A coumarin-based colorimetric fluorescent probe for hydrogen sulfide

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MS received 5 September 2014; revised 30 October 2014; accepted 23 December 2014

Abstract. A coumarin-based fluorescent probe for selective detection of hydrogen sulfide (H₂S) is presented. This ‘off–on’ probe exhibited high selectivity towards H₂S in aqueous solution with a detection limit of 30 nM. Notably, because of its dual nucleophilicity, the probe could avoid the interference of thiols and other sulfur containing compounds.

Keywords. Coumarin; reactive probe; H₂S probe

1. Introduction

Hydrogen sulfide (H₂S) is an endogenously produced gaseous signalling compound (gasotransmitter), along with nitric oxide (NO) and carbon monoxide (CO), which mediate a wide range of physiological and pathological effects, such as anti-oxidation, anti-inflammation, apoptosis, etc.^{1–3} Due to the important role of H₂S, developing a sensitive, rapid, and reliable detection technique for it is in high demand and has become a hot research area in the past few years. Many excellent fluorescent probes for the detection of H₂S have been reported based on different strategies including H₂S mediated reduction of azide and nitro compounds,^{4,5} an azamacrocyclic copper(II) ion complex,⁶ thiolysis of dinitrophenyl ether,⁷ and H₂S trapping by nucleophilic addition.⁸ The nucleophilic reactions of H₂S toward α , β -unsaturated acrylate methyl ester or an aldehyde group have been widely used to design H₂S-targeted chemodosimeters. The diversity of the reaction sites is beneficial for the design of fluorescence sensors, which result in ON-OFF, OFF-ON or ratiometric probes.⁸ But the selectivity and interference from other thiols (such as cysteine, homocysteine and glutathione) are still a challenge. Meanwhile, most of the probes need organic solvents as co-solvent, which improve the solubility of the probes or shorten the reaction time. To overcome the problems mentioned above, two-step nucleophilic reaction was introduced when designing probes for H₂S, which exhibit promising future with good anti-interference.⁹ More recently, Guo group exploited 2-(iodomethyl) benzoate as a new H₂S trap group,

and reported a fluorescein based probe for H₂S with high selectivity.^{9e} Inspired by this work, we wanted to develop a coumarin-based H₂S probe with similar mechanism.

Coumarin has been largely utilized as fluorophore in various fluorescent probes due to its large Stokes shift, good solubility and high quantum yield.¹⁰ Previously, we reported a coumarin-based chromogenic and ratiometric probe for hydrazine.¹¹ As we know, when the phenolic hydroxyl of a fluorophore is protected via esterification, no emission of the fluorophore would be found due to the electron-withdrawing property of acetate group. However, the removal of ester groups under suitable condition will lead to the fluorescence recovery of the fluorophore. Herein, we designed a two-step nucleophilic reaction-based H₂S probe, which employs 7-hydroxy-coumarin as fluorophore by protecting the hydroxyl via an ester group.

The fluorescent probe was generally constructed with a 7-hydroxy-coumarin as fluorophore and 2-(iodomethyl) benzoate as H₂S trap that has two electrophilic reaction sites. The initial nucleophilic attack of H₂S would lead to an intermediate thiol, which spontaneously undergoes a cyclization by forming benzo[c]thiophen-1(3*H*)-one and releases the fluorophore thereby eliciting the obvious fluorescent signal (scheme 1).

2. Experimental

2.1 General

¹H NMR, ¹³C NMR spectra were measured on a Bruker AM400 NMR spectrometer. ESI-MS and HRMS spectral data were recorded on a Finnigan LCQDECA and

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Scheme 1. The proposed sensing mechanism of probe **1** for H_2S .

a BrukerDaltonics Bio TOF mass spectrometer, respectively. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluorophotometer (HORIBA JobinYvon) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

2.2 Synthesis of 2-iodomethylbenzoic acid^{9e}

A solution of phthalide (1.34 g, 10 mmol) and iodo-trimethylsilane (3.00 g, 15 mmol) in CH_2Cl_2 (15 mL) was refluxed for 3 h, cooled to room temperature, quenched with water (10 mL) and the precipitate was filtered off, washed with water to give a white solid (2.4 g, 90%). The crude product was used directly without further purification.

