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The design of hydrogen sulfide fluorescence probe based on dual nucleophilic reaction and its application for bioimaging

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ABSTRACT

Hydrogen sulfide (H_2S) can undergo dual nucleophilic reaction, which is a wise and effective way to distinguish biothiols and H_2S. A novel H_2S fluorescence probe, 4-{2-[4-(2-disulfide pyridyl-benzoyloxy)-phenyl]-vinyl}-1-methyl-pyridinium[*e*]iodide (DSPBP), with two nucleophilic reaction sites has been developed. The spectra results showed that DSPBP could detect H_2S in ratiometric and colorimetric signals and has excellent selectivity and sensitivity. The fluorescence ratiometric signals (F_{520}/F_{450}) displayed a prominent increase from 0.74 to 7.08, the fluorescence color turned to yellow form blue simultaneously. The linear range was 2–14 μM and its detection limit was 25.7 nM. Moreover, the biocompatibility of DSPBP was fine and its toxicity was very low. It has been successfully used for imaging H_2S in cells.

1. Introduction

The detection of hydrogen sulfide (H_2S), especially the lower concentration detection in biosystems, has been caused more and more attention since it has been regarded as the third gaseous signal molecule [1–6]. At present, the biggest challenge for its detection is the interference of biothiols, in order to solve the problem, some H_2S fluorescence probes have been designed based on the difference of reactive ability between H_2S and biothiols, including stronger reduction ability of H_2S [7–10], better complex ability of H_2S with metal ions [11–15] and dual nucleophilic capability of H_2S [16–18]. In these reported literature, the probes of H_2S contained bis-electrophilic centers are more attractive and more advantageous in selectivity.

For most dual electrophilic centers of H_2S probes, fluorophore and H_2S trap site were linked by esterification. The H_2S trap site was the first nucleophilic center, such as aldehyde [19–21], a,β-unsaturated ester [22], benzyl iodide [23] and dithiopyridine [24–28],which can trap H_2S by nucleophilic addition or nucleophilic substitution and form intermediate mercapto compound. Then the intermediate mercapto compounds spontaneously react with the second nucleophilic site, ester bond, to release corresponding fluorophore. Obviously, the two nucleophilic reactions ensure the high selectivity of the type of H_2S probes. M. Xian's group first proposed the mechanism, used dithiopyridine as the trap site of H_2S and a series of fluorescence probe with excellent performance were developed [29,30]. Compared with other trap sites, the reaction speed of dithiopyridine towards H_2S was faster and accomplished completely in several minutes. Although these probes are innovative, most of them display turn-on signal on fluorescence spectra, which might be disturbed by many factors, including the temperature of detection, pH of solution, solvent polarity and instrument interference and so on [31–35]. Ratiometric fluorescent probes can avoid these influences and achieve more accurate detection results.

In our previous research, the H_2S ratiometric fluorescence probe based on the 4-hydroxy-1,8-naphthalimide fluorophore was reported [36], but the ratiometric effect, solubility and biocompatibility need to be further improved. Herein, the probe DSPBP adapt fluorophore, 4-[2-(4-Hydroxy-phenyl)-vinyl]-1-methyl-pyridinium[*e*]iodide, which is ionic compound with better water solubility and better biocompatibility, can show excellent ratiometric properties after reaction. As we anticipated, all the properties of DSPBP (selectivity, sensitivity, solubility and toxicity) were ideal and the imaging H_2S in living cells was successful and satisfying.

2. Experimental

2.1. Materials and Instruments

All materials were purchased from Aldrich. The solutions used for the spectral analysis were processed as required. UV–vis absorption spectra were obtained using a UV-2450 spectrophotometer (Shimadzu,

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Tokyo, Japan). Fluorescence spectra were measured with a F4500 fluorescence spectrometer (Hitachi, Tokyo, Japan). ^{1}HNMR and ^{13}CNMR spectra were performed on a 400 MHz spectrometer. High-resolution mass spectra were recorded using a mass spectrometer (Bruker Autoflex).

2.2. Spectra Measurement

Stock solutions of DSPBP (5 mM) were prepared in DMF. Aqueous Na_2S (H_2S donor) and other interference analytes solutions (all at 10 mM) were prepared using the deionized water. All UV–vis and fluorescence spectra were obtained in DMF: PBS (1:9 v/v, pH 7.4) solutions. Each spectra measurement was monitored after 15 min at 25 °C (E_x = 370 nm, E_m = 520 nm, silt: 10 nm/10 nm).

2.3. Cell Imaging Experiments With H_2S

HeLa cells were obtained from biological laboratory of Shanxi University. First, the adherent cells were washed once with FBS-free DMEM. Then the cell was treated with DSPBP (10 μM) in culture media for 30 min at 37 °C and washed three times with PBS (pH 7.4). Second, the cells were further incubated with Na_2S (20 μM) for 20 min. Third, cell imaging was carried out on a Zeiss LSM 880 confocal laser scanning.

2.4. Preparation of DSPBP

As illustrated in Scheme 1, compound 3 was got by esterification of compound 1 and 2, DSPBP was synthesized from methylation of compound 3. The detailed synthesis of compound 1 and 2 were illustrated in supporting materials and synthesized by the literature methods [29].

2.4.1. Synthesis of Compound 3

Compound 1 (79 mg, 0.4 mmol), compound 2 (105 mg, 0.4 mmol), EDC (76 mg, 0.4 mmol) and DMAP (5.0 mg, 0.04 mmol) were added in 6 mL mixed solution of CH_2Cl_2/DMSO (5:1) and the mixture was stirred for 10 h at 25 °C. Washed by water and dried, purified directly by column chromatography using CH_2Cl_2/CH_3OH (10:1, v/v) and lead to the desired compound 3 (115 mg, 65%).

