Pyrene Appended Hg^{2+}-selective Fluoroionophore Based upon Diaza-Crown Ether

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A new pyrene appended diaza-18-crown-6 ether derivative **1** has been prepared and its fluoroionophoric properties toward transition metal ions were investigated. Compound **1** exhibited a high Hg^{2+}-selectivity over other transition metal ions as well as alkali and alkaline earth metal ions in aqueous acetonitrile solution. The ratiometric analysis of the monomer and excimer emissions of pyrene successfully signals the presence of Hg^{2+} ions. The detection limit for Hg^{2+} ions was found to be 3.1×10^{-6} M in 50% aqueous acetonitrile solution at pH 8.1. Competition experiments also suggest that the compound could be utilized as a selective and sensitive fluorescent chemosensor for the analysis of micromolar Hg^{2+} ions in physiological and environmental samples.

Key Words : Diaza-crown ether, Hg^{2+}-selectivity, Pyrene, Fluoroionophore, Ratiometry

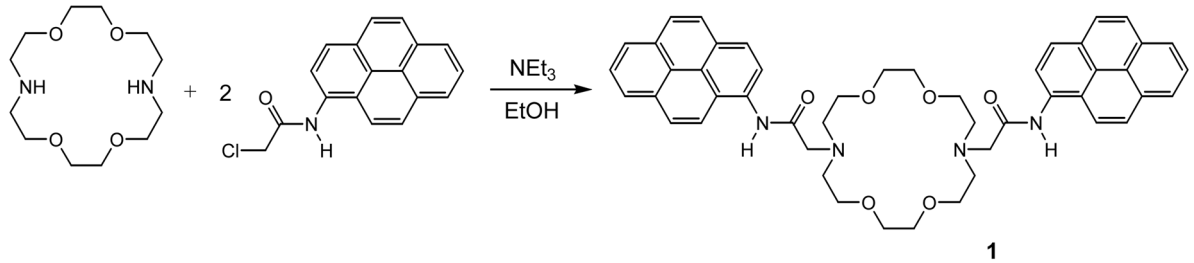
Introduction

The development of selective and sensitive chemosensors for the determination of biologically and environmentally important ionic guests of transition metal ions is one of the most essential research field in supramolecular chemistry.[1] Of particular interest is the determination of Hg^{2+} ions in physiological environments and a large number of Hg^{2+} selective functional ionophores having chromogenic or fluorogenic signaling ability have been developed.[2] They are generally based on the well-established structures of crown ethers,[3] calixarenes,[4] and more recently rhodamine and fluorescein based[5] molecular frameworks. In spite of numerous elaborate systems thus developed, however, new chemosensors for the Hg^{2+} determination are still required to meet the varying sample origins and concentration ranges. Crown ether moieties are particularly attractive for the recognition of alkali and alkaline earth metal ions and a large number of well-designed derivatives based upon various structural characteristics have been devised for the construction of new functional ionophores having useful optical responses.[6] Diaza-crown ethers also received much attention for the design of supramolecular systems due to their unique ionophoric properties and easy of derivatization utilizing secondary amino groups of the macrocycle moiety with a number of functional subunits.[7]

For the efficient signaling of the metal ion recognition event, fluorescence is particularly attractive in view of their sensitivity and easy of signal detection,[8] and pyrene fluorophore is one of the most widely employed functional subunits for the construction of fluorogenic chemosensor systems.[9] In this paper, we report a new diaza-18-crown-6 ether derivative having two appended pyrene moieties aiming for the selective signaling of Hg^{2+} ions in samples of physiological or environmental origin. The designed ionophore exhibited a pronouncedly selective signaling behavior toward Hg^{2+} ions over other physiologically and environmentally important metal ions in aqueous media by utilizing the ratiometric changes in monomer and excimer emissions of pyrene subunit.

Results and Discussion

Compound **1** was prepared by the reaction of diaza-18-crown-6 ether with 2-chloro-*N*-pyren-1-yl-acetamide[10] in the presence of triethylamine in EtOH.[11] The compound was designed by combining the two well-known molecular motifs of diaza-crown binding site and frequently employed signaling handle of pyrenylacetamide subunit. The structural characteristic of the compound **1** is a typical of bibracchial lariat ethers.[12] Thus the presence of amide carbonyl functions in the side chain of **1** provides an extra binding site in axial mode in addition to the crown framework, that might resulted in enhanced binding strength and selectivity for the complexation of target metal ions.[13] Furthermore, the possible perturbation exerted by the complexation with a specific metal ion on the interaction between directly conjugated amide function and pyrene moiety could be utilized as an



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Scheme 1

additional signaling tool for the sensing of guest binding event.

