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Discriminating three biothiols by using one fluorescent probe

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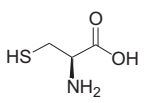
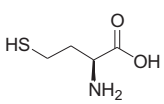
ABSTRACT

A 1,1′-bi-2-naphthol (BINOL) based dialdehyde (*R*)-**1** is found to exhibit selective fluorescent response towards cellular thiols, L-Cys, L-Hcy, and GSH. (*R*)-**1** reacts with L-Trp to form a Schiff base **2** which quenches the emission of L-Trp at λ = 340. Coordination of **2** with Zn(II) leads to greatly enhanced emission at λ = 530 nm. When the *in situ* generated **2** from the combination of the solution of (*R*)-**1** (1.0×10^{-5} M) + Trp (2 equiv) +$Zn(OAc)_2$ (2 equiv) is treated with L-Cys, L-Hcy, and GSH, different fluorescent responses at three emission wavelengths, including 340, 421 and 530 nm, are observed. This allows the *in situ* generated **2** to be used as a ratiometric fluorescent probe to discriminate these three biothiols. NMR study of (*R*)-**1** with D- or L-Cys in the presence of Zn(II) shows that the unique reactivity of Cys provides basis for the selective ratiometric fluorescent response. (*R*)-**1** + Zn(II) also exhibits enantioselective fluorescent response toward D- and L-Cys.

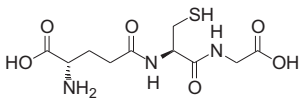
L-Cys, L-Hcy and GSH are intracellular biothiols which are associated with different crucial roles in biological systems [1–3]. A normal level of these biothiols is important in protein synthesis, xenobiotic metabolism, redox homeostasis and combating oxidative stress [4]. Abnormal level results in various diseases (Parkinson's disease, Alzheimer's disease, Cancer, AIDS) and disorders in vital organs (kidneys, liver, heart, brain) [4–12]. GSH being most abundant non-protein biothiol in living cells performs important functions including intracellular redox homeostasis, normalizing oxidative stress and intracellular signal transduction [4,10]. Aberrant level of GSH is a key indicator for risk of cancer, AIDS, liver damage and neural disorder [9–11]. Abnormal level of L-Cys is linked with slowed growth, skin lesion, hair depigmentation, muscle and fat loss, lethargy, cardiovascular, Alzheimer's and Parkinson's diseases [7,13–16]. In the same way abnormalities in the level of Hcy are responsible for skin sclerosis, cardiovascular complications and mental disorders [13,16–18].



L-Cys



L-Hcy



GSH

Need of the time is to regulate optimum level of these intracellular biothiols to reduce the risk of above mentioned chronic diseases and disorders in vital organs. Detection and discrimination of these closely related biothiols from each other and from other amino acids can provide a base to effectively deal with this problem. As these biothiols are closely related to each other, their detection and discrimination becomes a challenging task. Amongst several methods reported to selectively discriminate and measure these biothiols, fluorescent methods appear most promising because of high sensitivity, low detection limit, operational simplicity and fluorescent imaging with in living cells [19–21]. Based on nucleophilicity and high bonding affinity of thiol towards electrophilic substrates, a variety of reaction strategies including Michael addition, cyclization reactions, conjugate additions, cleavage reactions and nucleophilic substitutions have been applied to develop dozens of fluorescent probes capable of selective discrimination of these biothiols [22]. Most of the probes developed so far often show selectivity towards one biothiol, discriminating it from the rest. Only very few examples have appeared to distinguish these three biothiols [23]. Herein, we report an aldehyde-based fluorescent receptor capable of discriminating L-Cys, L-Hcy and GSH from each other.

