Long-Wavelength Fluorescent Chemosensors for Hg$^{2+}$ based on Pyrene

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Supporting Information

ABSTRACT: A novel long-wavelength turn-on fluorescent chemosensor CS based on pyrene was synthesized to detect Hg$^{2+}$. In the presence of other metal ions, CS could effectively recognize Hg$^{2+}$ and produce the turn-on fluorescent emission at 607 nm. Also, the absorption spectrum exhibited red-shift. Meanwhile, the change of the solution color from yellow to orange was directly observed by the naked eye. The interaction between CS and Hg$^{2+}$ was confirmed by the Job's plot, electrospray ionization mass spectrometry, scanning electron microscopy, and density functional theory calculations. It was found that the fluorescence of CS could be reversible when I$^-$ was added into the solution of CS and Hg$^{2+}$. CS illustrated high selectivity and good sensitivity for Hg$^{2+}$ with the limit of detection of 36 nm. Moreover, CS could be utilized as test strips and silica gel plates to identify Hg$^{2+}$.

INTRODUCTION

It is well known that Hg$^{2+}$ is harmful to human health and environment$^{7-13}$ because it can cause deadly damages to organs of the human body including the brain, nervous system, endocrine system, and kidneys.$^{5,6}$ Therefore, it is necessary to develop some rapid and efficient methods to identify Hg$^{2+}$.

Compared to others methods, fluorescent and colorimetric chemosensors have attracted more and more attention owing to their obvious advantages.$^{7-9}$ At present, a lot of chemosensors toward Hg$^{2+}$ have been designed, synthesized, and characterized.$^{10-13}$ In these chemosensors, there are three aspects to be improved: (1) many chemosensors recognize Hg$^{2+}$ through the “on-off” fluorescent type. However, the type is easy to give false positive results.$^{14-16}$ (2) Although some chemosensors can recognize Hg$^{2+}$ by the “off–on” fluorescent type, their emission wavelength is not long enough when they interact with Hg$^{2+}$. Hence, their selectivity and sensitivity have been restricted. It is essential to figure out certain chemosensors with long-wavelength emission when they combine with Hg$^{2+}$.17–22 (3) The structures of several chemosensors are too complicated to be synthesized.23,24 In order to solve the defects, it is necessary to seek turn-on, long-wavelength, and ease-synthesis chemosensors toward Hg$^{2+}$.

In this paper, a novel pyrene-based chemosensor CS (Scheme 1) has been synthesized by one-step reaction, by which it is convenient to be separated and purified. When CS interacts with Hg$^{2+}$, a new emission peak at 607 nm arises. Also, the absorption spectrum exhibits red-shift. The color change can be directly observed by the naked eye.

RESULTS AND DISCUSSION

By the absorption spectrum and the fluorescence spectrum, the effect of metal ions on the spectrum behavior of CS was studied. It was clear that only Hg$^{2+}$ could lead to the absorption change, the maximum absorption peak of which shifted from 427 to 470 nm. It was interesting that the solution color change directly from yellow to orange could be observed by the naked eye (Figure 1). Also, the solution of CS displayed weak fluorescence when it was excited by 470 nm. Once Hg$^{2+}$ was added into the CS solution, the new red emission peak at 607 arose immediately. The change did not happen as other metal ions were added except Hg$^{2+}$ (Figure 2). Based on the changes of spectra, it indicated that CS would recognize Hg$^{2+}$ selectively and sensitively.

According to competitive experiments, it was found that CS could efficiently identify Hg$^{2+}$ in the presence of other metal...
ions (Figures 3 and 4). These results suggested that CS could be used as the fluorescent chemosensor for detecting Hg$^{2+}$ with long-wavelength emissions.

