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Styryl-BODIPY based red-emitting fluorescent OFF–ON molecular probe for specific detection of cysteine

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ABSTRACT

We have synthesized a styryl boron-dipyrromethene (BODIPY)/2,4-dinitrobenzenesulfonyl (DNBS) dyad based red-emitting molecular probe for specific detection of cysteine among the biological thiols. The probe shows intensive absorption at 556 nm and the probe is non-fluorescent. The DNBS moiety can be cleaved off by thiols, the red emission of the BODIPY fluorophore at 590 nm is switched on, with an emission enhancement of 46-fold. The probe shows good specificity toward cysteine over other biological molecules, such as glutathione and amino acids. The emission of the probe is pH-independent in the physiological pH range. The probe is used for fluorescent imaging of cellular thiols. Theoretical calculations based on density functional theory (DFT) were used to elucidate the fluorescence sensing mechanism of the probe, which indicate a dark excited state (S_1) for the probe but an emissive excited state (S_1) for the cleaved product (i.e. the fluorophore).

1. Introduction

Selective detection of biological thiols, such as cysteine, homocysteine and glutathione, have attracted much attention due to the pivotal role of these –SH containing molecules in physiological pathways (Chen et al., 2010a; Weh et al., 2007; Tang et al., 2007; Refsum et al., 2004; Hong et al., 2009; Lee et al., 2010; Huo et al., 2010; Shang and Dong, 2009; Yu et al., 2007; Huang et al., 2010; Yi et al., 2009; Wang et al., 2009). For example, increased white cell cysteine concentrations are only observed in individuals with cystinosis. Despite improved genetic diagnosis in recent years, the measurement of white cell cysteine remains the easiest and quickest way to confirm or rule out a clinical diagnosis of cystinosis. Fluorescent molecular probes are the promising tools to detect thiols, especially for in vivo fluorescent analysis such as bioimaging, due to the high spatial and temporal resolution, high sensitivity and the easy-to-detect signal relay of the molecular probes (Wolfbeis and Narayanaswamy, 2004).

A wide variety of sensing mechanisms have been developed for molecular fluorescent thiol probes (Li et al., 2009; Chen et al., 2010a,b; Lin et al., 2010; Ruan et al., 2010; Yi et al., 2009; Amoroso et al., 2008; Lee et al., 2008; Tang et al., 2009; Zhu et al., 2010; Zhang et al., 2007, 2010a,b). One of the methods is to attach electrophilic group to the fluorophores (e.g. iodoacetamides and benzyl halides) (Amoroso et al., 2008). Alternatively dyads of fluorophore/maleimides were used as thiol probes (Matsumoto et al., 2007; Weh et al., 2007; Maeda et al., 2006). Fluorophores containing formyl (–CHO) group were also used as thiol probes, which are based on tuning the intramolecular charge transfer (ICT) effect by addition with thiols (Tang et al., 2007; Tanaka et al., 2004; Zhang et al., 2006; Lee et al., 2008; Chen et al., 2010b).

A photo-induced electron transfer (PET) thiol probe with boron-dipyrromethene (BODIPY) as the fluorophore/electron donor and maleimide as the electron acceptor is in particular interesting (Matsumoto et al., 2007). BODIPY is an ideal fluorophore for assembly molecular probes due to its characteristic photophysics, such as the intensive absorption at ca. 500 nm (large UV–vis molar extinction coefficients, ε), high fluorescence quantum yield (even in aqueous media), and pH-independent emission and excellent photostability (Peng et al., 2007; Cheng et al., 2008; Zhang et al., 2008; Bozdemir et al., 2010). However, the characteristic emission of the parent BODIPY fluorophore is centered at ca. 520 nm with a small Stokes shift (ca. 20 nm, Ulrich et al., 2008). Molecular probes with longer emission wavelength and excitation wavelength are desired because the biological tissues have a transmission window in the red/near IR region, not in the blue/green region of the spectrum. Furthermore, the biological samples can be excited with short excitation wavelength, thus the background fluorescence emission

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of the samples will impose significant interference on the fluorescence relay. However, to the best of our knowledge, no BODIPY fluorophore with longer emission (such as in the red) have been used as fluorophore for molecular thiol probes (Chen et al., 2010a).

