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A long-wavelength-emitting fluorescent turn-on probe for imaging hydrogen sulfide in living cells

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ABSTRACT

A long-wavelength-emitting fluorescent turn-on probe for H_2S based on the thiolysis of dinitrophenyl ether has been designed and synthesized facilely. The probe has a red emission at 655 nm in aqueous solution with high sensitivity (17-fold turn-on) to H_2S. Further, the probe exhibits a high selectivity for H_2S detection without interference from various biologically relevant species. Its potential utility for biological applications was confirmed by fluorescence imaging of H_2S in living cells.

1. Introduction

Hydrogen sulfide (H_2S), a toxic gas with the characteristic smell of rotten eggs, has been recognized as the third endogenous gaseous signaling molecules, along with the other two transmitters including nitric oxide (NO) and carbon monoxide [1–3]. H_2S is also a biological gas produced endogenously from cysteine after a series of reactions catalyzed by several enzymes such as cystathionine β-synthase and cystathionine γ-lyase [4]. Furthermore, recent studies show that H_2S is involved in various physiological processes including antioxidation [5], neuromodulation [6], angiogenesis [7], anti-inflammation [8], and ischemia reperfusion injury [9]. In addition, some other research studies also indicate that the endogenous concentration of H_2S is related to Down syndrome [10], Alzheimer's disease [11], and hypertension [12]. Since H_2S is related to many physiological and pathological processes, it is urgent to develop sensitive and convenient methods for monitoring H_2S in aqueous media and living systems.

In the past two decades, the detection of H_2S in living systems has attracted great attention. A large number of sensitive and selective methods have been developed for the detection of H_2S, such as colorimetry [13], electrochemical analysis [14], gas chromatography [15], and metal-induced sulfide precipitation [16]. However, these techniques often require post-mortem processing and/or destruction of tissues or cells [17]. In contrast, the fluorescence method provides more convenience, less invasiveness, high selectivity and sensitivity as well as real-time imaging [18]. Using the fluorescence method, the following characteristics are highly desirable for intracellular imaging: (i) selectivity and sensitivity toward H_2S; (ii) fluorescence maxima in the long-wavelength region (>650 nm) [19]. Recently, several groups have made great progress in the development of fluorescence probes for monitoring H_2S. These probes are constructed primarily based on the specific H_2S-induced reactions, including quencher removal [20,21], reduction of azides and nitros [22–24], nucleophilic reaction [25,26], and interaction with metal-containing compounds [27–29]. However, most of these probes require complicated synthesis, or have a slow response time of ~1–2 h, or have emissions in the ultraviolet or the visible region, in which the fluorescence imaging is easily interfered by cell auto-fluorescence. As we know, fluorescent probes with emission in the long-wavelength region are more suitable for biological imaging applications as a result of minimum interference from background auto-fluorescence and minimal photo damage

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to biological samples in living systems. Therefore, to develop new types of long-wavelength-emitting fluorescent probes for the non-destructive and rapid detection of H_2S in live cells or tissues is still highly demanded.

Herein, we present a new long-wavelength-emitting fluorescent turn-on probe NR-HS (Scheme 1) for H_2S based on the principle of photoinduced electron transfer (PET). Nile red is well known as an environment-sensitive fluorescent probe for labeling and sensing biomolecules, owing to its high fluorescence quantum yield, long-wavelength emission and good photostability [30]. Therefore, Nile red was used as a fluorescent moiety in the H_2S probe. After the incorporation of the dinitrophenyl ether group in the 2-position of Nile red, which acts as the H_2S reactive site [31], the probe was synthesized facilely and the fluorescence of the probe was quenched *via* the PET process between the fluorophore and the dinitrophenyl ether group. For example, the fluorescence quantum yield of probe NR-HS was 0.21 in DMSO and 0.054 in aqueous solution (DMSO/PBS = 1:9). However, that of the thiolysis compound NR increased to 0.83 in DMSO and 0.32 in aqueous solution, respectively. Obviously, the big difference between the fluorescence quantum yield of probe NR-HS and that of compound NR suggests that NR-HS is highly sensitive fluorescent turn-on probe for H_2S. As expected, NR-HS exhibits high sensitivity and selectivity for H_2S in the presence of other competitive ions due to the removal of the dinitrophenyl ether group by H_2S. The proposed mechanism of probe NR-HS for H_2S detection are shown in Scheme 1.

2. Materials and methods

2.1. Materials and instruments

Reagents were purchased from Aldrich Inc. and Adamas-beta Chemical Ltd. and used without further purification unless otherwise stated. Column chromatography was conducted with silica gel 60 (400 mesh). Twice-distilled water was used throughout all experiments. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz by a Bruker 400 spectrometer, respectively, using TMS as an internal standard. The mass spectra (MALDI-TOF) were recorded on a Voyager-DE STR mass spectrometer. Absorption and fluorescence spectra were acquired using a PerkinElmer Lambda-35 UV–vis spectrophotometer and a Varian Cary Eclipse spectrophotometer, respectively. Fluorescence quantum yields were determined in relative to that of cresyl violet in methanol (54.5% fluorescence yield) [32]. Cell imaging was performed with a Nikon Eclipse TE300 inverted microscope.

