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Pyridinium-Based Fluororeceptors As Practical Chemosensors for Hydrogen Pyrophosphate ($HP_2O_7^{3-}$) in Semiaqueous and Aqueous Environments

Kumaresh Ghosh,*,† Avik Ranjan Sarkar,† Asmita Samadder,‡ and Anisur Rahman Khuda-Bukhsh‡

Departments of Chemistry and Zoology, University of Kalyani, Kalyani-741235, India

ghosh_k2003@yahoo.co.in

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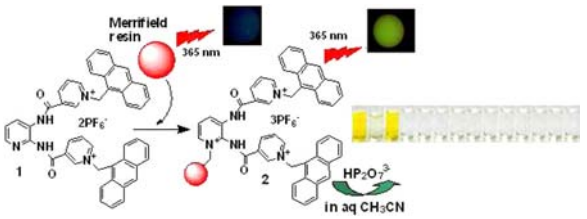
ABSTRACT



The pyridinium-based fluororeceptor 1 and the sensor bead 2 recognize hydrogen pyrophosphate effectively through the 'Indicator Displacement Assay' (IDA) technique over a series of other anions in aq CH_3CN (CH_3CN/H_2O = 4:1, v/v, pH = 6.5). The sensor bead 2 is also capable of sensing the same anion selectively in pure water.

The selective detection of pyrophosphate (PPi) is an important research topic in supramolecular chemistry. PPi is a biologically important target because it not only is the product of ATP hydrolysis under cellular conditions[1] but also is involved in DNA sequencing/replication, etc.[2] It has also physiological relevance in energy storage and signal transduction, in addition to being a structural component in bones and teeth.[3] Recently, the detection of PPi has become important in cancer research.[2,4] Patients with calcium pyrophosphate dehydrate (CPPD) crystals and chondrocalcinosis have been shown to have a high synovial fluid PPi level.[5] Sensing of this important anion in an aqueous or semiaqueous medium requires the precise design of receptor. In this context, the fluorescent and colorimetric sensors are found to be promising for their successful recognition.[6] Among the various designs, anthracene bearing a polyamine based receptor was reported to detect PPi in a 100% aqueous solution.[7] Metal ion

† Department of Chemistry.
‡ Department of Zoology.

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complexes have also been established as the ideal binding sites for PPi recognition in aqueous solution.[6a,8] It is to be mentioned that some metal complex systems that lack a fluorophore can be considered in the sensing process using the fluorescence competition assay.[6b,9] Besides the metal complexes, researchers have also used different H-bonding sites such as pyrene functionalized guanidinium,[10] calixpyrrole bearing thiourea groups,[11] dipyrrolyl quinoxalines,[12] pyrrole-based triazolophane,[6j] imidazolium-based macrocyclic/acyclic receptors,[13] and a triazole tethered ferrocene–pyrene dyad[14] for complexing PPi anions in organic solvents. To date, a pyridinium-based fluororeceptor for complexing hydrogen pyrophosphate anion is unknown. Pyridinium-based receptors with good electrophilic character[15a] bind anions strongly by involving H-bonding and charge–charge interactions. Also, they are easily synthesized and can easily be appended on the solid support to access practical-based supramolecular systems. The good response of pyridinium-based receptors in the visual sensing of anions in a semiaqueous environment through the IDA (indicator displacement assay) technique[15b] makes them appealing for existing systems.[6]

From our work on anion recognition by artificial designed receptors,[15] we report here the design and synthesis of pyridinium-based simple molecular architecture **1** and sensor bead **2** that respond competently in the IDA technique for naked-eye detection of hydrogen pyrophosphate over related analogues such as ATP, ADP, AMP, and other anions tested in CH_3CN/H_2O (4:1 v/v) at pH = 6.5 (10 mM Tris HCl buffer). Also, **2** allows detection of the same anion in pure water at pH 6.5.

