Selective Chemosensing of Hg^{2+} Ions by Diazatetrathia-crown Ether Having Nitrobenzoxadiazolyl Subunits

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A diazatetrathia crown ether derivative that has two appended nitrobenzoxadiazolyl moieties showed selective OFF-ON type fluoroionophoric signaling properties toward Hg^{2+} ions over other transition metal ions. The compound also exhibited a pronounced chromogenic behavior toward Hg^{2+} ions by changing the solution color from light orange to yellow, which can easily be detected with naked-eye. The detection limit for the analysis of Hg^{2+} ions in 90% aqueous acetonitrile was found to be 4.8×10^{-6} M, which suggests that the compound may be used as a chemosensor for analyzing sub-millimolar Hg^{2+} ions in aqueous environments.

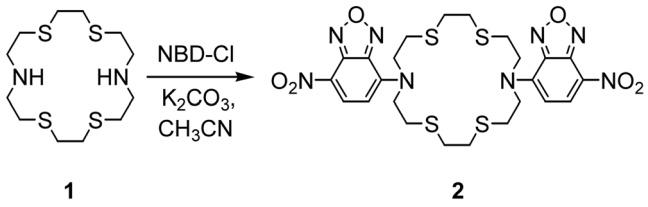
Key Words : Chemosensor, Fluoroionophore, Hg^{2+} ions, Diazatetrathia-crown ether, Nitrobenzoxadiazolyl subunit

Introduction

Over the years a large number of elaborate chemosensors have been developed to detect important ionic species by utilizing the selective signaling behaviors of designed supramolecular systems.[1] Particularly, selective determination of mercury ions is very important because of its extremely toxic impacts on our environments.[2,3] Among the numerous selective chemosensors developed for mercurial species to date, the chromogenic and fluorogenic systems[4] have distinct advantages in terms of their sensitivity, versatility, and ease of signal detection and visualization. The molecular framework of diazatetrathia crown ether has been successfully utilized as a platform for the construction of many transition metal ion selective supramolecular systems.[5] Particularly, the diazatetrathia crown ether ligand introduced on the 1,8-positions of anthracene scaffold has been synthesized, and its Hg^{2+}-selective CHEQ (chelation-enhanced quenching) type fluoroionophoric properties have been reported.[6] Our previous investigation has also discovered that the pyrene appended derivative of **1** also exhibits selective signaling of Hg^{2+} ions over other transition metal ions.[7] Recently, Sakamoto *et al.* have reported that the podand type acyclic tetrathiamonoaza derivative having nitrobenzoxadiazolyl (NBD) subunit exhibits Hg^{2+}-selective fluoroionophoric properties in aqueous acidic solution.[8] Based on these findings, we developed a Hg^{2+}-selective chemosensor by employing the cyclic molecular framework of diazatetrathia crown ether **1** and NBD[9] signaling unit expecting a more enhanced macrocyclic effect. In this study, the compound **2** thus prepared exhibited a pronounced fluorescence enhancement selectively in response to the Hg^{2+} ions over other transition metal ions in aqueous media.

Results and Discussion

The desired compound **2** having two NBD subunits directly linked to the diazatetrathia-crown ether was prepared by reacting 1,4,10,13-tetrathia-7,16-diazacyclooctadecane **1** with NBD-chloride (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole, K_2CO_3, CH_3CN) in good yield (75%).



1 **2**

Scheme 1. Preparation of chemosensor **2**.

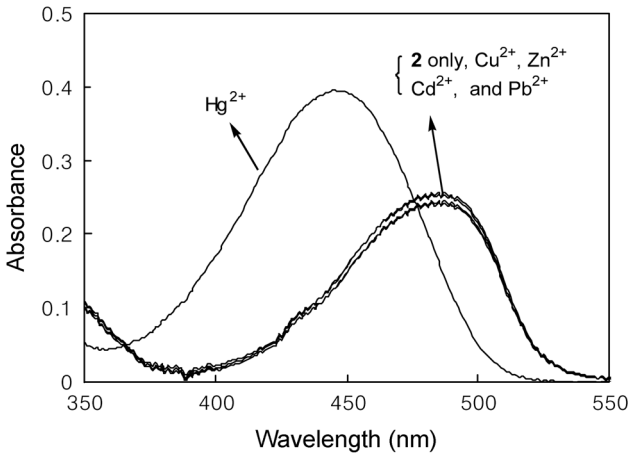


Figure 1. UV-vis spectra of **2** in the presence of varying metal ions. [**2**] = 1.0×10^{-5} M, [M^{2+}] = 1.0×10^{-3} M in 95% aqueous acetonitrile ($CH_3CN : H_2O$ = 95 : 5, v/v).

