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A triphenylamine as a fluorophore and maleimide as a bonding group selective turn-on fluorescent imaging probe for thiols

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ABSTRACT

With the biological importance of biothiols, the development of probes for thiols has been an active research area in recent years. Here, we report a novel thiol-reactive fluorescent probe based on Michael addition reaction for selectively detecting thiols over other relevant biological species. The thiol adduct was characterized using NMR and mass spectroscopy and detection mechanism was further confirmed. This sensor with excellent selectivity for biothiols over other amino acids features a rapid signal response time, a good linearity range and a low detection limit. For the practical application of the sensor, it can be used to monitor thiol in live cells with turn-on fluorescence imaging.

1. Introduction

Biological thiols including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) are components of many peptides, which play crucial roles in maintaining the biological redox homeostasis through the equilibrium of free thiols and oxidized disulfides in biological systems [1–3]. It is known that intracellular concentration of GSH is much higher than Cys (Cys: 30–200 μM; GSH: 1–10 mM) [4,5]. In contrast, in healthy human plasma, Cys concentration is typically 10 times that of GSH, 20–30 times that of Hcy, which normally presents below 12–15 μM [4]. However, the alterations in the level of thiols in biological fluids are implicated in a variety of diseases [6–8]. For example, Cys deficiency is involved in many syndromes such as slow growth in children, hair depigmentation, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and weakness [9]. At elevated levels in plasma, Hcy is a risk factor for Alzheimer's disease [10], folate and cobalamin (vitamin B12) deficiencies [11], and cardiovascular diseases [12]. GSH is the most abundant intracellular non-protein thiol [13], which serves many cellular functions, including maintenance of intracellular redox activities, xenobiotic metabolism, intracellular signal transduction, and gene regulation [14,15]. Owing to their important roles, sensitive and selective detection of thiols has received growing attention in recent years.

In recent times, fluorescent molecular probes have emerged as an attractive tool for selective detection of various chemical and biological components [16–19], including thiols [20–27]. Compared to traditional techniques, chemical probes based on absorption or fluorescence changes are more feasible for detecting analyses owing to their many appealing advantages such as low detection limit, high selectivity, its real-time monitoring and its potential for in vivo imaging of living cells. However, fluorescence quenching may be caused by a number of factors other than the target analyte, and thus the sensing behavior may be nonspecific [28]. Probes that rely on fluorescence quenching suffer from inherent drawbacks including low signal-to-noise ratio and non-specific quenching [29], so that "turn-on" type fluorescence probes are preferred [30–33].

Maleimide groups are known to react fairly selectively with thiols via addition reactions involving their C=C double bond. They are also known to quench fluorescence in their conjugated form, but not as their thiol adduct products [34]. These properties were demonstrated in the characterization of fluorophores bearing a maleimide group whose fluorescence increased dramatically

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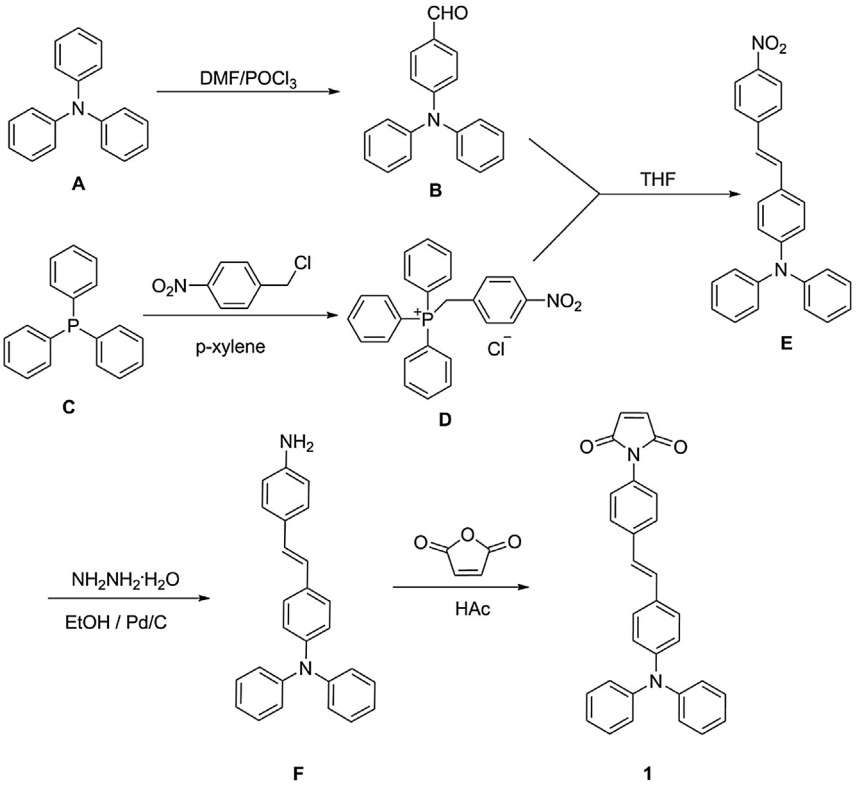