Previously we designed a fluorescent thiol probe based on the 2,4-dinitrobenzenesulfonyl (DNBS) protected ethynylated pyrene moiety (Ji et al., 2009). However, the fluorescence quantum yield (Φ) of our pyrene based thiol probe is low, especially in aqueous solution (Φ = 0.018), due to the quenching effect of the hydrogen bonding between the probe and the protic solvent molecules. Recently we devised a Ru(II) polyimine phosphorescent thiol probe, for which the emission is not quenched by protic solvents (Ji et al., 2010a). However, the excitation of this phosphorescent probe is in the blue region and the absorbance is only moderate (molar extinction coefficients ε = 18,610 M^{-1} cm^{-1} at ca. 450 nm) (Ji et al., 2010a). Molecular probes with intensive absorbance in the visible region are desired because the fluorescence detection sensitivity can be improved.

Moreover, very few cases have been reported to use theoretical approach to elucidate the sensing mechanism of the fluorescent molecular probes. Previously we used theoretical calculations based on density functional theory (DFT) and time-dependent DFT (TDDFT) to rationalize the sensing mechanism of thiol probes (Ji et al., 2009, 2010a). However, our strategy needs to be generalized to more molecular probes with different fluorophores.

In order to tackle the aforementioned limitations, herein we devised red-emitting BODIPY based thiol probe **1** based on BODIPY/DNBS dyad. The probe shows intensive absorption in green region. Probe **1** is non-fluorescent but intense red emission is switched on in the presence of cysteine. The probe was also used for fluorescence imaging of cellular thiols.

2. Experimental

2.1. Apparatus and general methods

A JASCO FP-6500 or a Sanco 970 CRT spectrofluorimeter was used for fluorescence measurements. The absorption spectra were recorded with a HP8453 UV–vis spectrophotometer. The fluorescence images of cells for the staining experiments were performed with Nikon ECLIPSE-Ti confocal laser scanning microscope.

2.2. Synthesis of ***1a***

2,4-Dimethyl pyrrole (2.48 g, 0.026 mol) and benzoyl chloride (1.83 g, 0.013 mol) were dissolved in 250 mL dry CH_2Cl_2 under argon atmosphere. One drop of trifluoroacetic acid (TFA) was added to the solution and reaction mixture was stirred at room temperature overnight. 10 mL anhydrous triethylamine was added to the mixture and after stirring for 15 min, 10 mL $BF_3{\cdot}Et_2O$ was added dropwise at 0 °C. The stirring was continued for 2 h and then the reaction mixture was washed with water. The organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified with column chromatography (silica gel, CH_2Cl_2). Red solid was obtained, 0.75 g (18.0%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.49–7.47 (m, 3H, Ar-H), 7.29–7.26 (m, 2H, Ar-H), 5.98 (s, 2H, Pyrrole-H), 2.56 (s, 6H, Pyrrole-CH_3), 1.37 (s, 6H, Pyrrole-CH_3).

