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Multisignaling Optical-Electrochemical Sensor for Hg^{2+} Based on a Rhodamine Derivative with a Ferrocene Unit

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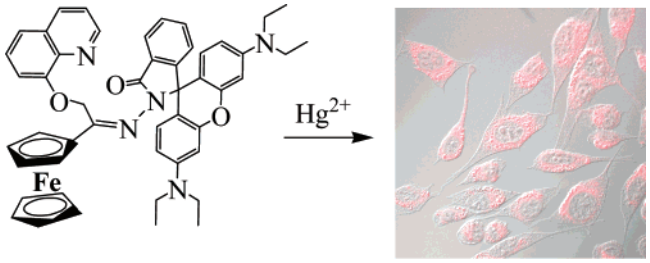
ABSTRACT



A new multisignaling sensor based on rhodamine B with a ferrocene substituent (1) has been synthesized and has been shown to display extreme selectivity for Hg^{2+} over other metal ions. Multisignaling changes are observed through UV/vis absorption, fluorescence emission, and electrochemical measurements. Furthermore, by means of confocal laser scanning microscopy experiments, it is demonstrated that 1 can be used as a fluorescent probe for monitoring Hg^{2+} in living cells.

Mercury, one of the most dangerous and ubiquitous of pollutants,[1] causes serious environmental and health problems because it can easily pass through skin, respiratory, and gastrointestinal tissues into the human body, where it damages the central nervous and endocrine systems.[2] Therefore, it is important to explore new methods for analyzing Hg^{2+} in vitro and in vivo. In the past few years, a number of Hg^{2+}-selective sensors have been devised by utilizing electrochemical,[3] chromogenic,[4] and fluorogenic[5] properties as output signals. The majority of the work on such Hg^{2+}-selective chemosensors has hitherto been focused on the development of individual optical signaling sensors, whereas there have been few reports concerning multichannel chemosensors for Hg^{2+} that are capable of both optical and electrochemical sensing.[6,7] Veciana et al. reported that two

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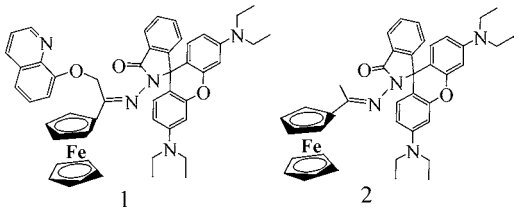
new sensors that operated through two different channels exhibited higher sensitivity and selectivity for Hg^{2+} in aqueous environments.[6a] Recently, we reported that a phosphorescent iridium(III) complex, $Ir(btp)_2(acac)$, could be used as a multisignaling chemosensor for Hg^{2+}, exhibiting suitable absorption, phosphorescence emission, and electrochemical properties.[7] However, such Hg^{2+} chemosensors are not suitable for monitoring intracellular Hg^{2+} as a result of their poor solubility in aqueous medium, especially in buffer solutions. We became interested in developing a multisignaling sensor for the in vitro monitoring of Hg^{2+}. In this regard, fluorescence bioimaging is well suited to meet the need for a highly sensitive, high-speed spatial analysis of cells.[8] The principal challenge to achieving this goal was to develop a fluorescent probe that exhibits increased visible fluorescence emission upon the addition of Hg^{2+} over other metal cations.

Rhodamine-based dyes are excellent candidates for the construction of an OFF/ON-type fluorescent probe due to their excellent spectroscopic properties of large molar extinction coefficients, high fluorescence quantum yields, and visible wavelength excitation. Several rhodamine-based fluorescent chemosensors, for Cu^{2+},[9] Pb^{2+},[10] Fe^{3+},[11,12] and Hg^{2+},[13,14] have recently been reported. Very recently, two rhodamine derivatives have been successfully fabricated as fluorescent probes for monitoring Fe^{3+} [12] and Hg^{2+} [14] in living cells. In the present case, our strategy for designing a multichannel molecular system has been to incorporate a reversible redox-active ferrocenyl group into the rhodamine fluorophore to form **1** (Scheme 1). To improve the complexation ability toward Hg^{2+}, 8-hydroxyquinoline was also introduced into **1** as a coordinating site.[15] As expected, upon addition of Hg^{2+}, **1** showed a significant switching-on of a fluorescent response and changes in its electrochemical properties. Furthermore, confocal laser scanning microscopy (CLSM) experiments demonstrated that **1** could be used as a fluorescent probe for Hg^{2+} in living cells.

