A heterocyclic-based bifunctional sensor for detecting cobalt and zinc ion

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Abstract

A new bifunctional chemosensor HBP based on heterocyclic compounds was designed and studied. HBP showed a successful detecting ability toward cobalt ion with a UV-visible red-shift and a color change of colorless to pink. Moreover, toward zinc ion, HBP showed an obvious fluorescence turn-on response. The binding ratio of HBP to cobalt and zinc ions was a 2 to 1, respectively. The detection limits were found to be 10 nM for Co^{2+} and 18 nM for Zn^{2+}. Based on UV-vis and fluorescent spectral variations, Job plots, ESI-MS, FT-IR and calculations, the binding mechanisms of HBP toward cobalt and zinc ions were proposed.

Keywords: heterocyclic compound, colorimetric and fluorometric analysis, cobalt ion, zinc ion, calculations
Introduction

Development of chemosensors for detecting a variety of metal ions has attracted great attention owing to their versatile applicability in pathological, biological, and industrial environments.\(^1\)–\(^3\) Among various analytical methods, colorimetric and fluorescent assays are considerably preferred as optical methods, because they offer versatile advantages like a fast response, high selectivity and sensitivity.\(^4\)–\(^8\) Therefore, a wide range of colorimetric and fluorescent sensors have been reported for detecting diverse metal ions.

Among a variety of metal ions, cobalt ion is a crucial micronutrient for plants and animals and participates in the production of vitamin B\(_{12}\) as well as other biological products. Zinc ion is well known as the second most plentiful transition metal ion in body. It is an important component in physiological processes like gene expression, DNA organization, and enzymatic reactions.\(^9\)–\(^13\) However, the disorder of the concentration level of these metal ions can adversely affect the human body and lead neurogenic diseases like Menkes’ and Alzheimer’s diseases.\(^14\)–\(^16\) Therefore, it is greatly important to developing chemosensors for detecting cobalt and zinc ions with low detection limits.

Up to now, a great number of chemosensors for cobalt or zinc ion have been reported.\(^17\)–\(^24\) However, the chemosensors capable of detecting both cobalt and zinc ions are very rare.\(^25\)–\(^28\) though multifunctional chemosensors have attracted much attention because of their advantages such as higher efficiency and applicability, low cost and easy preparation for sample.\(^29\),\(^30\) In addition, although a few sensors show the selective sensing abilities to cobalt and zinc ions, none of them have the nano molar level of
detection limit to both ions. For these reasons, there is still a need to develop chemosensors with the nano molar level of detection limits for detecting both of them. Since cobalt and zinc ions prefer to bind to N donor atom, we envisioned that a chemosensor having a nitrogen-rich environment might sense selectively the two metal ions.

Heterocyclic compounds like quinoline, pyridine and imidazole have a great ability to bind to metal ions like cobalt and zinc ions because they contain the electron-rich atoms like nitrogen atom.\(^{31-34}\) In particular, a quinoline moiety is a well-known fluorophore as well as a chromophore and is very interesting because it can induce the unique optical response to particular metal ions like Zn\(^{2+}\).\(^{35,36}\) Moreover, a pyridine moiety containing the electron-withdrawing atom bromine can induce the outstanding optical changes with the charge transfer.\(^{37,38}\) Therefore, we expected a chemosensor based on quinoline and pyridine moieties could be a bifunctional detector for metal ions like cobalt and zinc ions through the optical response.

Hence, we developed a heterocyclic-based bifunctional chemosensor HBP for detecting cobalt and zinc ions. HBP showed outstanding optical responses to cobalt and zinc ions, respectively, with colorimetry and fluorometry. The proposed binding mechanisms toward cobalt and zinc ions were explained by the various spectroscopic techniques, ESI-mass and DFT calculations.

**Experimental**

*Materials and machines*

All chemical reagents were commercially obtained. \(^1\)H and \(^{13}\)C NMR data were
recorded on a Varian spectrometer. Absorption and fluorescence spectra were recorded by using Perkin Elmer spectrometer (Lambda 25 UV-Vis and LS45). ESI-MS spectra were collected on a Thermo Finnigan ion trap machine.

