Dyes and Pigments 129 (2016) 156–162

Contents lists available at ScienceDirect

Dyes and Pigments

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

An ESIPT-based fluorescent probe for highly selective detection of glutathione in aqueous solution and living cells

Xintong Ren, Fang Wang, Jing Lv, Tingwen Wei, Wei Zhang, Yong Wang, Xiaoqiang Chen*

State Key Laboratory of Materials-Oriented Chemical Engineering College of Chemistry and Chemical Engineering, Nanjing Tech University, Nanjing, 210009, PR China

ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form
21 February 2016
Accepted 25 February 2016
Available online 3 March 2016

Keywords:
Fluorescent probe
Glutathione
ESIPT
Benzothiazole
Dinitrophenyl

ABSTRACT

In this paper, highly sensitive and selective fluorescent probe **1** for GSH is designed and synthesized based on modulation of the excited-state intramolecular proton transfer (ESIPT) process of 2-(2′-hydroxy-3′-ethoxyphenyl)benzothiazole. Upon introducing GSH in neutral solution containing CTAB micelles, dinitrophenyl, the protecting group of the probe, is removed via the nucleophilic substitution, thereby retrieving the ESIPT process of 2-(2′-hydroxy-3′-ethoxyphenyl)benzothiazole, which results in a fluorescence enhancement at 485 nm. Additionally, the fluorescence intensity of the probe is linearly proportional to GSH concentration ranging from 0 to 100 μM and the obtained detection limit is as low as 0.81 μM. The enhanced selectivity toward GSH over Cys and Hcy is also attributed to one more carboxyl group in GSH. Importantly, the probe is successfully utilized for monitoring GSH in living HeLa cells.

1. Introduction

Much attention has been focussed in the last two decades on detection of biological thiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) [1]. GSH plays various roles in reversible redox reactions and has vital cellular functions in living cells [2–4]. It is noteworthy that the abnormal concentration levels of GSH are more likely to bring about AIDS, cancer and Alzheimer's disease [5–7]. Hence, selective and sensitive detection of GSH, in biological systems particularly, is very important for monitoring these diseases.

Recently, extensive efforts have been made to develop fluorescent probe owing to its unique advantages such as high sensitivity, good specificity, real-time determination and simple operation. So far, many probes have been synthesized to detect S-containing compounds, especially for H_2S [8–10] and thiols [11–14]. Only a few fluorescent probes are available to detect GSH, Cys and Hcy, and the familiar detection mechanisms include Michael addition reaction [15–17], nucleophilic substitution [18,19], cleavage reaction [20–23] and ligand displacement of metal complexes [24–26]. Nevertheless, because of the similarities in both the structure and the reactivity, the target for distinguishing GSH from Cys and Hcy is still arduous to achieve. Nowadays, a number of excellent fluorescent probes have been developed for Cys and Hcy over GSH [27–31], while there are few probes that can efficiently tell GSH apart from Cys and Hcy. Thus, the development of probes with great selectivity to GSH is still in need.

2-(2′-hydroxyphenyl)benzothiazole and its derivatives have gained wide attention in recent years because they can undergo an excited-state intramolecular photon transfer (ESIPT) process upon photoexcitation [32–34]. Furthermore, the dinitrophenyl ether can be introduced to a fluorophore easily to get a new probe, which has very low background fluorescence due to the strong quenching effect of the nitro group [35,36]. Keeping these in mind, we designed and synthesized a highly sensitive and selective fluorescent probe **1** (Scheme 1). It can differentiate GSH from other thiols within 30 min in aqueous solution containing CTAB micelles. Moreover, it was also been successfully applied to monitoring GSH in living cells.

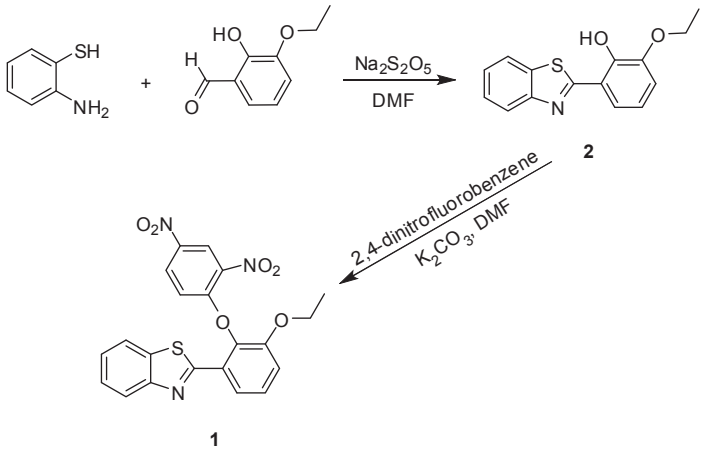
2. Experimental

2.1. Materials

2-Aminothiophenol was purchased from Aladdin Co., Ltd. (Shanghai, China). Lys-Cys-Gly and Glu-Cys-Glu were purchased

* Corresponding author.
E-mail address: chenxq@njtech.edu.cn (X. Chen).

http://dx.doi.org/10.1016/j.dyepig.2016.02.027
0143-7208/



Scheme 1. Synthesis of probe **1**.

from Sangon Biotech (Shanghai, China). Cetyltrimethylammonium bromide (CTAB) was purchased from Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). 3-Ethoxysalicylaldehyde, 2,4-dinitrofluorobenzene and other reagents of analytical reagent grade were purchased from Sigma-aldrich Co., Ltd. (Shanghai, China). All reagents were directly used for the following experiments without further purification. Unless otherwise noted, the aqueous solutions were prepared with deionized water. Chromatography was implemented on silica gel 60 (230–400 mesh ASTM).

