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A mitochondria-targeted turn-on fluorescent probe for the detection of glutathione in living cells

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ABSTRACT

A novel turn-on red fluorescent BODIPY-based probe (Probe **1**) for the detection of glutathione was developed. Such a probe carries a *para*-dinitrophenoxy benzyl pyridinium moiety at the *meso* position of a BODIPY dye as self-immolative linker. Probe **1** responds selectively to glutathione with the detection limit of 109 nM over other amino acids, common metal ions, reactive oxygen species, reactive nitrogen species, and reactive sulfur species. A novel electrostatic interaction to modulate the S_NAr attack of glutathione was believed to play significant role for the observed selective response to glutathione. The cleavage of dinitrophenyl ether by glutathione leads to the production of *para*-hydroxybenzyl moiety which is able to self-immolate through an intramolecular 1,4-elimination reaction to release the fluorescent BODIPY dye. The low toxic probe has been successfully used to detect mitochondrial glutathione in living cells.

1. Introduction

Reduced glutathione (GSH) as a tripeptide of glutamic acid (Glu), cysteine (Cys), and glycine (Gly), is the most abundant intracellular nonprotein thiol species (1–10 mM) and a biomarker of oxidative stress (Armstrong et al., 2002; Townsend et al., 2003). It has been revealed that GSH plays a critical role in controlling oxidative stress in order to maintain the redox homeostasis for cell growth and function (Dalton et al., 1999). Aberrant levels of GSH have been correlated with various diseases, *e.g.* AIDS, cancer, liver damage and neurodegenerative diseases (Tapiero et al., 2003). Modern intelligent drug delivery systems have been developed by taking advantages of the differences between intracellular and extracellular GSH concentrations in cancer cells (Bauhuber et al., 2009; Flygare et al., 2013). Abnormal levels of Cys have been associated with slow growth, hair depigmentation, liver damage, loss of muscle and fat, skin lesion and cancer *etc.* (Shahrokhian, 2001). Homocysteine (Hcy) has been implicated in various types of vascular and renal diseases and was regarded as a risk factor for disorders of cardiovascular diseases and Alzheimer's disease (Refsum et al., 1989; Seshadri et al., 2002). More importantly, many evidences have suggested that GSH and Cys/Hcy levels are interrelated in biological systems. GSH is contemplated as a putative intracellular reservoir of Cys in the liver of adult rats (Hidalgo et al., 1990). In addition, the synthesis of GSH is dependent on the *trans*-sulphuration of Hcy. Thus, the detection of thiols is highly important for early diagnosis of diseases and evaluation of disease progression. Mitochondria are membrane-bound organelles that account for as much as 20% of the total cell volume. The foremost function of mitochondria is to produce ATP, the major energy currency molecule of the cell. Moreover, mitochondria are also involved in ROS-induced apoptosis. In order to protect cells from the oxidative stress, mitochondria are equipped with a number of free radical scavengers and the mitochondrial glutathione (γ-glutamylcysteinylglycine or GSH) pool is a critical antioxidant reservoir within cells (Jocelyn, 1975; Wahllander et al., 1979). Therefore, it is urgent to develop a mitochondrial GSH probe. Among the reported detection methods, fluorescent sensing has attracted considerable attention due to the simplicity, low cost, selectivity and sensitivity of fluorescent probe. Pioneered by Strongin's group (Rusin et al., 2004), fluorescent probes for biothiols including GSH, Cys and Hcy, have been the focused area because of their significance in biological processes very recently (Chen et al., 2010; Zhou and Yoon, 2012; Peng et al., 2012; Yang et al., 2013; Yin et al., 2013; Jia et al., 2015). Among the fluorescent probes reported, discrimination of biothiols from other amino acids typically takes advantage of the unique nucleophilicity of the thiol group. However, it remains a challenge to discriminate individual biothiol species due to similar structures and reactivities

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of various biothiols.

