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Selective detection of cysteine over homocysteine and glutathione by a bis(bromoacetyl)fluorescein probe

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ABSTRACT

A bromoacetyl functionalized fluorescein chemodosimeter (**1**) was utilized as a fluorescent probe for cysteine. The probe showed a selective and sensitive response to cysteine over homocysteine and glutathione in aqueous buffer through a rapid cyclization reaction. When cysteine was added, a fast fluorescence turn-on change of **1** was observed, thus allowing cysteine to be discriminated from other biothiols and micromolar concentrations of cysteine to be detected by the naked eye.

A myriad of bioprocesses are related to biothiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH).[1] Cys and Hcy are involved in cellular growth, while GSH plays an important role in redox homeostasis.[2] Alterations in the biothiols are implicated in cancer and AIDS.[3] Therefore it is of growing interest to develop molecular probes for biothiols. The discrimination of biothiols with a similar functionality, however, is a difficult goal to achieve. Recently, rationally designed fluorescent probes for biothiols have been of considerable interest, and some probes showing a selective response toward Cys,[4] Hcy,[5] or GSH[6] were designed by employing smart strategies such as cyclization kinetics, thermodynamic stability, or micelle formation, respectively. However, the mechanistic rationale for their specificity was not clearly uncovered. In this letter, we report a selective fluorescent probe for Cys over Hcy and GSH by introducing a kinetic and thermodynamic discrimination strategy during the probe-cysteine cyclization step.

Probe **1** was prepared by treating fluorescein with 2 equiv of bromoacetylbromide in the presence of excess base. Chromatographic purification of the reaction mixture gave the desired product (**1**) in 48% yield (Scheme 1).[7]

The chemical reaction of **1** with Cys was monitored by UV–vis spectroscopy. Upon the addition of Cys (5 equiv) to **1** (20 μM) in DMSO/HEPES (1:1, v/v, 0.10 M, pH 7.4), the absorption maxima at λ 500 nm gradually increased and reached the saturation point within 10 min, whose kinetic analysis gave the second-order rate constant of $k_2 = 83\ M^{-1}s^{-1}$ at 25 °C (Fig. 1).

We investigated whether other biothiols such as Hcy and GSH could mediate the transformation. The reaction of **1** into fluorescein was also monitored in the absence or presence of 5 equiv of Hcy/GSH by UV–vis spectroscopy. Probe **1** did not induce any significant changes by itself, which meant that the background hydrolysis of **1** was suppressed under these reaction conditions. Cys showed such rapid saturation kinetics with **1** ($k_2 = 83\ M^{-1}s^{-1}$) that the reaction was complete within 10 min, whereas Hcy and GSH exhibited much slower reaction rates with $k_2 = 19\ M^{-1}s^{-1}$ and $6.9\ M^{-1}s^{-1}$, respectively (Fig. 2).

To further evaluate the selectivity of **1** for Cys against other natural amino acids (AAs), we measured the fluorescence intensity of **1** in the presence of various AAs (Pro, Ala, Val, Phe, His, Ser, Asn, Asp, Lys, and Arg) as well as Cys (100 μM) (Fig. 3A). The fluorescence maximum intensity of **1** at 525 nm was dramatically enhanced ($F/F_0 = 63$) by Cys (5 equiv), whereas no significant fluorescence changes were induced ($F/F_0 < 1.3$) by other AAs with neutral, acid, basic side chains. A competitive assay also showed quite consistent evidence that **1** was rapidly transformed only by Cys. The fluorescence intensity of **1** and the other AAs was restored as much as that of **1**-Cys by the addition of Cys to their mixture (Fig. 3B).

The prominent optical changes of **1** were also observable by the naked eye. Upon the addition of Cys to **1** (20 μM) in 50% DMSO/HEPES buffer (0.10 M, pH 7.4), the initial colorless solution turned

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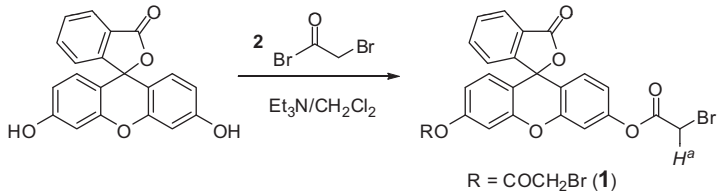

Scheme 1. Synthesis of **1**.

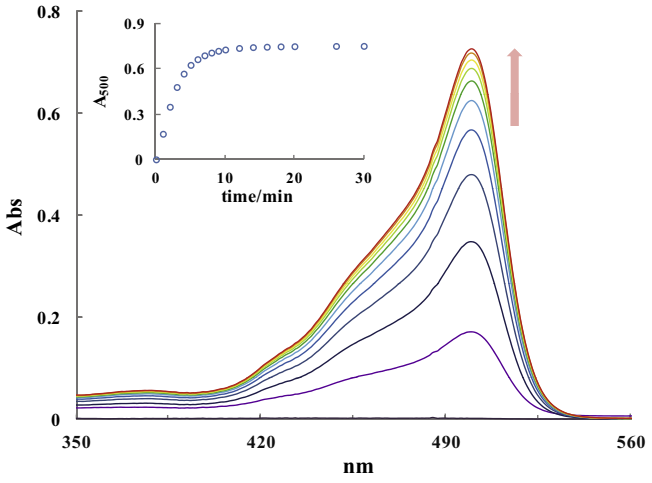

Figure 1. Time-dependent UV–vis spectra of **1** (20 μM) upon the addition of Cys (5 equiv) in 50% DMSO/HEPES (0.10 M, pH 7.4). Inset: its kinetics.

