RSC Advances

COMMUNICATION

View Article Online
View Journal | View Issue

Fluorogenic sensing of H_2S in blood and living cells *via* reduction of aromatic dialkylamino *N*-oxide†

Cite this: *RSC Adv.*, 2014, **4**, 30398

Zhisheng Wu,[a] Yongliang Feng,[b] Bin Geng,[b] Junyi Liu[a] and Xinjing Tang*[a]

Received 23rd April 2014
Accepted 29th June 2014

DOI: 10.1039/c4ra03677h

www.rsc.org/advances

A fluorescent chemodosimeter, *N*-oxide of 7-diethylamino-4-methylcoumarin, was developed for H_2S detection based on a new water soluble aromatic dialkylamine *N*-oxide group and their selectivity and sensitivity for fluorescent detection of H_2S were evaluated in aqueous solution and blood media. Fluorescence sensing of H_2S was also achieved in MCF-7 cells.

Hydrogen sulfide (H_2S) is a well-known toxic gas that is often present in the environment and industrial processing. Recent studies have demonstrated that endogenous H_2S in living systems is one of the gaseous transmitters, along with nitric oxide (NO) and carbon monoxide (CO) that exhibit beneficial effects in a number of pathophysiological conditions.[1] Different endogenous sulfide concentrations have been reported in the literature, suggesting large variation of the sulfide concentrations in blood due to the interference of other analytes and/or different sample processing methods.[2] Therefore, a direct and efficient method for sensitively and selectively probing H_2S in biological systems is highly required.

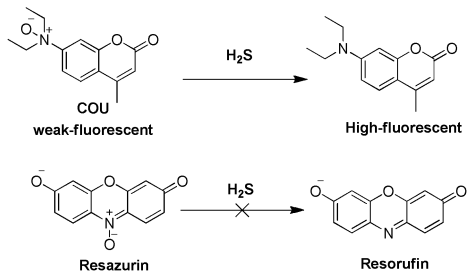
Current methods including colormetric,[3] electrochemical,[4] gas chromatography assays[5] and metal-induced precipitation[6] were reported in literature for H_2S detection. Due to the high sensitivity, nondestructivity, and high spatiotemporal resolution, fluorescence-based assays have exhibited widespread applications especially in biological systems. In the past few years, fluorescent probes used for fast and real time H_2S detection in biological systems were based on H_2S-triggered specific reaction including the reduction of azido,[7] the reduction of nitro or hydroxylamine groups,[8] the reduction of organic selenium oxide,[9] nucleophilic reaction,[10] high binding affinity towards copper ions[11] and efficient thiolysis of dinitrophenyl ether.[12] However, probes bearing these functional group usually have the low solubility in aqueous solution which needs organic solvents for H_2S detection. This limitation impeded their further applications in biological systems. Wu *et al.*[13] reported two kinds of H_2S sensors in which chemofluorophores were respectively conjugated to poly(ethylene glycol) and carbon dots to improve water-solubility. However, this conjugation increased the size of sensors. In addition, most previous sensors always involved two or more synthetic steps. Herein, we developed a new H_2S probe bearing a dialkylamine *N*-oxide group that was readily synthesized in single step with available fluorophores under a mild reaction condition and maintained high solubility in aqueous solution. Moreover, detection of H_2S based on reduction of aromatic dialkylamine *N*-oxide group was not reported.

The rational design of this new H_2S sensitive functional group for commercially available fluorophores is depicted in Scheme 1. Two kinds of N–O functional moieties were introduced to 7-diethyl-4-methylcoumarin and resorufin, respectively. Since the formation of N–O bond consumes the electron pair of nitrogen atom, it breaks the electron conjugation of nitrogen and aromatic moiety which can quench the fluorescence of fluorophores. Upon reaction with H_2S in pH 7.4 PBS buffer, aromatic dialkylamine *N*-oxide was subsequently reduced and highly fluorescent species were observed. To determine the fluorescent species, the product of **COU** reaction with NaHS was purified and then analyzed by ^{1}HNMR and ^{13}CNMR which were in consistent with the NMR spectra of 7-diethylamino-4-methylcoumarin (Fig. S2 and S3, ESI†). Further HRMS analysis confirmed the product to be 7-diethylamino-4-methylcoumarin (Fig. S4, ESI†). For commercially available resazurin, the addition of NaHS did not induce the change of absorbance and fluorescence spectra under physiological conditions, indicating that resazurin was more difficult to be reduced than aromatic dialkylamine *N*-oxide by NaHS and was not suitable for H_2S sensing (Fig. S7, ESI†).