The chemosensing properties of compound **1** were investigated by the fluorescence measurements. First, to have an insight into the optimized condition for the selective signaling toward specific metal ions, the fluorescence behaviors of free ionophore **1** in the presence of various metal ions in different aqueous organic solutions as well as buffers were studied. Preliminary survey in common organic solvent systems including acetone, THF, and DMSO having various water content and representative buffer systems of acetate (pH 5.0), HEPES (pH 7.0), and Tris (pH 8.1) indicates that the compound revealed a significant selectivity toward Hg^{2+} ions in aqueous acetonitrile solution at pH 8.1 (Tris buffer). For example, the diaza-crown based compound **1** exhibited a gradually decreasing fluorescence intensities at 385 nm as the water content increases, while those of **1**+Hg^{2+} system (obtained by treating **1** with 100 equiv of $Hg(ClO_4)_2$) showed a progressively increasing fluorescence over 20-80% water content. Based on this observation, the Hg^{2+}-selective sensing behavior of compound **1** was investigated in 50% aqueous acetonitrile solution at pH 8.1 (Tris, 0.01 M). In this solvent system, the chemosensor exhibited characteristic monomer emissions around 370-430 nm region along with a broad excimer band at 490 nm of pyrene. Upon interaction with metal ions, compound **1** revealed a pronounced selectivity toward Hg^{2+} and Cd^{2+} ions over other surveyed metal ions of alkali, alkaline earth, and transition metal ions (Figure 1). Addition of 100 equiv of Hg^{2+} ions resulted in efficient fluorescence reduction for the monomer emissions at 380-400 nm and almost complete disappearance of the excimer emission at 486 nm. With Cd^{2+} ions, the monomer emissions were similarly reduced, but the excimer band was not so significantly affected. Other metal ions showed almost no changes under the same experimental conditions manifesting a definite Hg^{2+} and Cd^{2+} selectivity of **1**.

The Hg^{2+}-selectivity of the compound **1** was more easily understandable by using ratiometric approach for the chemosensing behavior (Figure 2). That is the fluorescence

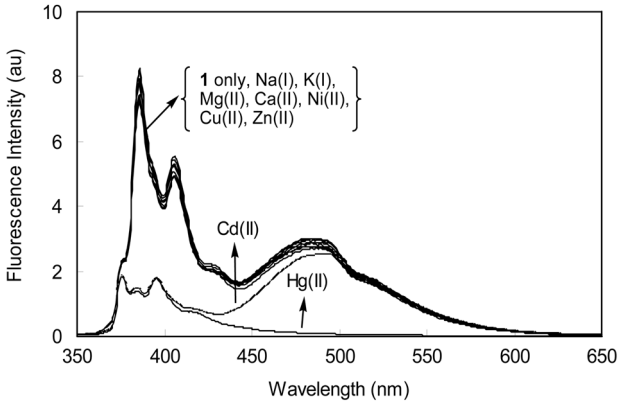

Figure 1. Fluorescence spectra of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M in aqueous acetonitrile solution (H_2O:CH_3CN = 50:50, v/v) buffered at pH 8.1 (10 mM of Tris). λ_{ex} = 340 nm. The pH value denotes that of water portion before mixing with acetonitrile.

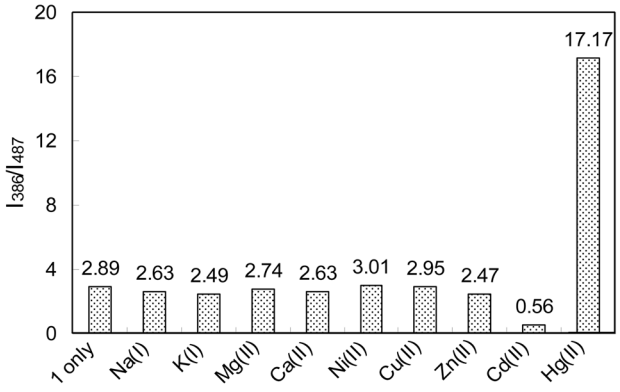

Figure 2. Changes in the fluorescence intensity ratio (I_{386}/I_{487}) of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M in aqueous 50% acetonitrile solution buffered at pH 8.1 (10 mM of Tris). λ_{ex} = 340 nm.

