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A colorimetric and near-infrared fluorescent probe for hydrogen polysulfides and its application in living cells

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ABSTRACT

The development of probes for rapid, selective, and sensitive detection of the hydrogen polysulfides is of great importance in biological science. We report here a new Near-infrared (NIR) fluorescent off-on probe for selective and sensitive detection of hydrogen polysulfides (H_2S_n) with colorimetric and NIR fluorescent dual signal changes. The probe was synthesized by an esterification reaction coupling between a dicyanomethylene-benzopyran (DCMB) dye and a bis-electrophilic H_2S_n capture group 2-fluoro-5-nitrobenzoic acid. The probe showed a specific off-on response to H_2S_n in aqueous solution with nanomolar LOD, and without interference by a range of competing sulfocompounds and several reactive nitrogen and oxygen species. Moreover, it can be used for imaging of endogenous H_2S_n in living cells.

1. Introduction

Reactive sulfur species (RSS) such as cysteine (Cys), homocysteine (Hcy), glutathione (GSH), hydrogen sulfide (H_2S), hydrogen polysulfides (H_2S_n) have received much attention due to their biological and physiological functions in redox biology [1–4]. Among these RSS molecules, H_2S has been extensively studied as a new signaling molecule in the past decade. Up or down level of H_2S in living biological systems can result in a range of deleterious effects including Down's syndrome, Alzheimer's disease, liver cirrhosis and diabetes [5]. Recent studies reveal that H_2S_n can be considered as the oxidized forms of H_2S. In this case, H_2S_n was likely to coexist with H_2S in living organisms and they work together as redox pairs [6–8]. However, as a class of important functional signaling molecules, the fundamental chemistry and physiological functions of H_2S_n are still poorly understood. So monitoring of H_2S_n in living biological systems with a simple, rapid and sensitive method is of great significance. The traditional methods for detection of H_2S_n mainly depend on the employment of mass spectrometry, UV spectroscopy [9–11]. Despite their accuracy and repeatability, these methods are limited by long detection time, high detection cost and complicated conducting procedures.

Compared with the traditional methods, fluorescence (FL) methods have received more attention due to their high sensitivity, selectivity and flexibility in structural modification [12–19]. Recently, a few of FL probes for H_2S_n have been developed, but some of which were limited by their short emission wavelength [20–26] and poor water solubility [27–29]. Furthermore, some probes suffer from long synthetic routes and low overall yields [30–33]. All these drawbacks restrict their applications in monitoring real samples.

To address the above issues, we report a novel Near-infrared (NIR) FL probe for selective and sensitive detection of H_2S_n with colorimetric and NIR fluorescent dual signal changes. The probe was synthesized by a esterification reaction coupling between a dicyanomethylene-benzopyran (DCMB) dye and a bis-electrophilic H_2S_n capture group 2-fluoro-5-nitrobenzoic acid. The probe showed a specific off-on response to H_2S_n in aqueous solution with nanomolar LOD, and without interference by a range of competing sulfocompounds and several reactive nitrogen and oxygen species. Moreover, it can be used for imaging of endogenous H_2S_n in living cells.

2. Materials and methods

2.1. General

All purchased chemicals and reagents are of analytical grade. Solvents were purified by standard procedures. Reactions were monitored by TLC (thin-layer chromatography) using E-Merck aluminum precoated plates of Silica Gel. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using tetram-

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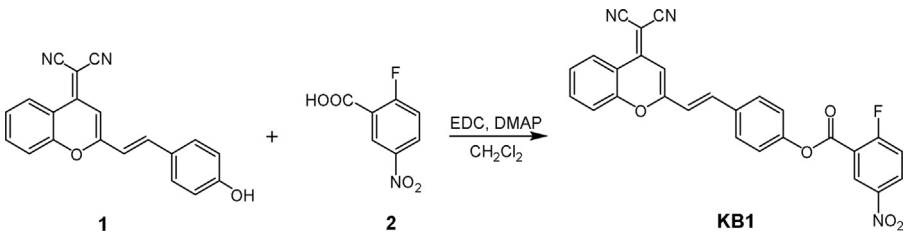

Scheme 1. Synthetic procedures for compound **KB1**.

ethylsilane (TMS) as the internal standard (chemical shifts in parts per million). High resolution mass spectra were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV). All fluorescence spectra were measured on a Varian Cary Eclipse Fluorescence spectrophotometer.

2.2. Spectroscopic measurements

Stock solution of **KB1** (5 mM) was prepared in DMSO. Stock solutions of 5 mM of $NaNO_2$, $NaNO_3$, Cys, GSH, Hcy, Na_2S, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, S_8, Na_2S_2, Na_2S_4 were all prepared in deionized water. The preparation of reactive nitrogen and oxygen species (RNS/ROS) such as NO, $ONOO^-$, H_2O_2, ClO^-, O_2^-, •OH, $^1O_2$ can be found in the Supporting information, and they were used immediately after being generated. The FL measurements were carried out with a path length of 10 mm and an excitation wavelength at 535 nm by scanning the spectra between 545 nm and 800 nm. The bandwidth for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, All FLs were excited at 535 nm and acquired in 10 mM HEPES buffer (pH 7.4, 0.5% DMSO) after incubation for 5 min at 25 °C.

