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A new chemosensing ensemble for fluorescent recognition of pyrophosphate in water at physiological pH

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ABSTRACT

A novel chemosensing ensemble that exhibits sensitive and selective recognitions of pyrophosphate in 100% aqueous solution at physiological pH has been developed. The chemosensing ensemble was constructed by a dinuclear Zn(II) complex of 2,6-bis[(bis(2-benzimidazolylmethyl)amino)methyl]-*p*-cresol and sodium fluorescein, the receptor–indicator pair is able to highly selectively discriminate pyrophosphate from phosphate and other anions in water at physiological pH.

1. Introduction

Recently, considerable efforts have been focused on the selective sensing of biologically important anions due to their important roles in various chemical and biological processes.[1] Fluorescent sensing of anions has become particularly attractive because of its simplicity and low detection limit.[1,2] Particularly, pyrophosphate (PPi) is a biologically important target because it participates in several bioenergetic and metabolic processes.[3] It is also well known that patients with calcium pyrophosphate dihydrate crystals and chondrocalcinosis have been shown to have high synovial fluid PPi levels.[4] Therefore, the detection of PPi has been the main focus of several research groups.[5,6] Among the various methods for the detection of PPi,[7] the most advantageous are the systems which can recognize PPi in an aqueous environment producing an optical signal. However, during the past decade, there are only few examples of effective fluorescent sensors in 100% aqueous solution which have been reported.[6,8] Among the numerous PPi receptors reported to date, few of them are able to effectively discriminate PPi from phosphate (Pi).[6a,d,8d,9] In general, it is challenging to develop receptors that bind tightly, reversibly, and selectively to small molecules in water for sensing purposes.[9] Others and we have demonstrated that some carefully designed dinuclear metallic receptor can bind a target anion selectively and tightly.[10] Recently, Smith and co-workers have studied a variety of dizinc enzyme model($Zn_2\mathbf{L}_1$)/complexometric indicator pairs in indicator displacement assays (IDAs) for phosphate (Pi) recognition under physiological conditions.[11] $Zn_2\mathbf{L}_1$ as an IDAs receptor with various commercial indicators showed similar binding affinity toward Pi and PPi, which makes their differentiation difficult. The Zn–Zn distance in $Zn_2\mathbf{L}_1$ (3.0 Å) is less than that in phosphotriesterase (3.5 Å), this may be the reason why $Zn_2\mathbf{L}_1$ and its analogue can bind both Pi and PPi.[8c,12] When an improved ligand $\mathbf{L}_2$ was synthesized by incorporating the di-(2-picolyl)amine (DPA) unit onto the *m*-terphenyl scaffolds, the Zn–Zn distance in $Zn_2\mathbf{L}_2$ is increased and can satisfy the requirement for PPi binding, and subsequently reduce the affinity of $Zn_2\mathbf{L}_2$ toward Pi due to the larger Zn–Zn separation. The $Zn_2\mathbf{L}_2$ complex performed an enhanced preference for binding PPi over Pi. Thus, the Zn–Zn distance played a key role in analyte preference.

Bearing this concept in mind, we selected bis(2-benzimidazolylmethyl)amino group as an analogue of the DPA unit, which can perform the similar chelating function to zinc as DPA does, what is more, the steric interactions between benzimidazole groups can play crucial roles in increasing the metal–metal distance. The Zn–Zn distance in the optimized molecular model of $[Zn_2(\mathbf{L_3})]^{4+}$ is calculated to be 3.964 Å (Fig. S1, Supplementary data). We reasoned that a couple of well-positioned metal complexes could cooperatively bind to four oxygens of PPi tightly, reversibly, and selectively over other oxyanions such as oxalate, CH_3COO^-, Pi, and HSO_4^-, and some simple anions such as F^-, Cl^-, Br^-, and I^-. Herein, we report the synthesis of dinuclear metal complex $[Zn_2(\mathbf{L_3})]^{4+}$ and its application on fluorescent recognition of pyrophosphate in 100% aqueous solution at physiological pH by a chemosensing ensemble approach.[13]