[a]*State Key Laboratory of Natural and Biomimetic Drugs, the School of Pharmaceutical Sciences, Peking University, Beijing 100191, China. E-mail: xinjingt@bjmu.edu.cn; Fax: +8610-82805635*

[b]*Department of Physiology and Pathophysiology, the School of Basic Medical Science, Peking University, Beijing 100191, China*

† Electronic supplementary information (ESI) available: The detailed synthesis and characterization of COU and data of fluorescence response to H_2S See DOI: 10.1039/c4ra03677h



Scheme 1 Reduction of N–O chemical bond of fluorophore for H_2S detection.

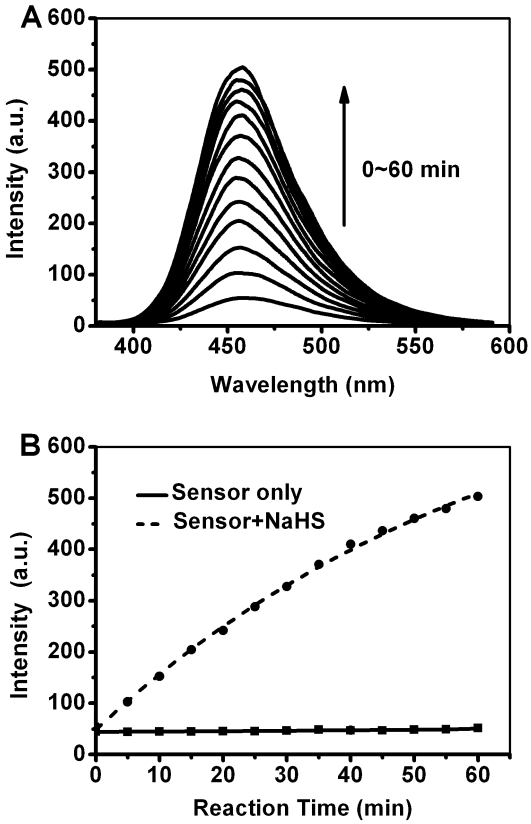


Fig. 1 (A) Fluorescence response of COU (5 μM) after the addition of NaHS (40 μM) in different incubation time; (B) time-dependent fluorescence intensity change in the absence (solid line) and presence (dash line) of 40 μM NaHS. Time points represent 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min. λ_{ex} = 360 nm, λ_{em} = 455 nm. Slits: 5/10 nm.

To access the applicability of H_2S sensing assay with dialkylamine *N*-oxide moiety, **COU** (5 μM) was incubated with NaHS (40 μM) in pH 7.4 solution. As shown in Fig. 1, a robust increase in fluorescence intensity was observed. Within 60 min of reaction time under above conditions, nearly 10-fold turn-on fluorescence response was observed with the probe **COU**.

Subsequently, we assayed the sensitivity of **COU** for H_2S detection. Various concentrations of NaHS were added to a series of degassed PBS solutions containing the probe (100 μM), and the fluorescence intensity of the resulting reaction

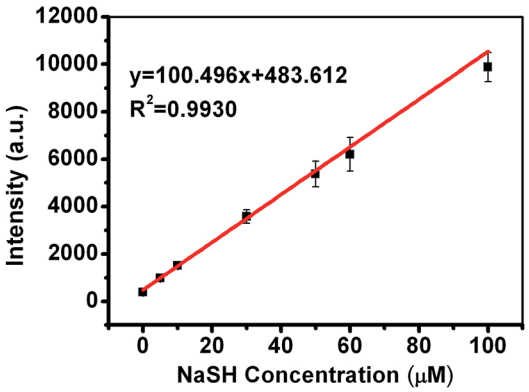


Fig. 2 H_2S concentration-dependent fluorescence intensity of COU (100 μM) to NaHS. The fluorescence intensity at 455 nm was recorded. Data was acquired after incubation 60 min. λ_{ex} = 360 nm, λ_{em} = 455 nm, Cut off: 420 nm, PMT: medium.

solutions was analyzed. Standard curve for **COU** in degassed PBS was gained between fluorescence signal at 455 nm and the concentrations (μM) of H_2S in 60 min incubation. The regression equation was $F_{Ex/Em(360/455\ nm)} = 100.496\ [H_2S] + 483.612$ with $R^2 = 0.9930$, suggesting a good linear relationship between turn-on fluorescence intensity of **COU** at 455 nm and NaHS concentration in degassed PBS buffer (Fig. 2). The detection limit is nearly down to 0.2 μM.

