A Ratiometric Tetrazolylpyridine-Based “Turn-On” Fluorescent Chemosensor for Zinc(II) Ion in Aqueous Media

Palanichamy Kaleeswaran,[a] Ismail Abulkalam Azath,[a] Vairaperumal Thamaraj,[a] and Kasi Pitchumani*[a, b]

The highly selective ratiometric “turn-on” fluorescent sensing of Zn$^{2+}$ ion involving 2-(1H-tetrazole-5-yl)pyridine (2PT) in aqueous medium is reported, which is not observed when other metal ions are present. Upon 2PT binding selectively with Zn$^{2+}$ ion, a fluorescence enhancement is observed that is attributed to an enhancement of localized emission and suppression of excited-state intramolecular proton transfer. The described sensing system involving 2PT is also successfully applied to the detection of Zn$^{2+}$ ion in real samples with a detection limit of 7.5 $\times$ 10$^{-7}$ M.

Introduction

Zinc, an essential trace element, is the second most abundant metal in the human body that plays a vital role in numerous biological processes, such as peptide synthesis, DNA synthesis, RNA transcription, metabolism of cells, metalloenzyme regulation, and neurophysiology, and it also induces the formation of β-amyloid, which is related to neurological function. More than 100 enzymes, such as peptidases, carbonic anhydrases, and alcohol dehydrogenases, require zinc for their catalytic activity. Labile zinc is found in the cells of mammalian brain, pancreas, and prostate. Zinc and its compounds are widely used in various industries, such as electroplating, rubber, dye, wood preservatives, ointments, batteries, paint, and pharmaceuticals.

Uncontrolled release of zinc from mossy fiber terminals causes brain injury, stroke, or neuronal death. Studies show that a lack of zinc in the body causes prostate cancer, diabetes, Alzheimer’s disease, night blindness, growth retardation, and skin lesions, and affects gene expression and enzyme activity. Thus, in view of its biological relevance and importance, a selective sensor for the monitoring of zinc is essential.

Although there are several analyses available to detect zinc ion, such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry, and voltammetry, they are all expensive and time-consuming. Recently, many zinc sensors have been developed using the quinoline moiety, cyclam, Znpyr, Schiff base, thiophene-based moieties, pyrazoline and pyrazole moieties, and rhodamine-based moieties. However, construction of selective and ratiometric sensors for Zn$^{2+}$ remains in high demand. Thus considerable attention has been focused on developing simple, inexpensive, ratiometric, “turn-on” sensors for Zn$^{2+}$ selective determination. Also, understanding the biological and environmental roles of Zn$^{2+}$ requires robust and versatile methods for quantification. The goal has been to devise “turn-on” fluorescent sensors for Zn$^{2+}$. However, these turn-on signals are insufficient for quantification. An alternative approach involves developing sensors that display a change in the ratio of multiple emission bands, thus providing quantification as a significant advantage, and only a few ratiometric fluorescent sensors for zinc were available recently.

Our interest in developing chemosensors for biologically important cations, anions, and neutral molecules prompted us to develop a simple ratiometric fluorescence chemosensor involving 2-(1H-tetrazole-5-yl)pyridine (2PT) for the selective sensing of Zn$^{2+}$, which involves excited-state intramolecular proton transfer (ESIPT). Though several ESIPT sensors for Zn$^{2+}$ have been reported, the present system is very simple and is employed in aqueous medium.

Results and Discussion

Synthesis and characterization of the probe

Compounds 2PT and 4-(1H-tetrazole-5-yl)pyridine (4PT) were prepared by following the literature procedure using picolinonitrile and sodium azide in the presence of ZnCl$_2$, as a catalyst (Scheme 1), and the products were characterized by NMR spectroscopy (Figures S1–S4 in the Supporting Information) and ESI-MS (Figures S5 and S6). As ZnCl$_2$ is used as a catalyst during the synthesis of the probe, care is taken to remove it completely from the reaction mixture and the synthesized 2PT...
is also analyzed by AAS for the absence of Zn$^{2+}$. The UV/Vis spectrum of 2PT shows absorptions at 274 and 232 nm (Figure S7). The emission spectra show two emission peaks for 2PT at 331 and 381 nm when excited at 275 nm (Figure S8), which are ascribable to localized emission (331 nm) and ESIPT emission (381 nm). [29]

Zn$^{2+}$ ion sensing studies

Compound 2PT contains pyridine and tetrazole nitrogen atoms, so it will provide a good platform for binding metal ions. To study the cation binding ability of 2PT, fluorescence titrations were performed with various cations, such as Li$^+$, Ca$^{2+}$, Ba$^{2+}$, Pb$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, and Hg$^{2+}$, in aqueous medium. Figure 1 shows that, except for the Zn$^{2+}$ ion, all the other metal ions show poor response.

