Selective Fe$^{2+}$ Ion Recognition Using a Fluorescent Pyridinyl-benzoimidazole-derived Ionophore

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Fluorescent organic molecules that respond to changes in the Fe$^{2+}$ concentration with selectivity to other abundant di-valent metal ions will offer the ability to understand the dynamic fluctuations of the Fe$^{2+}$ ion in interesting media. The use of 6-Br-ppmbi, derived from 2-pyridin-2-yl-benzimidazole, for metal ion-selective fluorescence recognition was investigated. Screening of the main group and transition metal ions showed exclusive selectivity for Fe$^{2+}$ ions even in the presence of competing metal ions. In addition, the requirement for low concentrations of probe molecules to detect certain amounts of Fe$^{2+}$ ions make this sensor unique compared to previously reported Fe$^{2+}$ ion sensors.

Key Words: Fluorescent chemical sensor, 6-Br-ppmbi, Fe$^{2+}$ cation

Introduction

Effective sensors for the detection of transition metal ions have been of interest in recent years due to the essential roles of transition metal ions in the structural and functional aspects of biological systems. Among the many analytic methods, many fluorescent metal ion probes have been reported because of the use of fluorescence as a detecting method has distinct advantages in terms of sensitivity. A number of organic ionophores bind extremely selectively to the targeted transition metal ions. Hence, the selective and sensitive fluorescent probes for detecting metal ions can be synthesized by chemically linking the part of the ionophores to the adequate fluorophore. Upon the binding of metal ions to the ionophores, the fluorophore showed an optical change. Using such design principles, very effective fluorescent sensors for Ag$^{+}$, Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Hg$^{2+}$, Fe$^{3+}$, etc. has been reported. In particular, Hg$^{2+}$ and Zn$^{2+}$ sensors are applied to image the respective metal ions in living cellular systems.

Iron is required by all living organisms owing to its potent redox chemistry and possibility to engage in catalytic activity. If the Fe$^{2+}$ concentration is not regulated, it can lead to uncontrolled oxidative chemistry causing tissue damage and fibrosis to various organs. Although biological iron are observed frequently in ferrous (Fe$^{2+}$) oxidation states bound tightly by enzymes and storage proteins, the existence of labile iron within cells is controversial, resulting in high demand for selective sensors for iron. Compared to the number of fluorescent sensors for other transition metal ions, iron-specific fluorescent sensors are quite rare. Currently, a few probes for Fe$^{2+}$ ion are available. Recently, “Calcein” with an EDTA-derived receptor has been reported to detect Fe$^{3+}$ ions with ten-fold higher selectivity for Fe$^{2+}$ over Fe$^{3+}$. On the other hand, this reagent shows significant interference from other divalent metal ions, including Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ cations. Fluorescent sensors that directly analyze the concentration of Fe$^{2+}$ ions over other divalent metal ions are unavailable.

Benzoimidazole and pyridine are well-known building blocks for constructing chelating ionophores for transition metal ions. In previous studies, pyridines derivatized positions with sterically hindered methyl groups have altered the binding properties to Fe$^{2+}$ cations. If the bulky and electron withdrawing -Br group, instead of a methyl group, is attached to pyridine, it is possible that it would alter the binding properties of pyridine-containing ionophore to transition metal ions, and discriminate Fe$^{2+}$ ions from other divalent metal cations. This paper reports the synthesis of 2-bromo-pyridinyl-benzoimidazole derivatives as well as their emission response toward Fe$^{2+}$ ions and other metal ions.

Materials and Methods

General. All solvents and reagents were obtained from commercial suppliers and used as received without further purification. Solutions were prepared using reagent grade chemicals and doubly distilled water was used throughout. Elemental analysis for carbon, nitrogen, and hydrogen was carried out by using an EA1108 (Carlo Erba Instrument, Italy) in the Organic Chemistry Research Center of Sogang University, Korea. H-NMR (300 MHz) spectra were recorded on a JEOL JNM-AL400 spectrometer. UV-Vis spectra were obtained using a Cary 3 spectrophotometer with a quartz cuvette (path length = 1 cm) and fluorescence was recorded on a Perkin Elmer LS45 fluorescence spectrometer.

Fluorescent organic molecules that respond to changes in the Fe$^{2+}$ concentration with selectivity to other abundant di-valent metal ions will offer the ability to understand the dynamic fluctuations of the Fe$^{2+}$ ion in interesting media. The use of 6-Br-ppmbi, derived from 2-pyridin-2-yl-benzimidazole, for metal ion-selective fluorescence recognition was investigated. Screening of the main group and transition metal ions showed exclusive selectivity for Fe$^{2+}$ ions even in the presence of competing metal ions. In addition, the requirement for low concentrations of probe molecules to detect certain amounts of Fe$^{2+}$ ions make this sensor unique compared to previously reported Fe$^{2+}$ ion sensors.

Key Words: Fluorescent chemical sensor, 6-Br-ppmbi, Fe$^{2+}$ cation
IR spectra were measured on a BIO RAD FTS 135 spectrometer as KBr pellets. ESI-MS spectra were measured on a Si-2/LCQ DecaXP mass spectrometer.

