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Dual-functional fluorescent probe responds to hypochlorous acid and SO_2 derivatives with different fluorescence signals

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ABSTRACT

In view of the important roles of reactive oxygen species (ROS) and reactive sulfur species (RSS) in the complex signal transduction and oxidation pathways, fluorescent probes that are able to display distinct signals to hypochlorous acid and SO_2 derivatives are highly valuable. Herein, a novel dual-functional probe **(DFP)** as an efficient single fluorescent-molecule which can respond to HClO and HSO_3^- with two different sets of fluorescence signals was presented. The **DFP** displayed desired properties such as high specificity, suitable sensitivity, appreciable water solubility and stability. The sensing mechanism was confirmed by high-resolution mass spectroscopy analysis and ^1H NMR spectrometry analysis. Moreover, as a biocompatible molecule, the **DFP** has been successfully applied for the detection of HClO in living cells with a dual-channel mode. Therefore, the present work established a novel strategy for monitoring the multiple ROS and RSS species using a single fluorescent probe and the **DFP** is promising as a molecular tool to investigate the production and dynamics of HClO and HSO_3^- in the complex interaction networks of the living system.

1. Introduction

It is known that reactive oxygen species (ROS) and reactive sulfur species (RSS) participate in a wide variety of biological events. Among the ROS, hypochlorous acid (HClO) is mainly produced in leukocytes (including neutrophils, macrophages, and monocytes) by myeloperoxidase (MPO)-catalyzed peroxidation of chloride ions and plays a vital role in killing a wide range of pathogens [1–3]. In living organisms, the highly active HClO can mediate the chemical modification of various biomolecules such as proteins, lipids, DNA and RNA [4]. Whereas, increasing evidence suggests that abnormal production of HClO is also involved in several human diseases, including osteoarthritis, kidney diseases, neuron degeneration, cardiovascular diseases, and cancer [5–8]. As a kind of RSS, sulfur dioxide (SO_2) can be endogenously generated through the oxidation of intracellular hydrogen sulfide or sulfur-containing amino acids by ROS, which could result in oxidative stress and aged-related diseases [9–12]. Moreover, SO_2 is recognized as a newly gaseous signal transmitter like nitric oxide (NO) and involves in many physiological processes, including increasing anti-oxidative capacity, regulation of cardiovascular smooth muscle tone and lowering blood pressure [13–16]. However, excessive intake of SO_2 caused severe adverse effects and acute symptoms, such as flushing, hypotension, diarrhea, urticaria, and abdominal pain [17–19]. Since SO_2 mostly exists in biological systems in the form of equilibrium of its derivatives bisulfite (HSO_3^-) and sulfite (SO_3^{2-}), the toxicity of SO_2 is considered to be caused mainly by these two anions [20].

In view of the important roles of ROS and RSS in the complex signal transduction and oxidation pathways, it is of great significance to develop a selective and sensitive method for the detection and imaging of hypochlorous acid and bisulfite. Recently, fluorescence sensing method combined with confocal laser imaging technology has emerged as one of the most powerful and versatile tools for monitoring the level, localization, and transportation of vital bio-molecules within the context of living systems [21–23]. To date, a number of well-designed synthetic fluorescent probes have been designed for the determination of HClO with some specific reactions, such as HClO-induced oxidation of *p*-methoxyphenol [24–26] and C=C unsaturated bond [27–29], deoximation reaction [30–32] and other reactions [33,34]. Meanwhile, many fluorescent probes for SO_2 derivatives (HSO_3^- and SO_3^{2-}) detection have been reported in recent years [35–37]. Not long ago, Yu and coworkers presented several mitochondria-targeted fluorescent probes for the detection of biological SO_2 derivatives [38–40]. Wang and coworkers prepared a reversible fluorescent probe for selective detection and cell imaging of HSO_3^- and H_2O_2 [41].

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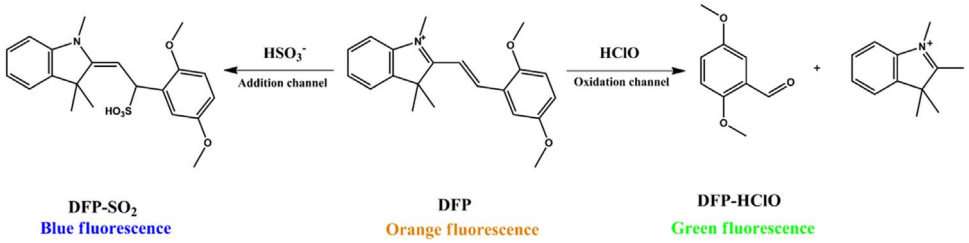

Scheme 1. Design of **DFP** and its reaction with HClO and HSO_3^-.

Although numerous fluorescent probes possessing specific and sensitive detection performance for HClO or SO_2 derivatives have been reported, the development of the dual-functional fluorescent probe responds to both HClO and SO_2 derivatives, to the best of our knowledge, is still an unmet challenge. A possible solution to the problem is to use several different types of fluorescent probes in one system, while this method produces cross-talk, a larger invasive effect, the different localization and the different metabolisms, making the scenario very complicated [42,43]. Thus, a single fluorescent probe which is capable of responding to HClO and SO_2 derivatives with distinct fluorescence signals is highly desirable for dissecting the complicated roles of these biomolecules in living systems.

