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

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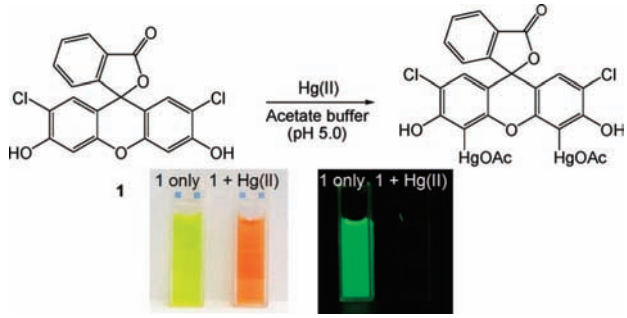
ABSTRACT



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 imaging[4] 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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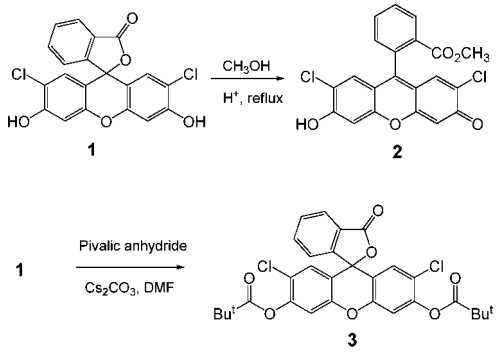
labeling of proteins by utilizing affinity for tetracysteine motifs.[7] However, despite the potential of mercurated fluoresceins for spectroscopy-based chemosensing, chemosensor studies using these compounds have not been reported, except for assays of sulfur compounds like hydrogen sulfide,[8] disulfide, or thiols by fluorescence quenching.[9] In this paper, we report the selective chemodosimetric Hg^{2+} signaling behaviors of simple structured fluorescein derivatives. Dichlorofluorescein and its methyl ester derivative (Scheme 1) showed pronounced Hg^{2+}-selective chromogenic and fluorogenic signaling behaviors in an aqueous environment via selective mercuration of the 4′,5′-position of the xanthene moiety. The development of selective and sensitive signaling systems for the determination of Hg^{2+} ions[10] is very important due to the toxic impact of mercury on the environment.[11]

Scheme 1. Structures of Dichlorofluoresceins Investigated



The surveyed compounds are dichlorofluorescein **1**, methyl ester derivative **2** (formed from the lactone moiety of **1**), and derivative **3** where the hydroxyl substituents on the xanthene 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 Hg^{2+} 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 Hg^{2+} 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 Hg^{2+} ions. The ratio of A_{533}/A_{483} was 1.02 for Hg^{2+} ions and varied in a limited range from 0.058 (Ni^{2+}) to 0.085 (Ag^{+}) for the other metal ions (Figure S2, Supporting Information).

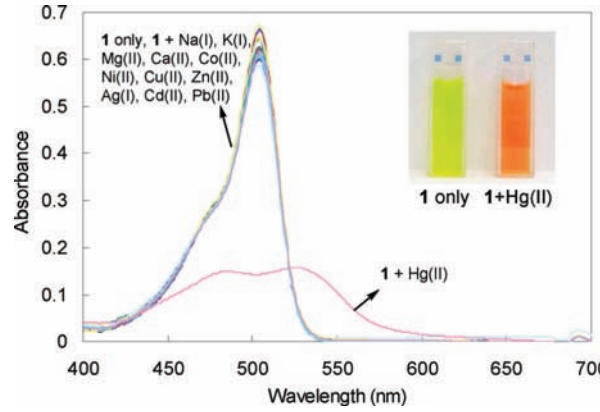


Figure 1. UV−vis spectra of dichlorofluorescein **1** in the presence of various metal ions in H_2O/DMSO (90:10) at pH 5.0 (10 mM acetate buffer). $[\mathbf{1}] = 1.0 \times 10^{-5}$ M, $[M^{n+}] = 1.0 \times 10^{-3}$ M. Inset: solution color of **1** in the absence and presence of Hg^{2+} ions.

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 Hg^{2+} ions. The quenching efficiency can be expressed by the ratio of I_o/I at 528 nm (I_o and I represent the fluorescence intensity of **1** in the absence and in the presence of metal ions, respectively); I_o/I was larger than 1900 for Hg^{2+} ions (Figure S3, Supporting Information). Other metal ions did not induce noticeable changes in the fluorescence emission of **1**; I_o/I ranged from 1.03 for K^+ to 1.27 for Ni^{2+}. 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 Hg^{2+} ions by the naked eye.

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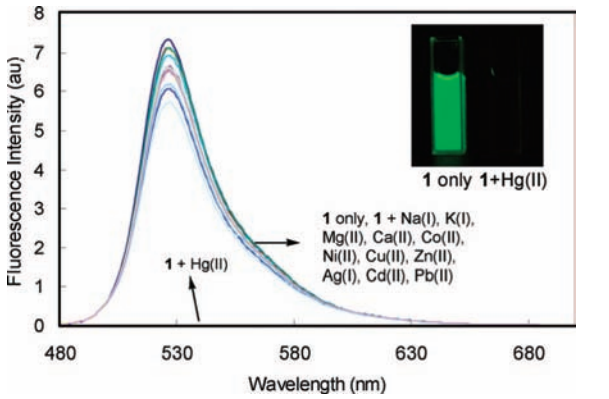
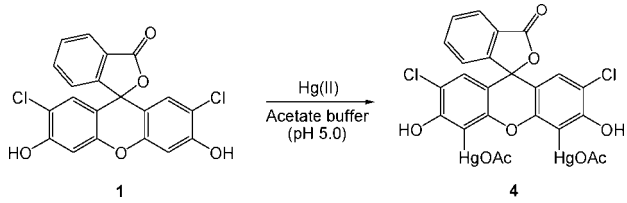