In order to discuss the interaction between CS and Hg$^{2+}$, the molar method and the continuous variation method were carried out to explore their complexation ratios. As a result, Job’s plot showed 1:2 stoichiometry for the interaction between CS and Hg$^{2+}$ (Figures S3–S6, Supporting Information). By mass spectral analyses, an ion peak was detected at m/z 811.0479, which was in accordance with [CS + 2Hg$^{2+}$ + H$_2$O]$^+$ (Figure S2, Supporting Information). On the basis of the data, it was clear that the stoichiometric ratio between them was 1:2 when CS interacted with Hg$^{2+}$. To make further efforts to understand the interaction between them, scanning electron microscopy (SEM) experiments were applied to examine the aggregation of CS and CS$^-$Hg$^{2+}$. To our surprise, the aggregation of CS transformed the spherical flower-type into layered porous structure after CS interacted with Hg$^{2+}$. Also, there were a lot of evident needle-like synapses on the layered structures (Figure 5). Besides, the limit of detection (LOD) of CS toward Hg$^{2+}$ was calculated to be 36 nm (Figure S7, Supporting Information). In addition, the comparative analysis of CS with chemosensors, which have been reported previously is displayed in Table 1.

To ensure the interaction between CS and Hg$^{2+}$, the density functional theory (DFT) calculations were adopted by use of the Gaussian 03 program. It was definite that the molecular orbital structure of CS–Hg$^{2+}$ was different from CS in Figure 6. The highest occupied molecular orbital (HOMO) was uniformly distributed over the entire molecule in CS–Hg$^{2+}$. Also, the lowest unoccupied molecular orbital (LUMO) was mainly concentrated around the CS–Hg$^{2+}$ binding point. The energy gaps between the HOMO and LUMO in CS and CS–Hg$^{2+}$ was calculated to be 2.9604 and...
2.7051 eV, respectively. The results also proved the interaction mode between \( \text{CS} \) and \( \text{Hg}^{2+} \) (Scheme 2).

For chemosensors to identify metal ions, reversibility was very important. Therefore, the anion fluorescence response experiment was performed by adding different kinds of anions. Interestingly, it was definite that none but I\(^-\) could reduce the fluorescent intensity of [CS–Hg\(^{2+}\)] at 607 nm to minimum when different kinds of anions (10 equiv) were added into the solution of [CS–Hg\(^{2+}\)] (Figure 7). It was because I\(^-\) might react with Hg\(^{2+}\), which makes the fluorescence of the solution turn off. Therefore, it hinted that CS could detect Hg\(^{2+}\) reversibly with an “off–on–off”-type fluorescent signaling behavior.

For inspecting CS practicability, test strips and silica gel plates were prepared by us in line with the literatures. When test strips were immersed in the solutions of Hg\(^{2+}\), the fluorescence enhanced at once under the 365 nm UV lamp irradiation (Figure 8a,b). Meanwhile, the obvious color change was perceived under sunlight (Figure 8c,d). In addition, under the 365 nm UV lamp irradiation, the red fluorescent “Hg\(^{2+}\)” image appeared immediately after the Hg\(^{2+}\) solution was dipped with a brush and written on a silica gel plate containing CS (Figure 7e,f). Consequently, chemosensor CS could be looked upon as the potential fluorescent probe in practice.

### Table 1. Comparison of Previously Reported Fluorescent Chemosensors with CS

| Probes name | LOD (nM) | Solvent | Refs |
|-------------|---------|---------|------|
| L1          | 152     | CH\(_3\)CN/\(\text{H}_{2}\)\text{O} = 9/1, v/v, buffered with Tris-HCl, pH = 7.0 | 25   |
| S1          | 522     | DMF/HEPES = 3/1, v/v, 10 mM, pH = 7.4 | 26   |
| PEG–DMS     | 646     | water   | 27   |
| NBDTe       | 4200    | \(\text{CH}_3\)\text{CN}/PBS = 4:1, v/v, 1 mM, pH = 7.4 | 28   |
| 1           | 5000    | 90/10 (v/v) DMF–H\(_2\)O solution (4 mM) | 29   |
| BN–S        | 72.7    | water   | 30   |
| 3           | 200     | 50% aqueous \(\text{CH}_3\)\text{CN} (acetate buffered at pH = 4.0) | 31   |
| CS          | 36      | HEPES buffer (10 mM, pH = 7.4)/\(\text{CH}_3\)\text{CN} (30:70, v/v) | this work |

### Scheme 2. Proposed Interaction Mode Between CS and Hg\(^{2+}\)

For chemosensors to identify metal ions, reversibility was very important. Therefore, the anion fluorescence response experiment was performed by adding different kinds of anions. Interestingly, it was definite that none but I\(^-\) could reduce the fluorescent intensity of [CS–Hg\(^{2+}\)] at 607 nm to minimum when different kinds of anions (10 equiv) were added into the solution of [CS–Hg\(^{2+}\)] (Figure 7). It was because I\(^-\) might react with Hg\(^{2+}\), which makes the fluorescence of the solution turn off. Therefore, it hinted that CS could detect Hg\(^{2+}\) reversibly with an “off–on–off”-type fluorescent signaling behavior.