Receptors **1** and **2** were accomplished according to Scheme S1 (Supporting Information (SI)). **1** was fully characterized by ^1H, ^{13}C NMR and mass spectroscopy. **2** was characterized by recording FTIR, fluorescence, and SEM images (SI).

The recognition ability of **1** toward a series of anions (taken as their tetrabutylammonium and sodium salts) was studied by fluorescence, UV–vis, and ^1H NMR methods. In fluorescence titration, carried out in CH_3CN/H_2O (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer), the emission intensity of **1** was significantly enhanced only in the presence of hydrogen pyrophosphate ($HP_2O_7^{3-}$). Other anions except ATP indicated less or negligible change in the emission of **1** (Figure S1). This is clearly understood from Figure 1. A similar feature was noted while the interaction was studied in CH_3CN/H_2O (4:1 v/v) at pH = 6.5 using 0.1 mM Tris HCl buffer (Figure S2). This signifies the key role of anions in the binding event. However, upon complexation of $HP_2O_7^{3-}$ the monomer emission of **1** increased considerably with a red shift of 5 nm without showing any other peak at higher wavelength (Figure S1l). Although no peak at higher wavelength for the excimer/exciplex was observed in aq CH_3CN, a peak at ~520 nm in the presence of $HP_2O_7^{3-}$ was noted when the titration was performed in pure CH_3CN (Figure S3b).

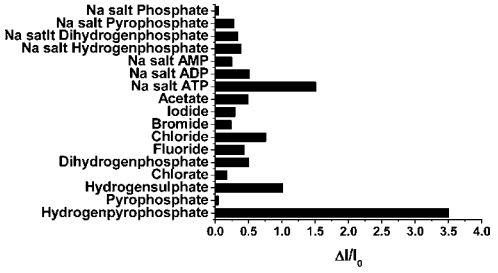

Figure 1. Fluorescence ratio of **1** ($c = 2.5 \times 10^{-5}$ M) at 412 nm upon addition of 10 equiv of tetrabutylammonium and sodium salts of a particular anion in CH_3CN/H_2O (4:1 v/v, 10 mM TrisHCl buffer, pH 6.5).

This is attributed to the role of solvent polarity for which a stronger interaction of $HP_2O_7^{3-}$ into the cleft of **1** in CH_3CN influences the formation of either an anthracene-stacked excimer or a pyridinium–anthracene exciplex in the excited state. Among the different ions examined in CH_3CN, only $HP_2O_7^{3-}$ perturbed the emission of **1** strongly (Figure S3a, c) and this complexation-induced increase in emission is attributed to the restricted rotation of the pyridinium amide groups[16] upon recognition for which the PET process occurring between the excited state of anthracene and the binding site is inhibited.

UV–vis titrations of **1** with the same anions were carried out in CH_3CN/H_2O (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer). The change in absorption of **1** upon complexation was also appreciable, and particularly, it was meaningful with $HP_2O_7^{3-}$ (Figure S4) involving 1:1 stoichiometry[17] (Figure S5a, b). Nonlinear curve fittings of both emission

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and absorbance data for **1** with $HP_2O_7^{3-}$ gave the binding constant values[18] $(9.59 \pm 1) \times 10^4$ and $(1.96 \pm 0.5) \times 10^5$ M^{-1}, respectively (Table S1 in SI). The binding selectivity was determined by observing the emission behavior of **1** upon addition of $(TBA)_3HP_2O_7$ to the solution of **1** in CH_3CN/H_2O (4:1 v/v; pH = 6.5) containing other interfering anions (Figure S6c). It is noteworthy that all the anions including ATP negligibly interfered in the binding of $HP_2O_7^{3-}$. Careful analysis of Figure S6c reveals that $HP_2O_7^{3-}$ shows an ~5 times higher increase in fluorescence ratio in the presence of ATP.

To understand the effect of pH in the sensing process, it was found that the change in emission of **1** at pH = 7.5 was noticeably less than the change occurring in an acidic medium (pH = 6.5) (Figure S7) suggesting less efficiency in a slightly alkaline medium.

