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A ratiometric colorimetric and fluorescent chemosensor for rapid detection hydrogen sulfide and its bioimaging

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ABSTRACT

A new selective colorimetric and fluorescent sensor for H_2S, diethylaminocoumarin–hemicyanine dye has been designed, synthesized and evaluated. In CH_3OH-HEPES (10 mM, pH 7.4) buffer solution containing the sensor, the addition of H_2S made the UV–vis absorption of the sensor taking place blue-shift from red to yellow, and the fluorescence of the sensor also changed from red to green. Other analytes did not disturb the determination of H_2S. The mechanism is based on the nucleophilic attack toward the polarized C=N and an electron-poor C=C bond of the sensor. Moreover, its adduct was proved by ESI-MS and NMR. The system was used to react with H_2S in the cell, which can provide a potentially powerful approach for probing H_2S chemistry in biological system.

1. Introduction

Development of molecular sensors for anions has been a subject of intense research interest, because anions play important roles in a wide range of environmental, clinical chemicals, and biological applications [1–8]. Sulfide is an inorganic anion used in a large number of applications by humans, for instance, conversion into sulfur and sulfuric acid, dyes and cosmetic manufacturing, production of wood pulp, etc. [9]. Although historically known for its characteristic rotten egg smell and as a poison for centuries [10,11], hydrogen sulfide (H_2S) is an endogenously produced gaseous signaling compound (gasotransmitter), along with nitric oxide (NO) [12] and carbon monoxide (CO) [13], which mediates a range of physiological and pathological effects [14–17]. H_2S contributes to a diverse array of physiological processes, including vasodilation [18], angiogenesis [19], oxygen sensing [20], apoptosis [21], inflammation [22], and neuromodulation [23], and it can also protect against ischemia/reperfusion injury [24]. On the other hand, H_2S levels are altered in diseases ranging from Alzheimer's disease [25] and Down's syndrome [26] to diabetes [27] and liver cirrhosis [28]. Thus, there is considerable interest in developing effective detection methods for sulfide anion.

There are various methods for H_2S detection such as gas chromatography [29], colorimetric assays [30], fluorescence probes and polarographic sensors [31]. The fluorescence probe based approach, because of its non-destructive and sensitive nature, is highly desirable for the selective analysis of H_2S. Fluorescent probes for H_2S are further subdivided into four different categories depending upon their reaction type [32], Azide-to-amine reduction approach [33–35], Nucleophilic addition approach [36–44], Copper displacement approach [45,46], Nitro-to-amine reduction approach [47].

Herein, we report a novel fluorescent probe based nucleophilic addition on the selective of HS^- to a specific coumarin–hemicyanine derivative in medium of near neutral pH value (Scheme 1). Probe was straightforwardly synthesized in 68% yield through a simple reaction of 7-diethylaminocoumarin-3-aldehyde with 1-methyl-2,3,3-trimethyl-3H-indolium in ethanol [48]. The product was characterized by electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy (Figure S1, Supporting Information). Noting the fact that the nucleophilic attack of HS^- group takes place addition fastly to an electron-poor C=C double bond and the polarized C=N of the probe will not only interrupt the π-electron conjugation, but also block the above-mentioned intramolecular charge transfer (ICT) progress (Scheme 1).

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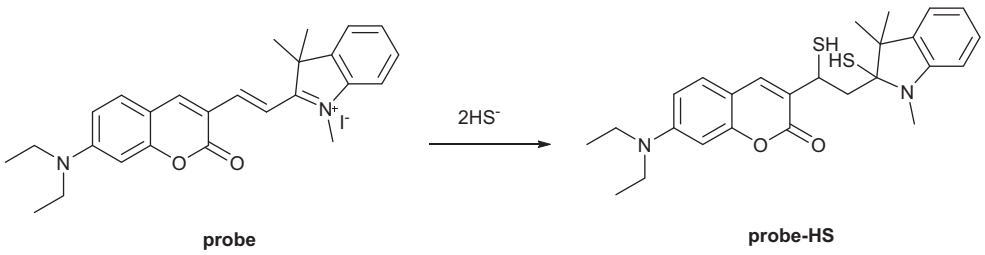

Scheme 1. The proposed H_2S sensing mechanism.

2. Experimental

2.1. Methods

All spectroscopic measurements were performed in CH_3OH-HEPES (10 mM, pH 7.4) buffer. HEPES buffer solutions were obtained by adding 1 M NaOH solution into 10 mM HEPES aqueous using a pH meter. The probe was dissolved in absolute CH_3OH to prepare the stock solutions with concentrations of 2.0 mM. The UV–vis spectra and fluorescence spectra were recorded at 25 °C. The decrease of the absorbance at 572 nm was followed.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on a Cary 50 Bio UV–visible spectrophotometer. Fluorescence spectra were measured on Cary Eclipse fluorescence spectrophotometer. All fluorescence and UV–vis spectra data were recorded at 10 s after the analytes addition. A PO-120 quartz cuvette (10 mM) was purchased from Shanhai Huamei Experiment Instrument Plants, China. ^1H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 MHz and 75 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Melting point (mp) was determined on WRS-2 digital melting point apparatus (Shanghai Physical Optical Instrument Factory). ESI-MS was measured with an LTQ-MS (Thermo) instrument. The ability of probe 1 reacting to hydrogen sulfide in the living cells was also evaluated by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope.

