

Chemodosimetric Hg^{2+} -Selective Signaling by Mercuration of Dichlorofluorescein Derivatives

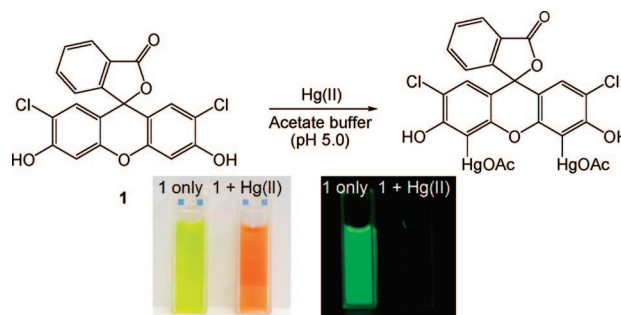
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Received June 14, 2008

ABSTRACT



The chemodosimetric behavior of dichlorofluorescein derivatives toward Hg^{2+} ions was investigated. Simple chemodosimetric systems showed selective and efficient signaling behaviors toward micromolar concentrations of Hg^{2+} ions over other common interfering metal ions in an aqueous environment. The signaling mechanism is selective mercuration of the 4',5'-position of the xanthene moiety, which results in efficient chromogenic and fluorogenic signaling of Hg^{2+} ions in aqueous environment.

Recently, a number of ingenious chemosensing systems to detect important chemical and biological species have been developed.¹ Among many widely used signaling approaches, chemodosimeters are particularly attractive due to their advantages of high selectivity and a characteristic accumulative effect for analyte determination. Based on the classic fluorescent chemodosimeter of rhodamine B hydrazide for selective Cu^{2+} signaling,² many unique signaling systems to detect Hg^{2+} , Cu^{2+} , and F^- ions have been successfully devised.³

Fluorescein and its related derivatives have been widely employed as signaling handles for molecular imaging⁴ and chemosensing applications,⁵ including the detection of metal ions like K^+ , Mg^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , and Hg^{2+} .⁶ Mercurated fluoresceins have been employed as key intermediates for the preparation of arsenic derivatives in the site-specific

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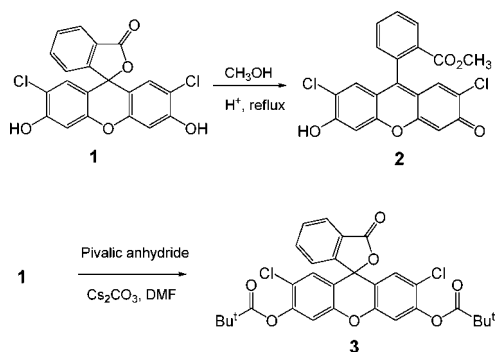
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labeling of proteins by utilizing affinity for tetracysteine motifs.⁷ However, despite the potential of mercurated fluoresceins for spectroscopy-based chemosensing, chemosensor studies using these compounds have not been reported, except for assays of sulfur compounds like hydrogen sulfide,⁸ disulfide, or thiols by fluorescence quenching.⁹ In this paper, we report the selective chemodosimetric Hg²⁺ signaling behaviors of simple structured fluorescein derivatives. Dichlorofluorescein and its methyl ester derivative (Scheme 1)

Scheme 1. Structures of Dichlorofluoresceins Investigated



showed pronounced Hg²⁺-selective chromogenic and fluorogenic signaling behaviors in an aqueous environment via selective mercuration of the 4',5'-position of the xanthere moiety. The development of selective and sensitive signaling systems for the determination of Hg²⁺ ions¹⁰ is very important due to the toxic impact of mercury on the environment.¹¹

The surveyed compounds are dichlorofluorescein **1**, methyl ester derivative **2** (formed from the lactone moiety of **1**),

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and derivative **3** where the hydroxyl substituents on the xanthere moiety of **1** are functionalized as pivaloyl esters. Dichlorofluorescein **1** exhibited characteristic absorption bands at 475 and 505 nm in acetate-buffered aqueous 10% DMSO solution (pH 5.0) (Figure 1). Upon treatment with

