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A Mononuclear Zinc Complex for Selective Detection of Diphosphate *via* Fluorescence ESIPT Turn-On

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Abstract

A mononuclear zinc complex has been found to exhibit unexpected selectivity for biologically important diphosphate anions (PPi and ADP). The diphosphate binding could turn-on the ESIPT, whose study reveals mechanistic insight to aid the future design of new sensors.

Phosphate-containing anionic species¹ are ubiquitous in biological systems and play important mediatory roles in signal transduction pathways, as well as in carrying genetic information.² Design of fluorescent chemosensors for biologically relevant anions remains to be a challenging topic.^{3–6} This is due to the lack of general design principle that can reliably translate an anion-binding event to a large fluorescent signal response.³ Most of the developed phosphate anion sensors are based on the binuclear Zn(II) complexes such as 1-Zn, where two zinc centers are properly positioned to provide improved selectivity for anions such as pyrophosphate (PPi)^{7–14} and adenosine 5'-triphosphate (ATP).^{3,10,15,16} The fluorescence response of these sensors, however, exhibit little spectral shift in the fluorescence signal, which lowers the sensitivity and hampers their practical applications. Continuing interests exist in searching for new sensor mechanisms that can generate a large spectral response upon selective recognition of PPi anion. While the binuclear Zn(II) complexes are widely regarded as a popular mechanism for selective detection of polyphosphates PPi and ATP, very few mononuclear Zn(II) complexes have shown useful fluorescence response for polyphosphates such as adenosine 5'-diphosphate (ADP),^{17,18} PPi^{19,20,21,22} and ATP.^{18,23} In addition, none of the existing sensors is able to induce a large spectral shift upon binding to polyphosphates, which limits their practical application. The most recent example of mononuclear-based PPi sensor is based on fluorescence turn-on.²²

Recently we reported the pyrophosphate sensor 2-Zn complex, whose blue emission (~420 nm) is shifted by about 100 nm to ~518 nm upon addition of PPi in HEPES buffer.²⁴ The unusually large bathochromic shift from 2-Zn is attributed to the PPi binding-induced turn-on of the excited state intramolecular proton transfer (ESIPT). While the binuclear complex 2-Zn has two Zn(II) sites, the site II linking to the phenoxide is responsible for the switch-on and off of ESIPT. A fundamental question is whether the 1,3-binuclear Zn(II) complex is

essential for the ESIPT turn-on mechanism. In an effort to elucidate the intriguing PPi binding-induced ESIPT turn-on mechanism, we decided to further examine the structure and PPi binding of **3**-Zn which only has one Zn(II) site.

Synthesis and structure of **3** and its zinc complex

Ligand **3** was synthesized as a white solid (m.p. 118–120°C) by using a modified procedure.²⁴ In order to gain a better understanding on the ESIPT ON and OFF, titration of **3** was studied by addition of Zn(NO₃)₂. The absorption peak of **3** in EtOH (λ_{max} =343 nm) was red-shifted to λ_{max} =361 nm, as a consequence of deprotonation Ph–OH → Ph–O[−] (Figure 1a). A clear isobestic point was observed at about 352 nm, in agreement with the proposed formation of **3**-Zn (Scheme 1). The assumption was supported by high resolution mass spectroscopy (HRMS) which detected the molecular ion at 535.075, corresponding to [**3**+Zn²⁺+Cl[−]]⁺ (the calcd mass for C₂₇H₂₄ClN₄O₂Zn: 435.0879) (ESI Figure S10). The mass spectroscopy also detected the dimer (**3**-Zn)₂, whose optical absorption could be very similar to **3**-Zn. When using PF₆[−] (KPF₆) as the counter anion, a single crystals of **3**-Zn was obtained from its acetonitrile solution by slow evaporation. The crystal structure of **3**-Zn revealed the zinc binding pattern, where Zn²⁺ cation is connected to the phenoxide oxygen (Figure 2). The Zn²⁺ center also contained weak ligands (halide and water), which might be easily replaced.

