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Zinc(II) and PPi Selective Fluorescence OFF−ON−OFF Functionality of a Chemosensor in Physiological Conditions

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Supporting Information

ABSTRACT: A fluorescent chemosensor based on a quinoline derivative, $\mathbf{L^2}$ (OFF state), selectively senses Zn^{2+} by effective chelate-enhanced fluorescence (ON state), which further shows selectivity toward PPi over competing anions like Pi, AMP, and ATP via fluorescence quenching (OFF state) in a 100% aqueous HEPES buffer (pH 7.4). A plausible mode for the selective binding of PPi to **1** has been demonstrated by quantum mechanical density functional theory calculations and high-resolution mass spectrometry analysis.

Detection of the zinc ion (Zn^{2+}) has shown considerable interest in the chemistry community for its biological relevance.[1] Because Zn^{2+} (d^{10} electronic configuration) is spectroscopically silent, many fluorescent-enhancement-based chemosensors for its detection have been studied.[2] Especially, quinolines and their derivatives are attractive fluorogenic agents for the quantitative chemical sensing of Zn^{2+} and other metal ions.[3] On the other hand, pyrophosphate (PPi), the product of adenosine triphosphate (ATP) hydrolysis under cellular conditions, is a biologically important target,[4] and its detection is being investigated as a real-time DNA sequencing method.[5] Thus, the fluorescence-based chemosensing of PPi has been one of the main focuses among different sensing studies.[6] For applications in biological systems, PPi sensing normally requires selectivity in the presence of phosphate (Pi), adenosine 5′-monophosphate (AMP), adenosine diphosphate (ADP), and ATP.[6] Thus far, the most successful strategy in the design of receptors for PPi has shown the incorporation of metal-ion complexes in the system because of the strong binding affinity between metal ions and PPi, which allows the detection of PPi.[7] Most receptors reported for PPi bear either two Zn^{2+} or Cu^{2+} binding sites,[7] with exceptions being two receptors that employ mononuclear Cu^{2+} complexes for selective PPi sensing.[8] Herein we report a simple quinoline derivative for selective and effective chelation-enhanced fluorescence (CHEF) sensing of Zn^{2+}, which further shows selective sensing toward PPi over competing anions like Pi, AMP, and ATP via chelation-enhanced fluorescence quenching (CHEQ) in physiological conditions. Moreover, we also structurally demonstrate the formation of a mononuclear distorted octahedral Zn^{2+} complex of $\mathbf{L^2}$.

trans-(1*R*,2*R*)-*N*′,*N*″-Bis[(quinolin-2-yl)methyl]cyclohexane-1,2-diamine ($\mathbf{L^2}$) was synthesized following a modified literature procedure.[9] A methanolic solution of $\mathbf{L^2}$ was reacted with an aqueous solution of $Zn(NO_3)_2\cdot 6H_2O$ to yield the complex $[\mathbf{L^2}\cdot Zn\cdot NO_3]NO_3$ (**1**) at room temperature (Chart 1). Single crystals of **1** suitable for X-ray analysis were obtained upon the slow evaporation of its aqueous solution. Figure 1 unambiguously illustrates 1:1 binding of $\mathbf{L^2}$ and Zn^{2+}. The unit cell contains three discrete $\mathbf{L^2}/Zn^{2+}$ complexes, one of which is depicted in Figure 1. The geometry of Zn^{2+} is distorted octahedral, where Zn^{2+} is strongly coordinated by four nitrogen atoms of $\mathbf{L^2}$ and weakly coordinated by two oxygen atoms of a nitrate ion.