First, the UV-vis spectral behavior of **2** was investigated in 95% aqueous acetonitrile. The compound **2** exhibited a broad absorption band around 486 nm and appeared in faint orange color. Upon interaction with Hg^{2+} ions, the absorption band changed to a new prominent one at 448 nm with somewhat increased absorbance (Figure 1). The significant blue shift ($\Delta\lambda$ = -38 nm) resulted in changes of

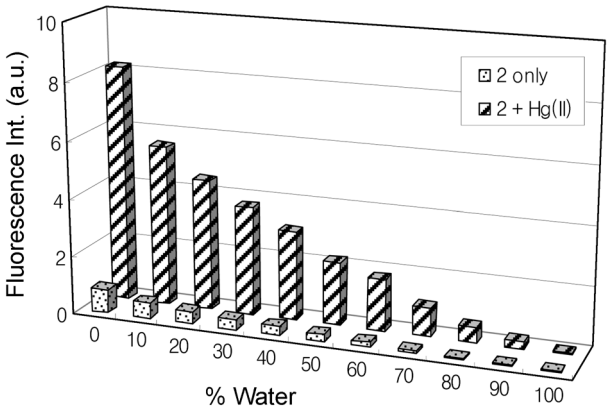


Figure 2. Effects of water content on the fluorescence intensity of free **2** and **2** in the presence of Hg^{2+} ions in aqueous acetonitrile. Fluorescence intensities were measured at 529 nm. [**2**] = 5.0×10^{-6} M, [Hg^{2+}] = 5.0×10^{-4} M, λ_{ex} = 470 nm.

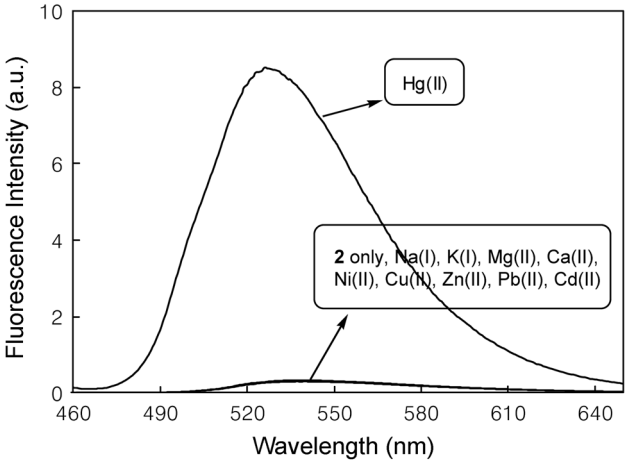


Figure 3. Fluorescence spectra of **2** in the presence of varying metal ions in aqueous acetonitrile solution. [**2**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, λ_{ex} = 470 nm, in CH_3CN-H_2O (90 : 10, v/v).

solution color from faint orange to yellow, which can be easily recognized with naked eye.[10] The association constant K_{assoc} was estimated from a non-linear curve fitting analysis of the UV-vis titration data of **2** with $Hg(ClO_4)_2$ in 95% aqueous acetonitrile and was found to be 3.6×10^4 M^{-1}.[11] Other representative metal ions of alkali (Na^+, K^+), alkaline earth (Mg^{2+}, Ca^{2+}), and transition metal ions (Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+}) revealed no significant changes in spectral behaviors.

Next, the fluoroionophoric properties of **2** toward the above mentioned representative metal ions were investigated in aqueous acetonitrile. To provide a more optimized condition for selectivity toward a specific target metal ion, the effects of water composition on the fluorescence behavior were scrutinized (Figure 2). As the water content increases, the fluorescence intensity of both free ligand **2** and the **2**-Hg^{2+} systems (obtained by the treatment with 100 equiv of Hg^{2+} ions) showed a nice and monotonously decreasing trend. Based on this behavior, the fluorescence measurements were carried out in 90% aqueous acetonitrile where high selectivity toward Hg^{2+} ions was observed. The pH of solution did not affect the fluorescence of **2** at the analytically useful pH range of 3 to 10, so all the experiments were performed without adding a buffer to fix solution pH.