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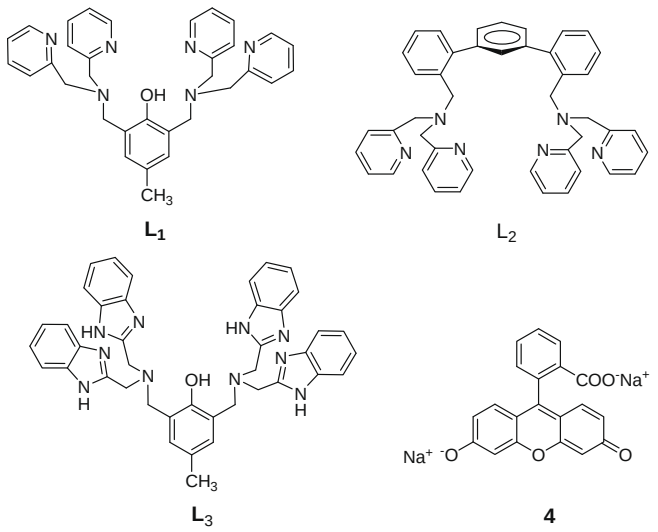


Structures of L_1, L_2, L_3 and fluorescein **4**

2. Results and discussion

The synthesis of L_3 and dinuclear zinc complex $[Zn_2(L_3)]^{4+}$ is shown in Scheme 1. L_3 was readily prepared in 80% yield by a modified method from the reaction of 2,6-bis(chloromethyl)-4-methylphenol **1**[14] and bis(2-benzimidazolylmethyl)amine **2**.[15] Reaction of ligand L_3 with $Zn(NO_3)_2{\cdot}6H_2O$ in dry methanol led to complex $[Zn_2(L_3)]^{4+}$.[16] The bis(2-benzimidazolylmethyl)amine moiety was selected in this study because it can not only perform a similar chelating function as DPA does, but also easier to be synthesized compared with DPA unit. Among the various metals that may bind ligating group with L_3, zinc is the reasonable choice for a physiological and reversible binding receptor due to its air stability and the relatively high kinetic lability of its complexes. In the API-ES mass spectrum (positive), the peak appearing at $m/z = 937.0$ can be assigned to $[Zn_2(L_3\text{-}H^+)(NO_3^-)_2]^+$ ion, which strongly supports the formation of dinuclear zinc complex.

After the screening of several of indicators for this chemosensing ensemble, fluorescein sodium salt (**4**) was selected as the indicator for this research. Upon addition of an aqueous solution of $[Zn_2(L_3)]^{4+}$ to a solution of **4** buffered by 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH 7.4, the fluorescence intensity of **4** sharply decreased and resulted in complete quenching of the emission when 100 equiv of $[Zn_2(L_3)]^{4+}$ was used (Fig. 1). Nonlinear least-squares fitting of the titration profiles (Fig. 1, inset) employing the 1:1 binding mode equation strongly support the formation of a 1:1 complex of $[Zn_2(L_3)]^{4+}$ and **4**, and the binding constant K_s was calculated to be $(4.1 \pm 0.1) \times 10^4\ M^{-1}$.[17]

The fluorescence emission changes of the chemosensing ensemble (an aqueous solution of **4** (1.0×10^{-6} M) and $[Zn_2(L_3)]^{4+}$ (1.0×10^{-4} M)) upon the addition of $(COO)_2^{2-}$, HSO_4^-, Pi, CH_3COO^-, I^-, Br^-, Cl^-, F^-, and PPi (for each anion, 100 equiv (relative to **4**) of the sodium salt was used) are illustrated in Figure 2. As shown in Figure 2, among the tested anions, only PPi is able to displace the indicator from the receptor–indicator complex and cause significant fluorescence revival, whereas, no noticeable changes were observed upon addition of other anions. This result demonstrated that the chemosensing ensemble has a high selectivity toward PPi.

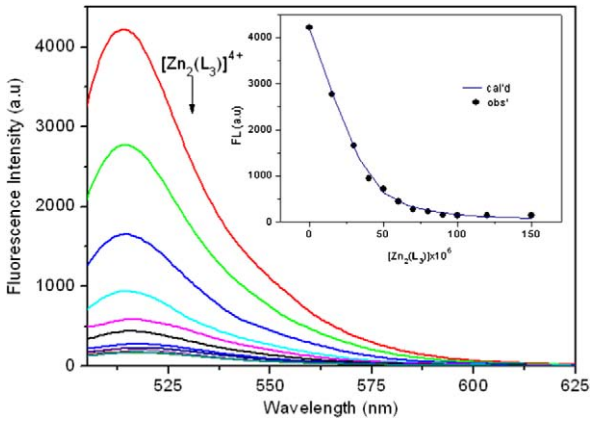


Figure 1. Fluorescence intensity of **4** by titration with $[Zn_2(L_3)]^{4+}$. The concentration of fluorescein is 1.0×10^{-6} M, all were aqueous solutions buffered by HEPES (10 mM, pH 7.4), excited at 489 nm. (Inset: plot of F_{514} vs equiv of $[Zn_2(L_3)]^{4+}$.)

