Chinese Chemical Letters 27 (2016) 540–544

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

A novel resorufin based fluorescent "turn-on" probe for the selective detection of hydrazine and application in living cells

Tun Tang, Yu-Qi Chen, Bo-Shi Fu, Zhi-Yong He, Heng Xiao, Fan Wu, Jia-Qi Wang, Shao-Ru Wang, Xiang Zhou *

College of Chemistry and Molecular Sciences, Key Laboratory of Biomedical Polymers of Ministry of Education, Wuhan University, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 29 December 2015
Accepted 14 January 2016
Available online 22 January 2016

Keywords:
Resorufin
Fluorescent probe
Hydrazine
Selectivity
Cell imaging

ABSTRACT

In this study, a resorufin derivative RTP-1, which is a novel fluorescent "turn-on" probe for sensitive detection of hydrazine within 30 min, is designed and synthesized. The selective deprotection of the ester group of the probe by hydrazine led to a prominent enhancement of fluorescent intensity, as well as a remarkable color change from colorless to pink, which could be distinguished by naked eye. The fluorescence enhancement showed decent linear relationship with hydrazine concentration ranging from 0 to 50 μmol/L, with a detection limit of 0.84 μmol/L. The specificity of RTP-1 for hydrazine to a number of metal ions, anions and amines is satisfactory. The sensing mechanism of RTP-1 and hydrazine was evaluated by HPLC, ESI mass spectrometry and density functional theory (DFT). Moreover, we have utilized this fluorescent probe for imaging hydrazine in living cells, and the fluorescence was clearly observed when the cells were incubated with hydrazine (100 μmol/L) for 30 min.

1. Introduction

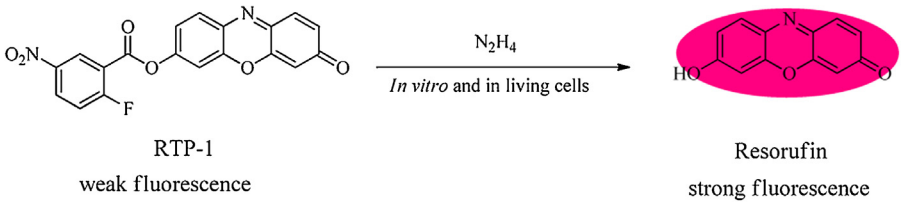
Among the factors that cause healthy and environmental problems, hydrazine (N_2H_4) is the one that cannot be ignored. It is a highly toxic and unstable compound which can cause severe accident during production, transportation or application. It can act as a highly reactive base and reducing agent in the synthesis of many chemicals [1–4]. It is also used as efficient fuels for various rockets and missiles [5,6]. Although there is no hard evidence for endogenous hydrazine in living cells, it can easily get into the body through oral, breathing and skin contact. It has been reported that various diseases disorders related to the lungs, kidneys, liver and the nervous system are caused by hydrazine contamination [7–9]. Therefore, the development of convenient, efficient and inexpensive methods to detect trace amount of hydrazine in environmental and biological samples is of great importance.

During the past decades, many methods were utilized to detect hydrazine levels, such as chromatography–mass spectrometric, titrimetric and electrochemical methods [10–12]. These methods are unsuitable for measuring hydrazine levels in cellular systems. In recent years, fluorescent probes have been gradually utilized for the detection of hydrazine [13–23]. Some of these systems are limited by onerous synthetic procedures, low pH reaction system, imperfect limit of detection (~2 μmol/L) or narrow linear response range (0–10 μmol/L), which restrict further applications. Therefore, it is necessary to develop a fluorescent probe which has advantages including simple synthetic process and good biocompatibility.

Herein, we designed and synthesized a novel resorufin based fluorescent "turn-on" probe for hydrazine detection *in vitro* and in living cells. Compared to other fluorophores, resorufin exhibits excellent physical and fluorescent properties including good water-solubility, high manoeuvrability and easy fluorescence quenching *via* 7-hydroxy substitution [24–28]. As illustrated in Scheme 1, the fluorophore resorufin is modified with 2-fluoro-5-nitrobenzoic acid, thus fluorescence of resorufin was quenched. Then, fluorescent probe RTP-1 is added to the detection system, incubated with hydrazine at ambient temperature. Hydrazine reacted with the ester group of fluorescent probe RTP-1 *via* nucleophilic addition–elimination reaction, accompanied by a distinct enhancement of the fluorescent intensity through the release of free resorufin.

* Corresponding author.
E-mail address: xzhou@whu.edu.cn (X. Zhou).

http://dx.doi.org/10.1016/j.cclet.2016.01.024



Scheme 1. Schematic illustration of the resorufin based fluorescent "turn-on" probe RTP-1 for detection of hydrazine levels.

