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A simple probe with visible color change for selective detection of cysteine

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ABSTRACT

In this work, we synthesized a simple fluorescent probe, which can selectively detect cysteine. With addition of cysteine, the probe solution showed marked color change from pale yellow to orange color by naked eye. There was an excellent linear relationship between fluorescence intensity of probe and cysteine concentration. Moreover, the probe displayed satisfactory detection limit. Low toxicity and cellular imaging offered the important conditions for detecting cysteine in biological system.

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KEYWORDS
Bioimaging; cysteine; fluorescence; probe; selectivity

Introduction

Biothiols, such as glutathione (GSH) and homocysteine (Hcy), as well as cysteine (Cys) play critical role in maintaining redox homeostasis and the regulation of important cellular processes.[1,2] GSH is the most abundant reductant in the biological systems. GSH insufficiency can result in oxidative stress in cells, which is linked to neuro degenerative disorders. In addition, abnormal level of GSH and Hcy are also related to cardiovascular disease, cancers and AIDS.[3,4] Among them, Cys, as an important amino acid, participates in many enzymatic reactions, regulates a lot of important cellular processes, and plays a critical role in the cellular antioxidant system.[5,6] Furthermore, the abnormal level of Cys may cause many human diseases. For example, elevated levels of Cys are closely related to cardiovascular complications, Parkinson's disease, and Alzheimer's disease, while low levels of Cys are associated with slow growth rate, hair depigmentation, muscle and fat loss, liver damage, skin lesions, weakness.[7–11] Therefore, selective detection of Cys in biological samples by convenient method is of great significance.

Among the various detection methods, the fluorescent probe detection has attracted much attention due to its high sensitivity and selectivity, and ease of handling.[12–14] With the efforts of researchers, many fluorescence probes have been developed for detection of biothiols. The reaction mechanisms included Michael addition, nucleophilic substitution, cyclization reaction, conjugate addition, disulfide exchange, and others.[15–25] However, it is difficult to distinguish Cys from Hcy and GSH because they have the same recognition site and similar chemical reaction activity. Although many fluorescence probes toward Cys were reported,[26–32] they more or less had some limitations in the practical application including low selectivity, poor sensitivity, use of surfactant, undesired spectra overlap. Moreover, shorter excitation and emission spectra all resulted in interferences for detection of Cys in biological systems. Therefore, development of fluorescence probes for efficient detection of Cys is urgently needed.

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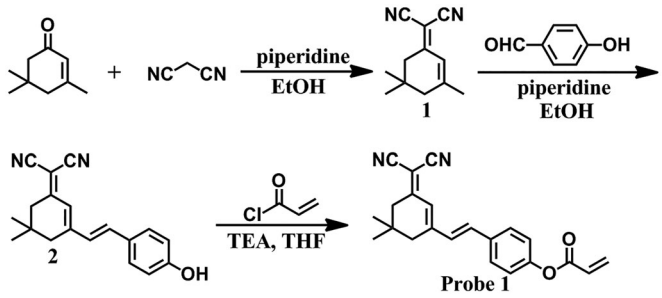
Supplemental data for this article can be accessed here.



Scheme 1. Synthesis of probe **1**.

Acrylate, as the recognition site, can selectively recognize Cys over Hcy and GSH by addition-cyclization mechanism. Therefore, in this work, we designed and synthesized the fluorescent probe **1** ((*E*)-4-(2-(3-(dicyanomethylene)-5,5-dimethylcyclohex-1-en-1-yl)vinyl)phenyl acrylate) for selective detection of Cys based on acrylate as the recognition site. Firstly, the probe showed a large Stokes shift (160 nm), which greatly reduces the interference of absorption spectrum to fluorescence intensity. In addition, larger emission wavelength (580 nm) reduces background interference of biological system. Meanwhile, the results showed that the probe **1** was able to determine selectively Cys over Hcy and GSH, and accompanied by color change by the naked eye, which facilitated the Cys identification. Low toxicity and cellular imaging offered the potential conditions for detecting cysteine in biological system.