**Synthetic procedure of HBP ((E)-2-(2-((5-bromopyridin-2-yl)methylene)hydrazinyl)quinoline)**

2-Hydrazinylquinoline (0.5 mmol) and 5-bromo-2-pyridinecarboxaldehyde (0.7 mmol) were dissolved in 5 mL of EtOH. With stirring for 3 h, ivory-colored powder was produced, filtered, and washed with hexane. Yield: 54 %. 1H NMR (DMSO-d6): 11.74 (s, 1H), 8.68 (s, 1H), 8.24 (d, J = 8.7 Hz, 1H), 8.09 (s, 1H), 8.05 (m, 1H), 8.00 (m, 1H), 7.82 (d, J = 8 Hz, 1H), 7.70 (m, 3H), 7.33 (m, 1H). 13C NMR (DMSO-d6): 155.3 (1C), 152.9 (1C), 149.9 (1C), 146.9 (1C), 139.2 (1C), 138.7 (1C), 138.2 (1C), 129.8 (1C), 127.9 (1C), 126.0 (1C), 124.4 (1C), 123.0 (1C), 120.6 (1C), 119.1 (1C), 109.4 (1C). ESI-MS: m/z calcd for [HBP + H+]+, 327.02; found, 327.33. Element analysis calcd (%) for C15H11BrN4 + 0.5 H2O: C, 53.59; H, 3.60; N, 16.67 %; found (%): C, 53.48; H, 3.21; N, 16.59 %.

**Fluorescence and UV-visible studies**

For cobalt (II) ion, all UV-visible studies were achieved in bis-tris buffer (10 mM, pH = 7.0). A HBP stock was made in DMSO at 5 mM concentration and the concentration of HBP in all experiments was set as 2 μM. All metal ion stocks including MNO3 (M = Na and K) or M(NO3)2 (M = Zn, Co, Cd, Cu, Mn, Ni, Mg, Ca and Pb) or M(NO3)3 (M = Al, Ga, In, Fe and Cr) or M(ClO4)2 (M = Fe) were dissolved in bis-tris buffer. Their UV-vis spectral changes were collected by adding corresponding amounts of Co²⁺ to HBP and blending them for 15 sec.
For zinc ion, all fluorescence and UV-visible studies were conducted in DMF. A HBP stock was prepared in DMSO at 5 mM concentration and the concentration of HBP in all experiments was set as 5 μM. All metal ion stocks containing MNO₃ (M = Na and K) or M(NO₃)₂ (M = Zn, Co, Cd, Cu, Mn, Ni, Mg, Ca and Pb) or M(NO₃)₃ (M = Al, Ga, In, Fe and Cr) or M(ClO₄)₂ (M = Fe) were prepared in DMF. Their fluorescence and UV-vis spectral changes were collected by adding corresponding amounts of Zn²⁺ to HBP and blending them for 15 sec.

Quantum yields

Quantum yields of HBP and HBP-Zn²⁺ were calculated with fluorescein (Φ = 0.79) as a reference fluorophore. Quantum yield was given by using the following equation:

\[
\Phi_F = \Phi_F \times \frac{A_S \times F_S}{A_R \times F_R} \times \left( \frac{n_S}{n_R} \right)^2
\]

(Φₖ=fluorescence quantum yield, A=absorbance, F=integrated fluorescence emission, S=test sample, n=refractive index of the solvent and R=reference material)

Job plot measurements

To prepare HBP and Co²⁺ solutions at the same concentration of 50 μM, 400 μL of a HBP stock (5 mM, DMSO) and 100 μL of Co²⁺ (2x10⁻² M, bis-tris buffer) were diluted to 39.6 mL and 39.9 mL of buffer, respectively. 2.7-0.3 mL of HBP solution and 0.3-2.7 mL of Co²⁺ solution were put into each UV-visible cell. Their total volume was set to be 3 mL. With blending them for 10 s, UV-vis measurements with different concentration ratios were carried out.
To make HBP and Zn\(^{2+}\) solutions at the same concentration of 50 μM, 400 μL of a HBP stock (5x10\(^{-3}\) M, DMSO) and 100 μL of Zn\(^{2+}\) (2x10\(^{-2}\) M, DMF) were diluted to 39.6 mL and 39.9 mL of DMF, respectively. 2.7-0.3 mL of HBP solution and 0.3-2.7 mL of Zn\(^{2+}\) solution were transferred to each fluorescent cell. Their total volume was set to be 3 mL. With blending them for 10 s, fluorescent measurements with different concentration ratios were carried out.

