Sensors and Actuators B 258 (2018) 42–49

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A simple two-output near-infrared fluorescent probe for hydrazine detection in living cells and mice

Zhengliang Lu[a,*], Xiaomin Shi[a], Yufan Ma[b], Wenlong Fan[a], Yanan Lu[a], Zhuo Wang[b], Chunhua Fan[a]

[a] Key Laboratory of Interfacial Reaction & Sensing Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China
[b] College of Science, State Key Laboratory of Chemical Resource Engineering, Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China

ARTICLE INFO

Article history:
Received 20 September 2017
Received in revised form
20 November 2017
Accepted 21 November 2017
Available online 22 November 2017

Keywords:
Fluorescence
Probe
Hydrazine
Cell
Mice

ABSTRACT

Hydrazine is an important reactive base in chemistry, pharmacy and agriculture and an effective propellant in aeronautics and astronautics. However, tiny leakage might induce serious environmental contamination and fatal biological damage. Thus, how to rapidly detect hydrazine with high selectivity and sensitivity is of significance and challenge. In this report, we demonstrate a hydrazine-specific fluorescent probe on the modulation of intramolecular charge transfer process. This probe contains a 4-bromobutyl group as a recognition site for hydrazine anchored on the fluorescent dye. Leaving of 4-bromotutylate moiety leads to a marked NIR fluorescent emission at 715 nm induced by hydrazine. Additionally, presence of hydrazine leads to a color change from green to blue-purple and a ratiometric UV–vis absorption change with a clear isosbestic point at 635 nm. The good linear relationship between fluorescence intensity and concentration of hydrazine results in a low detection limit of 5.09 ppb. The color and fluorescence two-output signals are preferable for qualitative and quantitative detection of hydrazine with high sensitivity and specificity. More importantly, the probe is also successfully applied to visualize hydrazine in living both cells and mice.

1. Introduction

Hydrazine has extensive applications in various fields, such as the pharmacy, chemistry, catalysis and agriculture due to its reducibility and alkalinity [1–3]. Additionally, its flammable nature makes it a suitable high-energy fuel propellant using in missiles, satellites and rocket propulsion systems [4–6]. However, hydrazine is carcinogenic and toxic, tiny leakage of which probably induces various environmental contamination and harm to biology during its manufacture, transportation, application, and disposal. The good water solubility and absorption ability of hydrazine through dermal and oral contact, and inhalation amplifies and accelerates mutagenic effects which further aggravate severe organ damage, including but not limited to the liver, kidneys, lungs, the central nerves and the respiratory system [7–9]. Therefore, the U.S. Environmental Protection Agency (EPA) suggested the threshold limit of hydrazine is as low as 10 ppb [10].

Compared with traditional analytical methods including chromatography [11], spectrophotometry [12], electrochemistry [13] and titrimetry [14], fluorescence methodology is a more reliable, sensitive, noninvasive imaging technique due to many advantages such as high temporal resolution, simple sample preparation, low requirement of expensive instruments and facilities and good compatibility in biological systems [15–18]. These features are extremely favorable for the rapid and convenient detection of interesting analytes in living cells and animals [19–24]. Therefore, numerous fluorescent probes for hydrazine detection have been developed based on various chemical strategies, such as hydrazinolysis of ester and imide [25–32], condensation of aldehyde with hydrazine [33–36], and transformation from malononitrile to imine [37–41], hydrogen bonding [42,43]. These hydrazine-induced structural changes could significantly modulate electron processes including excited-state intramolecular proton (ESIPT), photoinduced electron transfer (PET), or intramolecular charge transfer (ICT) [9,27,28,41,44]. It is to be pointed out that most of them showed at least one of the below defects, such as long response time, or high detection limit, especially short emission and excitation wavelength. Recently, NIR fluorescent probes have

* Corresponding author.
E-mail address: Zhengliang.lu@yahoo.com (Z. Lu).

https://doi.org/10.1016/j.snb.2017.11.125
0925-4005/

been attracting much interest due to emission of long wavelength light in the range of 650–900 nm, which led to outstanding performance such as reduced auto-fluorescence, low phototoxicity and light-bleaching and deeper penetration [45–51].

Unfortunately, to date only few near-infrared (NIR) probes have emerged as an efficient methodology to monitor hydrazine [29,52–55]. For example, Peng group developed a NIR fluorescent probe based on cyanine which can selectively detect in vitra or in vivo hydrazine at pH 4.5 [29]. A ratiometric pyridomethene-BF_2 derivative was developed for hydrazine detection only in 90% DMF aqueous solution [52]. Lin group synthesized a hemicyanine-containing NIR fluorescent probe for hydrazine working in HEPES/CH_3CN (7:3, v/v) [53]. With the similar fluorophore, Zhang group constructed a NIR fluorescent probe containing the acetyl moiety as the hydrazine recognition group working in 20% DMSO aqueous solution [54]. Very recently, another hydrazine-specific NIR fluorescent probe showing ratiometric responses was designed by Ni group according to hydrazine-induced decomposition from the cyanine derivative to the coumarin core in 50% DMSO HEPES buffer [55]. Apparently, Those NIR fluorescent probes could only exhibit good selectivity and sensitivity with deficiencies such as high amount of organic solvent or acidic environment, which significantly limit their extensive application in biology. Therefore, construction of NIR fluorescent probes suitable for hydrazine detection in bio-samples under physiological conditions is still of challenge and importance.

