J Fluoresc (2014) 24:1–5
DOI 10.1007/s10895-013-1296-5

RAPID COMMUNICATION

2,6-Dansyl Azide as a Fluorescent Probe for Hydrogen Sulfide

Ke Wang · Hanjing Peng · Nanting Ni · Chaofeng Dai · Binghe Wang

Received: 9 June 2013 / Accepted: 2 September 2013 / Published online: 1 October 2013

Abstract A second-generation sulfonyl azide-based fluorescent probe, 2,6-DNS-Az, has been developed for the quantitative detection of H_2S in aqueous media such as phosphate buffer and bovine serum. Compare to the first-generation 1,5-DNS-Az probe, this probe shows both high sensitivity in phosphate buffer without the need for addition of surfactant and selectivity for sulfide over other anions and biomolecules, and thus can be used as a useful tool for detection of H_2S in the biological system.

Keywords Hydrogen sulfide · Gasotransmitter · Fluorescent probe · Azide · Redox sensing

Introduction

The past decade has seen a boost of research interest in hydrogen sulfide (H_2S), which is recognized as one of the three important gasotransmitters including nitric oxide (NO) [1] and carbon monoxide (CO) [2]. H_2S is synthesized endogenously and is involved in the regulations of a series of important genes. Endogenous concentrations of H_2S is related to a number of diseases such as Down syndrome [3] and lung diseases [4]. H_2S was also found to show protective effects in the cardiovascular (CV) [5] and central nervous systems (CNS) [6] and to play a regulatory role in inflammation [7, 8]. Regulation of H_2S levels is also a potential drug development strategy [9, 10]. The importance of accurate detection of H_2S cannot be over-emphasized. However, this is not a trivial issue mainly due to the low stability of this gaseous molecule. H_2S is volatile and prone to oxidation. Fluorescent probes are emerging as important tools for the selective and sensitive detection of H_2S [11–13]. Chang and our group first reported that the reduction of an azido group attached to a fluorophore could be utilized for the design of H_2S-selective fluorescent probes [14, 15]. Recently, a number of fluorescent probes have been developed, including probes based on nucleophilic cyclization [16–20], redox reactions [15, 21–24], and metal-sulfide formation [25–27]. A redox-based fluorescent probe (DNS-Az), which was reported by our group, shows significant fluorescence "turn-on" effect in the presence of H_2S in aqueous solutions. Most importantly, the sensing reaction (completes in minutes) is the fastest among all redox-based fluorescent probes for H_2S. This might be attributed to the unique structural features of DNS-Az. Compared to other redox-based fluorescent probes bearing an azido group, DNS-Az includes a sulfonyl azide. We believe that the sulfonyl azide provides the probe with fast reaction rates and increased photo-stability. However, the quantum yield of the fluorescent species DNS-NH_2 is very low (< 0.05) in pure aqueous solutions. In order to reach higher sensitivity, a surfactant Tween-20 was added in previous experiments [14, 28]. Herein we describe the synthesis and evaluation of a new fluorescent probe, which shares structure similarity with the previously reported probe but emits at a different wavelength with a higher fluorescence quantum yield and detection sensitivity in phosphate buffer without the need for addition of any surfactant.

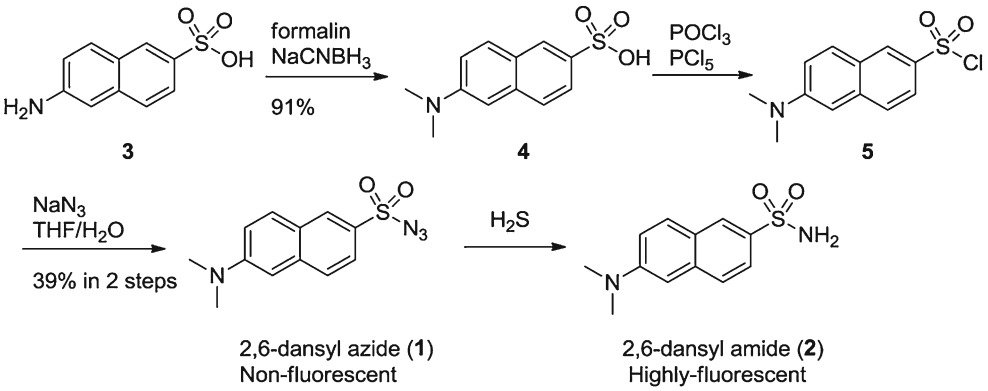
The structure of DNS-Az is based on a 1,5-dansyl fluorophore, which is known for its large Stokes shift (λ_{ex}= 330 nm, λ_{em}=517 nm in phosphate buffer/Tween-20). 2,6-Dansyl fluorophore has a smaller Stokes shift, however

Ke Wang and Hanjing Peng contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10895-013-1296-5) contains supplementary material, which is available to authorized users.

