A new fluorescent sensor for the detection of pyrophosphate based on a tetraphenylethylene moiety

Chuneung Park [a,b], Jong-In Hong [a,*]

[a] Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-747, Republic of Korea
[b] SK Holdings, Life Science Business Division, Drug Development Center, 140-1, Wonchon-dong, Yuseong-gu, Daejeon 305-712, Republic of Korea

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ABSTRACT

We have developed a new fluorescent sensor **1**–**2Zn** based on a tetraphenylethylene (TPE) moiety for the detection of PPi. This TPE-based chemosensor showed 'turn-on' fluorescence emission according to the concentration of PPi. The fluorescence enhancement upon binding of PPi to **1**–**2Zn** resulted from the restriction of intramolecular rotation of phenyl rings in **1–2Zn**.

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Biological anions such as pyrophosphate ($P_2O_7{}^{4-}$, PPi), adenosine 5′-monophosphate (AMP), and adenosine 5′-triphosphate (ATP) play important roles in a wide range of biological processes. In particular, PPi is known to be involved in several biochemical reactions, such as hydrolysis of ATP, DNA polymerization, and other metabolic processes.[1] Therefore, the selective detection of PPi has been a major research focus. Although traditional methods of anion sensing such as the use of ion-selective electrodes have already been discovered, there is an increasing need to find alternative means of analysis, including the use of selective fluorescent chemosensors.[2] Because the fluorescent chemosensors present many advantages such as high sensitivity, low cost, easy detection, and versatility, many research groups have made an effort to discover selective fluorescent probes for PPi.[3]

Usually, fluorescent probes are composed of a signaling subunit known as a fluorophore, and a binding site which is covalently linked to a fluorophore. Mechanisms which regulate the response of a fluorophore to the substrate binding include photoinduced electron transfer (PET), fluorescence resonance energy transfer (FRET), excimer/exciplex formation or extinction, and photoinduced charge transfer (PCT).[4] In addition to these mechanisms, a new photoluminescence (PL) process has been identified, known as 'aggregation-induced emission (AIE)'.[5] In this case, a non-emissive material is induced to emit fluorescence by aggregation. It is assumed that the AIE effect is mainly caused by the restriction of intramolecular rotation of the phenyl rings. For example, the compounds which have a tetraphenylethylene (TPE) moiety show weak fluorescence in solution but they show strong emission upon aggregation because of the restriction of intramolecular rotation of the TPE moiety. In fact, a chemosensor having a TPE moiety has been described recently as having the ability to detect Ag+ and Hg^{2+} ions selectively.[6]

Herein, we report a fluorescence 'turn-on' chemosensor (**1**–**2Zn**) which can be used for PPi sensing by means of combining a TPE moiety showing unique aggregation-induced emission and a dipicolylamine(dpa)–Zn(II) complex exhibiting high sensitivity and selectivity for PPi ions compared to other anions. It was reported that dpa could form a complex with Zn^{2+} ions and that this dpa–Zn(II) complex could be used as a binding module for PPi.[7] We expected that when dpa–Zn(II) moieties bind PPi, the rotational restriction of the phenyl rings of the TPE moiety would lead to fluorescence emission.

The synthesis of **1**–**2Zn** is described in Scheme 1. Compounds **2** and **3** were synthesized according to the reported methods.[6] Compound **1** was prepared by the S_N2 reaction of **3** with 2,2′-dipicolylamine in the presence of K_2CO_3 and KI in acetonitrile at reflux temperature. Sensor **1**–**2Zn**, dinuclear Zn^{2+} complex of compound **1**, is easily formed by the addition of a methanolic solution of **1** to an aqueous solution of 2 equiv of $Zn(NO_3)_2$.

