Dyes and Pigments 86 (2010) 87–92

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

The determination of thiols based using a probe that utilizes both an absorption red-shift and fluorescence enhancement

Baocun Zhu [a], Xiaoling Zhang [a,*], Hongying Jia [b], Yamin Li [a], Shutang Chen [a], Sichun Zhang [c,**]

[a] Department of Chemistry, School of Science, Beijing Institute of Technology, Beijing 100081, People's Republic of China
[b] Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, People's Republic of China
[c] Department of Chemistry, Key Laboratory for Atomic and Molecular Nanosciences of Education Ministry, Tsinghua University, Beijing 100084, People's Republic of China

ARTICLE INFO

Article history:
Received 7 October 2009
Received in revised form
29 November 2009
Accepted 30 November 2009
Available online 16 December 2009

Keywords:
Thiol probe
Fluorescence enhancement
Red-shift
Glutathione
Intramolecular charge transfer
Two-channel

ABSTRACT

A pyridylvinylene derivative containing piazselenole displayed high selectivity toward glutathione in the presence of other biorelevant analytes. The compound exhibited a 19 nm red-shift in absorption spectra and ~3-fold fluorescence intensity enhancement; in addition, it was possible to detect micromolar amounts of glutathione quantitatively using both red-shift absorbance and enhanced fluorescence. The mechanism of the reaction between the modified pyridylvinylene derivative and glutathione was confirmed using ESI-MS and absorption/fluorescence spectra.

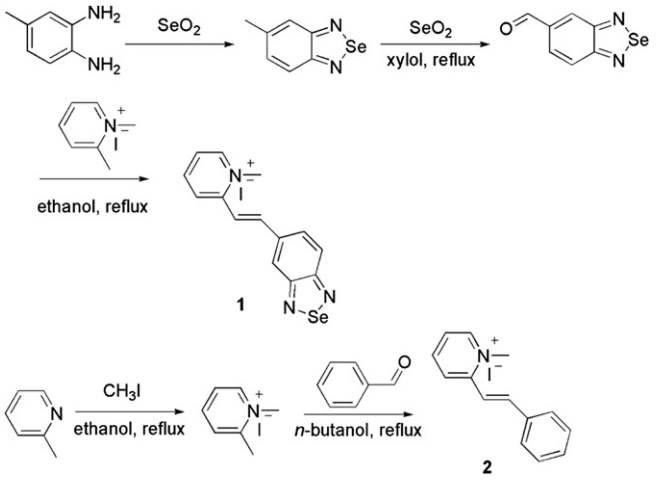
1. Introduction

Low M_r thiols, such as glutathione (GSH) and cysteine (Cys), play a crucial role in physiological systems owing to their participation in reversible redox reactions [1]. GSH is the most abundant cellar thiol and exists in a redox equilibrium between the reduced (GSH) and oxidized (GSSG) forms [2]. The ratio of GSH:GSSG is a key indicator for monitoring cellular oxidative stress and large changes can lead to some diseases and cancers [3–5]. Hence, the quantitative detection of physiological thiols is very important for investigating cellular functions. Of the various methods [6–15] for detecting thiols, great attention has attended the use of fluorescent probes owing to advantages over other methods, such as high sensitivity and operational simplicity [16–18]. Recently, attention has focused on the development of fluorescent probes for thiols, including maleimide-type [19–24], aldehyde-type [25–31], dithiol [32–34], 2,4-dinitrobenzenesulfonyl-protected [35–39] probes and other different response mechanism [2,40–53] probes. Despite the large number of fluorescent thiols probes, many of them can quantitatively detect nanomolar [23,45] or millimolar [52] concentrations of GSH. It is well-known that the concentration of GSH is about 2 μM in blood plasma [54], in which context, Yan et al. [50] and Wang et al. [51] developed two systems based on quantum dots for the detection of GSH in which GSH was determined by fluorescence intensity. Recently, Tian et al. have reported many colorimetric probes based on intramolecular charge transfer (ICT) mechanism for several analytes, such as mercury [55], pyrophosphate (PPi) [56]. However, to the best of our knowledge, few reports relating to the quantitative detection of GSH at micromolar level using red-shift absorption and enhanced fluorescence have appeared, with the exception of a merocyanine-containing chemodosimeter published by Zhengs et al. [57].

In this paper we describe the synthesis of probe **1**, which comprised an aminophenylpyridylvinylene derivative fluorophore and a piazselenole receptor (Scheme 1). Using an aminophenylpyridylvinylene derivative as fluorophore offers many inherent virtues, such as desirable spectroscopic properties and outstanding intramolecular charge transfer (ICT) [58,59]; the use of piazselenole as receptor was inspired from previous reports [10,45]. We hypothesize that piazselenole reacts with thiols to provide the stronger electron-donor, *o*-phenylenediamine, which induces changes in two-channel output signals, namely absorption red-shift and fluorescence enhancement.

* Corresponding author. Tel./fax: +86 10 88875298.
** Corresponding author.
E-mail addresses: zhangxl@bit.edu.cn (X. Zhang), sczhang@chem.tsinghua.edu.cn (S. Zhang).

0143-7208/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2009.11.011



Scheme 1. Synthesis of probe **1** and reference compound **2**.