2.2. Equipments and instruments

^{1}H NMR and ^{13}C NMR spectra were obtained using Bruker spectroscopy. Mass spectra were recorded on Agilent HPLC-MS. Ultraviolet–visible light (UV–vis) absorption spectra were carried out on α–1860A UV/vis spectrophotometer. Fluorescence emission spectra were collected using RF-5301/PC fluorophotometer. Single crystal X ray diffraction measurements were carried out on a Bruker SMART CCD diffractometer using a Mo K_α radiation ($\lambda = 0.71073$ Å). The cells were imaged by confocal laser scanning microscopy (Leica, TCS sp5 II).

2.3. Synthesis

Compound **2** was synthesized according to previous typical literature [37]. 2-aminothiophenol (0.391 g, 3.12 mmol) and 3-ethoxysalicylaldehyde (0.525 g, 3.16 mmol) were mixing together in 10 mL N,N-dimethylformamide (DMF). Then, sodium metabisulfite ($Na_2S_2O_5$, 0.61 g) was added to the stirring mixture. The reaction mixture was heated to 75 °C for 2 h under N_2 atmosphere and the progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature. 20 mL of H_2O was added and the product which precipitated as a yellow solid was collected. Recrystallization from methanol afforded pure product **2** (0.761 g, 2.81 mmol) in high yield (90%). Then, N,N- dimethylformamide (DMF) solution (10 mL) of compound **2** (0.271 g, 1 mmol) was mixed with 2,4-dinitrofluorobenzene (0.223 g, 1.2 mmol) in the present of K_2CO_3 to obtain compound **1**. The mixture was heated to 80 °C under N_2 atmosphere with monitoring by TLC in the progress of the reaction. After 3 h, 20 mL water was added and pale yellow sediment appeared. The sediment was then collected and dried in vacuo. Chromatography of the crude product on silica gel using CH_2Cl_2 as eluent afforded **1** (0.363 g, yield 83%). *Compound **2***: ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 12.75 (1H, s), 7.98 (1H, d, $J = 8.07$ Hz), 7.89 (1H, d, $J = 7.92$ Hz), 7.51 (1H, t, $J = 7.41$ Hz), 7.41 (1H, t, $J = 7.49$ Hz), 7.32 (1H, d, $J = 7.29$ Hz), 6.98 (1H, d, $J = 7.38$ Hz), 6.89(1H, t, $J = 7.95$ Hz), 4.21–4.14 (2H, m), 1.52 (3H, t, $J = 6.98$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm): 169.43, 151.76, 148.56, 148.28, 132.70, 126.69, 125.54, 122.23, 121.49, 120.05, 119.10, 116.93, 115.65, 64.75, 14.92. *Compound **1***: ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.94 (1H, d, $J = 2.72$ Hz), 8.25–8.22(1H, m), 8.12–8.05 (2H, m), 7.88–7.86(1H, m), 7.52–7.37 (3H, m), 7.15–7.12 (1H, m), 6.93–6.91 (1H, m), 4.07–4.01(2H, m), 1.19 (3H, t, $J = 6.96$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm): 160.96, 155.73, 152.53, 150.75, 141.60, 139.77, 138.92, 135.73, 128.63, 127.98, 127.64, 126.50, 125.66, 123.42, 122.04, 121.71, 121.54, 117.30, 115.79, 65.05, 14.49. LC-MS (ESI+): $m/z = 438.0692$ $[M + H]^+$, calc. for $C_{21}H_{16}N_3O_6S = 438.0760$; $m/z = 460.0511$ $[M + Na]^+$, calc. for $C_{21}H_{15}N_3NaO_6S = 460.0579$.

2.4. Crystal growth and X-ray structure determination

Single crystals of compound **1** suitable for X-ray analysis were grown from CH_3CN by slow evaporation at room temperature for a week. The well-shaped single crystals of **1** were selected for lattice parameter determination and collection of intensity data at 296 K on a Bruker SMART CCD diffractometer with a detector distance of 5 cm and frame exposure time of 10 s using a graphite-monochromated Mo K_α ($\lambda = 0.71073$ Å) radiation. The structures were all solved by direct methods and refined on F^2 by full-matrix least squares procedures using SHELXTL software [38]. All non-hydrogen atoms were anisotropically refined. All H atoms were located from a difference map and refined isotropically.

