RSC Advances

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Vitamin B_6 cofactor based fluorescent probe for sensing anion (F^-) and cation (Co^{2+}) independently in pure aqueous medium

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Abstract:

A new highly selective and sensitive bifunctional fluorescent probe for Co^{2+} and F^- ions has been derived from vitamin B_6 cofactor and the response mechanism has been analyzed by DFT calculations. The probe features facile synthetic protocols, good water solubility, high selectivity and sensitivity, and fluorescence turn-on response to F^-, ratiometric response towards Co^{2+} in aqueous medium.

Introduction

Fluorescent chemosensors can serve as effective tool in molecular sensing as evident from their prominent role in medicinal diagnostics, biological labeling and optoelectronic materials[1]. In particular, single molecular sensors with varied responses towards different analytes are cost effective and convenient for real applications. Among metal ions, sensing of cobalt has received increasing attention, since Co^{2+} is a component of vitamin B_{12}, a vitamin essential for DNA synthesis, formation of red blood cells, maintenance of the nervous system, growth and development of children[2]. On the other hand, fluoride is also an essential nutrient for the normal development and growth of human being[3]. Excess accumulation of cobalt in the body can result in cardiomyopathy, hypothyroidism, and neurological damage while high level of fluoride can cause dental and skeletal fluorosis[4].

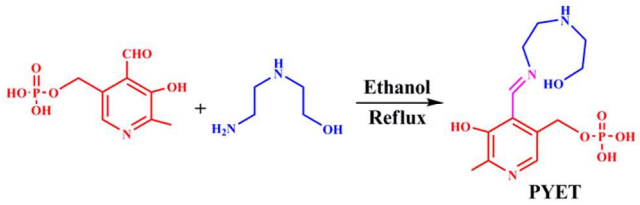
A number of fluorescent sensors have already been established for sensing individually either cobalt[5] or fluoride[6]. But reports about a single probe that senses fluoride and cobalt(II) ions independently are rare. Recently, chromogenic recognition of Co^{2+} or fluoride ion has been achieved in DMSO/CH_3CN system using calixarene based ditopic receptor by H. M. Chawla *et al*.,[7] However it suffers from interference of Ag^+ and Cu^{2+} while binding of fluoride ion with receptor. Furthermore, most of the reported sensors often feature tedious synthetic protocols, non-aqueous media requirement, lack of dual response and cross sensitivities towards other ions. All these limitations restrict their potential use in environmental and biological applications.

Amongst different organic scaffolds, the scaffold that facilitates Excited State Intramolecular Proton Transfer (ESIPT) as sensing mechanism are perfect candidates for fluorescence probes because of its significant photostability, large stoke shift and intense luminescence[8]. Pyridoxal phosphate (PLP), the active form of vitamin B_6 function as a coenzyme in numerous enzyme-catalyzed reactions such as transamination, α- and β-decarboxylations, β– and γ-eliminations, racemizations, and aldol reactions[9]. Although PLP is well known for its coordination and optical properties, only very few reports have been arisen in the design of fluorescent chemosensor by utilizing PLP platform[10]. Fluorescence detection of ions using small-molecule sensors in aqueous environment is still a difficult task. Only a small number of fluorescent probes that have been used successfully for this purpose appeared in the literature[11]. In continuation of our ongoing research for the development of fluorescent chemosensors[12], herein, we have designed a pyridoxal linked aminoethylaminoethanol (PYET) as a receptor for selective recognition of Co^{2+} and F^- in aqueous solution via enhancement of fluorescent emission. Interestingly, compared to various fluorescent chemosensors, probe PYET exhibit good water solubility, optical sensitivity in buffer medium, dual emission, low detection limit and visible strong fluorescence under UV light with the addition of guest species. To the best of our knowledge, probe PYET is the first bifunctional chromogenic and fluorogenic chemosensor that enables independent sensing of fluoride and cobalt(II) ions in aqueous medium.

Results and Discussion

Chemosensor PYET was synthesized from Vitamin B_6 cofactor pyridoxal phosphate with 2-(2-Aminoethylamino)-ethanol in methanol solution (Fig S1-S6). Aminoethylethanolamine has been selected as side chain because of its hydrophilic character, while Pyridoxal phosphate PLP acts as signalling moiety given its low fluorescence quantum yield and good binding sites (Scheme 1). Further, this type of structural arrangement can undergo keto-enol tautomerism through excited state intramolecular proton transfer (ESIPT) mechanism. The probe PYET was characterized by NMR and other spectroscopic

methods.