2. Experimental

2.1. General

All reactions were carried out under a nitrogen atmosphere. All the chemicals used in this paper were obtained from commercial suppliers. ^{1}H NMR spectra was taken on a Bruker AMX400 spectrometer. Chemical shifts (δ) were reported in ppm relative to a Me_4Si standard in $CDCl_3$ or DMSO-d_6. High-resolution mass data were measured with fourier transform ion cyclotron resonance mass spectrometer (APEX IV). Electrospray ionization (ESI) mass spectra were measured with an LC-MS 2010A (Shimadzu) instrument. Absorption spectra were recorded on TU-1901 UV–vis spectrophotometer. Fluorescence spectra were measured on Hitachi F-7000 fluorescence spectrometer. All pH measurements were made with a Sartorius basic pH-meter PB-10.

2.2. Synthesis of 2-methyl-N-methyl pyridinium iodide

2-Methylpyridine (932.2 mg, 10.01 mmol) and methyl iodide (4262.9 mg, 30.03 mmol) were dissolved in ethanol (20 mL). After stirred and refluxed for 4 h under N_2 atmosphere, the reaction mixture was cooled to room temperature and was filtered to give the product (1695.7 mg) in 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ($^*10^{-6}$): 2.82(s, 3H), 4.27(s, 3H), 7.97(t, J = 6.9 Hz, 1H), 8.09(d, J = 7.9 Hz, 1H), 8.50(t, J = 7.7 Hz, 1H), 9.03(d, J = 6.2 Hz, 1H).

2.3. Synthesis of 5-methyl-2,1,3-benzoselenadiazole

4-Methylbenzene-1,2-diamine (1.2217 g, 10 mmol) and selenium dioxide (1.1096 g, 10 mmol) were ground respectively, and then mixed in a mortar at room temperature. After 30 min grinding, the crude products were dissolved in *n*-hexane, and then filtered. The solvent was removed under reduced pressure to give the desired product (1.8133 g, 9.2 mmol, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ($^*10^{-6}$): 2.46(s, 3H), 7.30(d, J = 9.2 Hz, 1H), 7.57(s, 1H), 7.70 (d, J = 9.2 Hz, 1H).

2.4. Synthesis of 2,1,3-benzoselenadiazol-5-carbaldehyde

5-methyl-2,1,3-benzoselenadi-azole (985.8 mg, 5.00 mmol) and selenium dioxide (1110.8 mg, 10.01 mmol) were dissolved in methyltoluene (30 mL). After stirring under reflux for 4 h under

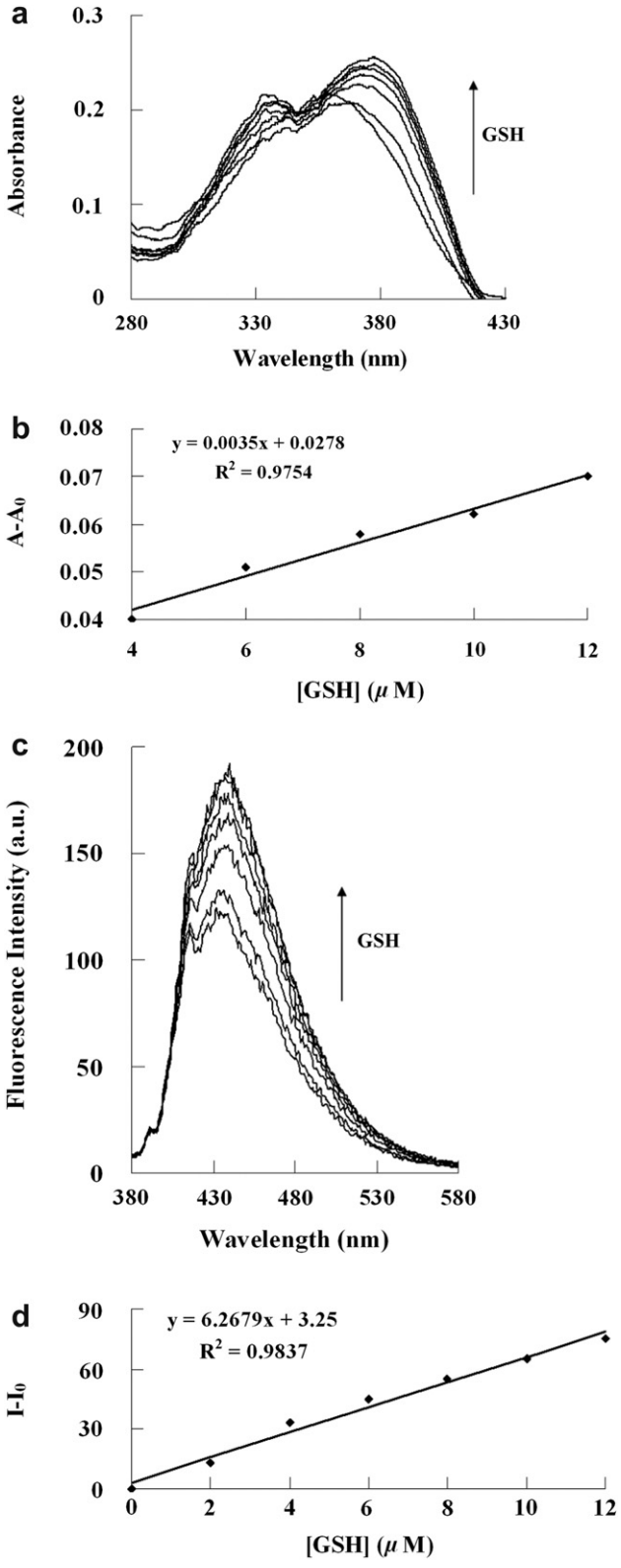


Fig. 1. Absorption (a) and fluorescence (c) spectra of **1** (10 μM) in the presence of GSH (0–12 μM), and plot of the absorbance (at 376 nm) (b) and the fluorescence intensity (at 440 nm) (d) of **1** as a function of the GSH concentration in a mixture of DMF and water (8:2, v/v) solution. Each spectrum was acquired 30 min after GSH addition at 25 °C. The excitation wavelength was 370 nm. The excitation and emission slit widths were 2.5 and 5.0 nm.

