Analytica Chimica Acta 969 (2017) 49–56

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Fluorescence probe for hypochlorous acid in water and its applications for highly lysosome-targetable live cell imaging

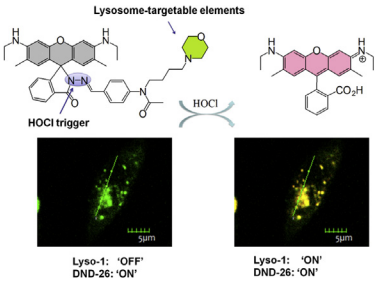
Xiaojie Jiao [a], Chang Liu [a], Qing Wang [b], Kun Huang [b], Song He [a], Liancheng Zhao [a, b], Xianshun Zeng [a, b, *]

[a] Tianjin Key Laboratory for Photoelectric Materials and Devices, Tianjin University of Technology, Tianjin 300384, China
[b] School of Materials Science and Engineering, Institute of Information Functional Materials & Devices, Harbin Institute of Technology, Harbin 150001, China

HIGHLIGHTS

- A lysosomal-targeting probe **Lyso-1** has been synthesized for lysosomal HOCl imaging.
- The probe showed a highly selective fluorescent off/on response to HOCl in water.
- The probe demonstrated a perfect lysosomal targetable ability.
- The probe has been successfully applied to image of lysosomal HOCl in live L929 cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form
1 March 2017
Accepted 3 March 2017
Available online 22 March 2017

Keywords:
Fluorescent probe
Rhodamine
Hypochlorous acid
Lysosome
Imaging

ABSTRACT

Hypochloric acid (HOCl) plays important roles in cell signaling and homeostasis, such as anti-inflammation and immune regulation, pathogen response and so on. Accordingly, direct detection of HOCl at the organelle level is important for investigation of the complex contributions of HOCl to human health. In the present study, a water soluble lysosome-targeting fluorescent probe **Lyso-1** bearing a hydrazone moiety as a HOCl-responsive site and a morpholine unit as a lysosomal-targeting group has been synthesized and evaluated for its ability to image lysosomal HOCl. The probe **Lyso-1**, based on a novel HOCl-promoted hydrazone oxidation strategy, showed a highly selective fluorescent off/on response to HOCl with the various reactive oxygen species in water. With increasing amount of ClO^- from 0.5 to 2.5 μM, a linear correlation between the fluorescence intensity (570 nm) of **Lyso-1** and [ClO^-] was found, and the regression equation was $y = 96.65 + 110.2068[ClO^-]$ with a linear coefficient R of 0.9920. The detection limit is determined to be 60 nM. **Lyso-1** demonstrated a perfect lysosomal targetable ability, and was successfully applied to image of exogenous, endogenous produced lysosomal HOCl in live L929 cells. The success of subcellular imaging indicated that the lysosome-targetable probe **Lyso-1** could be used in further applications for the investigation of biological functions and pathological roles of HClO at organelle levels.

1. Introduction

Hypochlorous acid (HOCl), one of the biologically significant ROS, is produced from peroxidation of chloride ions catalyzed by the enzyme myeloperoxidase (MPO) in activated leukocytes.

* Corresponding author. Tianjin Key Laboratory for Photoelectric Materials and Devices, Tianjin University of Technology, Tianjin 300384, China.
E-mail address: xshzeng@tjut.edu.cn (X. Zeng).

http://dx.doi.org/10.1016/j.aca.2017.03.020
0003-2670/

Cellular HOCl plays important roles in cell signaling and homeostasis, such as antiinflammation and immune regulation, pathogen response and so on [1]. Abnormal levels of HOCl are implicated with many inflammation-associated diseases including cardiovascular diseases, rheumatoid arthritis, and cancer [2–6]. As one of organelles in mammal cells, lysosomes are responsible for the degradation and recycling of macromolecules and play a pivotal role in normal cell processes. Lysosomes degrade cellular and extracellular materials by various internal routes, recycle unwanted or damaged materials, and provide intermediates for further metabolic functions [7,8]. It has been reported that aberrant level of HOCl could cause cell damage at the organelle level, such as lysosomal rupture through calcium dependent calpain activation [9,10], mitochondrial dysfunction via the HOCl-promoted loss of mitochondrial membrane potential [11,12]. Therefore, the development of sensitive, selective and reliable detection methods for investigation of the complex contributions of HOCl to human health is highly desirable.