Instead of PBS buffer, we also applied this fluorescent chemodosimeter to the determination of H_2S in blood. A standard addition procedure was used in the experiment (see ESI† for the detail). A linear relationship of **COU** turn-on fluorescence signal at 460 nm and the concentrations (μM) of H_2S in 60 min incubation was also obtained under 25 μM H_2S in mouse blood serum and human blood plasma (Fig. 3). The regression equations were $F_{Ex/Em(355/460\ nm)} = 67.672\ [H_2S] + 101.290$ with $R^2 = 0.9680$ and $F_{Ex/Em(355/460\ nm)} = 65.836\ [H_2S] + 66.867$ with $R^2 = 0.9922$ for mouse blood serum and human blood plasma, respectively.

To evaluate the specific selectivity of **COU** for H_2S, fluorescence enhancement (F/F_0) of **COU** incubated with relevant

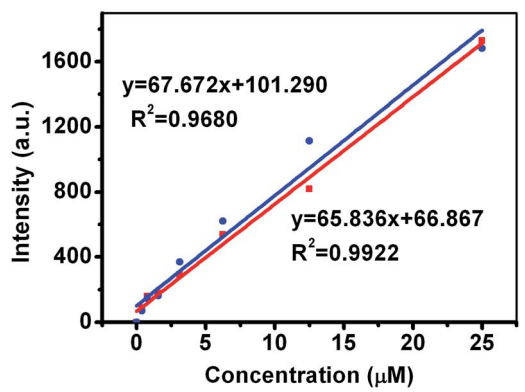


Fig. 3 H_2S concentration-dependent fluorescence intensity of COU (100 μM) in mouse blood serum (blue line) and human blood plasma (red line). λ_{ex} = 355 nm, λ_{em} = 460 nm; gain: 650.

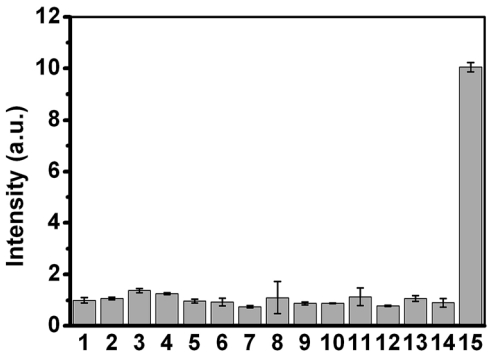


Fig. 4 Fluorescence enhancement (F/F_0) of **COU** (5 μM) in the presence of biological relevant species in pH 7.4 buffer (20 mM): (1): control, (2): Hcy (5 mM), (3): Cys (5 mM), (4): GSH (5 mM), (5): SO_4^{2-} (1 mM), (6): HCO_3^{2-} (1 mM), (7): N_3^- (1 mM), (8): $H_2PO_4^-$ (1 mM), (9): NO_2^- (1 mM), (10): CO_3^{2-} (1 mM), (11): Br^- (1 mM), (12): Cl^- (1 mM), (13): citrate (1 mM), (14): AcO^- (1 mM), (15): HS^- (40 μM). Data was acquired after incubation 60 min. $\lambda_{ex} = 360$ nm, $\lambda_{em} = 455$ nm, Cut off: 420 nm, PMT: medium.

species including various anions (1 mM) and biothiols (5 mM, such as Cys, Hcy and GSH) was investigated. As can be shown in Fig. 4, no significant fluorescence change was found upon reaction with these species and only NaHS (40 μM) was found to induce dramatic increase in fluorescence intensity, suggesting high selectivity for H_2S over other biothiols with concentration up to 5 mM.

Cell viability indicated that no obvious toxicity was observed up to 200 μM **COU** (Fig. S9†). To further evaluate the feasibility of H_2S detection in living cells, MCF-7 cells were incubated with **COU** for 30 min, and were then treated with NaHS 250 μM for 30 min. The cells were washed to remove extracellular reagents and then visualized by fluorescence microscopy. As displayed in Fig. 5, control cells treated with only **COU** remained very weak fluorescence in the absence of NaHS treatment. While fluorescence intensity in MCF-7 cells became much stronger after cells were treated with NaHS for 30 min, confirming the capability of **COU** in sensing of H_2S in living cells.

