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A fluorescent chemosensor for relay recognition of Fe^{3+} and PO_4^{3-} in aqueous solution and its applications

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ABSTRACT

A novel fluorescent probe XDS based on 4-methylumbelliferone and 2-picolylamine platforms has been designed and synthesized, which behaves fast relay recognition of Fe^{3+} and PO_4^{3-} via a fluorescence "on–off–on" response signal. Probe XDS exhibited very high sensitivity and unique selectivity for Fe^{3+} over other common metal ions, and the detection limit of was 3.2×10^{-7} M. Moreover, the addition of the PO_4^{3-} ions could cause the recovery of fluorescence. This relay recognition feature of probe XDS has potential applications in the determination of trace amount of Fe^{3+} and PO_4^{3-} in environmental systems. Interestingly, fluorescence imaging experiments demonstrated that the probe XDS can also be used to monitoring the intracellular Fe^{3+} in RAMOS cells.

1. Introduction

The design and development of chemosensors for the recognition of cations and anions have attracted noticeable attention due to their important roles in the wide range of environmental, clinical, and biological systems.[1–4] With the comparisons to other instrumental methods such as inductively coupled plasma atomic emission or mass spectroscopy (TCP-AES, ICP-MS), atomic absorption spectroscopy (AAS), electro-chemical methods, etc,[5–11] fluorescent detections have more practical value for sensing and detecting trace amounts ions owing to its favorable superiorities including non-destructive character, high selectivity and sensitivity, fast response and economical method for the detection without any tedious sample pretreatment.[12–14]

Therefore, considerable studies have concentrated on construction of highly efficient and convenient ion probe using spectrophotometric and spectrofluorometric methods.[15,16]

It is well known that iron is one of the most essential trace elements in human body. It is an indispensable ion for most organisms by exhibiting a crucial role in various biological and chemical processes at the cellular level such as oxygen uptake, oxygen metabolism, enzyme catalysis.[17–19] However, its deficiency or excess may lead to hypoferremia or hyperferremia, accordingly. In addition, it has recently been discovered that iron is another key limiting factor for the primary productivity of phytoplankton besides elemental nitrogen, phosphorus and silicon.[20,21] In contrast, phosphates and its derivatives (eg, adenosine triphosphate (ATP)) play a crucial role in signal transduction and energy storage in biological systems.[22,23] Phosphate anions are ubiquitous in biological systems and it plays vital functional roles in cell signaling, membrane integrity, bone mineralization, muscle function and other important biological processes.[24–26] Unfortunately, only a small number of fluorescent anion sensors can effectively distinguish F^-, AcO^-, phosphates and their derivatives.[27–31]

Thus, it is highly desirable to develop a highly selective and sensitive analytical method for the detection of Fe^{3+} and PO_4^{3-} for further study of their physiological and pathophysiological

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functions in living organisms.

Herein, we have prepared a simple and efficient fluorescent probe XDS based on 4-methylumbelliferone and 2-picolylamine platforms for relay recognition of Fe^{3+} and PO_4^{3-}. We chose coumarin as the fluorescent group because of its good fluorescence properties and water solubility. Meanwhile, as a recognition group, 2-aminomethylpyridine can provide the N atoms as excellent coordinating site. Thus, we chose Chloroacetyl chloride as a linker to effectively combine the 4-methylumbelliferone and 2-picolylamine to form a compound XDS with strong fluorescence emission. In this study, we also further investigated in detail the fluorescence performances of the probe XDS. The investigated results demonstrated that the probe XDS exhibits high selectivity, sensitivity and rapid fluorescence response to Fe^{3+} ion over other metal cations in aqueous solution. Moreover, we also explore anion-sensing properties of the in situ-formed [XDS-Fe^{3+}] complexes in the aqueous environment and test trips. As anticipated, the complex exhibited excellent properties for PO_4^{3-} detection according to the strong binding capacity of PO_4^{3-} to Fe^{3+}. At the same time, the performance of XDS for the fluorescence imaging in living cells was also evaluated.

2. Experimental

2.1. Materials and instruments

Unless otherwise noted, all reagents and solvents employed for synthesis were purchased from Aladdin Chemical Reagent Ltd., and used without further purification. All the metal chlorate salts (Al^{3+}, Sr^{2+}, Li^{+}, Cs^{+}, Ca^{2+}, Cd^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+},Fe^{2+}, Fe^{3+},K^{+}, Hg^{2+}, Mg^{2+}, Mn^{2+},Ni^{2+},Na^{+}, Pb^{2+} and Zn^{2+}.) and all anionic sodium salt (PO_4^{3-},F^{-}, Cl^{-}, Br^{-}, I^{-},SO_4^{2-}, SCN^{-}, ClO_4^{-}, CO_3^{2-}, NO_3^{-}, NO_2^{-}, $H_2PO_4^{-}$, HPO_4^{2-}, AcO^{-} and CN^{-}.) were purchased from Sinopharm Chemical Reagent Ltd.,

^1H NMR and ^{13}C NMR spectra were measured using an AVANCE II 400 MHz spectrometer (Bruker, Switzerland). Mass spectra were obtained using a Thermo LXQ Liquid chromatography ion trap mass spectrometer (USA). Fluorescence measurements were taken at a Cary Eclipse fluorescence spectrophotometer (Variance. LTD, Australia). Absorption spectra were recorded with a UV-2450 UV–Vis spectrophotometer (Shimadzu, Japan) at room temperature. Cell experiments were applied on an inverted fluorescence microscope (Leica DMI4000B, Germany).