a N_2 atmosphere, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated and column chromatographed on silica-gel (elution with chloroform) to give the product (532.6 mg) in 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ($*10^{-6}$): 7.93–8.00(m, 2H), 8.34(s, 1H), 10.20(s, 1H).

2.5. Synthesis of probe **1**

2,1,3-benzoselenadiazol-5-carbaldehyde (253.3 mg, 1.2 mmol) and 2-methyl-*N*-methyl pyridinium iodide (235.1, 1 mmol) were dissolved in absolute ethanol (25 mL). After stirring under reflux for 45 min under a N_2 atmosphere, the reaction mixture was concentrated under reduced pressure. The residues were added to a 40:1 mixture of chloroform:methanol (25 mL) and the product was collected by filtration and washed with 5 × 3 mL mixture solvents (chloroform:methanol = 40:1, v/v) to give a pure product **1**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ($*10^{-6}$): 4.45(s, 3H), 7.85(d, J = 16.0 Hz, 1H), 7.97–8.01(m, 2H), 8.14(d, J = 16.0 Hz, 1H), 8.20(d, J = 9.4 Hz, 1H), 8.26(s, 1H), 8.59(d, J = 4.2 Hz, 2H), 8.99(d, J = 6.2 Hz, 1H). HRMS (ESI positive) calcd for $C_{14}H_{12}N_3Se$ $[M]^+$ 302.01913, found 302.01860.

2.6. Synthesis of reference compound **2**

Benzaldehyde (318.4 mg, 3 mmol) and 2-methyl-*N*-methyl pyridinium iodide (235.1, 1 mmol) were dissolved in *n*-butanol (25 mL). After stirring under reflux for 2 h under N_2 atmosphere, the mixture was cooled to room temperature to afford crude product after filtration. The pure product was obtained by recrystallization from ethanol. HRMS (ESI positive) calcd for $C_{14}H_{14}N$ $[M]^+$ 196.11208, found 196.11161.

3. Results and discussion

3.1. Spectra titration of **1** with GSH

In this paper, GSH was selected as the representative thiol in the spectral experiments. The absorption and fluorescence spectra were determined in a mixture of DMF and water (8:2, v/v) solution.

As shown in Fig. 1a, **1** displayed a major absorption band centered at 357 nm corresponding to molar absorption coefficient (ε) of 2.60 × 10^{-4} M^{-1} cm^{-1}. Addition of GSH to the solution of **1** (10 μM) resulted in a remarkable red-shift (19 nm) in the absorption spectra and a gradual increase of the absorbance at 376 nm. There was a good linearity between absorbance and concentrations of GSH in the range of 4–12 μM (Fig. 1b), and the detecting limit was 0.5 μM.

In the fluorescence spectra (Fig. 1c), the mixture of DMF and water (8:2, v/v) solution of **1** exhibited a fluorescence emission

