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A sensitive and selective detection method for thiol compounds using novel fluorescence probe

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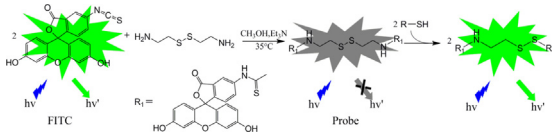
HIGHLIGHTS

- A sensitive and selective detection method for thiol compounds using novel fluorescent probe was developed.
- The fluorescence quenching of the synthesized probe was based on the fluorescence resonance energy transfer (FRET) effect.
- The synthesized probe containing a disulfide bond which can selectively react with thiol compounds by the thiol-disulfide exchange reaction.
- This method was successfully utilized to analyze Cys in the compound amino acid injection.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a sensitive and selective detection method based on fluorescence resonance energy transfer (FRET) was developed for analyzing thiol compounds by using a novel fluorescent probe. The new fluorescent probe contains a disulfide bond which selectively reacts with nucleophilic thiolate through the thiol-disulfide exchange reaction. An obvious fluorescence recovery can be observed upon addition of the thiol compound in the fluorescent probe solution due to the thiol-disulfide exchange reaction and the destruction of FRET. This novel probe was successfully used to determine dithiothreitol (DTT), glutathione (GSH) and cysteine (Cys). The limits of detection (LOD) were 2.0 μM for DTT, 0.6 μM for GSH, and 0.8 μM for Cys. This new detection method was further investigated in the analysis of compound amino acid injection.

1. Introduction

Thiol compounds, such as dithiothreitol (DTT), glutathione (GSH) and cysteine (Cys), are commonly used in the reduction of the disulfide bonds of proteins. In biological systems, thiol compounds play important roles in maintaining the biological redox homeostasis through the exchange reaction between thiols and disulfide bonds [1–4]. DTT is the most widely used dithiol reductant for protein disulfides [5]. However, DTT is a highly toxic substance [6]. GSH contributes to maintaining the normal functions of the immune system. It is an essential endogenous antioxidant which has often been used for detoxification and protecting the cell membrane from being oxidized [7–9]. Abnormal GSH levels often relate to some serious disease [10,11]. It has been

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demonstrated that a low GSH level leads to the development of autism in children [12]. Cys is one of the twenty amino acids used for protein biosynthesis. Cys deficiency is involved in hematopoiesis decrease, leukocyte loss, skin lesions, and weakness [13–15]. Elevated levels of Cys have been associated with neurotoxicity [16,17]. Therefore, it is very important to precisely measure these thiol compounds. The reported thiol detection methods include high-performance liquid chromatographic (HPLC) [18,19], liquid chromatography–mass spectrometry (LC–MS) [20], electrochemistry [21,22], colorimetric assays [23,24] and chemiluminescence [25] etc. Some of these methods require expensive equipment, complicated operating steps and are usually time-consuming. However, there are lots of concerns on fluorometric methods for thiols determination owing to its simplicity and sensitivity [26–30].

In recent years, a number of novel thiol sensing strategies based on the fluorescence resonance energy transfer (FRET) have been developed [31–35], which have largely improved the sensitivity of thiol detection. FRET will cause the quenching of donor fluorescence whereby an excited state donor D (usually a fluorophore) transfers energy to a proximal ground state acceptor A through long-range dipole–dipole interactions [36]. FRET usually occurs over distances comparable to the dimensions of most biological macromolecules, that is, about 10–100 Å [37]. Among the reported thiol sensing methods based on FRET, many fluorescence turn-off detection modes could considerably increase the likelihood of false positive signals [38]. Therefore, a sensitive and selective fluorescence turn-on thiol sensing technique based on FRET is of great interest.

A variety of sensing methods based on the thiol-disulfide exchange reactions for determination of thiol and disulfide redox state of proteins have been developed [39–43]. In the thiol-disulfide exchange reaction, a nucleophilic thiolate attacks one of the two sulfur atoms of the target disulfide bond and then generate a new disulfide bond and another thiolate leaving group [5]. The exchange reaction between thiol and disulfide bond possesses high specificity in sensing of thiol compound [28].