Experimental

$^{1}H/^{13}C$ NMR spectra were recorded on a Bruker AVIII-400/600 MHz spectrometer. High resolution mass spectra (HRMS) were measured with Thermo (orbitrap Elite). Absorption spectra were measured using a Thermo (BioMate 3S) UV/Vis spectrophotometer. Fluorescence measurements were carried out with a F97pro fluorospectrophotometer. Unless otherwise noted, materials were purchased from commercial suppliers and used without further purification. All the solvents were purified and dried according to general methods.

Results and discussion

The probe **1** was synthesized (Scheme 1, for details and characterization see the the Suppementary Material) and confirmed by NMR and HRMS (ESI). Keeping the probe **1** in hand, next we explored detailedly the optical properties of probe **1**. Firstly, we measured the absorption spectra of the probe 1 in presence or not of Cys to determine the excited wavelength (Fig. S1). The result showed that the probe 1 had strong absorption at 420 nm with the addition of Cys. Subsequently, we investigated the influence of solvent type (Fig. S2) and content (Fig. S3) on the fluorescence intensity of free probe **1** and probe **1** toward Cys. On the basis of fluorescence intensity (I) and relative value (I/I_0), we selected the aqueous solution (pH 7.4 PBS, containing 20% acetonitrile) as the spectral response. Subsequently, we surveyed the fluorescence spectrum response of probe **1** (20 μM) in the presence of different amino acids ($_{L}$-Cys, alanine (Ala), arginine (Arg), Aspartate (Asp), methionine ($_{DL}$-Met), Glutamate (Glu), glycine (Gly), histidine (His), isoleucine (Ile), methionine ($_{L}$-Met), leucine (Leu), lysine (Lys), N-acetylcysteine (NAC), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), valine (Val), Homocysteine (Hcy), 200 μM; glutathione (GSH), 1 mM) with incubation for 10 min at 37 °C (Fig. 1a). The results displayed the fluorescence intensity of probe solution was clearly strengthened at 580 nm with the addition of Cys. However, other analytes didn't cause obvious change of fluorescence intensity. Although Hcy induced the slight enhancement of fluorescence intensity, which was negligible compared with the fluorescence intensity increasement caused by Cys. More importantly, 1 mM GSH also didn't change distinctly the fluorescence intensity. The probe showed a large Stokes shift (160 nm), which greatly reduces the interference of absorption spectrum to fluorescence intensity. Thus,

