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Pyrophosphate-Selective Fluorescent Chemosensor at Physiological pH: Formation of a Unique Excimer upon Addition of Pyrophosphate

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Recently, sensors based on anion-induced changes in fluorescence have become particularly attractive due to their simplicity and the low detection limit of the fluorescence.[1] In particular, pyrophosphate (PPi) is a biologically important target because it is the product of adenosine triphosphate (ATP) hydrolysis under cellular conditions.[2] Furthermore, the detection of PPi release is being investigated as a real-time DNA sequencing method.[3] The detection of PPi is also considered important in cancer research.[4] Accordingly, detection and discrimination of PPi on the basis of fluorescence changes has been the main focus of many research groups.[5,6] Even though various types of fluorescent chemosensors that can selectively recognize PPi have been reported, only a few can function for PPi in an aqueous solution.[6] In 1994, Czarnik et al. reported the results of a pioneering study in which an anthracene derivative bearing polyamine groups was used as a PPi sensor in a 100% aqueous solution.[6g] Recently, Hong et al. reported azophenol-based fluorescent PPi sensors in water.[6c,d] Kikuchi et al. used a Cd^{2+}−cyclen−coumarin system as a fluorescent chemosensor for PPi in an aqueous solution.[6f] Our group reported a new fluorescein derivative that can display enhanced fluorescence with a significant red-shift upon the addition of PPi at pH 7.4.[6b] An acridine−$2Zn^{2+}$ system was also investigated by our group as a fluorescent chemosensor for PPi and phosphate (Pi) in 100% aqueous solution.[6a] A few indicator displacement systems for PPi using the changes in fluorescence have also been reported by Jolliffe et al.,[7a] Smith et al.,[7b] and Fabbrizzi et al.[7c]

However, only a couple of fluorescent chemosensors had sufficient selectivity for PPi over ATP or Pi in an aqueous solution.[6c,d] This paper describes a fluorescent chemosensor that is highly selective for PPi over ATP, adenosine diphosphate (ADP), adenosine 5′-monophosphate (AMP), or Pi in a 100% aqueous solution. The chemosensor can detect PPi through its unique 2+2 type excimer formation. In the presence of PPi, the sensor makes a unique dimeric system that can induce a selective excimer peak. Furthermore, this sensor can detect PPi in the presence of ATP or Pi. Therefore, it can be considered a potential fluorescent sensor for PPi.

For the synthesis of sensor **1**, 1,4,5,8-naphthalenetetracarboxylic dianhydride and (2-aminoethyl)bis(2-pyridylmethyl)amine[8] were refluxed in toluene for 5 h to give compound **2** in 42% yield, as shown in Scheme 1. The treatment of **2** with $Zn(NO_3)_2$ in CH_3CN−THF−MeOH afforded the desired sensor **1** in 90% yield. The X-ray crystal structure of **2** was obtained in ethanol (Figure 1). The experimental details and characterization data are given in the Supporting Information.

Figure 2 shows the fluorescence emission changes in complex **1** (6 μM) upon the addition of PPi, ATP, F^-, Br^-, I^-, Cl^-, $CH_3CO_2^-$, HSO_4^-, Pi, ADP, and AMP (10 equiv each) at pH 7.4 (0.01 M HEPES). As shown in Figure 2, there were unique changes

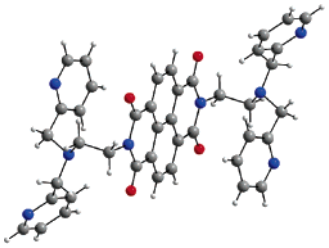


Figure 1. X-ray crystal structure of **2**.

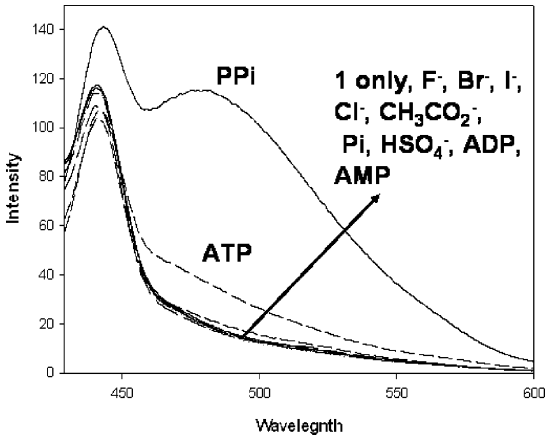
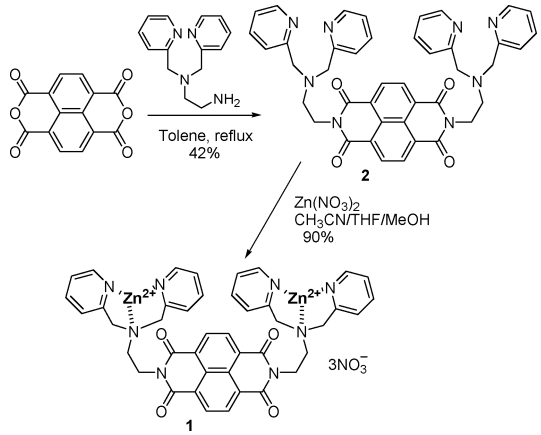


Figure 2. Fluorescent changes of **1** (6 μM) with various anions (10 equiv) at pH 7.4 (0.01 M HEPES) (excitation at 383 nm).

