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SHORT COMMUNICATION

Reduction of Nitro Group on Derivative of 1,8-Napthalimide for Quantitative Detection of Hydrogen Sulfide

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Abstract A fluorescence "turn-on" sensor (HSS) for detection of H_2S was developed on the basis of NO_2-NH_2 reduction. HSS showed a high affinity and sensitivity to H_2S over other reducing reagents, particularly for biothiols. Also, the short responding time and high linear dependence between fluorescence enhancement and H_2S concentration had HSS behave as a rapid sensor for quantitatively detection of H_2S in the biological level.

Keywords H_2S sensor · 1,8-naphthalimide · Reduction

Introduction

As a cytotoxic gas, hydrogen sulfide (H_2S) has recently been demonstrated as a novel gasotransmitter, exerting many biological functions for regulating endocrine, immune, cardiovascular and neuronal systems [1]. In the mammalian system, endogenous H_2S was mostly synthesized by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) [2]. The abundance of H_2S in different tissues and organs are varied in a large range and even reach to 50–160 μM in the central nervous system [3]. Maintaining the endogenous H_2S at certain level has been demonstrated to be critical for inhibition of apoptosis, regulation of inflammation and anti-oxidation [4]. Also, the abnormal H_2S level has been observed in many diseases, such as Down's syndrome, Alzheimer's disease, diabetes, and Parkinson's disease [5]. Therefore, developing a robust approach for quantitative detection of H_2S is critical to understand the biological functions of H_2S under physiological processes and disease states, which are essential issues prior to using H_2S as a therapeutic treatment for diseases [6].

Conventionally, many approaches including colorimetric assays, high pressure liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry and electrochemical methods have been used for quantitative H_2S analysis [7–11]. Each of these techniques showed certain advantages but also displayed unavoidable limitation, such as requiring costly instruments and complicated sample preparation, which significantly limited their applications [12]. Compared to traditional approaches, fluorimetric methods have been proven as useful assays for detection of various analyte and have attracted widespread interest in the biophysical, biochemical and biomedical science due to the high sensitivity and non-destructivity [13]. Recently, several reaction-based fluorescence chemosensors have made significant progress for detection of H_2S by using reduction of azide to amine, nucleophilic addition, and thiolysis of dinitrophenyl ether [14–16]. These sensors showed high selectivity to H_2S over other species containing sulfur atom or functioning as reducing reagents in biological system. However, the long responding time, even up to several hours, is still a challenge for reaction-based sensors [17–19].

In this paper, we reported a fluorescence sensor (HSS), a derivative of 1,8-naphthalimide, synthesized by using a one-step reaction for quantitative detection of H_2S based on reduction of nitro to amino group. HSS showed the high selectivity to H_2S as other reported reaction-based sensors, but with a short responding time in DMSO-H_2O media. In the presence of H_2S, HSS showed significant fluorescence enhancement at 538 nm (up to 30-fold) as a "turn-on" signal, quantitatively relating to the abundance of H_2S. HSS provide a robust approach for rapid detection of H_2S (Scheme 1).

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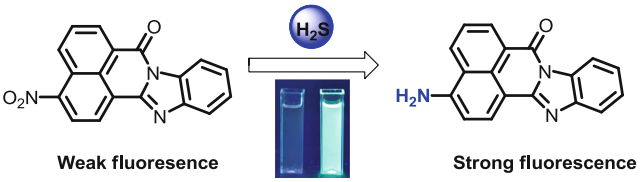


Scheme 1 The nitro group in HSS was reduced into an amino group by H_2S in DMSO/H_2O media, which led to a significant fluorescence enhancement at 538 nm

Experimental

Apparatus

Absorbance spectra were collected by Cary Series UV–vis Spectrophotometer (Agilent Technologies). Fluorescence measurements were all performed by using a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon, USA). All of fluorescence spectra were recorded in a 1 cm quartz cuvette at 25 °C. The excitation and emission slits were set at 2 nm. ^{1}H and ^{13}C NMR spectra were recorded on (^{1}H 300MHz, ^{13}C 75 MHz) Bruker 300 Ultra-Shield spectrometer at room temperature.

Reagents

All reagents used for synthesis and measurements were purchased from Sigma-Aldrich (MO, USA), Fisher Scientific (USA) and Acros Organics (USA) in analytical grade and used as received, unless otherwise stated.

