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A label-free turn-on fluorescence probe for rapidly distinguishing cysteine over glutathione in water solution

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ABSTRACT

A novel label-free fluorescent chemodosimeter (C1) was synthesized, based on coumarin and N-(4-aminobenzoyl)-β-alanine, for the selective detection of cysteine (Cys) over glutathione (GSH), which involved a click reaction of Cys to C═N of a Schiff base. The probe C1 featured a fast response (about 3 min), emission in the visible region, and high selectivity. Addition of Cys in HEPES-NaOH solution (pH 7.4) to C1 in water resulted in the appearance of a new emission peak at 445 nm, in company with remarkable enhancement of fluorescence intensity, while other amino acids did not induce any significant fluorescence change. Meanwhile, the addition reaction of Cys to C1 elicited 90.8-fold fluorescence intensity enhancement, which resulted in a change of emission color from orange to blue.

Introduction

As one of the most important components among the bio-relevant proteins, cysteine has attracted much attention in recent years due to its crucial roles in biological bodies, such as catalysis, heavy metal binding, protein turnover, and signal transduction [1–4]. A number of diseases can result from abnormalities in cysteine levels. Cysteine deficiency is known to be involved in edema, lethargy, hair depigmentation, liver damage, muscle and fat loss, slowed growth, and skin lesions [5,6]. Additionally, increased cysteine levels can lead to a higher risk of cancer, Alzheimer's, and cardiovascular diseases [7–10]. Therefore, it is of great importance to develop a rapid, sensitive, selective, and quantitative analysis of the cysteine level.

To date, many strategies developed for the measurement of cysteine have been studied, including mass spectrometry [11,12], high performance liquid chromatography (HPLC) [13,14], capillary electrophoresis [15,16], optical assays [17,18], and electrochemical analysis [19,20]. Nevertheless, most of these methods require professional experts, expensive instruments, or cumbersome separation and purification of samples. As a highly sensitive, nondestructive technique, fluorescence analysis has also been applied extensively for the measurement of cysteine [21–25]. However, some of these methods reveal limitations in the process of detection, including the introduction of heavy metal ions [26–28] or a long response time. More importantly, only a few sensors able to detect Cys over GSH have been reported to date [29–31], Therefore, it is still of interest to explore low-cost and time-saving methods for measuring cysteine.

Herein we report a new turn-on fluorescence probe C1 (Scheme 1) based on coumarin, which can effectively discriminate Cys from GSH in aqueous solution.

Experimental

Apparatus and chemicals

The melting point was determined using an SGW X-4 digital melting point apparatus (Shanghai Instrument Physical Optics Instrument Co., Ltd). The ^{1}H NMR spectrum was run on a Varian Mercury-Plus 400 NMR spectrometer using TMS as the internal standard (Varian, San Diego). Elemental analysis was performed with a Vario EL CHNS elemental analyzer (Elementar, Germany). The mass spectrum was recorded with a VG ZAB-HS double-focusing mass spectrometer (Thermo Onix Ltd, UK). Fluorescence spectra, fluorescence lifetime, and quantum efficiency were

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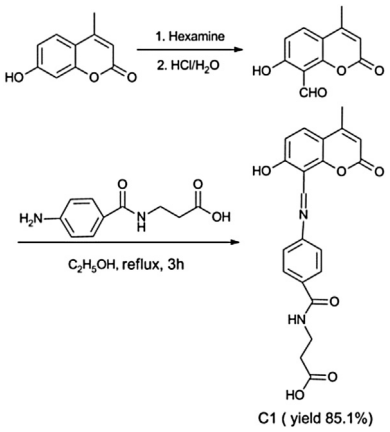

Scheme 1. Synthetic route of compound C1.

measured with a Fluorolog 3-TSCPC (Horiba Jobin Yvon Inc. France). All chemicals were purchased from Aladdin Industrial Corporation (China) and used without further purification. The solvents were purified using standard methods.

All solutions of C1 (5.0 μM) and various amino acids and Na_2S (10.0 μM) were prepared in HEPES–NaOH buffer solution (10.0 mM, pH 7.4) and allowed to equilibrate at room temperature for 3 min before spectral measurements. Every measurement was performed three times under the same testing conditions in a quartz cell with a 1 cm path length with a 380 nm excitation wavelength.

Synthesis of 8-formyl-7-hydroxy-4-methylcoumarin

A solution of 8-formyl-7-hydroxy-4-methylcoumarin was prepared by the known method [33]. 7-Hydroxy-4-methylcoumarin (5.0 g, 0.0284 mol) and hexamine (10.0 g, 0.071 mol) in acetic acid (37 mL) were stirred for 5.5 h at 95 °C. Then hydrochloric acid (75 mL, HCl:H_2O = 84:100, v/v) was added and the mixture was further heated for 45 min. After cooling, the mixture was poured into ice water (375 mL) and extracted with ethyl acetate (150 mL × 3). The organic layer was dried over sodium sulfate and the solvent removed. The residue was purified by column chromatography on silica gel using dichloromethane as eluent to provide the product as a light yellow solid. Yield 0.9 g (15.5%). ^{1}H NMR ($CDCl_3$, δ ppm): 2.45 (s, 3H); 6.22 (s, ^{1}H); 6.92 (d, J = 9.2 Hz, ^{1}H); 7.74 (d, J = 8.8 Hz, ^{1}H); 10.63 (s, ^{1}H); 12.23 (s, ^{1}H). MS *m/z*: 205$[M + H]^+$.

