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Short communication

A coumarin-boronic ester derivative as fluorescent chemosensor for detecting H_2O_2 in living cells

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ABSTRACT

A coumarin derivative **CBE** fluorescent chemosensor based on boronic ester elimination by introducing boronic ester moiety as sensing site for efficient detecting H_2O_2 in living cells was developed. The probe can specifically recognize H_2O_2 in PBS buffer solution (containing 1% DMSO, pH = 7.4) with fluorescence enhancement response at 454 nm without interference from other substances. After H_2O_2 addition, the absorption band is red-shifted from 360 nm to 415 nm, and fluorescence is enhanced with fluorescence quantum yield increasing from 5.1% to 58.7%. The response is sensitive with detection limit being 0.17 nM within 16 min. The mechanism is that H_2O_2 triggers oxidative hydrolysis of the boronic ester and subsequent 1,6-elimination, causing C-O bond to break, thereby forming 7-hydroxycoumarin-3-acetyl and 5-cyclohexadien-1-one, 4-methylene-. The probe also has high photostability and excellent potential application in monitoring H_2O_2 in living cells.

1. Introduction

H_2O_2, like other reactive oxygen species (ROS) such as ClO^- and O_2^-, is a high-powered oxidant that plays an important part in immune system's natural defense against pathogens [1]. H_2O_2 also plays an important part in cell proliferation, immune response, signal transduction and other physiological processes [2–4]. At the same time, H_2O_2 is also a metabolic by-product produced in many enzymatic reactions [5,6]. In addition, excessive production of H_2O_2 has implications for a number of diseases, such as cancer, cardiovascular disease, DNA damage, Alzheimer's disease, diabetes, and neurodegenerative disease [7,8]. Too much or too little hydrogen peroxide in cells will promote cell apoptosis, and more severely cause cell death [9]. Therefore, the development of optical probes capable of sensing and imaging H_2O_2 based on the merits of optical probes including high rate, sensitivity and no injury [10–13] has aroused great interest. In bioanalytical chemistry, a great deal of work has been done to detect H_2O_2 [14–22].

Coumarin is a large class of natural organic compounds that are very important in nature. Experimental studies have found that coumarin compounds are involved in various biological activities such as anti-HIV, anti-cancer, analgesic, and antibacterial activities [23–25]. The fluorophores with coumarin as the skeleton have the characteristics of high fluorescence intensity, good solubility, cell permeability, light stability, and have attracted more and more attention in the field of fluorescence sensors [25–29]. Based on the above advantages, it will be very meaningful to design this structure into a fluorescence sensor.

In this work, boronic ester moiety was introduced to synthesize a coumarin derivative **CBE** to detect H_2O_2. Boronic esters, especially the 4,4,5,5-tetramethyl-1,3,2-dioxaborolane group, are often used as sensing sites to identify H_2O_2 [1]. After probe **CBE** reacting with H_2O_2, the boronate group is cleaved to form 5-cyclohexadien-1-one, 4-methylene-, the ether bond is broken, and 7-hydroxycoumarin-3-acetyl is formed. The probe can specifically recognize H_2O_2 with blue fluorescence turn-on response at 454 nm in PBS buffer solution with 1% DMSO. Probe **CBE** is sensitive to H_2O_2 with detection limit being 0.17 nM and fast response rate (about 16 min), which is not affected by other substances. Moreover, probe **CBE** has high photostability and can be used to monitor H_2O_2 in living cells.

2. Experimental

2.1. Materials and instruments

7-hydroxycoumarin-3-acetyl was obtained from published literature [30]. 4-Bromomethylphenylboronic acid pinacol ester was purchased from Aladdin Company. The probe was characterized by using Nuclear

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magnetic resonance (NMR, Bruker Avance III 400 MHz spectrometer, Fig. S1) spectra and high resolution mass spectra (HR-MS, Agilent Q-TOF6510 spectrometer, Fig. S1).