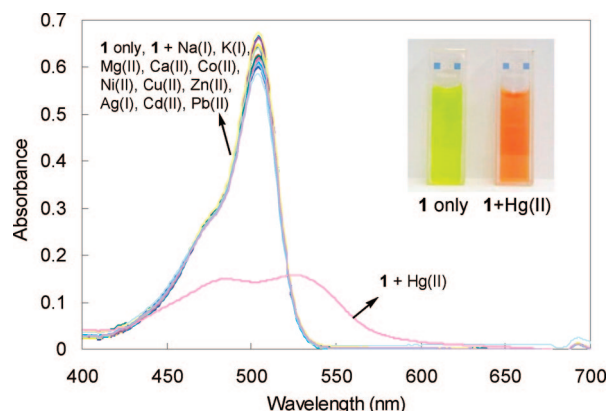


Figure 1. UV-vis spectra of dichlorofluorescein **1** in the presence of various metal ions in H₂O/DMSO (90:10) at pH 5.0 (10 mM acetate buffer). [**1**] = 1.0 × 10⁻⁵ M, [Mⁿ⁺] = 1.0 × 10⁻³ M. Inset: solution color of **1** in the absence and presence of Hg²⁺ ions.

Hg²⁺ ions, the absorption bands at 475 and 505 nm were gradually decreased and red-shifted to 483 and 533 nm (Figure S1, Supporting Information). The color of the solution changed from yellowish green to orange (inset of Figure 1). Other metal ions induced some variation in absorbance without significantly changing the absorption maximum. Selectivity toward Hg²⁺ ions was assessed by the ratiometric analysis of changes in absorption spectra. An absorbance ratio (*A*₅₃₃/*A*₄₈₃) of the two characteristic bands at 533 and 483 nm was used to illustrate the selective signaling of dichlorofluorescein toward Hg²⁺ ions. The ratio of *A*₅₃₃/*A*₄₈₃ was 1.02 for Hg²⁺ ions and varied in a limited range from 0.058 (Ni²⁺) to 0.085 (Ag⁺) for the other metal ions (Figure S2, Supporting Information).

The fluorogenic behavior of **1** was investigated under the same conditions, and **1** revealed a strong emission band around 528 nm (Figure 2). The characteristic fluorescence spectrum of **1** was effectively quenched upon treatment with Hg²⁺ ions. The quenching efficiency can be expressed by the ratio of *I*₀/*I* at 528 nm (*I*₀ and *I* represent the fluorescence intensity of **1** in the absence and in the presence of metal ions, respectively); *I*₀/*I* was larger than 1900 for Hg²⁺ ions (Figure S3, Supporting Information). Other metal ions did not induce noticeable changes in the fluorescence emission of **1**; *I*₀/*I* ranged from 1.03 for K⁺ to 1.27 for Ni²⁺. The color of the solution significantly changed from bright green to almost colorless when illuminated with a hand-held UV lamp (inset of Figure 2). Although this type of ON-OFF fluorescence signaling is not so appropriate for quantifying analytes, the chromogenic responses of this system facilitate the detection of Hg²⁺ ions by the naked eye.

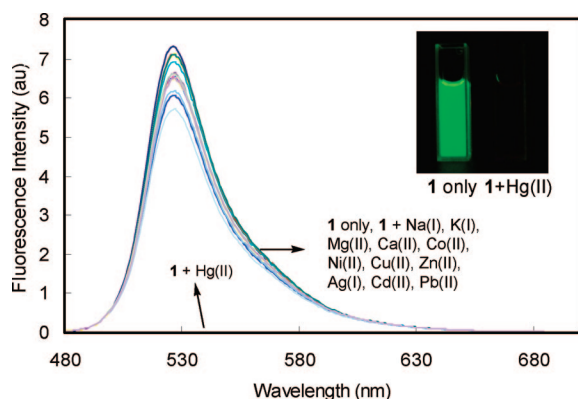
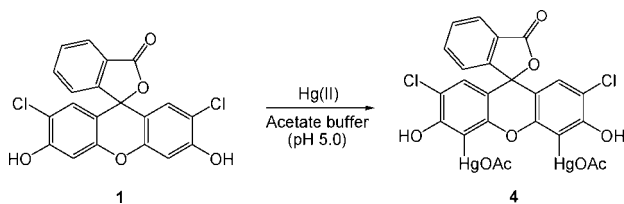


Figure 2. Fluorescence spectra of **1** in the presence of various metal ions in H₂O/DMSO (90:10) at pH 5.0 (10 mM acetate buffer). [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M. λ_{ex} = 470 nm (to obtain a full view of the fluorescence spectra of **1**). Inset: solution color of **1** in the absence and presence of Hg²⁺ ions under illumination with a UV lamp.

