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A high-resolution mitochondria-targeting ratiometric fluorescent probe for detection of the endogenous hypochlorous acid

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ABSTRACT

Hypochlorite anion, one of the biologically important reactive oxygen species, plays an essential role in diverse normal biochemical functions and abnormal pathological processes. Herein, an efficient high-resolution mitochondria-targeting ratiometric fluorescent probe for hypochlorous acid detection has been designed, synthesized and characterized. It is easily synthesized by the condensation reaction (C═C) of a 2-(2-hydroxyphenyl) quinazolin-4(3H)-one fluorophore and a cyanine group (mitochondria-targeting), which made the whole molecular a large Stokes shift (210 nm) and the two well-resolved emission peaks separated by 140 nm. As a result, it is considered as a good candidate for high resolution hypochlorous acid imaging in live cells. The ratiometric fluorescent probe exhibited outstanding features of high sensitivity, high selectivity, rapid response time (within 50 s), and excellent mitochondria-targeting ability. Moreover, the probe can also be successfully applied to imaging endogenously hypochlorous acid in the mitochondria of living cells with low cytotoxicity, and high resolution.

1. Introduction

Hypochlorous acid and its conjugate base ($HClO/OCl^-$) are essential oxygen metabolites in living systems [1], and endogenous HClO, which is produced from the mounting myeloperoxidase (MPO)-mediated peroxidation of chloride ions (Scheme 1), mainly by the mitochondrial electron transport chain [2], plays an important role in human immune defense system and contributes to destroying the invading bacteria and pathogens [3]. However, the over production of HClO and other reactive oxygen species (ROS) from the mitochondrial electron transport chain leads to oxidative stress [4], aberrant electron transport, disruption of calcium homeostasis, activation of apoptosis [5] and tissue damage. Accumulation of oxidative damage over time is connected to debilitating human diseases, including Alzheimer and some related neurodegenerative diseases, as well as cardiovascular disorders and cancer [6].

Recently, much effort has been focused on how to maintain the balance of HOCl between health and disease, and studying the acting mechanism of HOCl in life system [7]. In order to achieve this goal, several fluorescent probes have been developed for the detection of HClO, for instance, the p-methoxyphenol [8], ether [9], thioether [10], hydrazone [11], oxime [12], hydroxamic acid [13], hydrazide [14], and so on. Both of these probes utilized the strong oxidation property of HClO in design. However, there are some shortcomings such as limited sensitivity, the dependence on chemical environment and long reaction time [15]. Exploiting this, we use a 2-(2-hydroxyphenyl) quinazolin-4(3H)-one(HPQ) dye which is a class of well-known excited-state intramolecular proton transfer (ESIPT) fluorophore with widespread applications as the mother molecule due to its valuable characteristics [16–17], such as high fluorescence quantum yield leading to intense absorption, and fluorescence bands. Moreover, the keto form of the ESIPT is easy to make C═C cut off. Therefore, we designed and synthesized a novel and low-cost ratiometric fluorescent probe (HPQ-Cy_2) for selective and sensitive detection of HClO over other ROS, which used cyanine dye based on-ESIPT, HPQ-Cy_2 (Scheme 2), containing mitochondria-targeting. This ratiometric probe with two well-resolved emission peaks separated by 140 nm eliminates much interference causing by instrumental efficiency, environmental conditions, and affected concentrations [18]. Compared with other fluorescence probes [19], such probe has a built-in correction with two emission bands and is subsequently proved to be more favorable for intracellular imaging. The strong fluorescence of (HPQ-Cy_2) is restored after the oxidation of C═C bond by HClO. Furthermore, (HPQ-Cy_2) shows excellent cell membrane permeability and can also be applied to imaging HClO in the mitochondria of living cells with low cytotoxicity.

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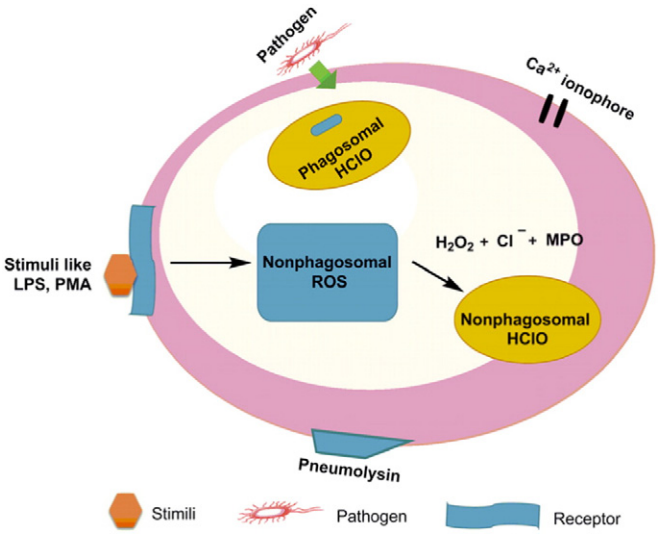


Scheme 1. Stimuli that induce intracellular HClO. Apart from ingested particles that give rise to phagosomal HClO (phHClO) production, certain stimuli, such as lipopolysaccharides (LPS), lead to nonphagosomal HClO (nphROS) production without being ingested. H_2O_2: Hydrogen peroxide; MPO: Myeloperoxidase.