We carried out the condensation of the 1,1′-bi-2-naphthol (BINOL)-based aldehyde (*R*)-**1** with L-tryptophan (L-Trp) in the presence of base to give the aldimine product **2** (Scheme 1). Fig. 1a gives the fluorescence spectra of (*R*)-**1** and Trp. It shows that L-Trp is strongly fluorescent at λ = 340 nm in methanol solution and (*R*)-**1** is almost nonfluorescent. When Trp condenses with

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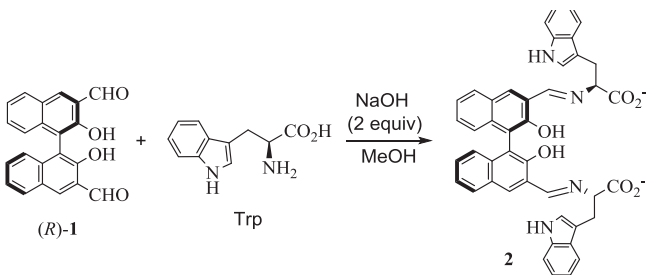

Scheme 1. Reaction of (*R*)-**1** with l-Trp to Form Compound **2**.

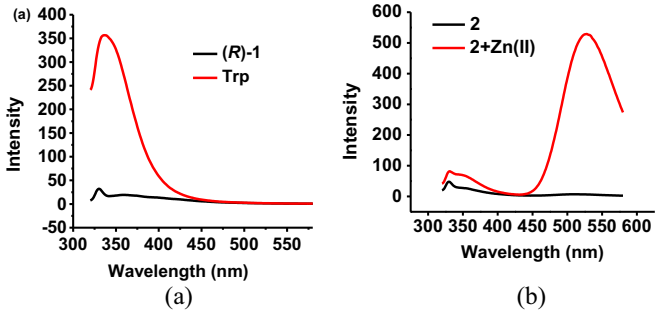

Fig. 1. Fluorescence spectra of (a) (*R*)-**1**, Trp, and (b) **2** with or without $Zn(OAc)_2$ (2 equiv) (Concentration: $(1.0 \times 10^{-5}$ M in methanol. λ_{exc} = 300 nm, slit = 5/5 nm).

(*R*)-**1**, the resulting product **2** gives little fluorescence (Fig. 1b). Apparently, this BINOL-based Schiff base has efficiently quenched the fluorescence of L-Trp. When compound **2** was treated with Zn $(OAc)_2$ (2 equiv), a large fluorescence enhancement at λ = 530 nm is observed (Fig. 1b) [24]. As demonstrated before, coordination of Zn(II) with the BINOL-based Schiff bases can greatly enhance their fluorescence by suppressing the excited state imine isomerization as well as the excited state proton transfer between the hydroxyl groups and the imine nitrogen [24]. The emission at 340 nm due to the L-Trp unit is still very low in this Zn(II) complex. The weak emissions at 340 nm in both **2** and the corresponding **2** + Zn(II) complex indicate an efficient energy transfer between the Trp units and the BINOL-aldimine unit which quenches the fluorescence of the Trp unit.

We have studied the fluorescence response of the *in-situ* generated **2** toward the three biothiols, L-Cys, L-Hcy and GSH. As shown in Fig. 2, when the solution of (*R*)-**1** $(1.0 \times 10^{-5}$ M) + Trp (2 equiv) +$Zn(OAc)_2$ (2 equiv) is treated with GSH, the fluorescence of **2** at λ_2 = 530 nm disappears with only the emission of the free Trp at λ_1 = 340 nm being observed. It is known that GSH can form stable Zn(II) complexes [25]. Hence, it is proposed that coordination of GSH with Zn(II) might have converted the **2** + Zn(II) complex back to **2** and free Trp, which diminishes the emission at λ_2. When the *in-situ* generated **2** is treated with L-Cys, a new emission signal at λ_3 = 421 nm is observed together with the emissions at λ_1 and λ_2, but no new signal with Hcy except intensity changes at λ_1 and λ_2. Previously, it was reported that a monoaldehyde analogue of (*R*)-**1** reacts with L-Cys and L-Hcy to form the corresponding thioazolidine (5 membered ring) and thiazinane (6 membered ring) respectively [26]. It is thus proposed that the thiazolidine product formed from the reaction of (*R*)-**1** with L-Cys should contribute to the new emission at λ_3 = 421 nm in the presence of Zn(II), but the thiazinane product from the reaction of (*R*)-**1** with Hcy does not show significant new emission in the presence of Zn(II) under the same conditions. Thus, the fluorescent probe based on **2** exhibits distinctively different responses toward these three biothiols.

