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Simple but Effective Way to Sense Pyrophosphate and Inorganic Phosphate by Fluorescence Changes

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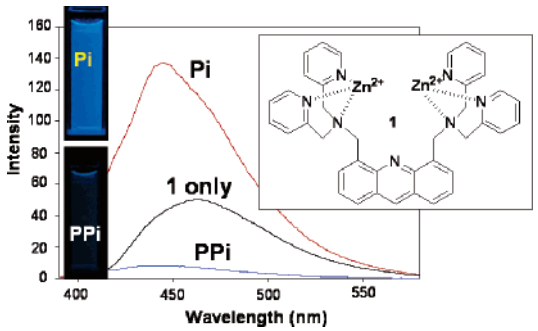
ABSTRACT



A new fluorescent chemosensor based on the acridine–Zn(II) derivative effectively recognizes pyrophosphate and inorganic phosphate at pH 7.4. Acridine derivative 1 displayed a fluorescent quenching effect with pyrophosphate; on the other hand, a large fluorescent enhancement was observed with inorganic phosphate.

Anions play an important role in a wide range of chemical and biological processes, and the development of anion selective receptors has been actively investigated.[1] Accordingly, sensors based on the anion-induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of the fluorescence.[1,2] Specifically, phosphate ions and their derivatives play important roles in signal transduction and energy storage in biological systems.[3] For example, pyrophosphate (PPi) can be a biologically important target because it is the product of ATP hydrolysis under cellular conditions.[4] Furthermore, the detection of pyrophosphate release is being investigated as a real-time DNA sequencing method.[5] On the other hand, many of the common enzymes, such as kinases and phosphatases, produce or consume inorganic phosphate (Pi), which is also related to the protein phosphorylation.[6] Accordingly, the detection of an increase or decrease of the phosphate concentration in the environment of these enzymes

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(2) (a) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551. (b) Martínez-Máñez, R.; Sancanón, F. *Chem. Rev.* **2003**, *103*, 4419. (c) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. A.; Huxley, T. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (d) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302. (e) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1994**, 197. (f) *Fluorescent Chemosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993.

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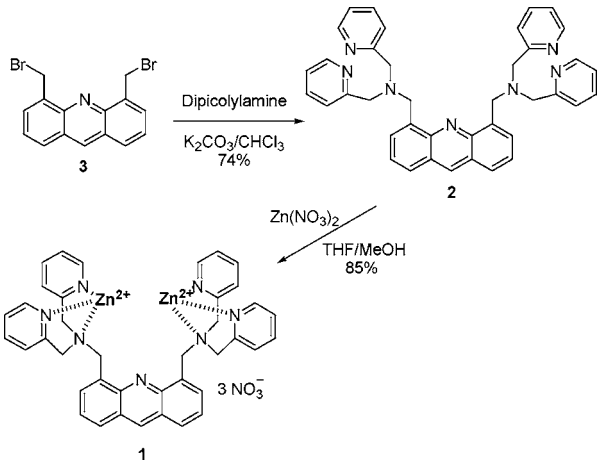
is a common way to monitor the enzyme activity or protein phosphorylation process.[7]

Scheme 1. Synthesis of Fluorescent Chemosensor **1**



In this regard, the detection and discrimination of these anions has been the main focus of the efforts of several research groups. Even though many fluorescent chemosensors, which can selectively recognize PPi[2d,8,9] or Pi,[2d,10,11] have been reported, only a few examples, which can function for PPi[9] and Pi[11] in aqueous solution, are available. In 1994, Czarnik et al. reported a pioneering work in which an anthracene derivative bearing polyamine groups was used as PPi sensor in 100% aqueous solution.[9g] Recently, Hong et al. reported azophenol-based fluorescent[9c] and colorimetric[12] PPi sensors in water. Kikuchi et al. utilized the Cd^{2+}−cyclen−coumarin system as a fluorescent chemosensor for PPi in aqueous solution.[9f] Jolliffe et al. utilized a cyclic peptide receptor bearing two Zn(II)−DPA as a receptor for PPi.[9a] Our group also reported a new fluorescein derivative that can display a fluorescent enhancement with a significant red-shift upon the addition of PPi at pH 7.4.[9b] A selective colorimetric Pi-sensing probe in water was recently reported by Kim et al.[13] On the other hand, Hamachi et al. recently reported anthracene derivatives as novel fluorescent chemosensors for phosphorylated peptides.[14]

However, as far as we are aware of, a fluorescent chemosensor, which can display different signal responses with pyrophosphate and phosphate in water, has not been reported. We report herein a simple acridine derivative that can display a selective CHEF (chelation enhanced fluorescence) effect with Pi and a selective CHEQ (chelation enhanced fluorescence quenching) effect with PPi in 100% aqueous solution. A large CHEF effect with Pi can be attributed to the additional hydrogen bonding between nitrogen on the acridine and hydrogen of Pi.

