Biosensors and Bioelectronics 72 (2015) 275–281

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A colorimetric and ratiometric fluorescent probe for selective detection and cellular imaging of glutathione

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ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form
10 May 2015
Accepted 11 May 2015
Available online 13 May 2015

Keywords:
Ratiometric fluorescent probe
Glutathione detection
Cysteine
Homocysteine
chlorinated coumarinyl aldehyde
Cell imaging

ABSTRACT

A new colorimetric and ratiometric fluorescent probe **1** based on a chlorinated coumarinyl aldehyde was developed for selective detection and cellular imaging of glutathione (GSH) over cysteine (Cys) and homocysteine (Hcy). Probe **1** exhibits a dramatic colorimetric and ratiometric fluorescence responses toward biothiols Cys, Hcy and GSH with high selectivity over other amino acids. Cys (or Hcy) induces a tandem S_NAr-rearrangement reaction to form the corresponding amino-coumarins (**2a** or **2b**), which result in about 75 nm and 35 nm blue-shifts in absorption and emission, respectively. By comparison, the thio-coumarin (**3′**) derived from the S_NAr reaction with GSH, which does not occur rearrangement because of steric hindrance, undergoes an intramolecular aldimine condensation lead to a cyclic iminium cation (**3**) with 47 nm and 39 nm red-shifts in absorption and emission, respectively. The significantly difference of photophysical properties enable excellent selectivity towards GSH over Cys and Hcy. Further application to cellular imaging indicates that the probe has appreciable cell permeability and is highly responsive to the changes of GSH level. As a result, it is applicable to monitor GSH level in living cells.

1. Introduction

Glutathione (GSH; γ-glutamylcysteinylglycine) is ubiquitous in mammalian and many prokaryotic cells and is the most abundant intracellular thiol (1–10 mM) (Meister, 1988). It is an essential endogenous antioxidant that plays a central role in cellular and defenses against toxins and free radicals (Miller et al., 2007). Aberrant levels of GSH have been associated with a number of diseases, including cancer, AIDS, Alzheimer's, cardiovascular disease and others (Townsend et al., 2003). Therefore, the accurate concentration measurement of GSH in physiological media has been considered as an essential factor in these diseases and their therapy because of its biological and clinical significance. Because of this, the considerable contemporary effort devoted to the development of an efficient method for the detection and quantification of GSH in living systems (Chen et al., 2010). Among the various analytical methods that are available, molecular imaging based on fluorescent probes is considered to be the most sensitive approach owing to its sensitivity and simplicity. Most of the existing probes utilize the strong nucleophilicity of the thiol group, operating by Michael addition (Guy et al., 2007), cleavage of disulfide (Pires and Chmielewski, 2008; Lee et al., 2012) and sulfonamide (Zhang et al., 2011; Shao et al., 2012), etc (Tang et al., 2007; Zhang et al., 2007). However, although these probes can highly selectively distinguish these biothiols from other amino acids, most of them cannot distinguish Cys/Hcy/GSH from each other due to their similar structure and reactivity. Up to now, the discrimination between them has been a focal point and also a tough challenge for researchers, albeit some advances have been obtained. By means of the cyclization of Cys/Hcy with aldehydes or acrylates, selective detection of Cys/Hcy over GSH was firstly achieved by the Strongin group (Rusin et al., 2004; Yang et al., 2011). Since then, as the extended version of the two strategies, some more specific probes for Cys or Hcy were also developed (Chen. et al. 2007; Li et al., 2014; Yuan et al., 2011; Yang et al., 2012b; Guo et al., 2012a; Yang et al., 2012a; Guo et al., 2012c). Recently, discrimination of Cys from Hcy/GSH was also achieved by taking advantage of either the Cys-induced substitution-rearrangement cascade reaction (Ma et al., 2012; Niu et al., 2013) or Michael addition combined with steric and electrostatic interactions (Jung et al., 2012a; Jung et al., 2012b; Zhou et al., 2012; Zhang et al., 2012). However, probes capable of discrimination of GSH from Cys/Hcy are still rare. As far as we know, only several strategies have been reported, including supramolecular interaction between a bis-spiropyran receptor and GSH (Shao et al., 2010), a micelle-catalyzed large ring formation of GSH with acrylate (Guo

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http://dx.doi.org/10.1016/j.bios.2015.05.030
0956-5663/

et al., 2012b), a specific reaction of GSH with o-phthalaldehyde (Xu et al., 2013), and those GSH-induced tandem reactions, such as S_NAr-rearrangement (Niu et al., 2012; Liu et al., 2014; Jia et al., 2015), S_NAr-rearrangement-cyclization (Liu et al., 2014; Liu et al., 2015), and nucleophilic substitution (Lim et al., 2014; Yin et al., 2014) as well as a native chemical ligation (NCL) reaction (Yang et al., 2014). Very recently, a novel strategy for selectively sensing GSH by a dual-response mechanism was reported (Wang et al., 2015). This strategy integrates two independent reaction sites with a disulfide linker and a thioether function into a fluorescent BODIPY-based probe to guarantee the synergetic dual-response in an elegant fashion to address the discrimination of GSH. Even so, there still are some limitations when considering the practical applications in biological systems, such as use of organic solvent or surfactant, long response time and require high equivalents of GSH to reach a maximal fluorescent signal, relatively poor selectivity, undesired spectra overlap, and so on. Furthermore, almost all of them are based on fluorescence measurement at a single wavelength, which means that GSH detection only depends on the changes of emission intensity, and could be significantly influenced by the excitation power and the detector sensitivity. Moreover, fluorescent intensity changes are not easy to observe by the naked eye directly (Hu et al., 2010). By contrast, ratiometric fluorescent probes allow the measurement of emission intensities at two wavelengths, which should provide a built-in correction for environmental effects and the fluorescence color change, which can be measured directly with a colorimeter or even distinguished easily by eye (Fu et al., 2011). Therefore, it is of high interest to develop new ratiometric fluorescent probes for GSH, in particular, with rapid response and high sensitivity.