2.3. Measurement procedure

The UV–vis procedures were shown as follows: into a CH_3OH-HEPES (10 mM, pH 7.4) buffer solution containing 5 μM probe, HS^- sample was gradually titrated. All UV–vis spectra data were recorded at 1 min after the HS^- addition. The fluorescence procedures were as follows: into a CH_3OH-HEPES (10 mM, pH 7.4) buffer solution containing 0.5 μM probe 1, HS^- sample was gradually titrated. All fluorescence spectra data were recorded at 1 min after the HS^- addition. The HepG2 cells were grown in 1× SPP medium (1% proteose peptone, 0.2% glucose, 0.1% yeast extract, 0.003% EDTA ferric sodium salt) at 30 °C. The HepG2 were treated with 20 μM of probe 1 in culture media for 30 min at 37 °C and washed three times with phosphate-buffered saline (PBS).

2.4. Preparation of ROS and RNS

Superoxide solution ($O_2^{\bullet -}$) was prepared by adding KO_2 (1.0 mg) to dry dimethylsulfoxide (1.0 mL) and stirring vigorously for 10 min [49]. Hydroxyl radical (•OH) was obtained from the Fenton reaction of Ferrous perchlorate and hydrogen peroxide. Single oxygen ($^1O_2$) was generated by mixing H_2O_2 with NaOCl sequentially [50]. ROO• was generated from 2,2′-azobis(2-amidinopropane)dihydrochloride. Nitric oxide was generated from SNP (sodium nitroferricyanide (III) dihydrate). SNP in deionizer water was added then stirred for 30 min at 25 °C [51].

3. Results and discussion

3.1. The selective response of probe to HS^-

Fig. 1 shows the absorbance and fluorescence changes that probe undergoes upon the addition of various analytes, including

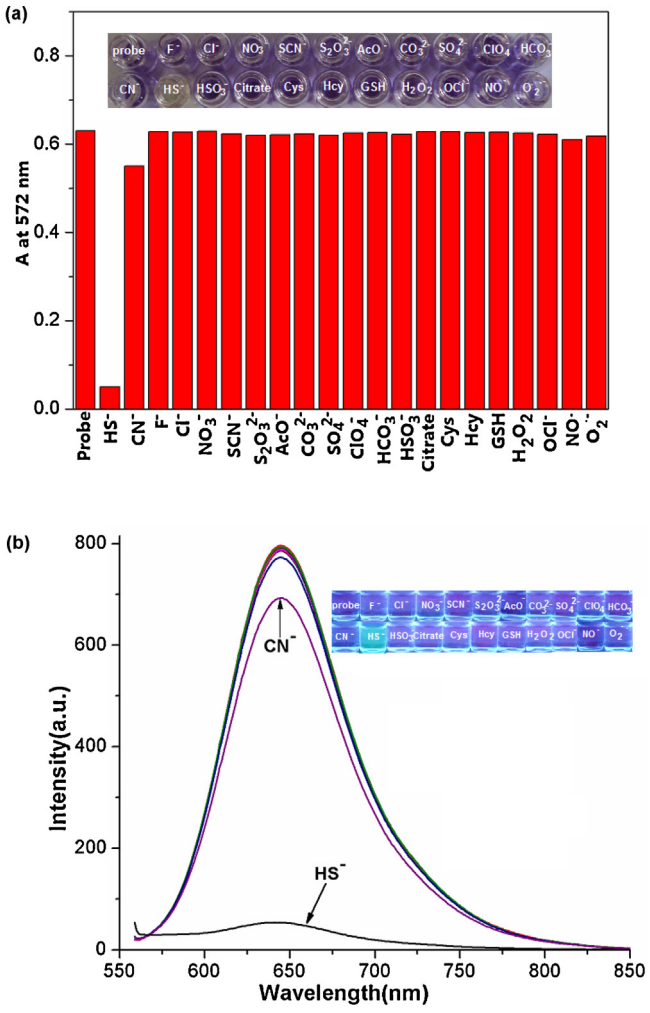

Fig. 1. (a) UV–vis absorption spectra of probe (5 μM) in CH_3OH-HEPES (10 mM, pH 7.4) in the presence of HS^- (20 μM), CN^- (20 μM), 200 μM of F^-, Cl^-, NO_3^-, SCN^-, $S_2O_3^{2-}$, AcO^-, CO_3^{2-}, SO_4^{2-}, ClO_4^-, HCO_3^-, HSO_3^-, Citrate, Cys, Hcy, GSH, H_2O_2, OCl^-, NO, O_2; each spectrum was recorded 3 min after HS^- addition; (b) fluorescence spectra of probe (0.5 μM) with various analytes (250 μM, 500 equiv. except 50 equiv. CN^-) in CH_3OH-HEPES (10 mM, pH 7.4) (λ_{ex} = 500 nm, slit: 5 nm/5 nm); Each spectrum was recorded 1 min after HS^- addition.