Selective detection of Cys/Hcy over GSH could be realized in some recent reports (Tsay et al., 2012; Wang et al., 2012a, 2012b; Guo et al., 2012b; Yang et al., 2012; Mei et al., 2013; Zhu et al., 2013; Hong et al., 2013; Niu et al., 2013; Murale et al., 2014), however, discrimination of GSH from Cys/Hcy was much more difficult and was addressed to much less extent due to the bulkiness of GSH with diminished nucleophilicity (Niu et al., 2012; Liu et al., 2014a, 2014b; Wang et al., 2014; Yang et al., 2014; Zhang et al., 2015b; Wang et al., 2015; Tang et al., 2007; Guo et al., 2012a; Yin et al., 2014; Lim et al., 2014; Işık et al., 2014). A noteworthy advancement reported recently was that differentiation of GSH from Cys/Hcy *via* different emission channels (Niu et al., 2012; Liu et al., 2014a, 2014b; Wang et al., 2014; Yang et al., 2014; Lim et al., 2014; Zhang et al., 2015b; Wang et al., 2015). However, to the best of our knowledge, relatively few probes have been developed that exhibit selective fluorescence response to mitochondrial GSH (Lim et al., 2014, 2011). Consequently, there is a pressing need for the identification of newer and more effective probes that could preferably detect GSH and target mitochondrial GSH.

Among the reported fluorescent probes for GSH detection, in most cases GSH displayed comparative or lesser extent rate due to the closely resembled reductivity/nucleophilicity however with more bulkiness for GSH. Only a few reports demonstrated that GSH displayed faster reaction rate than Cys/HCy and the typical reported structures were collected in Scheme S1 (Tang et al., 2007; Guo et al., 2012a; Yin et al., 2014; Lim et al., 2014; Işık et al., 2014). Tang et al. (2007) reported that Rhodamine-based fluorescent probe containing Se-N bond displayed higher fluorescence enhancement with GSH than Cys/Hcy *via* reduction mechanism, however, it was significantly affected by various protein thiols. Guo et al. (2012a) found that resorufin-acrylate probe detected GSH preferentially over other biothiols using cetyltrimethylammonium bromide (CTAB) micelles which bind GSH on the surface, however, a long reaction time and the need for surfactant media may limit its application in biological systems. Very recently, Yin et al. (2014) discovered that a cyanine dye containing 5-(dimethylamino)-naphthalene-sulfonamide unit, displays unique selectivity toward GSH over Cys and Hcy presumably assisted by hydrogen bonding or electrostatic interaction. In another report, Lim et al. communicated a cyanine dye containing a nitroazo aryl ether group exhibited dramatic off–on near-infrared (NIR) fluorescence response toward GSH over Cys/Hcy and was able to target mitochondrial GSH. However, it was also significantly affected by Cys and Hcy (Lim et al., 2014). An interesting publication from Işık et al. (2014) communicated a 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dye containing a crown ether moiety as a modulating group in the *meso* position to facilitate the attacking of GSH for selective GSH sensing, however it was effective in pH of 6.0. Until now, only a few fluorescent probes were documented to target mitochondrial GSH in living cell (Lim et al., 2014, 2011). Herein we report a BODIPY-based turn-on fluorescent probe **1** carrying a *para*-dinitrophenoxy benzyl pyridinium moiety at *meso* position as self-immolative portion which sacrifice itself to generate the fluorophore after initial recognition (Gnaim and Shabat, 2014; Xu et al., 2014) for selective GSH detection over Cys/Hcy on mitochondria (Xiao et al., 2015).