In this work, we designed a novel fluorescent probe for the detection of thiols based on FRET. The synthesis of probe was simple and the distance between two FITC groups in the probe was short which is in favor of high FRET quenching efficiency. The strategy is illustrated in Scheme 1. The probe was synthesized by the reaction of the N═C═S group of fluorescein isothiocyanate (FITC) with the amino groups of cystamine dihydrochloride. The two FITC groups of probe molecule are close to each other, causing a rapid quench of the fluorescence intensity at 517 nm of probe due to the FRET. When a thiol compound is added into the probe solution, the nucleophilic thiolate reacts with the disulfide bond in the probe molecule and generates a new disulfide which separates the two FITC groups. As a result, the fluorescence intensity at 517 nm of probe solution dramatically recovers due to the destruction of FRET. The new probe is readily soluble in water allowing the sensing to be performed in buffer solutions. Successful application of this new probe in determining Cys concentration in a compound amino acid injection has been demonstrated.

2. Experimental

2.1. Chemicals

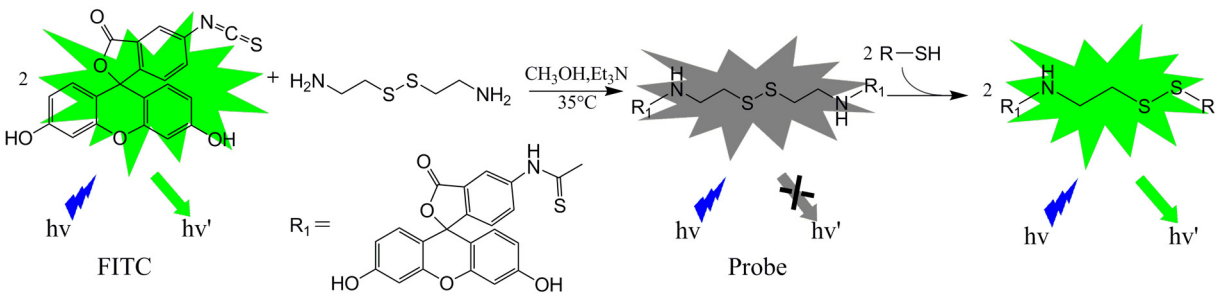
Fluorescein isothiocyanate (FITC), cystamine dihydrochloride, dithiothreitol (DTT), cysteine (Cys), glutathione (GSH), alanine, glycine, glutamine, cystine, serine and other amino acid were purchased from Sigma–Aldrich (Milwaukee, WI). Sodium borohydride ($NaBH_4$), ascorbic acid, methanol, triethylamine, sodium carbonate, sodium bicarbonate, deuterium oxide (D_2O), sodium hydrogen sulfite ($NaHSO_3$) and acetic acid were purchased from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China). Acetonitrile was purchased from Merck (New Jersey State, USA). Compound amino acid injection was purchased from Mitsubishi pharmaceuticals company (Guangzhou, China). All the chemicals were analytical-grade reagents except that acetonitrile was chromatographically pure. These chemicals were used without further purification. The water used throughout all experiments was purified by an Elix 5 Pure Water System of Millipore (Billerica, USA).

2.2. Apparatus

The fluorescence spectra were performed on a Shimadzu RF-5301PC fluorescence spectrophotometer. LC–MS analyses were carried out with an Agilent 1290 Infinity LC/6460 QQQ MS system. ^{1}H NMR spectrum was measured on a Bruker-600 NMR spectrometer with tetramethylsilane (TMS) as internal standard. Purification of the synthesized probe was performed with Waters 2998 preparative chromatograph system containing a Bridge preparative column (10 × 250 mm, 5 μm ODS).

2.3. Synthesis of probe

FITC (250.0 mg, 0.64 mmol) and cystamine dihydrochloride (67.2 mg, 0.30 mmol) were dissolved in 5 mL methanol/triethylamine (v:v = 100:1), respectively. The two solutions were mixed in the flask. The mixture was heated to 35 °C for 5 h under stirring and dark condition, and then the solvent was removed under reduced pressure. The residue was separated by preparative chromatograph with a Bridge preparative column. The mobile phase used in the preparative chromatograph consist of acetonitrile and ultrapure water (v:v = 6:4, 1% acetic acid). The flow rate was 5 mL min^{-1} and UV detection was performed at 254 nm. 1H NMR (600 MHz, D_2O/H_2O = 1:9): 1.85 (s, 1H), 2.98 (t, 2H), 3.89 (s, 2H), 6.33 (s, 3H), 6.53 (d, 2H), 6.92 (d, 2H), 7.32 (d, 1H), 7.50 (m, 1H), 3.29 (s, 1H) (Fig. S2).



Scheme 1. Schematic illustration of the mechanism of thiol sensing based on FRET.