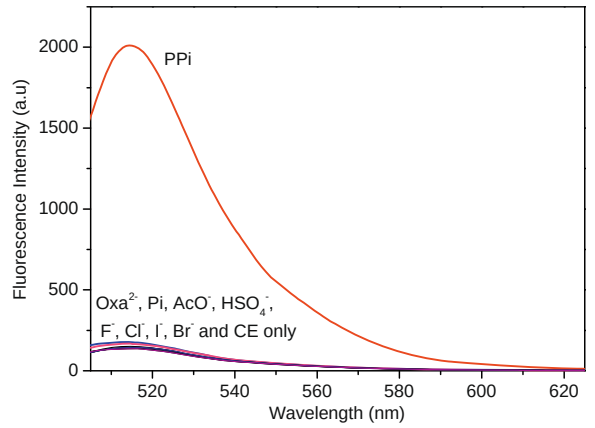
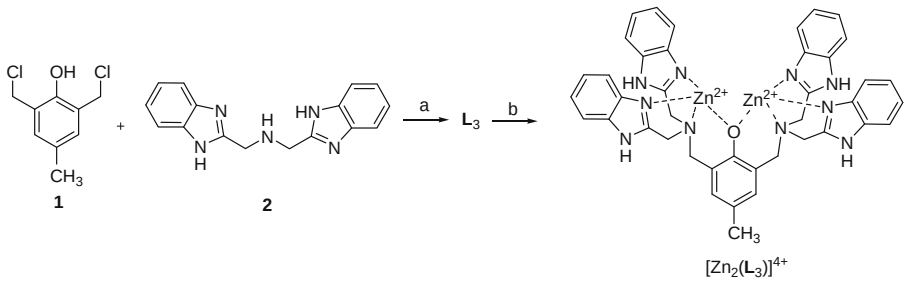


Figure 2. Fluorescence changes of the chemosensing ensemble upon the addition of 100 equiv of different representative anions.

The receptor–indicator pair was then subjected to titration by the indicator displacement method with some representative anions: $(COO)_2^{2-}$, HSO_4^{2-}, Pi, and PPi. Except PPi, all other tested anions cannot lead to significant fluorescence revival of the indicator,



Scheme 1. Synthesis of $[Zn_2(L_3)]^{4+}$. Reagents and conditions: (a) MeOH, triethylamine, ice/acetone bath; (b) $Zn(NO_3)_2{\cdot}6H_2O$, methanol.

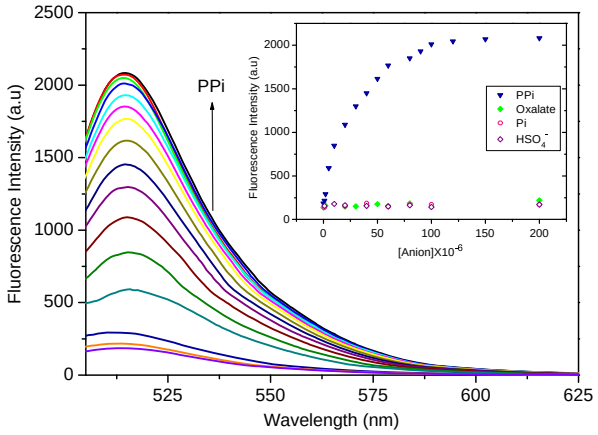


Figure 3. Competitive titration of an aqueous solution of fluorescein (1.0×10^{-6} M) and $[Zn_2(\mathbf{L}_3)]^{4+}$ (1.0×10^{-4} M) (pH 7.4, HEPES 10 mM) with standard solution of PPi. (Inset: Plot of relative $F_{514\ nm}$ vs concentration for four representative anions.)