Upon addition of Zn$^{2+}$ ion, the peak at 379 nm decreased in intensity and the peak at 331 nm exhibited increased intensity with a slight blueshift to 327 nm with an isoemissive point at 376 nm. This observation was ascribed to the formation of a five-membered ring complex between 2PT and Zn$^{2+}$, which was further confirmed by ESI-MS.

Fluorescence titration of 2PT with Zn$^{2+}$

To find out the sensitivity of Zn$^{2+}$ detection, the concentration of Zn$^{2+}$ was increased from 0 to 1×10$^{-5}$ M. The fluorescence intensity increased linearly with an increase in the concentration of Zn$^{2+}$ from 0 to 1×10$^{-5}$ M (Figure 2). The value of the linearly dependent coefficient ($R^2$) was found to be 0.997 and the limit of detection was 7.5×10$^{-7}$ mol L$^{-1}$ (3.3 s slope$^{-1}$). [30]

An isoemissive point appeared at 376 nm, which indicates that only a 1:1 complex was formed in fluorescence titration. The binding constant value was found to be 1.392×10^5 M$^{-1}$, which indicates strong binding between 2PT and Zn$^{2+}$.

The binding between 2PT and Zn$^{2+}$ is also confirmed by the Job’s plot and ESI-MS. The complex formation is in good agreement with a 1:1 stoichiometry by Job’s plot from the emission data (Figure S9). In ESI-MS, for 2PT an m/z value was observed at 146.19 (2PT·H). When 1.0 equivalent of Zn$^{2+}$ is added, a new peak appears at m/z 281.97 indicating formation of a Zn$^{2+}$–2PT complex (2PT + ZnCl$_2$·H; Figure 3).

Competitive binding study of 2PT

To investigate the efficiency of 2PT in detecting Zn$^{2+}$, a competitive assay was performed in the presence of other cations. The binding behavior investigation of 2PT towards different cations (Li$^+$, Ca$^{2+}$, Ba$^{2+}$, Pb$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, and Hg$^{2+}$) was investigated by fluorescence spectroscopy (Figure 4). Interestingly, 2PT senses Zn$^{2+}$ selectively even though other metal ions are present in the mixture, and a similar result is also observed in the literature. [31] On the addition of paramagnetic metals, such as Co$^{2+}$, Ni$^{2+}$, and Cu$^{2+}$, significant fluorescence quenching was observed. This is a result of the quenching pathway involving low-lying d–d states in the Co$^{2+}$, Ni$^{2+}$, and Cu$^{2+}$ and also the energy transfer from the...
photoexcited state of the ligand to the empty d orbital of the metals.

This finding indicates that the Zn$^{2+}$-dependent fluorescence response of 2PT is not affected by the presence of other metal ions.

NMR titration

The interaction of 2PT with Zn$^{2+}$ was also analyzed by $^1$H NMR spectroscopy to ascertain the binding between 2PT and Zn$^{2+}$. Significant chemical shift changes were observed for protons a, b, and c of 2PT in its $^1$H NMR spectra (all deshielded, which is indicative of binding between 2PT and Zn$^{2+}$) while adding Zn$^{2+}$, which is shown in Figure 5.

Cyclic voltammetric studies of 2PT

The selectivity for Zn$^{2+}$ was further confirmed by cyclic voltammetric study of 2PT (5 $\times$ 10$^{-5}$ mol L$^{-1}$) with various metal ions, such as Li$^+$, Ca$^{2+}$, Ba$^{2+}$, Pb$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, and Hg$^{2+}$ (5 $\times$ 10$^{-5}$ mol L$^{-1}$). Figure 6 shows that only in the presence of Zn$^{2+}$ ion does the reduction peak current increase and with other metal ions no significant changes are observed, which indicates that 2PT binds strongly with Zn$^{2+}$ ion (5 $\times$ 10$^{-5}$ mol L$^{-1}$).

