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Bromoacetylfluorescein monoaldehyde as a fluorescence turn-on probe for cysteine over homocysteine and glutathione

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ABSTRACT

A novel fluorescent probe possessing the two different functional groups of bromoacetyl ester and aldehyde functionality was designed as a selective chemodosimeter for cysteine (Cys). The initially nonfluorescent bromoacetyl-fluorescein monoaldehyde (**3**) was rapidly transformed into a strongly fluorescent molecule through the first nucleophilic substitution followed by the subsequent ester bond cleavage reactions by one Cys and the second oxazolidine formation reaction with another Cys. The molecular probe exhibited a rapid response toward Cys over homocysteine or glutathione owing to the favorable kinetic processes with Cys, of which property was successfully applied for live cell imaging with tunicamycin, an inducer of cytosolic Cys.

1. Introduction

A myriad of biological processes are closely related to cellular thiols such as cysteine (Cys), homocysteine (Hcy), and γ-glutamylcysteinylglycine (GSH). Cys and Hcy are involved in cellular growth [1], and GSH in redox homeostasis [2]. Alteration in the cellular thiols is also implicated in cancer, neurodegenerative disease, and AIDS [3]. Therefore, selective detection of the biothiols is of growing importance. The discrimination of these biothiols, however, is a difficult task to achieve due to their similarity in the functional groups. Recently, rationally designed fluorescent probes for biothiols have attracted considerable interest, and some probes exhibited a selective response towards Cys [4], Hcy [5], or GSH [6] by using smart strategies such as cyclization kinetics, thermodynamic stability, or micelle-catalyzed reaction, respectively. However, the mechanistic rationale for their specificity was not clearly understood. In this paper, we report a selective fluorescent probe (**3**) for Cys over Hcy and GSH by introducing a kinetic discrimination strategy and uncover the underlying mechanism.

Recently, we found that the introduction of a bromoacetyl group to fluorescein (**1**) could be utilized for the discrimination of Cys from the other structurally similar biothiols (Hcy, GSH) due to the thermodynamically stable and kinetically rapid formation of six-member lactam ring in aqueous solvent [7]. We also observed that the introduction of a favorable hydrogen bonding donor unit to the ortho position of a carbonyl group could accelerate the reaction rate of **2** toward Hcy up to 40-fold compared to a probe without the hydrogen bond [8]. We expected the combination of these strategies to allow the two different functional groups of bromoacetyl and ortho-hydroxyl formyl functionality to enhance both the selectivity and the reaction rate of **3** towards Cys (Scheme 1). Herein, we report a fluorescent probe (**3**) that displayed a selective and rapid detection of Cys over Hcy and GSH in aqueous buffer.

2. Materials and methods

2.1. General

All non-aqueous reactions were performed in flame-dried glassware under a positive pressure of nitrogen with all moisture excluded from reagents and glassware using standard techniques for manipulating air-sensitive compounds. Anhydrous solvents were obtained using standard drying techniques. Reactions were monitored by analytical thin-layer chromatography (TLC) on precoated, glass-backed silica gel plates. Visualization of the developed chromatogram was performed by UV absorbance. Flash chromatography was performed on 230–400 mesh silica gel with the indicated solvent. Nuclear magnetic resonance (NMR) spectra were recorded either on Varian Gemini 200 (200 MHz) or Varian Gemini 400 (400 MHz) spectrometers. Chemical shifts for ^{1}H NMR spectra are

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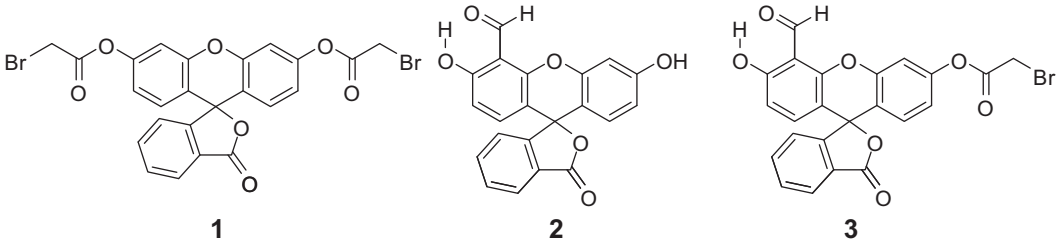

Scheme 1. Several cysteine-selective probes.

recorded in parts per million from tetramethylsilane with the solvent resonance as the internal standard (DMSO-d_6 δ 2.50 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, and br = broad), coupling constant in Hz, and integration. Chemical shifts for ^{13}C NMR spectra are recorded in parts per million from tetramethylsilane using the central peak of the solvent resonance as the internal standard (DMSO-d_6 δ 39.52 ppm). All ^{13}C NMR spectra were obtained with complete proton decoupling. All fluorescence and UV–vis absorption spectra were recorded in FP 6500 fluorescence and Agilent 8453 absorption spectrometers, respectively. Mass spectra were recorded on a G6401A MS-spectrometer. All experiments were carried out with commercially available reagents and solvents, which were used without further purification, unless otherwise stated.