2.5. Absorption and fluorescence measurements

The probe **1** (1 mM) was dissolved in acetonitrile and maintained at room temperature. Stock solutions (10 mM) of amino acids including glutathione (GSH), homocysteine (Hcy), cysteine (Cys), alanine (Ala), arginine (Arg), glycine (Gly), lysine (Lys), serine (Ser), phenylalanine (Phe), glutamic acid (Glu), histidine (His), glutamine (Gln), methionine (Met) and tyrosine (Tyr) were prepared in deionized water. Test solutions were prepared by placing 30 μL of the probe stock solution into a test tube, diluting the solution to 3 mL with HEPES buffer (20 mM, pH = 7.4) containing CTAB (1 mM) and then different analytes of corresponding concentrations were added. All UV–vis absorption and fluorescence measurements were measured at room temperature. For fluorescence study, the samples were excited at 385 nm and the fluorescence emission ranged from 400 nm to 650 nm. Both the excitation

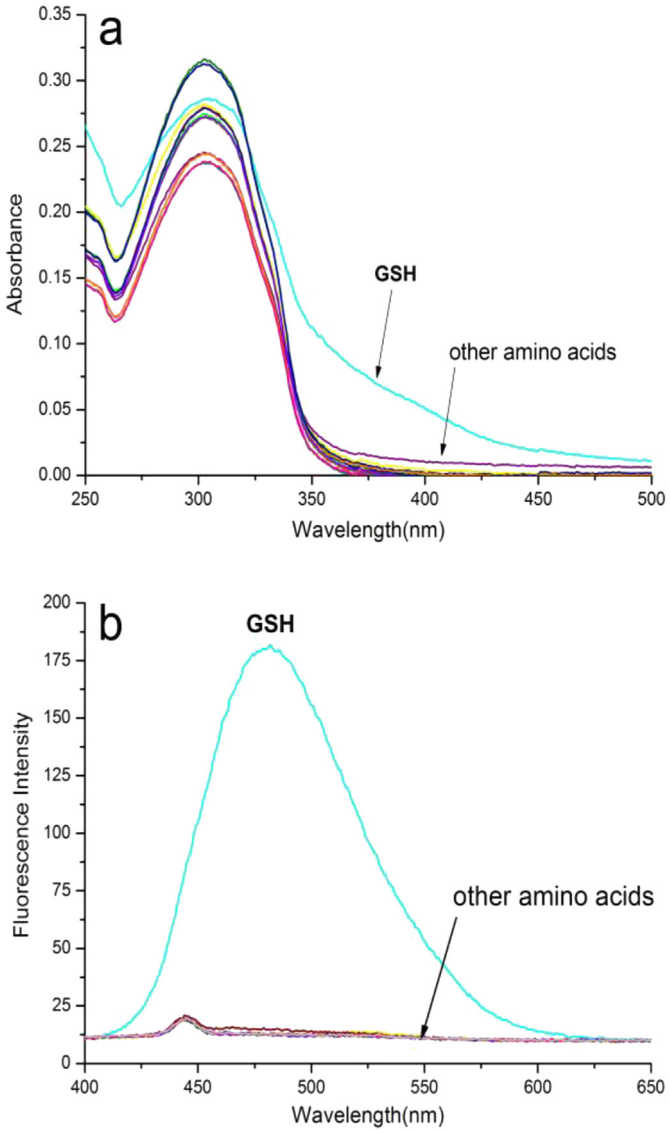


Fig. 1. (a) Absorption and (b) fluorescence spectra of **1** (10 μM) upon adding various analytes including GSH, Cys, Hcy, Phe, Ser, Glu, Arg, Ala, His, Lys, Gln, Gly, Tyr and Met (100 μM) in the HEPES buffer (20 mM, pH 7.4) solution. (λ_{ex} = 385 nm, slit: 5 nm/5 nm).

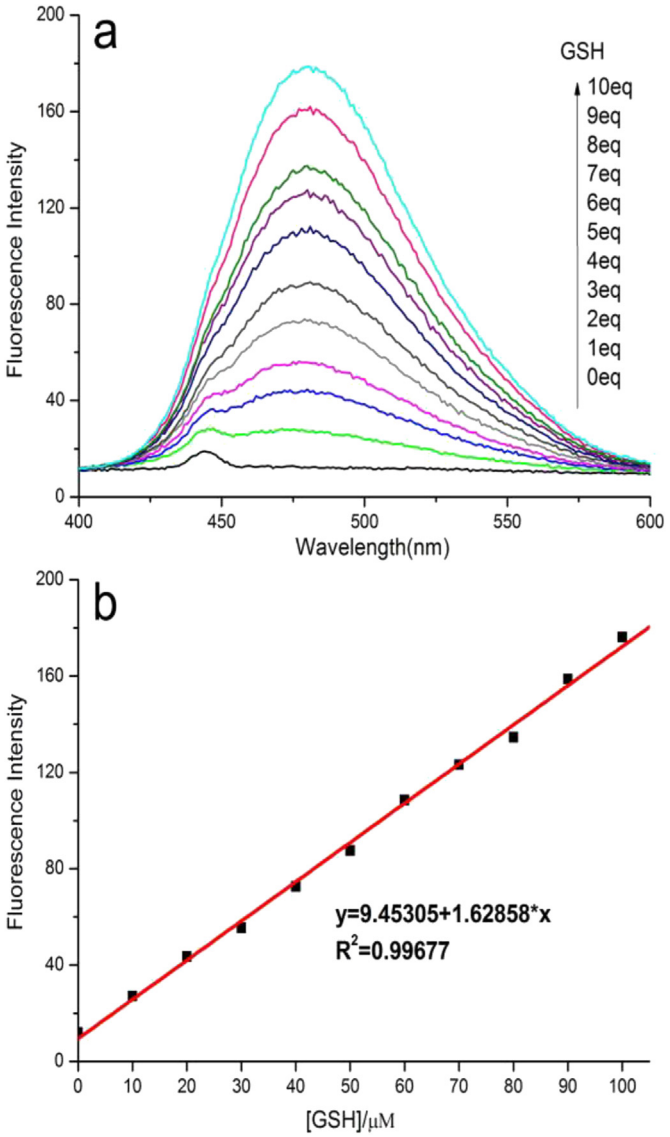


Fig. 2. (a) Fluorescence titration spectra of **1**(10 μM) with various concentrations of GSH in HEPES solution (20 mM, pH 7.4) and (b) the linear relationship between fluorescence intensity at 485 nm and GSH concentration. (λ_{ex} = 385 nm, slit: 5 nm/5 nm).

and emission slit widths were 5 nm. After the analyte adding into the tube, it would take 30 min for reaction.