Synthesis of 2-(6-bromopyridin-2-yl)-1-(6-bromopyridin-2-yl)methyl)-1H-benzoimidazole (6-Br-ppmbi).

To a solution of 4,5-dimethylbenzene-1,2-diamine (2.16 g, 20.0 mmol) in EtOH (40 mL) was added 6-bromopyridine-2-carbaldehyde (7.60 g, 40 mmol) (Scheme 1). Stirring was continued for a further 20 h at room temperature and the precipitate was collected by filtration, washed with cold EtOH and dimethyl ether, dried under vacuum, and weighed (Yield: 6.05 g, 68.1%). 0.015 g (0.050 mmol) of Zn(NO$_3$)$_2$ was dissolved in MeOH (4 mL) and mixed with MeOH solution (4 mL) of 6-Br-ppmbi ligand (0.02 g, 0.05 mmol). Slow evaporation of the difused mixture provided suitable crystals of 6-Br-ppmbi for X-ray analysis that were obtained in a week. Anal. Calcd for C$_{15}$H$_{12}$N$_4$Br$_2$: C, 48.68; H, 2.72; N, 12.62. Found: C, 48.60; H, 2.84; N, 12.77%.

Synthesis of 2-(6-bromopyridin-2-yl)-1-(6-bromopyridin-2-yl)methyl)-1H-benzoimidazole (6-Br-Me$_2$-ppmbi).

To a solution of 4,5-dimethylbenzene-1,2-diamine (2.72 g, 20.0 mmol) in EtOH (40 mL) was added 6-bromopyridine-2-carbaldehyde (7.60 g, 40 mmol) (Scheme 1). Stirring was continued for a further 20 h at room temperature. The reaction solution was then poured into 20 mL dimethyl ether and a dark green precipitate formed. The precipitate was filtered and washed with 2 × 20 mL dimethyl ether. The solid was dried under vacuum and weighed (Yield: 28.2 mg, 42.7%). For X-ray analysis, 3.3 mg (0.005 mmol) of the complex was dissolved in methylene chloride (3 mL) and carefully layered by petroleum ether (5 mL). Suitable crystals were obtained in 2 months.

X-ray Crystallographic Studies: Single crystals were mounted at room temperature on the tips of quartz fibers, coated with Partone-N oil, and cooled under a stream of cold nitrogen. Intensity data were collected on a Bruker CCD area diffractometer running the SMART software package, with Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods and refined on F$^2$ by using the SHELXTL software package. Empirical absorption corrections were applied with SADABS, part of the SHELXTL program package, and the structures were checked for higher symmetry by the program PLATON. All non-hydrogen atoms were refined anisotropically. In general, hydrogen atoms were assigned idealized position and given thermal parameters equivalent to 1.2 times the thermal parameter of the carbon atom to which they are attached. Data collection and experimental details for the compounds are summarized in Table S1.

Determination of the Apparent Fe$^{2+}$ Affinity of Ionophore 6-Br-ppmbi: The ionophore was dissolved in an aqueous solution at 0.050 µM. Upon the addition of a certain amount of Fe$^{2+}$, the fluorescence change was measured. By assuming a 1:1 metal-to-ionophore stoichiometry, the following relationship, Eq. (1), was found between the free Fe$^{2+}$ concentration, apparent dissociation constant $K_d$, and fluorescent intensity $F$:

$$F = \frac{[Fe^{2+}]F_{min} + K_d F_{max}}{K_d + [Fe^{2+}]}$$

where $F_{max}$ = fluorescence intensity of the free ionophore and $F_{min}$ = fluorescence intensity of the Fe$^{2+}$-bound ionophore. Nonlinear least-squares fit analysis of the experimental intensities versus the Fe$^{2+}$ concentrations allowed for a determination of $F_{max}$, $F_{min}$, and $K_d$.

Results

2-pyridin-2-yl-benzoimidazole is a versatile building block for the synthesis of transition metal complexes and has been used to prepare fluorescent materials owing to the strong fluorescent properties of benzoimidazole derivatives. Compound 6-Br-ppmbi was synthesized by the coupling of commercially available 1,2-phenylenediamine with 6-bromopyridine-2-carbaldehyde, followed by the rearrangement of resulting...
Crystallization successively was the Cu$_6$-Br-ppmbi species generated with divalent metal ions and derivatives of As complementary measures, attempts to crystallize the from the poor crystalline properties of the species generated. 

