

A highly selective fluorescent probe for pyrophosphate detection in aqueous solutions

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ABSTRACT: A novel and simple fluorescence enhancement method is introduced for selective pyrophosphate (PPI) sensing in an aqueous solution. The method is based on a 1:1 metal complex formation between tris(8-hydroxyquinoline-5-sulphonate) thulium(III) [Tm(QS)₃] and PPI ion. The linear response covers a concentration range of 1.6×10^{-7} – 1.0×10^{-5} mol/L PPI and the detection limit is 2.3×10^{-8} mol/L. The association constant of Tm(QS)₃–PPI complex was calculated as 2.6×10^5 mol/L. Tm(QS)₃ shows a selective and sensitive fluorescence enhancement toward PPI ion in comparison with I₃⁻, NO₃⁻, CN⁻, CO₃²⁻, Br⁻, Cl⁻, F⁻, H₂PO₄⁻ and SO₄²⁻, which is attributed to higher stability of the inorganic complex between pyrophosphate ion and Tm(QS)₃. Copyright © 2011 John Wiley & Sons, Ltd.

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Introduction

Anions are generally larger than cations such as metal ions, therefore anions are more subject to solvation than cations. In organic solvents, it is not difficult to capture and detect anions because the solvation energy is relatively small and electrostatic interactions can operate effectively. It is very difficult to recognize anions in aqueous solvents, which are relevant to biological applications, because of their strong hydration. To date, only a few anion fluorescent sensors that work in aqueous solution have been developed, while many anion fluorescent sensors are known for organic environments (1).

An anion fluorescent sensor for use in aqueous solution should have two requirements; one is a sufficiently strong affinity for anions, and the other is the ability to convert an anion recognition signal into a fluorescent signal. It is difficult to satisfy both requirements simultaneously. Most known anion sensors do not have a sufficiently strong affinity for anions in water, while they satisfy the latter requirement. Although some anion hosts can capture anions in aqueous solvent, they are only host molecules, not sensor molecules (1).

Metal-based sensing systems use metal–ligand interactions for the recognition of target anions, and thus result in high selectivity, a large binding constant and good solubility in aqueous solution (2,3). We have recently reported a number of highly selective and sensitive anion sensors based on metal complexes (4–7).

Since many organic and inorganic anions play important roles in living organisms, there is now an increasing interest in anion recognition and anion sensing (8–17).

Among the various anionic analytes, pyrophosphate (PPI) is a biologically important target because it is produced in ATP hydrolysis in cellular conditions (18). PPI also plays an important role in energy transduction in organisms and could control metabolic processes by participating in enzymatic reactions (19).

Until now, some examples of selective PPI fluorescent sensors have been reported but only a few display turn-on in emission

spectra (20–22). Here, we present a novel fluorescent turn-on PPI sensor using Tm(QS)₃ possessing three 8-hydroxyquinoline (Scheme 1).

Experimental

Reagents

All Chemicals were of the reagent grade from Fluka and Merck chemical companies. The fluorogenic reagent bis(8-hydroxyquinoline-5-sulphonate) thulium(III) chloride [Tm(QS)₃] was prepared as follows (23). An ethanolic solution of TmCl₃ was added to an ethanolic solution of HQS under stirring with the molar ratio of Tm³⁺:HQs being 1:2. Then the pH of the solution was adjusted to 7.0 by adding an ammonium hydroxide solution (2 mol/L) and an appropriate amount of water was added and the mixture was stirred at room temperature for 10 h. The obtained yellow precipitates were collected by filtration and washed with water and cold ethanol three times.

¹H-NMR (DMSO, 500 MHz): σ_{H} 6.16 (m, 1 H), 7.8 (m, 3 H), 8.6 (m, 1 H), 9.19 (m, 1 H). IR data: ν_{max} (KBr pellets)/cm⁻¹ 3175 (C–H str.), 1323, 1370, 1402, 1460 (C–C, C–N, CO str. and C–H bend.) 1498, 1577, 1604 (C–C str.) 754 (Al–N str.).

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Apparatus

All fluorescent measurements were carried out using a Perkin-Elmer LS50 luminescence spectrometer.

