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Selective detection of pyrophosphate anion by a simple Cd(II) based terpyridine complex

Aditya Kumar Purohit, Biswa Nath Ghosh, Pravin Kumar Kar *

Department of Chemistry, Veer Surendra Sai University of Technology, Burla, Sambalpur 768018, Odisha, India

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ABSTRACT

A simple ratiometric terpyridine-Cd(II) complex was synthesized by the treatment of $CdCl_2$ with terpyridine ligand 4′-(4-*N*,*N*′-dimethylaminophenyl)-2,2′:6′,2″-terpyridine. The synthesized complex was found to act as a selective fluorescent chemosensor for pyrophosphate $P_2O_7^{4-}$ (PPi) over other anions like F^-, Cl^-, Br^-, CO_3^{2-}, SO_3^{2-}, AcO^-, NO_2^-, and $H_2PO_4^-$. Furthermore, the receptor probe was also successfully employed in HeLa cell for PPi detection, which indicates this can be used as a chemosensor for cells.

1. Introduction

In the recent years, recognition of pyrophosphate $P_2O_7^{4-}$ (PPi) has been found to be an emerging area of research in recent years. It is the product of ATP hydrolysis and has a critical role to play in several biological processes such as energy transduction in organisms, skeletal structure, protein regulation, cell signalling and organelle membranes recognition [1,2]. Thus considerable efforts have been made to develop receptor probes for detection of PPi [3–6]. Although many techniques have been developed for its detection, fluorescence technique appears to be most widely used method due to its high sensitivity, fast response and in vivo and in vitro imaging facility [7–10]. However, one major problem associated with the receptor probes has been their solubility issue in aqueous medium. PPi detection of the receptor probes using fluorescence techniques have mostly been studied in organic medium and only limited number of studies in aqueous medium has been reported [11–15].

Considering the importance of PPi detection, several Zn based receptor probes of the nitrogen based ligands such as bis(2-pyridylmethyl)amine (DPA) [16], terpyridine (tpy) [17,15] and amide functionalized macrocycles [18] have been reported. Among the nitrogen based ligands, terpyridine are of special importance [19–23]. They play a prominent role in research owing to interesting photophysical and electrochemical properties of their transition metal complexes and have been used in a wide range of applications including the field of luminescent sensor material, self-assembled hydrogelation, halogen bonding [24–27], dye sensitized solar cells [28], two-photon luminescent systems [29] as well as organic light-emitting devices [30].

Although studies have been performed for Zn based terpyridine complexes as receptor for PPi detection, to the best of our knowledge PPi sensing studies based on Cd^{2+} receptor has been less explored [31]. Cadmium has wide application in industry and agriculture, such as special alloys, nickel–cadmium batteries and phosphate fertilizers and causes a high level contamination in soil, water and foods [32,33]. Furthermore, disease like chrondrocalcinosis or calcium pyrophosphate dehydrates (CPPD) crystal deposition diseases can be recognized by its identification and detection [34,35]. Also Cd^{2+} is known to stimulate cell growth and DNA synthesis significantly at low concentration down to 100 pM in living cells [36].

Herein we report the PPi detection in water at physiological pH and micromolar level by employing a simple fluorescent terpyridine-Cd receptor probe. The probe was also able to bind selectively towards PPi over other anions like F^-, Cl^-, Br^-, CO_3^{2-}, SO_3^{2-}, AcO^-, NO_2^-, and $H_2PO_4^-$. Furthermore, this chemosensor was used in living HeLa cells to detect PPi.

2. Experimental Section

2.1. Materials and Methods

All reagents and solvents were purchased from commercial sources and used without further purification. The terpyridine ligand 4′-(4-*N*,*N*′-dimethylaminophenyl)-2,2′:6′,2″-terpyridine **L** was synthesized following literature method [37]. ^1H NMR and Mass spectra were recorded on Bruker 400 MHz and Maxis impact Bruker mass spectrometer. UV–Vis absorption spectra were recorded on Hitachi U-3010

* Corresponding author.
E-mail address: pkkar_chem@vssut.ac.in (P.K. Kar).

