Dyes and Pigments 148 (2018) 353–358

Contents lists available at ScienceDirect

Dyes and Pigments

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

A mitochondria-targeted fluorescent probe based on fluorescein derivative for detection of hypochlorite in living cells

Jing Lv, Yahui Chen, Fang Wang**, Tingwen Wei, Zhijie Zhang, Jian Qiang, Xiaoqiang Chen*

State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemical Engineering, Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 17 August 2017
Received in revised form
14 September 2017
Accepted 15 September 2017
Available online 18 September 2017

Keywords:
Fluorescent probe
Fluorescein
Mitochondria targetable
Hypochlorite

ABSTRACT

In the present study, a mitochondria-targeted fluorescent probe **FL** based on fluorescein derivative for hypochlorite sensing was designed and synthesized. The probe **FL** showed highly selective to HOCl among various reactive oxygen/nitrogen species. With the increasing amount of HOCl from 0 to 50 μM, a linear correlation between the fluorescence intensity of **FL** at 530 nm and the concentration of HOCl was found. The detection limit is calculated to be 0.068 μM. Furthermore, the probe demonstrated a perfect mitochondria targetable ability and it was successfully applied to image mitochondrial HOCl in living HeLa cells. The success of subcellular imaging indicated that the mitochondria-targetable probe **FL** might be further used to investigate biological functions and pathological roles of HOCl at organelle levels.

1. Introduction

Hypochlorous acid or hypochlorite (HOCl) is one of the most important reactive oxygen spices [1], which mainly produced by the reaction of chloride ions (Cl^-) and hydrogen peroxide (H_2O_2) under the catalysis of myeloperoxidase (MPO) in life system [2–6]. Owing to the strong oxidizing effect of HOCl, it would defense the invasion of bacteria and regulate the lifecycle of the cell [7–10]. However, excessive HOCl can lead to tissue damage and diseases such as atherosclerosis, arthritis, cardiovascular disease, lung injury and neuron degeneration [11–16]. As fluorescent method is an effective way for small molecular detection, many fluorescent probes for HOCl sensing have been reported [17–19]. Mitochondria are vital sub-cellular organelles to eukaryotic cells, which not only provide energy for the whole life activity [20–27], but also play valuable roles in cellular signaling. Studies have shown that mitochondria are the principal source of most reactive oxygen species [28–33], mitochondrial HOCl is responsible for cell signaling and maintains normal cellular functions. Therefore, the development of an effective visualized agent which is able to sense HOCl in mitochondria is of great importance.

It is widely accepted that fluorescence techniques are the most important tools in the fields of modern bioanalysis and real-time bioimaging owing to their unique advantages [34]. Based on above facts, we decided to synthesize a fluorescent probe which not only can be used to detect HOCl but also be able to target mitochondria. Among lots of fluorophores, fluorescein possesses excellent properties such as good water solubility, visible excitation and emission, and maximum brightness at physiological pH [35,36]. In our previous works, we introduced an aldehyde group to fluorescein which made formylated fluorescein become a new platform for constructing various fluorescent probes [37–43]. According to previous reports, cationic pyridinium moieties can be introduced into fluorophore as a receptor for HOCl sensing [44–47], and it is also identified as a mitochondria targetable moiety [47–51]. Here we plan to introduce 1-methyl-4-picolinium iodide to fluorescein-monoaldehyde to construct a mitochondria targetable fluorescent probe for the detection of hypochlorite in living cells.

2. Experimental section

2.1. Material and instruments

Unless specially noted, all reagents and solvents were purchased from commercial suppliers and used without any purification. Deionized water was used throughout all experiments. ^{1}H NMR and

* Corresponding author.
** Corresponding author.
E-mail addresses: fangwang@njtech.edu.cn (F. Wang), chenxq@njtech.edu.cn (X. Chen).

https://doi.org/10.1016/j.dyepig.2017.09.037
0143-7208/

^{13}C NMR spectra were collected on Bruker spectroscopy (Bruker Ascend 400 M). Mass spectra were obtained from Q-Tof mass spectrometer (Agilent 6530). Fluorescent spectra were measured on Spectro-fluorophotometer (RF-5301/PC). Absorption spectra were recorded on UV-Vis spectrometer (α-1860A). The cell imaging experiments were carried out by using Confocal Scanning Microscopy (Leica, TCS sp5 II).

2.2. Synthesis of probe FL

The synthesis routine was showed in Scheme 1. Fluorescein-monoaldehyde (**1**) was prepared according to the previous report [52]. Compound **1** (180 mg, 0.5 mmol) and 1-methyl-4-picolinium iodide (55 mg, 0.51 mmol) were mixed in ethanol (30 mL). Piperidine (two drops) was used as catalyst. The mixture was refluxed at 80 °C under N_2. Along with the reaction, a large amount of precipitate produced. After 12 h, the brown precipitate was collected on a filter, washed by ethanol and dried in vacuum to yield a brick red solid (137 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.7 (d, 2 H, J = 5.25 Hz), 8.27–8.21 (m, 3 H), 8.04–8.01 (m, 2 H), 7.74–7.67 (m, 2 H), 7.28 (d, 1 H, J = 7.50 Hz), 7.00 (s, 1 H), 6.64–6.60 (m, 2 H), 6.53 (d, 1 H, J = 7.75 Hz), 6.48 (s, 1 H), 4.23 (s, 3 H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ(ppm): 168.84, 155.09, 154.48, 154.20, 143.90, 132.42, 130.84, 130.50, 129.18, 127.61, 122.23, 121.73, 110.94, 109.65, 102.79, 46.12, 40.06, 39.78, 39.50, 39.22, 38.94. TOF-MS (ESI) m/z calcd. For $C_{28}H_{20}NO_5$ $[M\text{-}I]^+$: 450.1341, found: 450.1332.