2.2. Methods

2.2.1. The detection limit

The detection limit was calculated based on the method reported in the literature [33]. The fluorescence emission spectrum of NR-HS was measured five times to obtain the standard deviation (σ) of blank measurement. The fluorescence intensity at 655 nm was plotted *versus* the concentration of NaHS, which gave the slope k. Then the detection limit was calculated by using the formula $3\sigma/k$.

2.2.2. Cell culture and fluorescence imaging

MCF-7 cells (human breast carcinoma) were cultured in Dulbecco's modified Eagle's medium (DMEM) 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 washed with PBS buffer before the experiments, then incubated with 5 μM of the probe NR-HS for 30 min at 37 °C. After being washed three times with PBS buffer, the cells were then incubated with NaHS (100 μM) for another 30 min. The fluorescence imaging was observed under a confocal microscopy Nikon Eclipse TE300. The fluorescence was collected at 600–630 nm upon excitation by a 488 nm laser.

2.3. Synthesis

2.3.1. Synthesis of compound NR

5-Diethylamino-2-nitrosophenol hydrochloride (2.30 g, 10 mmol) and 1,6-dihydroxynaphthalene (1.60 g, 10 mmol) were heated under reflux in DMF (100 ml) for 4 h. DMF was removed under reduced pressure. The crude mixture was purified by chromatography (ethyl acetate/isopropanol = 4/1 (v/v)) to yield a dark green solid (2.14 g, 64%). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS): δ = 10.41 (s, 1H), 7.97–7.88 (m, 1H), 7.87 (s, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.10–7.07 (m, 1H), 6.80–6.77 (m, 1H), 6.62 (s, 1H), 6.14 (s, 1H), 3.48 (q, J = 7.2 Hz, 4H), 1.16 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS): δ = 182.01, 161.05, 152.03, 151.12, 146.84, 134.20, 131.25, 127.90, 124.31, 118.80, 110.34, 108.57, 104.54, 96.49, 44.87, 12.91. MS (MALDI-TOF, m/z): calcd for $C_{20}H_{18}N_2O_3$ [M+1] 335.1; found, 335.1.

2.3.2. Synthesis of compound NR-HS

Compound NR (334 mg, 1 mmol) was dissolved in 5 ml of acetone, and 0.5 ml of triethylamine was pipetted into the reaction flask. 2,4-Dinitrofluorobenzene (186 mg, 1 mmol) was dissolved in 5 ml of acetone and added to the mixture. Then the solution was refluxed for 0.5 h. Acetone was removed by evaporation, followed by the addition of 10 ml of 5% HCl solution. The precipitate was filtered and washed several times with water. The crude product was purified by recrystallization in acetone to yield a dark green solid (355 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.96 (s, 1H), 8.51–8.48 (m, 1H), 8.28–8.25 (m, 2H), 7.61–7.59 (m, 2H), 7.39 (d, J = 9.2 Hz, 1H), 6.87–6.84 (m, 1H), 6.71 (s, 1H), 6.33 (s, 1H), 3.55–3.49 (q, J = 7.2 Hz, 4H), 1.17 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS): δ = 181.95, 160.98, 159.81, 157.12, 151.89, 151.01, 146.71, 143.80, 139.01, 137.21, 134.10, 131.64, 131.53, 131.16, 127.81, 124.21, 122.67, 120.79, 120.56, 118.68, 110.22, 108.48, 104.45, 96.33, 44.85, 12.87. HR-MS (MALDI-TOF, m/z): calcd for $C_{26}H_{20}N_4O_7$ [M^+] 500.1; found, 500.1. Elemental analysis (%): C 62.40, H 4.03, N 11.20; found: C 62.63, H 4.21, N 11.17.

2.3.3. Conversion of compound NR-HS to compound NR

To a solution of NR-HS (50 mg, 0.1 mmol) in acetone (5 ml) was added NaHS (6.0 mg, 0.11 mmol) in water (0.5 ml) with stirring at room temperature. After 15 min, the solvent was evaporated under reduced pressure. The mixture was purified by flash column chromatography (ethyl acetate/isopropanol = 4/1 (v/v)) to give a dark green solid (29 mg, 87%), which was characterized as compound NR according to NMR and mass spectrometry (See the data for compound NR).

3. Result and discussion

3.1. Synthesis

The synthetic route to the desired probe NR-HS is showed in Scheme 2. The probe was synthesized facilely by two steps. Firstly, the intermediate Nile red (NR) was synthesized by reacting 5-diethylamino-2-nitrosophenol hydrochloride with 1,6-dihydroxynaphthalene in 64% yield. Then, the probe NR-HS was obtained in 71% yield after the incorporation of the dinitrophenyl ether group in the 2-position of NR. Both compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS, the purity of the target compound NR-HS were further confirmed by elemental analysis.