K. Wang · H. Peng · N. Ni · C. Dai · B. Wang (✉)
Department of Chemistry, Center for Diagnostics and Therapeutics, and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30302-4098, USA
e-mail: wang@gsu.edu

Scheme 1 Synthesis of 2, 6-dansyl azide



exhibits a much higher fluorescence quantum yield in pure aqueous media [28]. Therefore, it is conceivable that 2,6-dansyl azide (2,6-DNS-Az, **1**) might serve as a fluorescent probe for H_2S with high detection sensitivity in pure aqueous media. Starting from commercially available 6-amino-2-naphthalenesulfonic acid (**3**), 2,6-dansyl azide was synthesized in 3 easy steps, including reductive alkylation to form dimethylamino sulfonate **4**, formation of sulfonyl chloride **5**, and substitution with sodium azide to afford the sulfonyl azide **1** in 35 % overall isolated yield. (Scheme 1)

2,6-Dansyl azide was evaluated as a fluorescent probe for H_2S. In all experiments, Na_2S was used as the sulfide source in aqueous solutions as reported previously [14] and elsewhere. As expected, 2,6-DNS-Az could be easily reduced to its corresponding sulfonamide after sulfide addition. 2,6-Dansyl amide **2** was isolated and identified using ^{1}H NMR, ^{13}C NMR and mass spectrometry (see supporting information). The quantum yield of 2,6-dansyl amide **2** was about 40-fold higher than that of 1,5-dansyl amide in deionized water (see supporting information). In a sulfide detection experiment, 20 μM of probe **1** showed an over 100-fold fluorescence increase in 60 min after addition of only 10 μM of sulfide in phosphate buffer without surfactant. This is a much more significant change compared to the 8-fold fluorescence increase observed for the first-generation probe 1,5-DNS-Az in phosphate buffer (Fig. 1b).

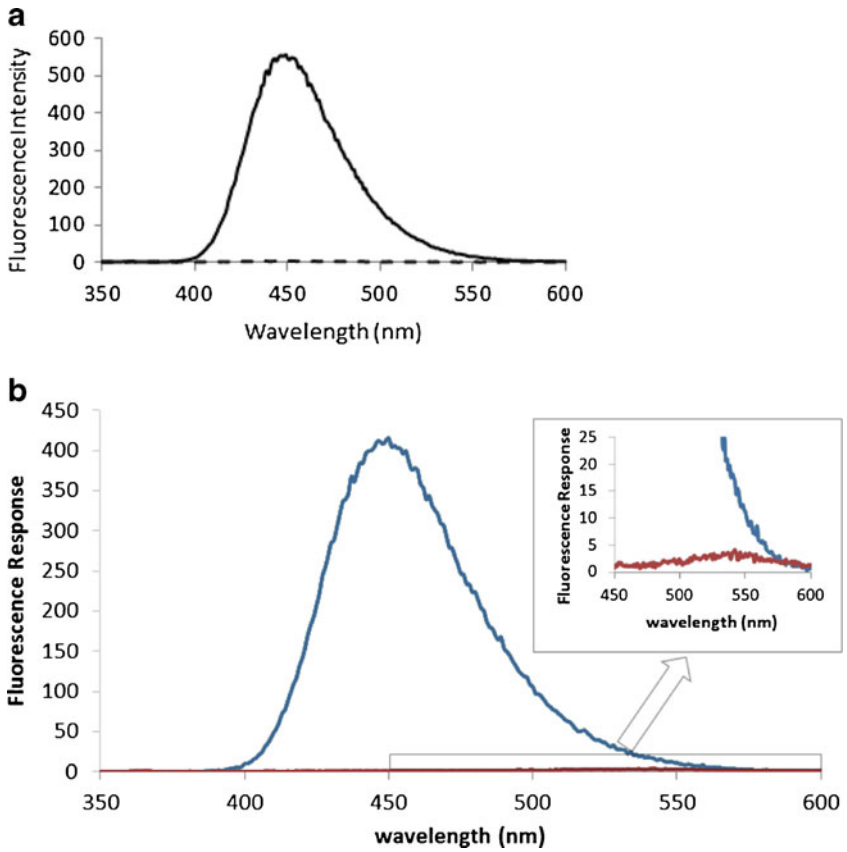


Fig. 1 **a** Fluorescence increase of 2,6-DNS-Az (**1**, 20 μM) with the addition of sulfide (10 μM) in 0.1 M sodium phosphate buffer (pH 7.4) at room temperature. Dashed line represents fluorescence spectrum of **1** alone. Solid line represents fluorescence spectrum of **1** with sulfide. **b** Comparison of fluorescence response of 2,6-DNS-Az and 1, 5-DNS-Az in phosphate buffer. Blue line represents fluorescence response of 2,6-DNS-Az (20 μM) to sulfide (10 μM); *red* line represents fluorescence response of 1,5-DNS-Az (20 μM) to sulfide (10 μM) in 0.1 M phosphate buffer (pH=7.4), λ_{ex}=325 nm