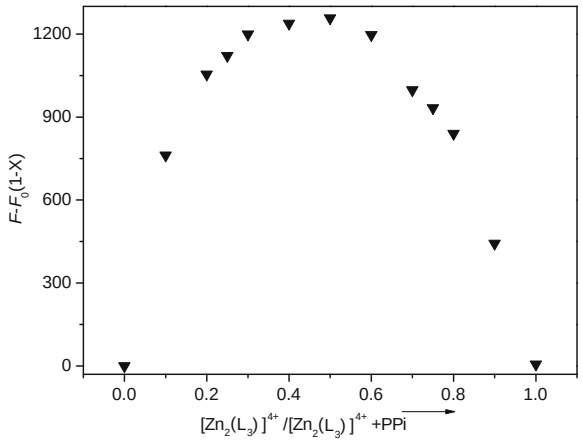


Figure 4. Job's plot examined between $[Zn_2(\mathbf{L}_3)]^{4+}$ + PPi and PPi in an aqueous buffer solution (pH 7.4, 10 mM HEPES).

even if 200 equiv of anion (relative to **4**) was used. In a typical experiment, increasing amounts of PPi was added to a chemosensing ensemble solution containing **4** (1.0×10^{-6} M) and $[Zn_2(\mathbf{L}_3)]^{4+}$ (1.0×10^{-4} M) in a buffered solution at pH 7.4 (10 mM HEPES), a significant revival of the indicator fluorescence intensity was observed upon addition of PPi (Fig. 3). This result indicates the successful competitive binding of PPi and displacement of the indicator from the receptor. The binding constant between PPi and $[Zn_2(\mathbf{L}_3)]^{4+}$ was measured to be $K_s = (1.3 \pm 0.1) \times 10^5\ M^{-1}$ by fitting the data with a standard method for competition assays.[18] The equilibrium constant for binding of other tested anions to the receptor is too small to be measured accurately by the above method.

To determine the binding stoichiometry of complex $[Zn_2(\mathbf{L}_3)]^{4+}$ with PPi, the continuous variation methods were carried out (Fig. 4). The results show that the fluorescent intensity of the receptor–guest complex reaches a maximum when the molar fraction of $[Zn_2(\mathbf{L}_3)]^{4+}$ is 0.5, indicating the formation of a 1:1 complex between $[Zn_2(\mathbf{L}_3)]^{4+}$ and PPi.

3. Conclusions

In summary, a new chemosensing ensemble which shows high selectivity toward PPi in 100% aqueous solution at physiological pH has been developed. Using the readily available bis(2-benzimidazolylmethyl)amine as the substitute of DPA unit, the anion receptor $[Zn_2(\mathbf{L}_3)]^{4+}$ is easily synthesized, and the chemosensing ensemble we presented herein can effectively differentiate PPi from Pi and other biologically important anions in water.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.09.133.

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15. *Synthesis of ligand* $\mathbf{L_3}$: To a solution of **2** (1.35 g, 4.9 mmol) in 20 ml of methanol at ice/acetone bath, 0.505 g of NEt_3 was added followed by dropwise addition of a solution of **1** (0.505 g, 2.5 mmol) in 5 ml methanol. The solution was allowed to warm to room temperature and then refluxed for 0.5 h. Cooling and stirring at room temperature for 24 h resulted in the precipitation of a fine white powder. The ^{1}H NMR and MS data were in full agreement with the presented structure. ^{1}H NMR (300 MHz, DMSO-d_6), δ: 7.5 (s, 8H), 7.15 (s, 8H), 6.96 (s, 2H), 5.7 (s, 1H), 4.3 (s, 8H), 3.9 (s, 4H), 3.3 (s, 4H), 2.0 (s, 3H); HRMS (ESI) calcd for $C_{41}H_{38}N_{10}O$ 686.3230, found 686.3237.

16. (a) *Synthesis of complex* $[Zn_2(\mathbf{L}_3)]^{4+}$: To a solution of $\mathbf{L}_3$ (160 mg, 0.29 mmol) in 10 ml of hot ethanol was added methanolic solution of $Zn(NO_3)_2{\cdot}6H_2O$ (88 mg, 0.58 mmol), the mixture was stirred at reflux for 30 min. After cooling to rt, the resulted white precipitate was collected by filtration, washed with cold ethanol, and dried to give 0.20 g of the dizinc complex. Anal. Calcd for $C_{41}H_{41}N_{13}O_{12}Zn_2$: C, 47.41; H, 3.98; N, 17.53. Found: C, 47.02; H, 4.03; N, 17.64.; (b) Zhou, C. Q.; Deng, X. H.; Yang, P.. *Chem. Res. Appl.* **2005**, *17*, 448–451.
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