Journal of Photochemistry and Photobiology A: Chemistry 299 (2015) 1–8

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Development of a selenide-based fluorescent probe for imaging hypochlorous acid in lysosomes

Zongjin Qu [a], Junxia Ding [a], Meiyu Zhao [b], Peng Li [a,*]

[a] State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[b] Institute of Theoretical Simulation Chemistry, Academy of Fundamental and Interdisciplinary Science, Harbin Institute of Technology, Harbin 150080, China

ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 15 October 2014
Accepted 20 October 2014
Available online 22 October 2014

Keywords:
Fluorescent probes
Naphthalimide
Lysosomes
Selenium
Hypochlorous acid

ABSTRACT

The development of fluorescent probes for hypochlorous acid (HOCl) has received intense attention because of the biological significance of HOCl. In this work, a novel fluorescent probe based on a selenide switch for the detection of HOCl in lysosomes has been designed and synthesized on a 1,8-naphthalimide scaffold. The probe exhibited a high selectivity for HOCl over various reactive oxygen species (ROS) with a fast response and a large fluorescence enhancement in aqueous media. Confocal microscopy imaging of living cells indicated that the probe was able to accumulate in lysosomes and was successfully applied to imaging exogenous HOCl in living cells. Attempts of using Lyso-NI-Se to image HOCl in stimulated RAW264.7 cells failed, probably due to the absence of endogenous HOCl in lysosomes or the undesirable detection limit.

1. Introduction

Due to their chemical reactivity, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are interrelated in innumerable ways with various physiological processes and health problems [1–6]. Hypochlorous acid (HOCl), an important member of ROS, plays essential roles in inflammation and the immune defence against microorganisms [7]. Nevertheless, persuasive evidences have shown that excessive or misplaced HOCl is implicated in various human diseases, such as neurodegeneration, cardiovascular diseases, arthritis and even cancers [8–12]. Besides, production of this highly reactive and powerful two-electron oxidizing agent can lead to downstream generation of RNS and other ROS which will cause cell injury by nitration, chlorination or oxidation of biomolecules [9]. Endogenous HOCl is produced by myeloperoxidase (MPO) from chloride and H_2O_2 within phagosomes [13–16]. In particular, HOCl could induce apoptosis of cultured cells through the rupture of lysosomes [17]; not only locales where ROS are generated, but also lysosomes play important roles in regulating ROS flux, which determines the ultimate biological outcome of ROS as well as the cross-talk between subcellular compartments [18]. In this regard, it is necessary to devise efficient ways to detect HOCl in lysosomes of living cells.

Fluorescent probes are powerful tools in cell biology and a variety of HOCl-sensitive probes have been developed for monitoring cellular HOCl under fluorescent microscopy [19–37]. However, there are still opportunities to improve these probes in terms of selectivity, sensitivity, response speed and subcellular localization. Recently, a novel nanodosimeter for HOCl with lysosome-homing specificity was reported and applied to imaging exogenous HOCl [33]. However, the area of small molecule HOCl-probes preferentially directed to lysosomes has not been explored.

In this work, we present Lyso-NI-Se, a lysosome-targetable fluorescent probe for imaging HOCl in living cells. The optical probe was designed and synthesized on a naphthalimide platform (Scheme 1). Besides the fluorophore, the structural key points of the probe are the selenide group and the (aminoethyl) morpholine moiety. Previous work has established that selenide could be selectively oxidized by HOCl [35–37], so we chose selenide as the fluorescent modulator. On the other hand, the morpholine moiety was expected to serve a function of leading this probe to lysosomes [38–40]. The probe was synthesised by a nucleophile substitution reaction between terminal bromine and aniline in the presence of KOH with CuI as the catalyst to afford a yield of 73%, and was well characterized by ^{1}H NMR, ^{13}C NMR, ^{77}Se NMR and HRMS.