¹H NMR titration of **3** by addition of Zn²⁺ revealed further details on the zinc complexation (Figure 3). Clear binding of Zn²⁺ to both pyridine rings was observed, as the chemical shift of the pyridyl protons *H_a* in **3** was shifted from 8.41 ppm to ~8.66 ppm. The phenyl protons *H_d* also exhibited significant downfield shift, in agreement with the zinc binding to the phenol group. When 1.0 equiv. of Zn²⁺ was added, the signal ratio of *H_a*:*H_d* was approaching 2:1, indicating that the reaction was complete (to form the complex **3**-Zn). The result showed that the species formed in solution was consistent with the structure found in the crystalline state (Figure 2). It was also noted that the ¹H NMR detected two −CH₃ signals: 2.3 (major) and 2.4 ppm (minor). The peak at 2.4 ppm was increasing when the Zn²⁺ cation was less than 0.5 equiv, then decreasing when the Zn²⁺ cation was larger than 0.5 equiv. It was likely that the chemical equilibrium “**3** + Zn²⁺” not only gave **3**-Zn as a major species (¹H signal at 2.3 ppm), but could also gave (**3**)₂-Zn such as **5** as the intermediate species, which was also detected in ESI-MS (Figure S10–S14). The sharp *H_d* and *H_{d'}* signals at 7.62 and 7.32 ppm were detected *only* after 0.5 equiv of Zn²⁺ was added, suggesting that significant **5** could be involved in the initial stage. When the Zn²⁺ concentration was increased (i.e. > 0.5 equiv), the equilibrium in the solution was driven toward the formation of **3**-Zn.

Addition of Zn²⁺ cation to **3** increased fluorescence intensity at 445 nm (Figure 1b). During the formation of **3**-Zn complex, the Zn²⁺ cation was connected to both dipicolylamine (DPA) and phenol segments. It should be noted that DPA is a stronger zinc-chelating group.²⁵ When Zn²⁺ cation was added to **3** in CH₂Cl₂, the cation was found to only bound to the DPA group (ESI Figure S5), while the fluorescence intensity at ~504 nm was increased by a factor of ~2 (ESI Figure S4). The origin of the observed fluorescence turn-on, therefore, was partially associated with Zn²⁺-binding to DPA.

Addition of Zn^{2+} cation also caused the emission color of **3** ($\lambda_{\text{em}} \sim 530$ nm, green color) to shift to blue ($\lambda_{\text{em}} \sim 445$ nm), as a consequence of forming **3-Zn**, which disabled the ESIPT as the phenol was connected to the Zn^{2+} cation (Figure 1). The fluorescent response of complex **3-Zn** was examined by addition of 5 equiv. of different anions (Figure 4). Addition of diphosphate anion ADP or hydrogen pyrophosphate H_2PPi to **3-Zn** in EtOH caused a notable spectral shift (from 447 to 504 nm), indicating the release of the phenolic group to turn on the ESIPT (Figure 4). The acidic species $\text{H}_2\text{P}_2\text{O}_7^{2-}$ was assumed to be in equilibrium with $\text{HP}_2\text{O}_7^{3-}$, i.e. $\text{H}_2\text{P}_2\text{O}_7^{2-} \leftrightarrow \text{HP}_2\text{O}_7^{3-} + \text{H}^+$, where the dissociated proton could facilitate the cleavage of phenoxide-Zn bond: $\text{PhO-Zn(II)} + \text{H}^+ \rightarrow \text{PhOH}$. Interestingly, the sensor was silent to monophosphate AMP and triphosphate ATP. The sensor was also silent to physiologically important anions such as acetate and citrate (ESI, Figure S3). The fluorescence turn-on at about 504–510 nm, however, was not observed when the “**3-Zn**” was examined in HEPES buffer under the same concentration.

Binding of PPi to **3-Zn** complex

From the ethanol solution containing **3-Zn** and H_2PPi , the mass spectrometry detected the cationic species $[\text{3-Zn} + \text{H}_2\text{PPi}^{2-} + \text{H}^+ + \text{Na}^+]^+$ (calcd for $\text{C}_{27}\text{H}_{26}\text{N}_4\text{NaO}_9\text{P}_2\text{Zn}$: 699.0364) (Figure 5a). ^1H NMR titration of H_2PPi further revealed that addition of H_2PPi caused H_a signal to shift downfield to ~ 8.75 ppm (Figure 6), indicating that the H_2PPi was bound to the Zn^{2+} center. Since no pyridyl signal was detected at ~ 8.4 ppm (for H_a in the ligand **3**), one can assume that the pyridine rings remained to be connected to the Zn^{2+} cation. On the basis of these experimental evidences, we proposed that the addition of H_2PPi to **3-Zn** initially formed adduct **6** via binding with one end of pyrophosphate. This step was relatively fast due to stronger electrostatic attraction between the cation and phosphate. In the second step, the other end of H_2PPi would attack the Zn^{2+} cation, leading to **7**, in which the phenoxide-Zn bond was weakened (Scheme 2). The intramolecular proton transfer in **7** could assist the formation of the phenol fragment in **8**, which is necessary for ESIPT turn-on. The proposed formation of **8** was consistent with the proton NMR (Figure 6), where the signal H_b of **3-Zn** was shifted upfield to ~ 7.84 ppm upon addition of PPi (as the binding environment at Zn^{2+} cation changed). The observed peak broadening in the ^1H NMR was in agreement with the proposed equilibrium between **7** and **8**. The ^1H signals H_d and $H_{d'}$ were notably shifted from 7.63 and 7.32 ppm to 7.58 and 7.27 ppm, respectively, in consistence with the detachment of Zn^{2+} from the phenolic oxygen. In the proposed mechanism, a diphosphate fragment was necessary to provide a strong phosphate-Zn interaction to weaken the phenoxide-Zn bond as shown in **7**, which could be the reason why the sensor **3-Zn** was not responding to monophosphate AMP.