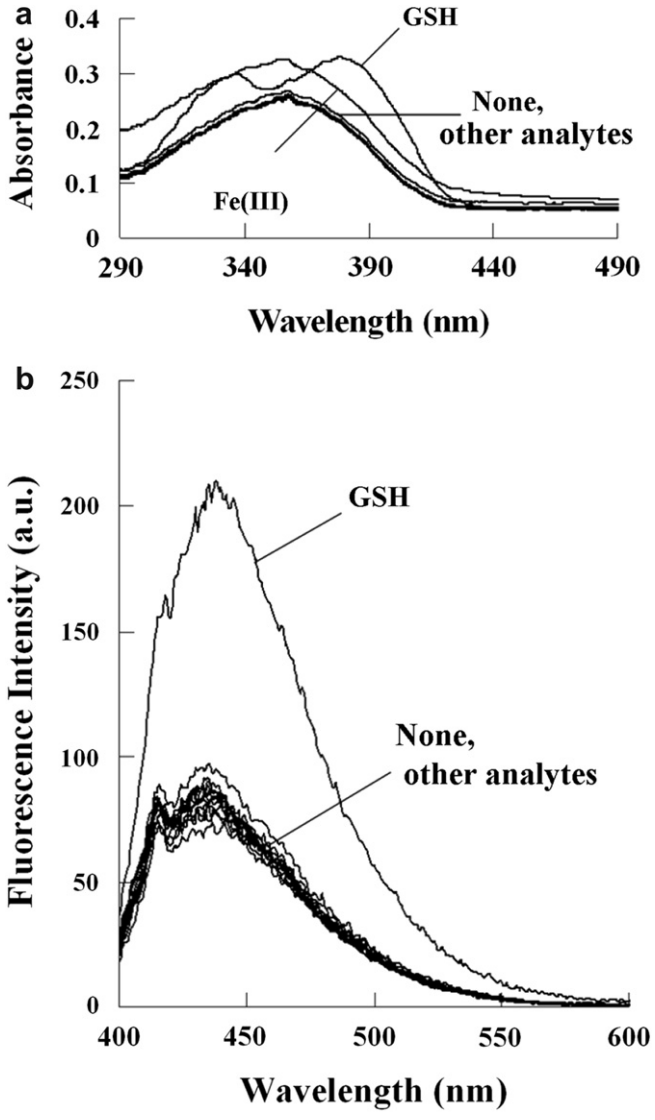


Fig. 2. Absorption (a) and fluorescence (b) spectra of **1** (10 μM) with or without GSH and a variety of biorelevant analytes including Al^{3+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, K^+, Mg^{2+}, Na^+, Zn^{2+}, H_2O_2, Fe^{3+} and ascorbic acid (Vc) (20 μM) in a mixture of DMF and water (8:2, v/v) solution. Each spectrum was acquired 30 min after various analytes addition at 25 °C. The excitation wavelength was 370 nm. The excitation and emission slit widths were 2.5 and 5.0 nm.

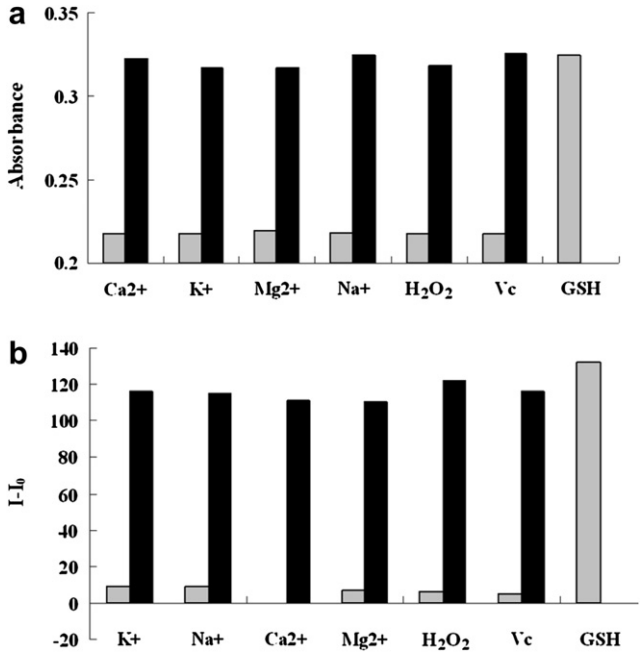


Fig. 3. Absorption (a) and fluorescence (b) responses of **1** (10 μM) to GSH (20 μM) in the absence and presence of biorelevant analytes (20 μM) in a mixture of DMF and water (8:2, v/v) solution. Each spectrum was acquired 30 min after various analytes addition at 25 °C. The excitation wavelength was 370 nm. The excitation and emission slit widths were 2.5 and 5.0 nm.

peak at 434 nm, with a fluorescence quantum yield of 0.0069 (see Supporting Information). Upon addition of GSH, the maximum emission peak underwent a red shift to 440 nm and the fluorescence quantum yield increased up to 0.0113. There was a good linearity between the fluorescence intensity and concentrations of GSH in the range of 2–12 μM (Fig. 1d), and the detecting limit was 0.03 μM.

Therefore, **1** could detect GSH qualitatively and quantitatively by both absorption and fluorescence spectrometry methods.

3.2. Spectra titration of *1* with biorelevant analytes

For an excellent probe, high selectivity is a matter of necessity. The specificity of **1** toward GSH was determined by the absorption and fluorescence titration experiments (Fig. 2a and b). Nearly no changes was observed in the absorption and fluorescence spectra with Al^{3+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, H_2O_2, Fe^{3+} and ascorbic acid (Vc). Next, the absorption and fluorescence responses of **1** toward GSH in the presence of physiological analytes were also investigated (Fig. 3a and b). The results showed that **1** possesses high selectivity toward GSH when present with other analytes.