2. Experimental section

2.1. Materials and apparatus

All chemicals were gotten from commercial suppliers and used without further purification. Water was doubly distilled and purified by a Milli-Q system (Millipore, USA). The analytical reagent of the hydrogen peroxide and sodium hypochlorite were obtained from the Sigma, before the experiments, we re-titrated the concentration of them. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer (Thermo Finnigan). NMR spectra were recorded on a Bruker DRX-400 spectrometer using TMS as an internal standard. All chemical shifts are reported in the standard δ notation of parts per million. UV–vis absorption spectra were recorded in 1.0 cm path length quartz cuvettes on a Hitachi U-4100 UV–vis spectrophotometer (Kyoto, Japan). The pH was measured with a Mettler-Toledo Delta 320 pH m. Fluorescence measurements were carried out on a Hitachi-F4500 fluorescence spectrometer with excitation and emission slits set at 5.0 nm and 5.0 nm, respectively. Fluorescence images of A357 cells were obtained using Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan).

2.2. Synthesis of compound probe HPQ-Cy_2

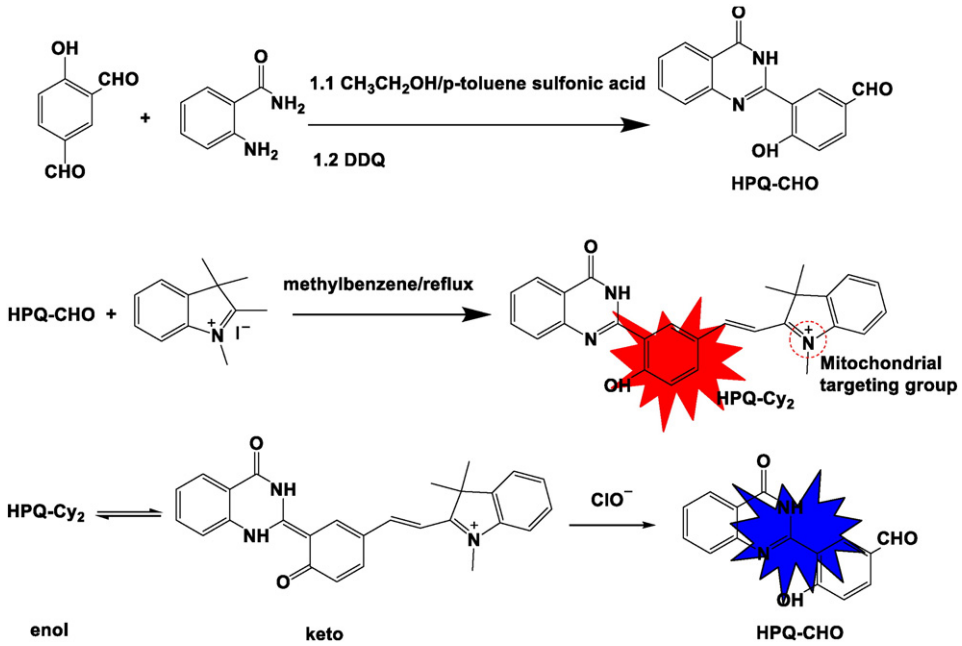
We synthesized the probe HPQ-Cy_2 with two steps. First, HPQ-CHO was synthesized by one-step reaction of 2-aminobenzamide and 4-hydroxybenzene-1, 3-dicarbaldehyde according to the literature method. Then aldehyde of HPQ with 1, 2, 3, 3-tetramethyl-3H-indolium iodide was added to produce final compound HPQ-Cy_2.

2.2.1. Synthesis of HPQ-CHO

To a solution of 2-aminobenzamide (2.0 g, 14.69 mmol) in absolute EtOH (30 mL) was added 4-hydroxybenzene-1, 3-dicarbaldehyde (2.2 g, 14.66 mol) giving a precipitate. The reaction was heated to 80 °C under a condenser open to the air for 30 min, then p-TsOH monohydrate (0.25 mmol) was added and the precipitate dissolved. The solution was heated to 80 °C for 1 h, then cooled to room temperature and DDQ (3.36 g, 14.8 mmol) added. The mixture was stirred at RT, open to the air, overnight. The solid was filtered on a porosity glass frit and air dried, rinsed twice with diethyl ether then dried at reduced pressure giving HPQ-CHO (3.14 g, 10 mmol, 80.36%). EI-$(M)^+$: 266.1, calcd for (M): 266.4.

2.2.2. Synthesis of HPQ-Cy_2

Cyanine (1.5 g, 5.0 mmol) and HPQ-CHO (1.46 g, 5.5 mmol) were dissolved in toluene (40 mL) with piperidine (0.5 mL) and acetic acid (0.5 mL) under argon protection at room temperature. Then the mixture was refluxed and stirred for 10 h. The solvent was evaporated in vacuo, and the crude solid was purified by column chromatography on silica gel eluting to afford a solid in 50% yield. ^{1}H NMR (400 MHz, d-DCl_3), δ_H: 8.299–8.279 (d, J = 4 Hz, 1H), 8.051–8.016 (d, J = 7 Hz, 1H), 7.710–7.674 (t, J = 7.4 Hz, 2H), 7.401–7.363 (t, J = 7.6 Hz, 3H), 7.251 (s, 5H), 7.158 (s, 1H), 6.767–6.744 (d, J = 4.6 Hz, 1H), 3.755



Scheme 2. Synthetic route for the ratiometric cassette HPQ-Cy_2 and the response mechanism for ClO^-.