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A fluorescent probe capable of discriminately and simultaneously detecting DL-dithiothreitol and single sulfhydryl-containing thiols

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ABSTRACT

DL-Dithiothreitol (DTT) is used mainly in clinical and laboratorial fields. It also participates in several cellular mechanisms and can be used in some diseases treatment like cystinosis or medical conditions resulting from ion or metal toxicity. Hence, DTT detection in biological milieu is of great significance. However, most of the current fluorescent detection systems cannot discriminate DTT from single sulfhydryl-containing biothiols. Herein, a fluorescent probe for DTT has been synthesized by linking maleimide to a pyrene fluorophore. It responds to DTT or single sulfhydryl-containing thiols at different emission bands, affording it the capability to discriminately detect DTT from those thiols with single —SH. The probe responds to dithiothreitol quickly and shows high selectivity through excimer emission of pyrene moieties. As for the fluorescence intensity of the probe versus DTT concentration, two linear ranges (0–3 and 4.5–6.0 μM) can be observed, and the detection limit for DTT is 0.07 μM. Furthermore, the probe exhibits low cytotoxicity and can be successfully used in fluorescence imaging in both L929 and Hela cell lines.

1. Introduction

DL-Dithiothreitol (DTT), also known as Cleland's reagent, is a protective reagent for sulfhydryl groups and has been widely applied in both peptide/protein chemistry and cell biology [1]. Discovered in the middle of last century, this small-molecular and water-soluble reagent is capable of reducing disulfides completely, protecting sulfhydryl groups from oxidation and preserving monothiols in the reduced state. As a potent reducing agent, it is used mainly in clinical and laboratorial fields, and it is also be used in some diseases treatment like cystinosis or medical conditions resulting from ion or metal toxicity [2–5,9]. Recently, DTT has been employed as a remarkable inhibitor for cell apoptosis and an inducer of unfolded protein as well as a protector for cell aging [6–8].

DTT itself prefers to form a six-membered ring with disulfide bridge, exhibiting a strong reducing ability to break up disulfide bonds in some biomacromolecules [9]. It is usually employed as the reducing agent for protecting proteins/peptides in the processes such as expression of recombinant proteins, proteins extraction and purification and storage with a relatively high level of 1-10 mM [10–12]. However, abnormal levels of DTT may cause oxidative damage in peptide, protein and DNA, resulting in destruction and dysfunction of some biomacromolecules [5,13–15]. The ubiquitous use of DTT in biology, biochemistry and ecology, in a way, declares the significance of DTT detection in biological milieu.

To date, there are a few quantitative detecting approaches for DTT [16,17]. For example, Cindric and coworkers employed HPLC–MS to detect DTT in complex protein mixtures [16]. On the other hand, fluorescence detection has proven to be highly selective, easy to operate and sensitive compared with other detection techniques [18–44], and several fluorescent probes for DTT have been successfully developed [17,45]. However, most of the probes cannot discriminate DTT from biothios. Recently, Zhang, Tan and coworkers synthesized a ratiometric fluorescent probe for detecting DTT, which could react with DTT, triggered the cleavage of piazselenole-based carbamate protecting group, and finally restored the green fluorescence of 4-aminonaphthalimide; and this probe was able to respond toward DTT but not single sulfhydryl-containing biothiols[17].

Since the thiol-disulfide interchange is essential for a wide range of biological systems, and in the meantime DTT plays critical roles in the protection of sulfhydryl groups and in the maintenance of biological functions for many biomacromolecules, the simultaneously discriminating detection of DTT and single sulfhydryl-containing biothiols may allow researchers to better understand the interplays of DTT and single sulfhydryl-containing biothiols in biological

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systems, so as to use DTT safely in laboratorial and medical applications.

Herein, we demonstrate a facile strategy to simultaneously and discriminately detect DTT and single sulfhydryl-containing thiols in aqueous media or inside cells by using a single fluorescent probe (Py-MA). The probe was synthesized by linking a pyrene fluorophore to a maleimide group (Scheme S1). For this DTT/thiol probe, the maleimide group is highly responsive to sulfhydryl-containing compounds through Michael addition; and it is also a strong electron withdrawing group, capable of quenching the emission of some fluorophores through photoinduced electron transfer (PET) process [46,47]. On the other hand, pyrene was selected as the fluorophore, which is famous for its monomer (emission at 350–400 nm)–excimer (emission at 450–500 nm) interconversion [48–50]. As shown in Fig. 1, the probe Py-MA is supposed to be non-fluorescent in the absence of DTT or thiols due to the PET effect. However, in the presence of single sulfhydryl-containing thiols, the PET process is hindered, thereby restoring the monomer fluorescence of pyrene moieties; while in the presence of DTT, Py-MA prefers to respond to both the sulfhydryls on DTT, resulting in π–π stacking of pyrene moieties and hence generating strong excimer fluorescence. Based on similar principles, some other pyrene derivatives have been applied in the study of the conformation and conformational changes of some proteins (Table S1). The main features of the sensing system herein include: sensitive fluorescent turn-on detection for DTT, distinguishing DTT from thiols, and discriminately and simultaneously detecting DTT and single sulfhydryl-containing thiols.