2.6. Dynamic assays

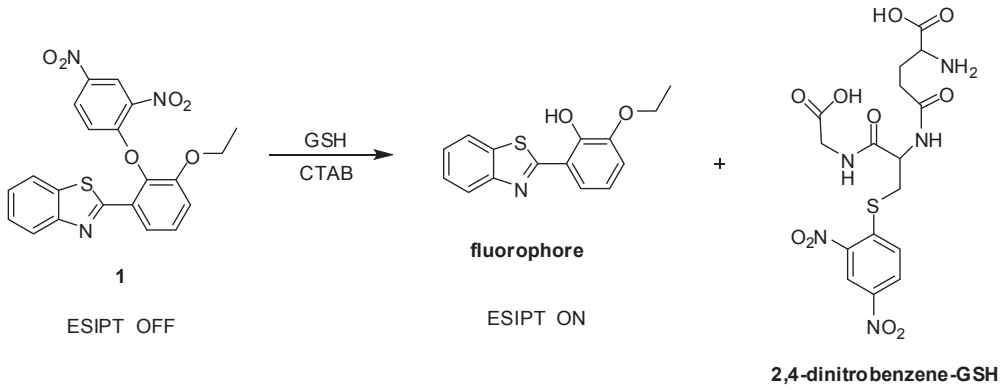
Glu-Cys-Glu, Lys-Cys-Gly, GSH, Cys and Hcy were stocked in deionized water. All the stock solutions (10 mM) were preserved at 4 °C. Test solutions were prepared by placing 30 μL of the probe stock solution into a test tube, diluting the solution to 3 mL with HEPES buffer (20 mM, pH = 7.4) containing CTAB (1 mM) and adding 30 μL of each analyte stock. Excitation and emission wavelengths were chosen at 385 nm and 485 nm, respectively. The excitation and emission slit widths were both 5 nm.

2.7. Mass checking

1 (43 mg), GSH (60 mg) were dissolved in 5 mL acetonitrile-HEPES buffer (20 mM, pH = 7.4, 1:4 v/v) containing CTAB (1 mM). After 3 h, 2 mg $NaClO_4$ was added to the reaction mixture. White precipitates came out gradually. Removing the precipitates, the product without CTAB was obtained for mass checking.

2.8. Fluorescence imaging of GSH in living cells

HeLa cells used in this study were purchased from Cobioer Biosciences Co., Ltd. (Nanjing, China). The cell lines were cultured in DMEM medium supplemented with 10% (v/v) calf serum, penicillin (100 UmL^{-1}), and streptomycin (100 mg/mL). The cells were seeded in laser confocal fluorescence microscope (LCFM) culture dishes and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. When the whole cells took up 60%–70% space of culture dishes, the cells were treated without or with 1 mM N-ethylmaleimide (NEM) in culture media for 30 min at 37 °C. After washing with phosphate buffered saline (PBS) to remove the remaining NEM, the cells were further incubated with 50 μM of **1** for 30 min at 37 °C. After treating with NEM and incubating with **1**,



Scheme 2. Proposed signaling mechanism of probe **1** to GSH.

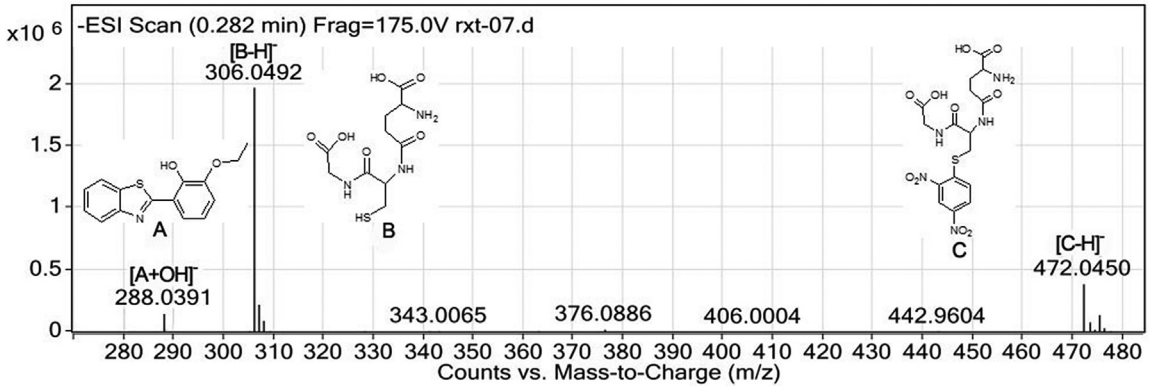


Fig. 3. ESI-MS spectra of the mixture of **1** and GSH. (A, B and C denote the compounds 2-(2′-hydroxy-3′-ethoxyphenyl)benzothiazole, GSH and 2,4-dinitrobenzene-GSH, respectively).