*Computational studies*

Gaussian 16 program was used for the theoretical calculations to study detection mechanisms.\(^{41}\) The optimized geometry and DFT calculations were performed on B3LYP/6-31G/LANL2DZ.\(^{42-48}\) IEFPCM (integral equation formalism polarizable continuum model) was employed for considering the influence of the solvent.\(^{49}\) Time-dependence DFT calculations were utilized to examine the electronic transition states of molecules. Among the 20 transition states, one calculation state, which best fits the experimental values, was chosen for the mechanism study.

*Results and discussion*

HBP was produced from the condensation reaction of 2-hydrazinylquinoline and 5-bromo-2-pyridinecarboxaldehyde (Scheme 1) and characterized by ESI-MS, \(^1\)H NMR and \(^{13}\)C NMR analysis (Fig. S1).

*UV-vis studies of HBP to cobalt ion*

Colorimetric response of HBP toward a variety of metal ions was studied to investigate the sensing ability of HBP in bis-tris buffer (Fig.1). HBP had a maximum absorption band at 365 nm. The addition of some metal ions like Cu\(^{2+}\), Hg\(^{2+}\), Ga\(^{3+}\), Ag\(^+\),
and Fe$^{2+}$ led slight spectral variations with neglectable color changes whereas most metal ions induced no spectral variation at 512 nm. In contrast, the addition of Co$^{2+}$ showed a marked spectral variation at 512 nm with a color change of colorless to pink. These outcomes meant that HBP had a colorimetric sensing ability to Co$^{2+}$.

To explore the complexation mode of HBP with Co$^{2+}$, Job plot analysis was carried out (Fig. S2). The maximum absorbance of 512 nm appeared at a mole fraction of 0.3, suggesting a 2 to 1 ratio of HBP to Co$^{2+}$. The result was verified by ESI-MS (Fig. S3). The peak of 709.67 ($m/z$) is indicative of [2·HBP-H$^+$ + Co$^{2+}$] (calcd; $m/z$ 709.96).

UV-vis spectral variations were examined to study the sensing properties of HBP to Co$^{2+}$ (Fig. 2). On the addition of Co$^{2+}$ up to 0.65 equiv, the gradual increase of absorption bands at 250 and 512 nm and the gradual decrease of the absorption band at 360 nm appeared with the formation of a single isosbestic point at 268 nm. It meant the production of a species between HBP with Co$^{2+}$. On basis of UV-vis spectra, the detection limit to Co$^{2+}$ was found to be 10 nM by definition IUPAC ($C_{DL} = 3\sigma/k$) ($R^2=0.9995$) (Fig. 3).$^{50}$ The value is considerably lower than WHO guideline (1.7 μM) and the lowest among sensors previously addressed for sensing both cobalt and zinc ions (Table S1).$^{51}$ The association constant was calculated to be $3 \times 10^{11}$ M$^{-2}$ with a suitable $R^2 = 0.9974$ by using Li equation (Fig. S4).$^{52}$

To investigate the binding mode between HBP and Co$^{2+}$, FT-IR study was conducted (Fig. S5). In the case of HBP, the band at 3030-3200 cm$^{-1}$ related to the -NH group was shown. Upon the binding with Co$^{2+}$, the band around 3200 cm$^{-1}$ disappeared and the band at 3030 cm$^{-1}$ moved to 3050 cm$^{-1}$. It implied the deprotonation of one -NH group of two HBP. Meanwhile, the band of 1607 cm$^{-1}$ related to the C= N bond showed a
slight shift to 1599 cm\(^{-1}\). These outcomes signified that the C=\(\text{N}\) and -\(\text{NH}\) groups of 2-HBP might be related to the binding to Co\(^{2+}\). Binding mechanism of HBP and Co\(^{2+}\) was proposed, based on Job plot, FT-IR spectra and ESI-mass (Scheme 2).