Upon addition of one equivalent PPi to “**3-Zn**”, UV-vis absorption revealed some residual absorption at ~ 365 nm (Figure 7a), indicating that the formation of phenoxide was not complete. Addition of excess PPi was necessary to completely drive the equilibrium to the final product. The observation was in agreement with the proposed process in Scheme 2, where the excess PPi would shift the equilibrium to **8**. Similar trend was also observed in the fluorescence response (Figure 7b), where the blue emission at ~ 445 nm was decreased by only about 50% with one equivalent PPi. Subsequent addition of more PPi decreased the emission at ~ 445 nm but did not increase the peak intensity at 512 nm. The emission band at

440 nm might be associated with **7**, which could have stronger fluorescence than **3-Zn**. The assumption of strong fluorescent **7** was understandable, as addition of an oxygen-containing species could significantly increase the fluorescence intensity of a HBO-zinc complex.²⁶

Binding of ADP to **3-Zn** complex

As seen in Figure 4, the sensor **3-Zn** exhibited the intriguing response to diphosphate ADP, while being silent to triphosphate ATP and monophosphates (AMP, K_3PO_4 and H_3PO_4 ; ESI Figure S3 and S4) under the same conditions. Upon addition of ADP, UV-vis absorption of **3-Zn** at 361 nm gradually decreased, which was accompanied with the new absorption peaks at 343 and 299 nm (Figure 8). The observed spectral response indicated the cleavage of ArO–Zn bond, thereby releasing the phenol group as shown in **11**. The mass spectrum detected the ADP adduct $[11 + Na^+]^+$ (from the solution of “**3-Zn** + ADP”), whose isotope pattern matched the calculated one (Calcd for $C_{37}H_{37}N_9NaO_{12}P_2Zn$: 948.1226) (Figure 5b). The formation of the protonated species **11** was consistent with the observed ESIPT turn-on (Figure 4). Under the same experimental conditions, the corresponding ATP adduct was not detected from the mass spectrometry study of “**3-Zn** + ATP” sample (See ESI Figure S17).

In the proposed mechanism for the fluorescence turn-on, the less hindered phosphate of ADP would attack the Zn^{2+} center of **3-Zn** to give **9**, attributing to the electrostatic attraction. Intramolecular addition of the adjacent phosphate to Zn^{2+} , in addition to the π - π interaction between HBO and adenosine units, could lead to **10**, where the phenoxide “oxygen” could be detached from the Zn^{2+} cation. Protonation of the phenoxide would then lead to **11** to give ESIPT turn-on.

1H NMR of the ADP adduct **11** revealed that the pyridyl proton H_a was shifted downfield to 8.82 ppm, when the Zn–phenoxide bond in **3-Zn** was replaced by the Zn–phosphate bond (with more ionic character) in **11**. The characteristic signals H_c and H_b from adenosine were notably shifted upfield, possibly due to the π - π stacking interaction between HBO and the aminopurine unit of adenosine. The proton H_d' on the phenol unit of **3-Zn** at 7.32 ppm was shifted to 7.25 ppm (for ADP adduct) and 7.15 ppm (for ATP adduct), respectively, indicating the possible different π - π interaction patterns for ADP and ATP adducts. The molecular modeling revealed that the aromatic fragment from the adenosine, i.e. aminopurine unit, adopted different π - π interaction geometries in the ADP and ATP adducts (Figure 10). In the adduct **10**, the aminopurine unit was *parallel* to HBO for stronger π - π interaction with the center of the HBO (Figure 10a). In contrast, the “aminopurine unit” in the corresponding ATP adduct **12** was *perpendicular* to HBO to interact with the phenol end of the HBO (Figure 10b). The estimated interplanar distances between the aminopurine and HBO were 4.3 Å and 4.6 Å for the ADP and ATP adducts, respectively, which are within the parallel π - π interaction distance ($<5\text{ Å}$).²⁷ In the ATP adduct (Figure 10b), the proton H_d' was located right above the aminopurine unit, which could give a stronger shielding effect (to shift the 1H NMR signal to upper field).

