Analytica Chimica Acta 893 (2015) 84–90

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Hydrazine selective dual signaling chemodosimetric probe in physiological conditions and its application in live cells

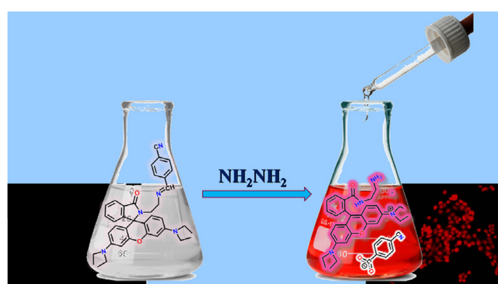
Sandip Nandi [a], Animesh Sahana [a], Sandip Mandal [a], Archya Sengupta [b], Ansuman Chatterjee [b], Damir A. Safin [c, *], Maria G. Babashkina [c], Nikolay A. Tumanov [c], Yaroslav Filinchuk [c], Debasis Das [a, **]

[a] Department of Chemistry, The University of Burdwan, Burdwan, 713104 West Bengal, India
[b] Department of Zoology, Visva Bharati University, Santiniketan, West Bengal, India
[c] Institute of Condensed Matter and Nanosciences, Molecules, Solids and Reactivity (IMCN/MOST), Université catholique de Louvain, Place L. Pasteur 1, 1348 Louvain-la-Neuve, Belgium

HIGHLIGHTS

- A selective rhodamine–cyanobenzene conjugate is synthesized.
- The conjugate is a selective dual signaling chemodosimetric probe towards hydrazine.
- Spirolactam ring opening of the probe, followed by its hydrolysis, is the sensing mechanism.
- The probe detects hydrazine in the human breast cancer cells MCF-7 imaging.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 31 May 2015
Received in revised form
16 August 2015
Accepted 24 August 2015
Available online 1 September 2015

Keywords:
Chemodosimetric probe
Hydrazine
Rhodamine
"Turn-on" fluorescence
Cell imaging

ABSTRACT

A rhodamine–cyanobenzene conjugate, (*E*)-4-((2-(3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthene]-2-yl)ethylimino)methyl)benzonitrile (**1**), which structure has been elucidated by single crystal X-ray diffraction, was synthesized for selective fluorescent "turn-on" and colorimetric recognition of hydrazine at physiological pH 7.4. It was established that **1** detects hydrazine up to 58 nM. The probe is useful for the detection of intracellular hydrazine in the human breast cancer cells MCF-7 using a fluorescence microscope. Spirolactam ring opening of **1**, followed by its hydrolysis, was established as a probable mechanism for the selective sensing of hydrazine.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Hydrazine (N_2H_4), a simple diamine and powerful reducing agent, has been used as a fuel and propellant in aircraft due to its flammable and detonable characteristics [1]. Hydrazine is widely used in many chemical, pharmaceutical and agricultural industries involving catalysts, metal anticorrosion, textile dyes and

* Corresponding author.
** Corresponding author.
E-mail addresses: damir.a.safin@gmail.com (D.A. Safin), ddas100in@yahoo.com (D. Das).

http://dx.doi.org/10.1016/j.aca.2015.08.041
0003-2670/© 2015 Elsevier B.V. All rights reserved.

pharmaceutical intermediates [2–4]. In contrast to its usefulness, toxic and carcinogenic effect of hydrazine potentially lead to serious environmental pollution during its production, purification, utilization and transportation [5,6]. It has been reported that hydrazine is a neurotoxin and has severe mutagenic effects causing infections of the respiratory tract and damage to the lungs, liver, kidneys and central nervous system [7–9]. Furthermore, hydrazine produces toxicity by interfering with a number of metabolic processes such as gluconeogenesis and glutamine syntheses [10]. Although there is no endogenous hydrazine in live cells, it is readily absorbed by oral, dermal or inhalation routes of exposure, being harmful to live cells. Thus, it is important to develop selective, sensitive and easy methods for the detection of hydrazine under biocompatible conditions at physiological pH [11]. Hydrazine can be routinely analyzed by some traditional method such as electrochemical analysis [12] and chromatography [13], including gas chromatography [14] and HPLC [15]. However, these techniques are often time consuming, require complicated sample processing and destructive for tissues or cells. Therefore considerable efforts have been made to synthesize probes that can detect hydrazine in a selective and sensitive manner. Probes based on changes in fluorescence induced by analytes are particularly attractive because of the simplicity of their utilization and lower detection limits [16,17]. Recently, few fluorescent probes, based on the coumarin [18–20], fluorescein [21] as well as other fluorophores [22–28], have been developed for the hydrazine detection. However, most of these reported fluorescent probes worked in pure organic solvents, that impeded their application for the hydrazine detection in living cells at physiological pH. Pronounced spectroscopic properties of rhodamine B, such as visible light excitation as well as long wavelength emission and high fluorescence quantum yield, make it a good choice for the designing of fluorescence probes [29]. Large fluorescence enhancement as well as colorimetric change with absorption at around 550 nm is due to spirolactam ring opening [30]. Careful observation of number of rhodamine based probes reported in the literature indicates that their sensing properties mostly depend on the appended functionality but not only on the rhodamine unit alone. This fact inspired us to design and synthesize a new rhodamine B based chemodosimetric probe (*E*)-4-((2-(3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthene]-2-yl)ethylimino)methyl)benzonitrile (**1**) having an electron withdrawing nitrile group at the *para*-position of the appended unit. Moreover, the probe **1** is also useful for intra-cellular hydrazine imaging and quantitative determination at physiological conditions. Another reference compound (*E*)-3′,6′-bis(diethylamino)-2-(2-(4-(dimethylamino)benzylideneamino)ethyl)spiro[isoindoline-1,9′-xanthen]-3-one (**2**) is also synthesized and studied to further strengthen the proposed sensing mechanism.