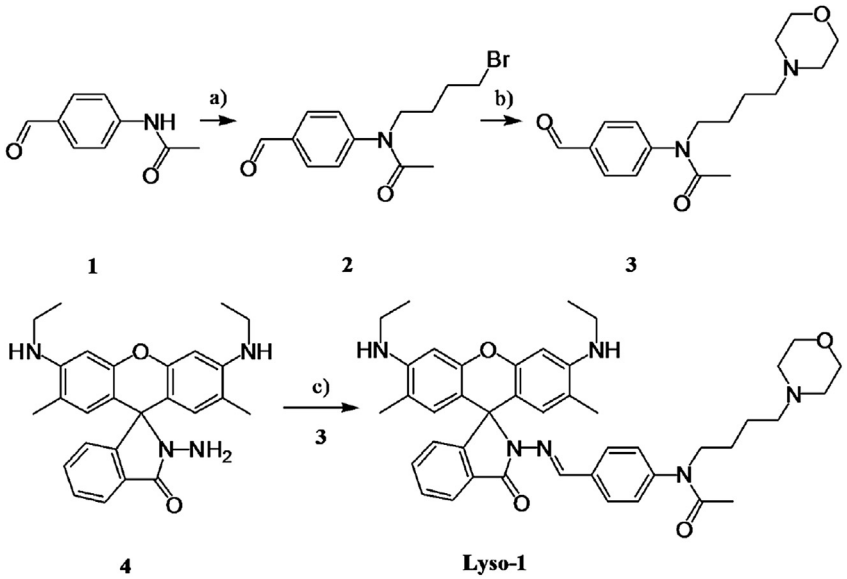
In the past decades, a number of methods for detection of HOCl have been developed [13–21]. Among them, fluorescence imaging methods are generally superior in terms of sensitivity, spatial and temporal resolution, and ease of use. They exhibit their uniqueness for the real-time monitoring of the physiological and pathological processes of HOCl at subcellular levels in living cells. To date, considerable endeavors have been devoted to the development of probes via distinct paradigms for HOCl detection and imaging in living cells [22–42], which have significantly enriched the knowledge about HOCl homeostasis and its critical roles in many biological processes. However, the distribution and function of HOCl in different organelles are still unclear, which caused difficulty to fully insight its biological functions in cellular signaling pathways and various diseases. To the best of our knowledge, reports for the detection of intracellular HOCl in lysosome [43–46] or mitochondria [47–52] are rare. Thus, the development of organelle-specific fluorescent probes to help understand the detailed network of HOCl biology at subcellular levels is still challenging.

With the described concerns in mind, we reported herein a novel lysosome-specific fluorescent probe **Lyso-1** for HOCl detection and imaging at subcellular levels in living cells (Scheme 1). The fluorescence turn-on effect of **Lyso-1** for the detection of HOCl was achieved by a HOCl-promoted specific oxidation of the hydrazone moiety which triggered the ring opening of the spirolactame. To achieve this organelle-targetable fluorescent probe, we introduced a morpholine unit [53], which is a lysosome-targetable element, onto a rhodamine 6G hydrazone, thereby, efficiently yielding the lysosomal-specific **Lyso-1** (Scheme 1). This cell-permeable probe is exceptionally bright, with quantum yields of ca. 0.77, and it is found to specifically localize in lysosomes of the living L929 cell with Pearson's and overlap coefficients over 0.93 via co-localization experiments.

2. Experimental

2.1. Materials and equipments

Unless otherwise stated, all starting materials and reagents were purchased from Tokyo Kasei Kogyo (TCI: Tokyo, Japan), AR grade or dry grade solvents were purchased from Alfa-Aesar, and used without further purification. Fresh solutions of $HOCl/OCl^-$ (pK_a = 7.6) were prepared by dilution of commercial NaOCl in 10 mM NaOH before use. The hypochlorite concentration was determined spectrophotometrically (ε_{292} = 350 M^{-1} cm^{-1}) after dilution with 10 mM NaOH [54]. The reactions were carried out in oven-dried glass wares with a magnetic stirring. NMR spectra were recorded on a Bruker spectrometer at 400 (^{1}H NMR) MHz and 100 (^{13}C NMR) MHz. Chemical shifts were reported in ppm down field from internal Me_4Si (^{1}H and ^{13}C NMR). High resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electro-spray ionization (ESI) source. All absorption spectra were recorded using a Shimadzu UV-2550 UV/Vis spectrophotometer with 1 cm quartz cell. In a similar manner, fluorescence spectra were recorded on a Hitachi F-4600 spectrofluorophotometer with a 1 cm quartz cell. For biological studies 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), dulbecco's modified eagle's medium (DMEM) trypsin-EDTA, dimethylsulfoxide (DMSO) for cell culture solution were purchased from Sigma Aldrich. Penicillin-Streptomycin, and fetal bovine serum (FBS), LysoTracker Green DND-26 were purchased from Invitrogen and Life technologies.



Scheme 1. The synthetic route of probe **Lyso-1**. Conditions: a) 1, 4-dibromobutane/K_2CO_3, CH_3CN, stirred at 75 °C for 2 d; b) morpholine/K_2CO_3, CH_3CN, stirred at room temperature for 27 h; c) THF, stirred at 85 °C.