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Highly Selective Fluorescent Recognition of Pyrophosphate in Water by a New Chemosensing Ensemble

Lijun Tang · Minghui Liu · Fangfang Li · Raju Nandhakumar

Abstract A new chemosensing ensemble that displays sensitive and selective fluorescent recognition of pyrophosphate in water at pH 7.4 has been developed. The ensemble is constructed by a copper complex (receptor) and eosin Y (indicator), the constructed ensemble is capable of highly selectively discriminate pyrophosphate from other common existing anions such as CH_3COO^-, HSO_4^-, NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, NCS^-, I^-, Cl^-, Br^-, F^- as well as some structurally similar carboxylates such as citrate, tartrate, oxalate, malonate, succinate and glutarate.

Keywords Pyrophosphate · Fluorescent recognition · Chemosensing ensemble · Dicarboxylate

Introduction

During the past two decades, considerable attention has been devoted to the development of chemosensors for anionic species, because they are ubiquitous in biological systems and play significant roles in a wide area of pharmacy, biology and environmental sciences [1–3]. In particular, selective detection of the anion pyrophosphate

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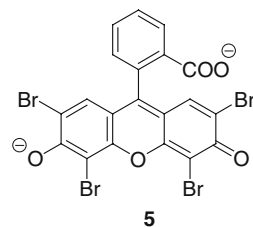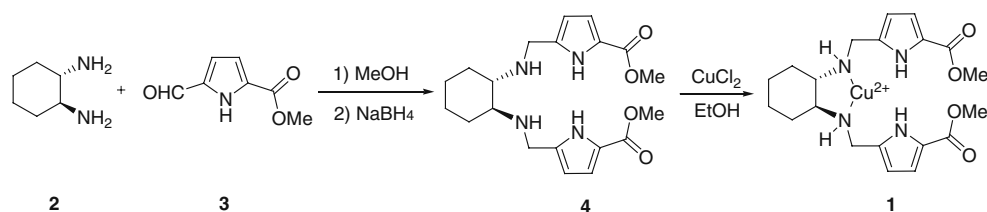Seoul 120-750, South Korea
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(PPi) is a major research focus, as it participates in several bioenergetic and metabolic processes [4]. It is also well known that patients with calcium pyrophosphate dihydrate crystals and chondrocalcinosis have been shown to have high synovial fluid PPi level [5, 6]. Moreover, the ability to detect PPi has become important in cancer research [7]. As a consequence, many researchers have made an effort to develop selective chemosensors for biologically potent PPi [8–24].

On the other hand, fluorescent sensing of PPi has become particularly attractive because of its simplicity and low detection limit [1–3, 25–30]. A convenient and increasingly popular method for sensing of anions is the displacement (ensemble) approach, which is based on competitive binding of an indicator and the analyte to a receptor [28, 31–33]. In this protocol, a signaling unit (indicator) is bound to a binding site (receptor) by non-covalent interactions to form a chemosensing ensemble. Treatment of the ensemble with the analyte results in displacement of the indicator from the receptor and restoration of the indicator's original spectral properties. This strategy avoids the complicated synthetic process for indicator-receptor chemically linked type sensors. Thus, one can focus on design of receptors having complementary shape with the target anion.

Anion sensing in water is generally regarded as a challenging aspect because of the strong hydration effects of anions as well as the ability to convert the anion recognition into a fluorescent or colorimetric signal [34]. Nevertheless, an effective strategy to solve this problem is by utilizing some of the carefully designed metallic receptors which can bind a target anion selectively and tightly in water [35–38]. Even though various types of chemosensors for selective recognition of PPi have been

Scheme 1 Synthesis of complex **1**

Structure of **5**

reported [8–24, 39], only a very few of them have taken structurally similar carboxylate anions as competing analytes [40, 41]. It is noteworthy that the sensors reported in literature 40 and 41 were all designed by the PET mechanism, they also have some disadvantages such as only could be used in pure organic solvent, and could not effectively distinguish PPi from dicarboxylates, for example, malonate and glutarate. As far as we are aware, there are no chemosensors that can distinguish PPi from competitive carboxylate anions in 100% aqueous solution at physiological pH has been documented.

Herein, we report a new chemosensing ensemble for fluorescent recognition of PPi in water at physiological pH. The ensemble is constructed by a copper complex **1** and eosin Y (**5**), which exhibits a high selectivity to PPi over other common existing anions and some structurally similar carboxylate anions.