Figure 2. Fluorescence spectra of **1** in the presence of various metal ions in H_2O/DMSO (90:10) at pH 5.0 (10 mM acetate buffer). [**1**] = 5.0×10^{-6} M, $[M^{n+}] = 5.0 \times 10^{-4}$ M. $\lambda_{ex} = 470$ nm (to obtain a full view of the fluorescence spectra of **1**). Inset: solution color of **1** in the absence and presence of Hg^{2+} ions under illumination with a UV lamp.

The Hg^{2+}-selective signaling of **1** is due to selective mercuration of the xanthene moiety of **1** by Hg^{2+} ions (Scheme 2).[12] In general, the mercuration of fluoresceins is

Scheme 2. Hg^{2+}-Selective Chemodosimetric Behavior of **1**



1 **4**

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 ^{13}C NMR spectra of the **1**−Hg^{2+} 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

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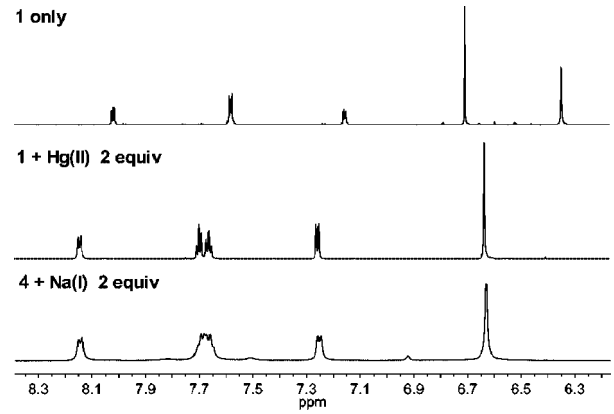

Figure 3. Partial ^1H NMR spectra of **1** only, **1** in the presence of $Hg(OAc)_2$, and **4** in the presence of NaOAc in deuterated acetate-buffered 1:1 D_2O/DMSO-d_6.

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 ^{13}C 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 Hg^{2+} signaling of **1** was not influenced by the presence of common coexisting metal ions. For example, the fluorescence intensity of the **1**−Hg^{2+} system, which was obtained by the addition of 10 equiv of Hg^{2+} ions, varied by less than 2-fold in the presence of 100 equiv of other, possibly interfering, metal ions (Figure 4 and Figure S7,

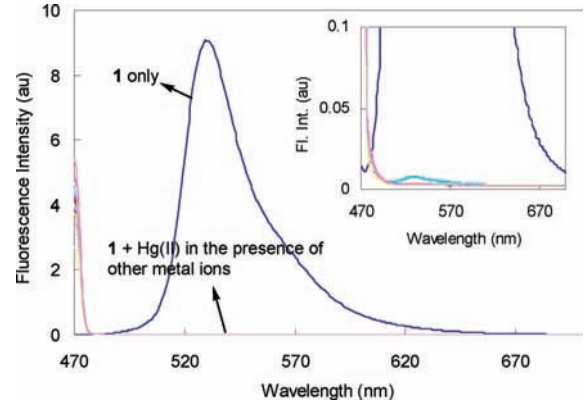

Figure 4. Fluorescence spectra of **1**−Hg^{2+} system in the presence of various metal ions in H_2O/DMSO (90:10) at pH 5.0 (buffered with 10 mM acetate). [**1**] = 2.5×10^{-5} M, $[Hg^{2+}] = 2.5 \times 10^{-4}$ M, $[M^{n+}] = 2.5 \times 10^{-3}$ M. $\lambda_{ex} = 470$ nm.

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

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 7.5×10^{-6} M.[14]

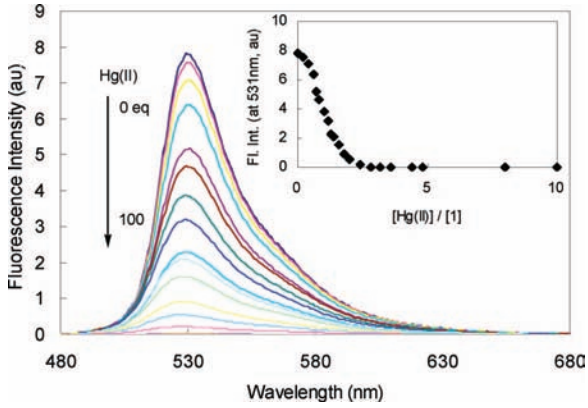

Figure 5. Fluorescence titration of **1** with Hg^{2+} ions. [**1**] = 2.5×10^{-5} M, H_2O/DMSO (90:10) at pH 5.0 (10 mM acetate buffer). λ_{ex} = 470 nm.

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 nm), and fluorescence was exclusively and efficiently quenched by Hg^{2+} ions (I_o/I at 534 nm = 501) (Figures S10−S12, Supporting Information). However, compound **2** showed somewhat less sensitive Hg^{2+} signaling (detection limit = 1.5×10^{-5} M) than **1**. In contrast, the responses of the pivaloyl derivative **3,** which could not be mercurated 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.

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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.

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