2.3. Fluorescence and absorbance measurements

The probe **FL** (0.5 mM) was dissolved by DMF/H_2O (v/v = 1/1) and kept at room temperature. Hypochlorous acid (HOCl) was provided by NaOCl (10 mM); hydroxyl radical (•OH) was generated by Fenton reaction (Fe^{2+}/H_2O_2 = 10 mM/10 mM); superoxide anion (O_2^-) derived from dissolved KO_2 (10 mM); alkyl peroxyl radical (ROO•) was obtained by thermolysis of 2, 2′-azobis-2-methyl-propanimidamide (AAPH, 10 mM) in air-saturated aqueous solution at 300 K; $^1O_2$ was generated by the reaction of H_2O_2 (10 mM) with NaOCl (5 mM); peroxynitrite ($ONOO^-$) was provided by 3-morpholinosydnonimine hydrochloride (SIN-1, 10 mM); nitric oxide (NO) derived from the solution of S-nitroso-N-acetyl-DL-penicillamine. Test solutions were prepared by adding 60 μL of the probe stock solution into a 3.5 mL test tube, diluting the solution to 3 mL with PBS buffer (10 mM, pH = 7.4), then different analytes of corresponding concentration were added. All UV-vis absorption and fluorescence spectra were measured at room temperature. For fluorescent study, the samples were excited at 490 nm and the slit widths were 5 nm.

2.4. Detection of fluorescence quantum yield

Fluorescence quantum yield of the probe with or without HOCl were measured by using fluorescein in 0.1 N NaOH (aq) ($\varphi_S = 0.925$) as a standard and calculated with the following expression:

$$\varphi_f = \frac{n_f^2}{n_s^2} \bullet \frac{A_S \bullet D_f}{A_f \bullet D_S} \bullet \varphi_S (A \leq 0.05)$$

While A represents absorbance, n represents the refractive index of the solution, D represents corrected fluorescence emission spectral integral area (integrated from 495 to 650 nm). Excitation was chosen at 490 nm.

2.5. Detection limit

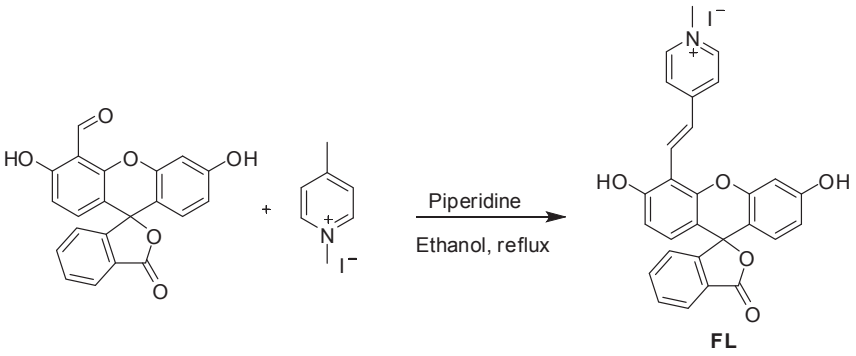
The fluorescence emission spectra of **FL** (10 μM) were measured by eleven times, thus the standard deviation of blank sample was obtained. After different equiv. of HOCl (0–50 μM) were added to the probe solution, the fluorescence intensity was recorded after 10 min. Then, the fluorescence intensity at 530 nm was plotted as the concentration of HOCl. The detection limit was calculated by using:

$$\text{Detection limit} = 3\sigma/k$$

where σ is the standard deviation of blank sample, k is the slope between the fluorescence intensity (I_{530nm}) versus the concentration of HOCl.

2.6. Cell culture and fluorescence imaging

HeLa cells were purchased from Nanjing Cobioer Biosciences Co., Ltd, incubated in Dulbeccos modified Eagles medium (DMEM) supplement with 10% (V/V) Fetal Fovine Serum (FBS, Gibco) at 37 °C with 5% CO_2 in appropriate humidity. These cells were passed on an uncoated 35 mm diameter glass-bottomed culture dish and allowed to adhere for 20 h. After being cleaned with PBS, the cells were incubated with DMEM containing 2.5% FBS and 10 μM of probe at 37 °C. In order to learn the mitochondria targeting function of the probe **FL**, co-localization images of the fluorescent probe with MitoTracker Red CMXRos (MTR) were studied. Three groups were conducted as follows: (I) Cells were incubated with **FL** (10 μM) for 10 min, then cells were treated with MTR (200 nM) for another 10 min (II) HeLa cells were pre-treated with the probe (10 μM) for 10 min and exposed to NaOCl (50 μM) for another 10 min, then cells were treated with MTR (200 nM) for another 10 min (III) HeLa cells pre-incubated with NaCl (250 μM) and the probe (10 μM) in MPO



Scheme 1. Synthesis routine of compound **FL**.