Compound **2** revealed a very broad emission band typical of NBD moiety[12] centering around 541 nm in 90% aqueous acetonitrile (Figure 3). Upon treatment with 100 equiv of varying metal ions, the ionophore **2** exhibited a sizable fluorescence enhancement exclusively with Hg^{2+} ions among the tested metal ions concomitantly with a small blue shift in the emission maximum from 541 nm to 529 nm. The large fluorescence enhancement (10.2-fold) was observed at 529 nm with Hg^{2+} ions. Other metal ions surveyed (Na^+, K^+, Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+}) showed almost negligible effects on the fluorescence behavior of **2**; the fluorescence changes, expressed as the ratio of fluorescence intensities in the presence and absence of metal ions (I/I_o), occurred in between 0.89 (for Ca^{2+}) and 1.05 (for Cd^{2+}). The large fluorescence enhancement observed with Hg^{2+} ions having an intrinsic quenching nature is favorable for practical application of the analysis of this metal ion.[13] Binding of Hg^{2+} ions resulted in perturbation of the NBD moiety and showed marked fluorescent responses as has been reported earlier for podand type ionophores and cyclam based NBD derivatives.[8,9b,14]

To gain an insight into the quantitative analytical possibility of **2** for the analysis of Hg^{2+} ions, the association constant (K_{assoc}) and detection limit were determined. The K_{assoc} for **2**-Hg^{2+} complex formation was also assessed from a non-linear curve fitting analysis of the fluorescence titration data and was found to be 4.5×10^4 M^{-1} in 90% aqueous acetonitrile solution, which is in good agreement with the results obtained by the UV-vis titrations.[11] Somewhat moderate K_{assoc} value of **2** might be attributed to the transformation of strongly complexing nitrogen ligating atom of macrocycle **1** into a relatively weak ligating group by appending NBD moiety.[14] Although the two nitrogen atoms of **2** exhibited a much reduced direct interaction with complexed metal ions compared with those of **1**, they still interacted strongly enough to signal the presence of Hg^{2+} ions, acting as a perturbation handle for the NBD function. The detection limit[15] for the analysis of Hg^{2+} ions in 90% aqueous acetonitrile was also estimated from Hg^{2+} ion dependent fluorescence intensity changes, and it was found to be 4.8×10^{-6} M.

Finally, the practicality of **2** for the analysis of Hg^{2+} ions in physiologically relevant sample was investigated. Fluorescence titration with Hg^{2+} ions was performed in the presence of physiologically important background metal ions.[16] Titration results shown in Figure 4 revealed a well-defined Hg^{2+} ion dependent profile. The result, although obtained in somewhat unrealistic environment of mixed solvent system, suggests the possible practical applicability of compound **2** for the analysis of Hg^{2+} ions in physiologically relevant samples.

In summary, we prepared a new selective and efficient

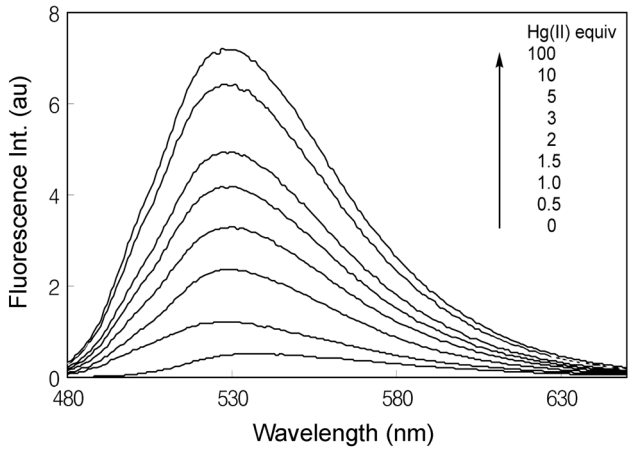


Figure 4. Titration of **2** with Hg^{2+} ions in the presence of physiologically relevant metal ions ([Na^+] = 138 mM, [K^+] = 4 mM, [Mg^{2+}] = 1 mM, [Ca^{2+}] = 3 mM, [Zn^{2+}] = 0.02 mM, and [Cu^{2+}] = 0.015 mM). [**2**] = 5.0×10^{-6} M, λ_{ex} = 470 nm, in CH_3CN-H_2O (90 : 10, v/v).

