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A simple and fast-response fluorescent probe for hypochlorite in living cells

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ABSTRACT

A novel fluorophore pyrido[1,2-*a*]benzimidazole was synthesized and used as a fluorescent probe for hypochlorite based on the oxidation of hydrazine to carboxyl group. The detection limit was measured to be as low as 7.0 nM. The probe can realize fast-detection for hypochlorite within 60 s. Furthermore, it could be used for imaging in living cells.

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Introduction

Hypochlorite anion (ClO^-), as one of the important reactive oxygen species (ROS), plays an essential role in many biological processes.[1–3] Endogenous ClO^- which is formed from the reaction between chloride ions and hydrogen peroxide by the catalysis of enzyme myeloperoxidase is essential to life and has important antibacterial properties.[4] However, abnormal level of ClO^- can cause some diseases such as atherosclerosis, arthritis, kidney disease, and cancer.[5–10] Therefore, accurate detection of ClO^- in living cells and organisms has become important for us to understand its role in biology.

To date, a number of fluorescent probes have been reported for specific detection of ClO^-. However, they are still restricted to the limited fluorophores such as coumarin,[11–14] BODIPY,[15–18] rhodamine[19–21]and cyanine.[22–25] Therefore, it is a great challenge to search for novel fluorophores with superior properties.

Previously, we successfully synthesized pyrido[1,2-*a*]benzimidazoles (PBI) via a tandem reaction.[26] The compounds were found to have good fluorescence properties such as high fluorescence quantum yield and used as a donor to construct FRET-based ratiometric probe for identifying Cu^{2+} and Hg^{2+}.[27,28] In order to extend the application of the new fluorescence, herein, we designed and synthesized a simple PBI-based probe bearing hydrazone moiety to detect ClO^-. The mechanism was based on the oxidation reaction of C═N by hypochlorite.

Experimental section

Materials and equipments

^{1}H NMR spectra were measured on a Bruker Avance 400 (400 MHz) spectrometer (DMSO d_6 as solvent and tetramethylsilane (TMS) as an internal standard). HRMS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). UV–vis spectra and FL spectra were recorded on a U-3900 UV–Vis spectrometer (Hitachi) and RF-5301PC luminescence spectrophotometer (Shimadzu) at room temperature, respectively. All reagents and solvents were purchased from commercial sources and used without further purification. Deionized water was used throughout the process of absorption and fluorescence determination.

Cell culture and imaging

Glioma cells were cultured in RPMI-1640 containing 10% fetal bovine serum at 37 °C in a 5% CO_2/95% air incubator. For living cells imaging experiments, the growth medium was removed and replaced with RPMI-1640 without FBS. The cells were treated and incubated with 2 μM of **PBI-HClO** at 37 °C under 5% CO_2 for 1 h. The cells were washed three times with PBS and then cell images were obtained via a confocal microscope from Radiance 2100 (Bio-Rad) at excitation of 405 nm.

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Synthesis of the probe ***PBI-HClO***

Compound **1** (0.27 g, 1 mmol),[29] 2-hydrazinylpyridine (0.11 g, 1 mmol) and piperidine (3 drops) was dissolved in absolute ethanol (20 mL). The mixture was refluxed for 4 h. After cooling to room temperature, the orange solid was filtered. Then, the crude solid was recrystallized from ethanol to obtain orange product in 86% yield. ^{1}H NMR (400 MHz, DMSO d_6) δ 11.07 (s, 1H), 8.92 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.21 (s, 1H), 8.10 (d, J = 4.0 Hz, 1H), 8.01 (s, 1H), 7.74–7.70 (m, 2H), 7.67–7.62 (m, 1H),7.58–7.53 (m, 4H), 7.44 (t, J = 8.0 Hz, 1H),7.35 (t, J = 8.0 Hz,2H), 6.79–6.76 (m, 1H); ^{13}C NMR (100 MHz, DMSO d_6) δ 157.0, 148.5, 148.3, 144.9, 138.5, 135.7, 134.3, 133.6, 131.4, 129.7, 128.7, 128.5 127.9, 125.7, 121.6, 119.5, 116.1, 112.2, 110.0, 107.4, 107.2; HRMS: 364.1570 ([M+H]$^+$); Calcd for $C_{23}H_{18}N_5$: 364.1562.

Results and discussion

Design and synthesis of the probe

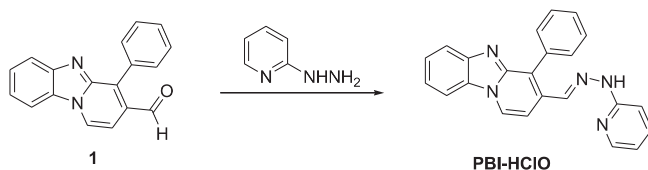
Previously, we successfully synthesized pyrido[1,2-*a*]benzimidazole acid and found that it exhibited strong fluorescence.[30] It was reported that the fluorophores with C=N structure was non-fluorescent.[31,32] Therefore, we designed a probe composed of pyrido[1,2-*a*]benzimidazole and 2-hydrazinylpyridine and expected that the hydrazone moiety in the probe could be oxidized by ClO^- to afford carboxyl group and recover fluorescence of pyrido[1,2-*a*] benzimidazole acid. The synthetic route is shown in Scheme 1. The reaction of compound **1** and 2-hydrazinylpyridine in C_2H_5OH afforded the probe as yellow powder in 86% yield. The probe was characterized by ^{1}H NMR and HRMS (see Supplementary Information).

