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Two-photon fluorescent probe derived from naphthalimide for cysteine detection and imaging in living cells

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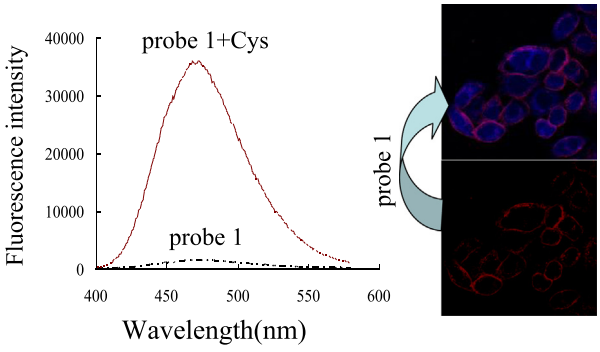
HIGHLIGHTS

- A maleimide bearing naphthalimide was proposed as new two-photon probe for Cys.
- Response mechanism was investigated by theoretical and experimental methods.
- Sensitive and selective detection of Cys over GSH and Hcy was achieved.
- The probe was successfully applied for two-photon imaging of intracellular Cys.

GRAPHICAL ABSTRACT

Two-photon fluorescence enhancement of probe 1 induced by Cys with excitation wavelength of 760 nm. Confocal fluorescence images of Hela cells incubated with probe 1 and a red emission membrane dye.



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ABSTRACT

A maleimide coupling naphthalimide was reported as new two-photon fluorescent (TPF) probe for cysteine (Cys). The probe was weakly fluorescent itself due to the donor-excited photoinduced electron transfer (d-PET). After reaction with Cys, d-PET process was blocked and fluorescence enhancement of the probe was observed at 470 nm. The d-PET principle was rationalized by theoretical calculations with density functional theory and time-dependent density functional theory. Thiol-maleimide addition between the probe and Cys was confirmed by ^{1}H NMR and mass spectrum measurements. TPF analysis demonstrated a 24.7-fold emission increase of the probe induced by Cys upon excitation at 760 nm. The two-photon action cross-section of probe-Cys adduct at 760 nm reached 42 GM compared to 1.7 GM for free probe. The probe showed high sensitivity and selectivity to Cys over other potential interferences; especially it had the capability to discriminate Cys from glutathione and homocysteine. Through TPF imaging, the probe was successfully applied in the detection of Cys in living cells.

Introduction

Cysteine (Cys) is an important thiol-containing molecule in biosystem. It plays versatile roles in a variety of physiological processes such as biocatalysis, providing resistance to the body

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against harmful effects, stabilizing protein structures and function [1–3]. It has been revealed that the disorder of intracellular Cys levels could result in slowed growth, liver damage, neurodegenerative diseases and other health problems [4,5]. Thus, the detection of intracellular Cys is very significant.

Fluorescent imaging (FI) is a powerful visualization technique dependent on fluorescent probes to sensitively detect biomolecules in living cells. For the detection of biothiols, various fluorescent probes have been developed on the basis of the reaction between thiol group and small molecules (e.g., borondipyrromethene, fluorescein, rhodamine B and coumarin derivates) [6–14]. However, most of these probes were implemented for FI with conventional one-photon microscopy (OPM). Due to adopting rather short excitation wavelength (usually <525 nm), OPM has some drawbacks such as photodamage, photobleaching and limited imaging depth. These problems could be effectively alleviated by two-photon microscopy (TPM), a new tool that employs two photons excitation in near-infrared spectral region (700–1100 nm). Over the past several years, a great deal of attention has been given to two-photon fluorescent (TPF) probes [15–19], but only a few of them were used for TPF imaging of thiols [20–23]. Furthermore, because of the similar structures of biothiols, TPF probe able to discriminate Cys from other biothiols such as glutathione (GSH) and homocysteine (Hcy) is still scarce.

1,8-Naphthalimide was a popular fluorophore in fluorescence sensing fields owing to its desirable photophysical properties of intense fluorescence, good photostability and insensitivity to pH [24–26]. It is also two-photon active and has been proved to be an efficient platform for TPF probe [27,28]. Recently, a dinitrophenyl ether bearing 1,8-naphthalimide has been reported as TPF probe for cell imaging of H_2S [28]. Herein, we propose a maleimide coupling naphthalimide derivate as new TPF probe for Cys detection and imaging in living cells. Its fluorescent turn-on response mechanism, that Cys-maleimide addition blocked the donor-excited photoinduced electron transfer (d-PET) process, was investigated by theoretical and experimental methods. TPF analysis demonstrated the feasibility of the probe for highly sensitive and selective detection of Cys over GSH and Hcy. The probe has been successfully applied for TPF imaging of intracellular Cys.

Experiments

Materials and apparatus

N-Butyl-4-amino-1,8-naphthalimide was synthesized according to the procedures reported in the literature [25]. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone Corp. 1,1′-dioctadecy1-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) and N-ethyl-maleimide (NEM) were obtained from AAT Bioquest Inc. Millipore-Q water was used throughout. All other materials were of analytical-reagent grade and used without further purification.