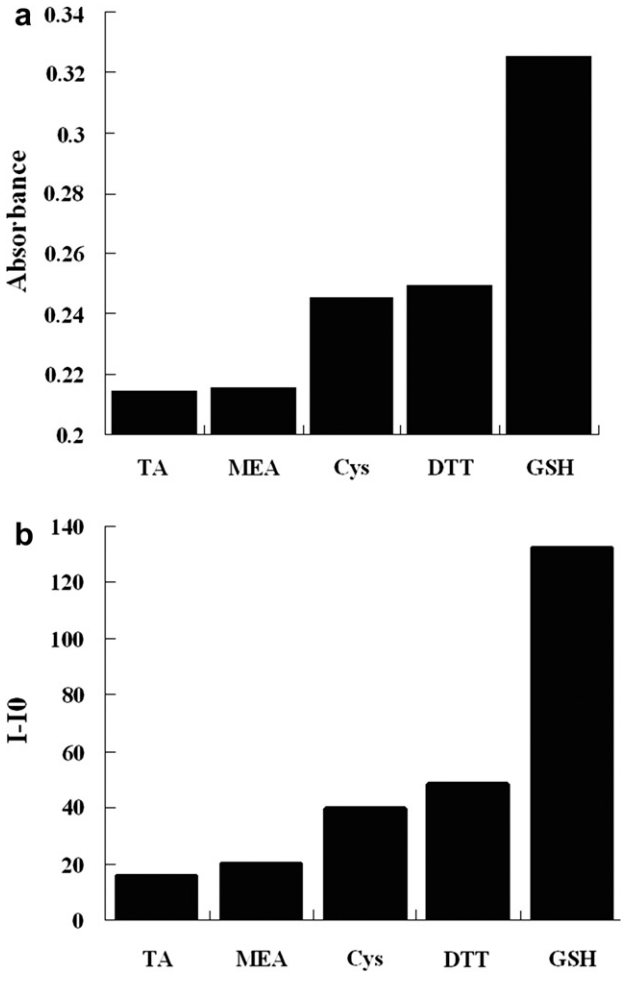


Fig. 4. Absorption (a) and fluorescence (b) responses of **1** (10 μM) to different thiols (including TA, MEA, Cys, DTT, and GSH (20 μM)) in a mixture of DMF and water (8:2, v/v) solution. Each spectrum was acquired 30 min after different thiols addition at 25 °C. The excitation wavelength was 370 nm. The excitation and emission slit widths were 2.5 and 5.0 nm.

3.3. Spectra of *1* in the presence of other thiols

The reactivity of **1** to other thiols was tested by the absorption and fluorescence titration experiments (Fig. 4a and b). The results demonstrated that **1** showed greater response to GSH than other nonprotein thiols such as thioglycolic acid (TA), β-mercaptoethylamine (MEA), L-cysteine (Cys) and dithiothreitol (DTT). This result is in good agreement with the conclusion reported by Tang et al. [45] and Zhang et al. [10].

3.4. The mechanism of *1* in sensing thiols

To understand the mechanism of **1** in sensing GSH, reference compound **2** was synthesized. When GSH was added to the solution of **1** and **2** respectively, the changes of the solution of **2** was ignored compared with those of the solution of **1** (Fig. 5a and b). This implied that the reaction of **1** with GSH was attributed to piazselenole reacting with GSH. The reaction products of **1** with GSH were subjected to electrospray ionization mass spectral analyses. The peak at *m*/*z* 226 corresponding to the compound **3** was observed (Fig. S6). Additionally, to further demonstrate the reaction of **1** with GSH by thiol, a mixture of *N*-ethylmaleimide (NEM, a known thiol-blocking agent) and GSH was added to the solution of **1**, no obvious changes in the absorption and fluorescence spectra was observed (Fig. 6a and b), implying the reaction of **1** to thiol of GSH. Therefore, a possible mechanism was proposed as shown in Scheme 2.

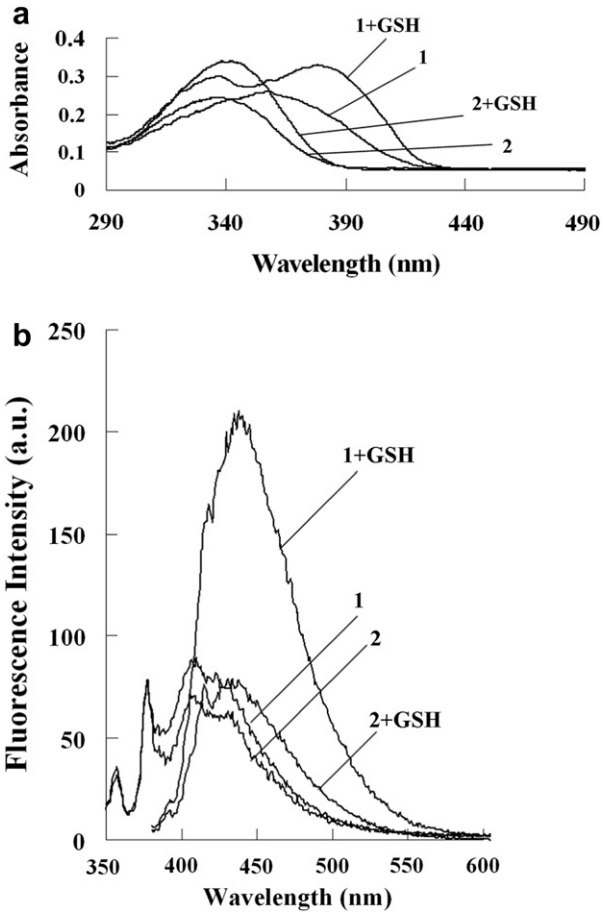


Fig. 5. Absorption (a) and fluorescence (b) responses of **1** and **2** (10 μM) to GSH (20 μM) in a mixture of DMF and water (8:2, v/v) solution. Each spectrum was acquired 30 min after GSH addition at 25 °C. The excitation wavelength was 370 nm. The excitation and emission slit widths were 2.5 and 5.0 nm.

