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A highly specific fluorescent probe for hypochlorite based on fluorescein derivative and its endogenous imaging in living cells

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ABSTRACT

A new fluorescein-based probe is found to be sensitive and selective for the detection of hypochlorite anion in aqueous buffer solution. The probe displays high water solubility, pH-independent fluorescence, and biocompatibility. A possible detection mechanism is that HOCl oxidizes the C=N bond into aldehyde, resulting in bright emission. Results show that the probe has a 7.3 nM detection limit to hypochlorite anion. In addition, the probe is successfully utilized for monitoring endogenously produced hypochlorite in living macrophage cells upon stimulation.

1. Introduction

Hypochlorite anion (OCl^-) is an oxidant that is widely encountered in our daily life, such as in household bleach and water disinfectant. As an important reactive oxygen species (ROS) in living organisms, hypochlorous acid (HOCl)/hypochlorite (OCl^-) performs a pivotal function in immune defense against microorganisms and also in inflammation [1–3]. The generation of endogenous hypochlorite is driven by heavy metal-catalyzed oxidation reactions and enzymatically by myeloperoxidase (MPO) [4], which is localized mainly in leukocytes, including neutrophils, macrophages, and monocytes [2,5]. MPO is a heme-containing enzyme that mediates the production of hypochlorious acid (HOCl/OCl^-) from chloride ion (Cl^-) and hydrogen peroxide (H_2O_2) [4,6,7]. Maintaining HOCl concentration within the physiological range is essential for numerous cellular functions. Nevertheless, an abnormal hypochlorite level can contribute to tissue damage and diseases [8,9], such as nephropathy [10], atherosclerosis [11,12], cardiovascular disease [13], and cancers [14]. Therefore, developing highly sensitive and selective methods for HOCl/ClO^- detection is urgently required for the further investigation of the pathogenic mechanism of hypochlorite in living systems and human diseases [15,16].

Various HOCl/ClO^- fluorescent probe have been developed in recent years [17–38], in which based on the C=N isomerization mechanism have received great attention [17–24]. The C=N bond is part of aldoxime that results from the reaction between aldehyde and NH_2OH, which are rapidly deprotected by hypochlorite under mild conditions. Upon oxidation with hypochlorite, aldoxime gives the corresponding aldehyde [19–21,24], carboxylic acid [17], or nitrile oxide [18,23]. Many probes for detecting hypochlorite have been developed based on the mechanism of the oxidization–hydrolysis of hydrazide [39–44]. Inspired by the two strategies mentioned above, a novel probe for hypochlorite was designed by modifying the aldehyde with hydrazine group. The probe possesses both C=N and hydrozine groups (Scheme 1), which is expected to have high sensitivity to ClO^-. To our best knowledge, this structure is used for the first time to sense hypochlorite.

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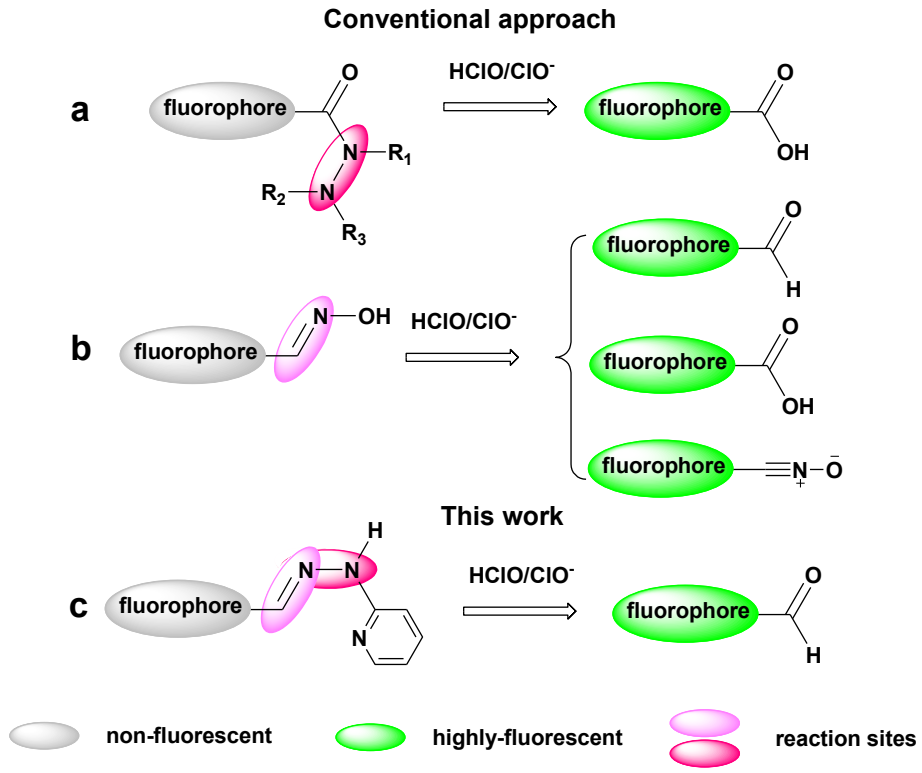

