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A sensitive BODIPY-based fluorescent probe suitable for hypochlorite detection in living cells

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ABSTRACT

A fluorescent probe composed of BODIPY and 2,4-dinitrophenylhydrazine was designed for the detection of ClO^-. The oxidizing reaction between probe and ClO^- resulted in a distinct fluorescence enhancement together with a color variation from pink to orange. This probe showed excellent selectivity to ClO^- among various ions including common reactive oxygen species and high sensitivity with a detection limit of 228 nM. A fast response (7 min) was observed, which made the probe a promising method in real-time detection. Furthermore, this probe was successfully applied to monitor ClO^- in real-life water and living cells.

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1. Introduction

Hypochlorite (ClO^-), as a biologically significant part of the reactive oxygen species (ROS), plays a vital role in various physiological processes [1]. Endogenous hypochlorite, which is produced mainly from the reactivity of H_2O_2 and Cl^- by myeloperoxidase(MPO) in leukocytes, has antibacterial effects on the biological immune-defense system [2]. A low concentration of hypochlorite can devastate invasive bacteria and pathogens, which is particularly crucial to physiological balances of proteins, DNA and RNA [3,4]. Unfortunately, the excessive hypochlorite may cause tissue damage and a variety of human diseases, including atherosclerosis, cardiovascular diseases, osteoarthritis, cystic fibrosis cancers and pulmonary lesions [5–9]. Hypochlorite is also widely used as disinfector in our daily life, for example, in swimming pool water and drinking water [10]. Owing to the biological importance of ClO^-, the development of detection methods for ClO^- in both real-life water and living cells has become an important issue.

Fluorescent probe is a kind of powerful tool for ion detection, due to the advantages of nontoxicity, spatial resolution capability, sensitivity, simple operation, potential biological value [11]. Recently, some fluorescent probes containing functional groups like amide, thiosemicarbazone, *p*-methoxyphenol, oxime have been developed to detect ClO^- [12–16]. Nonetheless, some limitations of these probes appeared during the detection process, such as delayed response time, low quantum yield, poor biocompatibility.

In this work, a new fluorescent probe (Probe 1) based on C=N bond transformation was designed and synthesized to detect ClO^-. BODIPY was selected for the basic fluorophore in consideration of excellent light stability, low molecular weight, high fluorescence quantum yield, admirable cells' penetration [17]. Probe 1 was non-fluorescent because C=N isomerization [18,19] quenched the fluorescence of fluorophore, but exhibited significant fluorescence enhancement in response to ClO^-. Excellent properties like high sensitivity and selectivity, rapid response, make Probe 1 a promising method for ClO^- detection in neutral water and biological cells.

2. Experimental

2.1. Chemicals and instruments

All chemicals used were of analytical grade, purchased form Aldrich and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Unless otherwise stated, these reagents were used without further purification and redistilled water were the main solvent. Mass spectrometry data were obtained with a Bruker ESQUIRE HPLC–MS AB 4000Q mass spectrometer. Ultraviolet-visible

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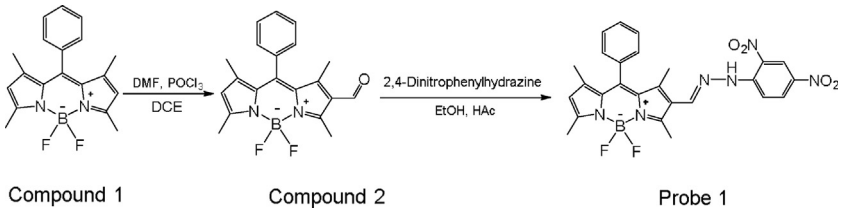

Scheme 1. Synthesis of Probe 1.

spectrum data were obtained with UV-2550 spectrophotometer and ^{1}H NMR spectra were recorded on an AVANCE II 400 MHz spectrometer (Bruker Bio Spin). Varian Cary Eclipse Fluorescence spectrophotometer was used to measure fluorescence spectra. An Olympus Zeiss 710 laser scanning confocal microscopy was used for Fluorescence image of cells. All the samples were tested in DMF-PBS solution (1:1 v/v, 0.01 M PBS, pH 7.4) at room temperature.

2.2. Synthesize of Probe 1

The intermediate products, Compound 1 and 2 were prepared according to our previous work [17]. As illustrated in Scheme 1, 69.3 mg(0.35 mmol)of 2,4-dinitrophenylhydrazine and 105.5 mg (0.3 mmol)of Compound 2 were added in the mixed solution (ethyl alcohol-ethyl acetate, v:v, 1:1). Then 5 drips of acetic acid were added and the mixture was stirred for 2 h at room temperature. The reaction progress was monitored by TLC (petroleum ether- ethyl acetate, v:v, 1:2) until Compound 2 was consumed. The crude product was recrystallized from ethyl alcohol and further purified by column chromatography (petroleum ether-dichloromethane, v: v, 1:1) to give Probe 1 (139.6 mg, 87.3%). ^{1}H NMR (400 MHz, $CDCl_3$, ESI) δ 11.21 (s, 1H), 9.12 (dd, J = 12.7, 2.5 Hz, 1H), 8.30 (dd, J = 9.6, 2.4 Hz, 1H), 8.09 (s, 1H), 7.80 (d, J = 9.6 Hz, 1H), 7.63-7.49 (m, 3H), 7.31 (dd, J = 6.5, 2.9 Hz, 2H), 6.11 (s, 1H), 2.84 (s, 3H), 2.62 (s, 3H), 1.60 (s, 3H), 1.41 (s, 3H). MS (ESI): 533.70 $[M+H]^+$.