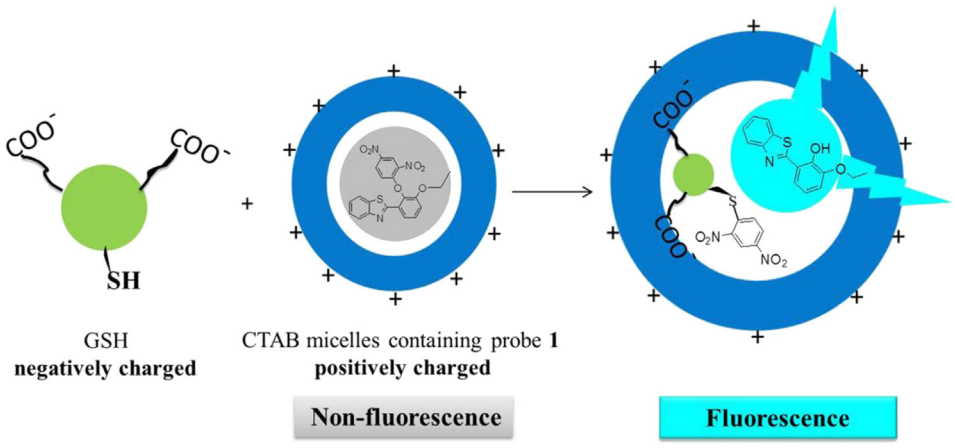


Fig. 4. Pictorial visualization of GSH attacking CTAB micelles and reacting with probe **1**.

the cells were subsequently treated with GSH (500 μM). The cells were imaged by confocal laser scanning microscopy.

3. Results and discussion

3.1. Synthesis

The chemical structure of the target probe 1 and its synthetic process were depicted in Scheme 1. After purification, probe 1 was obtained with a yield of 83% and characterized by X-ray crystallography, ^{1}H NMR, ^{13}C NMR and high resolution mass spectroscopy (Figs. S4–S9).

3.2. Spectral properties of probe *1* towards GSH

To study the selectivity of probe **1**, we examined 13 different amino acids including Cys, Hcy, Gly, Phe, Ser, Glu, Lys, Arg, His, Ala, Gln, Met and Tyr as the alternatives of GSH. First of all, we tested the

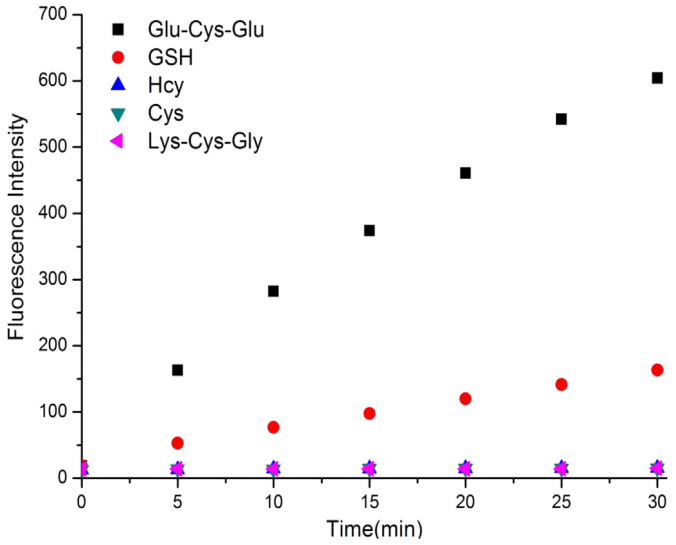


Fig. 5. Time-dependent fluorescence intensity of **1** (10 μM) at 485 nm in the presence of thiols (100 μM) and the two short SH-bearing peptides (100 μM). (λ_{ex} = 385 nm, slit: 5 nm/5 nm).

absorption spectral properties of probe **1** in 20 mM HEPES buffer solutions (pH = 7.4) containing 1 mM CTAB. As shown in Fig. 1a, an obvious change at ~380 nm was observed only after 10 equiv. GSH was added into probe **1** for 30 min. We next investigated the fluorescence spectra of the probe **1**. Notably, a sharp fluorescence intensity increase (about 15-fold enhancement) occurred at 485 nm when the probe **1** was incubated with 10 equiv. GSH for 30 min (Fig. 1b). Such cyan fluorescence could be detected by the naked-eye under UV light (Fig. S1).

The good selectivity inspired us to explore the concentration-dependent emission response of probe **1** toward GSH. We incubated our probe with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 equiv. GSH in 20 mM HEPES buffer (pH 7.4) containing 1 mM CTAB for 30 min. As shown in Fig. 2a, the fluorescence at 485 nm enhanced gradually with the increase of GSH concentration. A good fitted regression line was presented as well (Fig. 2b), and the detection limit was calculated as low as 0.81 μM (S/N = 3).