Synthesis and Characterization

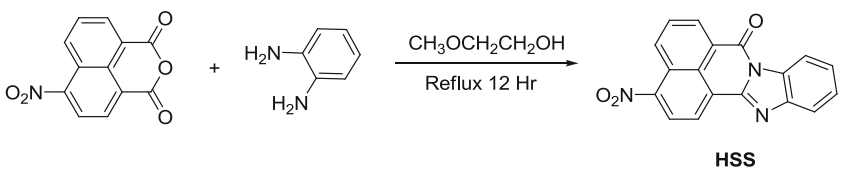
HSS was synthesized by a one-step reaction (Scheme 2). 4-nitro-1,8-naphthalic anhydride (243 mg, 1 mmol) was refluxed with *o*-phenylenediamine (108 mg, 1 mmol) in methoxyethanol (6 mL) for 12 hr. The reaction mixture was put into 6% HCl solution (25 mL) dropwise at 0 °C to collect yellow precipitate as the crude product. The crude product was recrystallized by using EtOAc/CH_2Cl_2 mixture to yield a yellow solid as product (211 mg, 67%). Melting point (m.p.): 247–249 °C. ^{1}H NMR (300 MHz, DMSO) δ: 7.56 (m, 2H), 7.97 (d, J=4.2 Hz, 1H), 8.19 (t, J=4.5 Hz, 1H), 8.46 (d, J=4.9 Hz, 1H), 8.65 (d, J=5.3 Hz, 1H), 8.86 (m, 3H); ^{13}C NMR (75 MHz, DMSO) δ: 122.9, 123.7, 126.7, 127.9, 128.9, 129.3, 129.7, 130.3, 131.5, 131.9, 132.1, 133.2, 133.4, 136.3, 142.3, 147.2, 148.7, 163.6. TOF MS EI$^+$: M^+ m/z 315.0644 (calcd.), 315.0646 (found).

Results and Discussions

As a derivative of 1,8-napthalimides (NIs), HSS showed similar spectroscopic features of NIs, which have been investigated and applied for sensor design in our research group [20–22]. The photophysical properties of NIs were significantly affected by substitution pattern on the naphthalene ring [23]. 4-nitro-1,8-naphthalimides gave a broad absorption band and a weak emission peak since the electron withdrawing group at position 4. In contrast, the reduction of nitro group ($-NO_2$) to an amino group ($-NH_2$), showing an electron donating character, led to an internal charge transfer (ICT) state with a strong emission between 450 and 550 nm [24]. To investigate photophysical properties of HSS, the UV–vis absorption spectra and fluorescence emission spectra were collected at 25 °C in DMSO-H_2O (9:1) media. As expected, weak fluorescence was detected for HSS due to the nitro group. The maximum absorption and emission of HSS were observed at 409 nm and 538 nm respectively. For the reduced form of HSS with the $–NH_2$ group, a blue-shift (5 nm) was observed in the absorption spectrum as well as a significant fluorescence enhancement in the emission spectrum, indicating that the fluorescence quenching caused by nitro group was interrupted (Fig. 1). The quantum yield of HSS and reduced HSS were calculated to be 0.008 and 0.282 respectively by using quinine sulfate as the reference.

As the sensor designed for quantitative detection of H_2S, HSS was investigated in the presence of Na_2S, which is able to rapidly generate H_2S and has been widely used to study the biological functions of H_2S in biological systems [25]. The fluorescence titration was carried out in DMSO-H_2O (9:1) media at 25 °C. As shown in Fig. 2, fairly weak fluorescence centered at 538 nm was observed for free HSS. Upon addition of Na_2S (0 to 20 equiv.) into HSS (1.0×10^{-5} M), significant fluorescence enhancement up to 30-fold was detected, giving a fluorescence "turn-on" signal. In the range of 0–1.0×10^{-4} M, the concentration of H_2S exhibited a high linearity to the fluorescence intensity at 538 nm (R^2=0.98), indicating that HSS can be used as an analytical means to quantitatively

