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Fluorescence sensor for sequential detection of zinc and phosphate ions

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ABSTRACT

A new, highly selective turn-on fluorescent chemosensor based on 2-(2′-tosylamidophenyl)thiazole (**1**) for the detection of zinc and phosphate ions in ethanol was synthesized and characterized. Sensor **1** showed a high selectivity for zinc compared to other cations and sequentially detected hydrogen pyrophosphate and hydrogen phosphate. The fluorescence mechanism can be explained by two different mechanisms: (i) the inhibition of excited-state intramolecular proton transfer (ESIPT) and (ii) chelation-induced enhanced fluorescence by binding with Zn^{2+}. The sequential detection of phosphate anions was achieved by the quenching and subsequent revival of ESIPT.

1. Introduction

Molecular fluorescent chemosensors for the recognition of cations and anions have attracted much attention for important and diverse ecological, biological, and clinical applications [1–4]. Fluorescent chemosensors have the advantages of real-time monitoring with fast response times, intrinsic high sensitivity, and ease of handling compared to other optical sensors [5,6]. The sensing of Zn^{2+} ions, the second most abundant transition metal in the human body, in an aqueous media is ecologically and biochemically relevant because they play crucial roles in biological systems. Zn^{2+} ions are vital for many cellular processes [7] such as apoptosis [8], DNA synthesis [9], neurotransmission [10,11], gene expression [12], modulation of diverse ion channels [13], and signal transduction [14]. Zn^{2+} ions are also an essential component of many enzymes, e.g., carbonic anhydrase, transcription factors, and zinc finger proteins, in which they play catalytic or structural roles [15]. Clinically, diverse Zn^{2+}-based compounds have been used as tumor photosensitizers [16], antibacterial/antimicrobial and anticancer agents [17], radioprotective agents [18], and antidiabetic insulin mimetics [19]. Moreover, the hepato and cardio toxicity induced by some anticancer drugs can be reduced by Zn^{2+} [20].

There is intense interest in the development of molecular systems capable of binding inorganic phosphate anions because of their crucial roles in signal transduction [21,22], energy storage in living organisms, eutrophication of water bodies [23–25], and catalysis [26,27]. Inorganic phosphates exhibit diverse shapes and sizes, hydrophobicity, and high hydration energies, and in some cases, exist only in a limited pH range because of protonation. One phosphate-recognition strategy is the use of metal complexes, mostly involving transition metals, in which the metal ion acts as an anchoring point for the phosphate species. Although different metal ions including transition, lanthanide, and main group metal ions have been used, one of the most commonly used ions for this purpose is Zn^{2+} [28,29].

Phosphate is an indispensable constituent of two important biopolymers—DNA and RNA—and many chemotherapeutic and antiviral drugs [30]. However, the excessive agricultural use of inorganic phosphates causes excessive algal growth, leading to decomposition and decreased dissolved oxygen levels [31]. Dihydrogen phosphate ($H_2PO_4^-$) is the predominant equilibrium species of inorganic phosphates at physiological pH. Therefore, $H_2PO_4^-$ sensing and detection methods have received much attention [32–35]. Phosphate oxoanions such as pyrophosphate play crucial roles in many bioenergetic and metabolic processes such as ATP hydrolysis and DNA or RNA polymerase reactions [36]. The detection of released pyrophosphate has been investigated as a real-time DNA sequencing method [37]. Studies on telomerase activity (a biomarker for cancer) by evaluating the amount of pyrophosphate are also important in cancer research [38]. Furthermore, the pyrophosphate level in synovial fluids has been correlated with the occurrence of calcium pyrophosphate dihydrate disease, a rheumatologic disorder [39]. This species can also be used as a potential biomarker for arthritis [40].

