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A cyanine-based colorimetric and fluorescent probe for highly selective sensing and bioimaging of phosphate ions

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ABSTRACT

This paper reports a near-infrared chemosensor for phosphate ions (Pi) based on Pi-controlled fluorescence off-on switching mechanism. The Pi sensor displayed colorimetric responses towards Pi with a distinct color change from green to yellow in aqueous media. Apyrase, a hydrolytic enzyme, was used to accelerate the endogenous Pi production for evaluating in vivo fluorescent sensing ability of the new cyanine based probe (**1**). In addition, the present study demonstrated potential of the newly developed chemosensor in bioimaging by performing experiments in Chinese hamster ovary (CHO) cells both in vitro and in vivo.

1. Introduction

Near-infrared (NIR) fluorescent probes can detect molecular activity in vivo because of the following advantages associated with such probes: deep penetration of NIR photons in tissues, weak photo-damage to biological samples, and low auto-fluorescence background [1]. Recently significant efforts have been successfully made towards the use of NIR probes for bioanalytical applications [2]. With the relatively large extinction coefficient and high quantum yield, cyanine dye is an ideal NIR fluorophore that has been widely employed for NIR in vivo imaging [3].

Phosphate ions (Pi, PPi, and nucleotides) play vital roles in living systems. For example, pyrophosphate (PPi), being the product of ATP hydrolysis under cellular conditions, is an important target in bioenergetic and metabolic processes [4]; phosphate ion (Pi), an important downstream metabolic product of nucleotides, plays pivotal roles in energy transduction and storage in biological systems [5]. However, compared with the numerous studies on PPi [6], the number of studies focusing on Pi is relatively low. Recently, a series of reaction-based chemodosimeters, displaying highly selective fluorescence changes towards Pi over PPi and ATP, was reported [7]. In our previous study, methoxy oxalyl group has been shown as an effective reaction site for the selective Pi sensing. Inspired by the results demonstrated by our previously developed chemodosimeters, we thought that a cyanine-based and Pi-targeted chemodosimeter with colorimetric or ratiometric NIR properties would be highly attractive. Herein, we designed a new cyanine based probe **1** for Pi sensing and examined its Pi sensing ability in vitro.

Probe **1** showed a strong NIR absorption band from 600 to 850 nm and exhibited specific recognition ability of Pi among all the tested anions and structural analogues including PPi, ATP, ADP, AMP, GTP, GDP, and GMP. Upon addition of Pi to the probe **1** solution (DMSO/HEPES = 4:6, pH = 7.4), a dramatic color change from deep green to light yellow, accompanied by an increase intensity of the fluorescence peak at 560 nm, was observed. In addition, the probe was tested for in vivo bioimaging of exogenous and endogenous Pis in Chinese hamster ovary (CHO) cells.

2. Experimental section

2.1. General procedures and materials

Unless otherwise specified, all chemicals were purchased from

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commercial sources and used without further purification. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker 500 MHz magnetic resonance spectrometer. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. Mass spectrometry was recorded with a Xevo TQ-S mass spectrometer and a Q-TOF B.05.01 mass spectrometer. The UV–Vis spectra were obtained using UV-240 IPC spectrophotometer. The fluorescence spectra were obtained with F-4500 FL spectrometer with a 1 cm standard quartz cell. Flash chromatography was carried out on silica gel (100–200 mesh).

2.2. Synthesis of the cyanine based probe 1

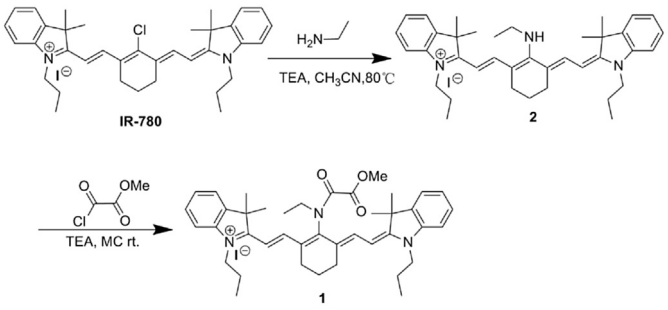
The compound **2** was synthesized following a previous report with modification [8], and the yield was 81%. Under argon atmosphere, compound **2** (100 mg, 0.15 mmol) was dissolved in 30 mL anhydrous dichloromethane (DCM), and the solution was kept in ice-bath for 20 min. To prepare the probe **1**, methyl oxalyl chloride (55 μL, 0.59 mmol) along with triethyl amine (Et_3N) (86 μL, 0.59 mmol) in 10 mL anhydrous DCM was added to the solution of **2** dropwise. The reaction mixture was stirred at 0 °C for 1.5 h and later kept at room temperature overnight. The solvent was removed under reduced pressure, and the resulting solid was purified by column chromatography (silica) by eluting with ethyl acetate and methanol mixture (EA: MeOH, 120:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.63 (d, J = 14.1 Hz, 2H), 7.38 (ddd, J = 21.9, 10.9, 4.0 Hz, 4H), 7.24 (d, J = 7.5 Hz, 2H), 7.16 (d, J = 7.9 Hz, 2H), 6.30 (d, J = 14.1 Hz, 2H), 4.27–4.16 (m, 4H), 3.84 (q, J = 7.2 Hz, 2H), 3.63 (s, 3H), 2.88–2.78 (m, 2H), 2.65–2.57 (m, 2H), 1.92 (dd, J = 14.8, 7.4 Hz, 6H), 1.69 (s, 6H), 1.66 (s, 6H), 1.33 (t, J = 7.2 Hz, 3H), 1.07 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 172.34, 162.10, 150.97, 142.33, 142.01, 141.27, 129.04, 128.76, 125.40, 122.21, 111.05, 102.35, 60.41, 53.43, 52.75, 49.28, 46.64, 45.22, 28.19, 27.83, 25.58, 21.02, 19.17, 14.21, 13.70, 11.72. MS m/z 634.38 M^+ (Calcd for $C_{41}H_{52}N_3O_3^+$ 634.40).

