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A fluorescein-based fluorescence probe for the fast detection of thiol

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ABSTRACT

A new turn-on fluorescent probe for the selective detection of thiol over other amino acids was synthesized. Probe possesses the widely-used thiol-selective 2,4-dinitrobenzenesulfonyl (DNBS) group which can react with thiol and release the fluorescein which has strong fluorescence. Fluorescein, a well known xanthene fluorescent dye, has two states at different environment. Fluorescein is in the state of spirocyclization when connected with 2,4-dinitrobenzenesulfonyl (DNBS) group which has no fluorescence. However, it is in the state of open form when it reacts with thiol which has a strong fluorescence. The transition of the two states can be used to selectively detect thiol and the color can change from colorless to yellow which can be differentiated by naked eyes. Upon the titration of thiol, the absorption band at 454 nm rises gradually and the fluorescence emerges at 521 nm and the detection limit can be as low as 0.16 μM. All of such good properties prove it to be a good sensor for the selective detection of thiol and it shows a potential use in bioimaging applications.

Introduction

Biological thiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), play essential roles in maintaining the appropriate redox status in physiological and pathological processes.[1,2] Generally, the abnormal level of cellular thiols has been related with numerous diseases such as psoriasis, slowed growth, liver damage, leukocyte loss, cancer, and AIDS.[3,4] Cysteine (Cys), one of the most abundant biothiols in living organisms, plays important roles in biological systems[5] and has been associated with neurotoxicity. Its abnormal level can cause hair pigmentation, retardation in growth, liver damage, and skin lesions.[4] Elevated level of Hcy is closely related to Alzheimer's disease,[6] cardiovascular,[7] neural tube defects, complications during pregnancy, and osteoporosis. GSH, the most abundant intracellular thiol with the concentration of millimolar range,[8] plays crucial roles in many cellular functions such as xenobiotic metabolism, maintenance of intracellular redox activity, and gene regulation.[9–11] An abnormal level of GSH is directly associated with cancer, aging, heart problems, and other ailments. Accordingly, the design and synthesis of a sensor for the detection of thiol is of significant importance.

Considering the vital important roles in human body, great efforts have been made for the detection of thiol.[12,13] Fluorescent probe, famous for its high sensitivity, relatively simple analysis protocols,[14] and far less expensive, has got much attention for the detection of metal ions,[15,16] hydrion,[17] anion,[18,19] and especially popular for the detection of biomolecule[20–22] in recent years. Lin[23] et al. designed a new NCL-mechanism-based ratiometric fluorescent probe with specificity toward aminothiols. The new fluorescent probe is capable of ratiometric detecting of aminothiols in newborn calf and human serum samples and is also suitable for ratiometric fluorescent imaging of aminothiols in living cells. Zhu[24] et al. applied fluorescein as fluorescent reporter, a 7-aminocoumarin as photo-trigger and a thiol-removable energy acceptor. Such doubly locked probes have bright prospect, promising biochemically and optically labeling cells with spatial precision for monitoring dynamic processes of cells in vitro or in vivo. Li[25] et al. used naphthalimide as the fluorescence source for the detection of thiol. The synthesized two naphthalimide-based fluorescent probes provided high on/off signal ratios and exhibited good selectivity toward thiols over other analytes and they were identified as good sensors for the detection of thiols both in living cells and in rabbit plasma samples.

Fluorescein is widely used as fluorescent sensors in biological systems.[26–28] Its relatively high molar absorption coefficients and quantum yields, high-intensity emission peaks, relative inertness under physiologically relevant conditions, and relative nontoxicity makes it an excellent dye for the design of sensors. On the other hand, fluorescence spectroscopy has become a powerful method for sensing and imaging trace amounts of samples because of its simplicity,[29,30] sensitivity, fast response times, and its application for not only in vitro assays but also in vivo imaging studies.[31,32]

As we all know, fluorescein exhibits strong fluorescence when it is in the state of open form while it has non-fluorescence when it is in the spirocyclic form. The spirocyclic form of fluorescein is

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colorless and nonfluorescent due to the break of the π-conjugation, whereas the ring opening form shows strong spectroscopic signals in both the absorption and fluorescence spectra because of the π-conjugation. Using the transformation of the two states can achieve the goal of testing different species. Here, we synthesized an asymmetric fluorescein-based probe for the detection of thiol using 2,4-dinitrobenzenesulfonyl (DNBS) group as the detection group. When it is connected with DNBS, the fluorescent probe shows either colorless in the absorption band nor non-fluorescence in the emission band because of the formation of spirocyclic form. However, it shows a color of yellow and a strong fluorescence which can be differentiated by naked eyes when it is treated with thiol. The detection limit can be as low as 0.16 μM.

Experiment

Chemicals and instrumentals

Fluorescein was purchased from Energy Chemical and used directly without any purification. The other chemicals were of the highest grade available and were used without further purification. All employed solvents were analytically pure and were employed without any further drying or purification.

Reactions were monitored by TLC using Merck Millipore DC Kieselgel 60 F-254 aluminum sheets. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker AM-400 MHz instruments with tetramethylsilane as internal standard. Low-resolution ESI mass analyses were performed on a Waters LCT Premier XE spectrometer. UV–vis absorption spectra were recorded on a SHIMADZU UV–Vis spectrophotometer. Fluorescence spectra were measured on a SHMADZU RF-5301PC Fluorescence spectrophotometer.