The photophysics of the cation-induced inhibition of excited-state intramolecular proton transfer (ESIPT) has been often used to develop Zn-selective ratiometric emission probes [41–43]. We have previously reported some thiazole-based chemosensors in which the thiazole ring was substituted with a phenol or tosylamide-protected aniline or naphthol at the position 2 and a pyridine, phenyl, or another thiazole

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phenol moiety at the position 4 as a ratiometric fluorescence sensor of Zn or dual chemosensor of Zn and Cu [44–50]. We also reported a thiazole-based compound containing a phenol or naphthol at the position 2 and an ester moiety at the position 4 as a very good Al^{3+} sensor [51,52]. Recently, we reported the synthesis of a phenol/naphthol-containing thiazole-based sensor for the sequential detection of Ga and HSO_4^- [53]. In this study, we prepared compound **1** containing a tosylamide-protected aniline at the position 2 of a thiazole ring and an ester moiety at the position 4 (Fig. 1) and investigated its sequential sensing abilities for different cations and anions based on two different mechanisms of fluorescence: ESIPT and chelation-induced enhanced fluorescence (CHEF) [54]. We also synthesized compound **2** to study the effect of the tosyl group in compound **1** on the sensing of cations and anions.

2. Experimental

2.1. General

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and uncorrected. The ^1H and ^{13}C NMR spectra were recorded using a Bruker AM-400 spectrometer and Me_4Si as the internal standard. The FAB mass spectra were obtained at the KBSI Daegu center. The UV–visible absorption spectra were determined using a Shimadzu UV-1650PC spectrophotometer. The fluorescence spectra were measured using a Shimadzu RF-5301 fluorescence spectrometer equipped with a Xe discharge lamp and 1-cm quartz cells with a 5-nm slit width. The IR spectra were recorded using a Shimadzu IR Prestige-21 FTIR spectrometer. All the measurements were carried out at 298 K. Analytical-grade ethanol was purchased from Merck. All other materials for the syntheses were purchased from Aldrich Chemical Co. and used as received without further purification. Compound **3** was obtained following a literature procedure [45], and the quantum yield (Φ) was calculated as reported [53]. The solutions of metal ions were prepared from their analytical-grade perchlorate salts, and those of the anions were prepared from their tetrabutylammonium (TBA) salts. The working solutions were prepared by further dilution of the stock solutions.

2.2. Synthesis

2.2.1. Synthesis of compound *2*

A mixture of compound **3** (200 mg, 0.72 mmol) and 5% Pd/C in ethanol (20 mL) was hydrogenated using H_2 gas for 10 h. After the removal of the solvent, CH_2Cl_2 was added, and the reaction mixture was filtered through a Celite pad to remove the catalyst. The filtrate was concentrated, and the residue was crystalized from a mixture of CH_2Cl_2/hexane, affording amino compound **2** (159 mg, 89% yield). ^1H NMR (DMSO-d_6) δ 1.33 (3H, t, $J = 7.1$ Hz, CH_3), 4.33 (2H, q, $J = 7.1$ Hz, CH_2), 6.62 (1H, ddd, $J = 7.2$, 6.9, 1.0 Hz, H_b), 6.83 (1H, dd, $J = 8.3$, 1.0 Hz, H_a), 7.11 (1H, s, NH), 7.18 (1H, ddd, $J = 7.1$, 7.0, 1.4 Hz, H_c), 7.59 (1H, dd, $J = 7.9$, 1.4 Hz, H_d), 8.47 (1H, s, H_e); 13C NMR (DMSO-d_6) δ 14.2, 60.9, 113.1, 115.7, 116.5, 126.7, 128.9, 131.4, 145.7, 146.7, 160.5, 169.3; HR-FAB MS calcd for $C_{12}H_{13}N_2O_2S$ $(M + H)^+$: 249.0698, found: *m/z* 249.0699.

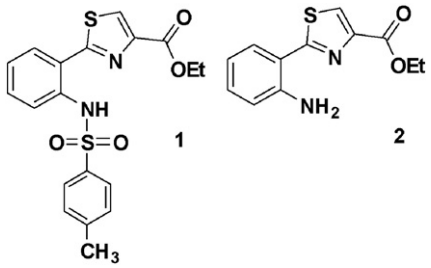

Fig. 1. Structures of chemosensors **1** and **2**.

