Tetrahedron Letters 49 (2008) 4879–4881

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Highly selective fluorescent sensor for homocysteine and cysteine

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ARTICLE INFO

Article history:
Received 29 April 2008
Revised 29 May 2008
Accepted 2 June 2008
Available online 5 June 2008

ABSTRACT

A new fluorescent sensor (**1**) based on a coumarin was synthesized for the selective detection of homocysteine (Hcy) and cysteine (Cys). The chemosensor has shown a selective response to Hcy or Cys over other various amino acids via a ring formation of thiazinane or thiazolidine. When Hcy or Cys was added, the fluorescent intensity of **1** was completely quenched through a photo-induced electron transfer with the sensitivity of sub-millimolar concentration.

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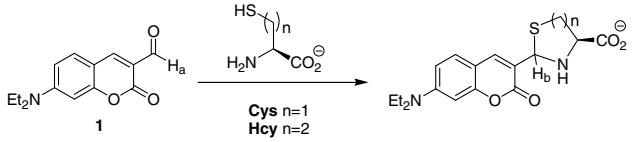
For their participation in the process of reversible redox reactions, homocysteine (Hcy), cysteine (Cys), and glutathione (GSH) play a crucial role in maintaining biological redox homeostasis.[1] Hcy is a risk factor for Alzheimer's[2] and cardiovascular diseases[3] and Cys is also related to slowed growth, liver damage, hair depigmentation, lethargy, muscle and fat loss, skin lesions, and weakness.[4] Therefore, selective detection of the intracellular thiols (Hcy, Cys, and GSH) in human body is of growing importance. HPLC combined with Ellman's reagent (DTNB) was widely used as a traditional method for detecting the biological thiols.[5] Such an analytical method is, however, inconvenient to operate and not suitable for in vivo imaging in cells. Recent advanced technology in fluorescence probes has shown that fluorescent probes could be applied to the selective and sensitive detection of the biological thiols because they are so simple to operate and very fast in their responses.

A variety of sensors for biologically important thiols such as Cys, Hcy, and GSH were developed as colorimetric,[6] fluorescent,[7a–e] and phosphorescent[7f] sensors, and applied to the intracellular thiol bioimaging in cells.[7c–e] Tedious synthetic routes to the chemosensors, however, and low yields made them difficult to their practical applications. Herein, we describe a coumarin-based fluorescent sensor (**1**) with aldehyde functionality, which is readily available in multi-gram scale through three-step synthetic routes and highly sensitive to Hcy and Cys. The sensor can form a rapid 6- or 5-membered ring with 1,3- or 1,2-aminothiols while other biothiols like GSH cannot form the ring, thus can discriminate Hcy and Cys from the other biological thiols.

We have studied on the reactivity of an aldehyde functional group activated by the intramolecular hydrogen bond of a phenol OH[8] and its application to the enantioselective recognition of amino acids or amino alcohols.[9] We assumed **1** could also be useful as a fluorescent chemosensor for Hcy or Cys owing to the reactivity of a coumarin aldehyde moiety (Scheme 1).



Scheme 1. Reaction scheme of **1** with Hcy or Cys.

Sensor **1** was synthesized from 4-(diethylamino) salicylaldehyde in three steps according to the literature procedure.[10] The sensor has a 'push-and-pull' type of coumarin fluorophore. Diethylamino group of **1** is pushing an electron, while aldehyde and carbonyl groups are pulling the π-electrons to afford a strong fluorescence emission at λ_{max} = 488 nm. When adding Hcy to 10 μM of **1** in ethanol, the fluorescence intensities of **1** decreased, reached a saturation point at 500 equiv guests with a hypsochromic shift to λ_{max} = 470 nm, and completely quenched at [G]/[**1**] ⩾ 500.

To analyze the reaction pathway and obtain a clue to the fluorescence quenching mechanism of **1**, we monitored ^{1}H NMR spectra of **1** in the absence and presence of Hcy (Fig. 1). Upon addition of Hcy (5.0 equiv) into **1** in DMSO-d_6, the aldehyde proton signal of

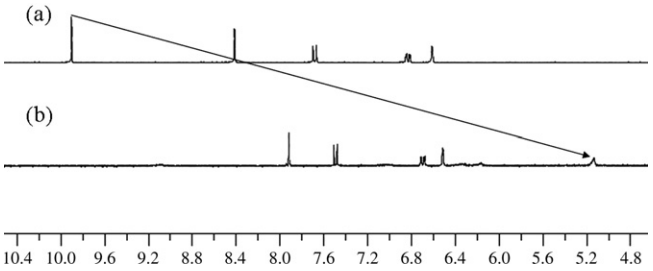


Figure 1. ^{1}H NMR spectral change of **1** (10 mM in DMSO-d_6) in the absence (a) and presence (b) of 5.0 equiv Hcy.