Scheme 1. Approaches for hypochlorite detection used in this work.

2. Experimental

2.1. Materials

Unless otherwise noted, the materials were obtained from commercial suppliers and used without further purification. NaOCl (reagent grade) and MPO (from human leukocytes) were purchased from Sigma–Aldrich. Fluorescein–monoaldehyde was synthesized according to our previous report [45].

2.2. Measurements

Column chromatography was performed on silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 300/500. Mass spectra were obtained using a Q-Tof mass spectrometer (Agilent 6530). Fluorescence emission spectra were obtained using RF-5301/PC spectro-fluorophotometer. UV absorption spectra were obtained on α-1860A UV–vis spectrometer. The imaging experiments were carried out using confocal laser scanning microscopy (Olympus FV-1000).

2.3. Synthesis of probe *1*

Exactly 5 mL of methanol solution of 1-(pyridin-2-yl)hydrazine (59 mg, 0.56 mmol) was added to a slurry of fluorescein–monoaldehyde (200 mg, 0.56 mmol) in 40 mL of ethanol over 30 min. The solution immediately changed from yellow to orange, and after stirring for 12 h at 25 °C, the product precipitated out of the solution. The yellow precipitate was collected on a filter, washed with n-pentane, and dried in vacuo to yield a brick red solid (137 mg, 55%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.18: (s, 1H), 11.31 (s, 1H), 10.24 (s, 1H), 8.87 (s, 1H), 8.22 (d, 1H, J = 4.4 Hz), 8.02 (d, 1H, J = 7.4 Hz), 7.80 (m, 3H), 7.33 (d, 1H, J = 7.5 Hz), 6.90 (m, 2H), 6.73 (m, 2H), 6.60 (d, 2H, J = 7.4 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 168.58, 159.52, 158.52, 155.03, 152.28, 151.23, 148.63, 148.27, 138.25, 136.35, 135.64, 130.16, 129.06, 128.82, 126.01, 124.65, 124.03, 115.73, 113.12, 109.64, 109.37, 106.66, 106.31, 102.09, 82.58. TOF-MS ES $C_{26}H_{18}N_3O_5$ $([M]+H)^+$ 452.1242, calculated: 451.1246.

2.4. Detection limit

Fluorescence titration was carried out in PBS-buffered solution (10 mM PBS, 1% (v/v) CH_3CN, pH = 7.4) to determine the detection limit, which was then calculated with the equation:

$$\text{detection limit} = 3\sigma_{bi}/m$$

where σ_{bi} is the standard deviation of blank measurements, and m is the slope between intensity and sample concentration.

2.5. Determination of the fluorescence quantum yields

The quantum yields of probe **1** and probe **1** after reaction with ClO^- were determined at room temperature, fluorescein in 0.1 N NaOH was used as a standard. Excitation was chosen at 494 nm; the emission spectra were corrected and integrated from 450 to 650 nm. The quantum yields were calculated with the following expression:

$$\varphi_{sample} = \varphi_{standard} \times \frac{\int F_{sample}}{\int F_{standard}} \times \frac{A_{standard}}{A_{sample}}$$

2.6. Cell culture and fluorescence imaging

MCF-7 cells and RAW264.7 macrophages were purchased from American Type Culture Collection (USA). The cells were seeded in laser scanning confocal microscope culture dishes with a density of