2.3. Preparation of test solutions

The corresponding amount of Probe 1 solid was dissolved in DMF to prepare a stock solution (1 mM). The test solutions were diluted with the mixture of DMF and 0.01 M PBS (phosphate-buffered saline, pH 7.4) (1:1, v/v) for the spectral analysis. The stock solutions of some ions (10 mM for F^-, Cl^-, NO_2^-, ClO_4^-, HCO_3^-, $H_2PO_4^-$, SO_4^{2-}, $S_2O_3^{2-}$, CO_3^{2-}, Fe^{3+}, Cu^{2+}, H_2O_2, $ONOO^-$, $ROO^\bullet$, $^\bullet OH$, $NO^\bullet$ and ClO^-) and the blank solution were prepared in deionized water. NaOCl and H_2O_2 stock solutions were respectively obtained by diluting the commercialization solutions (14.5% and 30%). Peroxynitrite ($ONOO^-$) was provided by nitrozation of $NaNO_2$ and H_2O_2. Peroxyl radicals ($ROO^\bullet$) was prepared from 30 min stirring of 2,2′-Azobis (2-amidinopropane) dihydrochloride(AAPH) solution at 25 °C. Hydroxyl radical ($^\bullet OH$) was derived from Fenton reaction with ferrous chloride and 10 equiv. H_2O_2 [20]. Nitric oxide ($NO^\bullet$) was obtained from sodium nitroferricyanide (III) dehydrate (SNP).

2.4. Calculation of fluorescent quantum yield [21]

Rhodamine B dissolved in EtOH (quantum yield $\Phi s = 0.97$) was used as the standard. The quantum yields of probe in the absence and presence of NaClO were estimated according to the following equations:

$$\Phi_X = \Phi_s \left(\frac{A_S}{A_X}\right)\left(\frac{F_X}{F_S}\right)\left(\frac{n_X}{n_S}\right)^2$$

Where Φ is the quantum yield; A is absorbance of excitation wavelength; F is integrated area under the corrected emission spectra; the subscript S and X stand for the standard and for the unknown, respectively.

2.5. Measurement of the detection limit [22]

The detection limit (D) based on the fluorescence titrations was estimated according to the following equations: Detection limit =

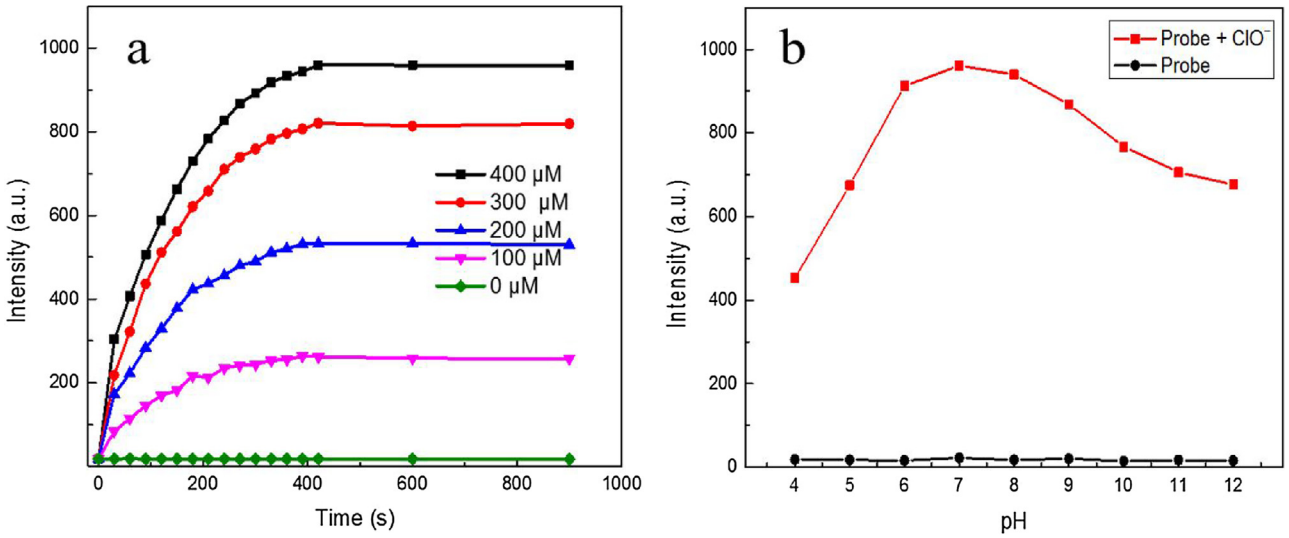

Fig. 1. (a) Fluorescence response of Probe 1 (10 μM) in presence of different concentrations of ClO^-; (b) Effect of pH value on the fluorescence intensity of Probe 1 (10 μM) in the absence and presence of 400 μM ClO^-. λex = 470 nm.

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