^{1}HNMR (400 MHz, DMSO, 25 °C, TMS) 8.54 (d, J = 6.0, 2H), 8.46–8.48 (m, 1H), 8.30–8.34 (m, 1H), 7.69–7.89 (m, 5H), 7.53–7.62 (m, 4H), 7.46–7.50 (m, 1H), 7.39–7.42 (m, 2H), 7.26–7.30 (m, 2H). ^{13}CNMR (100 MHz, CDCl3, 25 °C, TMS) δ 164.3, 157.3, 157.0, 150.4, 150.0, 149.9, 149.7, 144.2, 140.0, 139.5, 138.1, 134.4, 132.0, 128.3, 126.6, 126.4, 126.2, 125.9, 125.6, 122.4, 121.9, 121.8, 120.9, 119.8, 119.5 (Figs. S1–S2).

2.4.2. Synthesis of DSPBP

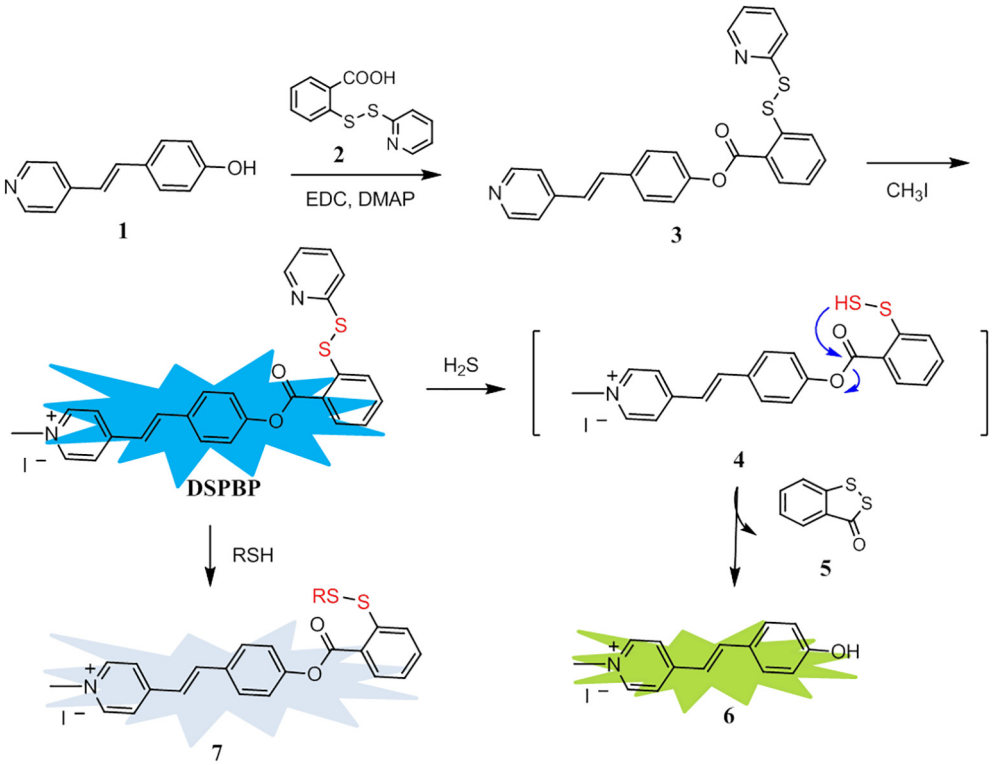
The compound 3 (44 mg, 01 mmol) was dissolved in 5 mL CH_3CN and 2 mL CH_2Cl_2, then 0.1 mL of CH_3I was added. The mixture was stirred at 25 °C for 2 days. Solvent was removed and the residue was washed with ether and CH_2Cl_2 to afford the probe DSPBP (38 mg, 65%).

^{1}HNMR(400 MHz, DMSO, 25 °C, TMS) δ 8.90 (d, J = 6.8, 2H), 8.51 (d, J = 4, 1H), 8.35 (d, J = 6.4, 1H), 8.26 (d, J = 6.8, 2H), 8.09 (d, J = 16.4, 1H), 7.90–7.93 (m, 3H), 7.76–7.84 (m, 2H), 7.51–7.61 (m, 5H), 7.30–7.33 (m, 1H), 4.29 (s, 3H). ^{13}CNMR (100 MHz, CDCl3, 25 °C, TMS) δ 169.4, 162.2, 157.6, 156.9, 155.2, 150.5, 150.4, 145.3, 144.8, 143.4, 139.8, 138.6, 137.5, 134.7, 131.9, 131.0, 130.8, 128.9, 128.0, 127.1, 125.1, 52.5. HRMS $[M + H]^+$ calcd for $C_{26}H_{21}N_2O_2S_2$, 457.1039, found, 457.1031 (Figs. S3–S5).

3. Results and Discussion

3.1. Proposed Mechanism

The reaction mechanism of DSPBP towards H_2S was interpreted in Scheme 1. Disulfide pyridine group of DSPBP was employed as H_2S receptor. After reacting, form —SH containing intermediate **4**, the ester cyclization of **4** should proceed swiftly to yield by-product **5** and the fluorophore **6**. Compound **6** showed strong yellow-green fluorescence emission due to the stronger electron mobility from hydroxyl group to pyridine salt. The mechanism was proved by the reaction of DSPBP



Scheme 1. The synthesis of DSPBP and mechanism against H_2S.