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A turn-on green fluorescent thiol probe based on the 1,2-addition reaction and its application for bioimaging

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ABSTRACT

The nucleophilic nature of thiols is used to develop a novel, sensitive, and highly effective sensor. In this work, we developed a new fluorescent probe for thiols based on fluorescein and maleic anhydride. Among the tested amino acid, only GSH, Cys, Hcy, could turn on the fluorescence emission suggesting that the system was a highly selective sensor for thiols. As a typical biological thiol, GSH was used for further examining the fluorescence response of probe. It is found that probe can detect GSH quantitatively with a detection limit as low as 0.026 μM in aqueous/DMSO solution. Moreover its potential for biological applications was confirmed by employing it for fluorescence imaging of thiol in living cells.

1. Introduction

Intracellular thiols such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) play many crucial roles in physiological systems [1–4]. For example, glutathione (GSH) is one of the most important thiolated biomolecules, playing a crucial role in mammalian and eukaryotic cells. It is the main non-protein biothiol found at high concentrations in the intracellular environment to protect cellular components from being damaged by reactive oxygen species (ROS) and toxins [5]. When cells are under oxidative stress, there exists an imbalance between GSH and its oxidized form, glutathione disulfide (GSSG). The latter then rapidly converts into GSH under the action of GSH reductase, increasing of the ratio of GSH to GSSG and relieving the oxidative stress of cells. The alteration of the intracellular redox status (ratio of GSH to GSSG) is associated with numerous clinical diseases [6–9], suggesting that intracellular GSH could be used as an important clinical biomarker [10–13]. Cys and Hcy are essential biological molecules required for the growth of cells and tissues in living systems. A deficiency of cysteine causes various health problems such as retarded growth, hair depigmentation, lethargy, liver damage, muscle and fat loss, and skin lesions [14]. An elevated level of Hcy in human plasma is a risk factor for Alzheimer's disease [2], cardiovascular disease [15], neural tube defect, inflammatory bowel disease, and osteoporosis [16]. Therefore, the detection and recognition of thiol-containion molecules in biological samples are of great importance. To date, several detection methods for thiols have been developed, such as HPLC [17], capillary electrophoresis [18], and UV–Vis detection/colorimetric assays [19–25]. Although these methods are useful to monitor thiols in vitro, only a few methods have been applied for intracellular detection due to their limitations to in vivo studies. Fluorescent methods are more desirable since they are simple, sensitive and efficient [26–29]. In the past few years, various fluorescent probes for thiols based on different mechanisms have been exploited [30–33], great attention has been paid to fluorescent probes for biothiol detecting or imaging owing to the apparent advantages of fluorescent probes over other methods in virtue of sensitivity and convenience. Among these probes, fluorophores attached with a maleimide unit as a bind were also used to identify thiols by 1,2-Michael addition [34–36], and fluorescein is usually used as a fluorophore [37–43]. Based on these issues, we synthesized a new thiol-reactive fluorescent probe containing fluorescein fluorophore and anhydride binding group (Scheme 1). The investigation showed that the fluorescence of this probe increased after reaction with thiol affording a 24-fold intensity increase, which can be used the detection of thiol. The result has a certain significance for further developing new chemosensors based on anhydride.

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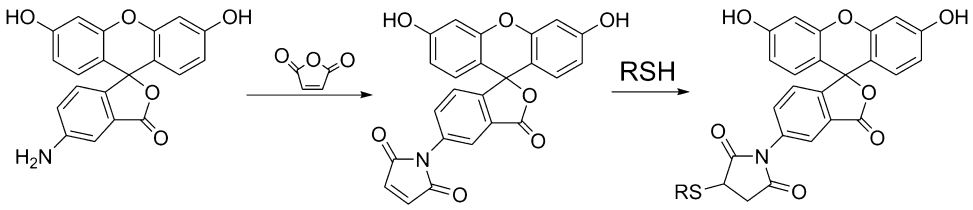

Scheme 1. Synthesis of probe and detection mechanism of the probe to thiol.

2. Experimental

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma–Aldrich (St. Louis, MO). Probe was synthesized using a modification of a literature method [44]. Sodium hydroxide solution (0.1 M) was added to aqueous HEPES (10 mM) to adjust the pH to 7.0. Reagents with analytical grades and demineralized water were used for preparing the solutions. Amino acids were purchased from Shanghai Experiment Reagent Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–Vis) spectra were recorded on a Cary 50 Bio UV–Vis spectrophotometer. Fluorescence spectra were measured on Cary Eclipse fluorescence spectrophotometer. A PO-120 quartz cuvette (10 mm) was purchased from Shanhai Huamei Experiment Instrrument Plants, China. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 MHz and 75 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). ESI was measured with an LTQ-MS (Thermo) instrument. The ability of probe reacting with thiol in the living cells was also evaluated by laser confocal fluorescence imaging using an Olympus FV1000 laser scanning microscope.