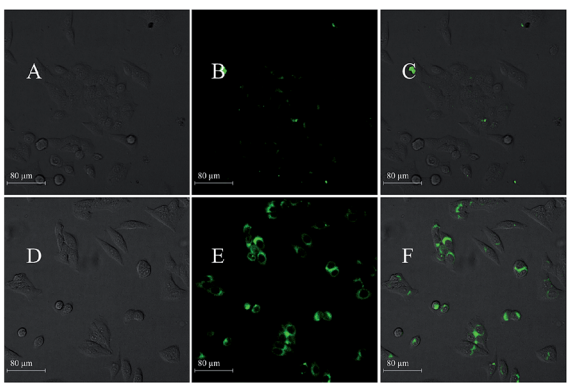


Fig. 5 Imaging of H_2S in MCF-7 cells with **COU** excitated at 405 nm. MCF-7 cells were seeded on glass coverslips and then incubated with **COU** (100 μM) for 30 min. The cells were washed and then treated with NaHS (250 μM) for 30 min. (A) DIC image of control cells with **COU**. (B) Fluorescence image of control cells with **COU**. (C) overlay of A and B. (D) DIC image of **COU**-loaded cells after treatment with NaHS. (E) Fluorescence image of **COU**-loaded cells in the presence of NaHS. (F) Overlay of D and E. Fluorescence images were set as pseudo-color green. Scale bar: 80 μm.

In summary, a novel selective fluorescent probe based on dialkylamino *N*-oxide was developed for the detection of H_2S in aqueous solutions. This new H_2S sensitive functional dialkylamine *N*-oxide group with positive charge facilitate its high solubility in aqueous buffer and accumulation in cells. Synthesis of this kind of probe needs only one-step from commercially available fluorophore under mild conditions in the presence of *m*-CPBA. The new sensor **COU** has the high solubility in aqueous solutions with no need of other soluble functionization. Fluorescence assay indicated that linear relationships between fluorescence intensity and sulfide concentrations were obtained in buffer, blood plasma or blood serum systems. This probe with high water solubility, sensitivity and selectivity has the potential to be a useful tool for detection of sulfide in many biological systems.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program; Grant no. 2013CB933800), the National Natural Science Foundation of China (Grant no. 21372018).

Notes and references

1 D. J. Lefer, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 17907–17908; W. Zhao, J. Zhang, Y. Lu and R. Wang, *EMBO J.*, 2001, **20**, 6008–6016; J. W. Calvert, S. Jha, S. Gundewar, J. W. Elrod, A. Ramachandran, C. B. Pattillo, C. G. Kevil and D. J. Lefer, *Circ. Res.*, 2009, **105**, 365–374.

2 R. Hyspler, A. Ticha, M. Indrova, Z. Zadak, L. Hysplerova, J. Gasparic and J. Churacek, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2002, **770**, 255–259; Y. H. Chen, W. Z. Yao, B. Geng, Y. L. Ding, M. Lu, M. W. Zhao and C. S. Tang, *Chest*, 2005, **128**, 3205–3211; L. Li, M. Bhatia, Y. Z. Zhu, Y. C. Zhu, R. D. Ramnath, Z. J. Wang, F. B. Anuar, M. Whiteman, M. Salto-Tellez and P. K. Moore, *FASEB J.*, 2005, **19**, 1196–1198; N. L. Whitfield, E. L. Kreimier, F. C. Verdial, N. Skovgaard and K. R. Olson, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, 2008, **294**, 16; J. Furne, A. Saeed and M. D. Levitt, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, 2008, **295**, 17.

3 M. N. Hughes, M. N. Centelles and K. P. Moore, *Free Radical Biol. Med.*, 2009, **47**, 1346–1353; D. Jimenez, R. Martinez-Manez, F. Sancenon, J. V. Ros-Lis, A. Benito and J. Soto, *J. Am. Chem. Soc.*, 2003, **125**, 9000–9001.

4 N. S. Lawrence, J. Davis, L. Jiang, T. G. J. Jones, S. N. Davies and R. G. Compton, *Electroanalysis*, 2000, **12**, 1453–1460.