Figure 2 shows emission spectra of $\mathbf{L^2}$ (1×10^{-5} M) and $\mathbf{L^2}$ in the presence of different metal ions in a HEPES buffer (10 mM, pH 7.4). $\mathbf{L^2}$ (1×10^{-5} M) showed a very weak fluorescence (OFF state) at 346 nm, characteristic of a quinoline monomer emission upon quinolinyl excitation (315 nm), due to an effective photoinduced-electron-transfer (PET) process. The addition of 10 equiv of various metal ions like Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Pb^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Ag^+ showed no change in the fluorescence intensity at 346 nm. A weak fluorescence quenching is detected upon the addition of Cu^{2+}. However, a strong fluorescence enhancement (ON state) and a remarkable red shift of 30 nm are observed for $\mathbf{L^2}$ upon the addition of Zn^{2+}. This could be due to the blocking of the PET pathway upon complexation with Zn^{2+} and the involvement of quinoline (chelating fluorophore) nitrogen in the complexation process. A blue-green emission of the solution can be easily observed by the naked eye upon UV irradiation (Figure 2, inset).

The binding affinity of $\mathbf{L^2}$ toward Zn^{2+} is performed in an aqueous buffer solution (pH 7.4) in both the absorbance and emission modes. UV−vis spectra of $\mathbf{L^2}$ showed two distinct peaks at 300 and 312 nm (Figure 17S, see the Supporting Information). Upon the addition of Zn^{2+} to the aqueous solution of $\mathbf{L^2}$ (1×10^{-4} M), a gradual increase in the absorbance at 300 nm, a simultaneous decrease in the absorbance at 312 nm, and an isosbestic point at 278 nm are observed, which signify the existence of an equilibrium in the solution. The absorbance at 300 nm increased linearly up to a mole ratio of 1:1, and it remained constant thereafter. Moreover, Job's plot analysis revealed a maximum at a 0.5 M fraction of Zn^{2+}, which further indicates 1:1 complex formation. Upon the addition of increasing amounts of Zn^{2+} to the solution of $\mathbf{L^2}$ (1×10^{-5} M), an ∼10-fold increase in the fluorescence intensity and a 30-nm red shift (from 346 to 376 nm) of fluorescence emission are observed

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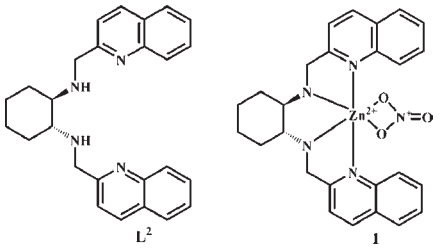
Chart 1. Chemosensor L^2 and 1 for Zn^{2+} and PPi Sensing, Respectively



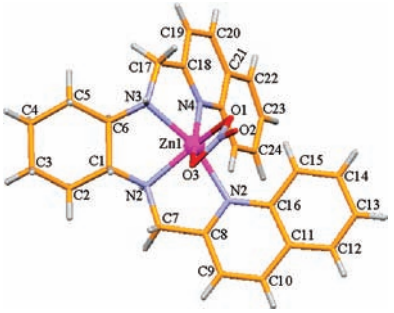


Figure 1. X-ray structure of **1**. Hydrogen atoms and two other units in the asymmetric unit are omitted for clarity.

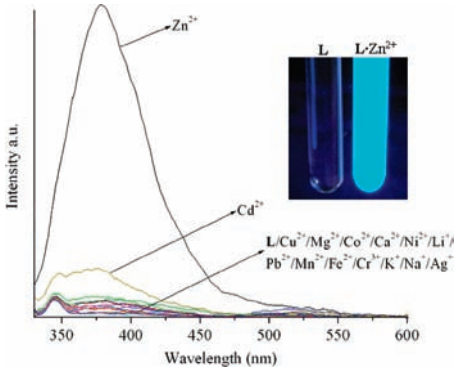


Figure 2. (a) Fluorescence changes of L^2 (1×10^{-5} M) upon the addition of different metal ions (10 equiv) at pH 7.4 (10 mM HEPES; excitation wavelength, 315 nm; excitation and emission slit width, 5.0 nm). Inset: Blue-green fluorescence of L^2 upon the addition of Zn^{2+} in the same experimental conditions.