2.2. Preparation of *1*

Dibromoacetyl-fluorescein (**1**) was synthesized according to the reported method [7]. Fluorescein (166 mg, 0.50 mmol) was dissolved in 3 mL of anhydrous CH_2Cl_2. Bromoacetyl bromide (300 mg, 1.50 mmol) and Et_3N (100 mg, 1.00 mmol) were added dropwise at 0 °C. The mixture was stirred from 0 °C to room temperature for 3 h. All the volatiles were removed under the reduced pressure and the residue was purified by column chromatography using CH_2Cl_2 as an eluent to afford the desired probe **1** as a light yellow solid (140 mg, yield 48%), whose spectral data were in good accordance with the previously reported data.

2.3. Preparation of *2*

The fluorescein monoaldehyde (**2**) was synthesized according to the reported method [8,9]. Fluorescein (1.0 g, 3.0 mmol), 5 g of NaOH solution (50% in water) and 2 mL of MeOH were placed in a 50 mL flask. Then 3 mL of $CHCl_3$ was carefully added while the reaction temperature was maintained at 55 °C. The reaction mixture was stirred overnight. After monitoring the completion of reaction on TLC, all the volatiles were removed under the reduced pressure. The residue was purified by column chromatography using CH_2Cl_2/MeOH (v/v 10:1, R_f = 0.40) to afford the desired monoaldehyde as a light yellow solid (848 mg, yield 26%), whose spectral data were in good accordance with the previously reported data.

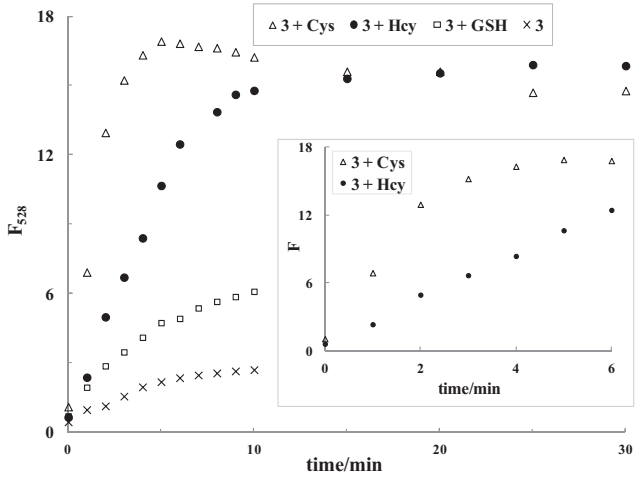

Fig. 2. Fluorescence kinetics of **3** (20 μM) upon the addition of biothiols in DMSO/HEPES buffer (6:4, v/v, 0.10 M, pH 7.4, 25 °C).

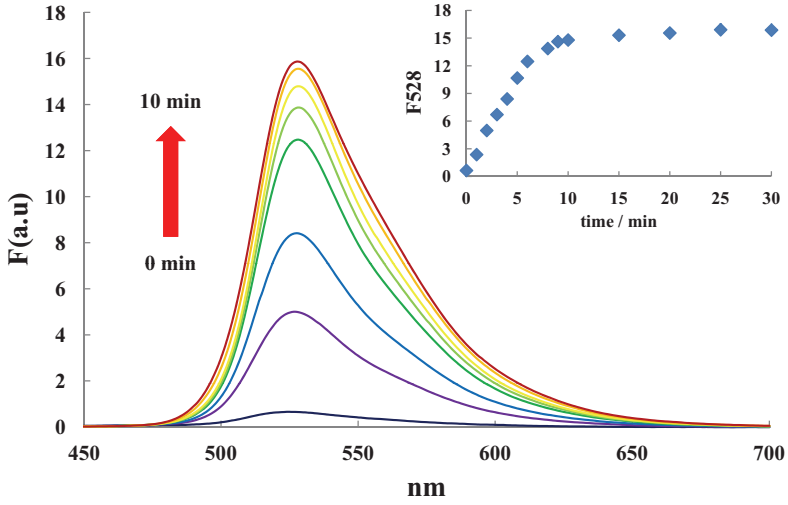

Fig. 1. Time-dependent fluorescence spectral changes of **3** (20 μM) with Cys (10 equiv.) in DMSO/HEPES buffer (6:4, v/v, 0.10 M, pH 7.4, 25 °C). Inset: its kinetics.