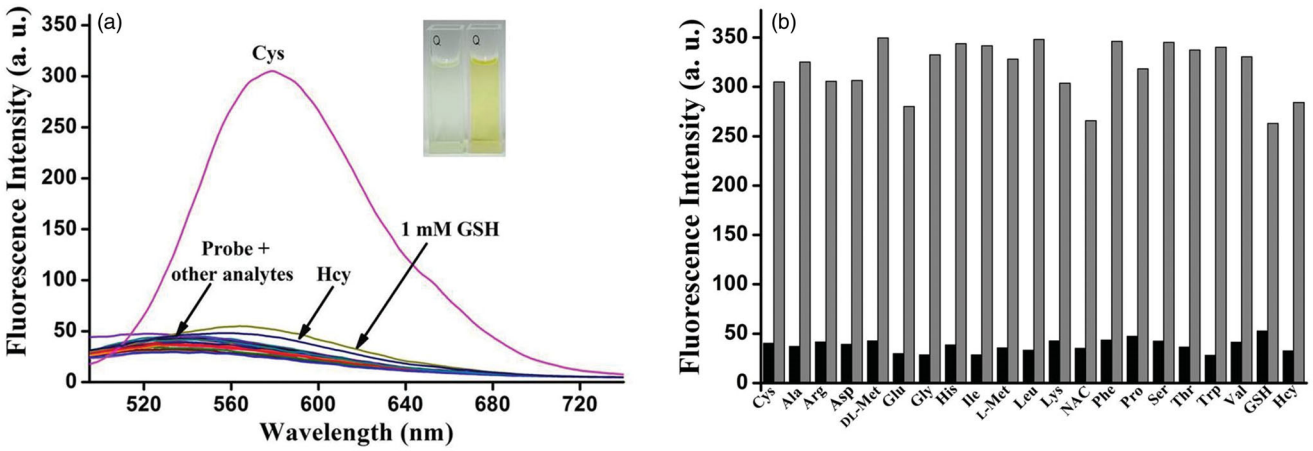


Figure 1. (a) Fluorescence spectra of probe **1** (20 μM) in the presence of various analytes in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile), $\lambda ex = 420$ nm; inset: color change of probe solution and probe toward Cys by naked eye. (b) Fluorescence intensity of probe **1** (20 μM) with various analytes (200 μM) in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile), $\lambda ex = 420$ nm. Black bar: probe **1** + analytes; Gray bar: probe **1** + Cys + analytes.

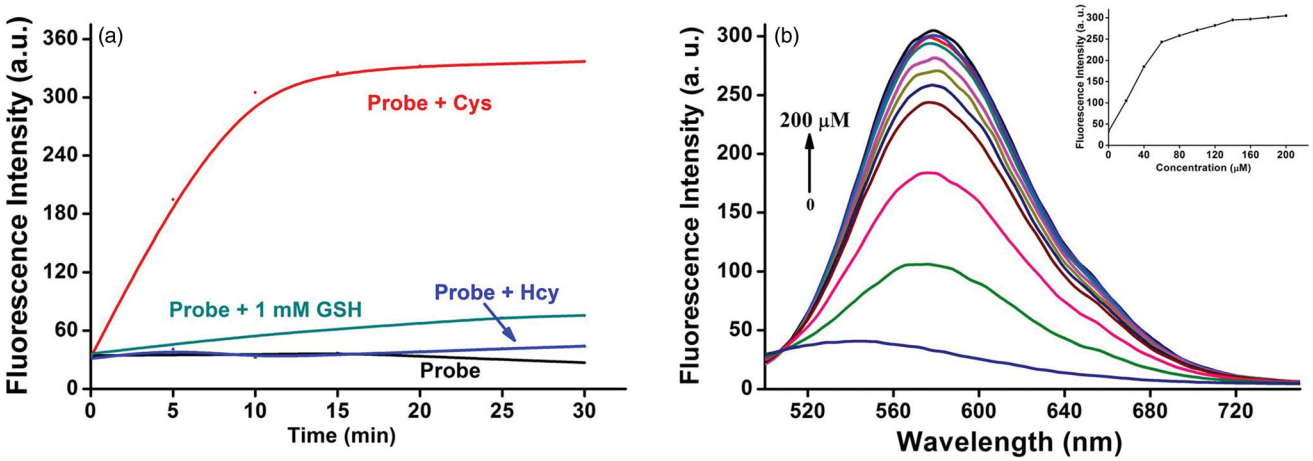


Figure 2. (a) Time-dependent fluorescence spectra of probe **1** (20 μM) in the presence of Cys/Hcy/GSH. (b) Fluorescence spectra response with increase of Cys concentration (from 0 to 10 equiv.) over probe **1** (20 μM) within incubation of 10 min at 37 °C; inset: Cys concentration-dependent change of fluorescence intensity.

probe **1** can detect selectively Cys. In addition, with the addition of Cys, the solution of probe **1** changed from pale yellow to orange color by naked eye, which provided the advantageous and convenient condition for practical application.

Encouraged by the above results, we carried out the competition experiment to investigate the interferences of other concomitant analytes for probe **1** toward Cys (Fig. 1b). The results showed the existence of other analytes didn't change obviously fluorescence intensity of probe **1** toward Cys, even including GSH and Hcy. In addition, we further lengthened the reaction time of probe **1** for Cys/GSH/Hcy (Fig. 2a). The fluorescence intensity increased gradually in presence of Cys and reached the plateau within 15 min, which is faster than that of previous reports.[33–35] Moreover, the fluorescence intensity enhanced slowly with increasement of GSH/Hcy incubation time, but the fluorescence intensity is far less than that of Cys. Therefore, the probe **1** is able to detect selectively Cys in view of above results.