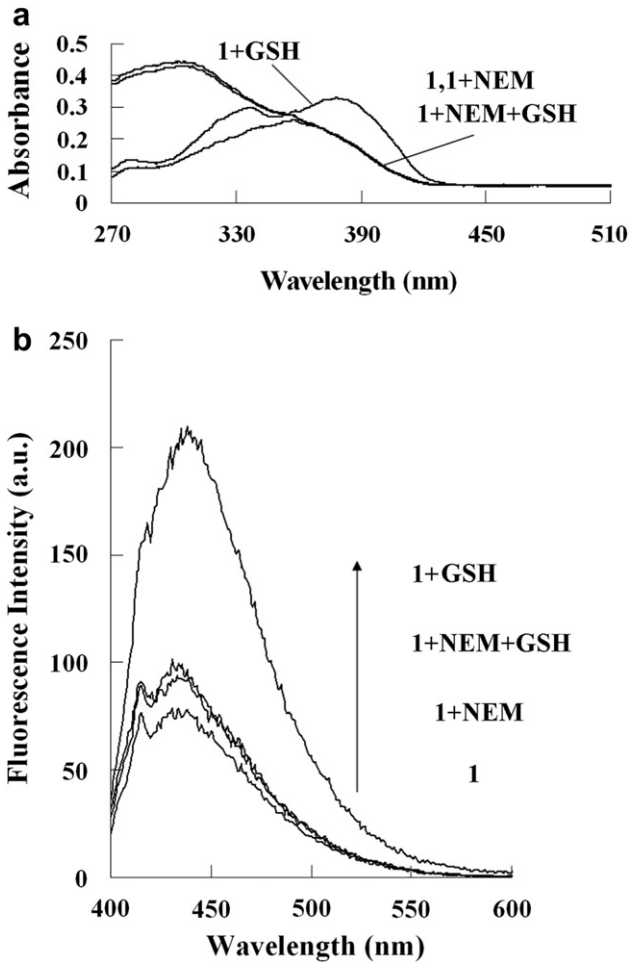
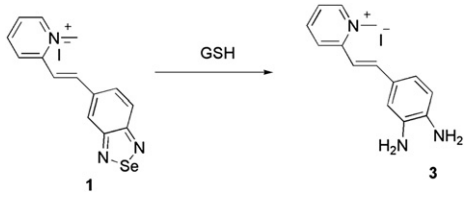


Fig. 6. Absorption (a) and fluorescence (b) responses of **1** with or without NEM (100 μM), a mixture of NEM (100 μM) and GSH, and GSH (20 μM) in a mixture of DMF and water (8:2, v/v) solution. Each spectrum was acquired 30 min after different analytes addition at 25 °C. The excitation wavelength was 370 nm. The excitation and emission slit widths were 2.5 and 5.0 nm.



Scheme 2. The possible mechanism of the reaction of **1** with GSH.

4. Conclusions

In summary, we have presented the synthesis and properties of a highly selective two-channel probe **1** for GSH and related biothiols. Addition of GSH to the solution of **1** induced the 19 nm red-shift in the absorption spectra and about 3-fold fluorescence intensity enhancement in the fluorescence spectra, which are due to the reaction of **1** with GSH to deliver the compound **3**. Moreover, probe **1** can be used for quantification of GSH in the range of 4–12 μM for red-shift absorbance and 2–12 μM for enhanced fluorescence. Experiments are underway to test the targetable capability of **1** to image thiols in living systems.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (No. 20975012) and the 111 Project (B07012).

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.dyepig.2009.11.011.

References

[1] Wood ZA, Schröder E, Harris JR, Poole LB. Structure, mechanism and regulation of peroxiredoxins. Trends in Biochemical Sciences 2003;28(1):32–40.
[2] Ahn Y-H, Lee J-S, Chang Y-T. Combinatorial rosamine library and application to in vivo glutathione probe. Journal of the American Chemical Society 2007;129 (15):4510–1.
[3] Rahman I, MacNee W. Regulation of reduction glutathione levels and gene transcription in lung inflammation: therapeutic approaches. Free Radical Biology and Medicine 2000;28(9):1405–20.
[4] Davies KJ. Oxidative stress: the paradox of aerobic life. Biochemical Society Symposia 1995;61:1–31.
[5] Miller EW, Bian SX, Chang CJ. A fluorescent sensor for imaging reversible redox cycles in living cells. Journal of American Chemical Society 2007;129 (12):3458–9.
[6] Ivanov AR, Nazimov IV, Baratova LA. Qualitative and quantitative determination of biologically active low-molecular-mass thiols in human blood by reversed-phase high-performance liquid chromatography with photometry and fluorescence detection. Journal of Chromatography A 2000;870(1–2): 433–42.
[7] Ivanov AR, Nazimov IV, Baratova L. Determination of biologically active low-molecular- mass thiols in human blood: I. Fast qualitative and quantitative, gradient and isocratic reversed-phase high-performance liquid chromatography with photometric and fluorescence detection. Journal of Chromatography A 2000;895(1–2):157–66.
[8] Inoue T, Kirchhoff JR. Determination of thiols by capillary electrophoresis with amperometric detection at a coenzyme pyrroloquinoline quinone modified electrode. Analytical Chemistry 2002;74(6):1349–54.
[9] Inoue T, Kirchhoff JR. Electrochemical detection of thiols with a coenzyme pyrroloquinoline quinone modified electrode. Analytical Chemistry 2000;72 (23):5755–60.
[10] Wang W, Li L, Liu SF, Ma CP, Zhang SS. Determination of physiological thiols by electrochemical detection with piazselenole and its application in rat breast cancer cells 4T-1. Journal of American Chemical Society 2008;130(33): 10846–7.
[11] Shao N, Jin JY, Cheung SM, Yang RH, Chan WH, Tian M. A spiropyran-based ensemble for visual recognition and quantification of cysteine and homocysteine at physiological levels. Angewandte Chemie International Edition 2006;45:4944–8.
[12] Durocher S, Rezaee A, Hamm C, Rangan C, Mittler S, Mutus B. Disulfide-liked, gold nanoparticle based reagent for detecting small molecular weight thiols. Journal of American Chemical Society 2009;131(7):2475–7.
[13] Sato Y, Iwata T, Tokutomi S, Kandori H. Reactive cysteine is protonated in the triplet excited state of the LOV2 domain in *Adiantum* phytochrome3. Journal of American Chemical Society 2005;127(4):1088–9.
[14] Chen S-H, Hsu J-L, Lin F-S. Fluorescein as a versatile tag for enhanced selectivity in analyzing cysteine-containing proteins/peptides using mass spectrometry. Analytical Chemistry 2008;80(13):5251–9.
[15] Lee J-S, Ulmann PA, Han MS, Mirkin CA. A DNA-gold nanoparticle-based colorimetric competition assay for the detection of cysteine. Nano Letters 2008;8(2):529–33.
[16] de Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley AJM, McCoy CP, Rademacher JT, et al. Signaling recognition events with fluorescent sensors and switches. Chemical Reviews 1997;97(5):1515–66.
[17] Rurack K, Resch-Genger U. Rigidization, preorientation and electronic decoupling – the 'magic triangle' for the design of highly efficient fluorescent sensors and switches. Chemical Society Reviews 2002;31:116–27.
[18] Callan JF, de Silva AP, Magri DC. Luminescent sensors and switches in the early 21st century. Tetrahedron 2005;61:8551–88.
[19] de Silva AP, Gunaratne HQN, Gunnlaugsson T. Fluorescent PET (photoinduced electron transfer) reagents for thiols. Tetrahedron Letters 1998;39:5077–80.
[20] Girouard S, Houle M-H, Grandbois A, Keillor JW, Michnick SW. Synthesis and characterization of dimaleimide fluorogens designed for specific labeling of proteins. Journal of American Chemical Society 2005;127(2):559–66.
[21] Guy J, Caron K, Dufresne S, Michnick SW, Skene WG, Keillor JW. Convergent preparation and photophysical characterization of dimaleimide dansyl fluorogens: elucidation of the maleimide fluorescence quenching mechanism. Journal of American Chemical Society 2007;129(39):11969–77.
[22] Matsumoto T, Urano Y, Shoda T, Kojima H, Nagano T. A thiol-reactive fluorescence probe based on donor-excited photoinduced electron transfer: key role of ortho substitution. Organic Letters 2007;9(17):3375–7.

