Sensors and Actuators B 188 (2013) 111–116

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Caged rhodamine-based fluorescent probe for biothiol: Selective detection of cysteine over homocysteine and glutathione in water

Soo-Yeon Lim[a], Doo-Ha Yoon[a], Do Yeon Ha[b], Jung mo Ahn[b], Dae Il Kim[c], Hyockman Kown[c,d], Hyun-Joon Ha[a,d,*], Hae-Jo Kim[a,*]

[a] Department of Chemistry, Hankuk University of Foreign Studies, Yongin, 449-791, Republic of Korea
[b] Korea Science Academy of KAIST, Busan, 614-822, Republic of Korea
[c] Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Yongin, 449-791, Republic of Korea
[d] Protein Research Centre for Bio-Industry, Hankuk University of Foreign Studies, Yongin, 449-791, Republic of Korea

ARTICLE INFO

Article history:
Received 28 February 2013
Received in revised form 27 June 2013
Accepted 28 June 2013
Available online 8 July 2013

Keywords:
Chemodosimeter
Cyclization
Cysteine
Fluorescence
Rhodamine

ABSTRACT

We report a rhodamine-based fluorescent probe (1) for cysteine (Cys). Masked with a para-hydroxybenzyl alcohol (HBA) unit, the probe's initial weak fluorescence is converted to strong fluorescence through a series of reactions of Michael addition and intramolecular cyclization of Cys, followed by deprotection of HBA. The caged probe (1) exhibits a selective and sensitive response toward Cys over homocysteine (Hcy) and glutathione (GSH) in HEPES buffer.

1. Introduction

Biothiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) are involved in a myriad of vital cellular processes, including redox homeostasis [1] and cellular growth [2]. In particular, Cys deficiency results in many syndromes such as slow growth in children, hair depigmentation, lethargy, liver damage, fat loss and skin lesions [3]. At elevated levels in plasma, Hcy is well known as a risk factor for Alzheimer's disease [4]. GSH is the most abundant cellular thiol with a concentration ranging from 1.0 to 15 mM [5]. It serves many cellular functions, including the maintenance of intracellular redox activities, xenobiotic metabolism, intra-cellular signal transduction, and gene regulation [6]. Although Cys, Hcy, and GSH possess similar structures with a thiol group, they are associated with different diseases. Therefore, it is of growing importance to develop a fluorescent probe capable of discriminating the structural difference between these biothiols. In recent few years, several fluorescent probes for biothiols were developed to exhibit a selective response toward Cys, [7] Hcy, [8] or GSH [9] by employing smart strategies such as cyclization kinetics, thermodynamic stability, or micelle formation structure, respectively. Nonetheless, it remains difficult to make a discrimination of these biothiols. In this article, we report a caged rhodamine probe with para-hydroxybenzyl alcohol (HBA), [10] whose phenol group is protected by an enone type of Michael acceptor. The fluorescent probe shows a selective and sensitive response for Cys by kinetic discrimination during the cyclization of **1**-Cys in HEPES buffer.

2. Experimental

2.1. General

All reagents and solvents were purchased from commercial sources and used without further purification, unless otherwise stated. All reactions were carried out on a magnetic stirrer and their reaction processes were monitored by thin-layer chromatography (TLC). The compounds were separated by flash chromatography on silica gel 60 (230–400 mesh). Absorption and fluorescence spectra were taken on an Agilent 8453 spectrophotometer and a JASCO FP-6500 fluorescence spectrometer, respectively.

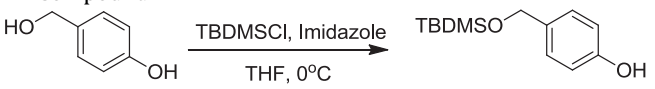
NMR measurement was performed with 200 or 300 MHz (^{1}H) and a 50 or 75 MHz (^{13}C) spectrometer. The solvent for NMR measurements was dimethyl sulfoxide (DMSO-d_6). All peaks were recorded as δ in ppm relative to the signals of residual non-deuterated solvent peaks. The following abbreviations were used to explain the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

* Corresponding authors. Tel.: +82 31 330 4703; fax: +82 31 330 4566.
E-mail addresses: hjha@hufs.ac.kr (H.-J. Ha), haejkim@hufs.ac.kr (H.-J. Kim).