With these considerations in mind, we developed a new colorimetric and ratiometric fluorescent probe **1** for discrimination of GSH over Cys and Hcy based on a chlorinated coumaryl aldehyde (Scheme 1). As we expected, probe **1** displayed a high selectivity for detection of GSH over Cys and Hcy based on a novel GSH-induced S_NAr-aldimine condensation strategy and that it can be used to monitor GSH in cells.

2. Experimental

2.1. Materials and apparatus

Commercially available compounds were used without further purification. The reaction solvents were dried according to standard procedures. Fluorescence spectra were carried out on a Shimadzu RF-5301PC fluorescence spectrophotometer. UV/vis spectra were recorded with a Shimadzu UV-2550 spectrophotometer. NMR spectra were recorded on a Bruker AV-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C), and chemical shifts were referenced relative to tetramethylsilane. Mass data were obtained by a Shimadzu AXIMA-CFR™ *plus* mass spectrometry using a 1, 8, 9-anthracenetriol (DITH) matrix. PBS buffer (10 mM, pH 7.4, 10% DMSO) prepared with deionized water and spectroscopic grade of DMSO were used as the solvent for all spectroscopic experiments. Stock solution of the probe (1×10^{-3} M) was prepared in DMSO, which was diluted to 1×10^{-5} M with the PBS buffer. Stock solution of the various amino acids were prepared with the PBS buffer for future use. For all fluorescence measurements, the excitations were at 476 nm for GSH and 385 nm for Cys and Hcy, and the excitation and emission slit widths were 3 and 5 nm, respectively.

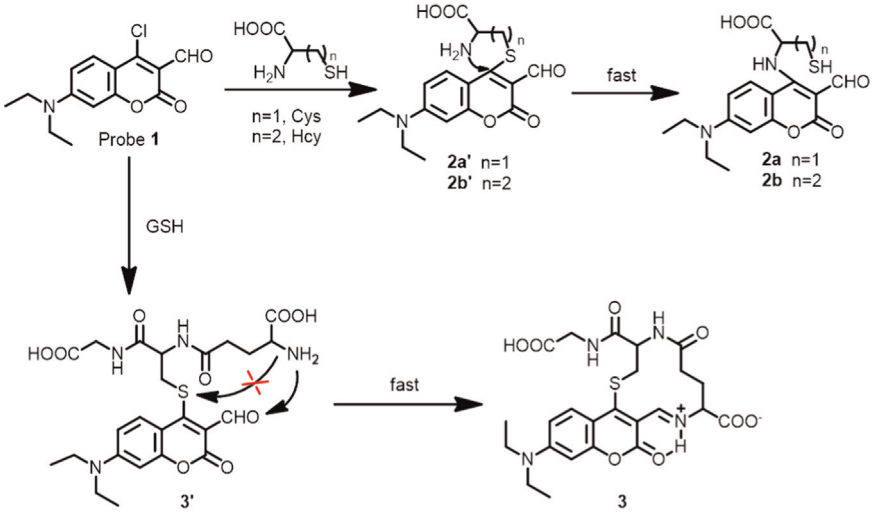
2.2. Methods for cell culture and fluorescent imaging

HeLa cells were obtained from American Type Culture collection and grown in Dulbecco's modification of Eagle's medium Dulbecco (Free DMEM/high: with 4500 mg/L Glucose, 4.0 mM L-Glutamine, and 110 mg/L Sodium Pyruvate). The cells were incubated in a 5% CO_2 humidified incubator at 37 °C and typically passaged with sub-cultivation ratio of 1:4 for two days. The HeLa cells were seeded in 6-well culture plate overnight. Stock solutions of probe (3 mM) and NEM (0.15 M) in DMSO, GSH, Cys (0.15 M) in PBS buffer (10 mM, pH 7.4) were prepared at the same day of experiment. Then, the cells were treated without or with GSH (0.1, 0.2, 0.5, 1.0, and 2.0 mM) or Cys (0.5 mM) for 30 min and NEM (0.1 mM) for 20 min at 37 °C, respectively, in culture media. After washing with phosphate buffered saline to remove the remaining GSH or Cys, the cells were further incubated with 10 μM of probe in the culture media for 30 min at 37 °C. The cells were imaged using an inverted microscope (OLYMPUS IX73) and Leica SP5II confocal microscope.

3. Results and discussion

3.1. Design concept of the probe

The design rationale is depicted in Scheme 1, and illustrated as follows. Chlorine lies in 4-position of probe **1**, where is doubly



Scheme 1. Proposed reaction mechanisms of probe **1** with Cys, Hcy and GSH.