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Double beam UV–Vis spectrophotometer, whereas fluorescence spectra were obtained on a Cary Eclipse Fluorescence spectrophotometer.

2.2. Synthesis of $[Cd(L)(Cl)_2]$

To an ethanolic (5 mL) solution of $CdCl_2$ (0.027 g, 0.15 mmol), Ligand L (0.052 g, 0.15 mmol) in 6 mL dichloromethane was added and the reaction mixture was stirred at 50 °C for 3 h. The resultant precipitate was filtered and washed with ethanol for several times. The solid product was re-dissolved in a minimal volume of acetonitrile and an excess of n-hexane was added. The precipitated was filtered, washed again several times with diethyl ether, and dried under vacuum to get the product. Yield (77%) ^{1}H NMR (400 MHz, DMSO d_6) δ/ppm: 8.96–8.90 (d, 4H), 8.77–8.76 (d, 2H), 8.30–8.17 (d, 4H), 7.84 (s, 2H), 6.90–6.88 (d, 2H), 3.06 (s, 6H). HRMS *m/z* calcd for $[M\text{-}Cl]^+$ $(C23H20N4ClCd)^+$: 501.0410; found: 501.0406.

2.3. Fluorescence Study

A 100 μL of $CdCl_2$ (25 mM) solution was mixed with 1 mL of **L** (2.5 mM), which was diluted to 50 mL, to this receptor stock solution 0.005 M HEPES buffer was added to maintain physiological pH of 7.4. To keep homogeneity, all solutions were sonicated for 5 min after mixing of receptor and analyte for fluorescence measurements.

2.4. Detection of PPi in Live Cells

HeLa cells were developed with MEM having 10% fetal bovine serum and 1× penicillin/streptomycin antibiotics for 12 h and used at subsidizely. At pH 7.4, the cells were first washed with 10 mM HEPES buffer, including 137 mM NaCl, then 10 μM $CdCl_2$L was treated for 30 min at 37 °C in the same buffer. The cells were washed repeatedly with buffer before imaging. After laser excitation at 488 nm images were recorded having emission wavelengths between 555 and 655 nm for orange-red fluorescence.

3. Results and Discussion

The terpyridine ligand 4′-(4-*N*,*N*′-dimethylaminophenyl)-2,2′:6′,2″-terpyridine **L**, was synthesized by the reaction of 2-acetylpyridine with 4-(dimethylamino) benzaldehyde in presence of KOH and aqueous ammonia in ethanol. The cadmium-terpyridine metal complex was obtained in quantitative yield by treatment of **L** with $CdCl_2$ in 1:1 M ratio (in dichloromethane ethanol mixture) and crystallized by slow diffusion of diisopropyl ether into the acetonitrile solution of the complex [37].

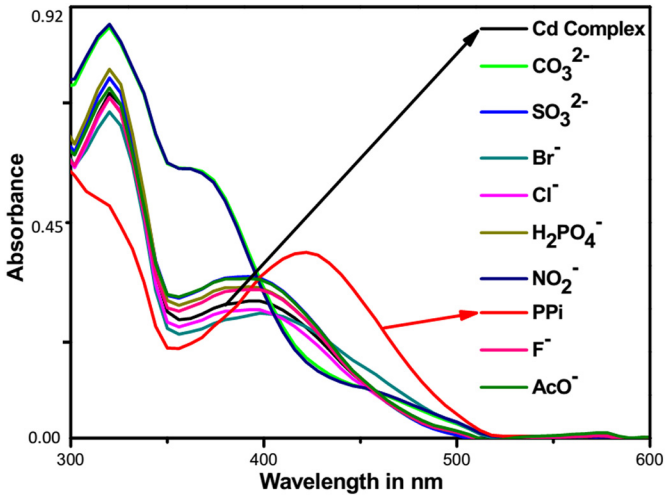

Fig. 1. UV–Vis plots of sensing of Cadmium complex with different Sodium anions.

