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A ratiometric fluorescent probe based on boron dipyrromethene and rhodamine Förster resonance energy transfer platform for hypochlorous acid and its application in living cells

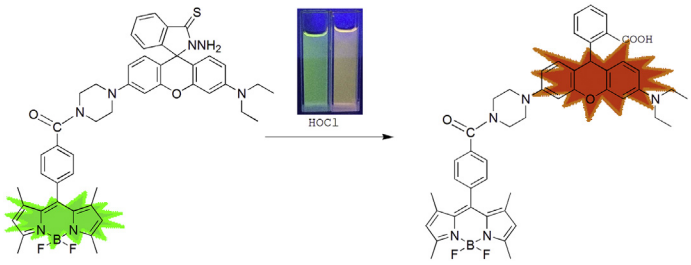
Ying Liu [a], Zhi-Min Zhao [b], Jun-Ying Miao [b, **], Bao-Xiang Zhao [a, *]

[a] Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, PR China
[b] Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, PR China

HIGHLIGHTS

- A probe based on BODIPY and rhodamine was developed for sensing HOCl.
- The probe could sense HOCl in a ratiometric manner based on the FRET platform in PBS buffer solution.
- The probe can detect HOCl in 15 s accompanied with a fluorescence colour change.
- This probe was successfully used to monitor HOCl in living RAW 264.7 cells.

GRAPHICAL ABSTRACT



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ABSTRACT

We have developed a ratiometric fluorescent probe **BRT** based on boron dipyrromethene (BODIPY) and rhodamine-thiohydrazide Förster resonance energy transfer (FRET) platform for sensing hypochlorous acid (HOCl) with high selectivity and sensitivity. The probe can detect HOCl in 15 s with the detection limit of 38 nM. Upon mixing with HOCl the fluorescence colour of probe **BRT** changed from green to orange. Moreover, probe **BRT** was applied to successfully monitor HOCl in living RAW 264.7 cells.

1. Introduction

Hypochlorous acid (HOCl)/hypochlorite ($^{-}$OCl) is known to be one of the reactive oxygen species (ROS) [1], which plays a vital role in many physiological processes. In our daily life, HOCl is used as disinfectant, deodorant, and bleaching agent [2–4]. Biologically, endogenous HOCl is generated from hydrogen peroxide and chloride ion by the enzyme myeloperoxidase (MPO) catalysis in

* Corresponding author.
** Corresponding author.
E-mail addresses: miaojy@sdu.edu.cn (J.-Y. Miao), bxzhao@sdu.edu.cn (B.-X. Zhao).

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activated leukocytes including neutrophils, macrophages and monocytes [5,6]. And it contributes to the human immune defence system, and plays an important role in killing a wide range of bacteria and pathogens in cells [7]. However, much evidence suggests that once the level of endogenous HOCl is higher than normal, that may cause some diseases, such as inflammatory, atherosclerosis, rheumatoid arthritis, respiratory distress, cardiovascular diseases and even cancer [8–15].

In the last decade, scientists have focused on much effort to the development of methods for the recognition of HOCl including chemiluminescence, electroanalysis and potentiometry [16–20]. Fluorescent probes have attracted great attention because of their high selectivity, high sensitivity, low cost, real-time essay and most important of all they can provide not only in vitro detection but also in vivo imaging studies [21–30]. A considerable number of fluorescent probes have been reported for the sensing of HOCl by the modification of common fluorophores such as fluorescein [31–33], coumarin [34,35], cyanine [36], BODIPY [37,38], rhodamine [39,40] and so on [41–47]. However, most of them are intensity-based fluorescent probes and these probes may be affected by the factors such as environment variations, instrumental efficiency and sensor concentrations. In contrast, ratiometric fluorescent probes employing the signal ratios at two wavelengths can alleviate the above problems and provide accurate analysis [48,49].

Inspired by the Förster resonance energy transfer (FRET) mechanism [50], we designed and synthesized a ratiometric fluorescent probe **BRT** for the detection of HOCl. In this new FRET system, BODIPY performed as the donor, rhodamine-thiohydrazide acted as the acceptor. Probe **BRT** can respond to HOCl under acid conditions by the reaction of thiohydrazide with HOCl [51], accompanied with the fluorescence intensity of BODIPY decreases and the fluorescence intensity of rhodamine gradually increases at the typical excitation of BODIPY fluorophore. Therefore, probe **BRT** can sense HOCl in a ratiometric manner by recording the ratios of emissions of the two fluorophores. Further, we found that probe **BRT** can be applied to monitor HOCl with high selectivity, sensitivity and low detection limit (38 nM). More importantly, probe **BRT** has been successfully used in imaging of endogenous HOCl in living cells.