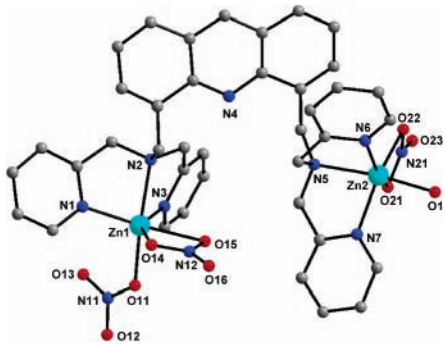


Figure 1. The crystal structure of the cation of **1**. All hydrogen atoms were omitted for clarity. Selected bond distances (Å) and angles (deg): Zn(1)−N(1) 2.062(5), Zn(1)−N(3) 2.065(5), Zn(1)−N(2) 2.231(5), Zn(2)−N(7) 2.062(5), Zn(2)−N(6) 2.073(5), Zn(2)−N(5) 2.192(4), Zn(1)−O(11) 2.047(4), Zn(1)−O(14) 2.135(4), Zn(1)−O(15) 2.346(4), Zn(2)−O(21) 2.268(4), Zn(2)−O(22) 2.217(4), Zn(2)−O(1) 2.038(5); O(11)−Zn(1)−N(1) 105.64(17), O(11)−Zn(1)−N(3) 98.37(17), O(11)−Zn(1)−N(2) 175.41(16), O(14)−Zn(1)−N(2) 94.72(16), N(2)−Zn(1)−O(15) 90.50(16), O(1)−Zn(2)−N(7) 92.7(2), O(1)−Zn(2)−N(6) 96.9(2), O(1)−Zn(2)−N(5) 170.27(18), N(5)−Zn(2)−O(22) 98.47(16), N(5)−Zn(2)−O(21) 90.16(16).

Figure 1 shows the crystal structure of the cation of **1**. A symmetric unit contains a Zn dinuclear cation, $[Zn(NO_3)_2(\mu\text{-DPA})Zn(NO_3)(H_2O)]^+$, a nitrate anion, and an ethanol solvent molecule. The geometry of each Zn^{2+} ion is distorted octahedral, where one of the Zn^{2+} ions (Zn1) is coordinated by the three nitrogen atoms of the DPA unit, two oxygen atoms of the nitrate ion, and one oxygen atom of the second nitrate ion; on the other hand, the second Zn^{2+} ion (Zn2) is coordinated by the DPA unit, nitrate ion, and water.

The fluorescence emission changes of **1** upon the addition of HSO_4^-, CH_3COO^-, I^-, Br^-, Cl^-, F^-, Pi, and PPi (100 equiv) are illustrated in Figure 2. The fluorescence spectra

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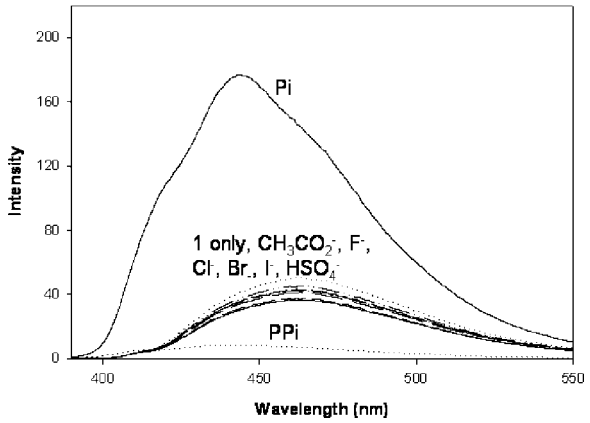

Figure 2. Fluorescent changes of compound **1** (3 μM) upon the addition of Pi, PPi, $CH_3CO_2^-$, HSO_4^-, F^-, Cl^-, Br^-, and I^- (100 equiv) at pH 7.4 (10 mM HEPES) (excitation at 359 nm, excitation and emission slit width = 3 nm).

were obtained by excitation into the acridine fluorophore at 359 nm. As shown in Figure 2, there were unique changes in its emission spectrum upon the addition of PPi and Pi at pH 7.4. A large fluorescence quenching effect with a bathochromic shift (~20 nm) was observed upon the addition of PPi; on the other hand, a large CHEF effect with a bathochromic shift (~20 nm) was observed with Pi. No significant change was observed upon the addition of other anions (100 equiv). A Job plot for the binding between **1** with PPi and Pi shows a 1:1 stoichiometry. From the fluorescence titration (Figure 2), the association constant of complex **1** with PPi was observed to be 4.85×10^7 M^{-1} (errors <10%) (excitation and emission slit width = 5 nm).[15] The overall emission change was over 4-fold. This observation means that **1** can even detect PPi at nanomolar concentrations in water. The fluorescent titration with a lower concentration of **1** is shown in the Supporting Information