We further explored the relationship between fluorescence intensity and Cys concentration by titration experiment (Fig. 2b). Results displayed the fluorescence intensity firstly heightened and arrived at saturation state with increase of Cys concentration from 0 to 200 μM. Meanwhile, there was excellent linear relation ($R^2 = 0.9963$) between fluorescence intensity and Cys

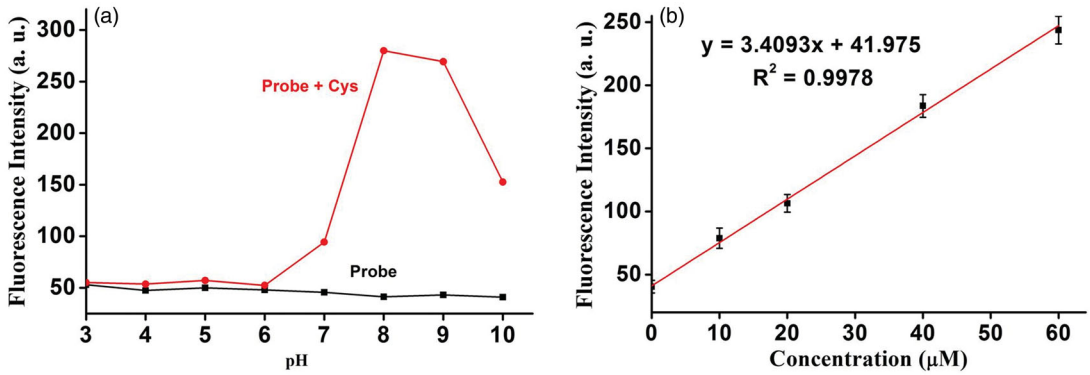


Figure 3. (a) pH-Dependent fluorescent intensity changes of probe **1** and probe **1** toward Cys at room temperature, λex = 420 nm. (b) Concentration-dependent fluorescence intensity response of probe **1** for Cys in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile) λex = 420 nm.

