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A New Highly Zn\(^{2+}\)-Selective and “Off-On” Fluorescent Chemosensor Based on Pyrene Group

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In this work, an “Off-On” type zinc ion fluorescent chemosensor \(\text{H}_2\text{L}\) with pyrene as fluorophore was investigated. The chemosensor \(\text{H}_2\text{L}\) demonstrates a \(\text{Zn}^{2+}\)-specific emission enhancement linearly with a 1:1 binding ratio even in the presence of a wide range of competing metal ions. This may be correlated to the efficient \(\text{Zn}^{2+}\) binding of \(\text{H}_2\text{L}\), which promotes a good chelation-enhanced fluorescence (CHEF) effect and prevents the photoinduced electron transfer (PET) effect, and \(\text{C}==\text{N}\) isomerization is inhibited. Importantly, the fabricated chemosensor can selectively distinguish \(\text{Zn}^{2+}\) from \(\text{Cd}^{2+}\). And the recognition behaviour has been proved experimentally and computationally.

1. Introduction

\(\text{Zn}^{2+}\) is an essential element for normal growth and development of human body\(^{1,2}\). It plays a key role in cellular processes such as DNA repair\(^3\) and apoptosis\(^4\) as well as in the synthesis of insulin and the pathological state of diabetes.\(^5\) Failing to maintain zinc homeostasis has been implicated in a number of severe neurological diseases.\(^6\) As a consequence, the development of novel fluorescent chemosensors for detecting \(\text{Zn}^{2+}\) accurately has received considerable attention.\(^7\)

Although, a number of fluorescent chemosensors for \(\text{Zn}^{2+}\) based on quinoline\(^8\), fluorescein\(^9\), coumarin\(^10\), and peptides\(^11\) have been reported to date, there is still a challenge to develop chemosensors that can selectively detect \(\text{Zn}^{2+}\) from \(\text{Cd}^{2+}\) as these two cations setting in the same group of the periodic table, have similar chemical properties, thus causing similar spectral changes after interacting with chemosensors.

Recently, particular attention has been paid to the synthesis and study of metal complexes of Schiff bases.\(^12\) Some of the Schiff base metal complexes possess antitumor properties,\(^13\) antioxidative activities,\(^14\) and attractive electronic and photophysical properties.\(^15\) In addition, Schiff base derivatives incorporating a fluorescent moiety are appealing tools for fluorescent sensing of metal ions. Schiff base is very easy to synthesize by reaction of aldehyde and amine, what is more, the nitrogen atom of amine could coordinate with metal ion. Consequently, organic molecule possessing Schiff base structure has potential application in sensors.

In this work, we designed chemosensor \(\text{H}_2\text{L}\) using pyrene as fluorophore, and 2-hydroxybenzohydrazide as ionophore to construct a small organic molecule containing Schiff base motif. Schiff base structure was obtained by the reaction of 1-hydroxypyrene-2-carbaldehyde and 2-hydroxybenzohydrazide in boiling ethanol with satisfactory yield. In this compound, the \(\text{Zn}^{2+}\) chelator 2-hydroxybenzohydrazide was incorporated with 1-hydroxypyrene-2-carbaldehyde at its formyl group position as the synergic \(\text{Zn}^{2+}\) coordination motif. Then, both 1:1 binding mode between \(\text{Zn}^{2+}\) and \(\text{H}_2\text{L}\) and higher \(\text{Zn}^{2+}\) binding affinity were expected. The synthetic route of \(\text{H}_2\text{L}\) is depicted in Scheme 1.

2. Experimental section

2.1. Materials, methods and instrumentation

All chemicals were purchased from commercial suppliers and used without further disposal. \(^1\)H NMR spectra were obtained at 400 MHz and \(^13\)C NMR spectra at 100 MHz with JEOL RESONANCE spectrometer in \(\text{d}_2\)-DMSO solution with DMSO as an internal standard and in CDCl\(_3\) solution with tetramethylsilane (TMS) as an internal standard. Elemental analysis were conducted using an
Elementar Vario EL. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 360 FT-IR spectrometer using KBr pellets in the range of 4000-400 cm⁻¹. UV-vis absorption spectra were determined on a Varian Cary-100 spectrophotometer. Hitachi F-4500 fluorescence spectrophotometer was used to record on steady state luminescence spectra. ESI-MS spectra were determined on a Bruker Daltonics Esquire 6000 spectrometer.