To affirm the sensing capability of HBP to Co\(^{2+}\) when other metal ions existed, a competition test was performed (Fig. 4). In the existence of Ga\(^{3+}\), Cr\(^{3+}\), and Fe\(^{3+}\), the sensing ability was disturbed by about 10 to 43%. However, most metal ions did not show any interference, meaning a great sensing ability of HBP to Co\(^{2+}\). The pH test of HBP to Co\(^{2+}\) was carried out at a pH range of 6 to 9 (Fig. S6). On the addition of Co\(^{2+}\), the immediate increase of absorbance at 512 nm and color change from colorless to pink were shown at a pH range of 6 to 9. It indicated that HBP could successfully detect Co\(^{2+}\) at biological pH range (6.0 - 7.6).

**Fluorescence and UV-vis studies of HBP to zinc ion**

To investigate the fluorescent sensing ability of HBP, fluorescent response of HBP to varied cations was studied in DMF (Fig. 5). HBP had no fluorescence emission at 560 nm and the addition of other metal ions except Zn\(^{2+}\) did no or neglectable spectral variations at 560 nm. In contrast, only Zn\(^{2+}\) showed an obvious fluorescence increasing at 560 nm (\(\lambda_{\text{ex}} = 480\) nm) with a yellow fluorescence color. It indicated that HBP had a fluorescent sensing response to Zn\(^{2+}\).

To investigate the complexation ratio of HBP and Zn\(^{2+}\), Job plot analysis was carried out (Fig. S7). The maximum fluorescence intensity at 560 nm appeared at a mole fraction of 0.3, which implied a 2 to 1 ratio of HBP to Zn\(^{2+}\). As shown in Fig. S8, it was further verified by ESI-MS. The peak of 715.25 (\(m/z\)) is indicative of [2-HBP-H\(^{+}\) + Zn\(^{2+}\)] (calcd; \(m/z\) 714.95).
Fluorescent and UV-visible variations were studied to explore the sensing properties of HBP to Zn$^{2+}$. Upon the addition of Zn$^{2+}$ up to 0.54 equiv (Fig 6), the gradual enhancement of fluorescent intensity at 560 nm was observed with an obvious change of quantum yield ($\Phi$) of 0.017 to 0.120. Under the same condition, the addition of Zn$^{2+}$ up to 0.5 equiv induced the gradual increase of absorbance at 300 and 515 nm and the decrease of absorbance at 355 nm with two marked isosbestic points at 315 and 394 nm (Fig. S9). The outcome meant the formation of a species of HBP with Zn$^{2+}$.

Based on the fluorescent spectra, detection limit for zinc ion turned out to be 18 nM by definition IUPAC ($C_{DL} = 3\sigma/k$) ($R^2=0.9977$) (Fig. S10). The value is significantly lower than WHO guideline (76 μM) and can be expressed down to nanomolar units.

The association constant was calculated to be $2\times10^{11}$ M$^{-2}$ with an adequate $R^2 = 0.9959$ with the determination of Li’s equation (Fig. S11).

To study the binding mode between HBP and Zn$^{2+}$, FT-IR spectra were obtained (Fig. S12). Similar to those of HBP-Co$^{2+}$, the band around 3030-3200 cm$^{-1}$ related to the -NH groups were broadened and the band of 3030 cm$^{-1}$ moved to 3050 cm$^{-1}$. It implied the deprotonation of one -NH group of two HBP. Meanwhile, the band at 1607 cm$^{-1}$ related to the C=N bond moved slightly to 1604 cm$^{-1}$. These results suggested that the C=N and -NH groups might be related to the binding to zinc ion. Binding mechanism of HBP and Zn$^{2+}$ was proposed, based on Job plot, FT-IR spectra and ESI-MS (Scheme 3).