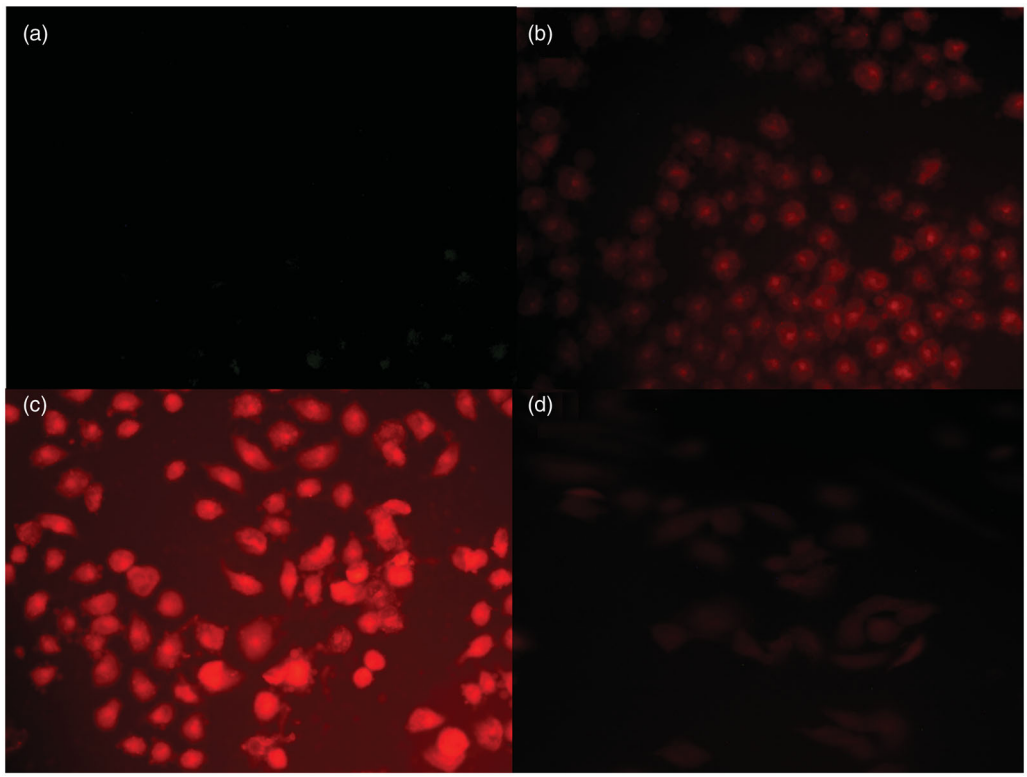


Figure 4. Cell imaging of probe **1**. (A) Hela cells; (B) Hela cells were incubated with probe **1** (20 μM) for 30 min; (C) Hela cells were pre-incubated with Cys (100 μM) for 30 min and then incubated with probe **1** (20 μM) for another 30 min; (D) Hela cells were pre-incubated with NEM (1 mM) for 30 min and then incubated with probe **1** (20 μM) for another 30 min. (λex = 400–460 nm, λem = 590 nm).

concentration from 0 to 60 μM, which offered possibility to analyze quantitatively the Cys content. On this same basis, the detection limit (Fig. 3b, 3s/m, $n = 20$) of Cys was evaluated to be 0.17 μM, which further guaranteed the practical application of the probe **1** in the biological system. Therefore, it is possible that probe **1** can detect Cys in biological systems.

Subsequently, we discussed the effect of pH value for fluorescence intensity (Fig. 3a). The result indicated the fluorescence intensity change of probe **1** toward Cys is obvious in pH 6.0–9.0, which is critical to the application of probe under the condition of physiological pH value. More interestingly, the fluorescence intensity of probe didn't present significant changes in pH 3.0–10.0,

which meant the probe **1** was stable adequately for application in biological systems.

We also investigated the reaction mechanism of probe **1** and Cys (Scheme S1). The reaction mechanism between probe **1** and Cys undergo conjugate addition and subsequent intramolecular cyclization two-step reaction.[36–39] which afforded the compound **2**, corresponding mass spectrum was observed (Fig. S4). Reaction of probe **1** and Cys formed seven numbered rings, which is more preponderant than that of Hcy and GSH in thermodynamics. Therefore, advantage of reaction rate played important role for selective detection of Cys.

Lastly, we completed the bioimaging of probe for detecting Cys in cells (Fig. 4) by fluorescence microscope. The cytotoxicity of the probe 1 was evaluated by MTT method before biological imaging. When the Hela cells was incubated with appointed concentrations of probe 1 for 12 h, the results showed that the probe (5–30 μM) did not cause cell death. Thus, the probe can be used to image cells. (Fig. S5). Hela cells were incubated with probe **1** (20 μM) for 30 min, and action of cellular thiols afforded moderate red fluorescence (λex = 400–460 nm, λem = 590 nm). The cells were pretreated with Cys (100 μM) and then incubated with probe 1 (20 μM) for 30 min, the red fluorescence was enhanced clearly. If the cells were pretreated with thoil scavenger (N-ethyl maleimide, NEM), the red fluorescence failed to appear. Thus, the probe **1** would be used for imaging of Cys in cells.

In conclusion, we designed and synthesized the simple probe **1**, which can detect selectively Cys and exhibit the good anti-interference ability. More importantly, the fluorescence intensity was evidently increased with addition of Cys and accompanied the obvious colur change by naked eye, which provided the advantage for practical application. Meanwhile, lower detection limit and bioimaging of probe were also the important guarantee for detecting Cys in cells. Therefore, probe **1** would afford the condition for understanding Cys in biosystem.

Disclosure statement

The authors have declared no conflicting interests.

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