(Figure 19S, see the Supporting Information). The emission intensity increases linearly with an increase in the Zn^{2+} concentration. From the UV−vis and fluorescence titration, the association constant of L^2 with Zn^{2+} is observed to be $\sim 1.4 \times 10^6$ M^{-1}.[10]

The complexation of Zn^{2+} and L^2 in an aqueous medium is also further supported by in situ NMR and MS experiments. The formation of a single binary complex in the solution is evident from the ^1H and ^{13}C NMR spectra of L^2 in the presence of equimolar amounts of $Zn(NO_3)_2 \cdot 6H_2O$ in D_2O (Figures 21S and 22S, see the Supporting Information). The aromatic signals (^1H and ^{13}C) of quinoline moieties in L^2 are shifted downfield upon the addition of Zn^{2+}, indicating the formation of a binary complex between L^2 and Zn^{2+}. Furthermore, electronspray

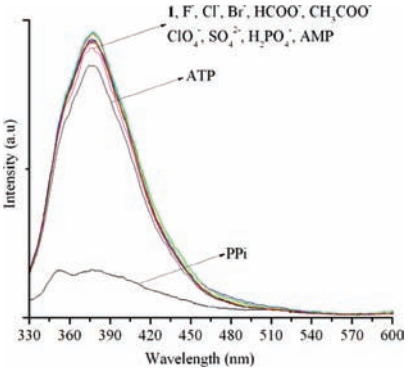


Figure 3. Fluorescence changes of **1** (1×10^{-5} M) upon the addition of different anions (100 equiv, as sodium salts) at pH 7.4 (10 mM HEPES; excitation wavelength, 315 nm; excitation and emission slit width, 9.0 nm).

ionization (HRMS) data of this solution also support the formation of a 1:1 complex between L^2 and Zn^{2+} (Figure 10S, see the Supporting Information).

The emission output of L^2 with Zn^{2+} in the presence of different competing ions is conducted to address the selectivity of L^2 toward Zn^{2+} (Figure 24S, see the Supporting Information). The fluorescence output of L^2 with Zn^{2+} is not influenced by other metal ions tested in this study. Most importantly, higher concentrations (1 mM) of Ca^{2+}, Mg^{2+}, Na^+, and K^+ (which generally coexist with Zn^{2+} in the biological systems)[2] do not interfere in the sensing of Zn^{2+} under physiological conditions.

To examine the effect of anions in the fluorescence output of L^2 and Zn^{2+} (ON state), different anions like F^-, Cl^-, Br^-, $HCOO^-$, CH_3COO^-, ClO_4^-, SO_4^{2-}, Pi, PPi, ATP and AMP (100 equiv, as sodium salts) were added to the above solution of L^2 and Zn^{2+} (Figure 20S, see the Supporting Information). In the case of the PPi input, a strong fluorescence quenching output (OFF state) was observed, whereas no appreciable change in the fluorescence emission was observed for all other anions. To verify the above findings of selective PPi sensing, we carried out a parallel experiment on isolated single crystals of a Zn^{2+} complex of L^2, **1**. In complex **1**, the weakly coordinated nitrate could be selectively replaced by an anion, which has a higher affinity toward the Zn^{2+} center. Selective binding of anion to the Zn^{2+} center could alter the emission output of **1** and that could help in the sensing of a specific anionic guest. Thus, fluorescence emission changes of **1** ($L^2 \cdot Zn^{2+}$) upon the addition of F^-, Cl^-, Br^-, $HCOO^-$, CH_3COO^-, ClO_4^-, SO_4^{2-}, Pi, PPi, ATP, and AMP (100 equiv, as sodium salts) were examined and are illustrated in Figure 3. In the absence of an anionic guest, the emission spectrum of **1** is characterized by an intense emission band (ON state) centered at 376 nm upon excitation of a quinoline fluorophore at 315 nm in a HEPES buffer (10 mM, pH 7.4). A large fluorescence quenching (OFF state) is observed only upon the addition of PPi due to CHEQ, although UV−vis absorbance does not show significant changes. A slight fluorescence quenching was observed with ATP upon complexation with **1**, whereas **1** does not exhibit any significant change upon the addition of AMP, $H_2PO_4^-$, SO_4^{2-}, and other monovalent anions, even when they are present in excess (0.01 M).