For naked-eye detection, we investigated the binding of **1** to $HP_2O_7^{3-}$ through the IDA technique[19] using uranine dye **3**. Upon addition of **1** ($c = 4 \times 10^{-4}$ M) to a solution of **3** ($c = 8.5 \times 10^{-5}$ M) in CH_3CN/H_2O (4:1 v/v; pH = 6.5, 10 mM Tris HCl buffer), both the absorption and emission of **3** were significantly reduced (Figures S9 and S10) and the resulting solution became colorless (Figure 2). The 1:1 stoichiometry of **3** with **1** was confirmed by Job's method[17] (Figure S11), and analysis of absorption and emission data gave the binding constants[17] $(6.34 \pm 0.5) \times 10^4$ and $(4.69 \pm 0.8) \times 10^4$ M^{-1}, respectively. These values are close in magnitude to the binding constant between **1** with $HP_2O_7^{3-}$, and thus combined **1**/dye **3** will be ideal for sensing $HP_2O_7^{3-}$. In practice, it was found to be true. While $HP_2O_7^{3-}$ was added to the **1**/dye **3** ensemble, dye was displaced from the binding cavity and its absorbance (Figure S9), fluorescence (Figure 3), and original color were retrieved. Other anions in the study did not restore the characteristic color of dye (Figure 2).

To understand the strong binding of $HP_2O_7^{3-}$ into the cleft of **1**, ^1H NMR of **1** itself and in the presence of an equivalent amount of $HP_2O_7^{3-}$ in d_6-DMSO was recorded (Figure S15). CD_3CN/D_2O was not used due to the presence of deuterium exchangeable amide protons in **1**. Yet, upon addition of an equivalent amount of $HP_2O_7^{3-}$ to the solution of **1** in d_6-DMSO, the amide protons were too broad to detect. Also, *ortho* protons (H_c, H_d) at 9.43 ppm moved downfield by 0.65 and 054 ppm as two different signals. Another set of *ortho* protons (H_e and H_f) also underwent a downfield shift by 0.21 ppm. During interaction, the central pyridine ring proton H_g moved downfield by 0.12 ppm.

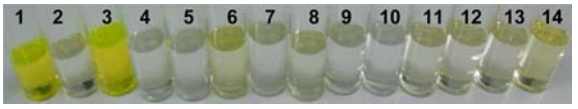

Figure 2. (1) **dye**, (2) **1** + dye **3** (1:1) = A, A with 1 equiv of (3) $HP_2O_7^{3-}$, (4) ATP, (5) ADP, (6) AMP, (7) $H_2PO_4^-$, (8) AcO^-, (9) ClO_4^-, (10) HSO_4^-, (11) Cl^-, (12) Br^-, (13) I^-, (14) F^-; [dye **3**] = 8.5×10^{-5} M, [**1**] = 4.0×10^{-4} M, [G] = 1.16×10^{-3} M.

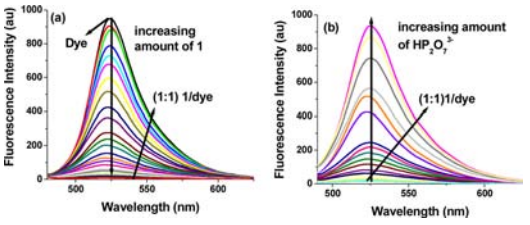

Figure 3. (a) Change in emission intensity of dye **3** ($c = 8.5 \times 10^{-5}$ M) upon increasing addition of **1** ($c = 4.0 \times 10^{-4}$ M) and (b) retrieval of emission upon gradual addition of $HP_2O_7^{3-}$ to the ensemble **1**/dye **3** in aq CH_3CN (4:1 v/v, 10 mM Tris HCl buffer, at pH 6.5).

The $-CH_2-$ protons labeled as H_h and H_i, originally appeared as two separate signals, showed a weak downfield shifting (0.12 ppm), and merged together. Also, in ^{31}P NMR, recorded in d_6-DMSO, signals for both the P-atoms of tetrabutylammonium hydrogen pyrophosphate underwent downfield shifting upon complexation (Figure 4A).