In summary, the zinc complex **3-Zn** responds selectively to diphosphate anions (PPi and ADP) to turn-on the ESIPT emission. On the basis of mass spectroscopy and spectroscopic evidences, the diphosphates bind to **3-Zn** in 1:1 ratio, forming the corresponding adducts

with defined chemical structures. Molecular modeling study, coupled with the ^1H NMR evidences, points to that the complex **3**-Zn binds ADP stronger than ATP, as the ADP adduct exhibits a more favorable parallel π - π interaction geometry than the ATP adduct. The overall study shows that the binding of the diphosphates anion can open the phenoxide-Zn bond, thereby turning on the ESIPT. The generated phenol group, however, appears to remain in the vicinity of the zinc cation (as shown in **11**), which gives the ESIPT fluorescence at ~509 nm (slightly less than the λ_{em} =530 nm for **3**).

The study demonstrates a new sensor for diphosphate anions by integrating the mononuclear Zn(II) complex with ESIPT. In comparison with the known sensors, which are mainly dependent on the fluorescence intensity changes, the new sensor is able to give a large spectral shift (by 57–67 nm) upon binding to PPi or ADP, which is useful for the naked eye detection. In addition, the induced fluorescence exhibits a large Stokes' shift (~169 nm). These attractive features, in addition to the excellent selectivity, would be useful for practical detection of PPi or ADP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

UV-vis (a) and fluorescence spectra (b) of **3** (10 μM) in EtOH upon addition of different equivalent of Zn(NO₃)₂. Fluorescence spectra were acquired while the sample being excited at the isobestic point ~352 nm.

Fig. 2.

X-Ray structure of **3-Zn** in the front view (a) and side view (b) of benzoxazole fragment, where the Cl, N, O, and C atoms are in green, blue, red and grey colors, respectively.

Fig. 3.
 ^1H NMR of **3** (10 μM) in CD_3OD upon addition of different equivalent of $\text{Zn}(\text{NO}_3)_2$.

Fig. 4.

Fluorescence response of **3**-Zn complex (10 μ M) with different phosphate anions (5 equivalents) in ethanol (top, excited at the isobestic point $\sim$ 352 nm). Fluorescence images of **3**, **3+Zn²⁺**, "**3+Zn²⁺ + PPi**," and "**3+Zn²⁺ + ADP**" in ethanol (bottom). And the structures of phosphate anions.

Fig. 5. ESI-MS spectra of $[3\text{-Zn} + \text{H}_2\text{PPi}^{2-} \text{H}^+ + \text{Na}^+]^+$ (a) and $[3\text{-Zn} + \text{ADP} + \text{Na}]^{++}$ (b); The samples were prepared by addition of diphosphates to **3-Zn** solution in ethanol.

Fig. 6.
 ^1H NMR titration of “**3**+ Zn^{2+} ” with hydrogen pyrophosphate H_2PPi in CD_3OD

Fig. 7.

Absorption (a) and fluorescence response (b) “ $3+Zn^{2+}$ ” (10 μM) in EtOH upon titration with PPI. The fluorescence spectra were acquired while the sample being excited at ~ 352 nm.

Fig. 8.
UV-vis (a) titration and fluorescence response (b) of **3**-Zn (10 μ M) with ADP in ethanol.

Fig. 9. ^1H NMR of zinc complex "3 + Zn^{2+} " in CD_3OD (a) upon addition of ADP (b) or ATP (c). In the spectra (b) and (c), the NMR solutions were saturated by the ADP or ATP (due to their poor solubility), respectively. The signals for H_d and $\text{H}_{d'}$ protons on the phenol segment are indicated by arrows. The ^1H NMR of ADP and ATP are acquired to aid the spectral assignment (d).

Fig. 10.

The molecular modeling of (a) “**3-Zn + ADP**” and (b) “**3-Zn + ATP**” adducts, showing the π - π interaction between HBO and aminopurine unit (of adenosine). The conformations were obtained by using Gaussian 09 (DFT with G-61 set) and plotted by using HyperChem software, where the P, N, O, and C-atoms are in yellow, blue, red and cyan colors, respectively.

Scheme 1.

An ESIPT sensor containing two Zn(II) sites (**2-Zn**) and one Zn(II) site (**3-Zn**). PPI binding to **3-Zn** leads to the recover of phenol, thereby turning-on the ESIPT.

Scheme 2.

The proposed binding between 3-Zn and pyrophosphate PPI.

Scheme 3.

The proposed binding structure between **3-Zn** and ADP.