BODIPY fluorescent dyes have found widespread applications because of many advantages, such as high molar absorption coefficients and quantum yields, elevated chemical and photo stability (Loudet and Burgess, 2007). We have rich experience on BODIPY dyes in recent years (Zhao and Carreira, 2005, 2006; Jiang et al., 2012a, 2012b, 2012c, 2012d), and we have been endeavouring to develop thiol-responsive fluorescent probes (Zhang et al., 2015b; Jiang et al., 2012e; Zhang et al., 2014; Shao et al., 2015; Lu et al., 2015). In our continuing interest in the discovery of new thiol-reactive BODIPY-based fluorescent probe, we speculated that once a cleavable self-immolative quinone-methide moiety was linked to a BODIPY dye containing a *meso* pyridine group at 4 position *via* a pyridinium component (Gnaim and Shabat, 2014; Xu et al., 2014), and a thiol-responsive recognition functionality linked to quinone-methide portion, a novel thiol-responsive fluorescent probe may be obtained. Moreover, the pyridinium component utilized may act as both the mitochondrial targeting moiety and electronic sink to turn-off the fluorescence of fluorophore (Xiao et al., 2015). To validate our concept, 2,4-dinitrophenoxy group which was used extensively in the past for selective recognition of H_2S (one of the three gasotransmitters) was adopted and we expected that a putative H_2S selective fluorescent probe might be obtained (Lin et al., 2015; Cao et al., 2012; Huang et al., 2014; Liu et al., 2013; Liu and Feng, 2014).

2. Results and discussion

2.1. Synthesis of probe *1*

Probe **1** was prepared conveniently as shown in Scheme S2. Reacting *p*-Cresol with 2,4-dinitrofluorobenzene resulted in 1-(4-methylphenoxy)- 2,4-dinitrobenzene (**2**) which was converted to 1-[4-(bromomethyl)phenoxy]- 2,4-dinitrobenzene (**3**) by bromination with N-bromosuccinimide (NBS) induced by

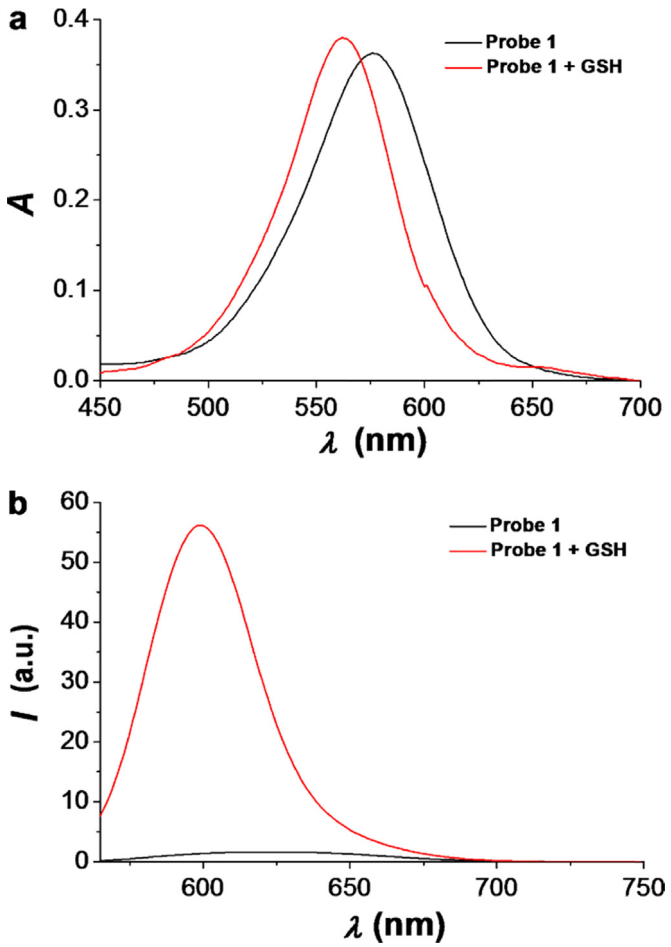

Fig. 1. Absorption (a) and emission spectra (b, λ_{ex}=550 nm) of probe **1** (10 μM, black curve) prior to and after addition of GSH (100 μM, red curve) and incubated for 120 min in DMSO/PBS buffer (1:1, v/v, 10 mM, pH 7.4) at 25 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)