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ARTICLE TYPE

Highly selective naphthalimide-based fluorescent probe for direct hydrogen sulfide detection in environment †

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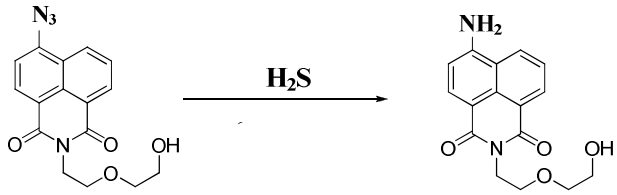
Here we report a highly selective and sensitive approach for monitoring of hydrogen sulfide (H_2S) in environment through an ingenious naphthalimide-based fluorescent probe. It shows 25-fold fluorescence enhancement in response to H_2S and has been realized to detect H_2S in aqueous with satisfactory results.

Development of special analytical methods for important chemical species detection in environment, or in vivo is a subject of considerable contemporary hot-spot research. Among numerous methodologies available, fluorescence based on its unique advantages such as inherent low detection limits, short response time and the possibility of imaging via fluorescence microscopy is becoming the focus of the study.[1] As a consequence, a variety of interesting molecules with versatile fluorophores have been purposefully designed to chemically and biologically sense some important species, especially metal ions and anions.[2]

In recent years, hydrogen sulfide (H_2S) has been considered the third most crucial gasotransmitter for regulating cardiovascular, neuronal, immune, endocrine, and gastrointestinal systems.[3] In contrast, H_2S in environment is a famously bad odor and a poisonous gas that is generated as a by-product in many industrial processes, in particular petroleum-related industries.[4] The toxicity of H_2S is comparable with that of hydrogen cyanide or carbon monoxideand at high enough concentrations may lead to death.[5] In addition, H_2S also controls the bioavailability of heavy metals in anoxic environments.[6] Up to now, although a number of techniques for hydrogen sulfide detection have been built, most of them are relatively complicated in terms of the requirement of a degree of sample preparation and the selectivity of some methods is poor. Fluorescence detection of H_2S seems to be a fine alternative to address those blocks because of its simplicity and high-efficiency, and these methods have been explosively investigated in biochemistry filed. However, unlike other extensively studied anions such as fluoride[7] or various forms of phosphate[8], the development of H_2S detection in aqueous solution has aroused little interest.



The probe 1 **The probe 2**

Scheme 1 Design of H_2S "off-on" fluorescent probe based on H_2S reductive property

Currently, there are two main pathways for sulfide fluorescent sensing. One is reactive probe which is based on the reduction ability and double nucleophilic character of H_2S, for example, the azido group can be irreversibly reduced to an amino group by H_2S;[9] the other one is competitive probe based on sulfide-specific binding affinity with metals.[10] In the present work, we designed a unique fluorescent probe 1 for detecting H_2S in environment based on the former route. Briefly, the probe 1 was designed by incorporating a reactive azido group as H_2S response site into a naphthalimide chromophore as fluorophore. And it was bonded a hydroxyl diethyl ether moiety which can increase the water solubility (see ESI). The strategy in the development of fluorescent sensor exhibiting changes in the emission profile is the manipulation of electronic features of substituents of a fluorophore through intramolecular charge transfer (ICT) pathways.[11] Prior to reaction with H_2S, non-fluorescent naphthalimide bears a strongly electron-withdraw group (an azido group) which occludes the ICT process. Due to the reducing ability of H_2S, the azido group on the probe could be reduced to an amino group and thus consequently affects its intramolecular electron density distribution, which leads to a dramatic fluorescence increase (Scheme 1).