2.3. Synthesis of compound *1*

Amino compound **2** (100 mg, 0.40 mmol) was added to *p*-toluenesulfonyl chloride (92 mg, 0.48 mol) and triethylamine (0.5 mL) in anhydrous CH_2Cl_2 (20 mL) and stirred at room temperature for 2 h. After the reaction was completed, the mixture was treated with $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was dried and concentrated. The residue was purified by column chromatography (SiO_2, 10% EtOAc in hexane), affording compound **1** (103 mg, 64% yield). Mp. 133 °C (CH_2Cl_2/hexane), TLC R_f 0.35 (10% EtOAc in hexane); ^1H NMR (DMSO-d_6) δ 1.36 (3H, t, $J = 7.1$ Hz, CH_3), 2.30 (3H, s, CH_3), 4.39 (2H, q, $J = 7.1$ Hz, CH_2), 7.24 (1H, d, $J = 7.6$ Hz, H_a), 7.27 (2H, d, $J = 8.4$ Hz, H_g), 7.42 (2H, t, $J = 7.4$ Hz, $H_{b,c}$), 7.57 (2H, d, $J = 8.4$ Hz, H_f), 7.90 (1H, d, $J = 7.6$ Hz, H_d), 8.64 (1H, s, H_e), 11.45 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.1, 20.9, 61.1, 121.8, 122.1, 125.3, 126.7, 129.2, 129.3, 129.7, 131.4, 134.9, 135.9, 143.7, 145.3, 160.2, 166.6; HR-FAB MS calcd for $C_{19}H_{19}N_2O_4S_2$ $(M + H)^+$: 403.0786, found: *m/z* 403.0788.

2.3.1. Synthesis of *1*-*Zn* complex

A mixture of compound **1** (40 mg, 0.10 mmol) and $Zn(NO_3)_2 \cdot 6H_2O$ (30 mg, 0.10 mmol) in ethanol/CH_2Cl_2 (5:5 v/v, 5 mL) was stirred for 1 h. The mixture was stand at room temperature, and the precipitated complex was filtered off. The filtered cake was washed thoroughly with ethanol and dried under vacuum, providing the complex (43 mg, 91% yield). HR-FAB mass: calcd for $[C_{19}H_{17}N_2O_4S_2Zn]^+$ 464.9921. Found: 464.9920.

2.4. UV–visible and fluorescence studies

A solution of host (30 μM) in EtOH was prepared, and the guest (300 μM) solution was added to the host solution for the UV–visible study. For fluorescence titration, a solution of host (3 μM) in EtOH was prepared, and the guest (30 μM) solution was added to the host solution. In a typical titration experiment, 2 mL of the host solution was transferred to a fluorescence cell, and the emission spectrum was recorded at a fixed wavelength. The guest solution (20 μL) was added through a microsyringe, and the amount was increased until 10 equiv of guest. The fluorescence spectrum of each solution was recorded after each addition. The association constants were determined by gnuplot using the following equation.

plot “gadata.dat” u 1:2 w lp.
f(x) = (a + b * c * x * * 1.00) / (1 + c * x * * 1.00).
fit f(x) “gadata.dat” u 1:2 via a, b, and c.

2.5. Theoretical calculations

The geometry of sensor **1** was optimized using gradient-correlated density functional theory (DFT) according to literature [55–59].

3. Results and discussion

3.1. Synthesis of sensor *1*

The required compound **2** was prepared by the reduction of ethyl 2-(2′-nitrophenyl)thiazoly-4-carboxylate, which was obtained by the reaction of 2-nitrothiobenzamide with ethyl bromopyruvate in ethanol [45]. Compound **1** was prepared by the reaction of compound **2** with *p*-toluenesulfonyl chloride in the presence of triethylamine in a good yield (Scheme 1). The ^1H NMR spectrum of sensor **1** in DMSO-d_6 showed CH_3CH_2 signals corresponding to the ethyl ester—a triplet at δ 1.36 and a quartet at δ 4.49—as well as one singlet corresponding to the tosyl CH_3 at δ 2.30 and one N–H singlet at δ 11.45. In the ^{13}C NMR