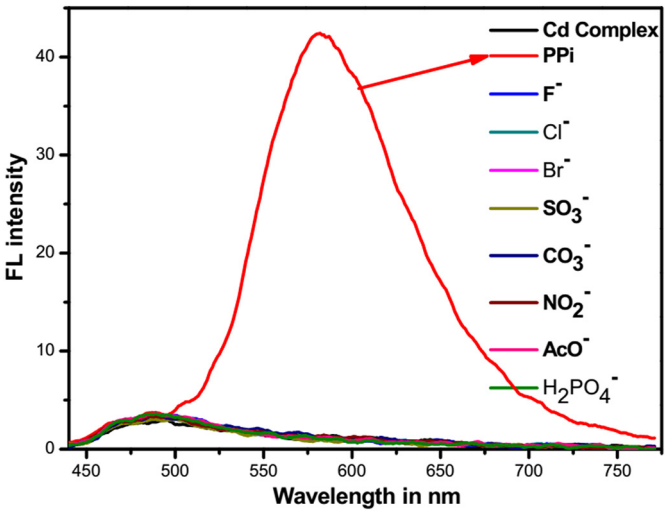

Fig. 2. Emission spectra of $CdCl_2$L (50 μM) in 10 mM HEPES buffer (pH 7.4) with different anion.

The complex was characterized by ^{1}H NMR spectroscopy and mass spectrometry. Also HeLa cells were examined using 1 × 71 Olympus fluorescence microscope. For all optical spectroscopic studies HPLC grade solvents were used.

The UV–Vis spectrum of the complex **$CdCl_2$L** (5.5×10^{-5} M) in HEPES buffer solution showed two major bands at 322 and 398 nm. In order to check the anion sensing capability of the parent Cadmium complex, sodium salts of several anions such as F^-, Cl^-, Br^-, CO_3^{2-}, SO_3^{2-}, AcO^-, NO_2^-, and $H_2PO_4^-$. (10×10^{-3} M) were added separately into the buffered complex solution. Although no prominent changes in the metal-complex spectra were observed upon addition of most of the anions, addition of sodium pyrophosphate to the metal complex solution showed a significant decrease of 322 nm peak and red shift of 398 nm peak in the complex to 410 nm (Fig. 1). Furthermore, upon addition of PPi to the metal complex, increase in the absorbance of the resulting solution was observed. This reveals the binding interaction of the receptor metal complex probe with PPi.

The anion recognition behaviour of the receptor complex (5.5×10^{-5} M) was also examined by studying fluorescence titrations in HEPES buffer with anions like F^-, Cl^-, Br^-, CO_3^{2-}, SO_3^{2-}, AcO^-, NO_2^-, $H_2PO_4^-$ and PPi. The receptor complex was found to be non-fluorescent in HEPES buffer and no significant changes in the fluorescence spectra of the receptor complex were observed upon addition of anions such as F^-, Cl^-, Br^-, CO_3^{2-}, SO_3^{2-}, AcO^-, NO_2^-, and $H_2PO_4^-$. However addition of 10 μM of PPi showed an ~40-fold enhancement in fluorescence intensity of $CdCl_2$L at 580 nm (Fig. 2). This observation was supportive to the results obtained from UV–Visible experiments. Furthermore, on fluorescence titration of the receptor complex with PPi (Fig. 4), it was observed that intensity of the peak at 580 nm gradually increases with increase in the amount of PPi in solution. This increase in the fluorescence intensity of 580 nm peak becomes saturated after addition of 460 μM of PPi to the

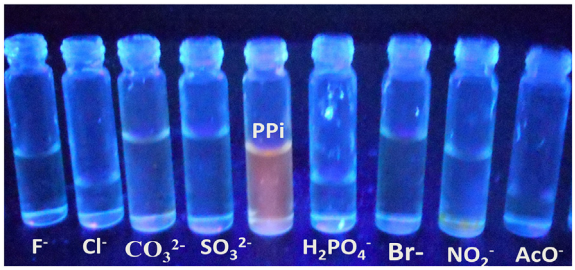

Fig. 3. Emission of $CdCl_2$L (50 μM) under 365 nm UV lamp with different anions.