intensity ratio of monomer and excimer at 386 nm and 487 nm (I_{386}/I_{487}), respectively, was found to be diagnostic. The ratio (I_{386}/I_{487}) was 2.89 for the compound **1** in the absence of any metal ion. Only Hg^{2+} ions induced a large enhancement in this ratio (17.17). The ratio was relatively constant from 2.47-3.01 for the other surveyed metal ions except for the Cd^{2+} ions which revealed somewhat reduced value of 0.56. With this ratiometric approach, the rather similar quenching behavior of Cd^{2+} ions in monomer emission compared with Hg^{2+} ions could be differentiated effectively. The discrimination between Hg^{2+} ions from Cd^{2+} ions was also possible by the comparison of the changes in solution color. Under illumination with a UV lamp, the solution color of **1** in the presence of Hg^{2+} ions was green, while those of **1** and **1** in the presence of Cd^{2+} as well as other metal ions were blue.

To have an insight into the possibility of using **1** in quantitative analysis of Hg^{2+} ions, the fluorescence titration with $Hg(ClO_4)_2$ was performed in 50% aqueous acetonitrile (Figure 3). The fluorescence intensity of **1** was progressively decreased with an increasing amount of Hg^{2+} ions in both monomer and excimer regions. The association constant K_{assoc} was determined from the titration results by a non-linear curve fitting procedure using Dynafit software[14] and

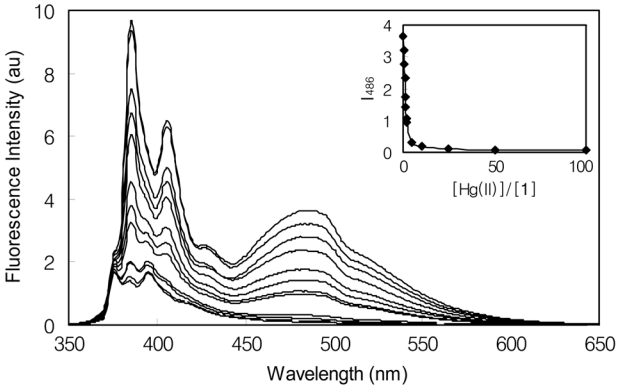

Figure 3. Fluorescence titration of **1** with Hg^{2+} ions in aqueous acetonitrile (H_2O:CH_3CN = 50:50, v/v). [**1**] = 5.0×10^{-6} M. λ_{ex} = 340 nm. In buffered solution at pH 8.1 (10 mM of Tris).

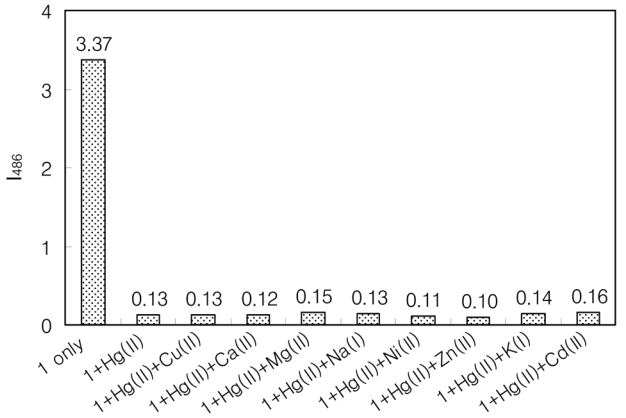

Figure 4. Changes in the fluorescence intensity of **1** at 486 nm in response to the Hg^{2+} ions in the presence of various coexistent metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, [Hg^{2+}] = 5.0×10^{-5} M, in aqueous acetonitrile solution (H_2O:CH_3CN = 50:50, v/v) buffered at pH 8.1 (10 mM of Tris). λ_{ex} = 340 nm.

found to be 2.2×10^5 M^{-1}. Detection limit[15] for the analysis of Hg^{2+} ions under the experimental condition was also assessed from the concentration dependent fluorescence changes and was found to be 3.1×10^{-6} M.