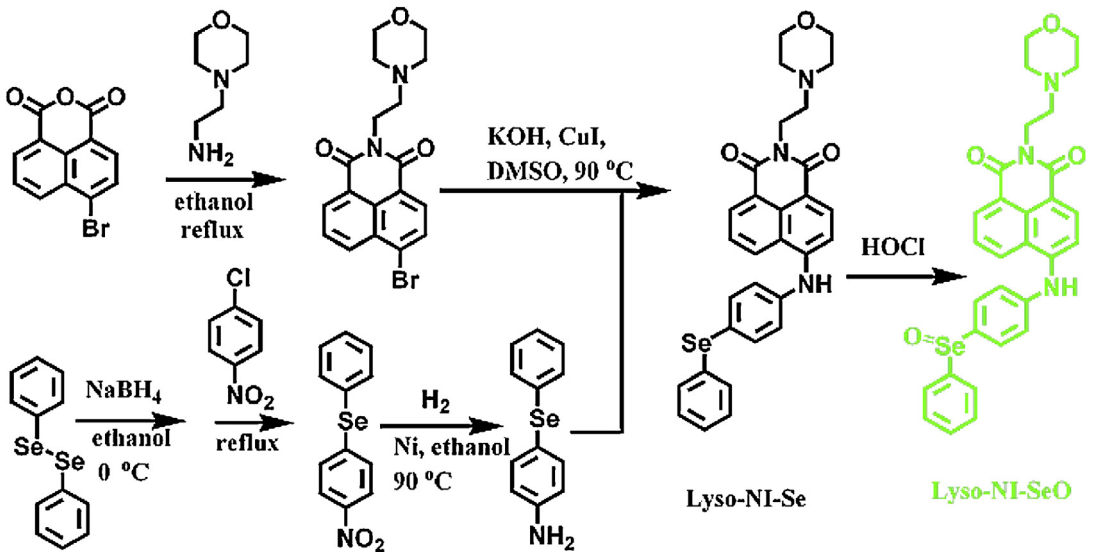
2. Experimental

2.1. Materials

Unless otherwise noted, all chemicals were analytical grade from commercial suppliers and used without further purification. The stock solution of the probe Lyso-NI-Se (acetonitrile or DMSO, 1.0 mM) could be maintained in refrigerator at 4 °C for more than

* Corresponding author. Tel.: +86 411 84379293; fax: +86 411 84675584.
E-mail address: pengl@dicp.ac.cn (P. Li).

http://dx.doi.org/10.1016/j.jphotochem.2014.10.015
1010-6030/



Scheme 1. Synthetic procedures of Lyso-NI-Se and the reaction scheme for the detection of HOCl.

2 months. Ultrapure water was used throughout. Hypochlorite acid (HOCl), *tert*-butylhydroperoxide (*t*-BuOOH) and hydrogen peroxide (H_2O_2) stock solutions were diluted by water from commercial NaClO solution, *t*-BuOOH (70%) and H_2O_2 (30%), respectively. Superoxide anion (O_2^-) was delivered by adding KO_2 dissolved in dry DMSO. Hydroxyl radical ($HO^\bullet$) was produced in situ by Fenton reaction between H_2O_2 (200 μM) and $FeCl_2$ (100 μM), and the concentration of $HO^\bullet$ was represented by the concentration of Fe^{2+}. Peroxynitrite ($ONOO^-$) was prepared from nitrosation of H_2O_2 by isoamyl nitrite [41]. NO was generated from sodium nitroprusside (SNP). The concentrations of $ONOO^-$ and NaClO were ascertained by absorbance spectra ($\varepsilon_{NaClO,292\ nm} = 350\ M^{-1}cm^{-1}$ and $\varepsilon_{ONOO^-,302\ nm} = 1670\ M^{-1}cm^{-1}$) just before use. DMSO solutions of Lyso-NI-Se (1 mM) and neutral red (NR, 1 mM) were used for cell-staining. Phosphate buffer saline (PBS) was aqueous solution containing phosphate (10 mM), NaCl (8.0 g/L) and KCl (0.2 g/L), pH 7.4.