Scheme 2 Synthetic route for HSS



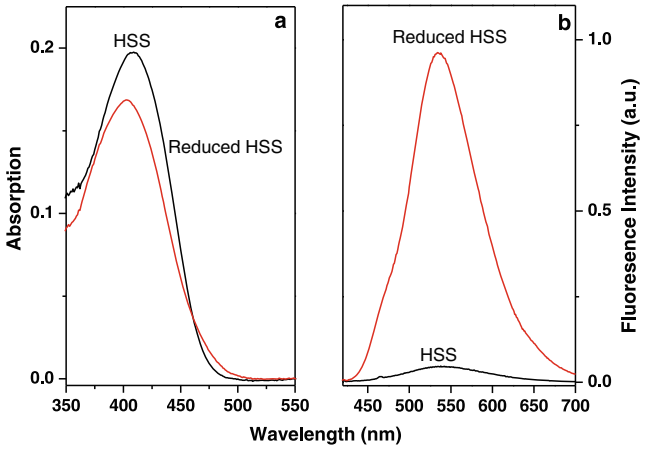


Fig. 1 The absorption spectra (**a**) and emission spectra (**b**) of HSS and reduced HSS in DMSO-H_2O (9:1) media

detect H_2S. With addition of H_2S to 1.0×10^{-5} M (10 equiv.) or more, the fluorescence enhancement achieved a plateau that suggested that the nitro group on HSS has been completely reduced.

In the presence of H_2S, the kinetics of reduction ($-NO_2$ to $-NH_2$) on HSS was investigated in the DMSO-H_2O (9:1) at 25 °C. After incubating HSS (1.0×10^{-5} M) with H_2S (1.0×10^{-4} M, 10 equiv.), the fluorescence intensity at 538 nm, which represented the reduction process, was monitored in 20 min. As shown in Fig. 3, the fluorescence intensity at 538 nm rapidly increased in 5 min and the maximum fluorescence was detected at 15 min, indicating the high reaction rate of reduction mediated by H_2S. Moreover, the observed first order rate constant (k_{obs}) was calculated to be 3.87×10^{-3} s^{-1}.

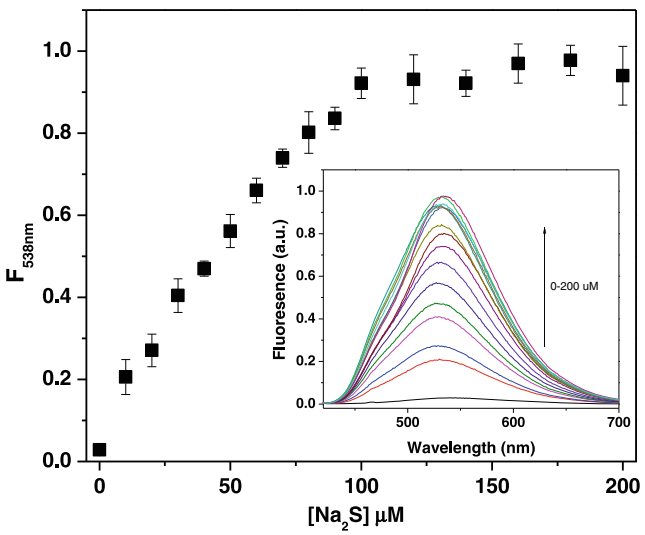


Fig. 2 In the presence of H_2S ($0–2.0\times10^{-4}$ M), a significant fluorescence enhancement centered at 538 nm was observed for HSS (1.0×10^{-5} M) in DMSO-H_2O (9:1) media at 25 °C (λ_{ex}=409 nm)

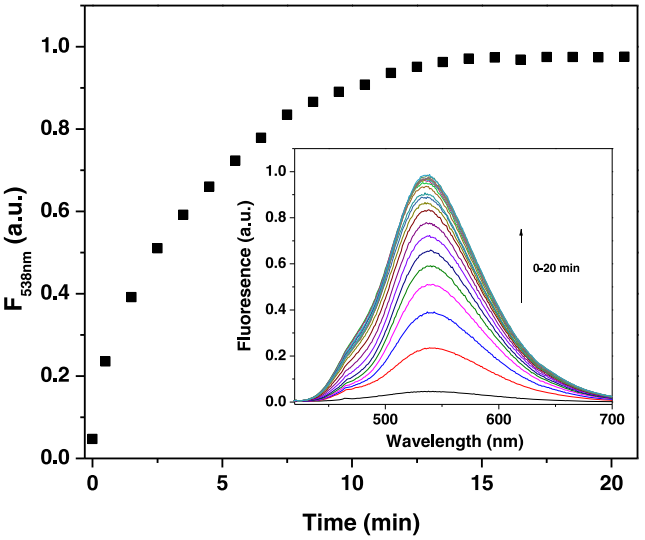


Fig. 3 HSS (1.0×10^{-5} M) showed significant fluorescence enhancement at 538 nm after incubation with H_2S (1.4×10^{-4} M, 10 equiv.) at 25 °C (λ_{ex}=409 nm)