2.3. Cell imaging assay

MCF-7 cells were cultured in DMEM supplemented with 10% FBS. Cells (1.5×10^4/well) were seeded on a black 24-well microplate with optically clear bottom overnight. For endogenous polysulfide labeling, the cells were incubated with 10 μM **KB1** for 30 min at 37 °C. For exogenous reactive sulfur species imaging, the four group of cells were preteated with 0.5 mM of N-methylmaleimide (NMM). After 30 min, 10 μM of **KB1** was added and incubated for anothor 30 min. Then 0.5 mM of Na_2S_2, Na_2S, Cys, GSH were added to the above group of cells respectively and incubated for another 30 min. For the thiol-consumed group, the cells were incubated with 0.5 mM NMM for 30 min and then treated with the same procedure. After three rinses in PBS, the fluorescence was eventually detected and photographed with a confocal laser scanning microscopy.

2.4. Cell viability assay

Cells were plated overnight on 24-well plates at 5000 cells per well in growth medium. After seeding, cells were maintained in growth media treated at increasing concentrations (25 μM, 50 μM, 100 μM, 200 μM, 500 μM, 1000 μM) of **KB1** (dissolved in DMSO, final concentration) for 72 h. 20 μL of MTS (Promega Corp) solution (2 mg/mL) was added to each well for 2 h at 37 °C, and then the absorbance was measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 490 nm with a reference at 690 nm. The optical density of the result in MTS assay was directly proportional to the number of viable cells. Each experiment was done in triplicate.

3. Results and discussion

In order to develop a NIR FL probe, a NIR fluorescent dye is needed. It was reported [34] that the dicyanomethylene-benzopyran (DCMB) derivatives not only show controllable emission intensity in the NIR region but also have high photostability. Therefore the target product **KB1** was prepared by the esterification reaction of the DCMB dye (compound 1 in Scheme 1) [34] as the fluorophore with a bis-electrophilic H_2S_n capture group 2-fluoro-5-nitrobenzoic acid (Scheme 1) [21,26].

With the probe in hand, its selectivity for H_2S_n was investigated using FL spectroscopic techniques. We observed that the presence of Na_2S_n cause a more than 30-fold increase in the FL intensity at 682 nm, whereas the presence of a variety of competing sulfocompound including cysteine (Cys), glutathion (GSH), Homocysteine (Hcy), Na_2S, S_8, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, and several reactive nitrogen and oxygen species (RNS/ROS) including NO, $ONOO^-$, H_2O_2, ClO^-, O_2^-, •OH, $^1O_2$ did not cause the same FL response (Figs. 1 a and S1). Additionally, the presence of some common metal ions also did not cause FL change of the probe (Fig. S5). More interestingly, even in the presence of all the above competing analytes, the FL change of **KB1** could be sensitively elicited by Na_2S_2. A colorimetric assay was also explored as it is simple. As shown in Fig. 2, the color of the probe **KB1** solution was obviously changed only upon addition of Na_2S_2 and Na_2S_4.

Next, we researched response of **KB1** to H_2S_2 in 10 mM HEPES buffer (pH 7.4, 0.5% DMSO). **KB1** exhibited an absorption peak centred at 410 nm. When 20 μM of Na_2S_2 was added to the solution of **KB1**, a new absorption peak appeared at 535 nm indicating that **KB1** had reacted with H_2S_2 (Fig. 1b). Simultaneously, a more than 30-fold increase in the FL spectra at 682 nm was observed (Fig. 1c). Plotting of the FL variation of **KB1** as a function of increasing Na_2S_2 (Fig. 1f) produced a linear range over 0–20 μM. The LOD of the probe for Na_2S_2 was determined to be 8.2 nM (LOD = 3σ/k). The results demonstrate that **KB1** can be applied for both qualitatively and quantitatively detection of hydrogen polysulfides. To test the detection kinetics, the FL response of **KB1** (FL at 682 nm) to Na_2S_2 was plotted as a function of incubation time (Fig. 1e). To our delight, the response could reach equilibrium within 6 min in the aqueous buffer.

Since pH may frequently impact the sensing ability of FL probes, we further tested the response of **KB1** to Na_2S_2 over a wide pH range from 4 to 10 (Fig. 1d). **KB1** was quite stable in aqueous media up to pH 10 and showed good sensitivity with the off-on response towards Na_2S_2 over the pH range from 6.0 to 10 with maximum changes at pH over 8. This clearly indicates that the probe can be used to detect H_2S_2 in the physiological pH range. Therefore, we decided to employ the probe for fluorescent imaging of cellular H_2S_n.

As illustrated in Scheme 2, H_2S_2 can firstly react with **KB1** through a nucleophilic aromatic substitution via replace F atom to form an intermediate containing free —SSH group. Then the free —SSH group continued a intramolecular cyclization with the ester group to release the DCMB fluorophore. This two-step reaction