Results and discussion

In order to evaluate whether $\text{Tm}(\text{QS})_3$ could be used as a selective fluorescent chemosensor for PPI ion, in preliminary experiments complexation of $\text{Tm}(\text{QS})_3$ with a number of anions was investigated spectrofluorometrically in an aqueous solution at $25.0 \pm 0.1^\circ\text{C}$. A 5.0×10^{-6} mol/L solution of $\text{Tm}(\text{QS})_3$ in aqueous solution was titrated with μM amounts of 1.0×10^{-4} mol/L solutions of anions spectrofluorometrically. Fluorescence titration experiments were recorded on excitation at 360 nm. As shown in Fig. 1, when titrations of ligand were performed with PPI a significant enhancement in its fluorescence intensity was obtained. No significant fluorescence changes were observed when F^- , H_2PO_4^- and other ions were tested.

The resulting mole ratio plot for PPI anion is shown in Fig. 2. As is obvious, the receptor forms a 1:1 complex with anions in aqueous solution. For the evaluation of formation constants of the resulting 1:1 complexes from fluorescence intensity vs mole ratio data, a non-linear least-squares curve-fitting programme, KINFIT, was used (24) and the results are given in Table 1. It is seen that, in aqueous solution, the stability of L complexes with anions decreases in the following order: $\text{PPI} > \text{H}_2\text{PO}_4^- > \text{F}^- > \text{CO}_3^{2-} > \text{Cl}^- > \text{SO}_4^{2-} > \text{NO}_3^-$. Thus, based on the relative stabilities of the resulting complexes, $\text{Tm}(\text{QS})_3$ was expected to act as a selective fluoroionophore in the preparation of an optical sensor for PPI ion.

The effect of PPI anion (sodium salt) on the absorption spectrum of ligand (5×10^{-5} M) was examined in an aqueous solution at 25°C (Fig. 3). As can be seen in Fig. 3, $\text{Tm}(\text{QS})_3$ was characterized by a broad absorption band centred at 250 nm and 360 nm, which can be contributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions.

Notably, titration of $\text{Tm}(\text{QS})_3$ against PPI ion led to a pronounced and two blue-shifted shoulders in UV spectra, with a well-defined three-isosbestic point at 244, 267 and 332 nm, respectively, indicating that the stable complex having a certain stoichiometric ratio between the receptor $\text{Tm}(\text{QS})_3$ and PPI had formed. $\text{Tm}(\text{QS})_3$ optical sensor did not show any obvious spectral changes upon the addition of H_2PO_4^- or other monovalent anions, such as CH_3CO_2^- , F^- , HCO_3^- and Cl^- , even up to an excess of 10 equiv.

As it can be seen from Fig. 4, the fluorescence emission spectrum of $\text{Tm}(\text{QS})_3$ is sensitive to PPI ion. In the presence of various concentrations of PPI ion in the range 1.6×10^{-7} – 1.0×10^{-5} mol/L,

significant fluorescence enhancement was observed. The fluorescence intensity was enhanced by increasing PPI concentration, which constitutes the basis for the recognition of PPI with the fluorescent probe proposed in this study.

The fluorescence quantum yield of $\text{Tm}(\text{QS})_3$ in water is 0.005, as determined by using QS in H_2O ($Q_F = 0.002$) as a reference (25). $\text{Tm}(\text{QS})_3$ shows larger enhancements (4.2-fold in Q_F) upon completely coordinating PPI.

The significant fluorescence enhancement might result from the electrostatic interaction between PPI and $\text{Tm}(\text{QS})_3$, in which two oxygen atoms of PPI coordinate with the centre of Tm^{3+} to

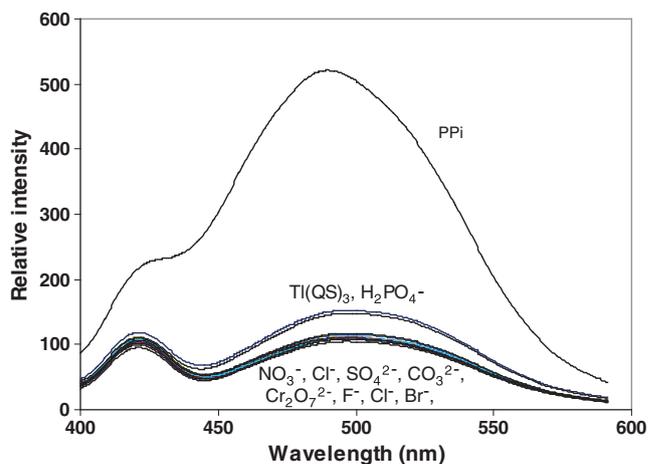


Figure 1. Fluorescence responses of $\text{Tm}(\text{QS})_3$ (3 mL, 1×10^{-6} mol/L) upon addition of different anions (50 μL , 5.0×10^{-3} mol/L) in aqueous solution ($\lambda_{\text{ex}} = 360$ nm).