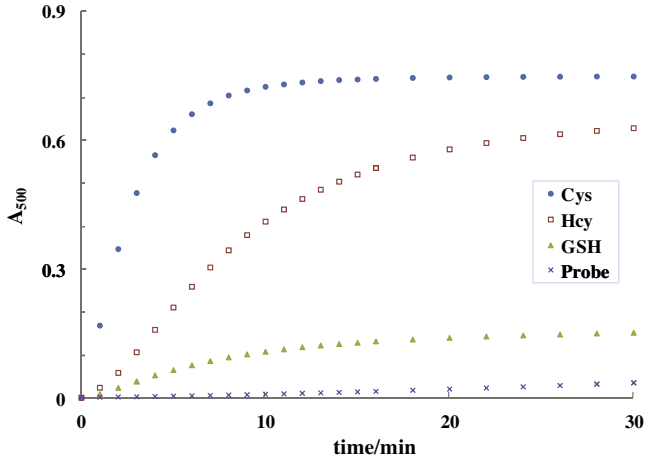

Figure 2. UV–vis kinetics of **1** (20 μM) in the presence of various biothiols (5 equiv) in 50% DMSO/HEPES (0.10 M, pH 7.4).

green. The resulting complex (**1**-Cys) exhibited a strong fluorescence, while the other AAs did not elicit any observable photophysical changes under a portable UV spectroscope (Fig. 4).

The stoichiometry between **1** and Cys was determined by a Job's plot, which showed a 1:2 reaction ratio between probe **1** and Cys (Supplementary Fig. S3).

The detection limit of Cys was measured by a fluorescence titration experiment. The standard deviation of the emission intensities of **1** without Cys was obtained as $\sigma = 0.453$ ($n = 5$). The fluorescence intensities at F_{525nm} were measured by the incremental addition of Cys to **1** (20 μM in 50% aqueous DMSO), the slope of which gave $m = 0.039$ (Supplementary Fig. S4). From the fluorescence experiment of **1**, we found that the limit of detection ($3\sigma/m$) of Cys was 35 μM.[8]

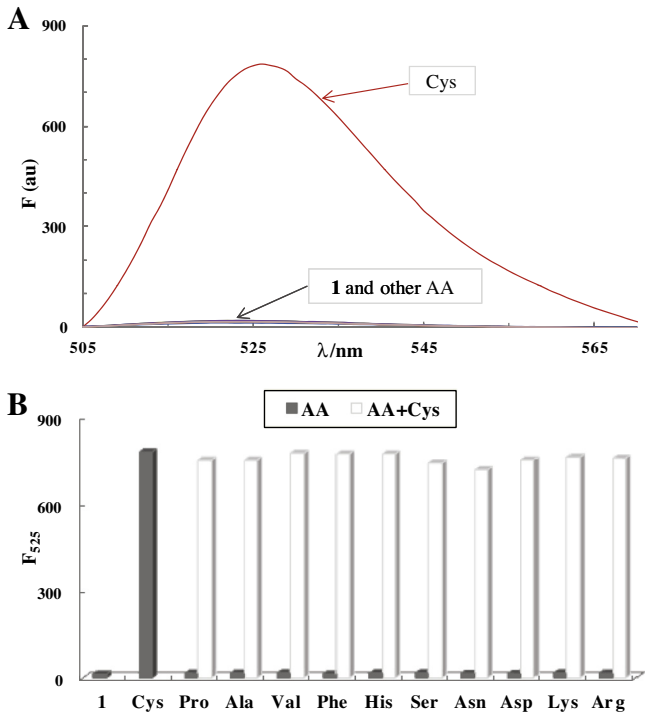

Figure 3. Fluorescence spectra (A) and competitive assay (B) of **1** (20 μM, λ_{ex} 505 nm) in the presence of various AAs (5 equiv) in 50% DMSO/HEPES (0.10 M, pH 7.4).

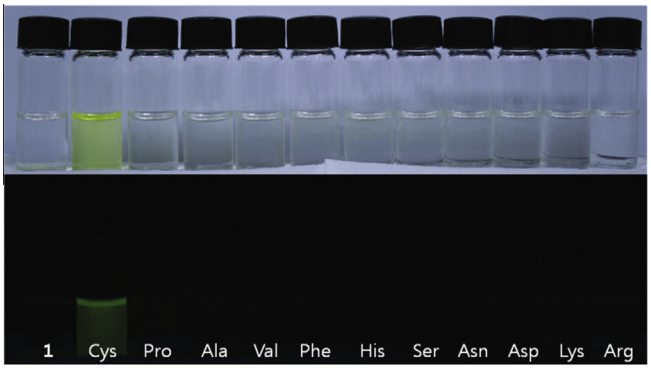

Figure 4. Fluorescence images of **1** (20 μM) upon the addition of various AAs in DMSO/HEPES (1:1, 0.10 M, pH 7.4).

In order to gain insight into the reaction mechanism, we investigated the ^1H NMR spectra after adding Cys to **1** and compared them with that of **1** itself. Upon the addition of Cys to **1**, a new set of ^1H NMR peaks appeared and the reaction was complete with