5 U. Hannestad, S. Margheri and B. Sorbo, *Anal. Biochem.*, 1989, **178**, 394–398.

6 M. Ishigami, K. Hiraki, K. Umemura, Y. Ogasawara, K. Ishii and H. Kimura, *Antioxid. Redox Signaling*, 2009, **11**, 205–214.

7 L. A. Montoya and M. D. Pluth, *Chem. Commun.*, 2012, **48**, 4767; G.-J. Mao, T.-T. Wei, X.-X. Wang, S.-y. Huan,

D.-Q. Lu, J. Zhang, X.-B. Zhang, W. Tan, G.-L. Shen and R.-Q. Yu, *Anal. Chem.*, 2013, **85**, 7875–7881; Q. Wan, Y. Song, Z. Li, X. Gao and H. Ma, *Chem. Commun.*, 2013, **49**, 502; W. Sun, J. Fan, C. Hu, J. Cao, H. Zhang, X. Xiong, J. Wang, S. Cui, S. Sun and X. Peng, *Chem. Commun.*, 2013, **49**, 3890; A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078–10080; H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. Wang, *Angew. Chem., Int. Ed.*, 2011, **50**, 9672–9675; Z. Wu, Z. Li, L. Yang, J. Han and S. Han, *Chem. Commun.*, 2012, **48**, 10120; B. Chen, W. Li, C. Lv, M. Zhao, H. Jin, J. Du, L. Zhang and X. Tang, *Analyst*, 2013, **138**, 946–951; C. S. Lim, S. K. Das, S. Y. Yang, E. S. Kim, H. J. Chun and B. R. Cho, *Anal. Chem.*, 2013, **85**, 9288–9295; B. Chen, C. Lv and X. Tang, *Anal. Bioanal. Chem.*, 2012, **404**, 1919–1923.

8 W. Xuan, R. Pan, Y. Cao, K. Liu and W. Wang, *Chem. Commun.*, 2012, **48**, 10669; R. Wang, F. Yu, L. Chen, H. Chen, L. Wang and W. Zhang, *Chem. Commun.*, 2012, **48**, 11757.

9 B. Wang, P. Li, F. Yu, J. Chen, Z. Qu and K. Han, *Chem. Commun.*, 2013, **49**, 5790–5792.

10 Y. Qian, L. Zhang, S. Ding, X. Deng, C. He, X. E. Zheng, H.-L. Zhu and J. Zhao, *Chem. Sci.*, 2012, **3**, 2920; C. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton and M. Xian, *Angew. Chem., Int. Ed.*, 2011, **50**, 10327–10329; C. Liu, B. Peng, S. Li, C. M. Park, A. R. Whorton and M. Xian, *Org. Lett.*, 2012, **14**, 2184–2187; J. Zhang, Y.-Q. Sun, J. Liu, Y. Shi and W. Guo, *Chem. Commun.*, 2013, **49**, 11305; Y. Qian, J. Karpus, O. Kabil, S. Y. Zhang, H. L. Zhu, R. Banerjee, J. Zhao and C. He, *Nat. Commun.*, 2011, **2**, 495–502; Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem., Int. Ed.*, 2013, **52**, 1688–1691; J. Liu, Y.-Q. Sun, J. Zhang, T. Yang, J. Cao, L. Zhang and W. Guo, *Chem.–Eur. J.*, 2013, **19**, 4717–4722; X. Wang, J. Sun, W. Zhang, X. Ma, J. Lv and B. Tang, *Chem. Sci.*, 2013, **4**, 2551–2556.

11 F. Hou, J. Cheng, P. Xi, F. Chen, L. Huang, G. Xie, Y. Shi, H. Liu, D. Bai and Z. Zeng, *Dalton Trans.*, 2012, **41**, 5799; F. Hou, L. Huang, P. Xi, J. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai and Z. Zeng, *Inorg. Chem.*, 2012, **51**, 2454–2460.

12 X. Cao, W. Lin, K. Zheng and L. He, *Chem. Commun.*, 2012, **48**, 10529; T. Liu, Z. Xu, D. R. Spring and J. Cui, *Org. Lett.*, 2013, **15**, 2310–2313.

13 C. Yu, X. Li, F. Zeng, F. Zheng and S. Wu, *Chem. Commun.*, 2013, **49**, 403; F. Zheng, M. Wen, F. Zeng and S. Wu, *Polymer*, 2013, **54**, 5691–5697.