2.3. Preparation of compounds

The fluorescent probe, N-(5-fluoresceinyl)maleimide required for these studies was synthesized as shown in Scheme 1. Fluorescein (2.5 g, 7.75 mM) and 3 mL of MeOH are placed in a 100 mL three-neck round-bottom flask. Next, 10 g of 50% NaOH solution, 2.42 mL of $CHCl_3$, and maleic anhydride (2.94 g 0.03 mol) are carefully added while the reaction temperature is maintained at 55 °C. The mixture is stirred at this temperature for 8 h. After cooling, the mixture is acidified with 10 M H_2SO_4. The product precipitates. The solid is filtered and dried in vacuum overnight. Chromatography on silica gel (15:85 EtOAc:DCM) yields a light yellow solid. Yield: 49%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.15 (d, 2H, J = 16.48), 7.99 (s, 1H), 7.78 (d, 2H, J = 8.07 Hz), 7.42 (d, 2H, J = 8.19 Hz), 7.28 (s, 2H), 6.69–6.50 (m, 4H, J = 56.90); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.0, 167.4, 159.0, 151.2, 150.5, 134.3, 132.9, 128.5, 126.2, 124.1, 121.4, 112.2, 108.6, 101.7, 82.8; ESI-MS (m/z): [probe + H]$^+$ calcd for $C_{24}H_{14}NO_7$ 428.08 Found 428.50, [probe + Na]$^+$ Calcd for $C_{24}H_{13}NO_7Na$ 450.06 Found 450.42, [probe + CH_3OH + H]$^+$ Calcd for $C_{24}H_{14}NO_7$ 460.10 Found 460.50, [probe + CH_3OH + Na]$^+$ Calcd for $C_{24}H_{13}NO_7Na$ 482.09 Found 482.33 (Fig. S1).

2.4. General fluorescence spectra measurements

Using the probe, thiols could be detected in buffered (10 mM HEPES, pH 7.0) aqueous DMSO solution (H_2O/DMSO = 1:3, v/v). Aqueous amino acid solutions were also prepared using deionized water. The procedure was as follows: into a buffered (10 mM HEPES, pH 7.0) aqueous DMSO solution (H_2O/DMSO = 1:3, v/v), containing 10 μmol/L probe (a non-fluorescence solution), a thiol sample was gradually titrated. At the same time, any changes in the fluorescence intensity were monitored using a fluorescence spectrometer (λ_{ex} = 420 nm, λ_{em} = 534 nm, slit: 5 nm/5 nm).

3. Results and discussion

3.1. Selectivity over other amino acid

The selectivity toward the thiol group is one of the most important criteria for probe design. A probe of biological importance must be able to discriminate the thiol group from other nucleophiles under physiological conditions. Probe was treated with various relevant each anion (100 equiv.) in buffered (10 mM HEPES, pH 7.0) aqueous DMSO solution (H_2O/DMSO = 1:3, v/v), then determinated their fluorescence emission on fluorescence spectrophotometer. The selectivity test was performed by incubating the probe with 18 kinds of natural amino acids including Ala, Arg, Asp, Gln, Glu, Gly, His, Asn, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. As shown in Fig. 1, a significant increase in fluorescence intensity was only observed with the GSH, Hcy, Cys. The other 18 amino acid samples exhibited no noticeable increase of the fluorescence signal. These experiments implied that the probe is highly selective toward the thiol group but not toward other biologically relevant nucleophiles

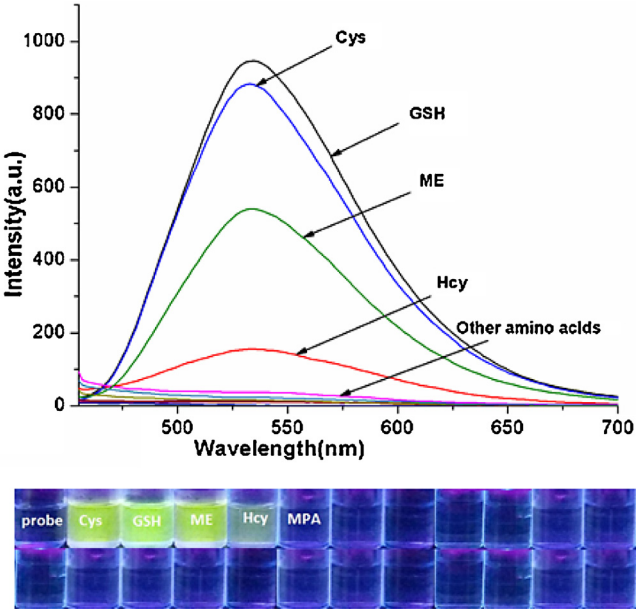

Fig. 1. (Above) Fluorescence spectra of probe (10 μM) with various analytes (500 μM) in buffered (10 mM HEPES, pH 7.0) aqueous DMSO solution (H_2O/DMSO = 1:3, v/v) (λ_{ex} = 420 nm, slit: 5 nm/5 nm). (Bottom) a visual fluorescence change photograph for thiols (green) and other animo acids (colorless) under illumination with a 365 nm UV lamp. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)