First, we investigated the fluorescence emission change of compound **1** according to Zn^{2+} concentration. Figure 1 shows the fluorescence emission changes (λ_{ex} = 320 nm) of **1** (100 μM) as Zn^{2+} (nitrate salt) concentration increases in H_2O/DMSO (10:1, v/v) at 25 °C. In the absence of Zn^{2+} ions, compound **1** showed strong emission at 472 nm. Because of the hydrophobic feature of a TPE moiety, compound **1** seems to be aggregated in an aqueous solvent system, which results in fluorescence emission due to the 'aggregation-induced emission (AIE)'. The higher the fraction of water in a mixed solvent system (water–DMSO), the stronger the fluorescence emission becomes, as a result of higher aggregation (Fig. S1). However, upon the addition of Zn^{2+} ions, the emission intensity of **1** gradually decreases as displayed in Figure 1. When about 3 equiv of Zn^{2+} ions was added, the emission was almost quenched. The decrease in fluorescence emission seems to be

* Corresponding author. Tel.: +82 28806682; fax: +82 28891568.
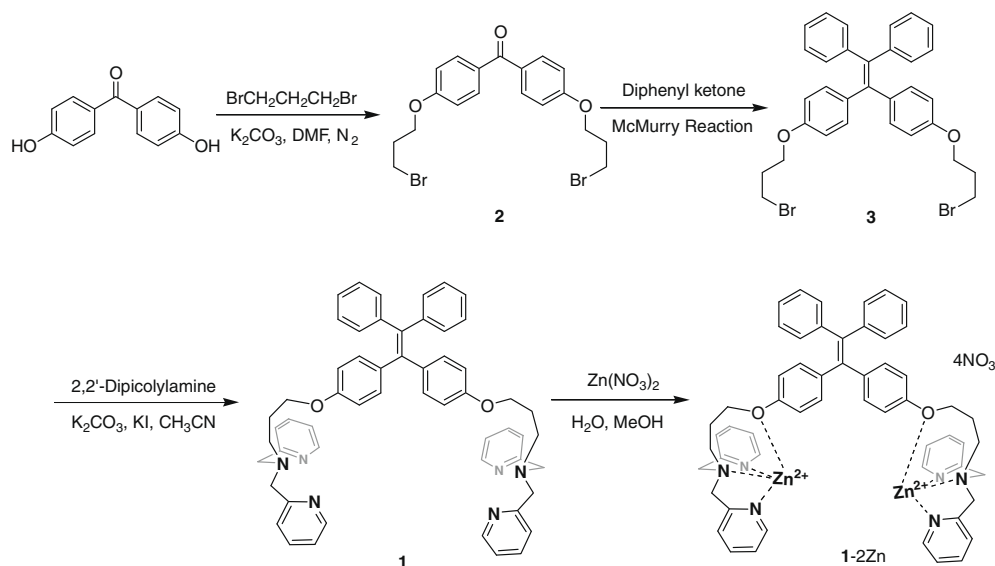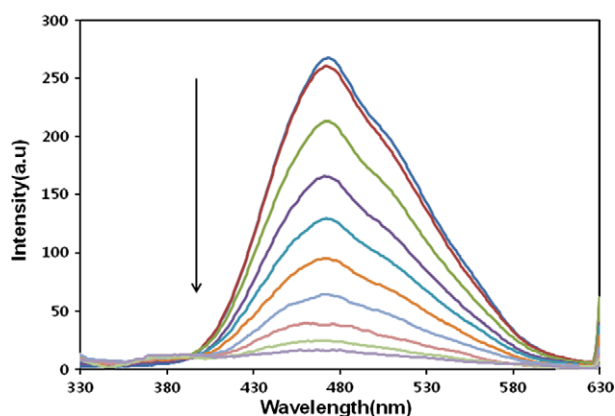E-mail address: jihong@snu.ac.kr (J.-I. Hong).

Scheme 1. Synthesis of chemosensor **1–2Zn**.

Figure 1. The fluorescence emission changes of **1** (100 µM, λ_{ex} = 320 nm) upon the addition of Zn^{2+} (nitrate salt) in H_2O/DMSO (10:1, v/v) at 25 °C: [Zn^{2+}] = 0, 47.6, 90.9, 130, 167, 200, 231, 259, 286, 310 µM.

Figure 2. The fluorescence emission changes of sensor **1–2Zn** (100 µM, λ_{ex} = 320 nm) upon the addition of PPi (sodium salt) in H_2O/DMSO (10:1, v/v) at 25 °C: [PPi] = 0, 9.90, 19.6, 29.1, 38.5, 47.6, 56.6, 65.4, 74.1, 82.6, 90.9, 99.1, 107, 115, 123, 130, 138, 145, 153, 167, 200 µM.

attributable to the degree of aggregation of compound **1**. As Zn^{2+} ions are added, the dpa–Zn(II) complex (**1–2Zn**) is formed and induces the destruction of aggregation which leads to the decrease in the emission.