Scheme 1: Synthetic route of probe PYET

The photonic properties of PYET were investigated by UV-vis and fluorescence measurements with different metal ions i.e. Na^+, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Ni^{2+}, Fe^{3+}, Cu^{2+}, Ag^+, Cd^{2+}, Cr^{3+}, Zn^{2+}, Fe^{2+}, Hg^{2+}, Pb^{2+}, Al^{3+} and anions such as Cl^-, Br^-, I^-, OAc^-, NO_3^-, HSO_4^-, $H_3PO_4^-$ and CN^- in aqueous solution in the presence of HEPES buffered at pH 7.4. The probe showed λ_{max} at 328 nm and 387 nm in its absorption spectrum. Systematic titration of sensor PYET with increasing concentrations of Co^{2+} revealed a new absorption band at 369 nm with disappearance of bands at 328 and 387 nm (Fig 1).

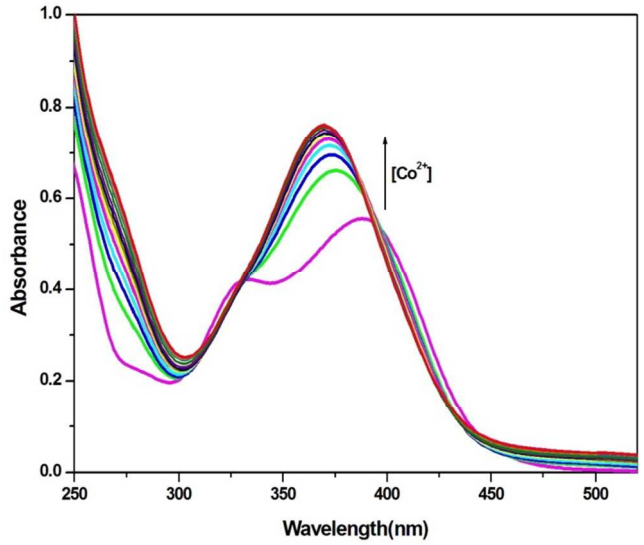


Fig 1: Absorption spectra of PYET (5μM) upon gradual addition of Co^{2+} in pH 7.4 HEPES buffered water.

Upon addition of F^- (Fig 2), intensity of the absorption band centered at 328nm decreased while that of the band at 387nm increased. Addition of other biologically important metal ions and anions led to no obvious change in ground state behaviour of probe PYET (Fig S7 and S8). When excited at 350nm in same solvent system, PYET exhibits fluorescence emission bands at 445nm and 510nm with a quantum yield of 0.010 at room temperature. The presence of dual emission indicates the possibility of the ESIPT mechanism. It also opens new channel for ratiometric analysis in which the ratio between the two emission intensities can be used to evaluate the analyte concentration and provide a built-in correction for receptor concentration, photobleaching and environmental effects[13]. The emission band at 445 nm was attributed to the enol form and the emission at 510 nm was assigned to the keto tautomer, produced by the ESIPT process (Scheme 2).[14]

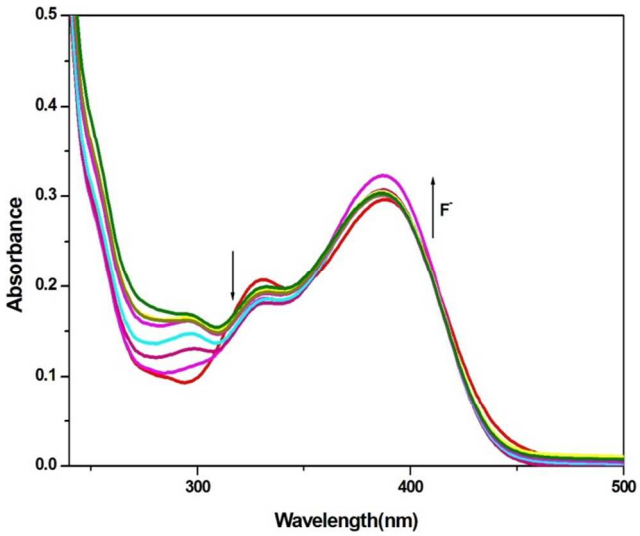
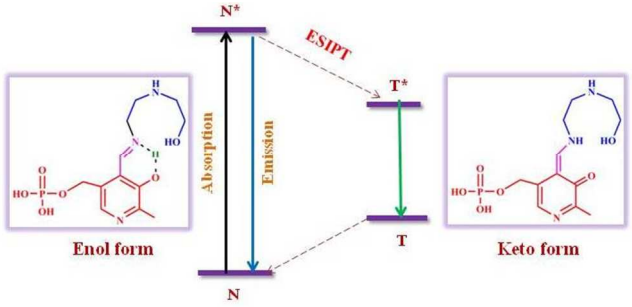


Fig 2: Absorption spectra of PYET (10μM) upon gradual addition of F^- in pH 7.4 HEPES buffered water.