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0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.06.003

1 at 9.90 ppm (H_a) disappeared with the concomitant appearance of a new peak at 5.13 ppm, which could be assigned to a methine proton (H_b) of the transformed thiazinane ring.[6a,b,7b] A similar thiazolidine ring formation was also observed upon addition of Cys. In the contrary to the amino acids with a thiol side chain, amino acids without a thiol group such as leucine and valine were observed to form imines (Fig. S5). The thiazinane/thiazolidine ring formation between **1** and Hcy/Cys seemed to be a critical factor for the fluorescence quenching of **1**.

To see the selectivity of **1** for other amino acids, we measured the fluorescence intensity changes after each addition of 500 equiv of amino acids with a neutral side chain (glycine: Gly, alanine: Ala, valine: Val, leucine: Leu, isoleucine: Ile, and proline: Pro), a polar group (serine: Ser, threonine: Thr) including dithiothreitol (DTT) and glutathione (GSH), an acidic (glutamic acid: Glu) and a basic (arginine: Arg, lysine: Lys) side chain. All the amino acids except Hcy and Cys did not induce any significant decreases in the fluorescence intensity of **1** (Fig. 2).

Fluorescence sensitivity, F_o/F, upon addition of 500 equiv of analytes was measured to be 54, 43, and 1.5 for Hcy, Cys, and GSH, respectively. The other analytes showed F_o/F values less than 1. High selectivity of sensor **1** was observed for Hcy and Cys, with a slight preference toward Hcy. Additionally, competitive binding experiments of the other amino acids against **1**–Hcy complex were performed under a mixture of **1**–Hcy and 5-fold other analytes, which did not show any significant changes in the fluorescent intensities (Fig. S10). These experiments suggested that **1** displayed a high selectivity for Hcy.

To determine affinities of **1** toward Hcy and Cys, fluorescence titrations were carried out in ethanol.[11] Fluorescence spectra of **1** were monitored after each addition of Hcy to 10 μM of **1**. Upon addition of Hcy, the fluorescence emission intensity of **1** decreased and eventually faded out with a saturation point around 500 equiv of Hcy (Fig. 3). Job's plot indicated the binding stoichiometry to be 1:1 between **1** and Hcy (Fig. S11). The dissociation constant (K_d) of **1**–Hcy was calculated to be $K_d = 3.23\ (\pm 0.20) \times 10^{-4}$ M (Fig. S8) on the basis of one-to-one binding stoichiometry and a reversible host–guest binding mode.[12] Addition of Cys to **1** also induced a fluorescence decrease with $K_d = 1.54\ (\pm 0.10) \times 10^{-3}$ M (Fig. S9).

In order for the naked-eye detection of Hcy and Cys, fluorescence intensity was monitored after each addition of analytes under a fluorescence microscope equipped with a WIB filter (excitation wavelength range: 430–450 nm, emission filter at 500 nm). Most of the amino acids bearing a neutral (Gly, Ala, Val, Leu, Ile, Pro) and a polar residue (Ser, Thr, Glu, Arg) did not induce any detectable fluorescence changes even after addition of 500 equiv of analytes. Complete quenching of the fluorescence intensity of **1**, however, was apparently observed upon the addition of Hcy or Cys (Fig. 4). It is noticeable that the addition of Lys with a primary amine increased the intensity. Although the excitation and emission wavelengths of the microscope were not perfectly matched with the photo-luminescence (PL) property of **1**, we could observe a consistent result with the fluorospectrometer. Sensor **1** exhibited selective fluorescence responses to Hcy and Cys.

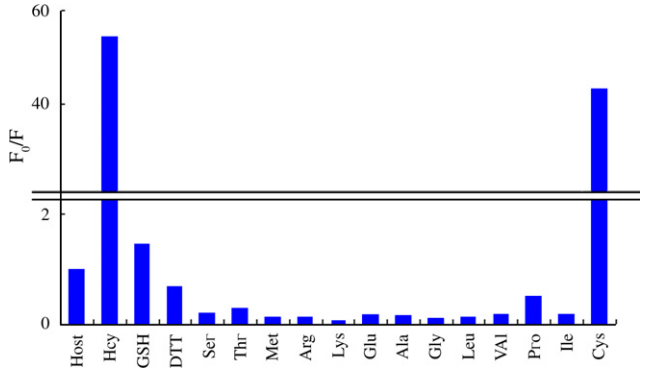


Figure 2. Fluorescence intensity at $\lambda_{em} = 488$ nm ($\lambda_{ex} = 448$ nm) of **1** (10 μM in EtOH) upon each addition of 500 equiv of various analytes, where F_o is a fluorescence intensity of **1** alone.