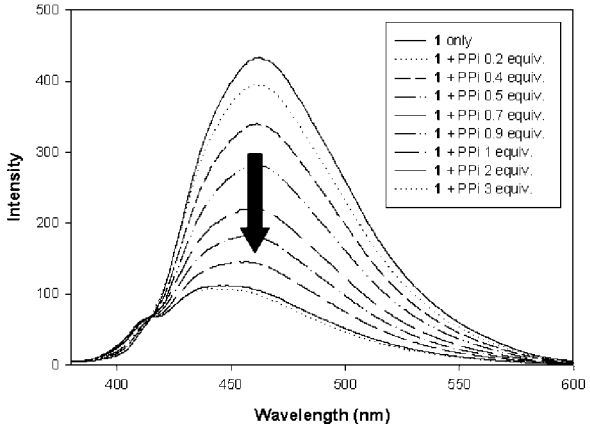

Figure 3. Fluorescent titrations of compound **1** (3 μM) with PPi at pH 7.4 (10 mM HEPES) (excitation at 359 nm, excitation and emission slit width = 5 nm).

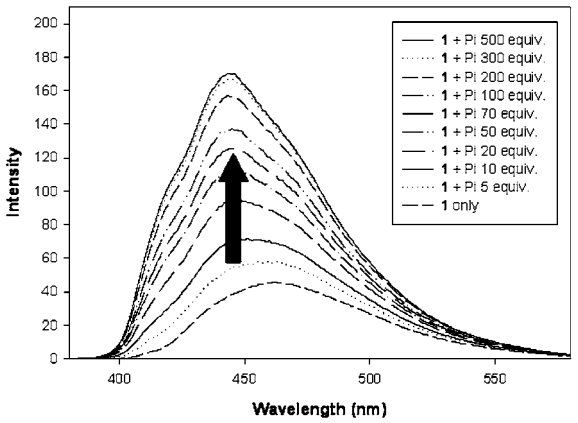

Figure 4. Fluorescent titrations of compound **1** (3 μM) with Pi at pH 7.4 (10 mM HEPES) (excitation at 359 nm, excitation and emission slit width = 3 nm).

(Figure S3). This CHEQ effect upon addition of PPi can be attributed to the possible PET (photoinduced electron transfer) mechanism from DPA unit. The binding of the pyrophosphate anion to the zinc center can induce a PET mechanism from benzylic amine to acridine moiety.[2c]

On the other hand, Pi induced a large CHEF effect. Figure 3 illustrates the fluorescence titration experiments of the complex **1** with Pi at pH 7.4 (10 mM HEPES) when the excitation wavelength was 359 nm. Upon the addition of Pi, the emission maximum of compound **1** gradually shifted from 462 to 444 nm and a large CHEF effect (~300%) was observed. The selectivity for Pi over other anions including sulfate, acetate, and halide anions can be attributed to a strong coordination of Zn^{2+} to the phosphate unit, which was also explained by Hamachi[14] and Kim.[13] From the fluorescence titration, the association constant of complex **1** with Pi was observed to be 9.36×10^4 M^{-1} (errors <15%).[16] The unique CHEF effect, which was induced by Pi, can be attributed to the additional hydrogen bonding between the hydrogen of OH in the Pi and nitrogen on the acridine moiety.[16] Similar hydrogen bonding between ammonium hydrogen and nitrogen acridine was reported by Hollósi et al.[17] Recently, the fluorescence enhancement due to the interaction between metal ions and a nitrogen on acridine was also reported.[18] Upon the addition of 1 equiv of complex **1** in D_2O, the chemical shifts due to the two different pyrophosphorous in

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(16) The hydrogen bonding in aqueous solution is a quite challenging process. However, in our case, the stronger interaction between the Zn center and the phosphate may induce the additional hydrogen bonding. There are few examples of sensors which utilize hydrogen bonding interactions in aqueous solution. For example, Czarnik et al. reported that the fluorescent change of their sensor with pyrophosphate in 100% aqueous solution was attributed to the hydrogen bonding between benzylic amine and pyrophosphate (ref 9g) Also, the fluorescent changes of dipyrrolyl quinoxaline derivatives with anions in aqueous solution were reported by Anzenbacher's group, in which the hydrogen bonding was the key interaction (ref 9d).

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PPi moved from −10.17 to −9.56 ppm (Supporting Informaion, Figure S2), which indicates that complex **1** directly interacts with the phosphate sites. In the case of Pi in D_2O, the chemical shift moved from 1.08 to 2.21 ppm (Supporting Information, Figure S1). The fluorescent change of **1** with PPi in the presence of excess Pi is shown in the Supporting Information (Figure S4).

In conclusion, an acridine−Zn complex **1** displays a selective CHEF effect with Pi and a selective CHEQ effect with PPi in 100% aqueous solution. The association constants for PPi and Pi were calculated as 4.85×10^7 and 9.36×10^4 M^{-1}, respectively. This observation means that **1** can even detect PPi at nanomolar concentrations in water. A large CHEF effect with Pi can be attributed to the additional hydrogen bonding between nitrogen on the acridine and hydrogen of Pi.

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Supporting Information Available: Experimental procedure and characterization data of compounds **1** and **2** and ^{31}P NMR spectra and CIF information (compound **1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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