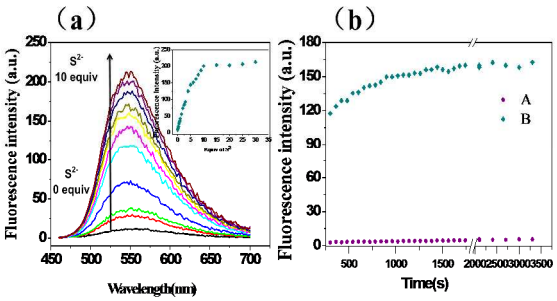


Fig. 1（a）Fluorescence spectra (λ_{ex}=440 nm) of the probe 1 (10 μM) with 0-100 μM Na_2S (10 equiv.) in 20 mM pH 7.4 HEPES buffer (containing 50% DMSO). The inset showed the corresponding relationship between the fluorescence intensity and 0-300 μM Na_2S (30 equiv.) (λ_{ex}=440 nm, λ_{em}=534 nm). (b) Time course of reactions of 10 μM probe1 with 50 μM Na_2S (5 equiv.) (B). The control (A) contained no Na_2S. Trials were carried out in the room temperature.

The proposed probe 1 was readily prepared in a two-step synthetic process (see ESI).With the probe 1 in hand, it was found that it indeed displayed weak fluorescence with excitation at 360 nm（ϕ=0.012）in aqueous DMSO solution (H_2O/DMSO =1:1, v/v, pH = 7.4) (Fig. S1-3, ESI). Upon interaction with Na_2S in the same medium (Na_2S was used as a hydrogen sulfide source in all experiments) in 60 min as probe 1 was fixed at 10 μM, a robust fluorescence turn-on response was produced, as shown in Fig. 1a. The probe 2 (λ_{ex}=440 nm, ϕ= 0.23) was separated and confirmed by NMR (see ESI). The intensity of response signals increased proportionally within the concentration range of 0 - 100 μM Na_2S and over 25-fold fluorescence enhancement can be obtained. As the concentration of Na_2S furth er increased, the fluorescence enhancement leveled off (the inset of Fig. 1a). A good linearity between the fluorescence intensity and the Na_2S concentration in the range of 0 - 40 μM was observed with the detection limit of 0.37 μM (S/N = 3). We also investigated the effect of reaction time and the time-dependent fluorescence changes was acquired with 50 μM Na_2S (Fig. 1b). We found that it took only a few minutes to trigger the conversion of the electron-withdrawing azido group to an electron-donor amino group in the probe. The fluorescence intensity increased quickly at first but gradually stabilized after 15 min. In the blank test, the probe was stable and only had a slight increase in fluorescence intensity over time change. Taken the sensitivity and analysis speed into consideration, 15 min was chosen to complete the reaction in the following experiments.

In addition to sensitivity, selectivity is another extremely significant parameter to assess the property of a new fluorescent probe. To evaluate the specificity of the probe for H_2S, different kinds of common species were examined in parallel under the same conditions, including cations, such as Co^{2+}, Mg^{2+}, Fe^{3+} and NH_4^+; common anionic analytes, such as Cl^-, Br^-, I^-, NO_3^- ,$N0_2^-$, HPO_4^{2-}, HCO_3^-, ClO_3^-, and OAc^-; reactive oxygen species (ROS), such as H_2O_2, hypochlorite (OCl^-); and also other members of the sulfide family, such as thiosulfate ($S_2O_3^{2-}$), thiocyanate (SCN^-), sulfite (SO_3^{2-}), dithionite ($S_2O_4^{2-}$). Most of