2.3 Synthesis of 7-hydroxy-3-methyl-coumarin¹²

2, 4-dihydroxybenzaldehyde (6.0 g, 43.2 mmol), sodium propionate (9.0 g, 93.6 mmol) and propionic anhydride (15 mL, 117 mmol) were placed in a 100 mL round-bottom flask. Triethylamine (6 mL, 43.2 mmol) was then added via the addition funnel, and the reaction mixture was heated to reflux for 6 h. After the reaction, water (30 mL) was poured and the resulting pink solid was collected by filtration and washed with cold water. Column chromatography (ethyl acetate: petroleum ether

= 1:4) of the crude product over silica gel gave a colourless powder. Yield: 3.63 g (47.7%).

2.4 Synthesis of probe **1**

To a solution of DCC (0.206 g, 1 mmol) in CH_2Cl_2 (10 mL) cooled at 0°C was added 2-iodomethylbenzoic acid (0.262 g, 1 mmol) followed by DMAP (4-dimethylaminopyridine, 0.012 g, 0.1 mmol) and 7-hydroxy-3-methyl-coumarin (0.176 g, 1 mmol). The mixture was stirred at room temperature for 6 h and then filtered, washed with CH_2Cl_2 . The filtrate was concentrated to afford the crude product, then purified by silica gel column chromatography (PE: EA = 20:1) to afford a white solid (0.15 g, 36%). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 8.21 (dd, 1H, J = 8.0 Hz, 1.0 Hz), 7.57 (s, 2H), 7.527–7.507 (m, 2H), 7.473–7.432 (m, 1H), 7.30–7.295 (m, 1H), 7.252 (dd, 1H, J = 8.0 Hz, 2.0 Hz), 4.987 (s, 2H), 2.261–2.258 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 164.5, 161.8, 153.8, 152.0, 142.4, 138.7, 133.7, 132.1, 131.5, 128.3, 127.7, 126.9, 125.4, 118.5, 117.6, 110.3, 17.2, 3.4. HRMS: m/z $\text{C}_{18}\text{H}_{13}\text{INaO}_4^+$ Calcd 442.9756, found 442.9758.

2.5 Determination of the fluorescence quantum yield

In this paper, the fluorescence quantum yields of probe **1** in the presence and absence of H_2S were determined in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB), using quinine sulfate (Φ = 0.542) as standard. The fluorescence quantum yield was calculated using the following equation:

$$\Phi_x = \Phi_s (A_s F_x / A_x F_s) \quad (1)$$

where, A_x and A_s are the absorbance of the sample and the reference, respectively, at the same excitation wavelength, F_x and F_s are the corresponding relative integrated fluorescence intensities. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

3. Results and Discussion

3-methyl-coumarin-7-yl 2-(iodomethyl)benzoate (probe **1**) could be easily prepared by the reaction of 7-hydroxy-

Scheme 2. Synthesis of probe **1**.

3-methyl-coumarin with 2-(iodomethyl)benzoic acid in CH_2Cl_2 at room temperature in a yield of 36% (scheme 2). Its structure was further confirmed by ^1H NMR, ^{13}C NMR and HRMS. 7-hydroxy-3-methyl-coumarin with 2-(iodomethyl) benzoic acid were synthesized according to the literature.^{9e, 12}

Firstly, we investigated the properties of probe **1** in PBS buffer. However, because of the poor solubility of probe **1**, only 10-fold fluorescence intensity enhancement was observed in the presence of large quantity H_2S (100 eq). Considering that the addition of excessive organic solvent might restrict the practical application of the probes, we envisioned that a rationally chosen micelle system could be substituted for the DMSO-PBS mixture. In the past decade, surfactants have been explored for modifying and improving the sensing capability of fluorescent probes.¹³ To explore the optimum condition of fluorescent turn-on probe for H_2S , cetyl trimethyl ammonium bromide (CTAB) was utilized to improve the solubility. We first evaluated the effect of CTAB concentration on the fluorescence of the probe. As shown in figure S1, the thiolysis reaction is restricted without CTAB, and upon increasing CTAB concentration, the reaction becomes fast and more than 60-fold fluorescence enhancement was achieved. The reaction is complete within 1 min using 1 mM CTAB (figure S1). The pH value also could affect the process (figure S2). The result showed that the action of the probe is rapid and highly sensitive selective for detection of hydrogen sulfide under optimum condition (10 mM PBS, pH 7.4, containing 1 mM CTAB). Acidic condition inhibits ionization of hydrogen sulfide, while probe is easy to hydrolyze under alkaline condition.

