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A reversible fluorescent probe for Zn^{2+} and ATP in living cells and in vivo

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ABSTRACT

A reversible fluorescein-based fluorescent probe which exhibits high sensitivity and selectivity for Zn^{2+} and ATP, has been designed and synthesized. The sensing process was completed via fluorescence variation induced by an opening and closing of the spiro-ring of fluorescein. Zn^{2+}/ATP-induced fluorescent intensity shows a good linear relationship with the concentration of Zn^{2+}/ATP in the range of 0–10 μM with a detection limit of 0.1 μM/0.5 μM. Moreover, the probe was further applied to visualize and detect $Zn^{2+}$and ATP *in vitro* and *in vivo*.

1. Introduction

Zinc ion is recognized as one of the most abundant transition metals ions in the environmental and biological systems [1]. It plays indispensable roles in various physiological and pathological processes, such as genetic expression, cell apoptosis, enzyme regulation and neural transmission [2–4]. In addition, the disorder of zinc ion in living organisms may associate with Pakinson's diseases, Alzheimer's diseases and immune dysfunction [5–7]. On the other hand, adenosine-5′-triphosphate (ATP) is an essential biomolecules in cell biology due to its energy production and storage for many cellular events [8,9]. ATP is crucial in energy metabolism, DNA replication and transcription, and other fundamental activities [10]. Therefore, it is of great importance to develop a compelling method to quantitatively monitor the concentration of $Zn^{2+}$and ATP in both the environmental and biological systems.

Compared with the traditional methods, fluorescence sensing shows obviously specific advantages in monitoring *in vitro* and *in vivo* biologically relevant species, such as metal ions, anions and active molecules due to its convenient operation and high sensitivity [11,12]. To date, numerous excellent fluorescein dyes have been developed to detect various target objects, *e.g.* Cu^{2+} [13,14], Hg^{2+} [15,16], Zn^{2+} [17,18], Fe^{3+} [19,20], NO [21,22], H_2S [23,24], HOCl [25,26] and pH [27,28]. Unfortunately, there is few ATP probes have been applied *in vitro* and *in vivo* in recent decades [29,30]. Consequently, it remains a great challenge to explore highly selective and sensitive probes for monitoring the concentration of Zn^{2+} and ATP *in vitro* and *in vivo*.

Recently, our group has designed and synthesized a series of fluorescein-based fluorescent probes for biologically relevant metal ions [31] and reactive oxygen [32,33] and sulfur species [34]. As a continuation of previous work, we report herein a fluorescein-containing fluorescent turn-on probe with demonstrated specific selectivity to Zn^{2+} via formation of zinc complexes. Moreover, we developed the zinc complexes as a fluorescent probe which displayed a turn-off fluorescence change for ATP detection. We believe that this probe provides a potential and powerful approach for monitoring and visualizing $Zn^{2+}$and ATP in living cells and in vivo Scheme 1.

2. Experimental

2.1. Materials and measurements

All reagents were obtained from J&K Scientific, Co., Ltd. (Shanghai China) and used directly without further purification

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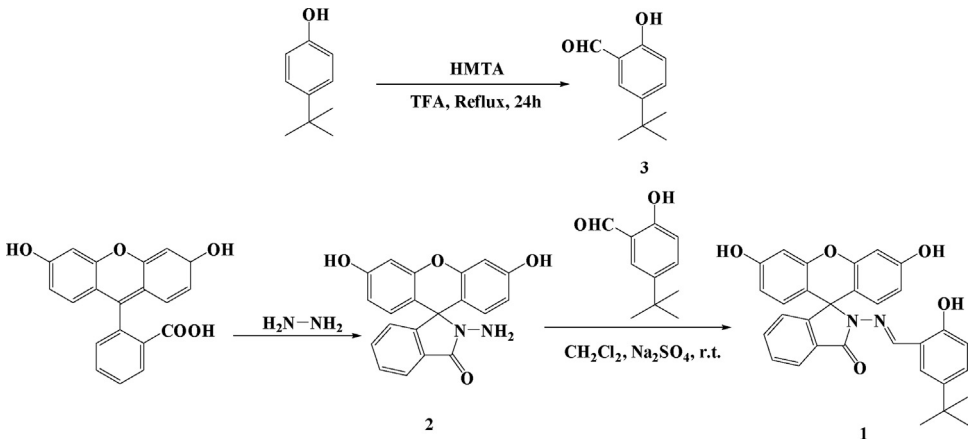

Scheme 1. Synthesis of probes **1.**

throughout the whole experiment. Fluorescence spectra measurements were performed on a Hitachi F-4500 fluorescence spectrophotometer equipped with a xenon discharge lamp, in a 1 cm quartz cell. UV–vis spectra were recorded with a Shimadzu UV-1700 spectrphotometer. Mass spectra were measured using a Brukermicro TOF-QII ESI-Q-TOF LC/MS/MS Spectrometer by means of the electronic spray ionization (ESI). NMR spectra were recorded on a Varian INOVA-400 MHz spectrometer (at 400 MHz for ^{1}H NMR) using tetramethylsilane (TMS) as internal standards. Fluorescent images were performed using a Leica SPE confocal laser scanning microscope with an excitation wavelength of 488 nm. In vivo fluorescence imaging analysis was carried out in an IVIS Kinetic imaging system. Scanningelectron microscope (SEM) images were obtained on a FEI Quanta 400F scanning electron microscope system at an acceleration of 20 kV. The samples were sputter-coated with gold in vacuum before observation.