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### Conclusions

In conclusion, long-wavelength chemosensor CS could effectively recognize Hg\(^{2+}\) with high selectivity and good sensitivity in the presence of other metal ions, which was synthesized easily by one-step reaction. Also, the LOD of CS toward Hg\(^{2+}\) was calculated to 36 nM. When CS interacted with Hg\(^{2+}\), the fluorescent emission peak appeared at 607 nm. Meanwhile, the color change of the solution was observed directly by the naked eye. The interaction between them was studied based on the Job’s plot, electrospray ionization mass spectrometry (ESI-MS), SEM, and DFT calculations. In addition, CS could detect Hg\(^{2+}\) with the “off–on–off” type fluorescent signaling behavior. Moreover, chemosensor CS showed good future applications in detecting Hg\(^{2+}\) in test strips and silica gel plates made by ourselves.
EXPERIMENTAL SECTION

Materials and Physical Methods. All the materials used for synthesis were procured from commercial suppliers and all solvents and materials used without further purification. NMR spectra were performed on a Bruker at 500 MHz using tetramethylsilane as an internal standard and DMSO-δ6 and CDCl3 as the solvents. UV–vis absorption spectra and luminescence spectra were recorded at room temperature on a Shimadzu UV-1601 spectrophotometer and a Horiba FluoroMax-4-NIR spectrometer. HRMS data were obtained with a Shimadzu mass spectrometer. SEM measurements were performed on a Carl Zeiss Sigma 500.

The solution of inorganic salt were prepared from the nitrate salts of K+, Na+, Ag+, Hg2+, Ni2+, Co2+, Cu2+, Mg2+, Cd2+, Zn2+, Ba2+, Ce3+, Pb2+, Fe3+, and Y3+. The solutions of anions (F−, Cl−, Br−, I−, S2−, SO4−2, SO3−2, HCO3−, H2PO4−, CO2−3, NO3−, NO2−, and CH3COO−) were prepared from their sodium or potassium salts. The solution of CS was prepared in HEPES buffer (10 mM, pH = 7.4)/CH3CN (30:70, v/v) and the ligand concentration was kept constant (1.0 × 10−6 M).

Synthesis and Characterization. 5-Phenyl-1,3,4-thiadiazol-2-amine (88.61 mg, 0.5 mmol) and pyrene-1-carbaldehyde (115.13 mg, 0.5 mmol) were dissolved in 30 mL of ethanol. A catalytic amount of glacial acetic acid was added into the solution and the mixture was refluxed for 12 h with stirring, during which an orange precipitate was formed. The crude product was filtered and recrystallized from ethanol to obtain the chemosensor CS. It was obtained in 63% yield, 1H NMR (500 MHz, DMSO): δ 9.99 (s, 1H), 9.33 (d, J = 9.4 Hz, 1H), 8.89 (d, J = 8.2 Hz, 1H), 8.53–8.47 (m, 4H), 8.44 (d, J = 8.9 Hz, 1H), 8.33 (d, J = 8.9 Hz, 1H), 8.21 (t, J = 7.6 Hz, 1H), 8.09–8.03 (m, 2H), 7.66–7.59 (m, 3H). 13C NMR (125 MHz, CDCl3): δ 174.41, 167.17, 164.86, 135.04, 131.92, 131.01, 130.72, 130.22, 129.97, 129.14, 128.95, 128.02, 127.65, 126.88, 126.71, 126.44, 126.27, 124.98, 124.63, 124.48, 124.16, 122.95, 122.04. HRMS (ESI) m/z: [M + H+] + calcd for C25H14N4S3, 390.1059; found, 390.1062.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02078.

Characterization of chemosensor CS, ESI-MS spectrum, absorption, fluorescence spectra and Job’s plot, and detection limits (PDF)

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Notes

The authors declare no competing financial interest.

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