Next, we monitored the fluorescence emission change of sensor **1–2Zn** according to the PPi (sodium salt) concentration. In contrast to the Zn^{2+} addition, the addition of PPi to **1–2Zn** resulted in a gradual increase in the emission band at 472 nm as shown in Figure 2. This fluorescence enhancement was almost saturated with the addition of 1 equiv of PPi. The fluorescence intensity of **1–2Zn** at 472 nm increases linearly with the concentration of PPi in the range of 0–60 µM as shown in the inset of Figure S4 (I_{472nm} = 5.62[PPi] + 32.9, r = 0.993, n = 7). The detection limit turned out to be 0.90 µM (Z = 2).

A putative sensing mechanism for this fluorescence OFF–ON is shown in Scheme 2. A presumed binding mode for PPi and **1–2Zn** is based on our previous work involving a structurally similar sensor and its X-ray crystal structure.[7] The proposed complex shows that the two sets of oxygen anions on each P atom of PPi bind to the dinuclear Zn complex by bridging the two metal ions to give rise to two hexacoordinated Zn^{2+} ions in **1–2Zn**. Therefore, when **1–2Zn** binds to PPi, two dpa–Zn(II) moieties are fixed by PPi. This fixation induces the restriction of intramolecular rotation of
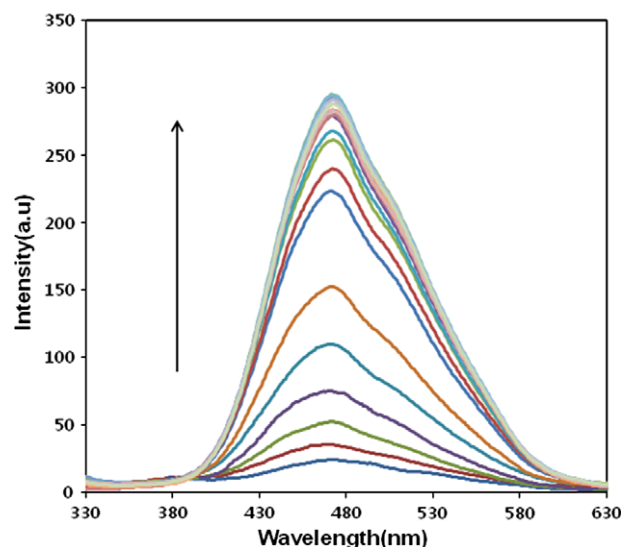
phenyl rings, which results in fluorescence enhancement. It is possible that two ways of coordination between **1–2Zn** and PPi can occur as expected in the previous report: intramolecular coordination and intermolecular coordination.[6] However, in any cases, the intramolecular rotations would be also restricted, leading to fluorescence enhancement.