## Experimental

Apparatus and Chemicals

^{1}H NMR spectra and ^{13}C NMR spectra were obtained on a Varian INOVA-400 MHz and Bruker AV-300 MHz Spectrometer, respectively. Chemical shifts were expressed in ppm and tetramethylsilane (TMS) was used as internal standard. High-resolution mass spectrometry (HRMS) was carried out on a UPLC/Q Tof mass spectrometer. Fluorescence spectra were obtained with a Hitachi F-4500 FL spectrophotometer at room temperature for aerated solutions.

Compound **3** was prepared according to a literature procedure [42]. Other reagents for synthesis obtained commercially were used without further purification. In the titration experiments, all the anions were added in the form of sodium salts.

Syntheses

Synthesis of Ligand 4

Compounds **2** (150 mg, 1.31 mmol) and **3** (500 mg, 3.27 mmol) were dissolved in anhydrous methanol (20 mL) in a 50 mL round bottomed flask and stirred under nitrogen atmosphere for 6 h at room temperature. Then the reaction mixture was cooled to 0 °C and $NaBH_4$ (160 mg, 4.10 mmol) was added portion-wise during 1 h, and the reaction mixture was allowed to stir for further 1 h. After the completion of the reaction, the solvent was removed under reduced pressure to give a white residue, which on recrystallization from ethyl acetate-hexane (1:19, v/v) gave **4** as white solid. Yield: 77%, mp: 107–108 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.83 (d, J=3.6 Hz, 2 H), 5.97 (d, J=3.6 Hz, 2 H), 4.26 (d, J=16 Hz, 2 H), 4.01 (d, J=16 Hz, 2 H), 3.78 (s, 6 H), 2.24
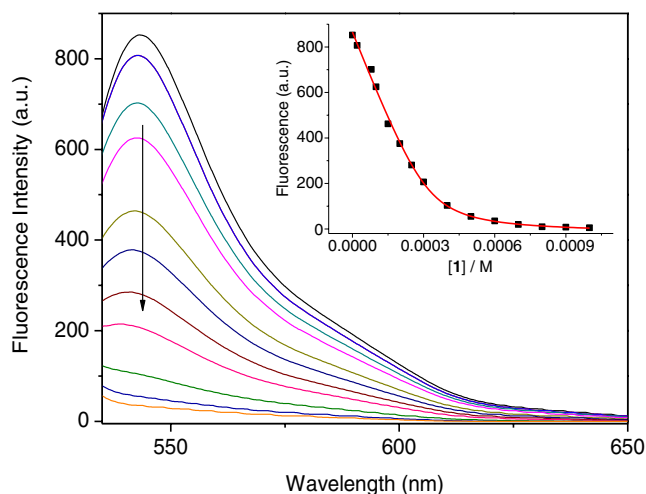
Fig. 1 Fluorescence changes of **5** (1.0×10^{-5} M, λ_{ex}=523 nm, λ_{em}=543 nm) upon the addition of complex **1** in aqueous buffer solution (HEPES 10 mM, pH=7.4). (Inset: plot of F_{543} vs concentration of **1**.)
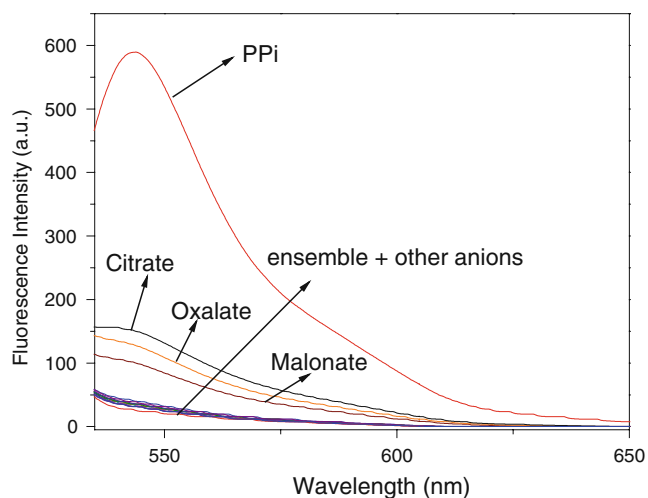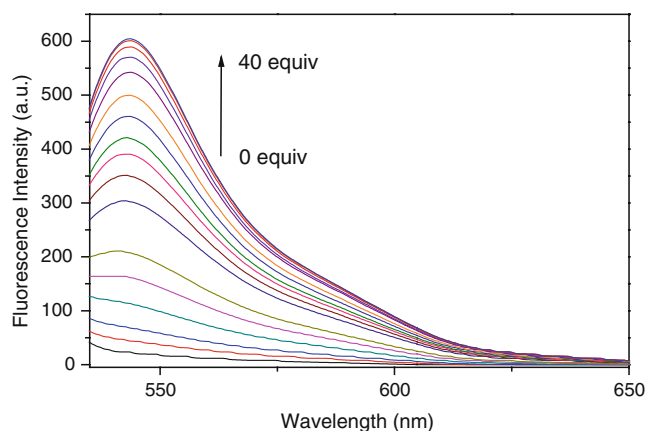
Fig. 2 Fluorescence changes of the ensemble upon the addition of various anions (4.0×10^{-4} M for each anion)
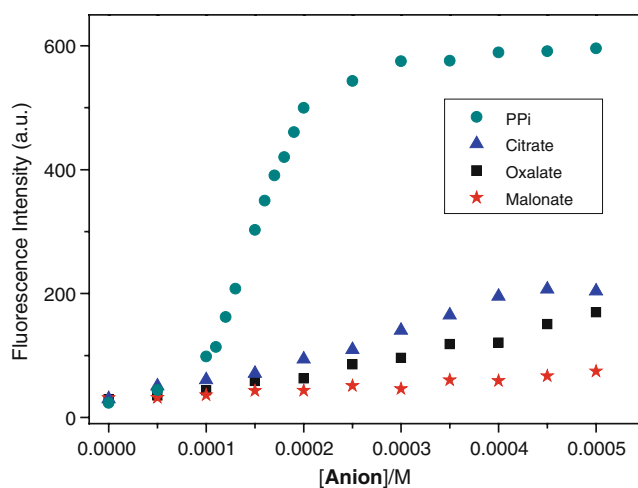

