058 ppm). The splitting of the chemical shift of H(18) after binding with Hg^{2+} suggested an asymmetric binding model between Hg^{2+} and two molecules of **1**. Meanwhile, the minor chemical shifts of H(12), H(13), H(14), and H(15) may be related to the weak binding of the indole rings to Hg^{2+}.

Slow evaporation of solvent from the sample which contained a complex of **1**–Hg^{2+} in ethanol and water at room temperature yielded buff-colored, strongly fluorescent crystals (Fig. S8, ESI†), which were analyzed by single crystal X-ray diffraction.‡ The complex comprises two molecules of **1** and one Hg^{2+} (Fig. 3b), in accordance with the data revealed by Job's plot and metal-binding fluorescence titrations. The diagram also reveals that the deprotonated amino group resulted in a super-strong interaction of nitrogen and Hg^{2+} defined by an averaged bond distance of 2.049(8) Å, and that the two sulfur and four oxygen atoms stabilize the complex through weak interactions. In addition, the weak interaction between Hg^{2+} and C(10), C(11) (for numbering see Fig. 1a) suggests that the two indole rings may play a role in stabilizing the **1**–Hg^{2+} complex (Fig. S9, ESI†). The crystallographic data thus supply solid evidence and confirm the ^1H NMR spectral data obtained in solution (Fig. 3a).

On the basis of these results, we confirmed that the blue-shift and enhancement of the emission band of **1** (Fig. 1b) originated from the strong binding between Hg^{2+} and the deprotonated amino group, which induced the disruption of ICT between the amino group and the dansyl moiety. The high stability of **1**–Hg^{2+} complex in aqueous solution could be attributed to the enhanced chelation of the nitrogen atom and Hg^{2+}, various weak interactions provided by multiple atoms, and also by the fixed placement in space of Hg^{2+} by the two indole rings.

Finally, **1** was used for imaging in HeLa cells to confirm that our novel fluorescent probe can detect Hg^{2+} in live cells (see ESI†). HeLa cells were incubated for 15 min with **1** (5.0 μM) to allow the probe to permeate into the cell. The increases in the fluorescence intensity in live cells were observed upon further addition of Hg^{2+} (5.0 μM) into the medium and incubation for another 15 min at 37 °C. The cells displayed

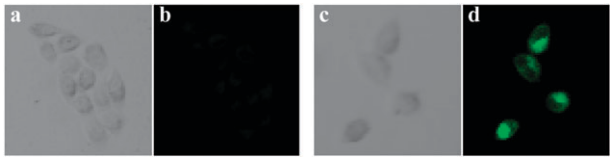

Fig. 4 Bright field (a, c) and confocal fluorescence microphotographs (b, d) of $HgCl_2$ uptake by live HeLa cells: (a, b) after 15 min exposure to 5.0 μM **1**; and (c, d) 15 min exposure to 5.0 μM **1** and further 15 min exposure to 5.0 μM $HgCl_2$. The emission was measured over the range of 475–505 nm, λ_{ex} = 405 nm.

markedly different brightness (Fig. 4) as the uptake of both **1** and Hg^{2+} in live cells proceeded. Notably, after thorough washing to remove free **1** in the extracellular medium, no background fluorescence can be observed in both cases. The results suggest that **1** can be used to image intracellular Hg^{2+} in live cells.

In summary, we have developed a hypersensitive fluorescent probe that can selectively detect low levels of Hg^{2+} in buffered aqueous solution. The novel probe exhibits characteristics of high affinity, fast and turn-on response to Hg^{2+}, and especially good water-solubility when compared to other reported probes. The detection of Hg^{2+} by **1** is evidence that specific properties can strongly influence the emission behavior of **1** both in aqueous solution and in live cells. Further investigations in live cells demonstrated the potential applications of this novel probe for the study of the toxicity or bioactivity of Hg^{2+} in live cells.

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Notes and references

‡ Crystal data for the complex **1**–Hg^{2+} ($C_{48}H_{48}HgN_6O_8S_2$): M_w = 1101.63, crystal dimensions: 0.22 × 0.10 × 0.05 mm^3, triclinic, space group: *P*1, *a* = 9.694(2) Å, *b* = 14.896(4) Å, *c* = 16.783(5) Å, α = 87.210(11)°, β = 87.610(11)°, γ = 85.290(11)°, *V* = 2410.8(12) $Å^3$, *Z* = 2, ρ_{calcd} = 1.518 g cm^{-3}, μ(MoKα) = 3.337 mm^{-1}, *F*(000) = 1108, *T* = 291(2) K, $2\theta_{max}$ = 55.02. 18 562 reflections measured, of which 13 763 were unique (R_{int} = 0.0565). Final R_1 = 0.0453 and wR_2 = 0.0789 for 10 458 observed reflections with $I > 2\sigma(I)$, Flack parameter = 0.000(5).

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