Scheme 1. Chemical Structure of **1** and **2**



Sensor **1** was synthesized by a condensation reaction between an intermediate product, 1-ferrocene-2-(quinolin-8-yloxy)ethanone, and the amino group of rhodamine B hydrozide[9] in a yield of 75%. The structure of **1** was confirmed by ^1H NMR, ^{13}C NMR, and MS data (Supporting Information).

An optimized ethanol/HEPES buffer (1:1, v/v, pH 7.2) solution was selected for the spectroscopic investigations. The UV/visible spectrum of **1** (20 μM) shows only a very weak band above 500 nm, which is ascribed to its spirolactam form predominating in solution. The characteristic peak at $\delta = 67.99$ ppm in the ^{13}C NMR spectrum of **1** (Supporting Information) also supports this conclusion. Upon addition of Hg^{2+} to a solution of **1**, the solution turned from colorless to pink (Figure 1), and the absorbance was significantly enhanced with a new peak appearing at around 560 nm (Figure 2a), clearly suggesting the formation of the ring-opened amide form of **1** as a result of Hg^{2+} binding.[10] The association constant for Hg^{2+} was estimated to be 3.7×10^3 M^{-1} in buffer solutions on the basis of nonlinear fitting of the titration curve assuming 1:1 stoichiometry. This binding mode was also supported by a Job plot (Figure 2a inset).

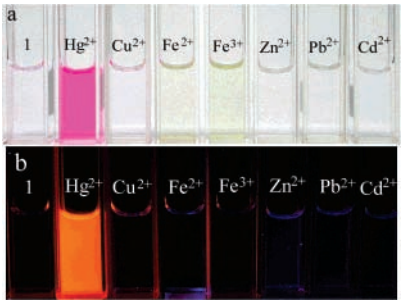


Figure 1. Photos of color changes (a) and fluorescent responses (b) of **1** (20 μM) upon addition of 100 μM different metal ions in ethanol/HEPES buffer (1:1, v/v, pH 7.2) solutions ($\lambda_{ex} = 365$ nm).

As expected, **1** shows an obvious change in its reversible ferrocene/ferricinium redox cycles. Differential pulse voltammetric (DPV) curves of **1** were recorded in ethanol solution containing 0.1 M *n*-tetrabutylammonium hexafluorophosphate (*n*-Bu_4NPF_6) as supporting electrolyte in the

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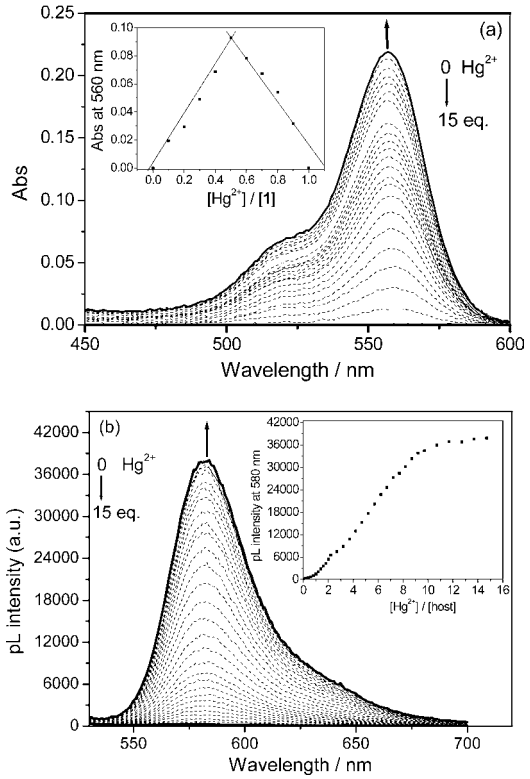

Figure 2. Changes in absorption (a) and fluorescence emission (b) spectra of **1** (20 μM) in ethanol/HEPES buffer (1:1, v/v, pH 7.2) solutions with various amounts of Hg^{2+} ions (0−15 equiv) (λ_{ex} = 520 nm). Each spectrum was acquired 2 min after Hg^{2+} addition. Inset (a): Job's plots of the complexation between **1** and Hg^{2+}. Total concentration of **1** + Hg^{2+} was kept constant at 10 μM. Inset (b): Fluorescence titration profile at 580 nm versus 1 equiv of Hg(II) in solution for **1**.

absence and presence of Hg^{2+}. As shown in Figure 3, upon addition of Hg^{2+}, a clear evolution of the oxidation peak ($E_{1/2}$) versus decamethylferrocene was observed from 0.40 to 0.15 V.