Scheme 1. The synthesis of the probe 1.

upon reaction with thiols [35–37]. Based on these issues, we synthesized a new thiol-reactive fluorescent probe containing triphenylamine and maleimide (Scheme 1). When the concentration of probe is low, Hcy/GSH induced a significant enhancement in fluorescence intensity whereas Cys induced almost no change, while increasing amount of probe, fluorescence enhancement induced by Cys was enough to make clear the recognition. Furthermore, this probe was successfully applied in fluorescent imaging in living cells.

2. Materials and methods

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma–Aldrich (St. Louis, MO). Sodium hydroxide solution (0.1 mol/L) was added to aqueous HEPES (10 mmol/L) to adjust the pH to 7.4. Amino acids were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on an Agilent 8453 UV–Visible spectrophotometer. Fluorescence spectra were measured on F-7000 FL Spectrophotometer. A PO-120 quartz cuvette (10 mm) was purchased from Shanghai Huamei Experiment Instrument Plants, China. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 MHz (and 600 MHz) and 75 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). ESI was measured with an LTQ-MS (Thermo) instrument. LC-MS was measured with Bruker solari X FTMS. The ability of probe reacting to thiols in the living cells was also evaluated by laser confocal fluorescence imaging using an Olympus FV1000 laser scanning microscope.

2.3. Preparation and characterization of probes

2.3.1. Preparation and characterization of B

The synthesis route is summarized in Scheme 1. $POCl_3$ (11 mL) was added to a DMF solution (250 mL) with stirring for 2 h in ice-water bath. After the color change to nacarat, added triphenylamine (100 mmol) to the solution with stirring at 40 °C. After the reaction was complete, the reaction mixture was poured into ice water (1000 mL) and adjusted pH to 9 with NaOH to separate faint yellow crystals out. The solid obtained by filtered, washed with water and recrystallized in ethanol to give compound B in 86% as a faint yellow powder. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 9.77 (s, 1H), 7.72 (d, 2H, J = 8.7 Hz), 7.43 (t, 4H, J = 15.6 Hz), 7.23 (m, 6H), 6.88 (d, 2H, J = 8.7 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 117.7, 125.0, 126.0, 128.1, 129.6, 130.9, 145.1, 152.3, 190.1; Elemental analysis (calcd. %) for $C_{19}H_{15}NO$: C, 83.49, H, 5.53, N, 5.12, Found: C, 83.46, H, 5.54, N, 5.14; ESI–MS m/z: $[B + H]^+$ Calcd for $C_{19}H_{16}NO$ 274.12, Found 273.92 (Fig. S1).

2.3.2. Preparation and characterization of C

A mixture of C (triphenylphosphine, 0.16 mol) and 4-nitrobenzyl chlorine (0.15 mol) in paraxylene (200 mL) was stirred and refluxed at 150 °C for 2 h. The mixture was then cooled to 0 °C and filtered. The taupe solid thus obtained was dried under vacuum to give compound D in 92%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 8.11 (d, 2H, J = 8.1 Hz), 7.93 (s, 3H), 7.75 (d, 12H, J = 9.9 Hz), 7.30 (d, 2H, J = 8.1 Hz), 5.60 (s, 1H), 5.54 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 116.0, 117.1, 122.9, 129.3, 129.5, 131.4, 133.2, 133.4, 134.5, 135.4, 135.6, 146.5; Elemental analysis (calcd. %) for $C_{25}H_{21}ClNO_2P$: C, 69.21, H, 4.88, N, 3.23, Found: C, 69.20, H, 4.90; N, 3.20 (Fig. S2).