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A near-infrared fluorescent probe for monitoring fluvastatin-stimulated endogenous H_2S production

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ABSTRACT

Most reported fluorescent probes have limitations in practical applications in living systems due to the strong autofluorescence background, construction of probes with near-infrared (NIR) fluorescence emission is an accessible approach for addressing this challenge. We here designed a NIR fluorescent probe for monitoring the endogenous production of H_2S in living cells. The designed probe showed significant NIR fluorescence turn-on response to H_2S with high selectivity, enabling the sensitive detection H_2S. Importantly, the probe could be applied in monitoring the endogenous production of H_2S in raw264.7 macrophages. This study showed that fluvastatin can promote the activity of cystathionine γ-lyase (CSE) for generation H_2S.

1. Introduction

Endogenous hydrogen sulfide, generated enzymatically by cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase, is an important gasotransmitter which is closely involved in a number of physiological functions and pathophysiological processes [1]. Therefore, development of valuable methods to interrogate the physiological and pathological functions of H_2S is in high demand [2]. In this context, fluorescent detection with H_2S-responsive fluorescent probe has been established as a promising tool due to its simplicity and high spatio-temporal resolution [3–5]. However, most of reported probes suffer from limitations imposed by their short emission wavelengths and thus retard their practical applications in living system due to the strong autofluorescence background from living tissues. To address this challenge, construction of probes with near-infrared (NIR) fluorescence emission is an accessible approach due to some advantages with NIR imaging, such as decreased autofluorescence, low light scattering, and high penetration depth [6].

Here, we developed a novel NIR probe (NIRDCM-H_2S) for H_2S, composed of dicyanomethylene-4*H*-pyran (DCM) chromophore as a NIR fluorescence reporter and a pyridine-disulfide-propionate group as the responsive site toward H_2S. The H_2S active trigger was specifically incorporated into DCM through esterification of a phenol conjugated into the dicyanopyran moiety through a vinyl bond. We reasoned that the cleavage of the disulfide (S–S) bond in NIRDCM-H_2S by H_2S could selectively liberate the phenol-based DCM (Scheme 1). Since ester is an electron withdrawing group while phenol is a strong electron donating unit, the transformation of ester to phenol would induce large spectral shifts into NIR region *via* modulation of the electron-donor ability of DCM derivatives. Furthermore, NIRDCM-H_2S was expected to be a promising probe in trapping endogenous H_2S produced by upregulation of CSE activity upon stimulation with fluvastatin.

2. Experimental

2.1. Synthesis. DCM-OH and PSSAcid were prepared according to our reported procedures [7,9]

2.1.1. Synthesis of NIRDCM-H_2S

To a solution of DCM-OH (34 mg, 0.11 mmol) and PSSAcid (25 mg, 0.11 mmol) in dry CH_2Cl_2 were added DPTS (218 mg, 0.70 mmol) and DIPC (88 mg, 0.70 mmol). The resulting mixture was stirred for 10 h at room temperature, then CH_2Cl_2 was removed under vacuum, and the product was purified by silica chromatograph to afford 50 mg NIRDCM-H_2S (90%). ^1H NMR

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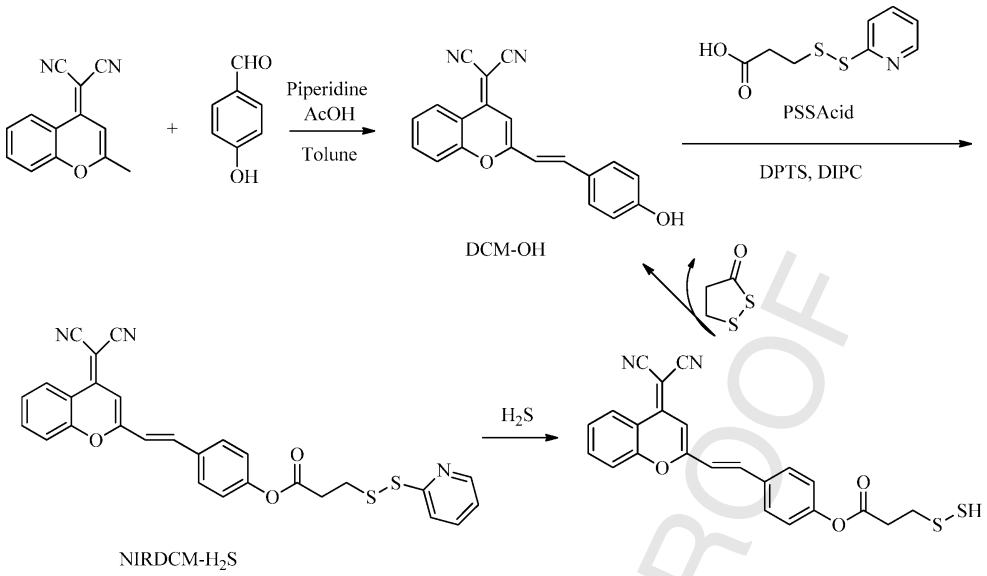

Scheme 1. The synthesis of NIRDCM-H_2S and the proposed reaction mechanism with H_2S.