All of the detections of metal ions were operated in tri(hydroxymethyl)aminomethane (Tris)-HCl (20 mM, pH 7.36), CH₃CN/H₂O (1:1, v/v). The stock solution of H₂L (1, 10 mM) was prepared in DMSO solution. Stock solutions of the perchlorate salts of Na⁺, K⁺, Mg²⁺, Ca²⁺, Cu²⁺, Zn²⁺, Al³⁺, Pb²⁺, Cr³⁺, Cd²⁺, Ag⁺, Fe³⁺, Mn²⁺, Ni²⁺ and Hg²⁺ (10 mM) were prepared in doubly distilled water, respectively. The volume of cationic stock solution added was less than 100 μL to maintain the concentration of H₂L unchanged. All fluorescence spectra were recorded at 25 °C with the excitation wavelength of 490 nm.

2.2. Calculation methods

To investigate the interaction mode, we carried out density functional theory (DFT) calculations with B3LYP/6-31G(d) method. The geometry of the molecules was optimized with Gaussian 0918 package at the B3LYP/6-31G(d) levels for C, H, O, N atoms and LANL2DZ17 levels for Zn.

2.3. Synthesis of chemosensor H₃L

The synthesis was performed according to literature18 as follows: 1-Hydroxyxyrrene-2-carbaldehyde (160 mg, 0.65 mmol) and 2-hydroxybenzoylhydrazide (98 mg, 0.65mmol) were mixed in boiling ethanol with stirring for 6 hours under Ar, then yellow precipitates was filtered, washed with ethanol and dried at 50 °C under vacuum to give H₃L (173 mg, 0.46 mmol, 70%) as an yellow solid. FT-IR (KBr disc, cm⁻¹): 3442, 3039, 16131 (C=O), 1608, 1561, 1542, 1492, 1459, 1348, 1205, 1218, 1160, 1096, 841, 751. Anal. Calcd for C₅H₁₈N₄O₄: C, 75.71; H, 4.21; N, 7.36. Found: C, 75.52; H, 4.29; N, 7.47. ¹H NMR (400 MHz, DMSO): δ 12.38 (1H, s), 11.70 (1H, s), 8.45 (1H, d), 8.37 (1H, s), 8.16 (3H, t), 8.05 (2H, m), 7.92 (2H, d), 7.50 (1H, t), 7.03 (2H, m). (Figure S1). ¹³C NMR (100 MHz, DMSO): δ 162.9, 160.4, 150.6, 141.6, 144.4, 134.5, 132.5, 132.1, 128.8, 127.5, 127.4, 126.7, 125.9, 124.3, 124.1, 123.9, 122.2, 121.7, 117.6. (Figure S2). MS (ESI+): m/z 381.1 (M+H)⁺ (Figure S3).