The influence of pH on the fluorescence process was further investigated. The fluorescence intensities at 485 nm of initial probe **1** in the absence and presence of GSH were collected, respectively. As seen from Fig. S2, the fluorescence spectra of probe **1** with or without GSH had no change in acidic condition. On the contrary, in

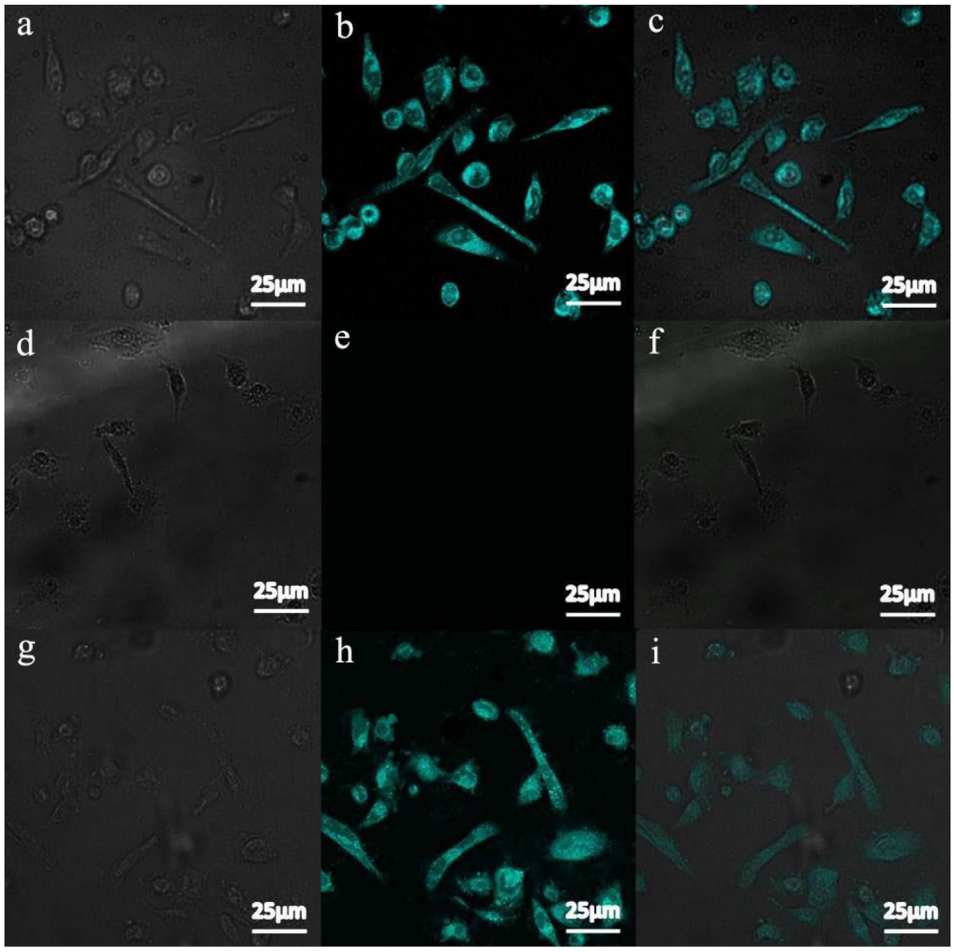


Fig. 6. Confocal fluorescence images of HeLa cells. (a) Cells incubated with **1** (50 μM) for 30 min (d) HeLa cells were pre-incubated with 500 μM NEM for 30 min and then treated with 50 μM of **1** for 30 min. (g) Subsequent treatment of the cells with GSH (500 μM) for 30 min (a, d and g, phase contrast images; b, e and h, fluorescence images; c, f and i, merged images).

neutral and alkaline conditions, fluorescence intensity of the solution containing probe and GSH had a significant enhancement, which indicated that the probe could be used in neutral and basic conditions. Time-dependent fluorescence intensity assay revealed that the reaction between probe **1** and GSH was completed on the whole within 2 h (Fig. S3).

3.3. Proposed mechanism

The fluorescence enhancement at 485 nm was attributed to the release of 2-(2′- hydroxy-3′-ethoxyphenyl)benzothiazole via the nucleophilic substitution reaction between probe **1** and GSH (Scheme 2). Once 2-(2′-hydroxy-3′-ethoxyphenyl)benzo-thiazole acted as fluorophore was free, an ESIPT process would come true, which ensured the strong fluorescence enhancement. To elucidate the details, an ESI-MS analysis of the reaction mixture of probe **1** and GSH was performed. It was noted that three distinct signals at $m/z = 288.0391$ $[A + OH]^-$, 306.0492 $[B-H]^-$ and 472.0450 $[C-H]^-$ appeared in the mass spectrum, where A, B and C denoted the compounds 2-(2′-hydroxy-3′-ethoxyphenyl)benzothiazole, GSH and 2,4-dinitrobenzene-GSH, respectively (Fig. 3). It was obvious that the probe **1** developed in this work provided a special signaling mechanism, which may contribute to the development of new fluorescent sensors for detection of GSH.

In this reaction system, CTAB micelles used as catalyst improved the reaction rate, which could be explained by the synchronous existences of electrostatic and hydrophobic interactions. GSH was negatively charged, so it was capable of closely binding with and breezily penetrating into positively charged CTAB micelles with hydrophobic substrate. In addition, the number of carboxyl group in thiols was considered to be another important reason for superior selectivity of probe **1** to GSH over Cys and Hcy. Compared to Cys and Hcy, GSH bearing two carboxyl groups which enhanced the ability of generating negative charge was more easily to have a strong affinity to positively charged CTAB micelles (Fig. 4). Thus, GSH was apt to attack probe substrate inside of the micellar aggregates, leading to the release of fluorophore. From the standpoint of the positive effect of carboxyl groups on nucleophilic substitution catalyzed by CTAB, we processed the reactions between probe **1** and thiols including GSH, Cys, Hcy and the other two short SH-bearing peptides (Glu-Cys-Glu and Lys-Cys-Gly). As we can see in Fig. 5, the reactivity of probe **1** to various thiols was manifested in the following order: Glu-Cys-Glu > GSH » Hcy, Cys and Lys-Cys-Gly. Obviously, Glu-Cys-Glu with three carboxyl groups demonstrated the highest reactivity to sensor **1**, while Hcy, Cys and Lys-Cys-Gly with only one carboxyl group showed little reactivity, which proved the validity of our original vision.