Then the selectivity of the probe was tested. As expected, probe **1** exhibited high selectivity towards H_2S over other biologically relevant reactive sulfur (RSS), oxygen (ROS) and reactive nitrogen species (RNS) under the same conditions. As shown in figure 1, most anions, ROS and RNS showed absolutely no change in the emission intensity compared with probe **1**. Other abundant thiols in cells, such as glutathione and cysteine, due to the absence of the dual nucleophilicity, they could not perform a second nucleophilic addition and exhibited limited fluorescence responses (100 eq. GSH or Cys). Additionally, a blue colour change was also observed in the presence of H_2S under UV light (365 nm). These results proved that probe **1** displayed excellent selectivity for H_2S over other abundant representative anions.

The time-dependent fluorescence spectra were then examined in the presence and absence of H_2S (figure S3). The results indicated that the process could be completed within 15 min with approximately 65-fold

Figure 1. Fluorescence response of probe **1** (5 μM) upon addition of various species in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB). $\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 466$ nm.

fluorescence enhancement. The fluorescence quantum yield (Φ) of probe **1** was determined as 0.04 in the absence of H_2S , and improved to 0.66 after incubation 10 min in the presence of H_2S .

More information on the optical responses of probe **1** towards H_2S was then studied using UV-Vis spectrum (figure S4). Probe **1** showed a moderately strong maximum absorption peak centred at 306 nm and the molar extinction coefficient values were determined as $1.6 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$. Upon addition of H_2S , this peak gradually decreased and red shifted to a new absorption band centred at 314 nm, which could be attributed to the removal of 2-iodomethylbenzoic acid group from probe **1**. To further confirm our proposed mechanism, HRMS was used to investigate the reaction process. After the reaction, the peak at 199.0368 was assigned as $[\text{M} + \text{Na}]^+$ (calc. 199.0371, figure S5).

Subsequently, the quantitative analytical behaviour of probe **1** for the analysis of H_2S was examined by fluorescence titration. As displayed in figure 2, upon gradual addition of H_2S to the probe, the emission intensity at around 466 nm increased significantly, while no change was found when 5 eq. H_2S or more was added. A good linear relationship was achieved with the H_2S concentration from 0 to 25 μM (figure S6), and the limit of detection (LOD) was calculated as 30 nM based on $\text{S/N} = 3$.

To further confirm the selectivity of this fluorescent probe for hydrogen sulphide, the competition experiments were performed in the presence of various anions. As displayed in figure 3, a similar fluorescence change was observed upon addition of 5 eq. H_2S in the presence of various anions (100 eq.), such as SO_3^{2-} , SO_4^{2-} , SCN^- , F^- , Cl^- , Br^- , I^- , PO_4^{3-} , HPO_4^{2-} , AcO^- , HCO_3^- , N_3^- , $\text{C}_2\text{O}_4^{2-}$. Meanwhile, probe **1** displayed

Figure 2. Fluorescence spectra of probe **1** (5 μM) upon addition of H_2S (0–100 μM) in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB). $\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 466$ nm.

Figure 3. Fluorescence response of probe **1** (5 μM) upon addition of various species in the presence of sulfide in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB). $\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 466$ nm. (1) probe, (2) S²⁻, (3) SO₃²⁻ + S²⁻, (4) SO₄²⁻ + S²⁻, (5) SCN⁻ + S²⁻, (6) F⁻ + S²⁻, (7) Cl⁻ + S²⁻, (8) Br⁻ + S²⁻, (9) I⁻ + S²⁻, (10) PO₄³⁻ + S²⁻, (11) HPO₄²⁻ + S²⁻, (12) AcO⁻ + S²⁻, (13) HCO₃⁻ + S²⁻, (14) N₃⁻ + S²⁻, (15) C₂O₄²⁻ + S²⁻, (16) Cys + S²⁻, (17) GSH + S²⁻.

strong fluorescence response upon addition of 5 eq. of H_2S in the presence of 100 eq. GSH and Cys. The good fluorescence recovery demonstrated the good selectivity for hydrogen sulfide.

4. Conclusions

A coumarin based, reaction-type fluorescent probe for detection of H_2S in aqueous solution was synthesized

and explored through a two-step nucleophilic reaction. The probe exhibited high selectivity towards H_2S and good anti-interference towards many anions. The sensing of H_2S was rapid and finished within 15 min with detection limit as 30 nM.

Supplementary Information

¹H NMR, ¹³C NMR and ESI-Mass spectra of probe and figures S1–S6 are available at www.ias.ac.in/chemsci.

Acknowledgement

This work was financially supported by the National Science Foundation of China (91026022) and China Post-doctoral Science Foundation funded project (2014M560718).

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