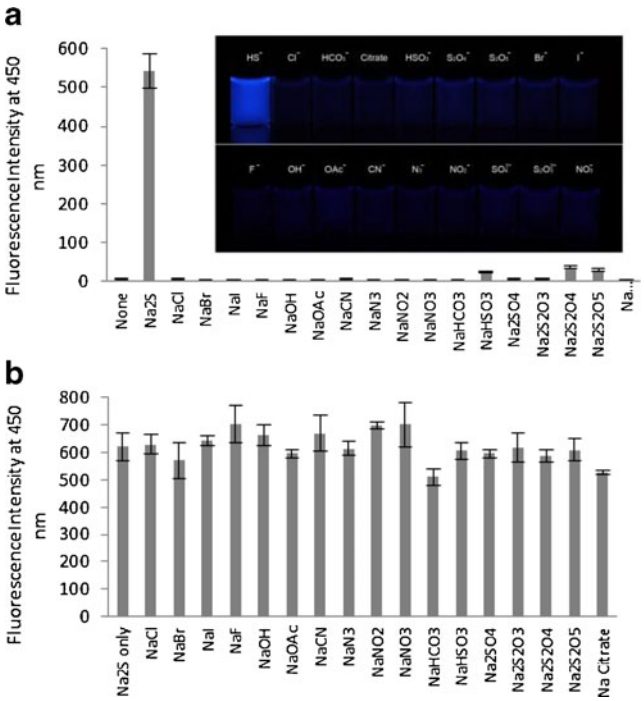


Fig. 2 **a** Fluorescence changes of 2,6-DNS-Az (**1**) in the presence of various anions; **b** fluorescence changes of 2,6-DNS-Az (**1,** 20 μM) in the presence of sulfide and various anions (HS^- 10 μM; HSO_3^-, $S_2O_4^{2-}$, and $S_2O_5^{2-}$ 20 μM; Cl^-, HCO_3^-, citrate 100 mM all other anions 1 mM in 0.1 M sodium phosphate buffer (pH 7.4), λ_{ex}=325 nm). Anions tested: Cl^-, Br^-, I^-, F^-, OH^-, OAc^-, CN^-, N_3^-, NO_2^-, NO_3^-, HCO_3^-, HSO_3^-, SO_4^{2-}, $S_2O_3^{2-}$–, $S_2O_4^{2-}$, $S_2O_5^{2-}$, and citrate. Data represents average of three independent experiments

After the fluorescence response of 2,6-DNS-Az to sulfide was demonstrated, a variety of 17 common anions, including sulfur-containing reducing anions, were used at concentrations above their physiological levels to test the selectivity of 2,6-DNS-Az. It was found that 2,6-dansyl azide showed almost exclusive response to sulfide over other anions (Fig. 2). For strong reducing anions such as HSO_3^-, $S_2O_4^{2-}$, and $S_2O_5^{2-}$, the selectivity was found to be 30 to 44-fold. Other reducing biological species, such as cysteine (Cys) and glutathione

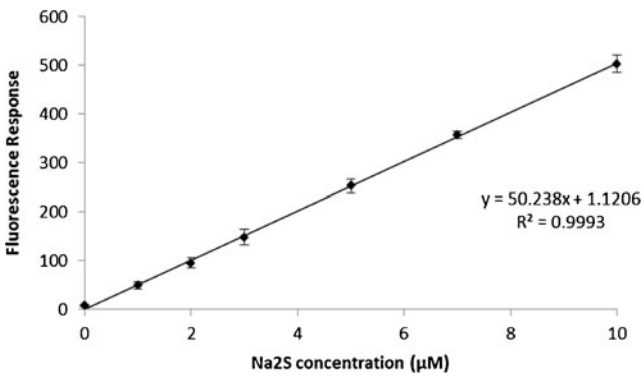


Fig. 3 Hydrogen sulfide concentration dependent fluorescence intensity changes: 2,6-dansyl azide 20 μM, Na_2S 0–10 μM in 100 mM phosphate buffer (pH 7.4, λ_{ex}=325 nm), Data represents average of three independent experiments

