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A Water-Soluble "Switching On" Fluorescent Chemosensor of Selectivity to Cd^{2+}

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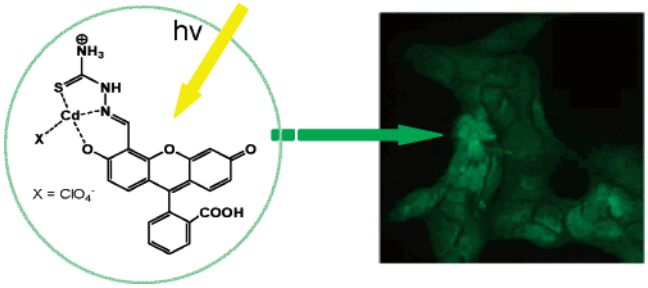
ABSTRACT



Compound 1, a new fluorescent chemosensor signaling via significantly enhanced fluorescence when bound with cation analytes, was synthesized and characterized. This fluorescent chemosensor exhibits its selectivity to Cd^{2+} among a series of cations in HEPES buffer solution. Its in vitro sensitivity to Cd^{2+} was demonstrated in the HK-2 cell line with use of confocal microscopy. The mechanistic selectivity and sensitivity of compound 1 to Cd^{2+} was discussed on the basis of fluorescence, ^1H NMR, and mass spectroscopic results.

Cadmium is extensively explored in the field of Ni−Cd batteries, phosphate fertilizers, pigments, and semiconducting quantum dots and rods.[1] The increasing exposure to these Cd^{2+} sources could cause diseases such as renal dysfunction, reduced lung capacity, and emphysema,[2] due to the accumulation of Cd^{2+} in organs such as the kidney, thyroid gland, hippocampus, and spleen.[3] Therefore, there is a clinical need to quantitatively monitor the existence of Cd^{2+} in vitro and in vivo. Fluorescent chemosensor has become an important diagnostic tool for biological and environmental concerns.[4] For these purposes, a fluorescent chemosensor is needed to be biocompatible in physiological conditions. Indeed, several groups have contributed to the exploitation of such types of fluorescent chemosensors for in vitro cation detection.[5] More recently, Chang and Pang's groups reported selective detec-

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tion of Pb^{2+} and Cd^{2+} in living cells using either fluorescein or BODIPY as optical reporter and dicarboxylate pseudocrown and *N*,*N*-bis(pyridin-2-ylmethyl)benzenamine as cationic receptors.[6] The turn on fluorescence in these in vitro sensing systems generally resulted from the suppression of photo-induced electron-transfer mechanism via chelating positive cations.

In the course of our continuing exploration for fluorescent probes/chemosensors,[7] we reported a novel switching on fluorescent system based on restricting the rotation of C═N bonds by complexation of cations. Empirically, compounds containing acyclic C═N bonds are usually nonfluorescent, and cyclic C═N bonds significantly fluorescent.[7d] Following this strategy, we designed a fluorescein-based chemosensor **1** transduced through the restriction on acyclic C═N, in which both the optical reporter fluorescein and cationic receptor thiosemicarbazide were water soluble and biocompatible. Experimentally, the fluorescence of compound **1** is largely enhanced by adding Cd^{2+}, differentiated from other cations such as NH_4^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Pb^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, and Zn^{2+}, indicative of its selectivity to Cd^{2+} in physiological conditions (50 mM HEPES, 100 mM KCl, pH 7.0). Moreover, compound **1** was also demonstrated in this contribution to image Cd^{2+} in living HK-2 cell lines with use of confocal microscopy.

Compound **1** was synthesized in two steps as shown in Figure 1. Following Reimer−Tieman reaction of fluorescein,[8]

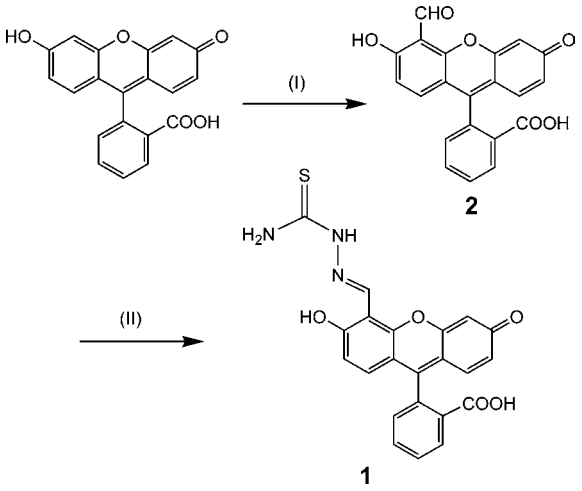

Figure 1. Synthesis of compound **1**: (I) $CHCl_3$, NaOH, MeOH/H_2O, 55 °C, 10 h, 31%; (II) $NH_2NHC{=}SNH_2$, ethanol, refluxing, 6 h, 70%.