The Hg²⁺-selective signaling of **1** is due to selective mercuration of the xanthene moiety of **1** by Hg²⁺ ions (Scheme 2).¹² In general, the mercuration of fluoresceins is

Scheme 2. Hg²⁺-Selective Chemodosimetric Behavior of **1**



known to be limited to the phenolic residues of the xanthene moiety, in positions ortho to the hydroxyl group,¹³ and two atoms of mercury could be substituted for **1**. The selective transformation of **1** to **4** was confirmed by comparing the NMR and fluorescence spectra of independently prepared dimercurated dichlorofluorescein **4** (Figures S4 and S5, Supporting Information). The ¹H and ¹³C NMR spectra of the **1**-Hg²⁺ system, obtained by treating **1** with 10 equiv of Hg(OAc)₂ under the same conditions, were almost identical to those of **4**.

The signaling process depicted in Scheme 2 could be followed by ¹H NMR measurements in deuterated acetate-buffered aqueous 50% DMSO-*d*₆ solution (Figure 3, and Figure S5, Supporting Information). Upon treatment with 2 equiv of Hg(OAc)₂, one of the characteristic aromatic signals corresponding to the xanthene moiety of dichlorofluorescein **1** at 6.37 ppm completely disappeared, leaving a slightly

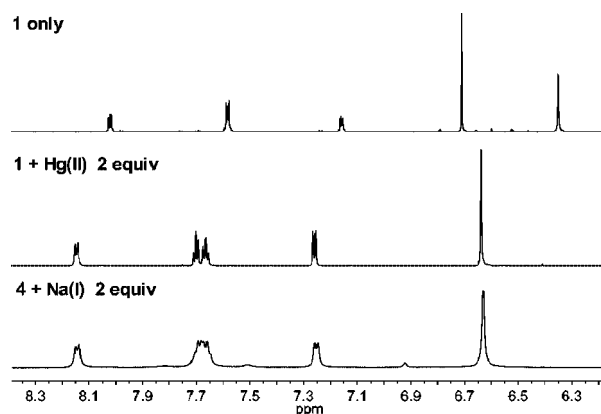


Figure 3. Partial ¹H NMR spectra of **1** only, **1** in the presence of Hg(OAc)₂, and **4** in the presence of NaOAc in deuterated acetate-buffered 1:1 D₂O/DMSO-*d*₆.

upfield-shifted singlet at 6.65 ppm, which is ascribable to the 1',8'-protons of the xanthene moiety. In contrast, the resonances of the phenyl ring bearing the carboxylic group around 7.1–8.1 ppm were not significantly affected. In the ¹³C NMR spectra, concomitantly, the resonance at 104.1 ppm, ascribable to the carbon atoms of the 4',5'-position, was prominently shifted to 120.8 ppm (Figure S6). These observations demonstrate that mercuration was effected selectively on the 4',5'-positions of the xanthene ring to yield dimercurated dichlorofluorescein **4**.

The selective Hg²⁺ signaling of **1** was not influenced by the presence of common coexisting metal ions. For example, the fluorescence intensity of the **1**-Hg²⁺ system, which was obtained by the addition of 10 equiv of Hg²⁺ ions, varied by less than 2-fold in the presence of 100 equiv of other, possibly interfering, metal ions (Figure 4 and Figure S7,

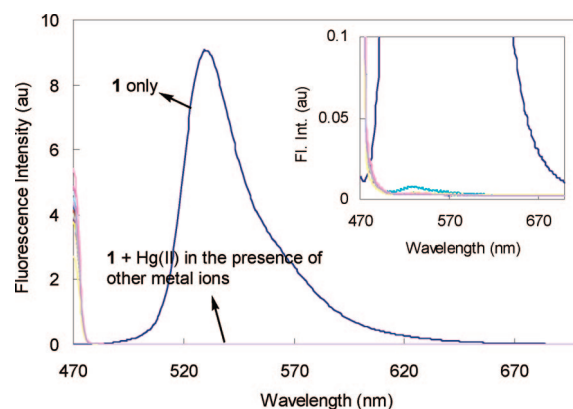


Figure 4. Fluorescence spectra of **1**-Hg²⁺ system in the presence of various metal ions in H₂O/DMSO (90:10) at pH 5.0 (buffered with 10 mM acetate). [**1**] = 2.5×10^{-5} M, [Hg²⁺] = 2.5×10^{-4} M, [M^{n+}] = 2.5×10^{-3} M. λ_{ex} = 470 nm.