[23] Guo X-F, Wang H, Guo Y-H, Zhang HS. Selective spectrofluorimetric determination of glutathione in clinical and biological samples using 1,3,5,7-tetramethyl-8-phenyl-(2-maleimide)-difluoroboradiaza-*s*-indacene. Analytica Chimica Acta 2009;633:71–5.
[24] Yi L, Li HY, Sun L, Liu LL, Zhang CH, Xi Z. A highly sensitive fluorescence probe for fast thiol-quantification assay of glutathione reductase. Angewandte Chemie International Edition 2009;48:4034–7.
[25] Tanaka F, Mase N, Barbas III CF. Determination of cysteine concentration by fluorescence increase: reaction of cysteine with a fluorogenic aldehyde. Chemical Communication 2004;15:1762–3.
[26] Zhang M, Li MY, Zhao Q, Li FY, Zhang DQ, Zhang JP, et al. Novel Y-type two-photo active fluorophore: synthesis and application in fluorescent sensor for cysteine and homocysteine. Tetrahedron Letters 2007;48:2329–33.
[27] Kim T-K, Lee D-N, Kim H-J. Highly selective fluorescent sensor for homocysteine and cysteine. Tetrahedron Letters 2008;49:4879–81.
[28] Duan LP, Xu YF, Qian XH, Wang F, Liu JW, Cheng TY. Highly selective fluorescent chemosensor with red shift for cysteine in buffer solution and its bioimage: symmetrical naphthalimide aldehyde. Tetrahedron Letters 2008;49:6624–7.
[29] Lee K-S, Kim T-K, Lee JH, Kim H-J, Hong J-I. Fluorescence turn-on probe for homocysteine and cysteine in water. Chemical Communication 2008;46:6173–5.
[30] Lin WY, Long LL, Yuan L, Cao ZM, Chen BB, Tan W. A ratiometric fluorescent probe for cysteine and homocysteine displaying a large emission shift. Organic Letters 2008;10(24):5577–80.
[31] Zhang XJ, Ren XS, Xu Q-H, Loh KP, Chen Z-K. One- and two-photo turn-on fluorescent probe for cysteine and homocysteine with large emission shift. Organic Letters 2009;11(6):1257–60.
[32] Pullela PK, Chiku T, Carvan III MJ, Sem DS. Fluorescence-based detection of thiols in vitro and in vivo using dithiol probes. Analytical Biochemistry 2006;352:265–73.
[33] Piggott AM, Karuso P. Fluorometric assay for the determination of glutathione reductase activity. Analytical Chemistry 2007;79(22):8769–73.
[34] Pires MM, Chmielewski J. Fluorescence imaging of cellular glutathione using a latent rhodamine. Organic Letters 2008;10(5):837–40.
[35] Maeda H, Matsuno H, Ushida M, Katayama K, Saeki K, Itoh N. 2,4-Dinitrobenzenesulfonyl fluoresceins as fluorescent alternatives to Ellman's reagent in thiol-quantification enzyme assays. Angewandte Chemie International Edition 2005;44:2922–5.
[36] Jiang W, Fu QQ, Fan HY, Ho J, Wang W. A highly selective fluorescent probe for thiophenols. Angewandte Chemie International Edition 2007;46:8445–8.
[37] Bouffard J, Kim Y, Swager TM, Weissleder R, Hilderbrand SA. A highly selective fluorescent probe for thiol bioimaging. Organic Letters 2008;10(1):37–40.
[38] Shibata A, Furukawa K, Abe H, Tsuneda S, Ito Y. Rhodamine-based fluorogenic probe for imaging biological thiol. Bioorganic & Medicinal Chemistry Letters 2008;18:2246–9.
[39] Ji SM, Yang J, Yang Q, Liu SS, Chen MD, Zhao JZ. Tuning the intramolecular charge transfer of alkynylpyrenes: effect on photophysical properties and its application in design of OFF–ON fluorescent thiol probes. The Journal of Organic Chemistry 2009;74(13):4855–65.
[40] Root P, Mutus B. *O*-Aminobenzoyl-*S*-nitrosohomocysteine, a fluorogenic probe for cell-surface thiol determinations via a microtiter plate assay. Analytical Biochemistry 2003;320:299–302.
[41] Ros-Lis JV, García B, Jiménez D, Martínez-Máñez R, Sancenón F, Soto J, et al. Squaraines as fluoro-chromogenic probes for thiol-containing compounds and their application to the detection of biorelevant thiols. Journal of American Chemical Society 2004;126(13):4064–5.