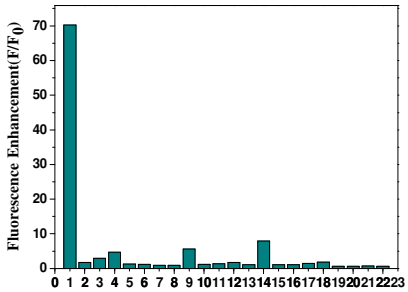


Fig. 2 Fluorescence responses of the probe (10 μM) with other testing species in buffered (pH 7.4) aqueous DMSO solution. Bars represent relative responses (λ_{ex} =440 nm, λ_{em}=534 nm) at 15 min after addition of RSS, RNS, or ROS: 1, 100 μM Na_2S; 2, 100 μM SO_3^{2-}; 3, 100 μM $S_2O_3^{2-}$; 4, 100 μM $S_2O_4^{2-}$; 5,1 mM SCN^-; 6, 1 mM SO_4^{2-}; 7, 1mM Cl^-; 8, 1 mM Br^-; 9, 1 mM I^-; 10, 1 mM NO_3^-; 11, 1 mM NO_2^-; 12, 1 mM HPO_4^{2-};13, 1 mM HCO_3^-; 14, 1 mM ClO_3^-; 15, 1 mMOAc$^-$; 16, 1 mM Citrate; 17, 1 mM Co^{2+}; 18, 100 mM HSO^{3-}; 19, 100 mM $S_2O_7^{2-}$; 20, 1 M ClO^{3-}; and 21, 1 M H_2O_2; 22, 1 M CrO_3.

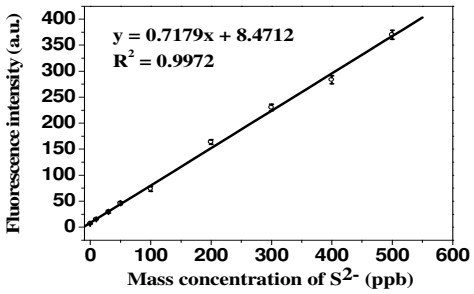


Fig. 3 Working curve of the developed method.

the anions were at a concentration of 1 mM, which was 10 - fold higher concentration than that of S^{2-}. Except for S^{2-}, among the detected anions, only $S_2O_3^{2-}$, $S_2O_4^{2-}$, I^-, ClO_3^- resulted in a certain amount of fluorescence intensity. However, the extent of the fluorescence increase was far smaller than that caused by sulfide even when the concentrations of those anions were same or ten-fold higher than that of sulfide. The results in Fig. 2 showed that only Na_2S caused apparent fluorescence enhancement while other 22 species were far weaker than sulfide anion in triggering fluorescence enhancement, which indicated that the probe exhibited a remarkably selective fluorescent response to H_2S.

The prepared probe was expected to achieve the analysis of H_2S in practical application. Prior to test, an acidification-blowing-absorption preconcentration step for H_2S in the water sample needs to be conducted (see ESI). In the concentration range of 0 - 500 ppb, calibration curve was established. High sensitivity and wide liner range can be obtained in the present method. The correlation coefficient (R^2) is 0.9972, showing a good linearity in the studied range. The detection limits calculated by the signal-to-noise ratio of 3 is 1.4 ppb.

Table 1 Detection results of sulfide anion in water samples using the proposed method.

Na_2S added[a] (ppb)	Tape water		Mineral water		Lake water	
	Na_2S found[b] (ppb)	Recovery (%)	Na_2S found (ppb)	Recovery (%)	Na_2S found (ppb)	Recovery (%)
0	Nd[c]	--	4±0.9	--	17±0.8	--
200	201±7.0	101	217±1.1	107	226±7.3	104
300	301±8.8	100	318±3.9	105	342±7.3	108
400	383±6.7	96	398±3.4	99	394±9.1	94

[a] Mean value (n=5). N_2S found ± standard deviation (n=5). [c] Not detected.

Afterwards, the proposed method was applied for the analysis of sulfide ion in nine water samples including three tap water samples, three mineral water samples and three lake samples. The detected concentration (N_2S found) and spiked recoveries of Na_2S in the water samples are shown in Table 1. Recoveries of Na_2S were in the range of 94 - 108% for all the spiked samples with standard deviation (0.8 - 9.1). According to the formula: Relative standard deviation RSD = SD/Mean value*100, low relative standard deviation (0.52% - 3.45%) was obtained, demonstrating the satisfactory recoveries and good method precision.

In conclusion, we synthesized a unique fluorescence probe based on the intramolecular charge transfer (ICT) pathways which showed the extremely high selectivity toward hydrogen sulfide. Meanwhile, the sensitivity of the probe 1 toward the hydrogen sulfide was nearly free from the interference of other anions and it also exhibited a remarkable sensitivity in the mild buffer-DMSO aqueous solution with a detection limit of 1.4 ppb by using a simple sample pretreatment method. Therefore, the probe1 has potential applications in environment for hydrogen sulfide detection.

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Notes and references

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