Preparation and characterization of 2

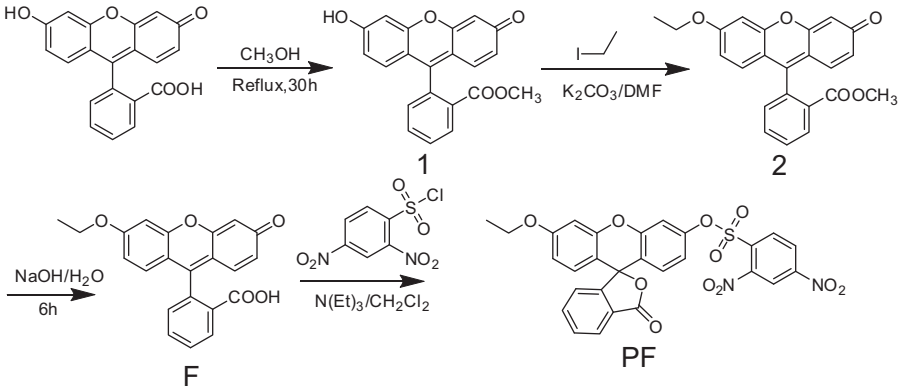
Fluorescein methyl ester **1** was synthesized according to the reported literature.[33,34] Then, compound **2** was synthesized according to the method of the reported Letter.[35] Compound **1** (1.6 g) and K_2CO_3 (0.5 g) were dissolved in 15 mL DMF. 0.5 mL iodoethane was added to the above solution. The whole mixture was heated at 65 °C for 6 h under stirring. After being cooled to room temperature, the solution was added to 80 mL 5% sodium chloride solution under stirring. The precipitates were collected and dried in vacuum. The targeted compound is isolated by flash column chromatography on silica gel using dichloromethane/methanol (20:3, v/v) for elution. The target products were in the color of yellow and in the state of solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J = 8 Hz, 1H), 7.71 (m, 2H), 7.30 (m, 1H), 6.89 (m, 3H), 6.74 (dd, 1H), 6.58 (m, 1H), 6.51 (d, J = 2 Hz, 1H), 4.14 (m, 2H), 3.63 (d, J = 8 Hz, 3H), 1.47 (m, 3H) (Fig. S1). ^{13}C NMR δ 185.74, 165.59, 163.70, 159.17, 154.45, 151.16, 134.56, 132.71, 131.14, 130.39, 129.56, 128.93, 117.34, 114.66, 114.06, 106.58, 100.59, 77.40, 77.08, 76.76, 64.52, 52.42, 14.54 (Fig. S2). Yield 1.2 g.

Preparation and characterization of F

Compound **3** was synthesized according to our previous work.[36] Compound **2** (1 g) was dissolved in 15 mL methyl alcohol and 20 mL sodium hydroxide solution at the concentration of 2 M was added to the above solution. The whole mixture was stirred overnight in room temperature. After that, 20 mL water was added and the pH was adjusted to 7 using HCl. The precipitates were filtered and dried in vacuum. The targeted compound is isolated by flash column chromatography on silica gel using dichloromethane/methanol (20:3, v/v) for elution. The compounds were in a yellow and in the state of solids. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.17 (s, 1H), 8.03 (dd, 1H), 7.80 (m, 1H), 7.72 (t, J = 4 Hz, 1H), 7.28 (m, 1H), 6.92 (m, 1H), 6.68 (m, 3H), 6.57 (d, J = 8 Hz, 2H), 4.06 (m, 2H), 1.39 (m, 3H) (Fig. S3). ^{13}C NMR δ 168.64, 159.51, 151.78, 135.61, 130.10, 128.98, 126.02, 124.62, 123.97, 112.73, 110.80, 109.40, 102.17, 101.12, 82.71, 63.60, 39.88, 39.67, 39.46, 39.25, 39.05, 14.42 (Fig. S4). HRMS: 361.1082, calcd for: 301.1076 (Fig. S7). Yield 0.8 g.

Preparation and characterization of PF

Probe PF was synthesized according to the methods of the reported papers.[37,38] Compound F (0.15 g) was dissolved in 15 mL anhydrous dichloromethane and kept in ice bath under stirring. Then, triethylamine (150 μL) was added to the above solution. 2,4-Dinitrobenzenesulfonyl chloride (0.133 g) was dissolved in 5 mL anhydrous dichloromethane and added to the above solution dropwise in 30 min. The whole mixture was stirred in ice bath for another 30 min and stirred in room temperature for 4 h. After that, the solvents were evaporated and the product was purified by flash column chromatography on silica gel using dichloromethane/alcohol (100:1, v/v) as the eluent. The probe was in the color of light yellow and in the state of solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, J = 4 Hz, 1H), 8.52 (dd, 1H), 8.25 (d, J = 12 Hz, 1H), 8.02 (d, J = 8 Hz, 1H), 7.67 (m, 2H), 7.19 (d, J = 4 Hz,1H), 7.15 (d, J = 8 Hz, 1H), 6.90 (m, 1H), 6.80 (d, J = 8 Hz, 1H), 6.75 (dd, 1H), 6.65 (m, 2H), 4.05 (q, 2H), 1.43 (t, J = 4 Hz, 3H) (Fig. S5). ^{13}C NMR δ 169.02, 161.03, 152.55, 152.13, 151.88, 149.41, 135.38, 134.08, 133.23, 130.10, 128.96, 126.73, 126.35, 126.29, 123.89, 120.49, 119.47, 117.19, 112.81, 110.93, 110.26, 101.35, 81.87, 77.36, 77.05, 76.73, 63.99, 14.62 (Fig. S6). HRMS: 591.0715, calcd for: 591.0710 (Fig. S8). Yield 0.1 g.



Scheme 1. The synthesis procedure of the probe PF.