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.06.101

2.2. Preparation of probe and intermediates

Compound 2

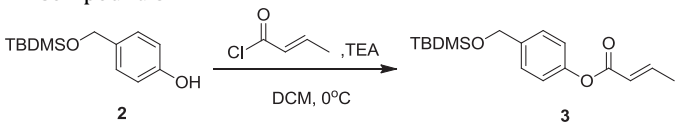


2

HBA (372 mg, 3.00 mmol), tert-butyl dimethylsilyl chloride (TBDMSCl, 452 mg, 3.00 mmol) and imidazole (40 mg, 2.0 mmol) were dissolved in tetrahydrofuran (THF, 12.0 mL) at 0 °C. The reaction mixture was stirred for 6 h. After completion of the reaction, all volatiles were removed under reduced pressure. The crude product was purified by column chromatography on silicagel using ethylacetate and hexane (1:5, v/v) as an eluent, to give compound **2** as a light yellow liquid (212 mg) in 30% yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.21 (d, ^3J = 8.6 Hz, 2H), 6.81 (d, ^3J = 8.6 Hz, 2H), 4.79 (s, 1H), 4.66 (s, 2H), 0.93 (s, 9H), 0.09 (s, 6H).

Compound 3

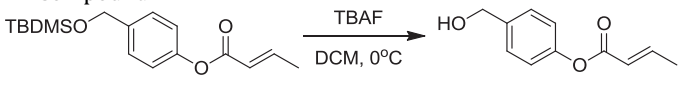


2 3

Compound **2** (212 mg, 1.77 mmol) and triethylamine (TEA, 0.591 mL, 2.65 mmol) were dissolved and stirred in anhydrous dichloromethane (DCM, 5 mL) at 0 °C for 30 min. Crotonylchloride (0.203 mL, 2.12 mmol) was slowly added to the stirred solution. The reaction mixture was further stirred at room temprature for 1 h. After completion of the reaction, all volatiles were removed under reduced pressure. The crude product was purified by column chromatography on silicagel using ethylacetate and hexane (1:5, v/v) as an eluent, to give compound **3** as a colorless liquid (376 mg) in 68% yield.

^{1}H NMR (DMSO-d_6, 200 MHz): δ 7.36 (d, ^3J = 8.6 Hz, 2H), 7.12 (m, 3H), 6.17 (dd, ^3J = 15.6 Hz, ^4J = 1.8 Hz, 1H), 4.71 (s, 2H), 1.96 (dd, ^3J = 7.0 Hz, ^4J = 1.8 Hz, 3H) 0.90 (s, 9H), 0.09 (s, 6H).

Compound 4

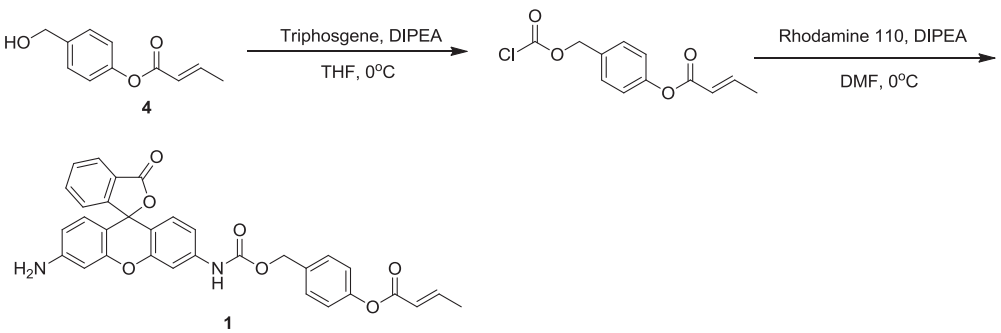


3 4

Compound **3** (376 mg, 1.23 mmol) was dissolved and stirred in anhydrous DCM (3 mL) at 0 °C. Tetra-*n*-butylammonium fluoride (TBAF, 1 M in THF, 2.46 mL, 2.46 mmol) was slowly added to the stirred solution. The reaction mixture was further stirred at room temprature for 6 h. After completion of the reaction, all volatiles were removed under reduced pressure. The crude product was purified by column chromatography on silicagel using ethylacetate and hexane (1:2, v/v) as an eluent, to give compound **4** as a colorless liquid (165 mg) in 70% yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.41 (d, ^3J = 8.4 Hz, 2H), 7.13 (m, 3H), 6.09 (dd, ^3J = 15.6 Hz, ^4J = 1.8 Hz, 1H), 4.71 (s, 1H), 4.68 (s, 2H), 1.99 (dd, ^3J = 7.0 Hz, ^4J = 1.8 Hz, 3H).