2.2. General procedure

Probes **1** stock solution (1 mM) was prepared in acetonitrile. The solutions of various testing species stock solutions (1 mM) were prepared in distilled water. During the titration experiments, different amounts of Zn^{2+} and 0.10 mL of 1000 μM probes were mixed and filled up with PBS to 10 mL in volumetric tubes. During the interference experiments, 30 μM Zn^{2+}, 0.10 mL of 1000 μM probe **1** and 1 mL of 1000 μM testing species were mixed and filled up with PBS to 10 mL in volumetric tubes. During the titration experiments of ATP, 0.10 mL of 1000 μM probes, 0.1 mL of 1000 μM Zn^{2+} and different amounts of ATP was mixed and filled up with PBS to 10 mL in volumetric tubes. 1 mL aliquots were pipetted into a 1 cm cuvette for spectral measurements. 5 nm bandpasses were used for both excitation and emission wavelengths. An excitation wavelength of 420 nm was used for the acquisition of emission spectra.

2.3. Synthesis of intermediate 3

4-tert-Butylphenol (5.14 g, 34.2 mmol) and hexamethylenetetramine (9.60 g, 68.5 mmol) were dissolved in anhydrous trifluoroacetic acid (60 mL) under N_2, and the reaction mixture was refluxed for 24 h. The mixture was poured into 4 M HCl (200 mL) and stirred for 10 min, after which it was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic extracts were washed with 4 M HCl(2 × 200 mL), water (200 mL), saturate brine (200 mL), then dried with Na_2SO_4 and concentrated in vacuum. The crude product was purified by silica gel column chromatography to give 4.58 g yellow viscous liquid, yield 64.88%.

2.4. Synthesis of probes 1

Fluorescein hydrazide was synthesized in a high yield according to the procedures reported in literature [32].

Fluorescein hydrazide (10 mmol, 3.46 g) was mixed with compound **3** (11 mmol, 1.70 g) in 30 mL of ethanol with 1 drops of CH_3COOH. The reaction mixture was refluxed for 24 h. After cooling to room temperature, the white crude product was filter off from the reaction mixture and purified by column chromatography on silica gel using MeOH: CH_2Cl_2 = 1: 30 as the eluent, to give 3.82 g pink power, yield 75.49%. ^{1}H NMR(DMSO-d_6, 400 MHz): δ (ppm) 1.21 (s, 9H), 6.49 (m, 4H), 6.65 (d, *J* = 2.0 Hz, 2H), 6.71 (d, *J* = 8.6 Hz, 1H), 7.19 (m, 1H), 7.26 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.37 (d, *J* = 2.5 Hz, 1H), 7.65 (ddd, *J* = 10.5, 7.3, 1.3 Hz, 2H), 7.94 (m, 1H), 9.26 (s, 1H), 9.92 (s, 2H), 10.02 (s, 1H). MS (ESI) *m*/*z* = 529.1876 [M+ Na]$^+$, calc. for $C_{28}H_{20}N_2O_6Na$ = 529.1734.

3. Results and discussion

3.1. Optical response towardsZn^{2+}

First of all, the time-dependent fluorescence response of the probe was investigated in the presence of 3.0 equiv. Zn^{2+} in CH_3CN-PBS (1/99, v/v, pH 7.4) solution. As demonstrated in Fig. S2, the maximal fluorescence signal at 527 nm reached the maximum within 5 min at room temperature, indicating probe **1** is adaptive for real-time detection of Zn^{2+} and the reaction time of 5 min was applied for the subsequent experiments.

The fluorescence titrations experiments of probe **1** with Zn^{2+} were then performed. As expected in Fig. 1a, the free probe (10 μM) exhibited almost no emission band (Φ = 0.0012) due to the closed fluorescein-spirolactam form. Upon the introduction of 0.0-4.0 equiv. of Zn^{2+}, a dramatic enhancement of the fluorescence intensity at 527 nm was observed, with the fluorescence quantum yield of Φ_f = 0.45 (using rhodamine 6G in ethanol). This phenomenon was ascribed to Zn^{2+} ions induced structural transformation from the colorless spirolactam ring to the colored ring-opening delocalized form of fluorescein.

As shown in Fig. 1b, the fluorescence titration curve revealed that the fluorescence intensity at 527 nm showed a good linear relationship against the concentration of Zn^{2+}in the range from 0.0 to 10.0 μM. The regression equation was y = 23.80 x–3.323 (R^2 = 0.997) and the detection limit (S/N = 3) of probe **1** for $Zn^{2+}$was found to be 0.1 μM. These results demonstrated that probe **1**could be potentially applicable for qualitative and quantitative analysis of Zn^{2+}.