A Ratiometric Fluorescence Sensor for Zinc in Neutral Solution Based on Thiourea Receptor

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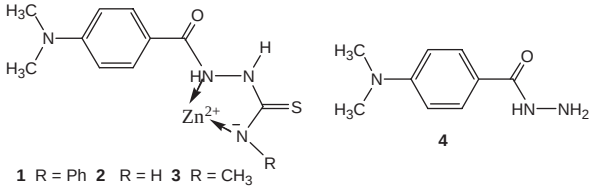
A simple structural receptor containing ICT fluorophore (*p*-(dimethylamino)benzamido) and binding moiety (thiourea) showed highly selective response to Zn^{2+} in pH 8.0 Tris-HCl buffer solution. The spectrum of **1** showed two emission peaks in the presence of Zn^{2+} and the fluorescence intensity ratio could serve as monitor index for the determination of Zn^{2+}.

As the second most abundant transition metal in human body, divalent zinc has played an essential role in the active sites of more than 300 enzymes.[1] What's more, zinc ion is critical for the growth and survival of cells.[2,3] The biological significance of the zinc ion has been recognized in neuroscience, DNA and RNA synthesis, gene expression, apoptosis, protein structure and function, the immune response, and mammalian reproduction,[4,5] as well as some diseases like Alzheimer's disease, epilepsy, ischemic stroke, and infantile diarrhea.[6,7] The relatively lower free zinc concentrations varying from about 1 nM in the cytoplasm of many cells to about 1 mM in some vesicles require a method of high selectivity and sensitivity for its detection under physiological conditions because other cations like calcium and magnesium might interfere. Therefore, development of useful zinc(II) sensors has recently been attracting much attention. Fluorescence spectroscopy offers high sensitivity and is believed to be suitable for the determination of trace metal cations in biological environment.[8] Therefore, a variety of fluorescent sensors for Zn^{2+} based on calix[4]arene,[9] diarylethene Schiff base,[10] bis(2-pyridyl)amine,[11–15] pyrrole,[16] naphthalimide,[17] peptide,[18] or quinoline-derivatized fluoresceins[19] have been reported. Among these, the binding sites focus on pyridylamine group which is a good ligand for metal ion especially for transition metal cations, such as Zn^{2+} and Cd^{2+}. Aminothiourea group, however, serving as binding site for zinc ion in neutral solution is very few.

Herein, we reported a novel sensor for Zn^{2+}. Our sensor is composed of two structural subunits: a fluorophore (for signal transduction) which is *p*-(dimethylamino)benzamido and an ionophore (for selective recognition of metal ion) which is aminothiourea. The binding with the target metal ion caused significant changes to the photophysical properties of the fluorophore. Receptors **1**–**4** were synthesized referring to the literature (Scheme 1),[20] and were characterized by ^{1}H NMR spectra, mass spectra, and elemental analyses.[21]



Scheme 1. Structures of receptors **1**–**4** and the binding model between thiourea derivatives and Zn^{2+}.

The fluorescence titration of receptor **1** with Zn^{2+} in pH 8.0 solution was presented in Figure 1. The maximum emission wavelength of **1** peaked at 383 nm. Upon addition of increasing amount of Zn^{2+}, the fluorescence intensity at 383 nm decreased and a new peak at longer wavelength of 455 nm appeared along with intensity increasing. An isoemission point at ca. 400 nm was observed which suggested the formation of a stoichiometric complex between **1** and Zn^{2+}. The ratio between intensity at 455 nm and that at 385 nm or the intensity at long wavelength alone could serve as monitor index for determination of Zn^{2+}. It was significant that the intensity ratio make the determination free of the fluctuation in the excitation source which has been a case in total intensity sensing mode. The inset plot in Figure 1 showed the intensity ratio change upon addition of Zn^{2+}. A good linear relationship between intensity ratio and zinc concentration ranged from 0 to 1.1×10^{-5} mol L^{-1} with a determination limit of 7.5×10^{-7} mol L^{-1}. The absorption titration of **1** with Zn^{2+} (see Figure S1) suggested zinc complex formation. Jobplot curve (see Figure S2) showed that 1:1 complex formation between **1** and Zn^{2+} and binding mode was assumed as that in Scheme 1. Meanwhile ESI-mass spectrum also confirmed the compound (**1**–Zn^{2+})Cl^-_2 formation (see Figure S3).