Keeping those drawbacks and the requirements in mind, in this report, a NIR fluorescent probe (**Hcy-OB**) for hydrazine detection is developed based on a modulation of an ICT process. This probe contains an excellent NIR fluorescent dye (**Hcy-OH**) developed by Lin's group, the fluorescence of which exhibits off-on response via release of the hydroxyl group of **Hcy-OH** [56]. The cleavage reaction of 4-bromobytyrate as a recognition site with hydrazine enables red fluorescence emission and leads to the distinct color change in 5% DMSO aqueous solution under physiological conditions. Furthermore, probe **Hcy-OB** has been successfully applied for visualization of hydrazine in living cells and mice.

2. Experimental section

2.1. Materials and instruments

All chemical reagents and solvents were obtained commercially and used as received without further purification unless otherwise stated. Ultrapure water was purified from Millipore. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 M spectrometer and referenced to the solvent signals. Mass spectra (ESI) were carried out on a Bruker Daltonics APEX II 47e FT-ICR spectrometer with ESI mode (America). UV–vis and Fluorescence spectra were performed on a Varian Cary 100 spectrophotometer and a hitachi F-7000 luminescence spectrophotometer, respectively. The pH value was measured using a digital pH-meter (PHSJ-3F, Leici, Shanghai, China). The fluorescent images of cells and mice were taken using a confocal laser-scanning microscope (TCS SP5, Leica, Germany) with an objective lens (×40). The absorbance measurements in MTT analysis were performed on a microplate reader. The Hemicyanine fluorescent dye (**Hcy-OH**) was prepared by a modified procedure according to literature [56].

2.2. Synthesis of probe hcy-OB

To a dichloromethane (50 mL) solution of 4-Dimethylaminopyridine (DMAP) (20 mg, 0.16 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) (101 mg, 0.52 mmol) were added 4-bromobutyric acid (60 mg, 0.36 mmol) in anhydrous under N_2 atmosphere and the mixture was stirred for 60 min at room temperature. After **Hcy-OH** (66 mg, 0.13 mmol) was added, the mixture was stirred overnight. Then the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10/1, v/v) to afford pure **Hcy-OB** as a green solid (52 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, J = 14 Hz, 2H), 7.34 (m, 4H), 7.17 (m, 4H), 6.05 (d, J = 15.2 Hz, 2H), 4.15 (q, J = 7.2 Hz, 4H), 3.63 (t, J = 6 Hz, 2H), 3.41 (t, J = 6.8 Hz, 1H), 3.03 (t, J = 7.2 Hz, 2H), 2.63 (s, 4H), 2.57 (t, J = 7.2 Hz, 1H), 2.32 (m, 2H), 2.24 (m, 1H), 2.10 (m, 1H),1.38 (t, J = 7.2 Hz, 6H), 1.29 (d, J = 16 Hz, 1H), 1.19 (s, 5H), 0.81 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 171.10, 170.11, 159.59, 141.63, 140.89, 140.11, 128.92, 125.37, 122.35, 122.26, 49.16, 39.76, 32.95, 32.86, 31.93, 29.66, 28.25, 27.39, 24.56, 20.67, 12.38. MS (ESI, m/z) Calcd for [$C_{38}H_{46}BrN_2O_2^+$]:532.14818, found: 532.14781.

2.3. Cell confocal imaging experiment

2.3.1. In vitro cytotoxicity

HeLa cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with heat-inactivated fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 °C in a 95% humidity atmosphere under 5% CO_2 environment. After washing with Dulbecco's phosphate-buffered saline (DPBS) twice, HeLa cells (1×10^4 cells/well) were seeded at a density of 1*10-6 cells in a flatbottom 96-well plate in 100 μL of culture medium and incubated in 5% CO_2 at 37 °C for 12 h. The cells were treated with different concentration probe **Hcy-OB** (0.0, 2.0, 5.0, 10.0, and 20.0 μM, respectively) for 24 h. The cytotoxicity of probe **Hcy-OB** was determined by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assays. MTT solution (5.0 mg/mL, PBS) was then added into each well (10 μMK/well, 0.5 mg/mL) and the residual MTT solution was removed after 4 h, and then 100 μM of DMSO was added to each well dissolve the formazan crystals. After shaking for 10 min, the absorbance values of the wells were recorded using a microplate reader. The cytotoxic effect (VR) of probe **Hcy-OB** was assessed using the following equation: VR = A/A0 × 100%. The assays were performed eight replicates. And the statistic mean and standard derivation were utilized to estimate the cell viability.

2.3.2. Confocal microscopy imaging

HeLa cells were seeded in 35 mm glass dishes at a density of 3×10^5 cells per dish in culture media. In a control experiment, after overnight culture, HeLa cells were incubated only with 10 μM probe **Hcy-OB** at 37 °C for 10 min. In another group experiment, HeLa cells were firstly incubated with 10 μM probe **Hcy-OB** for 10 min and then with 50 μM hydrazine for another 10 min. Fluorescence imaging was performed after the cells were washed with PBS twice.

2.4. In vivo imaging

Animal models and in vivo imaging. 4-week-old Balb/c mice were obtained from Laboratory Animal Center. All animal experiments were performed in accordance with the guidelines issued by The Ethical Committee of University of Jinan. Two sets of experiments were performed. In a control experiment, B/c mice were given a skin-pop injection of **Hcy-OB** (50 μL, 10 μM) into the peritoneal cavity. In other set of experiment, mice was given an injection of **Hcy-OB** (50 μL, 10 μM) into the peritoneal cavity and then followed by injection of 50 μL of 50 μM hydrazine at the same region. Fluorescent images were taken by confocal laser scanning microscopy.