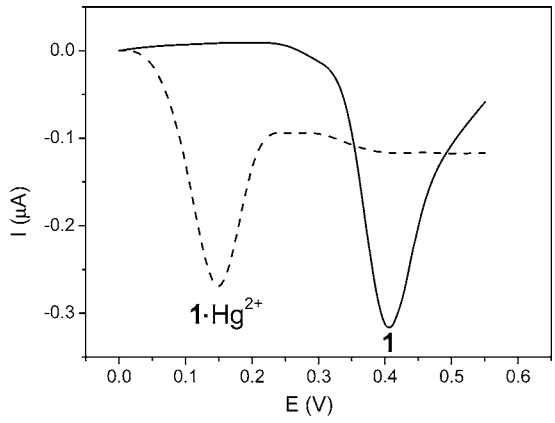

Figure 3. DPV of **1** (100 μM) in ethanol solution in the absence and presence of 1.3 equiv of Hg^{2+} with *n*-Bu_4NPF_6 as supporting electrolyte.

The shift in redox potential between the complex **1**·Hg^{2+} and the neutral ligand ($\Delta E_{1/2}$ = 250 mV) is indicative of a remarkably large value for the equilibrium constant for Hg^{2+} cation binding by the receptor **1**.[16]

The complexation of Hg^{2+} by **1** was also investigated by means of fluorescence titration in ethanol/HEPES buffer (1:1, v/v, pH 7.2). Upon addition of Hg^{2+}, a new emission band of **1** showing a maximum at 580 nm appeared (Figure 2b). Fluorescence titration profile at 580 nm versus 1 equiv of Hg(II) in solution for **1** is shown in Figure 2b inset. In the presence of 15 equiv of Hg^{2+}, the mixture showed an intense red fluorescence (Figure 1) with a quantum yield of 0.15, and an approximately 105-fold enhancement in the fluorescence intensity at 580 nm was estimated. This may be ascribed to the delocalized xanthene moiety of the rhodamine group. The introduction of a ferrocenyl group does not influence the typical emission of the rhodamine fluorophore, lending support to the rationality of our design strategy.

For a chemical sensor to be widely employed in the detection of specific analytes, the reversibility is an important aspect. In light of strong binding ability of the iodide anion (I^-) toward Hg^{2+},[17] the reversibility of the system was investigated by introduction of I^-. Upon addition of 20 equiv of KI, the color of the mixture of **1** (20 μM) and Hg^{2+} (15 equiv) changed from pink to almost colorless, and ~96% of fluorescent emission intensity of the system was quenched (Figure S3, Supporting Information) indicating that the anion I^- replaced the receptor **1** to coordinate Hg^{2+}. Thus, reversible response toward Hg^{2+} implies that **1** is a chemosensor not a chemodosimeter of Hg^{2+}.

To obtain an excellent chemosensor, high selectivity is a matter of necessity. In the present work, studies of selective coordination of cations of **1** by means of fluorescence spectroscopy were then extended to related heavy, transition, and main group metal ions. Only the addition of Hg^{2+} resulted in a prominent fluorescent change in fluorescence, whereas only very weak variations of fluorescent spectra of **1** were observed upon the addition of excesses of other metal ions such as Ag^+, Cr^{3+}, Cu^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Na^+, Zn^{2+}, Cd^{2+}, Al^{3+}, Mn^{2+}, Pb^{2+}, and Co^{2+} (Figure S6, Supporting Information).