Since the sensing mechanism used for HSS was on the basis of NO_2-NH_2 reduction, many ions and molecules are able to reduce $-NO_2$ group to give a fluorescence enhancement. To evaluate the selectivity of HSS to H_2S, several reducing reagents including HSO_4^-, $S_2O_4^{2-}$, SO_3^{2-}, $C_2O_4^{2-}$, ascorbic acid, GSH, and Cys were used for the comparison (Fig. 4). HSO_4^-, $S_2O_4^{2-}$, SO_3^{2-}, $C_2O_4^{2-}$ and ascorbic acid (2.0×10^{-4} M, 20 equiv.) were incubated with HSS (1.0×10^{-5} M) for 20 min in DMSO-H_2O media, but no significant fluorescence enhancement was observed. Due to the high level in biological systems, GSH and Cys were examined by using high concentration (1.0×10^{-3} M). Although Cys showed more fluorescence enhancement than other reducing reagents, it was obviously less than H_2S. These results strongly suggested that HSS showed a high affinity to H_2S over other reducing reagents, particularly for distinguishing H_2S from other biothiols.

Conclusions

In summary, we reported a facile H_2S sensor (HSS) designed on the basis of H_2S-induced NO_2-NH_2 reduction reaction. HSS was simply prepared by a one-step reaction with a high reaction yield. High sensitivity and selectivity to H_2S over other reducing reagents were observed for HSS. In the presence of H_2S, significant fluorescence enhancement (30-fold) was measured as a "turn-on" signal that showed liner function to the concentration of H_2S in the range of 0–100 μM, which is the major range of H_2S in biological systems. Compared to other reported H_2S sensors using the same strategy, HSS

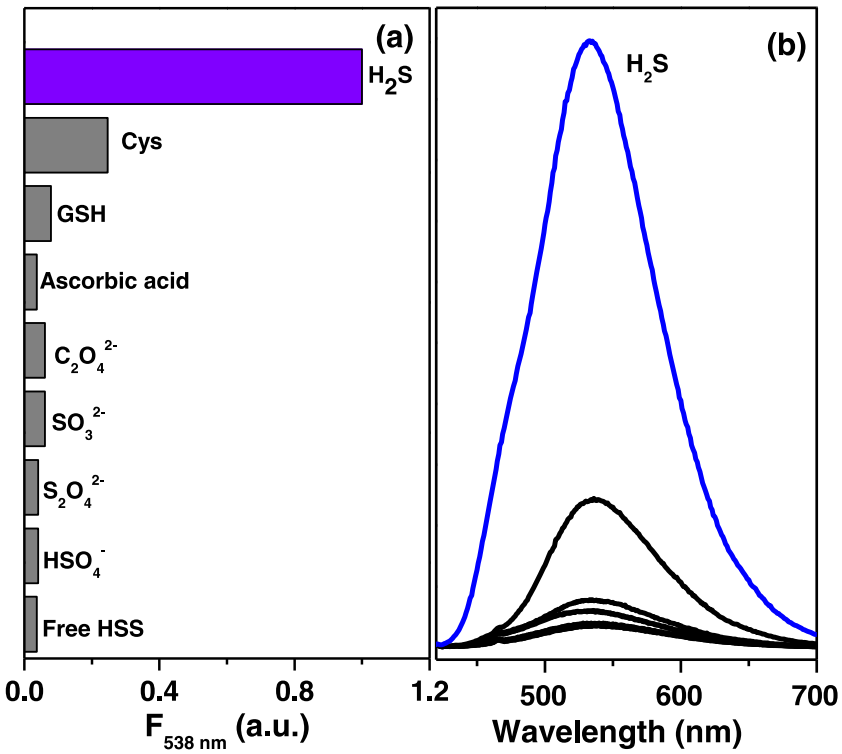


Fig. 4 (**a**) HSS showed high affinity to react with H_2S over other reducing reagents (HSO_4^-, $S_2O_4^{2-}$, SO_3^{2-}, $C_2O_4^{2-}$, ascorbic acid, GSH, and Cys), showing a 30-fold fluorescence enhancement at 538 nm. (**b**) Fluorescence emission spectra of HSS (1.0×10^{-5} M) in the presence of 20–100 equiv. various reducing reagents in DMSO-H_2O media

showed a short responding time that the reduction reaction was completely accomplished within 15 min in aqueous media. These results clearly indicated that HSS could be used as a robust approach for quantitative detection of H_2S and has potential for biological application.

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