Thus, based on NMR study a binding structure is proposed in Figure 4B. DFT calculation using the 6-31G(d) basis set and B3LYP functional clearly elucidates the H-bonded network in the cleft (Figure S16). In the complex, a separation of 5.85 Å between two anthracenes reveals that $HP_2O_7^{3-}$ binding induced signal in the emission at ~520 nm is presumably due to the exciplex formation between pyridinium and anthracenes.

For practical application,[20] sensor bead **2** (Scheme S1) was studied next as in the case of **1** in CH_3CN/H_2O (4:1 v/v; pH = 6.5, 10 mM Tris HCl buffer). In the IDA technique, 5 mg of **2** were added to a 2.3 mL solution of dye **3** ($c = 8.5 \times 10^{-5}$ M) (CH_3CN/H_2O = 4:1 v/v; at pH = 6.5) and the change in absorption spectra was recorded with time. After 30 min, the characteristic absorption peak at 502 nm for **3** was considerably reduced and the resulting solution became colorless (Figure S17a). Upon addition of $(TBA)_3HP_2O_7$ to the **2**/dye **3** ensemble, adsorbed **3** is displaced from the binding zone of **2** for which its absorbance is retrieved (Figure S17b). Similar findings were noticed in the emission (Figure S18a, b). The color of the solution turned

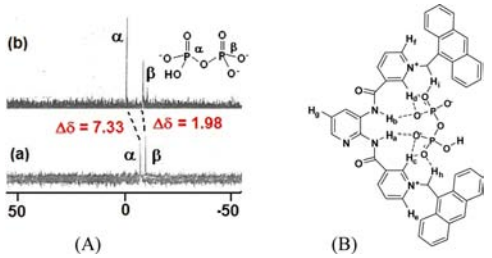

Figure 4. (A) ^{31}P NMR of (a) $(TBA)_3HP_2O_7$ and (b) in presence of equivalent amount of **1**. (B) Proposed structure of complex of **1** with $HP_2O_7^{3-}$.

Figure 5. (1) **dye**, (2) [dye **3** + **2**] = A, (3) A + $HP_2O_7^{3-}$, (4) A + $H_2PO_4^-$, (5) A + AcO^-, (6) A+ I^-, (7) A + Br^-, (8) A + F^-, (9) A + ClO_4^-, (10) A + $P_2O_7^{2-}$, (11) A + ^-OH, (12) A +ATP, (13) A + ADP, (14) A + AMP. ([dye **3**] = 8.5×10^{-5} M).

into the original color of the **3** (Figure 5). Other anions were unable to displace dye from **2** (Figure S19). Even in the presence of all anions with **2**/dye **3**, $HP_2O_7^{3-}$ was capable of restoring the characteristic absorption and fluorescence intensities of dye **3** and also the color of the dye (Figures S20−S23). In the IDA experiment the adsorption of the dye onto the bead **2** was confirmed by recoding both absorption and emission in the solid state (Figure S24). Figure 6 corroborates the change in color of sensor bead **2** during the IDA experiments while irradiating the beads at 365 nm. The SEM images of the beads of **2** as shown in Figure S25 also clearly indicated the change in morphologies of the beads during adsorption and release of the dye. It is to be mentioned that the recovered beads of **2** from the IDA experiment were usable five times. Further use was impossible as the beads became brittle. Importantly, **2** also detected $HP_2O_7^{3-}$ by the IDA technique in water at pH 6.5, and Figure S28 displays the colorimetric change. In Figure 5, the dye starts to release from the ensemble at 2×10^{-4} M $HP_2O_7^{3-}$ and then a linear increase in absorbance with [$HP_2O_7^{3-}$] (Figure S29), which can be useful for determining [$HP_2O_7^{3-}$], is noted.

