Dyes and Pigments 103 (2014) 9–20

Contents lists available at ScienceDirect

Dyes and Pigments

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

A facile ratiometric fluorescent chemodosimeter for hydrazine based on Ing–Manske hydrazinolysis and its applications in living cells

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ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form
11 November 2013
Accepted 12 November 2013
Available online 21 November 2013

Keywords:
Charge transfer
Fluorescent probe
Ing–Manske hydrazinolysis
pH effects
Semi-empirical calculations
Time resolved photoluminescence spectra

ABSTRACT

A facile and sensitive fluorescent probe for hydrazine was successfully constructed, displaying excellent colorimetric and ratiometric responses towards hydrazine via Ing–Manske hydrazinolysis conditions in semi-aqueous buffer solution. Semi-empirical calculations as well as spectroscopic results revealed the signalling mechanism of the current probe under hydrazinolysis conditions, in which hydrazine exclusively deprotected the phthalimide group by an intermediate of phthalhydrazide. Extensive screening of pH effects on the probe with the aid of proton nuclear magnetic resonance and mass spectrometry supported the distinctive and diverse ratiometric responses under hydrazinolysis and basic hydrolysis conditions. Time resolved photoluminescence measurements of the probe further confirmed its discernible ratiometric responses probed at respective wavelengths. A distinctive ratiometric response under basic hydrolysis conditions and a successful utilization of probe towards hydrazine detection in living cells are demonstrated.

1. Introduction

Developing efficient and reaction specific synthetic probes with better sensitivity for the detection of small molecule based analytes is of pivotal research interest owing to the toxic effects of many small molecules to humans and the environment [1]. Hydrazine is a strong reducing agent and highly reactive base [2]; moreover, its widespread usage is inevitable due to its vital roles in chemical, pharmaceutical, and agricultural industries involving catalysts, corrosion inhibitors, and pesticides [3]. Hydrazine is a well-known high-energy fuel in rocket propulsion and missile systems due to its improved detonable properties [4]. However, hydrazine is extremely toxic and easily absorbed by oral, dermal, and inhalation exposure routes. Previous studies on laboratory animals suggested that hydrazine is highly neurotoxic, mutagenic, and carcinogenic [5]. Thus, developing reliable and real-time fluorometric detection methods for the specific detection of hydrazine is warranted.

Conventionally, hydrazine was analysed by electrochemistry [6], chromatography-mass spectrometric [7], titrimetric [8] and gas chromatography [9] methods. However, those methods were often suffered in detecting hydrazine with low sensitivities. Despite their ease in detections with a trace amount of analytes by fluorometric methods possessing high sensitivity and selectivity functions; only a limited number of fluorescent small molecule based probes for hydrazine have been reported. Swager et al. developed the first fluorescent conjugated polymer for turn-on detection of trace amounts of hydrazine. [10] Chang et al. reported a selective detection of hydrazine by deprotection of a levulinate group [11]. Recently, Sessler and co-workers reported a trifluoroacetyl acetonate naphthalimide derivative that was formed a five membered heterocyclic compound, giving rise to a fluorescent turn-on response exclusively in the presence of hydrazine [12].

Developing ratiometric and reaction specific fluorescent chemodosimeters are often beneficial due to their specificity and built-in correction for quantitative measurement by the ratio of fluorescence intensities at two different wavelengths [13]. Chemodosimeters appended with specific protection groups for selective detections via target specific deprotection for various analytes have often been utilized effectively [14–16]. However, to date there are only two reported ratiometric probes based on hydrazine mediated ester deprotection [17] and hydrazone formation [18]. However, to the best of our knowledge a renowned NH_2 functional group

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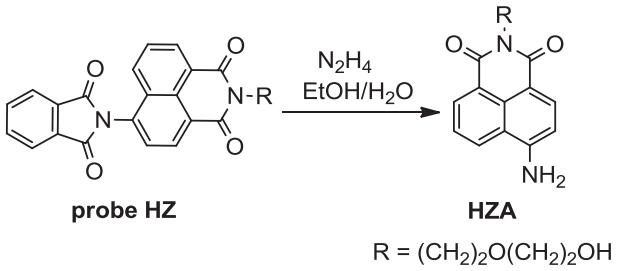
http://dx.doi.org/10.1016/j.dyepig.2013.11.015

synthon phthalimide [19] has never been explored in the specific ratiometric detection of hydrazine. Excellent photophysical properties and outstanding intramolecular charge transfer (ICT) structures of hydrophilic 4-aminonaphthalimide make them expedient candidates in designing novel fluorescent probes [20]. However, facile ratiometric probes for hydrazine with selective and discriminative functions from other amine sources having potent biocompatibility within the biological pH range are required.