Scheme 1. Synthesis of Compound **1**



in the emission spectrum upon the addition of PPi. A new peak at 490 nm can be attributed to excimer formation. Because there was no significant change in its UV absorption (Supporting Information (SI) S-Figure 3) when PPi was added, a charge-transfer mechanism can be excluded. Furthermore, electrospray ionization data also

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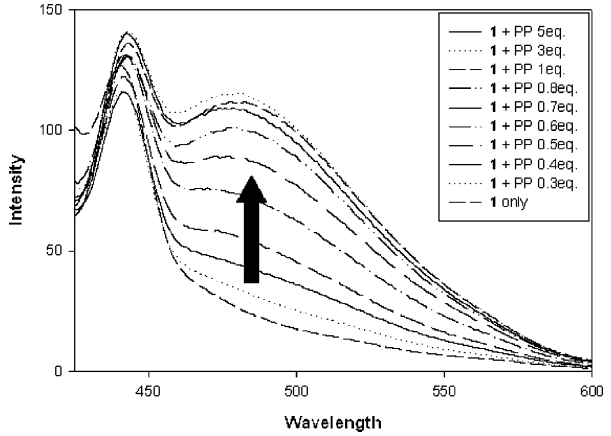
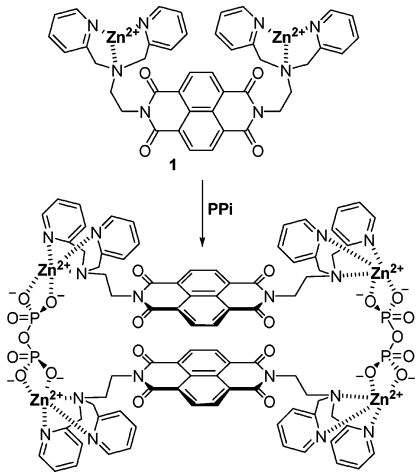


Figure 3. Fluorescent titrations of **1** (6 μM) with PPi at pH 7.4 (0.01 M HEPES) (excitation at 383 nm).

Scheme 2. Proposed Binding Mechanism of Chemosensors **1** with PPi



support this excimer formation (SI S-Figure 4). As shown in S-Figure 4 (SI), three sets of peaks were observed in the range of $m/z = 1000-1050$ when the sodium salt of PPi (1.1 equiv) was added. These peaks correspond $[C_{84}H_{74}N_{16}O_{22}P_4Zn_4]^{2+}$ (=[2**1** + 2PPi]$^{2+}$), $[C_{84}H_{73}N_{16}NaO_{22}P_4Zn_4]^{2+}$ (=[2**1** + 2PPi + $Na^+ - H^+$]$^{2+}$), and $[C_{84}H_{72}N_{16}Na_2O_{22}P_4Zn_4]^{2+}$ (=[2**1** + 2PPi + $2Na^+ - 2H^+$]$^{2+}$), which were confirmed by isotope calculations (SI S-Figures 5−7). There was a severe broadness in the ^{1}H NMR spectrom of **1** in D_2O when PPi was added (SI S-Figure 8). These results suggest the binding of compound **1** with PPi as a 2+2 complex, as explained in Scheme 2.

From the fluorescent titration experiments (Figure 3), the association constant was calculated as 4.1×10^5 M^{-1} (error <15%).[9] The titration experiments were also repeated in the presence of 100 equiv of Pi or 10 equiv of ATP (SI S-Figures 3 and 4).

There are two previous examples of PPi fluorescent sensors based on the formation of an excimer. Teramae et al. utilized a guanidium−pyrene system to detect PPi.[5g] However, this system was examined only in methanol, and the selectivity for PPi was compared only with Pi. On the other hand, Hong et al. reported a pyrene−Zn^{2+} complex as a PPi-selective fluorescent chemosensor based on excimer formation.[6d] However, in that system, the presence of a small amount of ATP (0.4 equiv) also induced a considerable excimer emission. In our case, the large association constant and selectivity of PPi can be explained by two cooperative factors. As explained by Hong et al., the total anionic charge density of the four O−P oxygen atoms involved in the complexation between ATP and the two Zn sites is smaller than that of the four O−P oxygen atoms of PPi.[6c,d] In addition to the four zinc binding sites for PPi, the favorable $\pi-\pi$ interaction of two flat aromatic center moieties can induce tight 2+2 type binding between compound **1** and PPi, which makes this the first example of a PPi fluorescent sensor based on 2+2 type excimer formation.

In conclusion, we report a highly selective fluorescent chemosensor for PPi that can function in a 100% aqueous solution. This sensor shows an excimer peak at 490 nm only in the presence of PPi. Four zinc sites as well as a $\pi-\pi$ interaction induced the unique 2+2 type excimer in the presence of PPi. Furthermore, the detection of PPi is selective over ATP or Pi.

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Supporting Information Available: Experimental details of the synthesis, fluorescence spectra, UV absorption spectra, and NMR spectra (PDF); X-ray crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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