In view of these findings, a novel probe **(DFP)** was presented here as a single fluorescent-molecule, which can respond to HClO and HSO_3^- with two different sets of fluorescence signals (Scheme 1). In our design, 2,5-dimethoxybenzaldehyde was introduced to the indolium-based hemicyanine molecular skeleton with a double bond as a linker. It is known that HClO shows strong reactivity to double bonds, which can destroy the large π-conjugation of the probe [29,44]. In addition, the unsaturated double bond between indolium and dimethoxybenzene group is selectively reactive with HSO_3^- through nucleophilic addition to produce the addition product [36,39]. Compared to the **DFP** of weak orange fluorescence, the reactions of probe with HClO and HSO_3^- generate two different products with strong green and blue emissions, respectively, which can effectively achieve the dual-functional detection of the two important biomolecules. On the basis of these interesting investigations, the **DFP** was further applied for detecting HClO in living cells by confocal fluorescence imaging. The novel dual-functional fluorescent probe is promising as an efficient molecular tool to report on the production and dynamics of HClO and HSO_3^- in the complex interaction networks of signal transduction and oxidative pathways.

2. Experimental

2.1. Materials and instruments

All chemicals were purchased from J & K Scientific Ltd. or Sigma-Aldrich reagent Co., and were used without further purification except special instruction. All the organic solvents were of analytical grade. HeLa cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell culture media were purchased from Thermo Scientific HyClone. Ultrapure water was obtained from a Millipore Direct-Q purification system (18.2 MΩ).

^{1}H and ^{13}C NMR spectra were measured on a Varian INOVA 400 spectrometer, using tetramethylsilane (TMS) as internal reference. Mass spectra were recorded on a Bruker micrOTOF-Q II mass spectrometer. UV–visible spectra were measured on a Perkin Elmer Lambda-35 double beam scanning spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer LS 55 scanning spectrofluorometer equipped with a xenon flash lamp as the excitation source. Samples for absorption and fluorescence measurements were contained in 1 cm×1 cm quartz cuvettes. Fluorescence imaging experiments were performed on the confocal laser scanning microscope (Fluoview 1000, Olympus, Japan). All pH measurements were made with a Sartorius basic pH-Meter. All experiments were carried out in DMF/HEPES buffer (25:75 v/v, 1.0×10^{-2} mol/L, pH 7.4) and at room temperature.

2.2. Procedure for hypochlorous acid and bisulfite detection

A stock solution of probe **DFP** (1.0×10^{-3} mol/L) was prepared in 100% DMF and was subsequently diluted to appropriate concentration (1.0×10^{-5} mol/L) with DMF/HEPES buffer (25:75 v/v, 1.0×10^{-2} mol/L, pH 7.4). Sodium hypochlorite (NaClO) and sodium bisulfite ($NaHSO_3$) stock solutions were freshly prepared prior to each experiment. For the fluorescence titration experiments, a mixture containing the corresponding-fold molar ratio of NaClO and $NaHSO_3$ was respectively added to the solution of **DFP**. After stirring for 90 min and 30 min, respectively, the fluorescence spectra were measured at room temperature.

2.3. Cell culture and fluorescence imaging

HeLa cells were cultured in the Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin at 37 °C under 5% CO_2 and 95% air environment. Cells were seeded into a 35-mm confocal dish (15-mm glass bottom well) with appropriate density and incubated for 24 h, and then the well-grown cells were selected and treated accordingly. For fluorescence imaging, the cells were further incubated with the probe (1.0×10^{-5} mol/L) for 30 min at 37 °C. And then, a fresh prepared solution of NaClO and $NaHSO_3$ was added for 90 min and 30 min incubation, respectively. Fluorescence imaging was then carried out after washing cells three times with PBS.

2.4. Synthesis of 1,2,3,3-tetramethyl-3H-indolium iodide

2,3,3-trimethylindolenine (1.2 g, 7.5 mmol) and iodomethane (1.3 g, 9.2 mmol) were dissolved in toluene (30 mL), and the solution was heated at 110 °C for 48 h. After the reaction mixture was cooled to room temperature, the methylated product crystallized out. The residue was filtered and washed with toluene three times, then dried to afford product as a brown solid (1.9 g, 84%). The crude product was directly used in the next reaction without further purification.

2.5. Synthesis of *DFP*

1,2,3,3-tetramethyl-3H-indolium iodide (2 mmol, 0.60 g) and 2,5-dimethoxybenzaldehyde (2 mmol, 0.33 g) were mixed in ethanol (20 mL), and then piperidine (100 μL) was added to the solution. The reaction mixture was heated to reflux and stirred overnight. The residue was obtained after rotary evaporation, and then was dissolved in CH_2Cl_2. The organic layer was washed three times with water, and dried over anhydrous $MgSO_4$. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10/1, v/v) to afford a red solid (0.43 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J=16.4 Hz, 1 H), 7.81 (d, J=16.3 Hz, 1 H), 7.72 (d, J=2.9 Hz, 1 H), 7.62–7.45 (m, 4 H), 7.07 (dd, J=9.1, 2.9 Hz, 1 H), 6.86 (d, *J*=9.2 Hz, 1 H), 4.40 (s, 3 H), 3.95 (s, 3 H), 3.89 (s, 3 H), 1.77 (s, 6 H). ^{13}C NMR (100 MHz,