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A quick response fluorescent probe based on coumarin and quinone for glutathione and its application in living cells

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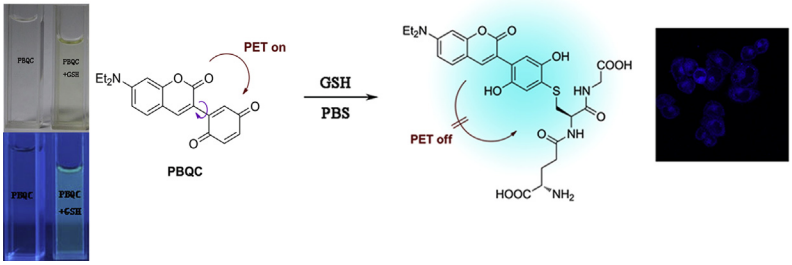
HIGHLIGHTS

- This probe can discriminate glutathione from sulfhydryl compound by PET process.
- This probe can be used to determine glutathione in aqueous solution within 1 min.
- This probe has been successfully applied in living cell image.

GRAPHICAL ABSTRACT



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ABSTRACT

We have designed and synthesized a simple but effective fluorescent probe for sensing glutathione (GSH) by PET process based on coumarin and quinone, which worked as fluorophore and reaction site, respectively. The probe could discriminate GSH from cysteine and homocysteine within 1 min in PBS-buffered solution. The sensing mechanism was confirmed by density functional theory (DFT), viscosity test, fluorescence spectrum analysis and HRMS, respectively. The probe has a low limit of detection (0.1 μM) and finally been used in cell imaging successfully.

1. Introduction

Small biothiols, mainly cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), are present in nearly all cells and mediate numerous cellular processes. As we all know, GSH plays an important role in various physiological processes such as helping anti-aging and advancing the immunity [1–3]. What's more, GSH can prevent damage caused from free radicals and peroxides as well as heavy metals. The abnormal level of GSH in human body has a close relationship with diseases such as cancer, Alzheimer's and different kinds of cardiovascular diseases [4–6]. Therefore, the detection of GSH both in academic research and clinical applications is important.

Fluorescent probes are widely used in the detection of thiols because of their simplicity, low cost, sensitivity, selectivity and live

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cell imaging [7–12]. However, only a few fluorescent probes could distinguish GSH, Cys and Hcy which are similar in structure. So far, some fluorescent probes for the detection of GSH have been reported based on different mechanisms, such as redox reaction [13,14], nucleophilic substitution reaction [15–23], Michael addition reaction [24–28] and others [29–31]. All these strategies are based on the nucleophilicity of mercapto group. Therefore, we selected quinone as the reaction site because of its excellent Michael acceptor feature and fluorescent quenching property [32,33]. Sulfhydryl thiols could induce benzoquinone reduction, and hydroquinone/benzoquinone had been extensively applied in the electroanalytical approach for thiols [34–37], delivery prodrugs under thiol-rich population of tissue and mimicking metabolic processes [38]. However, fluorescent probes based on quinone are seldom reported, and they always respond to biothiols [39–43]. Therefore, there are a great potential to develop fast-response and sensitive probes based on quinone for the detection of GSH.

Herein, we have designed and developed an effective fluorescent probe, 2-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)cyclohexa-2,5-diene-1,4-dione (**PBQC**), which is constructed by 7-diethylamino coumarin and quinone. **PBQC** could discriminate GSH from Cys and Hcy in 30 s, and successfully used to living imaging.

2. Experimental

2.1. Apparatus and chemicals

Thin-layer chromatography (TLC) involved silica gel 60 F_{254} plates (Merck KGaA) and column chromatography involved silica gel (mesh 200–300). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were acquired on a Bruker Avance 400 spectrometer, with DMSO-d_6 used as a solvent and tetramethylsilane (TMS) as an internal standard. Melting points were determined with an XD-4 digital micro-melting–point apparatus. IR spectra were recorded with the infra-red (IR) spectrophotometer VERTEX 70 FT-IR (Bruker Optics). HRMS spectra were obtained on a Q-TOF6510 spectrograph (Agilent). UV–vis spectra were measured by the use of a Hitachi U-4100 spectrophotometer. Fluorescence measurements were performed on a Perkin–Elmer LS-55 luminescence spectrophotometer. Quartz cuvettes with a 1-cm path length and 3-mL volume were used for all measurements. The pH was determined with a model PHS-3C pH meter. Unless otherwise stated, all reagents were purchased from Aladdin or Sinopharm Chemical Reagent Co. and used without further purification. Twice-distilled water was used throughout all experiments. The salts used in stock aqueous solutions of metal ions were KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $NaNO_3$ and $Fe(NO_3)_3{\cdot}9H_2O$.