3.4. Cell imaging experiments

In consideration of the excellent performance of probe **1** under physiological pH, confocal microscopy experiments were carried out to further investigate the permeability and the real-time monitor of GSH in living cells. Once HeLa cells were incubated with **1** (50 μM), strong fluorescence was observed inside the cells due to the high level concentration of thiols in living cells (Fig. 6b). In the control experiment, when cells were pre-treated with excess N-ethylmaleimide (NEM), a trapping reagent for thiol species, followed by treatment with **1** (50 μM), the confocal microscopic image showed a negligible fluorescence signal (Fig. 6e). That verified the specificity of probe **1** for thiols over other analytes in living cells. Subsequently, with addition of 500 μM GSH to NEM-treated cells for 30 min, the fluorescence signal was restored (Fig. 6h), which indicated the rapid response of probe **1** toward GSH in living cells.

4. Conclusion

In summary, we have successfully developed probe **1** for specific detection of GSH over Cys and Hcy based on ESIPT. Additionally, probe **1** exhibits excellent sensitivity toward GSH in aqueous solution under physiological pH condition. The addition of GSH to the probe solution induces 15-fold fluorescence intensity enhancement and the detection limit is calculated as low as 0.81 μM. One more carboxyl group in GSH is the reason for the enhanced selectivity to GSH over Cys and Hcy. What's more, probe **1** is demonstrated with good application in the study of GSH in living cells and shows potential biological significance.

Appendix A. Supplementary data

CCDC 1454726 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found in the online version.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21376117, 21406109), the Jiangsu Natural Science Funds for Distinguished Young Scholars (BK20140043), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (14KJA150005), the Qing Lan Project and the Project of Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). Thank Prof. Dunru Zhu for the help of X-ray structure determination.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2016.02.027.

References

[1] Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3–32.
[2] Rusin O, St Luce NN, Agbaria RA, Escobedo JO, Jiang S, Warner IM, et al. Visual detection of cysteine and homocysteine. J Am Chem Soc 2004;126:438–9.
[3] Wang WH, Escobedo JO, Lawrence CM, Strongin RM. Direct detection of homocysteine. J Am Chem Soc 2004;126:3400–1.
[4] Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Asp Med 2009;30:1–12.
[5] Biswas SK, Rahman I. Environmental toxicity, redox signaling and lung inflammation: the role of glutathione. Mol Asp Med 2009;30:60–76.
[6] Zhang Y, Li Y, Yan XP. Photoactivated CdTe/CdSe quantum dots as a near infrared fluorescent probe for detecting biothiols in biological fluids. Anal Chem 2009;81:5001–7.
[7] Liu J, Sun YQ, Huo YY, Zhang HX, Wang LF, Zhang P, et al. Simultaneous fluorescence sensing of Cys and GSH from different emission channels. J Am Chem Soc 2014;136:574–7.
[8] Li DW, Qu LL, Hu K, Long YT, Tian H. Monitoring of endogenous hydrogen sulfide in living cells using surface-enhanced Raman scattering. Angew Chem Int Ed 2015;54:12758–61.
[9] Zhao CC, Zhang XL, Li KB, Zhu SJ, Guo ZQ, Zhang LL, et al. Förster resonance energy transfer switchable self-assembled micellar nanoprobe: ratiometric fluorescent trapping of endogenous H_2S generation via fluvastatin-stimulated upregulation. J Am Chem Soc 2015;137:8490–8.
[10] Li HD, Yao QC, Fan JL, Jiang N, Wang JY, Xia J, et al. A fluorescent probe for H_2S in vivo with fast response and high sensitivity. Chem Commun 2015;51:16225–8.
[11] Chen X, Zhou Y, Peng XJ, Yoon J. Fluorescent and colorimetric probes for detection of thiols. Chem Soc Rev 2010;39:2120–35.
[12] Yin J, Kwon Y, Kim D, Lee D, Kim G, Hu Y, et al. Cyanine-based fluorescent probe for highly selective detection of glutathione in cell cultures and live mouse tissues. J Am Chem Soc 2014;136:5351–8.
[13] Jung HS, Chen X, Kim JS, Yoon J. Recent progress in luminescent and colorimetric chemosensors for detection of thiols. Chem Soc Rev 2013;42:6019–31.