Probe 1



4 1

N,*N*-Diisopropylethylamine (DIPEA, 44.0 μL, 254 μmol) was added to a solution of **4** in anhydrous THF (0.50 mL) at 0 °C for 30 min under nitrogen. Triphosgene (37 mg, 127 μmol) dissolved in THF (0.50 mL) was slowly added to the stirred solution over 10 min. The reaction mixture was monitored by TLC to afford the chloroformate intermediate.

Rhodamine 110 (45 mg, 123 μmol) and DIPEA (80.0 μL, 738 μmol) were dissolved in dry dimethylformamide (DMF, 1.0 mL) at 0 °C for 30 min. The crude chloroformate was slowly added over 10 min to the solution of rhodamine. The reaction mixture was stirred at room temprature for 3 h. After completion of the reaction, all the volatiles were removed under reduced pressure. The crude product was purified by column chromatography on silicagel using DCM/methanol (50:1, v/v) and ethylacetate/hexane (1:2, v/v) as gradient eluents, to give probe **1** as a pink powder (8.0 mg) in 12% yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.01 (d, ^3J = 7.4 Hz, 1H), 7.65(t, ^3J = 7.4 Hz, 1H), 7.58 (t, ^3J = 7.4 Hz, 1H), 7.51 (d, ^4J = 2.4 Hz, 1H), 7.42 (d, ^3J = 8.6 Hz, 2H), 7.25–7.08 (m, 4H), 6.93 (s, 1H), 6.87 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 1H), 6.67 (d, ^3J = 8.6 Hz, 1H), 6.55 (d, ^3J = 8.6 Hz, 1H), 6.50 (d, ^4J = 2.4 Hz, 1H), 6.33 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 1H), 6.08 (dd, ^3J = 15.6 Hz, ^4J = 1.8 Hz, 1H), 5.17 (s, 2H), 3.91 (s, 2H), 1.98 (dd, ^3J = 7.0 Hz, ^4J = 1.8 Hz, 3H)

^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.68, 164.80, 153.25, 152.96, 152.52, 152.08, 150.78, 148.85, 147.31, 139.75, 134.94, 133.28, 129.58, 129.10, 128.73, 126.85, 124.92, 124.00, 121.90, 114.04, 113.98, 111.67, 108.45, 106.33. 101.49, 83.57, 77.24, 66.59, 29.72, 18.26 (30 carbon peaks)

HRMS (MALDI$^+$, DHB): *m*/*z* obsd 549.1650 ([M + Na]$^+$, calcd 549.1657 for $C_{32}H_{24}N_2O_7$)

2.3. UV–vis and fluorescence measurements

Stock solution (10 mM) of **1** in DMSO was prepared and used by dilution with HEPES buffer (0.10 M, pH 7.4). For UV–vis measurement, the sample solutions were prepared by mixing appropriate amounts of stock solution of **1** (10 mM in DMSO) with appropriate amounts of each amino acid (AA) and finally diluted with HEPES buffer to afford the desired concentration of **1** and AA. The fluorescence was measured similarly with a slit width of 3 nm × 3 nm.

2.4. Fluorescence imaging of HeLa cells

For the detection of biothiols in live cells, HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal bovine serum. The cells were seeded on a Ø 35 mm glass-bottomed dish at a density of 0.8×10^5 cells in a culture medium overnight for live-cell imaging by confocal laser scanning microscopy (CLSM). The HeLa cells were treated with 2 μM of probe **1** in 2 mL of 1 × PBS for 10 min and washed with twice with pre-warmed 1× PBS before imaging by CLSM.