Competition test was conducted to check the sensing ability of HBP toward Zn$^{2+}$ (Fig. S13). Most metal ions showed no inhibition, whereas some metal ions like Hg$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, and Ni$^{2+}$ inhibited from 30 to 50 %, and Fe$^{2+}$ and Co$^{2+}$ completely interfered due to their paramagnetic properties.
Computational studies to Zn$^{2+}$

The optimized structures of HBP and HBP-Zn$^{2+}$ were obtained based on experimental results such as Job plot, ESI-mass, and FT-IR (Fig. 7). HBP and HBP-Zn$^{2+}$ exhibited planar and crossed-planar structures with dihedral angles of 0.000° and 1.968° (1N, 2N, 3C, 4N), respectively.

The possible transition states and molecular orbitals were explored using time-dependence DFT calculations. For HBP, the main absorption at 373.27 nm stemmed from HOMO → LUMO transition, which presented π-π* transition characteristic (Figs. S14 and S15). The red-shifted major transition of HBP-Zn$^{2+}$ derived from HOMO → LUMO+1 transition, which also displayed π-π* transition character (Figs. S15 and S16). Considering the similarity in transition properties and structures of HBP and HBP-Zn$^{2+}$, the fluorescent turn-on process may be a CHEF (chelation enhanced fluorescence) effect. As zinc chelated, non-radiative transitions such as rotation and vibration would be suppressed and switched into radiative transitions. Based on these results, we proposed a feasible zinc detection mechanism of HBP (Scheme 3).

Conclusion

A new heterocyclic-based bifunctional sensor HBP was developed. HBP had successful responses to cobalt in bis-tris buffer and zinc ions in DMF, showing the outstanding color and fluorescence change, respectively. On the other hand, HBP can detect only Co$^{2+}$ in aqueous solution. The significantly low detection limits were calculated as 10 nM for cobalt ion and 18 nM for zinc ion. Especially, the detection
limit for cobalt was the lowest among the sensors formerly addressed for detecting both cobalt and zinc ions. In particular, HBP is the first chemosensor that can have the detection limits down to nanomolar units for both cobalt and zinc ions. The binding mechanisms to cobalt and zinc ions were demonstrated with UV-vis and fluorescent spectral variations, Job plot, FT-IR, ESI-MS and calculations.

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Scheme 1. Synthesis of **HBP**.

Scheme 2. Proposed binding of **HBP** to **Co**\(^{2+}\).

Scheme 3. Proposed binding of **HBP** to **Zn**\(^{2+}\).
Figure Captions

Fig. 1 Colorimetric response of HBP (2 µM, DMSO) to varied cations (0.65 equiv) in bis-tris buffer.

Fig. 2 UV-vis spectral variations of HBP upon the addition of Co$^{2+}$ up to 0.65 equiv. Inset: plot of absorbance at 512 nm as a function of Co$^{3+}$ equiv.

Fig. 3 Determination of detection limit of HBP toward Co$^{2+}$.

Fig. 4 Competitions test of HBP to Co$^{2+}$ with other cations.

Fig. 5 Fluorescent response of HBP (5 µM, DMSO) to varied cations (0.54 equiv, DMF) in DMF. Inset: fluorescent photograph of HBP and HBP-Zn$^{2+}$.

Fig. 6 Fluorescence titrations of HBP with different equivalents of Zn$^{2+}$ up to 0.54 equiv.

Fig. 7 Energy-optimized forms of (a) HBP and (b) HBP-Zn$^{2+}$. 
Fig. 1.
Fig. 2.
Fig. 3.

\[ y = 0.0203x + 0.0016 \]

\[ R^2 = 0.9995 \]

D.L. = 10 nM
Fig. 4
Fig. 5
Fig. 6
Fig. 7

(a) 

Dihedral angle (1N, 2N, 3C, 4N) : 0.000°

(b) 

Dihedral angle (1N, 2N, 3C, 4N) : 1.968°
Graphical Index