compound **2** was obtained in 31% yield. The coupling reaction of compound **2** and thiosemicarbazide was refluxing in ethanol, to yield compound **1** in 70%. The detailed procedures and their characterizations are described in the Supporting Information (Figure S1). The UV−vis absorption and fluorescence spectra of fluorescein, **1**, and **2** in HEPES buffer solutions are compared in Figure 2. Spectrally, the featureless absorbance and emission of fluorescein were

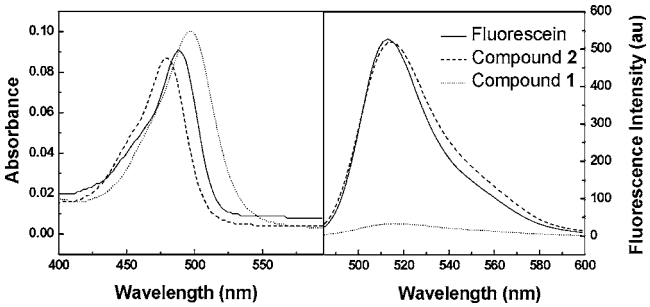

Figure 2. UV−vis absorption and fluorescence spectra (excited at 480 nm) of fluorescein, **1**, and **2** in HEPES solution.

observed for compounds **1** and **2**. Their maximum emissions remain the same; however, the absorbance peak shifts to the blue 9 nm for compound **2** and to the red 9 nm for compound **1**, reflecting their corresponding Stoke shifts via the structure modification of fluorescein. The fluorescence quantum yield of compound **2** ($\Phi = 0.659$) is comparable to that of fluorescein ($\Phi = 0.634$). In contrast, the fluorescence quantum yield of compound **1** ($\Phi = 0.00638$) is about 1000 times lower than that of fluorescein. We tentatively ascribed such significantly reduced fluorescence intensity of compound **1** to the involvement of acyclic C═N, though other mechanisms such as photoinduced electron transfer may also contribute.

The fluorescence spectra of compound **1** upon addition of diverse cations were recorded in HEPES buffer solution. The fluorescence response of compound **1** to different cations is displayed in Figure 3a. The fluorescence intensity of compound **1** in HEPES buffer solution is increased by adding Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, and Cd^{2+}, and

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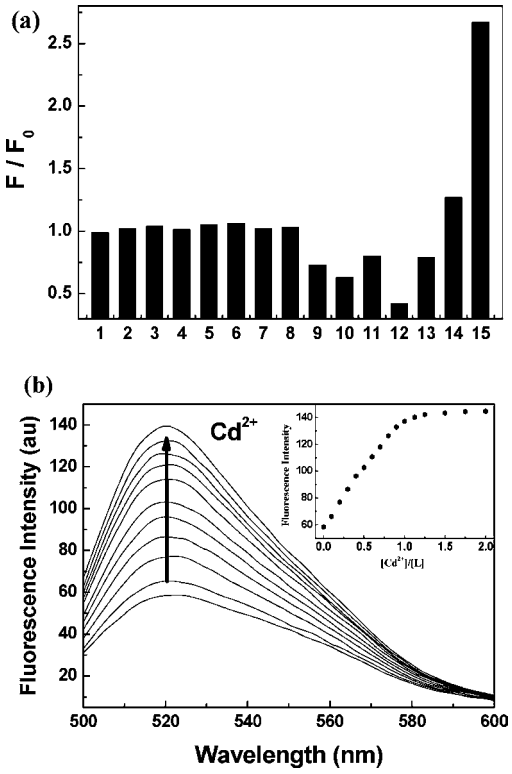