Fig. 4 Fluorescence changes of the ensemble recorded at 543 nm upon titration by some representative anions in buffered water solution (HEPES 10 mM, pH=7.4). PPi (*black circle*), Citrate (*black up-pointing triangle*), Oxalate (*black square*), Malonate (*black star*)

(d, J=9.2 Hz, 2 H), 2.18 (d, J=13.2 Hz, 2 H), 1.81 (b, 4 H), 1.72 (d, J=8.4 Hz, 2 H), 1.17 (t, J=10 Hz, 2 H), 1.01 (d, J=8.4 Hz, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 163.4, 137.5, 121.4, 116.2, 106.8, 61.4, 51.1, 43.6, 32.9, 25.2. HRMS (ESI+) m/z calcd. for $C_{20}H_{29}N_4O_4$ [**4**+H]$^+$: 389.2189 and found: 389.2193.

Synthesis of Copper Complex 1

To an ethanol solution of **4** (100 mg, 0.26 mmol), an ethanol solution of CuCl$_2$·2H$_2$O (56 mg, 0.33 mmol) was added and stirred for 1 h at room temperature. The blue precipitate formed was filtered and washed thoroughly with cold diethyl ether to give complex **1** in 70% yield (102 mg). UPLC/Q-TOF MS (ESI+) m/z calcd. for $C_{20}H_{27}CuN_4O_4$ [**1**-H]$^+$: 450.1317 and found: 450.2875. Scheme 1.

Results and Discussion

Determination of Chemosensing Ensemble

After the screening of several of fluorescent indicators for this chemosensing ensemble, eosin Y (**5**) was finally selected for this study. The fluorescence emission of **5** was quenched when it was mixed with copper complex **1**, which is attributed to the energy or electron transfer effect between **1** and **5** [43]. Fluorescence changes of **5** (1.0×10^{-5} M, HEPES 10 mM, pH=7.4) upon the addition of an aqueous buffer solution (HEPES 10 mM, pH=7.4) of complex **1** was firstly investigated (Fig. 1). Upon incremental addition of copper complex **1** to solution **5**, the fluorescence intensity of **5** gradually decreased at 543 nm (excited at 523 nm) and completely quenched when 60 equiv of complex **1** was used, accompanied with an obvious color change from orange to pink. Nonlinear least-squares fitting of the titration profiles (inset of

