Sensors and Actuators B 258 (2018) 72–79

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Two-stage ratiometric fluorescent responsive probe for rapid glutathione detection based on BODIPY thiol-halogen nucleophilic mono- or disubstitution

Deyan Gong[a], Jiaxi Ru[b], Ting Cao[a], Jing Qian[a], Wei Liu[a], Anam Iqbal[a], Weisheng Liu[a], Wenwu Qin[a,*], Huichen Guo[b,*]

[a] Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province and State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, PR China
[b] State Key Laboratory of Veterinary Etiological Biology and Key Laboratory of Animal Virology of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Xujiaping 1, Lanzhou, Gansu Province 730046, PR China

ARTICLE INFO

Article history:
Received 29 June 2017
Received in revised form
16 November 2017
Accepted 17 November 2017
Available online 21 November 2017

Keywords:
BODIPY
Two-stage ratiometric fluorescent probes
Nucleophilic mono- or disubstitution
Glutathione
Bioimaging

ABSTRACT

3,5-dichloro-8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (**BOD-2Cl**) (**1**) as a classical fluorescent dye was synthesized for the purpose of functionalization of BODIPY dye. Herein, it was applied for the purpose of achieving two-stage ratiometric fluorescent detection of glutathione based on the two-step thiol-halogen substitution reaction with the sulfhydryl group of glutathione at the 3 (mono-substitution) and 3,5-position (disubstitution) of **1** in CTAB micelles. The first stage induced fluorescent color change from green (λ_{em} = 530 nm) to yellow (λ_{em} = 562 nm) (I_{562nm}/I_{530nm}, mono-substitution), excess addition of glutathione caused the second stage fluorescent color change from yellow to red (λ_{em} = 597 nm) (I_{597nm}/I_{562nm}, disubstitution). It exhibits excellent properties with specific colorimetric and ratiometric fluorescent change to glutathione over cysteine and homocysteine. Further application to cellular ratiometric fluorescence imaging indicated that the probe was highly responsive to the glutathione in cells.