Another possible sensing mechanism would be induced charge transfer via the conjugation between a phenolic oxygen atom and a TPE moiety. Weakening the bond between the phenolic oxygen and Zn^{2+} at the binding of PPi induces more negative charge character on the phenolic oxygen atom and thus the fluorescent enhancement occurs due to a more extended π-conjugation.[7] Although the significant spectral shift is not shown in Figure 2, we can find the gradual formation of the shoulder peak at around 510 nm with increasing PPi concentration. Therefore, we think that the induced charge transfer may have a partial influence on the sensing event.
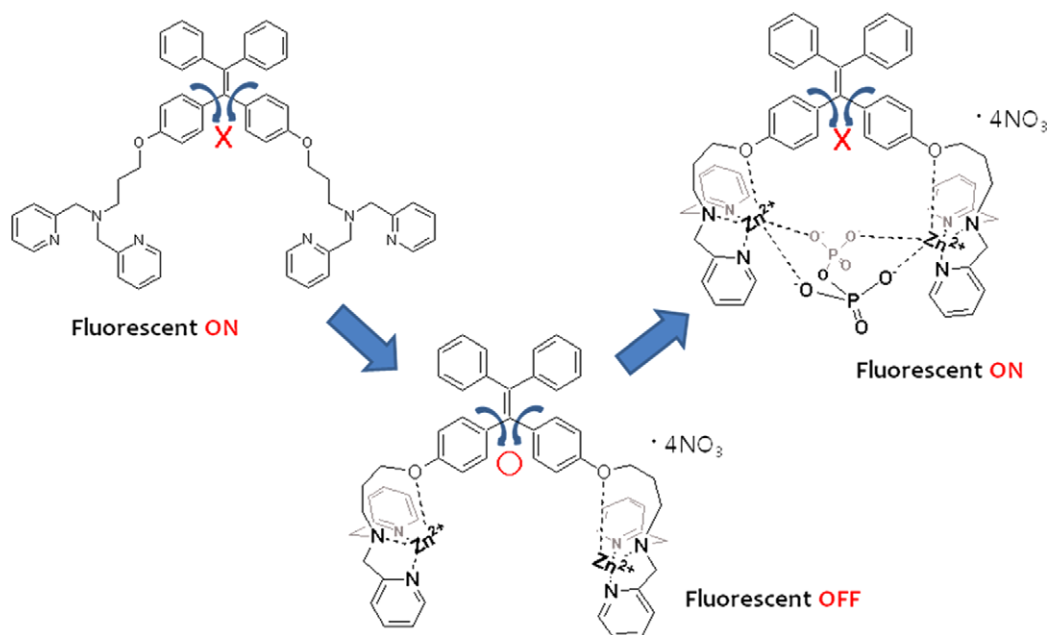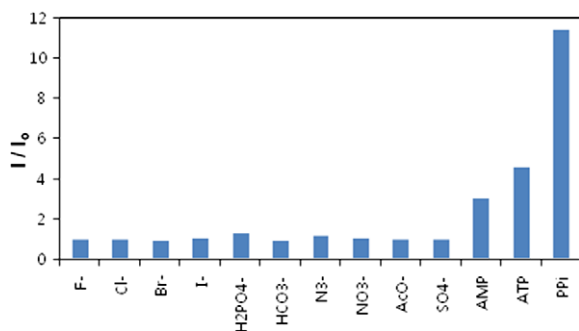
Scheme 2. The putative sensing mechanism.

Figure 3. The comparison of the fluorescence emission intensity (I) at 472 nm of **1–2Zn** after the addition of 1 equiv of each anion with the emission intensity (I_o) at 472 nm of **1–2Zn** (100 μM, λ_{ex} = 320 nm) before the addition of each anion.

Supplementary data

Supplementary data (synthesis, ^{1}H, ^{13}C NMR and HRMS data of compound **1**, **1–2Zn**, the fluorescence emission change of **1–2Zn** with different water fractions, and the comparison of the emission intensity of **1** and **1–2Zn** with other metal ions and anions) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.009.

Unlike the strong fluorescence enhancement observed upon the addition of PPi, relatively small fluorescence changes are seen with other anions. The fluorescence spectrum of sensor **1–2Zn** was measured in the presence of other anions including Br^-, Cl^-, F^-, $H_2PO_4^-$, HCO_3^-, I^-, N_3^-, NO_3^-, $CH_3CO_2^-$ and SO_4^- under identical conditions. As shown in Figure 3, enhancement of the fluorescence intensity of **1–2Zn** at 472 nm was rather small compared to that upon the addition of PPi after the addition of 4 equiv of these anions. Competition experiments between PPi and these anions also indicate that **1–2Zn** shows good selectivity toward PPi (Fig. S6). However, the addition of AMP and ATP also enhanced the fluorescence intensity although the intensity was relatively small compared to that after the addition of PPi.

In summary, we have developed a TPE-based fluorescent chemosensor **1–2Zn** which exhibits fluorescence 'turn-on' upon binding with PPi anions. This study demonstrates that chemosensor **1–2Zn** can be used as a fluorescence turn-on sensor for PPi. Fluorescence enhancement upon the addition of PPi to **1–2Zn** results from the restriction of intramolecular rotation and/or induced charge transfer. Further studies will include the design of new analogues of **1–2Zn** with enhanced sensitivity and selectivity for PPi over AMP and ATP to enable the practical application of this type of sensor to the monitoring of enzyme reactions.

References and notes

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