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Rational development of a highly selective ratiometric fluorescent probe for hydrogen polysulfides

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ABSTRACT

A novel ratiometric fluorescent probe, ACC-Cl, was developed by virtue of a unique H_2S_n-mediated coumarindithiolone formation under mild condition. The probe ACC-Cl exhibited excellent sensitivity and selectivity toward H_2S_n over other RSS such as biothiols and H_2S. More importantly, titration of H_2S_n to the solution of ACC-Cl under the existence of 1 mM GSH, the obvious ratiometric fluorescence response still can be observed. Significantly, preliminary biological experiments indicated its potential for sensing H_2S_n in living cells.

1. Introduction

Reactive sulfur species (RSS) are a family of sulfur-containing molecules widely existing in biological systems. Many RSS, such as thiols, hydrogen sulfide (H_2S) and sulfur dioxide (SO_2), play crucial roles in many physiological processes [1,2] and have attracted increasing attention in the field of biomedical research or clinical diagnosis. Very recently a new research hotspot in RSS which focuses on the chemical biology of hydrogen polysulfides (H_2S_n, $n \geq 2$) has emerged [3–6]. It was found that H_2S_n exhibit higher potency than H_2S in activating ion channels, transcription factors and tumor suppressors [3–7]. Thus, H_2S_n might be the real signal molecules in those above-mentioned physiological processes. The formation of endogenous H_2S_n may be from various pathways in biological systems: (1) the oxidation of endogenous H_2S by reactive oxygen species (ROS) like ClO^- [8]. (2) subsequent conversion from persulfides (e.g. CysSSH, GSSH, GSSSH, etc.) which are produced through cystathionine-β-synthetase (CBS) and cystathionine γ-lyase (CES) mediated cysteine trans-sulfuration [9,10]. Though some advances about the biological effects of H_2S_n have been obtained, the underlying physiological properties of H_2S_n remain largely unknown. One bottleneck might be the lack of effective methods for accurately detecting H_2S_n in biological systems.

Given its advantages of excellent sensitivity and high spatiotemporal resolution, fluorescent probes now have been recognized as the effective molecular tools that can help to detect or visualize biologically active molecules in vivo [11]. Pioneered by Xian's group, the first fluorescent probe for H_2S_n was developed by taking advantage of H_2S_n-mediated benzodithiolone formation (Scheme 1) [12]. From then on, this strategy was used to construct more specific fluorescent probes for H_2S_n [13–24]. While these probes can selectively distinguish H_2S_n from other RSS, most of them respond to H_2S_n in a fluorescence "turn-on" manner. It's known that "turn-on" fluorescent probes usually suffer from data distortion in the quantitative measurements caused by environment changes [25,26]. By contrast, ratiometric fluorescence probes allow the measurement of the fluorescence intensity at two different wavelengths, which can solve the problems arising from environmental factors and can also increase the dynamic range of fluorescence measurement [27–30]. Unfortunately, ratiometric fluorescent probes for H_2S_n are rare up to now [18,19,31].

In this work, we have developed a novel ratiometric fluorescent probe for H_2S_n detection by taking advantage of a unique H_2S_n-mediated coumarindithiolone formation under mild condition. The

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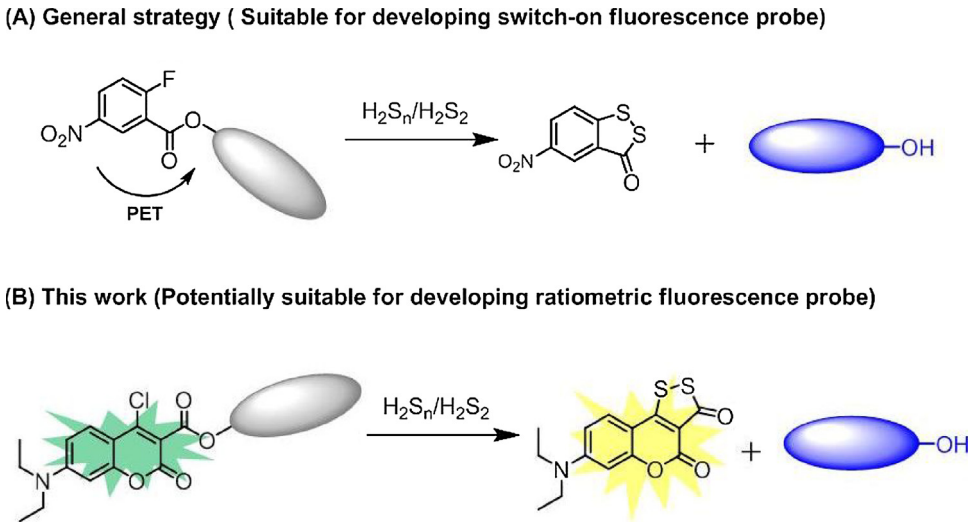