Figure 3. (a) Fluorescence responses of **1** (5 μM) in buffer solution (50 mM HEPES, 100 mM KCl, pH 7.0) to the addition of 20 equiv of (1) NH_4ClO_4, (2) $LiClO_4$, (3) $NaClO_4$, (4) $KClO_4$, (5) $Mg(ClO_4)_2$, (6) $Ca(ClO_4)_2$, (7) $MnSO_4$, (8) $Pb(ClO_4)_2$, (9) $Fe(ClO_4)_2$, (10) $CoCl_2$, (11) $NiCl_2$, (12) $Cu(ClO_4)_2$, (13) $Hg(ClO_4)_2$, (14) $Zn(ClO_4)_2$, and (15) $Cd(ClO_4)_2$ (λ_{ex} = 480 nm, λ_{em} = 521 nm). (b) Fluorescence spectral variation of **1** (10 μM) upon addition of Cd^{2+} in HEPES buffer solution from 0 to 10 μM excited at 480 nm. The inset shows the plot of fluorescence intensity vs the ratio of Cd^{2+} to **1**.

decreased in varied degrees upon the addition of Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Hg^{2+}. In general, some open-shell transition and post-transition cations often quench the fluorescence of fluorophores through the electron or energy transfer between these metal cations and fluorophores, resulting in fluorescence decrease.[5c] In contrast, cations like NH_4^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, and Cd^{2+} cannot form low-energy metal-centered or charge-separated excited states so that energy and electron-transfer processes cannot take place. The fluorescence enhancement of compound **1** upon addition of these cations could be interpreted as somewhat inhibited rotation of the acyclic C═N bond in the complex of compound **1** and these cations.

As shown in Figure 3a, the fluorescence enhancement of compound **1** is much more significant when Cd^{2+} is added. And the presence of some cations such as NH_4^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, and Pb^{2+} (Figure S2, Supporting Information) does not interfer with the Cd^{2+}-enhanced fluorescence. When 20 equiv of Cd^{2+} is added to the solution of compound **1** in the presence of 20 equiv of Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, or Zn^{2+}, however, no fluorescence response was observed, possibly because the formed complex between these cations and compound **1** was too stable to be replaced by Cd^{2+} as reported in the literature.[9] To understand the interaction between compound **1** and Cd^{2+}, the fluorescence variation of compound **1** was measured upon the addition of Cd^{2+} from 0 to 10 μM (Figure 3b). Their fluorescence spectra are the same when normalized. However, the UV−vis absorption spectrum of compound **1** shifts 4 nm to the blue when 20 μM of Cd^{2+} is introduced (Figure S3, Supporting Information). These results reveal the certain structural modification on compound **1** by adding Cd^{2+}, indicative of fluorescence enhancement induced by binding Cd^{2+}. The fluorescence intensity of **1**-Cd^{2+} solution decreased to its original intensity of free ligand upon addition of excess EDTA because EDTA is a very strong cation chelating agent, serving as a Cd^{2+} chelator superior to compound **1**. This result further confirms that the process is reversible.

The plot of fluorescence intensity change against the ratio of Cd^{2+} to compound **1** is presented in the inset to Figure 2b. A turning point appears at the 1:1 ratio of Cd^{2+} to compound **1**, indicating the formation of 1:1 complex of compound **1** and Cd^{2+}. Simultaneously, the complex constant of compound **1** and Cd^{2+} is estimated to be $(8.14 \pm 3.01) \times 10^6$ M^{-1} (R = 0.998) by using nonlinear least-square analysis (Figure S4, Supporting Information).[10] The further supporting evidence for the formation of a 1:1 complex of compound **1** and Cd^{2+} is the mass spectroscopy. The ESI-TOF result shows a maximum signal at m/z 546.1, the exact mass of [**1** + Cd − H]$^+$ (Figure S5, Supporting Information).

To illustrate the structure of the complexation of compound **1** and Cd^{2+}, ^{1}H NMR spectra of compound **1** and its complex with Cd^{2+} in DMSO-d_6 were measured as shown in Figure 4a. The active protons are assigned as displayed in Figure 4a referring to literature[11] as well as ^{1}H NMR of compound