Synthesis of 7-hydroxy-4-methyl-8-(4'-(N-carboxylethylformamide) phenylimino)methyl-2H-1- benzopyran-2-one (C1)

8-Formyl-7-hydroxy-4-methylcoumarin (0.21 g, 0.1 mmol) and N-(4-aminobenzoyl)-β- alanine (0.20 g, 0.11 mmol) were dissolved in anhydrous ethanol (20 mL). The reaction mixture was refluxed for 2 h; then the mixture was cooled to room temperature. The precipitate was filtered off, washed twice with cold ethanol, and dried under vacuum to give the desired product as a yellow solid. Yield 0.34 g (85.1%). Melting point 265.4–267.2 °C. ^{1}H NMR (d_6-DMSO, 400 MHz, δ ppm): 2.33 (s, 3H); 2.51–2.55 (t, J = 8.0, 2H); 3.44–3.48 (t, J = 8.0, 2H); 6.17 (s, ^{1}H); 6.84–6.86 (d, J = 8.0, ^{1}H), 7.50–7.52 (d, J = 8.0, 2H); 7.70–7.72 (d, J = 8.0, ^{1}H); 7.92–7.94 (d, J = 8.0, 2H); 8.60 (s, ^{1}H); 9.14 (s, ^{1}H); 12.20 (s, ^{1}H), 14.65 (s, ^{1}H). ^{13}C NMR (d_6-DMSO, δppm): 18.3, 33.7, 35.6, 106.0, 110.4, 110.9, 114.0, 121.2, 128.6, 130.6, 133.0, 148.5, 153.8, 154.0, 157.6, 159.0, 165.2, 165.2, 172.9.

Results and discussion

Selective response of probe C1 to Cys

In order to investigate the effect of various amino acids on the fluorescence spectra of C1, the amino acids Cys, His, Lys, Arg, Cys–Cys, Thr, Gly, Ala, Asp, Val, Met, Glu, Ser, GSH, and Tyr and Na_2S were used to evaluate the response properties of C1 in aqueous solution (HEPES-NaOH, 10.0 mM, pH 7.4). Fig. 1 shows the changes in the fluorescence spectra of C1 upon addition of various analytes. Compound C1 had a large effect only with Cys among the analytes examined. In the presence of Cys, C1 showed strong fluorescence enhancement (about 90.8-fold) and a dramatic fluorescence color change from orange to light blue, which could easily be identified by the naked eye under a UV lamp during the detection process. Other analytes gave no distinct response to C1 in the fluorescence spectra. This obvious feature reveals that compound C1 has high selectivity for Cys.

Sensitivity of C1 for Cys detection

The fluorescence spectra of C1 upon titration with Cys were then recorded (Fig. 2). The fluorescence intensity of C1 (5.0 μM) at 445 nm was gradually increased with the addition of an increasing amount of Cys (0–11.0 μM). The enhanced intensity of C1 displayed a good linear regression relationship (Stern–Volmer equation) [32]: $y = 1.84 \times 10^4[\text{Cys}] + 3.50 \times 10^3$ ($R^2 = 0.996$) with the concentration of Cys in the range 0–7.0 μM based on the titration experiment (Fig. 3). The limit of detection (LOD) of C1 for Cys was estimated to be as low as 0.15 μM based on 3σ/*S*, where σ is the standard deviation of blank measurements, and *S* is the slope for fluorescence intensity versus sample concentration. The results indicate that the probe C1 can be used as a sensitive chemosensor for the detection of Cys in aqueous solution.

Competition experiments

To further investigate the interference of other analytes with the detection of Cys, competition experiments were performed in which various analytes (10.0 μM) were added to a solution of C1 (5.0 μM) in the presence of Cys. As shown in Fig. 4, the competing

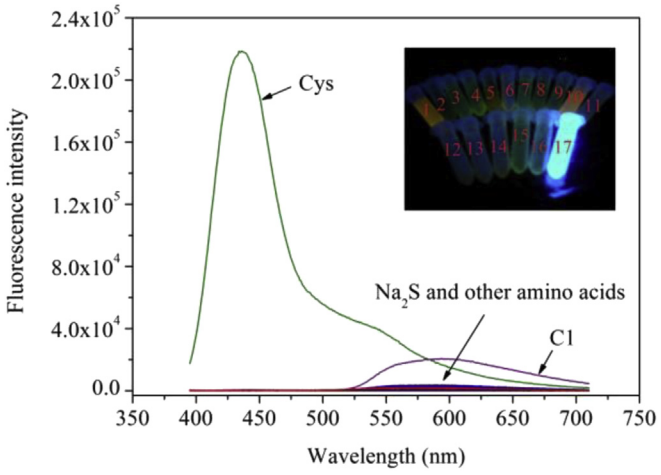

Fig.1. Fluorescence emission and fluorescence color changes (insert) of C1 (5.0 μM) upon addition of various analytes (10.0 μM) in aqueous solution (HEPES–NaOH 10.0 mM, pH 7.4, λ_{ex} = 380 nm). Analytes: 1: C1, 2: His, 3: Lys, 4: Arg, 5: Cys–Cys, 6: Thr, 7: Gly, 8: Ala, 9: Asp, 10: Na_2S, 11: Met, 12: Glu, 13: Ser, 14: GSH 15: Val, 16: Tyr, 17: Cys. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)