1. Introduction

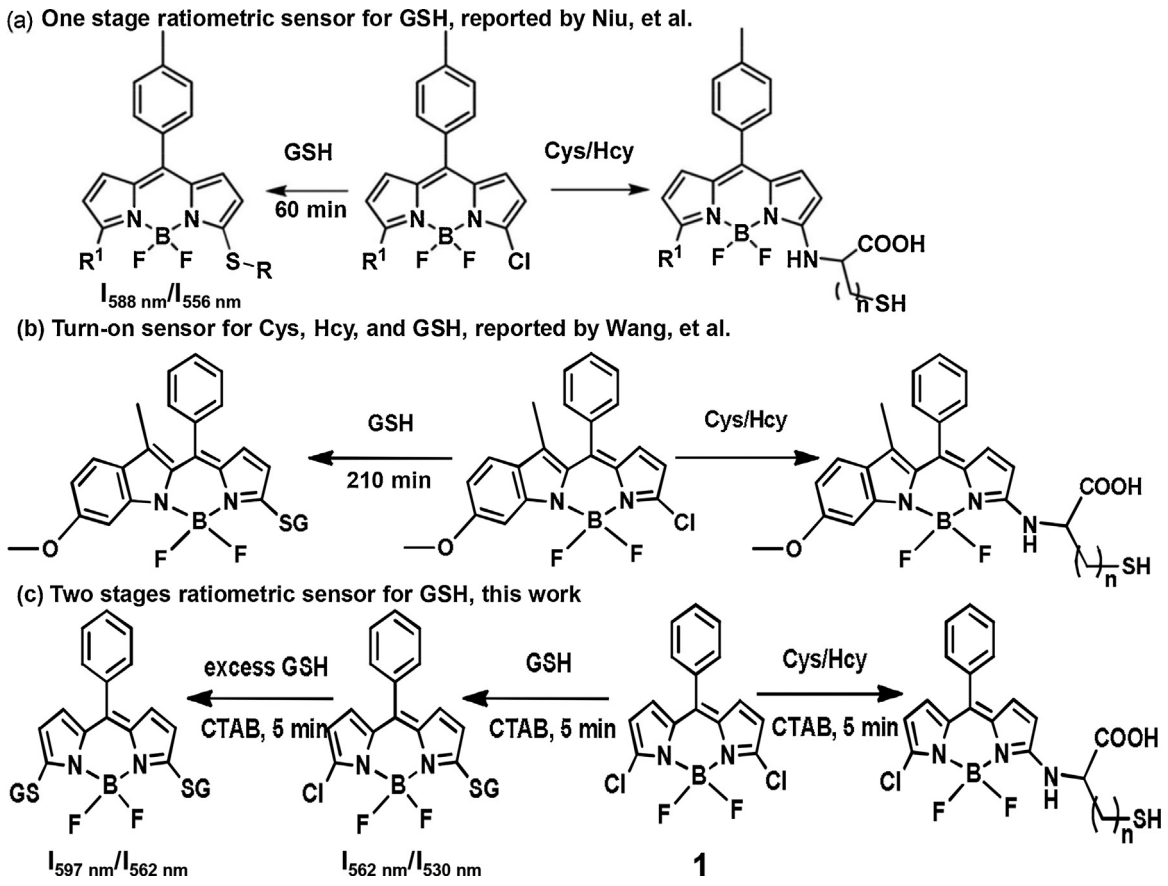
Biothiols including glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) have key roles in a lot of physiological and pathological processes [1,2]. It is well known that GSH acts an significant position in many physiological processes such as advancing the immunity and helping anti-aging [3,4]. In the meantime, an unusually descending level of GSH is directly related to retarded increase of children, hepatic impairment, dermatosis and even diseases such as AIDS, Alzheimer disease, cancer, diabetes mellitus, cardiovascular disease [5,6]. Thus, it is important to expand novel strategies for detection of GSH with the purpose of diagnosing the disease previous and assess the disease progression better [7].

Fluorescence probe is well matched for SH-containing life molecules detection *in vivo* and *in vitro* [8–11]. However, because of the similar reactivity and structures of GSH/Cys/Hcy, fluorescent sensors could discriminate GSH from Cys and Hcy only represent a small fraction [10,12–14]. Ratiometric measurement could offer a built-in correction for the environmental effects and provide a facile means for visualizing intricate life processes at the molecular level [15]. So far, only a few probes can afford ratiometric fluorescent selective detection for GSH [16–18]. Compared to single-stage response, multi-stage fluorescent response provides higher sensitivity and wider application [19,20].

For GSH-responsive fluorescent sensors, the mechanism of nucleophilic substitution is one of the most broadly employed design principles [21–23]. In Scheme 1, Niu et al. originally performed systematic effort on the reactivity of monochlorinated BODIPY derivatives with biothiols. Scheme 1a demonstrates a one stage ratiometric fluorescent probe for the discrimination of GSH over Cys and Hcy. The chlorine of the monochlorinated BODIPY is replaced by biothiols through thiol–halogen nucleophilic substitution. The amino groups of Cys/Hcy but not GSH further replace the thiolate via a five- or six-membered cyclic transition state to form aminosubstituted BODIPY [24]. Wang et al. reported a monochlorinated BODIPY molecule probe achieving simultaneous discrimination of Cys, Hcy, and GSH, which can allow Cys, Hcy, and GSH to be simultaneously discriminated on the basis of three distinct fluorescence turn-on responses (Scheme 1b) [25]. However, these organic molecules required at least 1 h to reach the full reaction state with GSH, which limited their further applications. As a

* Corresponding authors.
E-mail addresses: qinww@lzu.edu.cn (W. Qin), ghch-2004@hotmail.com (H. Guo).

https://doi.org/10.1016/j.snb.2017.11.092
0925-4005/



Scheme 1. Substitution reaction with GSH on different chromophores.