The pH sensitivity of the probe was tested by recording fluorescence spectra over different pH values (Fig S9). At low pH, the dual emission undergoes significant red shift with increase in fluorescent intensity due to the protonation of probe. However at high pH, the emission bands at 445nm and 510nm disappeared and a striking new peak emerged at 457nm due to the deprotonation of hydroxyl group. Fluorescence intensity remains weak at intermediate pH (HEPES buffer (20 mM, pH 7.4). The pH titration indicates that probe exhibits remarkable pH-dependent behaviour in fluorescence spectra.



Scheme 2: Schematic illustration of photophysical cycle of probe PYET.

Interestingly, addition of Co^{2+} to the solution of PYET caused a marked fluorescence enhancement ($\Phi_f = 0.28$), with the increase in intensity of emission band at 445 nm along with concomitant decrease of the band at 510 nm, thus allowing rapid quantification of Co^{2+} based on intensity ratio of enol and keto tautomer (Fig 3 and S10a). These changes led to the blocking of intramolecular hydrogen bonds by coordination of phenolic O-H with Co^{2+}. This in turn led to the inhibition of ESIPT feature. Therefore, the two nitrogen atoms (N-H group, CH=N). Phenolic O-H and methylene O-H play a crucial role in efficient binding of PYET with Co^{2+}. From Job's plot analyses and mass spectrum, these spectral changes were also attributed to the formation of 1:1 complex (Fig S5 & S11a). It is noteworthy to mention that the detection limit[15] and binding constant[16] of this probe for Co^{2+} is 6.42×10^{-7} M and 6.89×10^4 M^{-1} in pure aqueous medium. The probe as expected was insensitive to addition of other metal ions

(Fig S12).

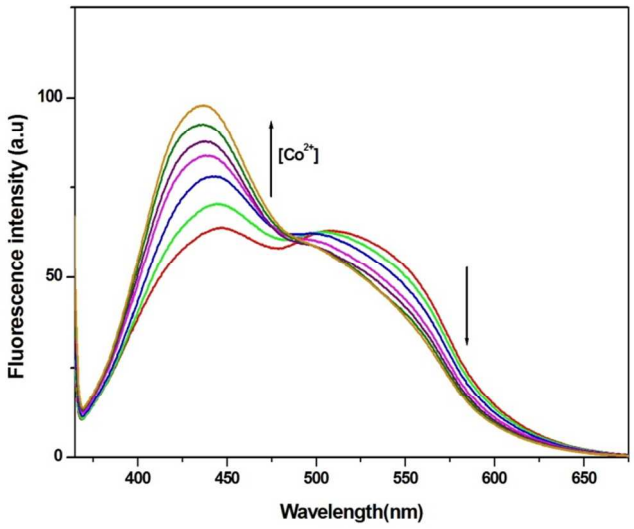


Fig 3: Fluorescence spectra of PYET (5μM) upon gradual addition of Co^{2+} in pH 7.4 HEPES buffered water. Excitation at 350nm. Slit width is 5nm.

In contrast, the addition of F^- resulted in a slight change of the keto emission band at 510 nm accompanied by a strong increase of enol emission band at 445 nm with a quantum yield of ($\Phi_F = 0.48$) (Fig 4 and S10b). Hence it is inferred that ESIPT is inhibited by interaction of F^- anion with the O-H group of PYET. The binding constant of the PYET-F^- system was estimated to be $1.46x10^5$ M^{-1} by linear fitting of the fluorescence titration curve. The Job's plot (Fig S11b) and mass spectrum (Fig S6) of [PYET–F^-] supports 1:1 binding stoichiometry. The detection limit was measured to be $7.77x10^{-8}$ M. Furthermore, the optical response of PYET is insignificant for anions other than fluoride under identical conditions (Fig S13).

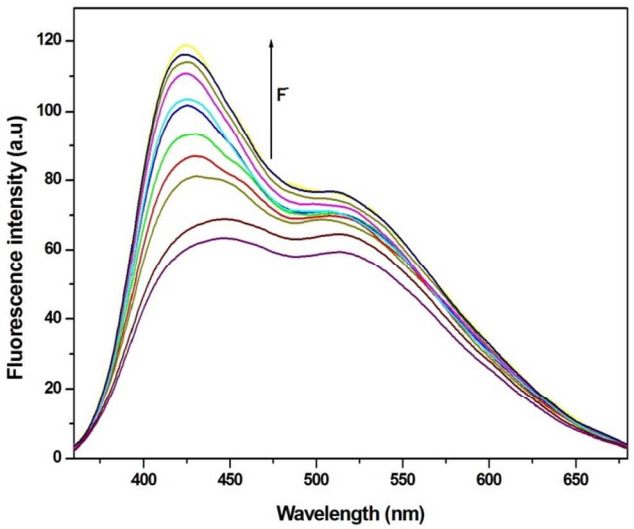


Fig 4: Fluorescence spectra of PYET (10μM) upon gradual addition of F^- in pH 7.4 HEPES buffered water. Excitation at 350nm. Slit width is 5nm.