[42] Fu YY, Li HX, Hu WP, Zhu DB. Fluorescence probes for thiol-containing amino acids and peptides in aqueous solution. Chemical Communication 2005;25:3189–91.
[43] Sudeep PK, Joseph STS, Thomas KG. Selective detection of cysteine and glutathione using gold nanorods. Journal of American Chemical Society 2005;127(18):6516–7.
[44] Zhang M, Yu MX, Li FY, Zhu MW, Li MY, Gao YH, et al. A highly selective fluorescence turn-on sensor for cysteine/homocysteine and its application in bioimaging. Journal of American Chemical Society 2007;129(34):10322–3.
[45] Tang B, Xing YL, Li P, Zhang N, Yu FB, Yang GW. A rhodamine-based fluorescent probe containing a Se–N bond for detecting thiols and its application in living cells. Journal of American Chemical Society 2007;129(38):11666–7.
[46] Sreejith S, Divya KP, Ajayaghosh A. A near-infrared squaraine dye as a latent ratiometric fluorophore for the detection of aminothiol content in blood plasma. Angewandte Chemie International Edition 2008;47:7883–7.
[47] Li HB, Xu J, Yan HJ. Ratiometric fluorescent determination of cysteine based on organic nanoparticles of naphthalene–thiourea–thiadiazole-linked molecule. Sensors and Actuators B: Chemical 2009;139(2):483–7.
[48] Shang L, Dong SJ. Sensitive detection of cysteine based on fluorescent silver clusters. Biosensors and Bioelectronics 2009;24:1569–73.
[49] Lin WY, Yuan L, Cao ZM, Feng YM, Long LL. A sensitive and selective fluorescent thiol probe in water based on the conjugate 1,4-addition of thiols to α,β-unsaturated ketones. Chemistry A European Journal 2009;15:5096–103.
[50] Zhang Y, Li Y, Yan XP. Photoactivated CdTe/CdSe quantum dots as a near infrared fluorescent probe for detecting biothiols in biological fluids. Analytical Chemistry 2009;81(12):5001–7.
[51] Han BY, Yuan JP, Wang EK. Sensitive and selective sensor for biothiols in the cell based on the recovered fluorescence of the CdTe quantum dots-Hg(II) system. Analytical Chemistry 2009;81(13):5569–73.
[52] Banerjee S, Kar S, Perez JM, Santra S. Quantum dot-based OFF/ON probe for detection of glutathione. The Journal of Physical Chemistry C 2009;113(22):9659–63.
[53] Hong V, Kislukhin AA, Finn MG. Thiol-selective fluorogenic probes for labeling and release. Journal of American Chemical Society 2009;131(29):9986–94.
[54] Kostiainen MA, Rosilo H. Low-molecular-weight dendrons for DNA binding and release by reduction-triggered degradation of multivalent interactions. Chemistry A European Journal 2009;15(23):5656–60.
[55] Liu B, Tian H. A selective fluorescent ratiometric chemodosimeter for mercury ion. Chemical Communication 2005;25:3156–8.
[56] Huang XM, Guo ZQ, Zhu WH, Xie YS, Tian H. A colorimetric and fluorescent turn-on sensor for pyrophosphate anion based on a dicyanomethylene-4H-chromene framework. Chemical Communication 2008;41:5143–5.
[57] Wang SP, Deng WJ, Sun D, Yan M, Zheng H, Xu JG. A colorimetric and fluorescent merocyanine-based probe for biological thiols. Organic & Biomolecular Chemistry 2009. doi:10.1039/b909760k.
[58] Wang H, Helgeson R, Ma B, Wudl F. Synthesis and optical properties of cross-conjugated bis(dimethylaminophenyl)pyridylvinylene derivatives. The Journal of Organic Chemistry 2000;65(18):5862–7.
[59] Rosania GR, Lee JW, Ding L, Yoon HS, Chang YT. Combinatorial approach to organelle-targeted fluorescent library based on the styryl scaffold. Journal of American Chemical Society 2003;125(5):1130–1.