Next, the chemosensing behaviors of diaza-crown **1** in aqueous acetonitrile solution in the presence of common interfering metal ions were also studied. As can be seen from Figure 4, the fluorescence spectra of **1** measured in the presence of both 100 equiv of coexistent metal ions and 10 equiv of Hg^{2+} ions were not so different from that obtained in the presence of 10 equiv of Hg^{2+} ions only. This observation suggests that the compound could be used as a selective Hg^{2+} sensor for the analysis of metal ions in the presence of other coexistent physiologically or environmentally important metal ions.

We finally tested the applicability of **1** in Hg^{2+} analysis in physiological samples by measuring the fluorescence responses in simulated physiological environment. That is the fluorescence titration of **1** with Hg^{2+} ions was performed in the presence of physiologically important metal ions ([Na^+] = 138 mM, [K^+] = 4 mM, [Mg^{2+}] = 1 mM, [Ca^{2+}] = 3 mM, [Zn^{2+}] = 0.02 mM, [Cu^{2+}] = 0.015 mM, and [Co^{2+}] = 0.002 mM) as background.[16] Compound **1** exhibited a well defined titration curve in response to the changes in the concentration of Hg^{2+} ions. All these observations suggest that the diaza-crown derivative **1** could be used as a selective chemosensor for the analysis of micromolar concentration of Hg^{2+} ions in samples of physiological systems.

The selective fluorescence changes of **1** in response to the Hg^{2+} ions in aqueous acetonitrile solution seems to be partly due to the metal ion induced perturbation of the electronic state of the pyrene-amide fluorophore.[17] Along with the perturbation, the complexation of amino group with metal ion in diaza-crown ether moiety might affect the fluorescence emissions of the pyrene by the suppression of the well-known photoinduced electron transfer process.[18] Upon interaction with metal ions the two factors interposed variously in the present system, and we could obtain the resultant selective ON-OFF type responses toward Hg^{2+} ions.

In summary, a new fluorescent diaza-crown ether derivative having two appended pyrenylacetamide subunits was prepared and the fluoroionophoric behaviors toward transition metal ions were investigated. Compound **1** exhibited a pronounced Hg^{2+}-selectivity over other environmentally and physiologically important metal ions in aqueous media by using the ratiometric analysis of the variation in monomer and/or excimer emissions of pyrene. The prepared compound could be utilized as a new fluorescent chemosensor for the analysis of micromolar Hg^{2+} ions in samples of physiological or environmental origin.

Experimental Section

General. 1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane (diaza-18-crown-6 ether) and 1-aminopyrene 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. UV-Vis spectra were recorded with a Jasco V-550 spectrophotometer. Fluorescence spectra were measured on an Aminco-Bowman Series 2 Spectrophotometer. All solvents used for the measurements of UV-vis and fluorescence spectra were purchased from Aldrich Chemical Co. as 'spectroscopic grade'. Mass spectral data were obtained with a Micromass Autospec mass spectrometer. 2-Chloro-*N*-pyren-1-yl-acetamide was prepared by the reaction of 1-aminopyrene with chloroacetyl chloride following the reported procedure.[10]

Synthesis of 1. A mixture of 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (262 mg, 1 mmol), 2-chloro-*N*-pyren-1-yl-acetamide (646 mg, 2.2 mmol), triethylamine (506 mg, 5 mmol) in ethanol was refluxed under N_2 atmosphere. After 24 h of reaction, the reaction mixture was evaporated and the residue was partitioned between CH_2Cl_2 and water. The organic layer was separated and the water phase was extracted with CH_2Cl_2. The combined organic solution was evaporated and the product was purified by the column chromatography (silica gel, CH_2Cl_2-MeOH) followed by the crystallization from CH_2Cl_2/MeOH to yield light amber colored product. Yield 72%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 2H), 8.45 (d, J = 8.4 Hz, 2H), 8.27 (d, J = 9.0 Hz, 2H), 8.17-7.94 (m, 14H), 3.48 (s, 8H), 3.42 (t, J = 5.0 Hz, 8H), 3.36 (s, 4H), 2.64 (t, J = 5.0 Hz, 8H). ^{13}C NMR (DMSO-d_6, 70 °C, 75 MHz) δ 170.1, 131.2, 130.6, 130.2, 127.9, 126.8, 126.0, 125.8, 124.7, 124.5, 124.4, 123.8, 123.1, 121.7, 121.3, 69.7, 68.5, 59.1, 54.8. Mass (FAB, *m*-NBA) Calcd for $C_{48}H_{48}KN_4O_6$ $[M+K]^+$, *m/z* = 815.3. Found 815.4.

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