The fluorescence response of **1** to various cations is shown in Figure 2. It was obvious that **1** showed highly sensitive and selective response to Zn^{2+}. Under the same experiment condition, only addition of Cd^{2+} or Zn^{2+} resulted in a new fluorescent peak along with intensity increasing. The emission wavelength of metal complex were at 455 and 475 nm for Zn^{2+} and Cd^{2+},

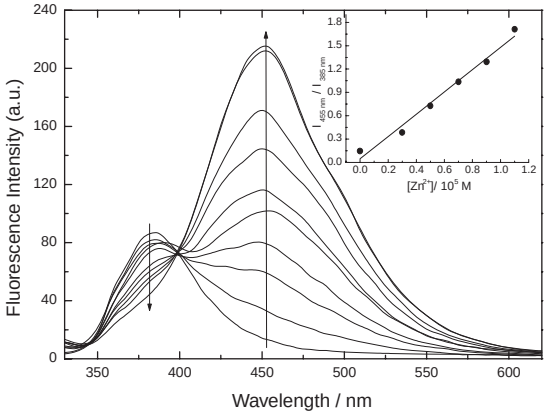


Figure 1. Fluorescence spectra changes of **1** (2.0×10^{-5} mol L^{-1}) upon addition of increasing amount of Zn^{2+} in pH 8.0 Tris-HCl buffer ($\nu_{ACN}/\nu_{H2O} = 1/9$). The concentrations of Zn^{2+} for curves 1 to 9 were 0, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 2.5×10^{-5} mol L^{-1}, respectively. The excitation wavelength was at 290 nm.

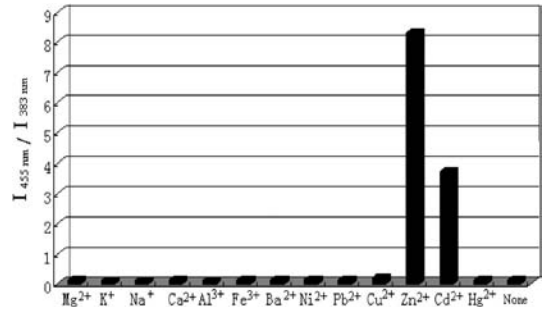


Figure 2. Fluorescence intensity ratio response of **1** (2.0×10^{-5} mol L^{-1}) to various cations (2.0×10^{-5} mol L^{-1}) in pH 8.0 buffer.

respectively. The experiment results revealed that a 1000-fold excess of Na^+, a 800-fold excess of Ba^{2+}, I^-, Br^-, and NH_4^+, a 500-fold excess of Mg^{2+}, $S_2O_3^{2-}$, and $CH_3CO_2^-$, a 400-fold excess of F^-, a 300-fold excess of K^+, Ca^{2+}, SO_4^{2-}, and ClO_3^-, 0.5-fold excess of Fe^{3+} and Al^{3+} existence (in each case compared with Zn^{2+}) resulted in less than $\pm 10\%$ relative error. But the presence of a 1-fold Cd^{2+}, Ni^{2+}, Cu^{2+}, and Hg^{2+} caused interference.