Herein, we developed a novel phthalimide protected 4-aminonaphthalimide for the specific and sensitive ratiometric detection of hydrazine via the Ing–Manske hydrazinolysis method [21], a key step in Gabriel amine synthesis [22] and thus, enabling ICT as well as living cell permeability. Probe **HZ** was synthesized by appending phthalimide group via CuI promoted aryl halide nucleophilic substitution of compound **2** with potassium phthalimide in high boiling dimethylacetamide (DMA) solvent in a moderate yield as depicted in Schemes 1 and 2.



Scheme 1. Hydrazine mediated phthalimide deprotection of probe **HZ** to form **HZA**.

2. Experimental

2.1. General characterization methods

NMR spectra were recorded on Bruker Avance DRX300 Series (^{1}H: 300 MHz; ^{13}C: 75 MHz) at a constant temperature of 25 °C. Chemical shifts were reported in parts per million from low to high field and referenced to residual solvent ($CDCl_3$, d_6-DMSO: ^{1}H δ = 7.26, 2.49 ppm and ^{13}C δ = 77.23, 39.52 ppm, respectively). Coupling constant (J) were reported in hertz (Hz). UV–Vis spectra were recorded on the Jasco UV-600 spectrophotometer using 1 cm quartz cuvette. Fluorescence measurements were conducted with HITACHI 7000 Series Spectrophotometer. All emission and excitation spectra were corrected for the detector response and the lamp output. Melting points were determined using a Fargo MP-2D apparatus and are uncorrected. Elemental analyses were conducted on HERAEUS CHN-OS RAPID elemental analyser. Infrared spectroscopy data were collected using Perkin Elmer IR spectrophotometer. Solid sample were analysed using KBr pellet method. Time resolved photoluminescence (TRPL) spectra were measured using a home built single photon counting system with excitation from a 400 nm diode laser (Picoquant PDL-200, 50 ps fwhm, 2 MHz). The signals collected at the excitonic emissions of all sample solutions were connected to a time-correlated single photon counting card (TCSPC, Picoquant Timeharp 200). The emission decay data were analysed for **HZ**-hydrazine and **HZ**-hydroxide complex with biexponential kinetics, from which two decay components were derived; the lifetime values of (τ_1, τ_2) and pre-exponential factors (A_1, A_2) were determined. Confocal imaging was carried out using Leica TCS SP8 confocal fluorescence microscope, confocal fluorescence imaging with using 60× times oil objective. Semi-empirical PM3 calculations were calculated using Gaussian-09 suite [23].

2.2. Materials

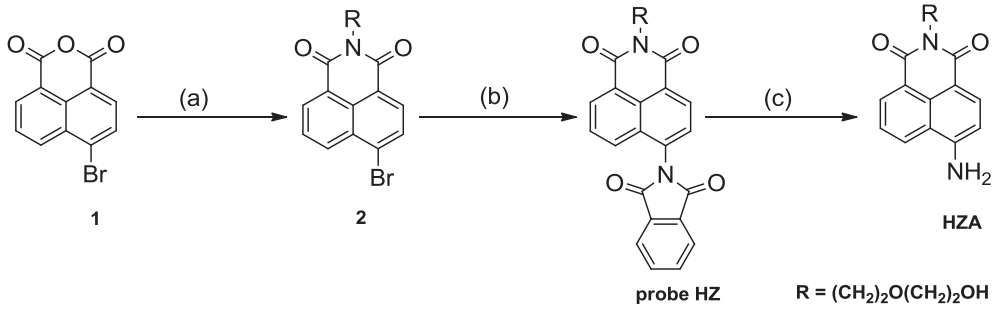
All the reagents were purchased from commercial sources and used without further purification. All the solvents were HPLC grade; anhydrous solvents were obtained by passing through activated alumina column purification system, further dried by standard drying procedures. Solvents were degassed by freeze/thaw/pump cycle technique prior to use. 6-bromo-2-(2-(2-hydroxyethoxy)ethyl)-1*H*-benzo[de]isoquinoline-1,3(2*H*)-dione was prepared with a slight modification of previous literature [24].

2.3. Stock solutions

Standard solution of probe **HZ** (100 μM) were prepared in (1:9, v/v) in a mixture of water and ethanol solution. Prior to analysis the stock solution was diluted and pH of the solution was adjusted to about 7.2 using phosphate buffer saline (PBS) solution to deliver the final concentration of the probe (5 μM, pH = 7.2) in PBS-EtOH (1:9, v/v) solution. Hydrazine, other primary amines, metal ions, and anion stock solutions with concentration of (10 mM) were prepared, respectively in water. Before the titrations analytes were diluted to their desired volumes.

2.4. Cell culture and imaging

The human cervical cancer cell line (HeLa cells) were seeded onto cover slips at a concentration of (2×10^5 cells/mL) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) and 10%



Scheme 2. Synthesis of Probe **HZ**. Reagents and Conditions: (a) 2-(2-aminoethoxy)ethanol, EtOH, reflux, 4 h, 88%; (b) potassium phthalimide, CuI, DMA, reflux, 1 day, 55%; (c) N_2H_4, EtOH/H_2O, 15 min, 70%.