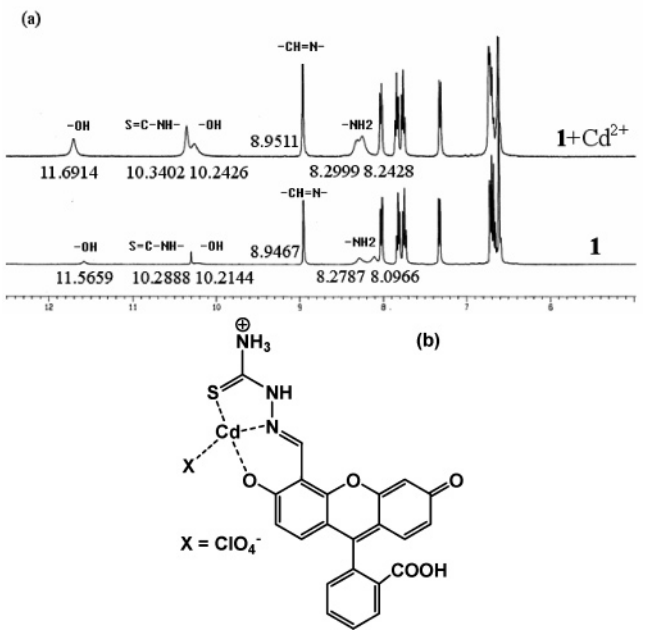

Figure 4. (a) ^{1}H NMR spectra of **1** and **1** + Cd^{2+}. (b) A proposed structure for **1** + Cd^{2+}.

2. Apparently, the signals for these active protons shift to downfield in certain degrees, associated with the enhancement of signal intensity in the **1** + Cd^{2+} complex, in comparison to those of **1**. These results indicate the electron-shielding effect of Cd^{2+} on protons in its proximity, as well as the reduced proton exchanges in a tight complexation form. Accordingly, the structure of **1** + Cd^{2+} is proposed as in Figure 4b, in which Cd^{2+} coordinates with thiocarbonyl, phenolic hydroxyl, and Schiff base.

The sensitivity of compound **1** to Cd^{2+} was examined in living cells by using confocal microscopy. The qualitatively in vitro results are exhibited in Figure 5. After HK-2 cells were incubated with 10 μM of compound **1** for 30 min at 37 °C, no obvious fluorescence can be imaged (Figure 5a). At the same experimental conditions, the strong fluorescence was imaged 10 min after 20 μM of Cd^{2+} was introduced into the same HK-2 cells as in Figure 5a (Figure 5b). In a parallel experiment, the fluorescence image of HK-2 cells incubated with 10 μM of compound **1** for 60 min at 37 °C remains the same as in Figure 5a (Figure S6a, Supporting Information). The imaged fluorescence thus resulted from the complexation of compound **1** and Cd^{2+}, instead of compound **1** itself. The bright field transmission images of these HK-2 cells in Figure 5c is exactly the same as the fluorescence image in Figure 5b, confirming that the imaged fluorescence is intracellular, instead of extracellular. It can also be observed that the imaged fluorescence intensity is increased in HK cells pretreated with 10 μM **1** with introduction of Cd^{2+} from 0, 5, 10, to 20 μM (Figure S6, Supporting Information).

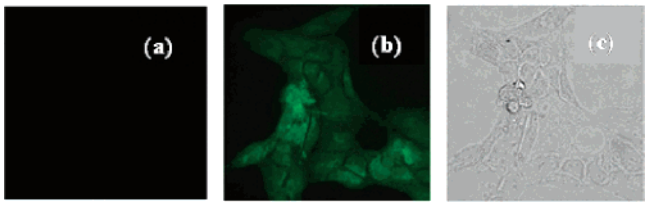

Figure 5. The intracellular Cd^{2+} was imaged in living cells at 37 °C with use of confocal microscopy. (a) HK-2 cells incubated with 10 μM of compound **1** buffer solution for 30 min. (b) HK-2 cells in part a 10 min after being treated with 20 μM of Cd^{2+} solution. (c) Bright field image of living HK-2 cells in parts a and b.

In summary, a fluorescein-based chemosensor **1** exhibiting good water solubility and biocompatibility was synthesized and characterized. The selectivity of compound **1** to Cd^{2+} in HEPES buffer solution from a series of cations is evidenced as its exceptional fluorescence enhancement. This significantly enhanced fluorescence is probably due to the formation of a 1:1 complex **1**-Cd^{2+} in which the rotation of acyclic C=N is frozen. The sensitivity of compound **1** to Cd^{2+} was demonstrated in living cells, indicating its potential application for Cd^{2+} diagnoses in clinics.

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Supporting Information Available: Method and materials, ^{1}H NMR and ^{13}C NMR of **1**, fluorescence response of **1** to a variety of cations, UV−vis absorption comparison of **1** and **1** + Cd^{2+}, ESI-MS of **1**+Cd^{2+}, and confocal images of living HK-2 cells in the presence of compound **1** treated with different concentrations of Cd^{2+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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