The binding affinity of **1** (1×10^{-5} M) with PPi at pH 7.4 (10 mM HEPES) is evaluated using the fluorescence titration experiment (Figure 23S, see the Supporting Information). Upon

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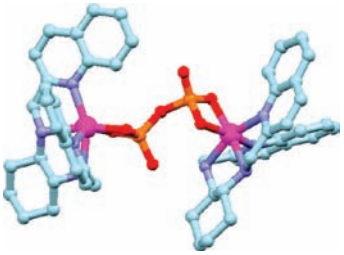

Figure 4. Calculated structure for the $[(\mathbf{1})_2 \cdot \mathrm{PPi}]$ complex.

the addition of PPi, the fluorescence intensity gradually decreased and reached a plateau. The fluorescence data gave the best fit for the 1:2 model, and the association constant was determined from a Benesi−Hilderbrand plot for 1:2 complexes.[10] From the fluorescence titration, the association constant of complex **1** with PPi is estimated as $4.68 \times 10^5\ M^{-2}$. Furthermore, electronspray ionization (HRMS) data of this solution support the formation of 1:2 complex of PPi and **1** (Figure 25S, see the Supporting Information). This plausible mode of binding for PPi to **1** was investigated by quantum-chemical calculations at the density functional theory (DFT) B3LYP level, which shows a minimal optimization for 1:2 complex $[(\mathbf{1})_2 \cdot P_2O_7]$, with the PPi anion bridging between two units of **1**. The Zn^{II} center is in a distorted octahedral geometry coordinated to four nitrogen atoms from **L** units (average distances $N_{cyclohexyl} \cdots Zn^{II} = 2.233$ Å and $N_{quinoline} \cdots Zn^{II} = 2.302$ Å) and two oxygen atoms (average distances $O_{PPi} \cdots Zn^{II} = 2.102$ Å) from PPi units (Figure 4). Frequency analysis on this complex showed only real vibrational frequencies, indicating a minimal optimum state for the complex. The selectivity of **1** toward PPi over other relevant anions like ATP, AMP, and Pi could also be explained by the fact that only PPi could be capable of bridging the two receptors effectively rather than other relevant anions.

The fluorescence titration experiments are also performed to probe the selectivity of **1** toward PPi over other anions in physiological conditions (Figure 26S, see the Supporting Information). The selective fluorescence quenching of **1** by PPi is not influenced by any other anions investigated, even at their higher concentration (0.01 M). Most importantly, the fluorescence pattern reveals that **1** could be employed in the selective sensing of PPi even in the presence of interfering anions like $H_2PO_4^-$, AMP, and ATP, which exist as high concentrations in physiological conditions.

In conclusion, this quinoline-based receptor is established as a selective fluorescent chemosensor for Zn^{2+} over other competing cations via fluorescence turn-"ON", and this turn-"ON" species shows selectivity toward PPi over competing anions like Pi, AMP, and ATP via fluorescence turn-"OFF" in a 100% aqueous HEPES buffer (pH 7.4). Thus, a simple quinoline derivative, $\mathbf{L}^2$, acts as an "OFF−ON−OFF" molecular switch by Zn^{2+} and PPi inputs, respectively. The binding mode of $\mathbf{L}^2$ and Zn^{2+} is also confirmed by single-crystal X-ray analysis of **1**. Thus, this receptor could be of potential biological application for the selective sensing of Zn^{2+} and PPi simultaneously.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental procedure and characterization data for receptors $\mathbf{L}^2$ and **1** and additional spectral and crystallographic information ($\mathbf{L}^1$ and **1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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