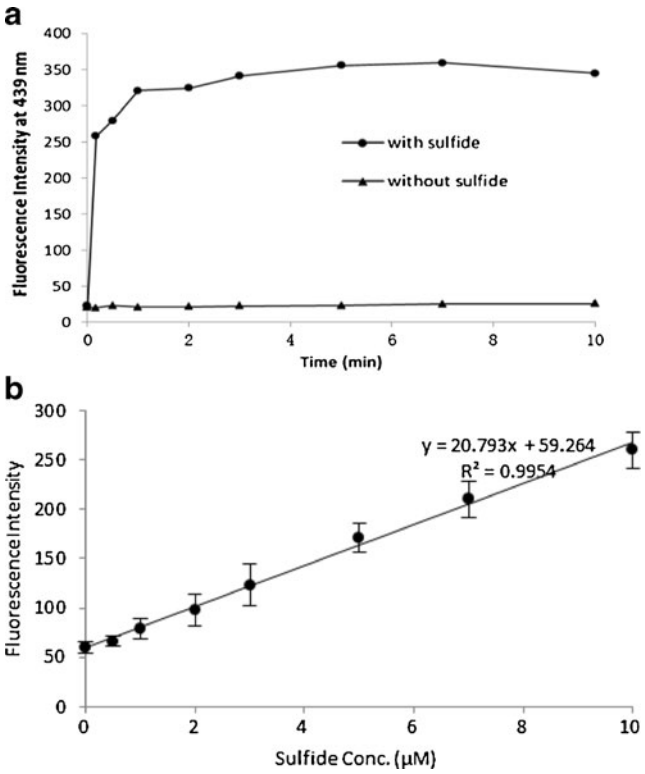


Fig. 4 **a** Reaction time profile of 2,6-DNS-Az (**1,** 20 μM) and H_2S (10 μM) in FBS; **b** H_2S concentration dependent fluorescence intensity changes, determined using a fluorometer: 2,6-DNS-Az 20 μM, Na_2S 0–10 μM in FBS (λ_{ex}=325 nm). Concentration dependence data represents average of three independent experiments

(GSH) at 100 μM, were also tested (Figure S3). The probe did not show fluorescence response to Cys and GSH. A slight increase in fluorescence response to sulfide was found in the presence of Cys and GSH. This could be due to antioxidative effect of Cys and GSH, which prevented undesired oxidation of sulfide in the reaction media. Selectivity over nucleophilic species was also tested by using high concentrations (1 mM) of glycine and lysine. These results have indicated that 2,6-DNS-Az shows sufficient selectivity for the detection of H_2S in a complex biological sample.

For all quantitative analytical methods, a linear calibration curve is always desired because it allows easy calculation. After confirming the selectivity of 2,6-DNS-Az, this probes was evaluated for quantitative measurement of H_2S by testing concentration-dependent response of 2,6-DNS-Az **1** to sulfide. As shown in Fig. 3, a linear correlation (R^2=0.9993) was found for sulfide in phosphate buffer. As determined by 3:1 signal/noise ratio, the detection limit for sulfide in phosphate buffer is 1 μM (Figure S4). This indicates that 2,6-DNS-Az could be used for quantitative measurement of H_2S in pure aqueous media.

Due to the biological significance of H_2S, its detection in biological systems such as blood serum is of great importance in both research and clinical applications. Therefore, we are also interested in using 2,6-DNS-Az for sulfide detection in

serum. The probe was evaluated in fetal bovine serum (FBS). First, a reaction time profile was tested for 2,6-DNS-Az and sulfide. As shown in Fig. 4, although FBS shows some background fluorescence, a very significant increase was still observed after addition of sulfide. Over 10-fold fluorescence increase was observed for only 10 μM of sulfide. This is more than 2-fold higher sensitivity compared to 1,5-DNS-Az reported earlier by our group. The reaction of 2,6-DNS-Az with sulfide is very fast and completes in about 2 min in FBS. This is very important considering that H_2S is very unstable and easily consumed in biological systems. We also tested concentration-dependent fluorescence changes of 2,6-DNS-Az in FBS. The linear calibration curve (R^2=0.995) obtained in FBS has also indicated that 2,6-DNS-Az could serve as a useful tool for H_2S detection. The detection limit of H_2S in FBS is about 6 μM.

Conclusions

The biological significance as well as difficulty in accurate detection of H_2S demonstrates the importance of finding new detection methods. We have reported previously a fluorescent probe (1,5-DNS-Az) for H_2S detection, which requires the addition of Tween-20 to achieve sufficient sensitivity when used in buffer solutions. The present work reports the development and evaluation of a new fluorescent probe for H_2S, 2,6-DNS-Az. This probe shows much higher fluorescence change in pure aqueous media (>100-fold for 10 μM of sulfide) compared to the probe described earlier (8-fold) without addition of any surfactant. High detection sensitivity, almost exclusive selectivity and excellent linear correlation for sulfide in aqueous solution and blood serum make this probe a useful tool for quantitative detection of H_2S.

Acknowledgment We are grateful for the financial support from Chinese Scholarship Council (KW), Molecular Basis of Disease for a fellowship (HP), and Center for Diagnostics and Therapeutics for a CDT/University fellowship (HP).

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