As shown in Figure 1, 6-Br-ppmbi exhibited strong fluorescent emission at 390 nm upon excitation at 320 nm. The fluorescent intensity of 6-Br-ppmbi decreased dramatically upon the titration of Fe$^{2+}$ ions. Saturated spectroscopic emission was reached after the introduction of more than 10000 equiv. of Fe$^{2+}$ ions, indicating very loose binding between Fe$^{2+}$ ions and the 6-Br-ppmbi sensor in solution. To identify the stoichiometry of the complex with Fe$^{2+}$ and 6-Br-ppmbi, repeated attempts to crystallize the resulting compound was performed albeit unsuccessfully. This may originate from the poor crystalline properties of the species generated. As complementary measures, attempts to crystallize the species generated with divalent metal ions and derivatives of 6-Br-ppmbi were made. The only complex that could be crystallized successively was the Cu$^{2+}$ complex with 6-Br-Me2-ppmbi, a derivative of 6-Br-ppmbi with two additional methyl groups in benzoimidazole. The octahedral copper(II) center in [Cu(6-Br-Me2-ppmbi)(NO$_3$)$_2$] have an N$_2$O$_4$ coordination environment with chelating terminal nitrate anion ligands (Fig. S2). As expected, 6-Br-Me2-ppmbi chelates Cu$^{2+}$ site with 6-bromo pyridine and imidazole units. This indicates that the 6-Br-ppmbi organic motif can generate a one to one complex with divalent metal ions.

Owing to the extremely low affinity of 6-Br-ppmbi for Fe$^{2+}$ ions, it was feasible to calculate the binding constant directly using Eq. (1), based on fluorescent titration, assuming 1:1 stoichiometry for Fe$^{2+}$ ions and sensor 6-Br-ppmbi (Fig. 1). The apparent dissociation constant of 6-Br-ppmbi was found to be $K_d = 1.06 \times 10^2$ by a nonlinear curve fit shown in the inset of Figure 1. This $K_d$ value was significantly larger than that of previously reported molecular sensors, possibly due to the steric hindrance rendered by the bulky bromine in the adjacent place of the coordinating nitrogen atom in the pyridine moiety. An extremely small amount of sensor, 0.05 µM of 6-Br-ppmbi in this case, was sufficient to detect more than 170 µM of Fe$^{2+}$ ions, which make this molecular sensor potentially useful in environmental practice.

In contrast, the addition of 6-Br-ppmbi to each solution of Ca$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Hg$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$, Ni$^{2+}$, and Zn$^{2+}$ ions caused the minor changes in the fluorescent character of 6-Br-ppmbi, compared to the ca. 93% decrease in fluorescence intensity in the case of the addition of the Fe$^{2+}$ (Fig. 2). These results show the highly selective response of 6-Br-ppmbi to Fe$^{2+}$ ions compared to other metal ions. There are two possible explanations for this unusual selectivity. One is that only Fe$^{2+}$ could quench the fluorescence of 6-Br-ppmbi, compared to the other ions despite the similar binding properties of the other cations with the 6-Br-ppmbi sensor. The other one is that the selective binding affinity of 6-Br-
**ppmbi** to the Fe$^{2+}$ ion, compared to the much low affinity to the other metal cations, may also explain the observed selectivity.

The fluorescence responses of **6-Br-ppmbi** (5.0 × 10$^{-2}$ μM) upon the subsequent addition of Fe$^{2+}$ (3.2 × 10$^{-2}$ μM) to solutions with the selected metal ions (3.2 × 10$^{-2}$ μM) were measured to determine the correct mechanism for the selective fluorescence quenching response to the addition of Fe$^{2+}$ (Fig. 3). Interestingly, the fluorescence was reduced to the same level upon the addition of Fe$^{2+}$ despite the existence of other cations at similar concentrations. This suggests that only Fe$^{2+}$ ion binds to **6-Br-ppmbi** ligand strongly compared to the other cations. If **6-Br-ppmbi** has similar binding affinity to Fe$^{2+}$ and other cations, the level of fluorescence in the case of the above mixed cation experiments must be higher than the one in the case of addition of Fe$^{2+}$ cations only.

Furthermore, the fluorescent color change can be used for the naked-eye detection of Fe$^{2+}$ ions, as shown in Figure 4. The selectivity of **6-Br-ppmbi** for Fe$^{2+}$ over the other cations is a significant advantage compared to the other fluorescent sensors reported thus far.

**Conclusions**

The syntheses of water-soluble fluorescent benzoimidazole molecules, which contain sterically hindered coordination moiety, are described. The fluorescence of the molecule was quenched when added to a solution of Fe$^{2+}$ ions. The synthesized **6-Br-ppmbi** bound selectively to Fe$^{2+}$ ion, reducing the luminescent character of its own, which makes the sensor molecule highly valuable in probing the Fe$^{2+}$ concentration. In addition, the naked-eye detection of Fe$^{2+}$ ions over other divalent cations and the requirement of low concentrations of sensor molecules to detect certain amounts of Fe$^{2+}$ ions make this sensor unique compared to previously reported Fe$^{2+}$ ion sensors.

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**Supporting Information Available.** Table S1 with the Summary of X-ray Crystallographic Data and Selected interatomic distances and angles for the Cu(II) complex. Figure S1, and S2 showing ORTEP drawing of **6-Br-ppmbi** and the Cu(II) complex. CCDC 842612 and 842613 contain the supplementary crystallographic data for the complexes. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk.

**References**


