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Short note

A novel near-infrared fluorescent probe for cysteine in living cells based on a push-pull dicyanoisophorone system

Yuewen Yu[a], Hongping Xu[a], Wang Zhang[a], Qiaorong Han[a,b], Bingxiang Wang[a,b,*], Yuliang Jiang[a,b,*]

[a] College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, PR China
[b] Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and Jiangsu Key Laboratory of Biofunctional Materials, PR China

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ABSTRACT

The near-infrared (NIR) fluorescent probe for rapid, highly sensitive and selective detection of cysteine (Cys) has great significance in both biological and environment sciences. In this work, a novel and specific dicyanoisophorone-based NIR probe for Cys was developed. This probe features remarkably large Stokes shift, with turn-on fluorescence property for highly sensitive and selective detection of Cys over other amino acids including the similar structured homocysteine (Hcy) and glutathione (GSH). The probe based on the conjugate addition-cyclization reaction, and has low detection limit of Cys. Furthermore, this probe shows great potential for Cys detection, which was successfully applied to bioimage Cys in living cells with low cytotoxicity.

1. Introduction

Detection of sulfydryl-containing amino acids is being watched by more and more researchers, and has become a popular research nowadays. The sulfydryl-containing amino acids are also known as biothiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH). They are commonly found in biological systems and possess different crucial roles in cellular processes [1–6]. Among these biothiols, Cys is the least abundant thiol inside the cell, its concentration being in the micromolar range [7,8]. It has been reported that the abnormal levels of Cys could induce many diseases such as decreased hematopoiesis, psoriasis, leucocyte loss, edema, neurotoxicity, liver damage, and Parkinson's disease [9–12]. Thus, discrimination of Cys is of great interest and importance in biochemistry and biomedicine fields.

Over the past decades, several methods have been utilized to detect Cys [13–17]. Among them, fluoresce-based methods are usually preferred due to their high sensitivity, ease of implementation, fast-response, cost-effectiveness, noninvasiveness, real-time detection capability and good biocompatibility [18–21]. Until now, a few fluorescent probes have been developed for Cys through various mechanisms, including Michael addition [22–26], cyclization reaction with aldehyde [27–30], cleavage of disulfide and sulfonamide [31–33] and others [34–38].

In recent years, several excellent Cys-specific probes based on various traditional fluorophores have been developed [39–47]. For example, Strongin's group reported a HMBT-based probe for Cys detection, which can undergo an excited-state intramolecular photontransfer (ESIPT) process [44]. Chen's group developed a fluorescein-based probe, which displays excellent selectivity and sensitivity for Cys over Hcy and GSH [46]. However, most of them have emission wavelength and absorption wavelength within the ultraviolet or visible region (300–650 nm), which will be detrimental to its use in biological imaging. Near-infrared (NIR) probes are widely accepted as an ideal candidate in biological system studies, because it works at 650–900 nm (NIR region), and possesses several advantages, such as lower energy, better tissue penetration as well as minimum background interference from autofluorescence [48,49]. Although NIR probes have made some progress in rescent years, but some limitations including low sensitivity, complex synthesis and/or low yield were still existed (Table S1) which could hinder its further application [50–52]. Therefore, it is still imperative to develop novel near-infrared fluorescent probes for rapid, highly selective and sensitive detection of Cys.

Herein we report a noticeable NIR fluorescent probe **DP-NIR** for the first time as shown in Scheme 1. The probe **DP-NIR** is composed of a novel fluorophore (dicyanoisophorone) as a reporter and acrylate moieties as a triggered moiety for Cys, and has high

* Corresponding authors at: College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, PR China
E-mail address: wangbingxiang@njnu.edu.cn (B. Wang).

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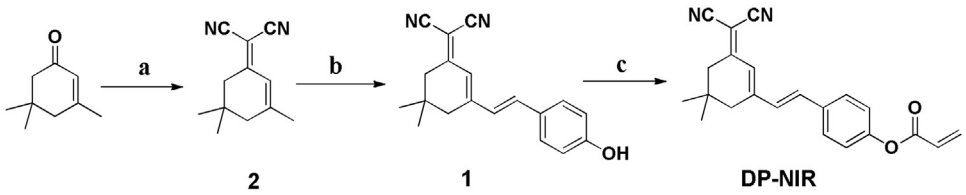

Scheme 1. The synthetic routes of probe **DP-NIR**. Reagents and conditions: (a) malononitrile, ethanol, piperidine; (b) *p*-hydroxybenzaldehyde, acetonitrile, piperidine; (c) acryloyl chloride, CH_2Cl_2, Et_3N, 0 °C.

selectivity and sensitivity for Cys over Hcy and GSH. In addition, this probe can be easily synthesized from cheap commercial raw materials with good yield. It shows almost none background fluorescence, and a rapid fluorescent turn-on response for Cys with a large stokes shift. Moreover, the probe was successfully applied for imaging Cys in living cells. The synthetic route of probe **DP-NIR** is shown in Scheme 1.