(400 MHz, $CDCl_3$, ppm): δ 8.91 (d, 1H, J = 8.0 Hz), 8.50 (d, 1H, J = 4.0 Hz), 7.78-7.54 (m, 7H), 7.46 (t, 1H, J = 8.0 Hz), 7.19 (d, 2H, J = 8.0 Hz), 7.15-7.10 (m, 1H), 6.87 (s, 1H), 6.78 (d, 1H, J = 16.0 Hz), 3.17 (t, 2H, J = 8.0 Hz), 3.07 (t, 2H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 169.88, 159.48, 157.15, 152.77, 152.30, 152.07, 149.84, 137.64, 137.15, 134.75, 132.47, 129.07, 126.05, 125.83, 122.35, 121.01, 119.99, 118.99, 118.64, 117.80, 116.68, 115.61, 107.05, 63.13, 34.01, 33.11, 29.70, 23.47; HRMS (ESI^+) calcd. for $C_{28}H_{19}N_3O_3S_2$ $[M+H]^+$: 510.0946; Found: 510.0940.

2.2. Confocal image of cells

Raw264.7 macrophage cells were cultured within a humidified atmosphere of 5/95 CO_2/air incubator in Roswell Park Memorial Institute 1640 medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) at 37 °C. Then the cells were seeded in a glass bottom dish for 24 h, followed by loading with NIRDCM-H_2S (5 μmol/L) in culture medium for 30 min at 37 °C, washed with D-Hanks. For imaging exogenous H_2S, Cells incubated with NIRDCM-H_2S (5 μmol/L) for 30 min at 37 °C, followed by the addition of 1 mmol/L NaHS for another 30 min. For imaging endogenous H_2S, Cells pretreated with 2 μmol/L fluvastatin for 48 h were loaded with 5 μmol/L NIRDCM-H_2S for 30 min at 37 °C. For evaluation the inhibitory effect, Cells were firstly incubated with 2 μmol/L fluvastatin and 1 mmol/L PAG for 48 h, then treated with 5 μmol/L NIRDCM-H_2S for 30 min at 37 °C. The excitation wavelength was 488 nm, images were collected at 620–750 nm.

3. Results and discussion

NIRDCM-H_2S was readily prepared in two steps commencing from DCM by known chemistry including Knoevenagel condensation and esterification in the presence of *p*-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) and *N*,*N*'-diisopropylcarbodiimide (DIPC) [7]. The yield is 90% and NIRDCM-H_2S was fully identified by ^1H NMR and ^{13}C NMR and high-resolution mass spectrometry (HRMS).

The reaction of NIRDCM-H_2S between H_2S was firstly monitored with fluorescence spectra. As shown in Fig. 1, when 10 μmol/L NIRDCM-H_2S in CH_3CN-PBS buffer (1:1, v/v, pH = 7.4) was treated with NaHS as a H_2S donor, the dramatic enhancement of an NIR emission falling into the range of 620–750 nm was observed. Obviously, such time-dependent NIR fluorescence increase resulted from H_2S-triggered modulation of the electronic characteristics of the substituents, which is well known in controlling the optical properties of DCM derivatives [8]. According to our previous results and NMR as well as HRMS characterization [9], it can be deduced that the S-S bond in NIRDCM-H_2S could be selectively cleaved by H_2S, followed by an intramolecular nucleophilic attack on the carbonyl function by the strong nucleophilic SH (Scheme 1, Fig. S1 and S2 in Supporting information), inducing the liberating DCM-OH through a five-membered cyclic transition state.

NIRDCM-H_2S was found to afford high selectivity toward H_2S over other biologically relevant species (Fig. S3 in Supporting information). Only H_2S led to a significant NIR emission increase, while minimal NIR fluorescence enhancement was observed upon incubation of NIRDCM-H_2S with various analytes, including glutathione, cysteine, glycine, Cl^-, Br^-, H_2O_2, $O_2^{\cdot-}$, ClO_4^-, ClO^-, NO_2^-,NO_3^-, N_3^-, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_3^{2-}$, HCO_3^-, HPO_4^{2-}. These results indicated that the good selectivity of NIRDCM-H_2S toward H_2S, which facilitates the accurate detection under complex biosystem.

It was also noticed that the NIR emission response to H_2S is dose dependent (Fig. 2). There was a good linear relationship between the fluorescence intensity and H_2S concentration in the 5−60 μmol/L range, resulting in a detection limit to be 2.5 μmol/L. These results inferred that NIRDCM-H_2S is sufficiently sensitive for detection endogenous H_2S in living systems.

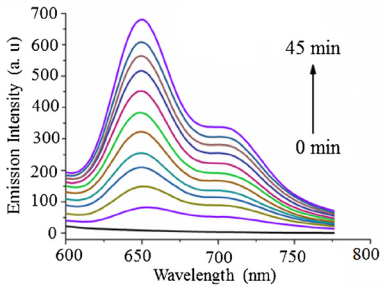

Fig. 1. Time-dependent fluorescence profiles of NIRDCM-H_2S (10 μmol/L) in the presence of 100 μmol/L NaHS in CH_3CN-PBS buffer (1:1, v/v, pH 7.4). Spectra were acquired at 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 45 min after addition of NaHS. λex = 450 nm.

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