2.3. UV/vis and fluorescence measurements

Stock solutions (1×10^{-2} M) of the sodium salts of Pi, $P_2O_7^{4-}$, ATP, ADP, AMP, GTP, GDP, GMP, TTP, TDP, TMP, UTP, UDP and UMP were prepared in deionized water. Fluorescence spectra were recorded with the slit width 5/5 nm.

2.4. Culture of CHO cells and fluorescent imaging

CHO was cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 °C under 5% CO_2. Then the cells were incubated with 500.0 μM Pi and ATP in an atmosphere of 5% CO_2 and 95% air for 2 h at 37 °C, respectively. Then the cells treated with ATP were incubation with 1 U and 1.5 U and 2 U of apyrase for 1 h, respectively. Then the cells were incubation with 20.0 μM **1** for 1 h. Wash cells twice with 1 mL deionized water at room temperature, cells were imaged using an Olympus BX51 inverted fluorescence microscopy.



Scheme 1. Synthesis route of compound **1**.

3. Results and discussion

3.1. Synthesis and characterizations

Scheme 1 depicts the synthesis route for probe **1**. With commercially available IR-780 as starting materials, a precursor **2** first prepared following a previously reported method with some modification [8]. Next, the target probe **1** was easily prepared in the presence Et_3N in dry DCM by acylation of methoxy oxalyl chloride with **2**, followed by column purification. The probe **1** was characterized using different spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, and ESI-MS. The detailed synthetic procedure and characterization of the new compound are provided in the Supporting Information (SI).

3.2. UV–vis absorption and fluorescent emission spectra

To investigate the selectivity of probe **1** for Pi ions, the spectral changes (UV–Vis and fluorescence) of **1** were monitored in the presence of various anions (as sodium salts) in DMSO-HEPES buffer

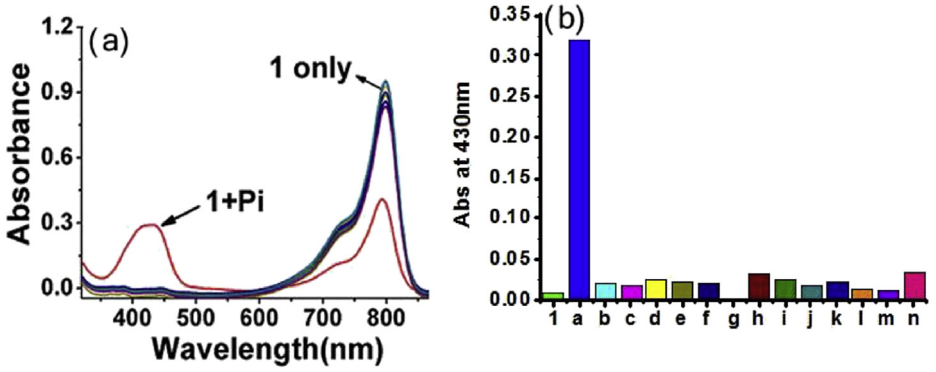


Fig. 1. (a) Absorbance spectra of **1** (2.0×10^{-5} M) in DMSO-HEPES buffer (0.02 M, pH 7.4) (V/V = 4:6) before and after the addition of 250 equiv of Pi, $P_2O_7^{4-}$, ATP, ADP, AMP, GTP, GDP, GMP, TTP, TDP, TMP, UTP, UDP and UMP. (b) Absorbance intensity of **1** (2.0×10^{-5} M) at 430 nm in DMSO-HEPES buffer (0.02 M, pH 7.4) (V/V = 4:6) after the addition of 250 equiv of selected molecules: **1**: blank, a: Pi, b: $P_2O_7^{4-}$, c: ATP, d: ADP, e: AMP, f: GTP, g: GDP, h: GMP, i: TTP, j: TDP, k: TMP, l: UTP, m: UDP n: UMP.