2. Experiment

2.1. Materials and instruments

All chemical reagents and solvents were purchased from commercial suppliers and used without further purification. Double distilled water was used throughout the work. All reactions were monitored using thin-layer chromatography (TLC). ^{1}H NMR and ^{13}C NMR spectra were recorded on the Bruker AN-400 spectrometer with chemical shifts reported in ppm (TMS as internal standard). Electron impact mass spectra were run on MAT-212 spectrometer. Ultraviolet-visible (UV-vis) absorption spectra were measured with a Varian Cary 50 spectrophotometer at 1 cm of the light path length. Fluorescence spectra were recorded on Varian Cary Eclipse fluorescence spectrophotometer with an excitation wavelength of 557 nm. High-resolution EI mass spectra were recorded on a MALDI-TOF/TOF Ultrafle Xtreme (Bruker USA) mass spectrometer.

2.2. Synthesis of compound ***2***

A solution of malononitrile (1.98 g, 30 mmol) in dry ethanol (25 mL) was added to a mixture of piperidine (0.25 mL, 2.5 mmol) and isophorone (3.46 g, 25 mmol). The solution was stirred at 50 °C for 10 h. After cooling to room temperature, the black solution was slowly poured into water (200 mL) and the precipitated solid was filtered. Recrystallization from *n*-hexane afforded a brown solid. Mp: 71–73 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.56 (d, J = 1.3 Hz, 1H), 2.53 (s, 2H), 2.24 (s, 2H), 2.05 (s, 3H), 0.96 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.82, 162.87, 119.87, 113.88, 113.09, 76.54, 45.26, 42.41, 32.33, 27.81, 25.43.

2.3. Synthesis of compound ***1***

To a stirred solution of Compound 2 (1.0 g, 5.4 mmol) and *p*-hydroxybenzaldehyde (0.7 g, 5.4 mmol) in dry acetonitrile (60 mL) was added several drops of piperidine. The mixture was stirred at 80 °C for 6 h. The resulting residue was dissolved in 40 mL dichloromethane, washed with water (3 × 40 mL), The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether: ethyl acetate, 20:1, v/v) to afford compound **1** as an orange solid (1.34 g, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.56 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 2.7 Hz, 2H), 6.79-6.81 (m, 3H), 2.60 (s, 2H), 2.53 (s, 2H), 1.01 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.79, 159.79, 157.23, 138.79, 130.35, 127.58, 126.72, 121.83, 116.33, 114.62, 113.80, 75.22, 42.78, 38.64, 32.15, 27.91.

2.4. Synthesis of probe ***DP-NIR***

Compound **1** (0.29 g, 1 mmol) and Et_3N (0.23 mL, 2 mmol) were dissolved in 20 mL anhydrous dichloromethane, the solution became dark red. The resulting mixture was stirred for 30 min in the ice bath at 0 °C. Then, the solution of acryloyl chloride (0.16 mL, 2 mmol) in 3 mL dichloromethane was added dropwise. The resulting mixture was stirred for 1 h, and then was allowed to react at room temperature until the reaction was completed (monitored by TLC). The reaction mixture was added with 25 mL water to wash the solution three times, and then the dichloromethane phase was dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, the crude solid product was further purified by silica column chromatography (petroleum ether: ethyl acetate = 10/1, v/v) to afford the pure product **DP-NIR** (0.15 g, 91% yield). Melting point: 166–168 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (d, J = 8.7 Hz,2H), 7.45 (d, J = 16.2 Hz, 1H), 7.32 (d, J = 16.2 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 6.91 (s, 1H), 6.56 (dd, J = 17.3, 1.4 Hz, 1H), 6.43 (dd, J = 17.3, 10.2 Hz, 1H), 6.18 (dd, J = 10.2, 1.4 Hz, 1H), 2.63 (s, 2H), 2.56 (s, 2H), 1.03 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.85, 164.46, 156.18, 151.46, 136.91, 134.35, 128.01, 123.42, 122.78, 114.31, 113.48, 76.99, 42.76, 38.61, 32.17, 27.91. HRMS (ESI): ($C_{22}H_{20}N_2O_2$) m/z: calculated for $[M + Na]^+$: 367.1422; found 367.1411.

2.5. Sensing mechanism of probe ***DP-NIR*** with Cys

Cys (78 mg, 0.5 mmol) was added to a solution of DP-NIR (172 mg, 0.5 mmol) in 10 mL DMSO-PBS(v/v = 1/1). Then the reaction mixture was stirred at room temperature until the reaction was completed (monitored by TLC). The mixture was extracted with dichloromethane for three times. The combined organic layers were removed under reduced pressure. The residue was purified by silica column chromatography (petroleum ether: ethyl acetate = 20/1, v/v) to afford compound **A**.

2.6. UV–vis and fluorescence measurements

Stock solution of **DP-NIR** (1 mM) was prepared in analytical grade DMSO. Other analytes including Alanine (Ala), arginine (Arg), asparagine (Asn), aspartic(Asp), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), cysteine (Cys), homocysteine (Hcy), glutathione (GSH), phenylalanine (Phe), proline (Pro), serine (Ser), tyrosine (Trp), tyrosine (Tyr) were dissolved in deionized water to afford 10 mM aqueous solution. The stock solutions were made freshly and were diluted to desired concentrations. For a typical optical study, a **DP-NIR** (10 μM) solution in PBS buffer (10 mM, pH 7.4, with 50% DMSO) was prepared. One-time glue dropper was used to absorb 3.0 mL of **DP-NIR** solution to a quartz