3. Results and discussion

The perchlorate salts of different metal ions were used to evaluate the binding property between the metal ions and chemosensor H₂L. The fluorescent spectroscopic properties of H₂L were studied in Tris–HCl (20 mM, pH 7.36), CH₃CN/H₂O (1:1, v/v) solution after systematically looking for selective signalling towards various tested metal ions for potential applications. As shown in Fig. 1, the emission spectrum of H₂L exhibited the emission maximum at 556 nm with low fluorescence intensity when pyrene as fluorophore was excited at 495 nm (Φ = 0.033)9. A significant fluorescence emission intensity enhancement was observed after addition of 1.0 equiv of Zn²⁺ to CH₃CN/H₂O buffer solution containing equivalent molar of H₂L (Φ = 0.279). The increase in emission intensity may be attributed to the formation of the L–Zn complex. When H₂L was coordinat ed with zinc ion, a large CHEF effect was observed because the stable complexion between H₂L and Zn²⁺ prohibited the PET process from the electron-donating group to the pyrene fluorophore (“On” state). In addition, the stable chelation of H₂L with zinc ion not only inhibited the C=N isomerization but also enhanced fluorescence intensity of H₂L. The emission intensity of H₂L was slightly quenched upon addition of Cu²⁺, Co²⁺ and Ni²⁺, which may be attributed to intrinsic paramagnetism of these metal ions15. Other metal ions such as Na⁺, K⁺, Mg²⁺, Al³⁺, Ca²⁺, Cr³⁺, Cd²⁺, Mn²⁺, Fe³⁺, Hg²⁺ and Ag⁺ had little effect on the fluorescence spectra of H₂L. These results showed the capability of chemosensor H₂L for distinguishing Zn²⁺ from other metal ions, in particular from Cd²⁺. Moreover, the stability constant value of H₂L for Zn²⁺ was determined to be 7.01×10⁵ M⁻¹ (Figure S4). Therefore, chemosensor H₂L showed a significant fluorescence enhancement only with Zn²⁺ among the various metal ions examined including Cd²⁺, indicating the higher selectivity of H₂L towards Zn²⁺.

To further explore the selectivity of H₂L to Zn²⁺, the intensity of H₂L in the presence of Zn²⁺ mixed with plentiful of various metal ions in CH₃CN/H₂O (1:1, v/v) solution at pH 7.36 in Tris–HCl (Fig. 2) buffer was measured. A range of competing metal ions were mixed with the H₂L prior to addition of 1 equiv of Zn²⁺. The test results of the above system showed that 5 equiv Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe³⁺, Hg²⁺, Ag⁺, Cd²⁺, Mn²⁺, Cr³⁺ and Ni²⁺ had no or little effect on the chemosensor H₂L in detecting Zn²⁺. However, Zn²⁺-induced fluorescence enhancement of H₂L was prevented by the addition of 1 equiv Cu²⁺ and Co²⁺. This may be ascribed to these two metal ions would remain binding with the sensor H₂L and thus preventing the fluorescence enhancement21, even in the presence of 1 equiv of zinc ions.

![Fig. 1. Fluorescence spectra of H₂L in the presence of various metal ions such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Cu²⁺, Zn²⁺, Al³⁺, Pb²⁺, Cr³⁺, Cd²⁺, Ag⁺, Fe³⁺, Mn²⁺, Co²⁺, Ni²⁺ and Hg²⁺ in Tris–HCl (20 mM, pH 7.36), CH₃CN/H₂O (1:1, v/v). Excitation wavelength was 490 nm. [H₂L] = 50 μM, [M²⁺] = 50 μM.](image-url)
Fig. 2. Selectivity of $\text{H}_2\text{L}$ for Zn$^{2+}$ in the presence of other metal ions in Tris–HCl (20 mM, pH 7.36), CH$_3$CN/H$_2$O (1:1, v/v), Excitation wavelength was 490 nm. Gray bars represent the addition of an excess of the appropriate metal ion (5.0 equiv Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Cd$^{2+}$, Cr$^{3+}$, Al$^{3+}$, Mn$^{2+}$, Fe$^{3+}$, Hg$^{2+}$, Ag$^{+}$, Pb$^{2+}$, Ni$^{2+}$, 1.0 equiv Cu$^{2+}$, Co$^{2+}$) to a 50 $\mu$M solution of $\text{H}_2\text{L}$. Black bars represent the subsequent addition of 1.0 equiv Zn$^{2+}$ to the solution.

Fig. 3. UV–vis spectra of $\text{H}_2\text{L}$ (10 $\mu$M) upon the titration of Zn$^{2+}$ (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 equiv) in Tris–HCl (20 mM, pH 7.36), CH$_3$CN/H$_2$O (1:1, v/v) at room temperature. The inset shows the plot of absorbance of $\text{H}_2\text{L}$ at 478 nm versus Zn$^{2+}$ concentration.