Blood serum samples (taken from mice) which usually contain phosphate and various carboxylates were supposed to interact with the receptor tagged bead **2** in water. We introduced bead **2** into serum samples (50 μL) (pH 7.4) which were incubated in 96 well plates for 30 min at 37 °C. Milli Q water (50 μL) was used as the control. The experimental series included groups, namely, water plus **2**, water plus **2**/**3**, serum plus **2**, and serum plus **2**/**3** (Figure 7). As can be seen from Figure 7e, in the serum containing dye-adsorbed beads of **2**, an intense release of green fluorescing dye from the outer surface of the beads was clearly noticed. Indeed, we carried out a similar experiment by contaminating the serum (1 μL) with hydrogen pyrophosphate at different concentrations (10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M). It was observed that the dye was released from the surface of the beads, present in the serum contaminated with 10^{-3}, 10^{-4}, and 10^{-5} M $HP_2O_7^{3-}$ but not from the serum with 10^{-6} M $HP_2O_7^{3-}$ (Figure S30). In a similar study, dye is slightly released from the surface of the bead in serum contaminated with ATP in a neutral medium (Figure S31). The change in pH of the medium showed that a slightly acidic medium (pH = 6.5) hindered the release of dye from the surface of the bead whereas neutral pH accelerated its release (Figure S32).

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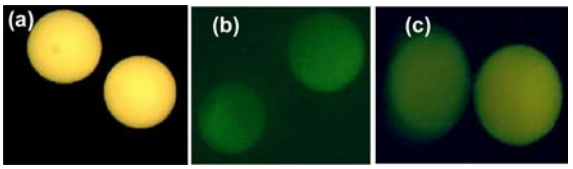

Figure 6. Fluorescence microscopic images of (a) **2**, (b) **2** after incubation in 2.3 mL of dye **3** solution ($c = 8.5 \times 10^{-5}$ M), and (c) **2** when $(TBA)_3HP_2O_7$ was added into the solution of **2**/dye **3** ensemble while irradiating the beads at 365 nm.

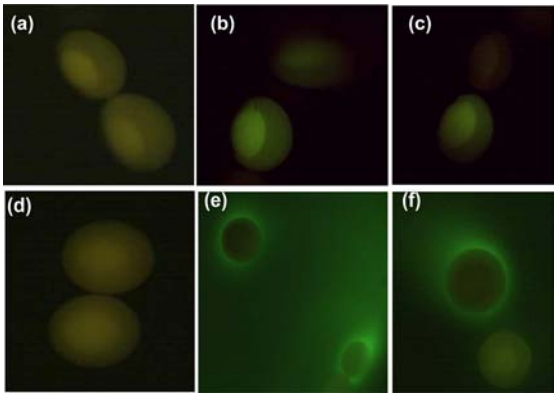

Figure 7. (a) Water (control) + **2**, (b) water (control) + dye/**2**, (c) comparison of **2** and dye/**2** in water, (d) **2** in serum, (e) dye/**2** in serum, (f) comparison of **2** and dye/**2** in serum.

In conclusion, we established that the selective sensing of $HP_2O_7^{3-}$ is possible by exploiting our pyridinium amide based receptor **1** in both CH_3CN and CH_3CN/H_2O (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer). H-bonding, charge−charge interaction, and the dimension of the cleft play a pivotal role in providing selectivity in the recognition process. The chemosensor **1** responds in the IDA technique to report naked-eye detection of $HP_2O_7^{3-}$ in solution. To make **1** more practical, the use of solid support is possible. In this regard, beads of **2** efficiently recognize $HP_2O_7^{3-}$ in CH_3CN/H_2O (4:1 v/v) and pure water at pH 6.5 *via* the IDA technique. Moreover, the experiments with blood serum suggest that the sensor bead **2** is capable of selectively sensing $HP_2O_7^{3-}$ in complex biological milieu.

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Supporting Information Available. Synthesis of **1** and **2**, figures showing absorbance and emission changes, Job plots, binding constant tables, and also other selected curves. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.