surfactant, cetyltrimethylammonium bromide (CTAB) micelles catalyze the nucleophilic substitution reaction of GSH obviously, it improves not only the water-solubility of the probe, but also the reaction rate and sensitivity with biothiols [26,27]. CTAB also has been utilized as media to detect hydrogen sulphide [28].**BOD-2Cl** (**1**) was synthesized as depicted previously [29]. Its derivatives may be favored as fluorescent chemosensors which have been applied to detect a variety of analytes, such as pH [30], cations [31,32], biothiols and so on[24,33–35]. Thus, we employed **BOD-2Cl** for the goal of realizing highly selective and rapid detection of GSH in CTAB media (Scheme 1c). The 3-position chlorine of the dichlorinated BODIPY would be rapidly substituted by thiolates of GSH (mono-substitution), excess addition of GSH promoted further substitution at the 5-position of the probe (disubstitution). Because of the exceptional properties of the BODIPY dye, the thioether substituents red-shift of both the absorption and emission spectra [24,36], excellent selective GSH ratiometric fluorescence detection is achieved. Interestingly, the two-step substitution of the chlorine of **1** by GSH exhibited two-stage ratiometric fluorescence change. The first stage induced fluorescent color change from green to yellow; excess addition of GSH caused the second stage fluorescent color change from yellow to red. So far, no report on two-stage ratiometric fluorescent responsive probe for detection of GSH has been reported.

2. Experimental

2.1. Instruments and reagents

All chemicals and solvents were analytical and used without further purification. ^{1}H NMR spectra were received with a Bruker DRX-400 and DRX-400/4 spectrometer. Mass spectra were detected in E.I. Mode. All pH measurements were finished with a pH-10C digital pH meter. All titration and selectivity experiment of **1** was diluted in DMSO/PBS buffer (1:9, v/v, 20 mM, pH 7.4) with 3 mM CTAB and then kept at 37 °C in a thermostatic waterbath.

2.2. The synthesis of probe ***BOD-2Cl***

As shown in Scheme 1, **1** was prepared according to the known procedure [29]. ^{1}H NMR ($CDCl_3$): δ 6.42 (d, 2H, J = 4.4 Hz, H-a), 6.83 (d, 2H, J = 4.4 Hz, H-b), 7.46–7.51 (m, 5H (Ph)). Mass spectrum (ESI), m/z 336.99 (M); 316.99 (M-HF, 100%) ($C_{15}H_9BCl_2F_2N_2$ requires m/z 336.9592).

2.2.1. The synthesis of BOD-GSH (mono-substituted BOD-2Cl with GSH)

According to the known procedure [37], we prepared *N*-*t*-butoxycarbonylglutathione (Boc-GSH), which is amine-protected GSH. The mixture of **1** (20 mg, 0.06 mmol) and Boc-GSH (26 mg, 0.07 mmol) was stirred in 10 mL of MeOH at 25 °C, then triethylamine (100 μL, 0.72 mmol) was added. After about 2 h, the solvent was evaporated to dryness under reduced pressure. The solid residue was dissolved in 5 mL MeOH with 4 mL HCl (3 M) added, and then was stirred for 1 h at 25 °C for acid hydrolysis reaction. After evaporation of the solvent *in vacuo*, the residue was refined by silica gel chromatography with MeOH as eluent to afford **BOD-GSH** as a reddish-brown solid (19 mg, 0.03 mmol, 52% yield). R_f 0.76 (MeOH/H_2O = 10:1). Mass spectrum (ESI), m/z 554.28 (M-Cl-F); m/z 574.28 (M-Cl + 1, 100%); 608.24 (M + 1) ($C_{25}H_{25}BClF_2N_5O_6S$ requires m/z 607.1275). HRMS: calcd for **BOD-GSH** ($C_{25}H_{25}BClF_2N_5O_6SNa$, M + Na) 630.1167, found 630.1169.