To investigate the binding property of chemosensor $\text{H}_2\text{L}$ for Zn$^{2+}$, the UV–vis spectra of $\text{H}_2\text{L}$ (10 $\mu$M) in the presence of a wide range of concentrations of Zn$^{2+}$ (0–20 $\mu$M) was measured. Fig. 3 showed the absorbances at the 254, 370 and 480 nm bands increased while the band at 312 nm decreased. Two isosbestic points were observed at 269 and 342 nm, clearly indicating that the $\text{H}_2\text{L}$ and the Zn$^{2+}$ formed only one visible active zinc complex. The spectra change showed the binding mode between $\text{H}_2\text{L}$ and Zn$^{2+}$ was a 1:1 stoichiometry. Furthermore, the Job’s plot suggested the stoichiometry of complex formed by $\text{H}_2\text{L}$ and Zn$^{2+}$ was also 1:1 (Fig. S5), this result further indicated that only a 1:1 complex was formed in the media. Free $\text{H}_2\text{L}$ in CH$_3$CN/H$_2$O (1:1, v/v) solution at pH 7.36 in Tris–HCl (Fig. 4) buffer showed weak fluorescence with the maximum emission band at 556 nm. Upon addition of Zn$^{2+}$ (0–1.0 equiv) to the above buffer, the emission intensity increased linearly. When the ratio reached to 1:1, however, higher [Zn$^{2+}]_{tot}$ did not lead to any further fluorescence enhancement. The fluorescence addition result indicated the chemosensor $\text{H}_2\text{L}$ can preferentially recognise Zn$^{2+}$, and exhibited a turn “On” state, which further demonstrated the complex formed between $\text{H}_2\text{L}$ and Zn$^{2+}$ was 1:1 stoichiometry. The limit of detection (LOD) for Zn$^{2+}$ was measured to be 1.38×10$^{-6}$ M (Figure S6).

In order to investigate the coordination configuration of $\text{H}_2\text{L}$ to Zn$^{2+}$, we carried out theoretical calculations of binding energy of $\text{H}_2\text{L}$ and Zn$^{2+}$. In theoretical calculations, the geometry of the molecules was optimized with Gaussian 09 package at the B3LYP/6-31G(d) levels for C, H, O, N atoms and LANL2DZ levels for Zn. The minimum nature of the structure was confirmed by frequency calculations at the same computational level. Fig. 4 showed the optimized configurations of binding mode of $\text{H}_2\text{L}$–Zn$^{2+}$. All atoms of C, N, O were nearly in a plane, which showed that only one Zn$^{2+}$ occupied the coordination centres of $\text{H}_2\text{L}$ well and one cavity was formed by the chemosensor, suitable for coordinating Zn$^{2+}$, irrespective to the impact of solvent molecule and perchlorate ion. The bond lengths were obtained from the natural bond orbital (NBO) analysis. The Zn–N bond length was 2.043Å and the two Zn—O bond lengths were 1.856Å, 1.883Å, respectively. The interaction energy of between L$^2$ with Zn$^{2+}$ was −830.52 kcal mol$^{-1}$. The result demonstrated the chemosensor can effectively detect Zn$^{2+}$.
4. Conclusions

In conclusion, we have successfully developed a new highly selective and sensitive fluorescent chemosensor for Zn²⁺ over other competing cations including Cd²⁺. DFT calculations also demonstrated that the chemosensor had high selectivity for Zn²⁺. The fluorescent enhancement may be attributed to chelation-enhanced fluorescence, which is resulted from the strong coordination ability of Zn²⁺ with H3L, the PET process and the inhibition of C = N isomerisation. These results indicated that pyrene group as fluorophore was valuable for designing fluorescent chemosensor.

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Notes and references


A highly selective for zinc ion fluorescent chemosensor $\text{H}_2\text{L}$ based on pyrene derivative was described. Importantly, this chemosensor can clearly distinguish $\text{Zn}^{2+}$ from $\text{Cd}^{2+}$. 