We also investigated the time course of the response of **1** to 15 equiv of Hg^{2+} in ethanol/HEPES buffer (1:1, v/v, pH 7.2). We found that the interaction of **1** with Hg^{2+} was completed in less than 2 min. Thus, this system might be used for the real-time monitoring of Hg^{2+} in cells and organisms. To verify the binding sites for Hg^{2+} in **1**, a control molecule **2** (Scheme 1) was synthesized. Upon addition of 15 equiv of Hg^{2+}, no obvious variation in the absorbance at 560 nm was observed even over a period of 2 h, indicating a lack of interaction between **2** and Hg^{2+} (Figure S7, Supporting Information). These observations show that the 8-hydroxyquinoline moiety in **1** plays a key role in the metal

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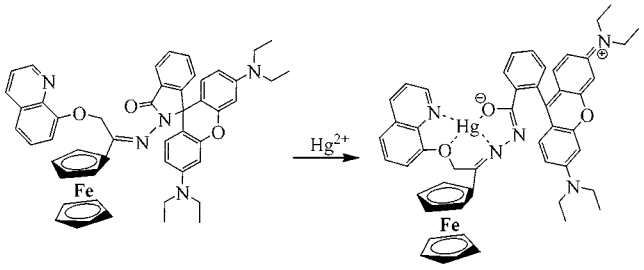
binding. It is clearly evident that the carbonyl O, imono N, and quinoline N, O atoms all participate in the coordination of Hg^{2+} (Scheme 2).

Scheme 2. Possible Proposed Binding Mode of **1** with Hg^{2+}



Further fluorescent response to Hg^{2+} for **1** with a low concentration of 5 μM could be performed in the ethanol/PBS (1:49, v/v) solution (Supporting Information). Owing to its favorable spectroscopic properties and the rapid kinetics of the response to Hg^{2+}, **1** should be ideally suited for fluorescence imaging in living cells. As determined by laser scanning confocal microscopy, staining Caov-3 ovarian carcinoma cells with a 5 μM solution of **1** in ethanol/PBS (1:49, v/v) buffer for 10 min at 25 °C led to very weak intracellular fluorescence (Figure 4a). The cells were then supplemented with 10 μM $Hg(NO_3)_2$ in the growth medium for 2.5 h at 37 °C and loaded with **1** under the same conditions, whereupon a significant increase in the fluorescence from the intracellular area was observed (Figure 4b). Bright-field measurements after treatment with Hg^{2+} and **1** confirmed that the cells were viable throughout the imaging experiments (Figure 4c). As depicted in Figure 4d, the overlay of fluorescence and bright-field images reveals that the fluorescence signals are localized in the perinuclear area of the cytosol, indicating a subcellular distribution of Hg^{2+}. These results demonstrate that **1** might be used for detecting Hg^{2+} within biological samples.

In summary, we have presented a highly selective and multisignaling optical-electrochemical chemosensor for Hg^{2+} based on a rhodamine dye bearing both a ferrocenyl group and an 8-hydroxyquinoline moiety. The structural features of the rhodamine and ferrocene moieties in this probe play

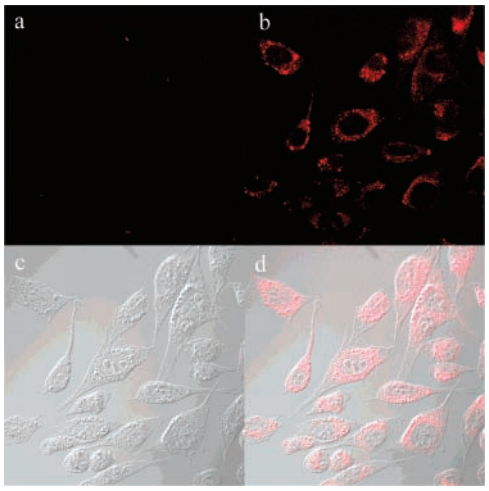


Figure 4. Confocal fluorescence and bright-field images of Caov-3 cells. (a) Cells stained with 5 μM solution of **1** in ethanol/PBS (1:49, v/v) buffer for 10 min at 25 °C (λ_{ex} = 515 nm). (b) $Hg(NO_3)_2$ supplemented cells loaded with 5 μM **1** for 10 min at 25 °C. (c) Bright-field image of cells shown in panel b. The overlay image of b and c is shown in d.

very important roles in the design of the multisignaling chemosensor. Confocal laser scanning microscopy experiments have shown that **1** can be used to detect Hg^{2+} in living cells and map its subcellular distration. The results provide a useful design strategy for the synthesis and application of new fluorescent sensors for other transition metal ions in living cells.

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Supporting Information Available: Synthetic details, NMR spectra, and additional spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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