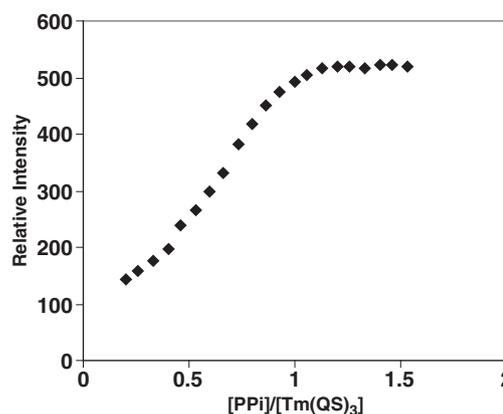
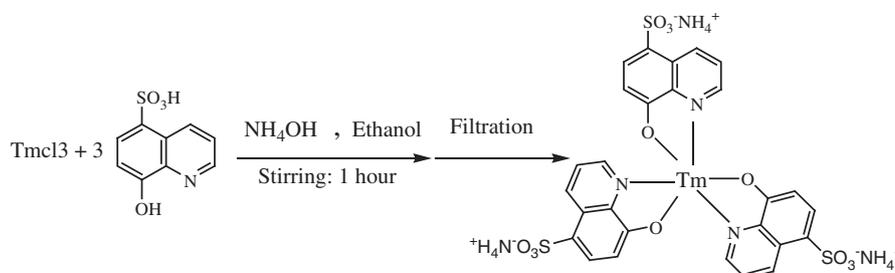


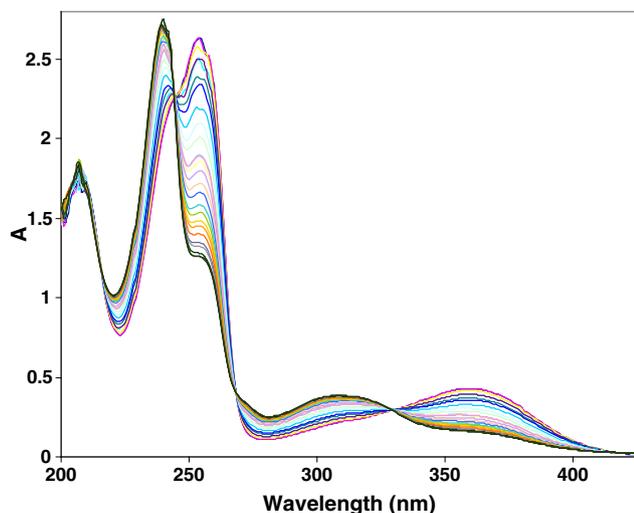
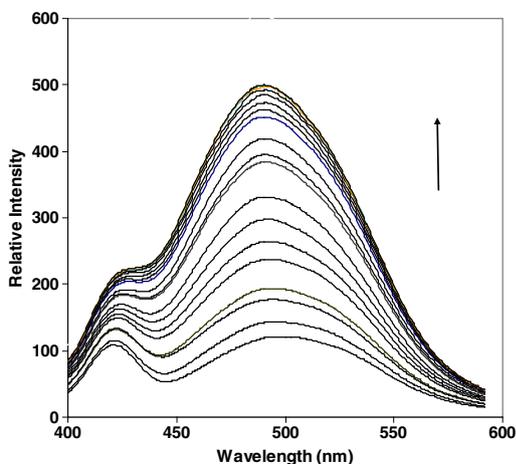
Figure 2. Fluorescence intensity vs $[\text{PPI}]/\text{Tm}(\text{QS})_3$ mole ratio plot in aqueous solution for PPI ions.



Scheme 1. Procedure of $\text{Tm}(\text{QS})_3$ synthesis.