[14] Balkrishna SJ, Hodage AS, Kumar S, Panini P, Kumar S. Sensitive and regenerable organo-chalcogen probes for the colorimetric detection of thiols. RSC Adv 2014;4:11535–8.
[15] Chen X, Ko SK, Kim MJ, Shin I, Yoon J. A thiol-specific fluorescent probe and its applica- tion for bioimaging. Chem Commun 2010;46:2751–3.
[16] McMahon BK, Gunnlaugsson T. Selective detection of the reduced form of glutathione (GSH) over the oxidized (GSSG) form using a combination of glutathione reductase and a Tb(III)-cyclen maleimide based lanthanide luminescent 'switch on' assay. J Am Chem Soc 2012;134:10725–8.
[17] Kim GJ, Yoon DH, Yun MY, Kwon H, Ha HJ, Kim HJ. Ratiometric fluorescence probes based on a Michael acceptor type of coumarin and their application for the multichannel imaging of in vivo glutathione. RSC Adv 2014;4:18731–6.
[18] Niu LY, Guan YS, Chen YZ, Wu LZ, Tung CH, Yang QZ. BODIPY-based ratiometric fluorescent sensor for highly selective detection of glutathione over cysteine and homocysteine. J Am Chem Soc 2012;134:18928–31.
[19] Yin J, Kwon Y, Kim D, Lee D, Kim G, Hu Y, et al. Preparation of a cyanine-based fluorescent probe for highly selective detection of glutathione and its use in living cells and tissues of mice. Nat Protoc 2015;10:1742–54.
[20] Long L, Lin W, Chen B, Gao W, Yuan L. Construction of a FRET-based ratiometric fluorescent thiol probe. Chem Commun 2011;47:893–5.
[21] Shao J, Sun H, Guo H, Ji S, Zhao J, Wu W, et al. A highly selective red-emitting FRET fluorescent molecular probe derived from BODIPY for the detection of cysteine and homocysteine: an experimental and theoretical study. Chem Sci 2012;3. 1049– 61.
[22] Cao X, Lin W, Yu Q. A ratiometric fluorescent probe for thiols based on a tetrakis(4-hydroxyphenyl)porphyrin-coumarin Scaffold. J Org Chem 2011;76:7423–30.
[23] Lee MH, Han JH, Kwon PS, Bhuniya S, Kim JY, Sessler JL, et al. Hepatocyte-targeting single galactose-appended naphthalimide: a tool for intracellular thiol imaging in vivo. J Am Chem Soc 2012;134:1316–22.
[24] Zhou L, Lin Y, Huang Z, Ren J, Qu X. Carbon nanodots as fluorescence probes for rapid, sensitive, and label-free detection of Hg^{2+} and biothiols in complex matrices. Chem Commun 2012;48:1147–9.
[25] Liang W, Zhao Z, Zhang Y, Wang Q, Zhao X, Ouyang J. Highly selective detection of gluta- thione using a NIP/Cu^{2+} complex fluorescent probe. J Lumin 2012;132:1160–5.
[26] Wang H, Zhou G, Chen X. An iminofluorescein-Cu^{2+} ensemble probe for selective detection of thiols. Sensors Actuators B 2013;176:698–703.
[27] Wang H, Zhou G, Gai H, Chen X. A fluorescein-based probe with high selectivity to cysteine over homocysteine and glutathione. Chem Commun 2012;48:8341–3.
[28] Yang X, Guo Y, Strongin RM. Conjugate addition/cyclization sequence enables selective and simultaneous fluorescence detection of cysteine and homocysteine. Angew Chem Int Ed 2011;50:10690–3.
[29] Guo Z, Nam S, Park S, Yoon J. A highly selective ratiometric near-infrared fluorescent cyanine sensor for cysteine with remarkable shift and its application in bioimaging. Chem Sci 2012;3:2760–5.
[30] Jung HS, Pradhana T, Han JH, Heo KJ, Lee JH, Kang C, et al. Molecular modulated cysteine-selective fluorescent probe. Biomaterials 2012;33:8495–502.
[31] Shiu HY, Wong MK, Che CM. "Turn-on" FRET-based luminescent iridium(III) probes for the detection of cysteine and homocysteine. Chem Commun 2011;47:4367–9.
[32] Majumdar P, Zhao JZ. 2-(2-Hydroxyphenyl)-benzothiazole (HBT)-rhodamine dyad: acid-switchable absorption and fluorescence of excited-state intramolecular proton transfer (ESIPT). J Phys Chem B 2015;119:2384–94.
[33] Sahana S, Mishra G, Sivakumar S, Bharadwaj PK. A 2-(2′-hydroxyphenyl) benzo- thiazole (HBT)–quinoline conjugate: a highly specific fluorescent probe for Hg^{2+} based on ESIPT and its application in bioimaging. Dalton Trans 2015;44:20139–46.
[34] Huang Q, Yang XF, Li H. A ratiometric fluorescent probe for hydrogen sulfide based on an excited-state intramolecular proton transfer mechanism. Dyes Pigments 2013;99:871–7.
[35] Huang Z, Ding S, Yu D, Huang F, Feng G. Aldehyde group assisted thiolysis of dinitrophenyl ether: a new promising approach for efficient hydrogen sulfide probes. Chem Commun 2014;50:9185–7.
[36] Wang L, Chen H, Wang H, Wang F, Kambam S, Wang Y, et al. A fluorescent probe with high selectivity to glutathione over cysteine and homocysteine based on positive effect of carboxyl on nucleophilic substitution in CTAB. Sensors Actuators B 2014;192:708–13.
[37] Khan KM, Rahim F, Halim SA, Taha M, Khan M, Perveen S, et al. Synthesis of novel inhibitors of β-glucuronidase based on benzothiazole skeleton and study of their binding affinity by molecular docking. Bioorgan Med Chem 2011;19:4286–94.
[38] Sheldrick GM. A short history of SHELX. Acta Crystallogr A 2008;64:112–22.