2. Experimental

2.1. Materials and equipment

All solvents and reagents were obtained from Alfa Aesar or Sigma–Aldrich, and the solvents were treated as required prior to use. Other chemicals were commercially available and used without further purification unless for special needs. HeLa cells were purchased from China Center for Type Culture Collection. UV/vis absorption spectra were collected on a Shimadzu UV-2550 spectrophotometer from 400 nm to 700 nm with 600 μL quartz cuvettes. Fluorescent emission spectra were collected from 570 nm to 650 nm on PerkinElmer LS 55 with an excitation wavelength of 550 nm, the excitation and emission slit widths were 15 nm and 3 nm, respectively. Quartz cuvettes with 2 mL volume were used for emission measurements. For determination of the fluorescence quantum yield (Y_u), rhodamine B in water (Y_s = 0.31) was used as a standard. Values were calculated according to the following equation.

$$Y_u = \frac{Y_s \times F_u \times A_s}{F_s \times A_u}$$

where s means standard, u means sample, *A* means absorbance at the excitation wavelength, *F* means integrate area under the fluorescence spectra on an energy scale. Unless otherwise specified, all spectra were taken at an ambient temperature. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 spectrometers, respectively. Data for ^{1}H NMR spectra were recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), integration, coupling constant (Hz). HPLC data was collected with Lambo Model 2000. Mass spectrometry (MS) was recorded on Shimadzu LCMS-2010. High resolution mass spectrometry (HRMS) was recorded on Agilent 6224 Accurate-Mass Time-of-Flight (TOF) LC/MS.

Synthesis of probe RTP-1: Resorufin (213 mg, 1 mmol), 2-fluoro-5-nitrobenzoic acid (220 mg, 1.2 mmol) and 4-dimethylaminopyridine (DMAP, 12 mg, 0.1 mmol) were dissolved in dry dichloromethane (20 mL) and stirred at 0 °C. Then *N,N*-dicyclohexylcarbodiimide (DCC, 309 mg, 1.5 mmol) dissolved in dichloromethane (5 mL) were added to the solution dropwise and stirred at room temperature for 16 h. The precipitate of urea was removed by filtration and the filtrate was concentrated in high vacuum to give an oily residue. This residue was purified by silica gel column chromatography with eluent dichloromethane/ethyl acetate (v/v 20:1) to give RTP-1 (130 mg, 34%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.04 (d, 1H, J = 3.6 Hz), 8.56 (d, 1H, J = 9.6 Hz), 7.90 (d, 1H, J = 8.4 Hz), 7.48–7.42 (m, 2H), 7.33–7.26 (m, 2H), 6.91 (d, 1H, J = 9.9 Hz), 6.36 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 186.4, 152.6, 149.2, 148.8, 135.4, 134.9, 131.7, 131.4, 130.8, 130.7, 128.7, 119.1, 118.8, 109.8, 107.5. HRMS calcd. for $C_{19}H_9FN_2O_6$ [M+H]+: 381.05174; found: 381.05177.

2.2. Cell culture

HeLa cells (CCTCC, China) were cultured in DMEM medium (Hyclone, Thermo Fisher Scientific Inc., MA) supplemented with 10% FBS (Hyclone, Thermo Fisher Scientific Inc., MA), 1% penicillin and streptomycin. For all cell experiments, cells were trypsinized and seeded in a 96-well culture plates, and for confocal experience, cells were seeded in 35 mm glass-bottomed dishes, then incubated in a humidified 37 °C incubator supplied with 5.0% CO_2.

2.3. MTT assay

HeLa cells were digested by trypsin and washed with PBS buffer (10 mmol/L, pH 7.4). The cells were seeded into a 96-well plate and incubated in a 5% CO_2-containing incubator at 37 °C overnight for attachment. Then the cells were treated with RTP-1 (1–500 μmol/L) for 48 h. Cell viability was evaluated *via* MTT assay. At first, freshly prepared MTT solution (10 μL, 5 mg/mL in PBS) was added to each well. After incubated for 4 h, the medium was removed and DMSO (100 μL) was added to each well. The optical density values were detected at 492 nm and the cytotoxicity data was expressed as relative cell viability.

2.4. Confocal imaging

When the cells grew to the 50% confluence, RTP-1 (2 μmol/L, final concentration) was added to the complete culture medium from a 10 mmol/L stock in DMSO. After incubating with RTP-1 for 30 min, cells were washed 3 times in PBS followed by incubation with hydrazine (100 μmol/L) for another 30 min. Confocal fluorescence microscopy images of RTP-1-labeled cells were obtained by Laser Scanning Confocal microscope (Nikon, TE2000, Japan) with an oil objective lens (×60). The fluorescence emission at 560–610 nm was recorded using an excitation wavelength of 532 nm.

3. Results and discussion

3.1. Synthesis and characterization

In our initial study, resorufin was used as the starting material for the synthesis of the fluorescent probe RTP-1 (Scheme 2). Resorufin reacted with 2-fluoro-5-nitrobenzoic acid using 4-dimethylaminopyridine (DMAP) as catalyst and *N,N*-dicyclohexyl-carbodiimide (DCC) as dehydrating agent. After one-step synthesis, the fluorescent probe RTP-1 was obtained and characterized by NMR and HRMS (Figs. S9–S11 in Supporting information).

3.2. Absorption and emission spectroscopic properties of RTP-1 toward hydrazine

Firstly, the time-dependent fluorescent property of the probe RTP-1 toward hydrazine was evaluated. Since the pH value in