2. Experimental

2.1. Reagents and materials

3-Aminopropanoic acid, maleic anhydride, 1-pyrenemethanol, amino acid: L-alanine (Ala), L-arginine (Arg), L-aspartic acid (Asp), cysteine (Cys), L-valine (Val), L-glutamine (Gln), L-histidine (His), L-isoleucine (Ile), glycine (Gly), L-serine (Ser), L-asparagine (Asn), L-leucine (Leu), L-lysine (Lys), L-phenylalanine (Phe), L-glutamic acid (Glu), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr), L-methionine (Met), L-proline (Pro), glutathione (GSH), homocysteine (Hcy) and bovine serum albumin (BSA) were purchased from Aladdin Reagents. Dithiothreitol, 4-dimethylaminopyridine (DMAP) and N,N-diisopropylcarbodiimide (DIC) were purchased from Alfa Aesar. Thioredoxin (Trx) from Escherichia coli and alkaline phosphatase (ALP) from bovine intestinal mucosa were purchased from Sigma–Aldrich. NaCl, KCl, $MgSO_4$, $FeCl_3$, $ZnCl_2$, $CaCl_2$, $Fe(NH_4)_2{\cdot}(SO_4)_2{\cdot}6H_2O$, $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}7H_2O$ were purchased from Guangzhou Chemical Reagent Factory.

All solvents (acetic acid, dichloromethane, ethyl acetate, dimethylsulfoxide) were of analytical grade. The water used in the experiments was the triple-distilled water treated by ionexchange columns and then by a Milli-Q water purification system.

2.2. Measurements

^1H NMR and ^{13}C NMR spectra were obtained on a BrukerAvance 600 MHz NMR spectrometer (acquisition software: Topspin; processing programs: MestReNova). Mass spectra were measured through a Bruker Esquire HCT Plusmass spectrometer. UV–vis spectra were recorded by using a HitachiU-3010 UV–vis spectrophotometer. Fluorescence spectra were measured by using a Hitachi F-4600 fluorescence spectrophotometer. The fluorescence lifetime data were obtained by an Edinburgh Instrument FLS920 fluorescence spectrometer equipped with a nF900 nanosecond flash lamp. HPLC data were acquired from Agilent 1260 Infinity liquid chromatograph (stationary phase: Eclipse plus C18, 4.8 mm × 100 mm; solvents: acetonitrile: methanol: water = 7:2:1).

2.3. Synthesis of 3-maleimidopropionic acid

3-Aminopropanoic acid (890 mg, 10 mmol) was dissolved in acetic acid (15 mL) in a round-bottomed flask. Maleic anhydride (980 mg, 10 mmol) was dissolved in acetic acid (6 mL) and added dropwise slowly by syringe. The solution became cloudy and was kept stirring at ambient temperature for 4 h. Then the reaction mixture was refluxed overnight under nitrogen. The mixture was cooled to room temperature next day and the solvent was evaporated under reduced pressure. The product was purified on a silica gel column chromatography (1000:100:1 = dichloromethane:methanol:acetic acid) to yield a white solid (1.15 g, yield 62%). ^1H NMR (600 MHz, $CDCl_3$) δ 6.73 (s, 2H), 3.84 (t, J = 7.2 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.18, 170.36, 134.28, 33.30, 32.50. ESI-MS: m/z 167.80 $[M-H]^-$.

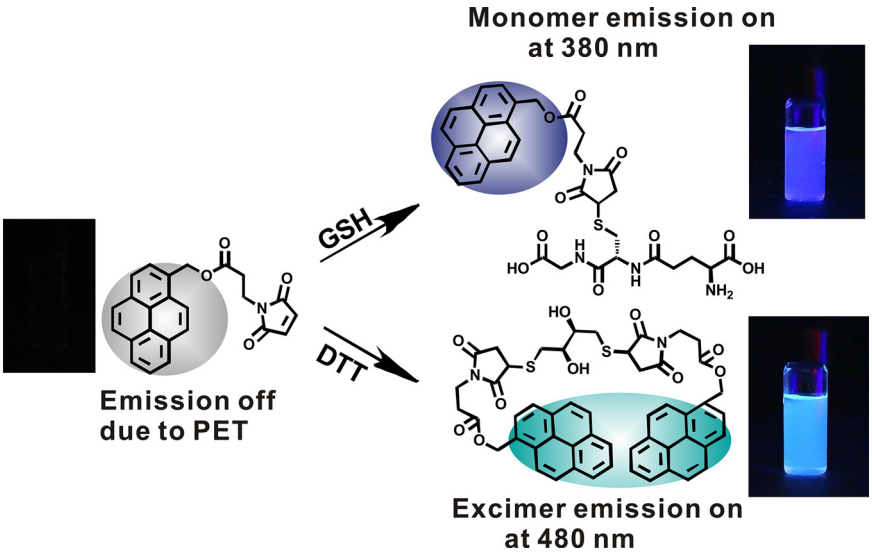

Fig. 1. Schematic illustration for Py-MA and its detection of DTT and thiol at different emission wavelengths.