Hg^{2+}-sensing ionophore based upon the binding motif of diazatetrathia-crown ether conjugated with signaling handle of NBD subunits, and its chromogenic and fluorogenic chemosensing behaviors were investigated. The prepared compound exhibited a highly selective OFF-ON type fluorescence enhancement behavior as well as a naked eye detectable chromogenic behavior toward Hg^{2+} ions over other transition metal ions. The compound may be used as a novel chemosensor to analyze the sub-millimolar concentration range of Hg^{2+} ions in the samples of aqueous environments.

Experimental Section

General. All solvents were purchased from Aldrich Chemical Co. as 'anhydrous' or 'spectroscopic grade'. 1,4,10,13-Tetrathia-7,16-diazacyclooctadecane (Lancaster) and NBD chloride (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole, Aldrich) were purchased and used without further purification. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian Gemini-2000 spectrometer. Mass spectral data were obtained with a Micromass Autospec mass spectrometer. TLC was carried out using precoated aluminum TLC plate (silica gel, 60 F254). UV-Vis spectra were recorded with a Jasco V-550 spectrophotometer. Fluorescence spectra were measured on an Aminco-Bowman Series 2 Spectrophotometer.

Preparation of 2. A mixture of 1,4,10,13-tetrathia-7,16-diazacyclooctadecane (0.31 mmol, 100 mg), NBD chloride (1.24 mmol, 240 mg) and K_2CO_3 (1.24 mmol, 170 mg) in acetonitrile was refluxed for 24 h. The precipitate formed was filtered and washed with water several times and dried under vacuum to obtain orange colored solids. The product was crystallized from DMF. Yield, 75%. ^{1}H NMR (DMSO-d_6) δ 8.49 (d, J = 9.3 Hz, 2H), 6.49 (d, J = 9.3 Hz, 2H), 4.23 (br m, 8H), 2.99 (m, 8H), 2.92 (s, 8H). ^{13}C NMR (CD_3CN/DMSO-d_6, 80 °C) δ 145.1, 144.9, 144.8, 136.0, 122.3, 103.2, 54.6, 32.8, 29.2. MS (MALDI) Calcd for $C_{24}H_{28}KN_8O_6S_4$ $[M+K]^+$ 691.0652. Found 691.0562.

UV-Vis Spectra Measurements. Stock solution of chemosensor **2** was prepared in acetonitrile (1.0×10^{-3} M) and stock solutions of metal ion (Na^+, K^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} Cd^{2+}, Pb^{2+}, and Hg^{2+} in perchlorate) were prepared in water (0.1 M). To a stock solution of **2** in acetonitrile (0.03 mL) were added stock solutions of metal ion (0.015 mL) and diluted with calculated amount of acetonitrile and water to obtain 5% aqueous acetonitrile solution. Final concentrations of **2** and metal ions were 1.0 x 10^{-5} M and 1.0×10^{-3} M, respectively.

Fluorescence Measurements. All the absorption and fluorescence measurements were carried out using spectroscopic grade solvents. Stock solution of chemosensor **2** was prepared in acetonitrile (5.0×10^{-4} M) and stock solutions of metal ion (Na^+, K^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} Cd^{2+}, Pb^{2+}, and Hg^{2+} in perchlorate) were prepared in water (0.1 M). To a stock solution of **2** in acetonitrile (0.03 mL) were added stock solutions of metal ions (0.015 mL) and diluted with calculated amount of acetonitrile and water to obtain 10% aqueous acetonitrile solution. Final concentrations of **2** and metal ions were 5.0×10^{-6} M and 5.0×10^{-4} M, respectively. Fluorescence titration of **2** with Hg^{2+} ions was carried out in 10% aqueous acetonitrile solution. Calculated amounts of water and acetonitrile were added to the solution containing **2** and metal ions (0-100 equiv) to obtain 10% aqueous acetonitrile solution. The fluorescence changes of the resulting solution ([**2**] = 5.0×10^{-6} M and [Hg^{2+}] = 0- 5.0 $\times 10^{-4}$ M) were measured. The same titration was also performed in the presence of physiologically important metal ions ([Na^+] = 138 mM, [K^+] = 4 mM, [Mg^{2+}] = 1 mM, [Ca^{2+}] = 3 mM, [Co^{2+}] = 0.002 mM) as background.

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