2.2. Synthesis of XDS

2.2.1. Compound **R**: 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetyl chloride

Chloroacetyl chloride (0.35 mL) was dissolved in dry CH_2Cl_2 at 0 °C, and then added dropwise to a cooled stirred mixture of 4-MethylμMbelliferone (0.70 g, 4 mmol) and triethylamine (Et_3N) (0.3 mL) in CH_2Cl_2 under N_2 within 1 h, After being stirred over night at room temperature, the reaction mixture was quenched with distilled water and then was extracted three times with 20 mL of CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and removed under reduced pressure to obtain a white solid product. The crude product was purified by recrystallization from ethanol (methanol) to give analytically pure compound **R** (0.93 g) in 93% yield.

2.2.2. Compound XDS: 4-methyl-2-oxo-2H-chromen-7-yl 2-((pyridine-2-ylmethyl) amino)acetate

R (1.006 g, 4 mmol) was dissolved in anhydrous CH_3CN and the solution was added dropwise to a stirred mixture of 2-aminomethylpyridine (0.86 g, 8 mmol), $NaHCO_3$ (0.69 g), K_2CO_3(1.1 g) and KI (1.3 g) at room temperature under N_2 over a period of 30 min and the resulting mixture was stirred for an additional 12 h. Then the resulting mixture was cooled to room temperature, filtered over gravity and the solvent was removed under reduced pressure to afford solid product, which was purified by recrystallization from ethanol to get analytically pure compound XDS (4-methyl-2-oxo-2H-chromen-7-yl 2-((pyridin-2-ylmethyl) amino)acetate) (1.1 g) in 86% yield (Scheme 1). The product is verified by ^1H NMR, ^{13}C NMR, MS (Figs. S1–3). ^1H NMR (400 MHz, DMSO): $\delta = 8.87$ (t, $J = 5.7$ Hz, 1H), 8.51 (d, $J = 4.5$ Hz, 1H), 7.74 (dd, $J = 12.8$, 5.1 Hz, 2H), 7.35–7.21 (m, 2H), 7.09–6.97 (m, 2H), 6.24 (s, 1H), 4.75 (s, 2H), 4.46 (d, $J = 5.9$ Hz, 2H), 2.37 (d, $J = 29.8$ Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO): $\delta = 40.62$, 40.41, 40.20, 39.89, 39.75, 39.58, 39.37, 18.61 ppm.

MS: $[M + H]^+$ calcd for $C_{18}H_{16}O_4N_2$: 325.33, found: 325.60.

2.3. Fluorescence spectroscopy

Unless otherwise noted, materials were of analytical grade from commercial suppliers and were used without further purification. Deionized water was used throughout all experiments.

Stock solutions (10 mM) of the various anions of F^{-}, Cl^{-}, Br^{-}, I^{-}, SO_4^{2-}, SCN^{-}, ClO_4^{-}, CO_3^{2-}, NO_3^{-}, $H_2PO_4^{-}$, HPO_4^{2-}, NO_2^{-}, AcO^{-} and CN^{-} in deionized water, were prepared. Stock solutions (10 mM) of various metal ions were prepared from NaCl, CsCl, $PbCl_2$, $CoCl_2$, $ZnCl_2$, $CuCl_2$, $NiCl_2$, $HgCl_2$, $CdCl_2$, $CrCl_3$, $FeCl_2$,$FeCl_3$, LiCl, $MgCl_2$, $CaCl_2$, $AlCl_3$, $SrCl_2$ and $MnCl_2$.

Stock solution of probe XDS (1 mM) was also prepared in deionized water and diluted to prepare the analytical solution (10 μM). For fluorescence measurements, both the excitation and emission slit widths were 5 nm. The excitation wavelength was set at 320 nm.

2.4. Cell culture and imaging studies

The RAMOS cells (Human B lymphocyte tumor cells) were seeded on a 24-well plate and were incubated in medium (supplementing with RPMI 1640, 10% FBS) at 37 °C for 24 h. Subsequently, the cells were incubated with 20 μM of probe XDS at 37° C for 0.5 h and then washing with three times to remove the remaining probe XDS. The cells were then incubated with Fe^{3+}(20 μM) at 37° C for an additional 0.5 h, then 20 μM of PO_4^{3-} were added and incubated for another 0.5 h. The treated cells were rinsed three times with buffered saline. The cell imaging in different stages were obtained by an inverted fluorescence microscope.

2.5. Computational methodology

The density functional theory (DFT) calculations were perused to get the theoretical aspects of the coordinating mode of the probe XDS and Fe^{3+}. The DFT optimizations of probe XDS and XDS-Fe^{3+} complex were carried out using the Gaussian 09 program,[32] in which the B3LYP function was used. The 6-311 + G* and LanL2DZ basis sets were used for the probe XDS and the metal ions, respectively.[33] Besides, the distribution of electronic clouds on both HOMO and LUMO of probe XDS and XDS-Fe^{3+} complex were also studied.

3. Results and discussion

3.1. Fluorescence characterization and selectivity of XDS toward Fe^{3+}

The effect of metal ions on the fluorescence properties of XDS