Table 1. Association constants for various anions toward receptor L in aqueous solution

Cation	$\log K_f$
AcO ⁻	2.25 ± 0.10
H ₂ PO ₄ ⁻	3.45 ± 0.11
F ⁻	2.95 ± 0.10
PPI	6.61 ± 0.11
Br ⁻	<2.0
CO ₃ ²⁻	<2.0
BrO ₃ ⁻	<2.0
NO ₃ ⁻	<2.0
SO ₄ ²⁻	2.05 ± 0.17
Cr ₂ O ₇ ²⁻	2.08 ± 0.17
Cl ⁻	2.15 ± 0.11

**Figure 3.** Changes in the UV-vis spectra of Tm(QS)₃ (5×10^{-5} mol/L) upon addition of PPI (5×10^{-3} mol/L) in aqueous solution.**Figure 4.** Fluorescence titration of Tm(QS)₃ with PPI in an aqueous solution: Tm(QS)₃ = 5×10^{-6} mol/L; [PPI] = 5×10^{-4} mol/L; λ_{ex} = 360 nm.

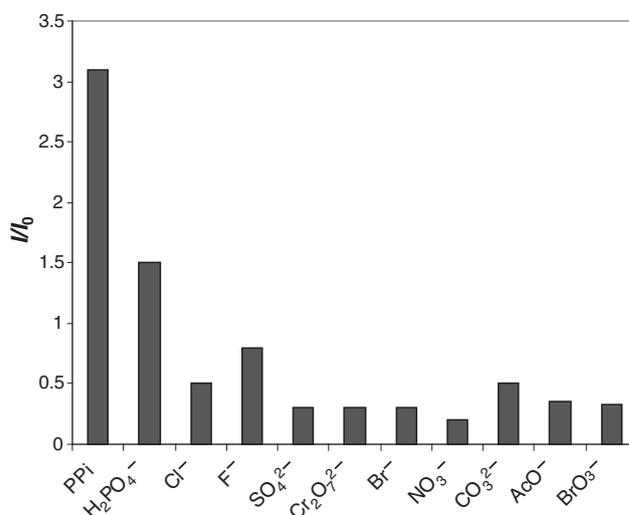
reduce the magnitude of the electron withdrawal via partial neutralization of the charge on the Tm³⁺ ion, thus increasing the electron-donating character of the QS moiety and finally resulting in an increased efficiency of ICT (1,20,25–27).

The detection limit using Tm(QS)₃ as a fluorescent sensor for the analysis of PPI ion was also determined from the plot of the fluorescence intensity as a function of the added anion concentrations. It was found that Tm(QS)₃ has a detection limit of 2.3×10^{-8} mol/L for PPI ions, which is quite low for the detection of PPI ions found in many chemical and biological systems.

Selectivity behaviour, i.e. the relative sensor response for the primary ion over other ions present in solution, is one of the most important characteristics of a chemosensor. To examine the selectivity of Tm(QS)₃, we investigated its affinity in the presence of PPI at 60 μ mol/L mixed with 120 μ mol/L other background anions, such as F⁻, Cl⁻, Br⁻, I⁻, H₂PO₄⁻, HSO₄⁻, CH₃COO⁻, NO₃⁻, SO₄²⁻ and P₂O₇⁴⁻. The influence of the intensity of solutions containing both background anions and PPI is shown in Fig. 5. As can be seen, other background anions had small or no obvious interference with the detection of PPI ions. All these results indicated that Tm(QS)₃ could be used as a potential candidate for a fluorescence chemosensor for PPI ion with very high selectivity.

Conclusion

A novel enhancement fluorescent chemosensor, Tm(QS)₃, for PPI detection in aqueous solution, was synthesized and investigated. It showed selective and sensitive fluorescence response to PPI in aqueous solution. The fluorescence enhancement of Tm(QS)₃ is attributed to 1:1 complex formation between Tm(QS)₃ and pyrophosphate, and this has been utilized as the basis for the selective detection of pyrophosphate over a wide PPI concentration range, and with no interference of background anions.

**Figure 5.** Fluorescence responses of Tm(QS)₃ (3 mL, 5×10^{-6} mol/L) containing 60 μ mol/L PPI and the background anions (120 μ mol/L) (λ_{ex} = 360 nm).

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