To test the practical applicability of PYET, a competitive binding experiment was carried out in the presence of varying concentration of Co^{2+}/F^- (0-20μM), treated with 100 μM of competing analytes. No significant variation was observed in the presence of other competitive ions in comparison to solution containing only Co^{2+}/F^- (Fig S14). These results suggest that the Co^{2+}/F^- recognition by probe is barely interfered by other coexisting metal ions/anions.

Further, the binding mode of probe with F^- was investigated by running proton NMR titrations in the presence and in the absence of F^- in DMSO-d_6 (Fig S15). Upon addition of F^-, the signals on the aromatic rings changed slightly and resonance signals corresponding to N-H and phenolic O-H at 6.5ppm and 10.4ppm shifted downfield and eventually disappear upon increasing concentration of F^- ion. The fact supported the deprotonation interaction among F^-, phenolic O-H and N-H group.

To provide an insight into the photonic properties of probe, DFT studies were performed. The optimized structures of tautomers of probe PYET, PYET-Co^{2+} and PYET-F^- (Fig 5) were obtained using DFT/B3LYP-6-31G and B3LYP/LanL2DZ basis set[17] respectively. As shown in Fig S16, HOMO positioned on pyridoxal scaffold while LUMO spreads over pyridoxal phosphate with imine group. After the appendage of Co^{2+} ion to probe, HOMO spreads over both pyridoxal scaffold and metal center whereas pyridoxal phosphate with imine group still retains its LUMO character. Upon addition of F^- to probe, aminoethylethanolamine with fluoride behaves as HOMO, while pyridoxal scaffold with aminoethylethanolamine behaves as LUMO. The HOMO-LUMO energy difference of probe PYET is 5.17eV and the binding of Co^{2+}/F^- results in lowering of energy gap to 1.86eV and 3.33eV.

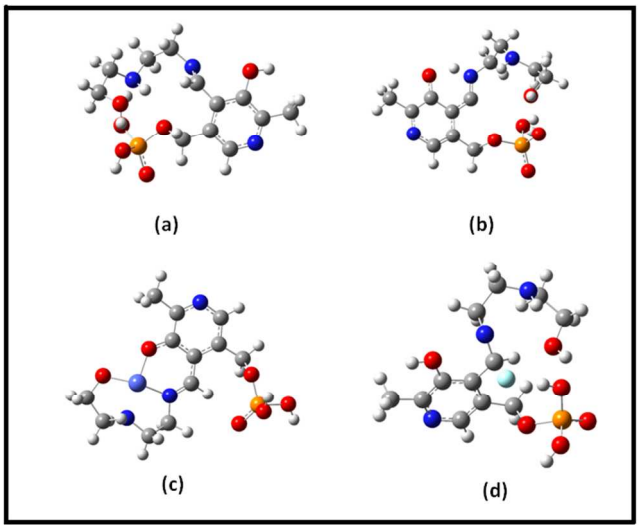


Fig 5: Optimized structures of (a) PYET-enol form (b) PYET-keto form (c) PYET-Co^{2+} (d) PYET-F^-

Conclusion

In summary, fluorescent chemosensor based on vitamin B_6 cofactor has been designed and synthesized. Probe PYET reveals fluorescence turn-on response to F^-, while ratiometric response towards Co^{2+} in aqueous medium. Notably, absorption change

and turn-on fluorescence response renders the sensor suitable for detection of Co^{2+} and F^- by simple visual inspection. Hence the promising characters such as facile synthetic methodology, good water solubility, ratiometric response and high selectivity constitute desirable features for the chemosensor reported in this manuscript.

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ACKNOWLEDGEMENT

M.I., D.J and K.K thank UGC-BSR for research fellowship. M.I., D.J., K.K. and D.C. also acknowledge DST-IRHPA, FIST and PURSE for instrumental facilities. The authors gratefully acknowledge DBT-IPLS, School of Biological Sciences, MKU for providing instrumentation facilities.

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†Electronic Supplementary Information (ESI) available: [NMR and MS Spectral data, UV-vis data and computational details]. See DOI: 10.1039/b000000x/

Graphical abstract