Supporting Information). Furthermore, signaling was completed within 10 min after the sample preparation (Figure

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S8, Supporting Information). Other metal ions, such as Ag^+ , Cd^{2+} , and Pb^{2+} , did not induce any significant responses even 24 h after the sample preparation. From the changes in Hg^{2+} -dependent fluorescence intensity (Figure 5), the detection limit was estimated to be $7.5 \times 10^{-6} \text{ M}$.¹⁴

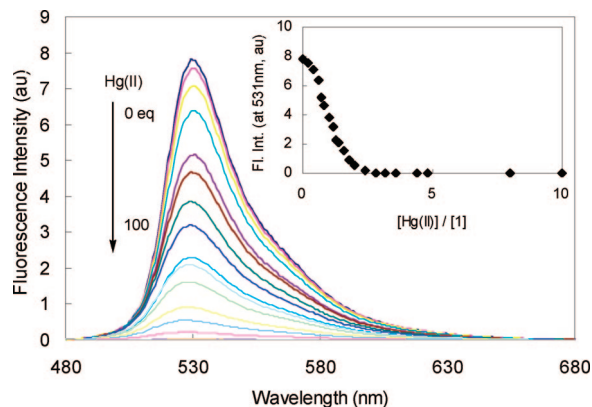


Figure 5. Fluorescence titration of **1** with Hg^{2+} ions. $[1] = 2.5 \times 10^{-5} \text{ M}$, $\text{H}_2\text{O}/\text{DMSO}$ (90:10) at pH 5.0 (10 mM acetate buffer). $\lambda_{\text{ex}} = 470 \text{ nm}$.

To gain more insight into the general signaling behavior of the fluorescein moiety, we also investigated other representative fluorescein derivatives. Methyl ester **2** exhibited similar chromogenic and fluorogenic behaviors to **1** (Figure S9, Supporting Information): the absorption band was red-shifted ($\Delta\lambda = 35 \text{ nm}$), and fluorescence was exclusively and efficiently quenched by Hg^{2+} ions (I_0/I at $534 \text{ nm} = 501$)

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(Figures S10–S12, Supporting Information). However, compound **2** showed somewhat less sensitive Hg^{2+} signaling (detection limit $= 1.5 \times 10^{-5} \text{ M}$) than **1**. In contrast, the responses of the pivaloyl derivative **3**, which could not be mercuroated on the xanthene moiety, were insignificant (Figure S13, Supporting Information), highlighting the importance of the phenolic moieties of the xanthene in the signaling process. Fluorescein itself exhibited similar chromogenic and fluorogenic behaviors to **1**; however, a disadvantage of signaling using fluorescein is that it involved the multimercuration of the xanthene moiety, which would consume up to four mercury ions under the present experimental conditions.

In summary, we have developed a simple chemodosimetric signaling system, based on a readily available dichlorofluorescein backbone, for the selective determination of Hg^{2+} ions in aqueous solutions. In acetate buffered solution, dichlorofluorescein and its methyl ester derivative exhibited selective and sensitive Hg^{2+} -induced chromogenic and fluorogenic behaviors. Signaling is due to the selective mercuration on the 4',5'-positions of the xanthene ring. Simple fluorescein-based Hg^{2+} detection systems could be used to measure the concentration of Hg^{2+} ions in the micromolar range in aqueous environment.

Acknowledgment. This work was supported by a KBSI grant (T27041) to K.S.H. and by the Bio-MR Research Program (E27071) from the Korean Ministry of Science and Technology.

Supporting Information Available: Experimental details and characterization for new compounds, NMR spectra, UV–vis, and fluorescence data are reported. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL8013446