Scheme 1. Proposed Strategy for Ratiometric H_2S_n Probes.

probe exhibited excellent sensitivity and selectivity toward H_2S_n over other RSS such as biothiols and H_2S. Preliminary biological experiments indicated its potential for sensing H_2S_n in living cells.

2. Materials and methods

2.1. Materials and instruments

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Solvents were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. NMR spectra were recorded on a BRUKER 300 spectrometer with TMS as an internal standard. All accurate mass spectrometric experiments were performed on a Xevo G2 QTof MS (Waters, USA). UV–vis absorption spectra were recorded on a TU-1901 (Puxi, P.R. China) spectrophotometer. Fluorescence spectra were recorded at room temperature using a HITACHI F-4600 fluorescence spectrophotometer with both the excitation and emission slit widths set at 5.0 nm. Cell imaging was performed with a Zeiss LSM 710 laser scanning confocal microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted using silica gel (mesh 200–300), both of which were obtained from Qingdao Ocean Chemicals, China.

2.2. General procedure for spectral measurements

A stock solution of ACC-Cl was prepared at 1 mM in CH_3CN. Solutions of various testing species were prepared from H_2S_n, glutamate (Glu), proline (Pro), serine (Ser), glutathione (GSH), cysteine (Cys), L-Cystine, DL-dithiothreitol (DTT), Na_2S, Na_2SO_3, $NaSO_4$, NaS_2O_3, NaClO, H_2O_2, $CaCl_2$, $MgCl_2$ and $ZnCl_2$, in twice-distilled water. Homocysteine (Hcy) was prepared in PBS buffer (10 mM, pH = 7.4). A typical test solution (10.0 mL) was prepared by placing 0.05 mL of ACC-Cl (1 mM), 2.95 mL of CH_3CN, and an appropriate aliquot of each analyte stock solution into an appropriate amount of PBS buffer. The resulting solution was shaken well and kept at room temperature (25 °C) for 120 min before recording its fluorescence spectra.

2.3. Cell culture and fluorescence imaging

RAW264.7 cells were seeded in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin. The cells were incubated under an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. Cell imaging was performed with a Zeiss LSM 710 laser scanning confocal microscope. Before each experiment, cells were washed with PBS buffered solution 3 times. Excitation wavelength: 405 nm. Emissions were collected at 440–460 nm for blue channel and 520–560 nm for green channel.

2.4. Cell viability assay

RAW264.7Cells were grown in CMEM medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 °C under an atmosphere of 5% CO_2. Immediately before the experiment, the cells well placed in a 96 well plate, followed by addition of increasing concentrations of ACC-Cl. The final concentrations of the probe were kept from 0 to 12 μM. The cells were then incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h, followed by MTT assays (n = 5). Untreated assay with CMEM (n = 5) was also conducted under the same conditions.

2.5. Determination of the fluorescence quantum yield

Fluorescence quantum yields for 4, 7, and ACC-Cl were determined by using fluorescein (Φ_f = 0.79 in ethanol) [32] as a fluorescence standard. The quantum yield was calculated using the following formula (1):

$$\Phi_{F(x)} = \Phi_{F(s)}(A_S F_X / A_X F_S)(n_X / n_S)^2 \quad (1)$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts $_S$ and $_X$ refer to the standard and the unknown, respectively.

2.6. Synthesis of compound 1

Compound **1** was synthesized according the literature method [33]. The spectroscopic characteristics were in good agreement with those found in the literature. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.32 (s, 1H), 7.87 (d, J = 9.3 Hz, 1H), 6.74 (dd, J = 9.3, 2.5 Hz, 1H), 6.48 (d, J = 2.5 Hz, 1H), 3.51 (q, J = 7.2 Hz, 4H), 1.29 (d, J = 7.1 Hz, 6H).