We then studied the effect of the biothiol concentrations on the fluorescence response of the *in-situ* generated **2** and plotted the fluorescence intensity ratios, I_{421}/I_{340}, I_{530}/I_{421} and I_{340}/I_{530}, versus the concentrations in Fig. 3a-c respectively. Fig. 3a shows that L-Cys greatly increases I_{421}/I_{340} between 0 and 4 equiv but L-Hcy and GSH do not significantly change this ratio in the same concentration range. That is, using I_{421}/I_{340} allows selective detection of Cys. Fig. 3b shows that L-Hcy greatly increases I_{530}/I_{421} from 0 to 1 equiv which remains to be high at 5 equiv, but L-Cys and GSH show decreasing in this fluorescence ratio. That is, using I_{530}/I_{421} can selectively detect L-Hcy. Fig. 3c shows that GSH greatly increases I_{340}/I_{530}, but L-Cys and L-Hcy only give small changes for the fluorescence ratio. That is, using I_{340}/I_{530} can selectively recognize GSH.

In order to gain further understanding on the new emission signal at λ_3 = 421 nm when **2** is treated with Cys, we studied the reaction of (*R*)-**1** with both L- and D-Cys in the presence of Zn(II). As shown in Fig. 4, when (*R*)-**1** (1.0 x 10^{-5} M) was treated with Zn $(OAc)_2$ (2 equiv) and L- or D-Cys (5 equiv), two emission signals at λ = 421 and 530 nm were observed. The two enantiomers of Cys give different intensity at these two wavelengths. That is, the fluorescence response of (*R*)-**1** toward Cys is enantioselective. We also studied the fluorescence responses of (*S*)-**1**, the enantiomer of (*R*)-**1**, with L- and D-Cys which show mirror image relation with those

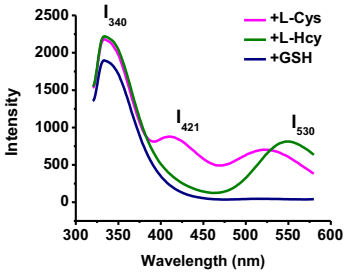

Fig. 2. Fluorescence spectra of (*R*)-**1** $(1.0 \times 10^{-5}$ M) + Trp (2 equiv) + $Zn(OAc)_2$ (2 equiv) with L-Cys, L-Hcy, GSH $(1.0 \times 10^{-5}$ M in methanol). (λ_{exc} = 300 nm, slit = 5/5 nm).

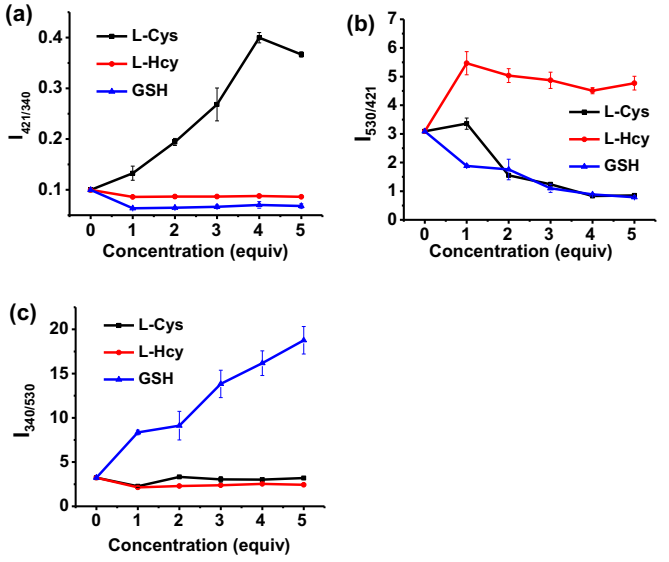

Fig. 3. Fluorescence response of (*R*)-**1** $(1.0 \times 10^{-5}$ M) + Trp (2 equiv) + $Zn(OAc)_2$ (2 equiv) versus the concentrations of L-Cys, L-Hcy, GSH $(1.0 \times 10^{-5}$ M in methanol) at (a) $I_{421/340}$ (b) $I_{530/421}$ (c) $I_{340/530}$ (λexc = 300 nm, slit = 5/5 nm).