2. Experimental

2.1. Materials and instrumentations

All reagents were purchased from commercial suppliers and used without further purification. TLC was conducted on silica gel 60 F_{254} plates (Merck KGaA). ^{1}H NMR and ^{13}C NMR spectra (400 and 100 MHz) were recorded on a Bruker Avance 400 spectrometer using tetramethylsilane (TMS) as an internal standard with DMSO-*d*6 as a solvent. IR spectra were determined with an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). UV–vis spectra were recorded on a U-4100 spectrophotometer (Hitachi). Fluorescence measurements were recorded on a Perkin–Elmer LS-55 luminescence spectrophotometer. HRMS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). All pH measurements involved the use of a Model PHS-3C pH meter (Shanghai) at room temperature about 298 K. Deionized water was used throughout the experiment.

2.2. Preparation of ROS and RNS

Various ROS and RNS including NaOCl, H_2O_2, *t*-BuOOH, •OH, *t*-BuO·, NO, $ONOO^-$, $^-O_2$, $^1O_2$ were prepared according to the following methods. Sodium hypochlorite (NaOCl), H_2O_2 and *tert*-butylhydroperoxide (*t*-BuOOH) were diluted from the commercially available solution to 0.1 M in water. Hydroxyl radical (·OH) was generated by Fenton reactions by mixing $FeSO_4{\cdot}7H_2O$ with 1 equivalent of H_2O_2, the concentration of ·OH was estimated from the concentration of Fe^{2+}.

Tert-butoxy radical (*t*-BuO·) was prepared according to Fenton reactions by mixing $FeSO_4{\cdot}7H_2O$ with 1 equivalent of *tert*-butylhydroperoxide (*t*-BuOOH), the concentration of *t*-BuO· was estimated from the concentration of Fe^{2+}.

Nitric oxide (NO) was generated from potassium nitroprusside dehydrate. Peroxynitrite ($ONOO^-$) was prepared according to the reported method [52]. Superoxide ($^-O_2$) was generated from KO_2 according to the literature [53]. Singlet oxygen $^1O_2$ was generated on mixing of NaOCl with 2 equivalent of H_2O_2 according to the literature [54].

2.3. General procedure for the measurement of absorption and fluorescence spectra

A 1.0×10^{-3} M stock solution of probe **BRT** was prepared in EtOH. Proper amounts of probe **BRT** solution were added directly with a micropipette to 10 mL glass tube, the solutions were diluted to 10 mL with PBS (pH = 5) and EtOH (v/v, 4:6), different amounts of NaOCl were added directly with a micropipette to the above solutions. Then the absorption and fluorescence measurements were run.

2.4. Detection limit calculation and the efficiency of energy transfer (EET)

Detection limit for HOCl was calculated by Eq. (1):

$$\text{Detection limit} = 3\sigma/\text{m} \tag{1}$$

where σ = standard deviation of 10 blank measurements and m = slope obtained from the graph of fluorescence intensity ratio vs concentration of HOCl [55].

The efficiency of energy transfer (EET) was calculated according to Eq. (2):

$$\eta_{EET} = 1 - \Phi_{F(\text{donor in FRET system})} \Big/ \Phi_{F(\text{donor})}. \tag{2}$$

where η_{EET} is the efficiency of energy transfer. $\Phi_{F(\text{donor in FRET system})}$ is the fluorescence quantum yield of the donor part in FRET system (BODIPY part in compound **BRA** in this work), $\Phi_{F(\text{donor})}$ is the fluorescence quantum yield of the donor when the FRET system is blocked (BODIPY part in probe **BRT** in this work) [56] and the fluorescence quantum yield was calculated according to the literature [57].

2.5. Cytotoxicity assay

The standard sulforhodamine B (SRB) assay was performed to examine the cytotoxicity of probe **BRT** in RAW264.7 cells. Briefly, RAW264.7 cells were cultured in 96-well culture plates for 12 h, and then incubated with DMEM containing different concentration of probe **BRT** (1, 5, 10 μM) for 12 h. The cells were fixed with 4% TCA for 1 h at 4 °C, then washed 5 times with deionized water. SRB (50 μL) was added to each well, and after sufficient reaction with cells, the remaining SRB was removed by washing each well with 1% acetic acid solution, then 100 μL Tris–HCl was used to dissolve the SRB. Absorbance at 540 nm was measured in a 96-well multi well-plate reader (TECAN).