2.2. Instruments

Steady-state UV/vis spectra were measured on a Lambda 35 UV–vis spectrophotometer (PerkinElmer). Fluorescence spectra were obtained from Fluoromax-4 spectrofluorometer (Horiba-Jobin Yvon, monochromator calibrated) with the widths of the excitation and emission slits 5 nm and 4 nm, respectively. ^1H, ^{13}C, and ^{77}Se NMR spectra were taken on a Bruker 400 MHz or 500 MHz NMR spectrometer. For ^1H and ^{13}C NMR, TMS in the solvents indicated was internal reference; for ^{77}Se NMR, external reference PhSeSePh was used. Multiplicities of signals are described as follows: s — singlet, br — broad, d — doublet, t — triplet, m — multiplet. High resolution mass spectra were obtained by an Agilent Q-TOF 6540 spectrometer. Element analyze was carried out on vario EL III (Elementar). Florescent images were taken by FV1000 confocal laser-scanning microscope (Olympus).

2.3. Absorption and fluorescence analysis

Absorption and fluorescence spectra were recorded at room temperature with 10-mm glass cells. The probe Lyso-NI-Se (acetonitrile, 1.0 mM) was diluted by acetonitrile and aqueous buffer. Typically, Lyso-NI-Se (acetonitrile, 50 μL, 1.0 mM), acetonitrile (4950 μL) and phosphate buffer (PB, 5000 μL, 0.1 M, pH 5.0) were added into a 10-mL glass comparison tube to get a probe solution (5.0 μM); then, NaClO (10 μL, 5.0 mM) was added and the absorption or fluorescence spectra were measured immediately.

2.4. Cell culture

MCF-7 or RAW264.7 cells (ATCC, USA) were maintained following protocols from the American Type Culture Collection. Cells were subcultured at a density of 1×10^6 cells mL^{-1} by seeding on 35 mm × 12 mm glass bottom cell culture dishes and cultured in

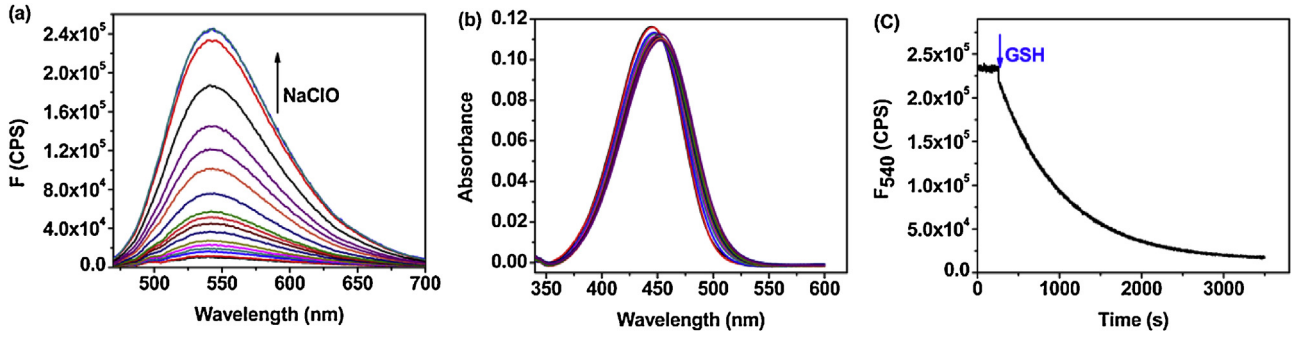


Fig. 1. Fluorescence (a) and absorption (b) spectra of Lyso-NI-Se (5.0 μM) treated with NaClO (HOCl source, 0–8.0 μM). (c) GSH can reduce the signal (the arrow represents the addition of 123 μM GSH). Data were acquired in 1:1CH_3CN–H_2O solutions at pH 5.0 (50 mM phosphate buffer) with λ_{ex} = 430 nm.