^{1}H NMR spectra were collected on an INOVA400 (Varian Inc., USA) at 400 MHz. IR spectra were obtained on a Nicolet 7199 spectrometer (Nicolet Co., USA) as KBr disks. Mass spectra were conducted by using a Thermo Fisher LCQ Fleet Ion trap mass spectrometer. One-photon fluorescence measurements were recorded using a PerkinElmer LS55 fluorescence spectrometer. TPF analysis was carried out by exciting with a mode-locked Ti:sapphire pulsed laser (Chameleon Ultra II, Coherent Inc.) and then recording with a DCS200PC single photon counting (Beijing Zolix Instruments Co., Ltd.). Absorption spectra were measured with a UV2600 spectrophotometer (Shimadzu Co., Japan). Hela cells were cultured in a SANYO CO_2 incubator. A Zeiss Confocal Microscope LSM 710 was employed for cell imaging.

Preparation of probe 1

N-Butyl-4-amino-1,8-naphthalimide (150 mg) and maleic anhydride (165 mg) were dissolved in 10 ml of $CHCl_3$, and the solution was refluxed for 24 h. Then the sediment was filtered, washed with CH_2Cl_2 and dried to obtain 1a. Melting Point: 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d6+D_2O) δ(ppm): 8.68 (d, J = 8.7 Hz, 1 H), 8.53 (dd, J = 11.8, 7.8 Hz, 2 H), 8.27 (d, J = 6.9 Hz, 1 H), 7.90 (t, J = 7.9 Hz, 1 H), 6.76 (d, J = 12.1 Hz, 1 H), 6.42 (d, J = 12.2 Hz, 1 H), 4.05 (t, J = 6.9 Hz, 2 H), 1.61 (d, J = 7.2 Hz, 2 H), 1.35 (dd, J = 14.3, 7.4 Hz, 2 H), 0.93 (t, J = 7.2 Hz, 3 H). IR(KBr) cm^{-1}: 3400–2800, broad peak (COOH); 2958, 2871 (butyl, C—H); 1722, 1698 (C═O); 1636 (alkenyl, C═C); 1590, 1539 (Ar, C═C). MS calc. for $C_{20}H_{18}N_2O_5$ [M]$^+$ 366.12, found 365.91.

The mixed solution of 1a (150 mg), sodium acetate (21.2 mg) and 10 mL acetic anhydride was heated at 50 °C for 1.5 h, followed by rotary evaporation. The residue was purified by silica column chromatography (diethyl ether/petroleum ether = 99:1) to get probe 1. Melting Point: 129–132 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ(ppm): 8.60 (d, J = 7.8 Hz, 1 H), 8.55 (d, J = 7.2 Hz, 1 H), 8.28 (d, J = 8.5 Hz, 1 H), 7.88 (dt, J = 7.9, 4.0 Hz, 2 H), 7.37 (s, 2 H), 4.07 (m, 2 H), 1.64 (dt, J = 14.9, 7.5 Hz, 2 H), 1.37 (m, 2 H), 0.93 (t, J = 7.3 Hz, 3 H). IR(KBr) cm^{-1}: 3090 (Ar, C—H); 2960, 2933, 2874 (butyl, C—H); 1720, 1660 (C═O); 1622 (alkenyl, C═C); 1590, 1515 (Ar, C═C). MS calc. for $C_{20}H_{16}N_2O_4$ [M+1]$^+$ 349.11, found 349.46.

Theoretical calculations

The ground state geometries of probe 1 and 1-Cys (the adduction product of probe 1 with Cys) were optimized by density functional theory (DFT) at the B3LYP/6-31G(d) level. The excited state related calculations were performed with time-dependent density functional theory (TDDFT) based on the optimized ground state geometries. All these calculations were carried out with Gaussian 09W software and used water as solvent.

Spectral measurements

The stock solutions of probe 1 (in ethanol), amino acids (in water) and metal ions (in water) were stored at 4 °C and appropriately diluted with water for use. Test solution was prepared by placing buffer solution, probe 1 solution and an appropriate aliquot of each analyte into a tube, which was diluted with water to a volume of 10 mL. The resulting solution was well mixed and then incubated at 37 °C prior to the absorption and fluorescence spectra measurements. Fluorescence quantum yield was detected in 20.0 mM of pH 7.4 phosphate buffer containing 0.1% of ethanol with the solution of quinine sulfate in 0.05 M H_2SO_4 as the standard.

Determination of TP absorption cross section

The TP absorption cross section (σ) was obtained by using the TP induced fluorescence method. It was calculated by using the equation of $\sigma = \sigma_r(S_s\phi_r\varphi_r c_r)/(S_r\phi_s\varphi_s c_s)$, where the subscripts s and r denoted the sample and reference molecule, respectively. Rhodamine B in MeOH was used as a reference, whose TP properties have been well characterized in the literature [29]. The TPF intensity was denoted as S. ϕ was the fluorescence quantum yield. φ was the overall fluorescence collection efficiency of the experimental apparatus. c was the concentration of the molecule in solution. TPF intensities of the reference and sample were determined at the same excitation wavelength.