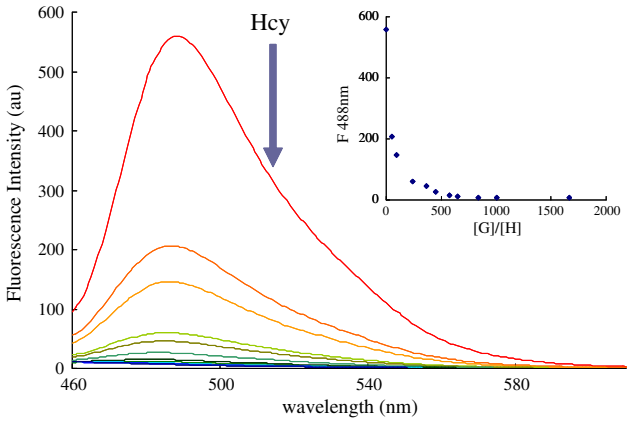


Figure 3. Fluorescence titration spectra of **1** upon addition of Hcy. Inset: $F_{488\ nm}$ versus [Hcy]/[**1**]·[**1**] = 10 μM in EtOH ($\lambda_{ex} = 448$ nm).

On the basis of the observed NMR and PL data, we propose a fluorescence mechanism of **1**, quenched upon the addition of Hcy or Cys (Scheme 2). When Hcy or Cys is added to **1**, six or five-membered ring can be rapidly formed. This thiazinane/thiazolidine has a lone pair electron at nitrogen atom near the fluorophore, which can block or quench the fluorescence of **1** by a photo-induced electron transfer (PET) mechanism.[13] The PET mechanism was confirmed by addition of trifluoroacetic acid (TFA) to **1**-Hcy complex. Upon addition of TFA (ca. 4 equiv), fluorescence intensity of **1**-Hcy complex increased 6 times compared to the complex by plausible protonation to the nitrogen atom (Fig. S12).

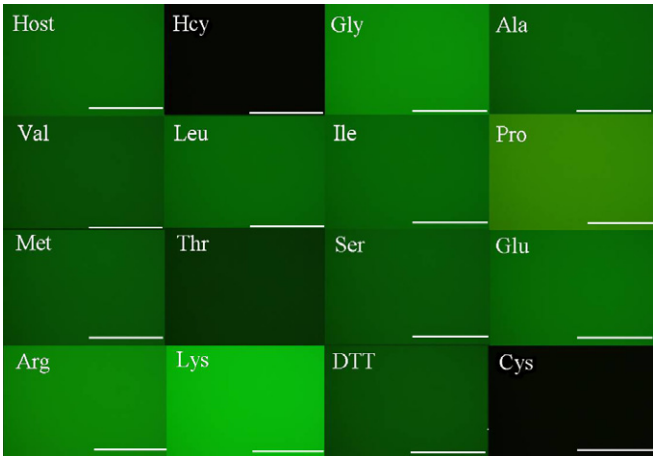
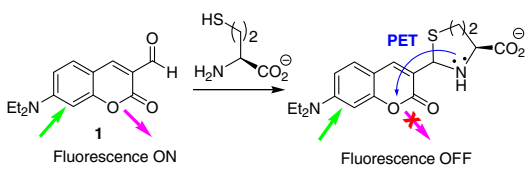


Figure 4. Fluorescence microscope images of **1** in the presence of 500 equiv of guests in EtOH. λ_{ex}: 430–450 nm, λ_{em}: 500 nm. (Scale bar: 200 μm).



Scheme 2. Proposed fluorescence mechanism of **1** upon addition of Hcy.

In summary, we have developed a chemosensor (**1**) based on a coumarin with an aldehyde functionality. The sensor selectively reacted with Hcy or Cys via a thiazinane or thiazolidine ring in a polar protic solvent. Its fluorescence was faded out due to a PET from a neighboring nitrogen atom of the thiazinane/thiazolidine ring. Sensor **1** showed a high selectivity and affinity toward Hcy or Cys over other amino acids. This selectivity could be easily observed in a naked eye using a fluorescence microscope. Further study on the Hcy/Cys sensor via the thiazinane/thiazolidine formation in water is on the progress.

Acknowledgements

Financial supports of this work from the Korean Science and Engineering Foundation for the Scientists Exchange Program (Grant F03-2008-000-10027-0) and 2007 Kyonggi University Specialization Program are gratefully acknowledged.

Supplementary data

Experimental data including NMR/mass for **1**, fluorescence titration and competition graphs, and Job's plot for **1**–Hcy (PDF). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.003.

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11. The ring formation was too slow ($\tau_{1/2} \approx 8$ h) in aqueous HEPES buffer (50 mM, pH 7.4).
12. When D_2O was added to **1**-Hcy, the thiazinane was reverted to free **1** and Hcy. Therefore, the reaction was assumed to be reversible and we called **1** as a sensor.
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