UV–vis and fluorescence spectra

As shown in Fig. 1, **PBI-HClO** was characteristic of high selectivity toward ClO^- over other ROS, RNS (t-BuO·, H_2O_2, KO_2, $NaOONO_2$, NO·, $^1O_2$, ·OH, t-BuOOH) and metal ions (K^+, Na^+, Zn^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}). The competitive ROS did not lead to any significant changes. As expected, the probe emitted weak fluorescence, while ClO^- caused a dramatic fluorescence enhancement (16.5-fold) at 463 nm (Fig. S1).

The absorption and fluorescence spectra of **PBI-HClO** with varying ClO^- concentrations (0–4.5 equiv) in C_2H_5OH/PBS (1/9, v/v pH = 7.3) were also recorded to further investigate the interaction of **PBI-HClO** and ClO^- (Figs. 2 and 3). When ClO^- was added to the solution of **PBI-HClO** (5 μM), absorption bands (382 nm, 403 nm and 429 nm) enhanced consistently. The intensity of absorption bands reached plateau when 4.5 equiv. ClO^- was added to the solution. The fluorescence intensity at 463 nm increased gradually upon addition of ClO^- until 4.5 equiv. and a 16.5-fold fluorescence enhancement was observed. These results show that probe responds well toward hypochlorite.

In addition, there was a linear correlation between the fluorescence intensity and the concentrations of ClO^- from 0.2 to 2.0 equiv., and the limit of detection (LOD) was calculated to be 7.0 nM (Fig. 4).



Scheme 1. Synthesis route to probe **PBI-HClO**.

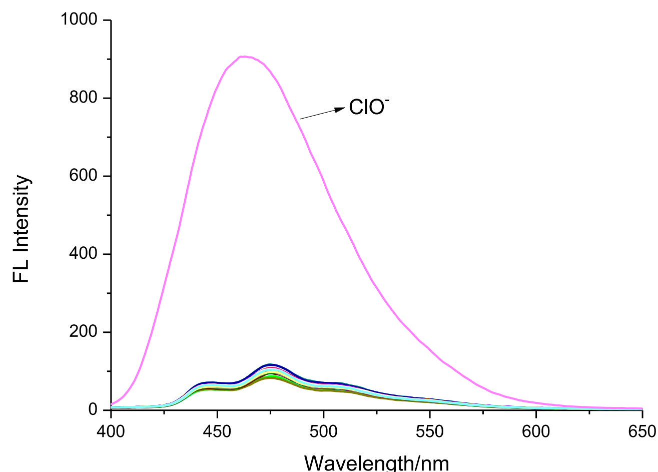


Fig. 1. Fluorescence spectra of **PBI-HClO** (5 μM) in the absence and presence of 4 equiv of other species in C_2H_5OH/PBS (1/9, v/v, pH = 7.3) solution (λ_{ex} = 380 nm, slit = 5 nm/3 nm).

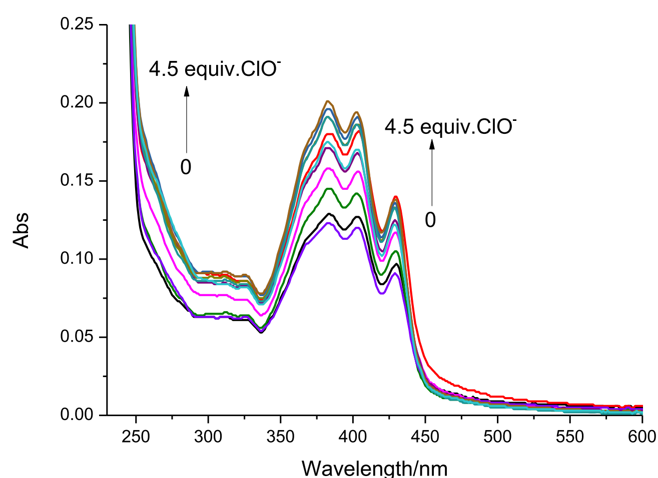


Fig. 2. Absorbance spectra of **PBI-HClO** (5 μM) in response to ClO^- (0.0–4.5 equiv.) in C_2H_5OH/PBS (1/9, v/v, pH = 7.3) solution.

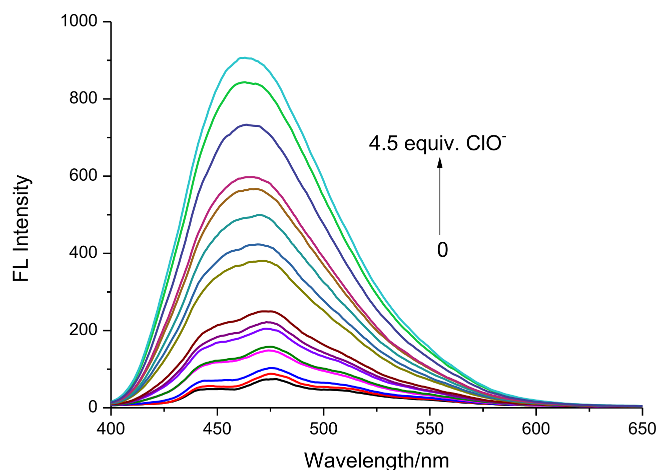


Fig. 3. Fluorescence spectra of **PBI-HClO** (5 μM) in response to ClO^- (0.0–4.5 equiv.) in C_2H_5OH/PBS (1/9, v/v, pH = 7.3) solution (λ_{ex} = 380 nm, slit = 5 nm/3 nm).

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