Effect of pH

As the influence of pH on the probe is very important for biological applications, in the present study it is noted that effective binding is observed at pH 6–7. At low pH values, the binding of Zn$^{2+}$ is decreased owing to the competitive binding between H$^+$ and Zn$^{2+}$. When the pH was increased beyond 7, a negligible effect was observed on the fluorescence intensity. The fact that the probe operates effectively in the pH range

Figure 3. ESI-MS spectrum of 2PT with Zn$^{2+}$ (2PT + ZnCl$_2$ – H$_2$O).

Figure 4. Bar chart showing the fluorescence selectivity of 2PT (5 $\times$ 10$^{-5}$ mol L$^{-1}$) towards Zn$^{2+}$ in the presence of other metal ions (5 $\times$ 10$^{-5}$ mol L$^{-1}$). The red bars represent the fluorescence intensity of 2PT in the presence of one equivalent of other metal ions. The green bars represent the fluorescence intensity of one equivalent of 2PT containing one equivalent of other metal ions and Zn$^{2+}$ ions.

Figure 5. $^1$H NMR spectra of 2PT with one equivalent of Zn$^{2+}$ in [D$_6$]DMSO.
around 6–7 indicates that it is suitable for biological applications.

**Mechanism of Zn\(^{2+}\) selective sensing**

The remarkable fluorescence enhancement in the localized emission (LE) band is attributed to the complexation of 2PT with Zn\(^{2+}\). According to Pearson’s hard and soft acids and bases theory, Zn\(^{2+}\) (a borderline acid) can interact preferentially with pyridine nitrogen atoms (a borderline base) in both pyridine and tetrazole rings of 2PT. In the absence of Zn\(^{2+}\), an excited-state intramolecular hydrogen atom transfer between the tetrazole N–H and N atom of the pyridyl ring occurs (ESIPT). Upon Zn\(^{2+}\) binding with 2PT, ESIPT is suppressed with a concomitant increase in the LE band of 2PT, which is aided by the planarity of the Zn\(^{2+}\)–2PT complex (Figure 7).

The above mechanism is further supported by using 4PT as a probe. In the case of 4PT, no change in fluorescence is observed upon addition of metal ions (Figure S11). This study supports the finding that the N atoms of the pyridyl ring and the tetrazole ring are responsible for the complexation of 2PT with Zn\(^{2+}\).

**Theoretical calculation**

The geometries of the sensor 2PT and of 2PT with Zn\(^{2+}\) ion were optimized using DFT at the B3LYP/6-31G and B3LYP/LANL2D(d) levels, respectively, by using the Gaussian 03 package.\(^{[34]}\) The optimized geometries of 2PT and its 1:1 complex with Zn\(^{2+}\) are given in Figure 8.

In the HOMO and LUMO of the energy level diagram of 2PT, the electron densities are distributed over the 2-pyridyl moiety in the HOMO and the tetrazolyl moiety in the LUMO (Figure 9). Upon binding of Zn\(^{2+}\) with 2PT, the electron density distributed over the 2-pyridyl moiety still has the HOMO character whereas the Zn\(^{2+}\) has the LUMO character. This observation strongly supports the proposed mechanism.

**Real sample analysis**

To evaluate the practical applicability of probe 2PT to real samples, analysis was performed for the detection of Zn\(^{2+}\) in well water and drinking water, which...
Table 1. Comparison of the 2PT probe with reported sensor systems for Zn$^{2+}$ detection.