2.2. Synthesis of *PBQC*

7-Diethylamino coumarin (1.52 g, 7 mmol) and benzoquinone (2.27 g, 21 mmol) were dissolved in acetonitrile (40 mL), and then zinc chloride (2.86 g, 21 mmol) was added as catalytic. The mixture was refluxed for 18 h. The mixture was cooled to room temperature and concentrated under reduced pressure. Water (80 mL) was added to the residue and then was extracted with ethyl acetate (50 mL × 3). The combined organic layer was washed with water (50 mL × 3) and dried over anhydrous $MgSO_4$. The organic layer was concentrated under reduced pressure to give crude product. The crude product was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (4:1, v/v). The target compound **PBQC** was obtained as purple black solid in 20% yield; m.p. 176–178 °C. IR (KBr, cm^{-1}): 3075, 2972, 2920, 1712, 1648 and 1595. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.13 (t, 6H, J = 7.0 Hz, CH_3), 3.46 (q, 4H, J = 7.0 Hz, CH_2), 6.56 (d, 1H, J = 2.2 Hz, 8H-coumarin), 6.75 (dd, 1H, J = 8.9 and 2.2 Hz, 6H-coumarin), 6.89 (dd, 1H, J = 10.2 and 2.6 Hz, Ar–H), 6.96 (d, 1H, J = 10.2 Hz, Ar–H), 7.14 (d, 1H, J = 2.6 Hz, Ar–H), 7.51 (d, 1H, J = 8.9 Hz, 5H-coumarin), 8.05 (s, 4H-coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): 12.28 (2C), 44.21 (2C), 96.08, 107.69, 109.58, 110.81, 130.61, 132.38, 136.14, 137.30, 141.09, 146.03, 151.60, 156.31, 159.31, 185.71, 187.57. HRMS: m/z calcd for $[C_{19}H_{17}NO_4 + H]^+$: 324.1236, found 324.1232.

2.3. Absorption and fluorescence spectroscopy

In order to avoid toxic organic solvent, we selected phosphate buffered saline (PBS) for the spectral response. **PBQC** was dissolved in DMSO for a stock solution (1 mM). The amino acids (GSH, Hcy, Cys, arginine, aspartic acid, glutamic acid, histidine, sarcosine, lysine, threonine, tryptophan and valine), cationic (Ca^{2+}, Fe^{3+}, K^+, Mg^{2+}, Na^+ and Zn^{2+}), Na_2S, glucose, 2-aminopyridine, 2-mercaptoethanol (MCH) and H_2O_2 stocks were all in deionized water at 10^{-2} M for absorption and fluorescence spectrum analysis. Test solutions were prepared by displacing 100 μL of the stock solution and an appropriate aliquot of each testing species solution into a 10 mL volumetric flask, and the solution was diluted to 10 mL with PBS at pH 8.0. The resulting solution was shaken well and recorded after 10 min at room temperature. Measurements of UV–Vis absorption spectra were performed at wavelength 408 nm; measurements of fluorescence spectra were performed at excitation wavelength 410 nm and emission wavelength 485 nm, scan speed 900 nm min^{-1}and slit 10.0/5.0 nm.

Fluorescence quantum yield was determined by the relative comparison with quinine sulfate (Φ_s = 0.54 in 0.1 N H_2SO_4 aqueous solution) as standard, and it was calculated by the Equation (1) [27].

$$\Phi = \Phi_S\left(IA_S/I_SA\right)\left(\eta^2/\eta_S^2\right) \quad (1)$$

in which, A is the absorbance, I is the integrated fluorescence intensity, and η is the refractive index of the solvent.

2.4. Theoretical calculations

All the calculations were implemented with the Gaussian09 program package. The initial geometries of the compounds were generated by the Gauss View software. The ground state structures of the compounds were optimized using the density functional theory (DFT) using a B3LYP/6-31G* level of theory. The solvent effects were modeled with the polarizable continuum model (PCM).

2.5. Cell culture and cell imaging

PBQC was dissolved in DMSO at a storage concentration of 10 mM. HeLa cells were cultured in DMEM-H with 10% CS at 2×10^4 cells per well for 12 h. HeLa cells were washed from the culture medium with PBS, incubated with **PBQC** (0, 1.0, 5.0 and 10.0 μM) at 37.5 °C for 1 h, respectively. Then washed 3 times with PBS and underwent imaging measurement by ultraviolet light with a confocal microscope (LSM700). Imaging analysis involved the use of ImageJ. The exciting light was 405 nm for the emission range of the blue channel (405–640 nm).

2.6. Cytotoxicity assay

The in vitro cytotoxicity of **PBQC** to HeLa cells was measured by a standard sulforhodamine B (SRB) assay. Briefly, HeLa cells were loaded in 96-well culture plates at 4×10^4 cells per well. After culture for 24 h, cells were incubated with fresh RPMI 1640