Fig. 3 Titration of an aqueous solution of the ensemble (HEPES 10 mM, pH=7.4) with standard PPi solution
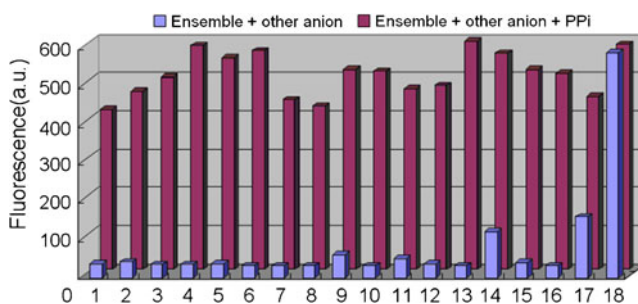
Fig. 5 Fluorescence response of the ensemble to selected anions in buffered water solution (HEPES 10 mM, pH=7.4). From left to right: I$^-$, Cl$^-$, Br$^-$, F$^-$, CH$_3$COO$^-$, HSO$_4^-$, HPO$_4^{2-}$, PO$_4^{3-}$, malonate, SCN$^-$, glutarate, NO$_3^-$, H$_2$PO$_4^-$, oxalate, succinate, tartrate, citrate and PPi

Fig. 1) employing the 1:1 binding mode equation strongly support the formation of a 1:1 complex of **1** and **5** and the association constant K_a was calculated to be 5.15×10^4 M^{-1} [44, 45]. Thus, a chemosensing ensemble solution (HEPES 10 mM, pH=7.4) composed of **5** (1.0×10^{-5} M) and complex **1** (6.0×10^{-4} M) can be readily prepared.

Selective Fluorescent Recognition of PPi

Fluorescence intensity changes of the prepared chemosensing ensemble upon addition of various anions (4.0×10^{-4} M for each anion) are illustrated in Fig. 2. A significant fluorescence revival of **5** was observed upon addition of PPi. This result indicates the successful competitive binding of the PPi anion and displacement of **5** from the receptor. Some carboxylate anions such as citrate, oxalate and malonate can also induce, to some extent, fluorescence revival of **5**, but the revival levels are very small compared with that of PPi. Whereas, no noticeable fluorescence changes were observed upon addition of other anions such as CH_3COO^-, HSO_4^-, NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, NCS^-, I^-, Cl^-, Br^-, F^- as well as other dicarboxylates such as succinate, glutarate and tartrate. The PPi induced displacement of indicator **5** from the receptor also can be easily conformed by visual detection, upon the addition of PPi, the pink color of the ensemble changed to orange. These results revealed that the chemosensing ensemble has a high selectivity toward PPi.

Fluorescence Titrations

To gain further insight into the sensing ability of the ensemble to PPi, a titration experiment was subsequently carried out (Fig. 3). Upon incremental addition of PPi to the ensemble solution, a fluorescence emission peak appeared at 543 nm and gradually developed with increase in PPi concentration. The fluorescence revival of **5** reached the saturation point when 40 equiv (relative to **5**) of PPi was added. The binding constant between PPi and **1** was measured to be $K_s=1.17 \times 10^5$ M^{-1} by fitting the data with the standard method for competition assays [44, 45].

Furthermore, to verify the high selectivity of the ensemble to PPi, the ensemble was subjected to titration using some representative anions such as oxalate, citrate and malonate. As shown in Fig. 4, compared with PPi, these three tested anions can only lead to relatively small fluorescence revival of **5** when the titration reaches the saturation point. This demonstrates that the constructed ensemble has a high selectivity toward PPi over other anions.

Competitive Experiments

In order to test the anti-jaming ability of the ensemble, competitive experiments in the presence of some potentially competitive anions were carried out. As shown in Fig. 5, except PPi, other anions (40 equiv to **5**) did not produce dramatic fluorescence emission changes. However, upon addition of PPi (40 equiv to **5**) to the solution containing both ensemble and other anion, a significant fluorescence emission enhancement is observed. These results indicate that the recognition of PPi by the ensemble is not significantly influenced by other coexisting anions, which strongly proves that the ensemble exhibits a high selectivity toward PPi.

Conclusions

In summary, we have developed a new chemosensing ensemble which exhibits high selectivity toward PPi in 100% aqueous solution at pH 7.4. The readily prepared chemosensing ensemble can effectively differentiate PPi from other common existing anions such as CH_3COO^-, HSO_4^-, NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, NCS^-, I^-, Cl^-, Br^-, F^- and some potentially competitive carboxylates such as citrate, tartrate, oxalate, malonate, succinate and glutarate. Moreover, the PPi recognition process by the ensemble is proved to be hardly influenced by other coexisting anions.

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