2. Experimental

2.1. Materials and methods

Rhodamine B, 4-cyanobenzaldehyde, 4-(dimethylamino)benzaldehyde and high-purity HEPES were purchased from Sigma Aldrich (India). Spectroscopic grade solvents have been used. Other chemicals were of analytical reagent grade and used without further purification. Mili-Q 18.2 MΩ cm^{-1} water has been used throughout all the experiments. A Shimadzu Multi Spec 1501 UV–vis spectrophotometer was used for recording absorption spectra. ^1H NMR titration in CD_3CN was recorded using a Bruker Avance 500 (500 MHz). Steady-state fluorescence experiments were performed using a Hitachi F-4500 spectrofluorimeter. The electrospray ionization (ESI^+) mass spectra were measured with a Finnigan-Mat TCQ 700 mass spectrometer. pH measurements were performed with a Systronics digital pH meter (model 335). Elemental analyses were performed on a Perkin Elmer 2400 CHN analyzer.

2.2. In vitro cell imaging

Human breast cancer cell line MCF-7 was grown in DMEM (Sigma, St. Louis, USA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, USA), 2 mM glutamine, 100 U m/L penicillin-streptomycin solution (Gibco, Invitrogen, USA) in the presence of 5% CO_2 at 37 °C. For *in vitro* imaging studies, cells were seeded in 6 well culture plates with a seeding density of 10^5 cells per well. After reaching 60–70% confluence, the previous media was replaced with serum free media, supplemented by hydrazine and **1** at 50 μM and 20 μM, respectively, and incubated for 2 h to facilitate hydrazine or **1** uptake by cells. Then cells were placed under an inverted microscope (Dewinter, Italy) at different magnifications to examine any adverse effect on cellular morphology. **1** treated cells were then incubated with hydrazine for 15–30 min and observed under an inverted fluorescence microscope at different magnifications with a blue filter. Images were taken through an attached ccd camera with help of the Bio-Wizard 4.2 software. A control experiment was done using medium devoid of hydrazine.

2.3. Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on the mouse bone marrow cells [31–33]. Cells were seeded into the 24-well tissue culture plate in the presence of 500 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere for overnight and then incubated for 6 h in the presence of **1** at different concentrations (10, 20 and 40 μM). Then cells were washed with PBS buffer and 500 μL supplemented DMEM medium was added. Subsequently, 50 μL MTT (5 mg/mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 500 μL sodium dodecyl sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 558 nm using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **1**.

2.4. UV–vis and fluorescence titration

The path length of cells used for the absorption and emission studies was 1 cm. For UV–vis and fluorescence titrations, a stock solution of **1** (10 μM) was prepared in the HEPES (0.1 M) buffered $CH_3CN–H_2O$ (9:1, v/v) solution at pH 7.4. Working solutions of **1** and hydrazine were prepared from their respective stock solutions.

2.5. Quantum yield measurements

The fluorescence quantum yields were determined using Rhodamine B as a reference with a known ϕ_{ref} value of 0.65 in basic EtOH [34]. The area of the emission spectrum was integrated using the software available in the instrument and the quantum yield was calculated according to the following equation [35]: $\phi_{sample} = \phi_{ref} \times [A_{sample}/A_{ref}] \times [OD_{ref}/OD_{sample}] \times [(\eta_{sample})^2/(\eta_{ref})^2]$, where ϕ_{sample} and ϕ_{ref} are the fluorescence quantum yield of the sample and reference, respectively; A_{sample} and A_{ref} are the area under the fluorescence spectra of the sample and the reference, respectively; OD_{sample} and OD_{ref} are the corresponding optical densities of the sample and the reference solution at the wavelength of excitation; η_{sample} and η_{ref} are the refractive index of the sample and reference, respectively.

Download English Version:

https://daneshyari.com/en/article/1163337

Download Persian Version:

https://daneshyari.com/article/1163337

Daneshyari.com