2.3. Synthesis of BODIPY ***1b***

A mixture of compound **1a** (486.0 mg, 1.5 mmol) and 4-hydroxylbenzaldehyde (183.0 mg, 1.5 mmol), dry toluene (15 mL), piperidine (0.7 mL) and galactic acid (0.7 mL) were refluxed for 9 h. Water formed during the reaction was removed azeotropically by a Dean-Stark apparatus. After completion of the reaction, the mixture was extracted with dichloromethane, then the organic phase was washed with brine (3× 10 mL), the organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residual was purified with column chromatography (silica gel, petroleum ether/ethyl acetate, 3:7, v/v). Red solid was obtained, 67.0 mg (10.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.44 (m, 6H, 5H for Ar-H, 1H for vinylic bond), 7.31 – 7.29 (m, 2H, Ar-H), 7.20 – 7.16 (d, 1H, J = 16.0 Hz, vinylic), 6.84–6.82 (d, 2H, J = 8.0 Hz, Ar-H), 6.58 (s, 1H, Pyrrole-H), 6.00 (s, 1H, Pyrrole-H), 5.48 (s, 1H, OH), 2.60 (s, 3H, Pyrrole-CH_3), 1.42 (s, 3H, Pyrrole-CH_3), 1.39 (s, 3H, Pyrrole-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 154.9, 153.7, 142.9, 142.6, 140.3, 136.4, 135.3, 133.0, 131.9, 129.6, 129.4, 129.3, 129.1, 128.4, 121.3, 117.7, 117.1, 116.0, 46.3, 14.8, 14.5. TOF HRMS (EI) ($[C_{26}H_{23}BF_2N_2O+H]^+$) calcd. 428.1872, found 428.1911.

2.4. Synthesis of probe ***1***

BODIPY **1b** (64.0 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (5 mL). Then dry triethylamine (0.1 mL) was added to the solution, the mixture was stirred for 5 min. Then 2,4-dinitrobenzeneslfonyl chloride (120.0 mg, 0.45 mmol) in CH_2Cl_2 (15 mL) was added dropwise to the above solution at 0 °C. The reaction mixture was stirred for 6 h at 50 °C. The solvent was removed under reduced pressure and the crude product was subjected to column chromatography (silica gel, petroleum ether/ethyl acetate, 3:7, v/v). Probe **1** was obtained as a red solid (28.0 mg, 28.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H, Ar-H), 8.50 (d, 2H, J = 8.0 Hz, Ar-H), 8.15 (d, 1H, J = 8.0 Hz, vinylic), 7.63 – 7.50 (m, 6H, 5H for Ar-H, 1H for vinylic), 6.57 (s, 1H, Pyrrole-H), 6.04 (s, 1H, Pyrrole-H), 2.59 (s, 3H, Pyrrole-CH_3), 1.42 (s, 3H, Pyrrole-CH_3), 1.40 (s, 3H, Pyrrole-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.1, 151.3, 150.3, 149.0, 148.6, 141.6, 136.7, 134.7, 134.4, 134.2, 132.5, 131.9, 131.1, 129.8, 129.8, 129.3, 128.4, 128.0, 127.5, 123.2, 122.5, 121.5, 120.5, 120.0, 118.6, 42.4, 14.6, 14.5. TOF HRMS EI ($[C_{32}H_{25}BF_2N_4O_7S+Na]^+$) calcd. 681.1403, found 681.1415.

2.5. Fluorescence quantum yields

The fluorescence quantum yields of the probe **1** and the BODIPY **1** were measured with quinine sulfate as standard (Φ = 0.546 in 0.05 M H_2SO_4), according to Eq. (1), where Φ is the quantum yield, I is the measured integrated emission intensity, and A is the optical density (absorbance). The u refers to the fluorophore of unknown quantum yield, and s refers to the reference fluorophore (quinine sulfate) of known quantum yield:

$$\Phi_u = \Phi_s \times \frac{I_u}{I_s} \times \frac{A_s}{A_u} \quad (1)$$

2.6. DFT calculations

The ground state geometries of probe **1** and BODIPY **1b** were optimized using density functional theory (DFT) with B3LYP functional and 6-31G(d) basis set. The excited state related calculations were carried out with the time-dependent DFT (TDDFT), based on the optimized ground state geometry. All these calculations were performed with Gaussian 09 (Frisch et al., 2009).

2.7. Fluorescent imaging of the cellular thiols

The fluorescent imaging of intracellular thiols of SGC-H446 cells and NCI-H446 cells were obtained using a Nikon ECLIPSE-Ti confocal laser scanning microscopy. The cells were incubated with probes in PBS buffer solution for 10 min at 37 °C, and red emission was observed with 543 nm laser excitation. In the control