<table>
<thead>
<tr>
<th>Sensing probe</th>
<th>Method</th>
<th>Limit of detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-aminophenol-functionalized silica nanoparticle</td>
<td>fluorescence</td>
<td>0.1 µM</td>
<td>[35]</td>
</tr>
<tr>
<td>microfabricated bismuth electrodes</td>
<td>electrochemical</td>
<td>0.24 µM</td>
<td>[20]</td>
</tr>
<tr>
<td>pyrazole and pyrazoline</td>
<td>fluorescence</td>
<td>1.0 × 10^{-4} M</td>
<td>[36]</td>
</tr>
<tr>
<td>solid-state ion-selective electrode based on polypropylene conducting polymer nanofilm</td>
<td>potentiometric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phenylbenzoxazole-amide-cyclen linkage as a ratiometric fluorescent receptor</td>
<td>fluorescence</td>
<td>0.6 µM</td>
<td>[23]</td>
</tr>
<tr>
<td>click-functionalized poly(p-phenylene ethynylene)s</td>
<td>fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tripodal zinc sensor</td>
<td>fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ordered mesoporous silica material (MCM-41) functionalized with N-(quinolin-8-yl)-2-(3-(triethoxysilyl)propylamino)acetamide</td>
<td>fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>di- and tripyrins</td>
<td>fluorescence</td>
<td>2.7 × 10^{-7} M</td>
<td>[39]</td>
</tr>
<tr>
<td>cyclam-based “clickates”</td>
<td>fluorescence</td>
<td>75 µM</td>
<td>[40]</td>
</tr>
<tr>
<td>click-generated cyclam-based zinc(II) sensor</td>
<td>fluorescence</td>
<td>2 µM</td>
<td>[41]</td>
</tr>
<tr>
<td>porphyrin-based sensor</td>
<td>fluorescence</td>
<td>1.8 µM</td>
<td>[42]</td>
</tr>
<tr>
<td>2PT</td>
<td>fluorescence</td>
<td>7.5 × 10^{-7} M</td>
<td>this study</td>
</tr>
</tbody>
</table>

Table 2. Results of Zn$^{2+}$ sensing in drinking water and well water.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn$^{2+}$ spiked [µM L$^{-1}$]</th>
<th>Zn$^{2+}$ found [µM L$^{-1}$]</th>
<th>Recovery [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well water</td>
<td>1 0</td>
<td>100.2(1) ± 0.43(1)</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td>3 200</td>
<td>200.1(1) ± 0.30(1)</td>
<td>101.3</td>
</tr>
<tr>
<td>Drinking water</td>
<td>1 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 100</td>
<td>100.7(1) ± 0.21(1)</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>3 200</td>
<td>200.6(1) ± 0.14(1)</td>
<td>99</td>
</tr>
</tbody>
</table>

[a] Mean values of three determinations. [b] Related standard deviation.

Conclusion

We have reported 2-(1H-tetrazole-5-yl)pyridine (2PT) as a simple, ratiometric, selective, and rapid “turn-on” chemosensor for Zn$^{2+}$ ions based on an excited-state intramolecular proton transfer mechanism at the micromolar level in aqueous medium. Compound 2PT shows selectivity toward Zn$^{2+}$ in the presence of other metal ions, such as Li$^+$, Ca$^{2+}$, Ba$^{2+}$, Pb$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, and Hg$^{2+}$. The ligand forms a 1:1 complex with Zn$^{2+}$, which is evident from fluorescence titration, the Job’s plot, and ESI-MS. The high binding constant value (1.392 × 10$^{10}$ M$^{-1}$) indicates that strong binding occurs between 2PT and Zn$^{2+}$. With a detection limit of 7.5 × 10^{-7} mol L$^{-1}$, 2PT senses Zn$^{2+}$ effectively at a pH range of 6–7 in aqueous solution, which is suitable for biological studies. The 2PT is also applicable to real sample analyses for Zn$^{2+}$ in well water and drinking water.

Experimental Section

UV/visible absorption spectra were recorded by using the JASCO Spectra Manager (V-550) in a 1 cm path length quartz cuvette. All fluorescence measurements were performed on a Fluoromax-4 spectrophotometer (Horiba Jobin Yvon) with excitation slit set at 5.0 nm band pass and emission at 5.0 nm band pass in a 1 cm quartz cell. Electrospray ionization mass spectrometry (ESI-MS) analysis was performed in the positive and negative ion modes on a liquid chromatography–ion-trap mass spectrometry instrument (LCQ Fleet, Thermo Fisher Instruments Limited, USA). The samples were introduced into the ion source by the infusion method at flow rate 1 µL min$^{-1}$. The capillary voltage of the mass spectrometer was 33 V, with source voltage 4.98 kV for the mass scale (m/z 50–400). A CH1205 electrochemical analyzer (CH Instruments, USA) and a standard three-electrode cell containing a platinum wire auxiliary electrode, a saturated calomel reference electrode, and a platinum electrode as working electrode were employed for electrochemical studies. DFT calculations were performed at the B3LYP/LANL2DZ(d) level by using the Gaussian 03 program.

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