In order to explain the mechanism of **1**'s selective response to Zn^{2+}, the control compounds **2**–**4** were investigated via fluorescence spectral titrations. For compound **2**, it emitted strong fluorescence peaked at 440 nm when excited wavelength was set as 355 nm. In the presence of Zn^{2+} the fluorescence spectrum was red shifted to 454 nm and the intensity increased (see Figure S4). However, upon addition of Zn^{2+} to **3**'s or **4**'s solution (see Figures S5 and S6), neither fluorescence spectral profile nor fluorescence intensity changes were observed. It was assumed that zinc complex with five-membered ring formed as shown in Scheme 1. For compound **3**, methyl group served as electron donor compared with phenyl group. The pK_a of **1** and **3** were estimated as 8.35 and 8.79, respectively, via absorption spectral pH titration. ^{1}H NMR data also demonstrated that the acidity of NH in **3** was weaker than that in **1**.[21] That is, it was difficult for N atom of NH linking with methyl to bind Zn^{2+} through coordinate bond. Consequently no obvious spectral changes appeared while adding Zn^{2+} to **3**'s solution. The effect of pH value on the **1**–Zn^{2+} complex formation was also studied (see Figure S7). The optimal pH value ranged from 7.0 to 9.0. When pH value was lower than 6.5, no fluorescence spectral changes were observed in the presence of Zn^{2+}. It means that the group NH of aminothiourea can easily lose proton and the electron density of N increased in basic solution, which was beneficial for aminothiourea bonding with Zn^{2+}. In IR spectrum of **1** there were three peaks at 3346, 3291, and 3245 cm^{-1}, respectively, which were assigned to be ν_{NH}. However, the relative intensity of ν_{NH} varied in the IR spectrum of **1**–Zn, at the same time, the spectrum of **1** range from 1108 to 1137 cm^{-1} which was assigned to be $\nu_{-N-C=S}$ also exhibited distinct changes (see Figure S8). Above changes further supported the proposed binding mode shown in Scheme 1.

In summary, a highly selective ratiometric fluorescence assay for Zn^{2+} was developed in neutral solution. The long wavelength emission was ascribed to charge transfer of L $\rightarrow$ M. Mass spectra and IR spectra also gave evidences for 1:1 zinc(II) complex formation. Moreover, this method successfully avoided the fluctuation in the excitation source that suffered in total intensity sensing mode and the background interference leading hopefully to the detection of Zn^{2+} in biological samples.

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21 Receptors **1**–**4** were characterized by ^{1}H NMR and ESI mass data, which were consistent with proposed structural formula. **1**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.98 (6H, s), 6.73 (2H, d, $J = 9$ Hz), 7.14 (1H, t, $J = 8$ Hz), 7.31 (2H, t, $J = 8$ Hz), 7.45 (2H, s), 7.81 (2H, d, $J = 9$ Hz), 9.57 (s, NH), 9.71 (s, NH), 10.14 (s, NH); ESI mass: m/e calcd for $C_{16}H_{19}N_4OS$ $[M + H]^+$ 315.12, found $[M + H]^+$ 314.89; Anal. calcd for $C_{16}H_{18}N_4OS$: C, 61.12; H, 5.77; N, 17.82%. Found: C, 61.03; H, 5.58; N, 17.77%. **2**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.97 (6H, s), 6.70 (1H, d, $J = 8.5$ Hz), 6.79 (1H, d, $J = 9$ Hz), 7.47 (s, NH), 7.69 (1H, d, $J = 8.5$ Hz), 7.75 (1H, d, $J = 8.5$ Hz), 9.19 (s, NH), 10.01 (s, NH); ESI mass: m/e calcd for $C_{10}H_{15}N_4OS$ $[M + H]^+$ 239.09, found $[M + H]^+$ 238.96; Anal. calcd for $C_{10}H_{14}N_4OS$: C, 50.40; H, 5.92; N, 23.51%. Found: C, 49.94; H, 5.84; N, 23.47%. **3**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.86 (3H, s), 2.98 (6H, s), 6.71 (2H, d, $J = 9$ Hz), 7.78 (2H, d, $J = 8.5$ Hz), 7.95 (1H, s, NH), 9.18 (1H, s, NH), 9.96 (1H, s, NH); ESI mass: m/e calcd for $C_{11}H_{17}N_4OS$ $[M + H]^+$ 253.10, found $[M + H]^+$ 252.89; Anal. calcd for $C_{11}H_{16}N_4OS$: C, 52.36; H, 6.39; N, 22.20%. Found: C, 51.87; H, 6.43; N, 22.38%. **4**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.95 (6H, s), 4.33 (s, NH), 6.68 (2H, d, $J = 9$ Hz), 7.69 (2H, d, $J = 9$ Hz), 9.36 (s, NH); ESI mass: m/e calcd for $C_9H_{14}N_3O$ $[M + H]^+$ 180.11, found $[M + H]^+$ 179.76; Anal. calcd for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45%. Found: C, 60.31; H, 7.22; N, 23.47%.

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