2.2. Synthesis of probe CBE

7-hydroxycoumarin-3-acetyl (0.31 g, 1.5 mmol) was added to a mixture of 4-bromomethylphenylboronic acid pinacol ester (0.45 g, 1.5 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 20 mL of DMF with nitrogen at room temperature for 6 h. Then 50 mL deionized water was added to the mixed system and the system was separated with ethyl acetate. The organic phase was separated, dried with $MgSO_4$, and removed by vacuum distillation. The product was obtained as a yellow solid with a yield of 80%. ^{1}H NMR (400 MHz, DMSO, Fig. S1), δ: 1.29 (s, 12H), 2.55 (s, 3H), 5.31 (s, 2H), 7.09 (dd, J = 8.8, 2.0 Hz, 1H), 7.13 (d, J = 2.0 Hz, 1H), 7.48 (d, J = 7.6 Hz, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.88 (d, J = 8.4 Hz, 1H), 8.63 (s, 1H). ^{13}C NMR (101 MHz, DMSO), δ: 24.66, 30.04, 69.92, 83.70, 101.18, 112.00, 114.03, 120.55, 127.06, 132.23, 134.62, 139.28, 147.52, 157.01, 158.90, 163.64, 194.65. For $[M+H]^+$ m/z 421.1822. Found: $[M+H]^+$ m/z 421.1817.

2.3. Spectral response to H_2O_2

In spectroscopic experiments, all the solvents were chromatographic grades. UV–vis absorption spectra were tested on Shimadzu UV2550 spectrophotometer. Fluorescence spectra were tested on Horiba Fluoromax-4 fluorescence spectrometer.

The stock solution of probe **CBE** was prepared at 1 mM in THF. The solutions of various testing species, such as NaF, NaCl, NaBr, NaI, Na_2S, $NaHSO_3$, Na_2SO_3, $NaNO_3$, $NaNO_2$, KCN, NaSCN, Na_2SO_4, Na_2CO_3, Na_3PO_4, NaClO, H_2O_2 were prepared in 10 mM deionized water solution. $ONOO^-$, $O_2^{\bullet-}$, $^1O_2$, $^{\bullet}OH$, $ROO^{\bullet}$ were prepared according previous method [31]. The detection environment of UV–vis absorption and fluorescence spectra is PBS buffer solution with 1% DMSO (pH = 7.4).

2.4. Cell imaging study

HeLa cells were cultivated with probe **CBE** (10 μM) for 30 min at 37 °C and then washed with 20 mM PBS (pH = 7.4) three times. Next, the cells were incubated with H_2O_2 at different concentrations (30 μM, 60 μM, 90 μM) in PBS buffer solution for 16 min (λ_{ex} = 405 nm, λ_{em} = 450 ± 20 nm). The imaging experiments were measured through confocal microscopy (Nikon A1MP confocal fluorescent microscope) immediately.

3. Results and discussion

3.1. H_2O_2 titration test and response dynamics

The spectral titration of **CBE** (10 μM) was explored in 20 mM PBS buffer solution with 1% DMSO. The original absorption peak at 360 nm gradually decreases and a new absorption band centered at 415 nm gradually increases upon the addition of H_2O_2 (60 μM) over 16 min (Fig. 1A). The probe shows a weak single-peak fluorescence emission spectrum at 454 nm (Fig. 1B). After the addition of H_2O_2, the fluorescence emission of the probe at 454 nm increases and the fluorescence intensity has 9.4-fold enhancement after 20 min of H_2O_2 treatment, and the fluorescence quantum yield increases from 5.1% to 58.7%. The fluorescence response spectra of H_2O_2 concentration also shows that with the addition of H_2O_2 increasing from 0 to 40 μM, there is a good linear relationship between fluorescence intensity and H_2O_2 concentration (Fig. 1B). When sensitive probe is incubated at different concentrations of H_2O_2, the slope of ln (I-I_0) vs log([H_2O_2]) is 2.30 for 16 min (Fig. 1C), indicating an approximate first order dependence on H_2O_2 concentration, and the probe can detect H_2O_2 quantitatively. The detection limit is 0.17 nM, indicating that probe **CBE** is highly sensitive to H_2O_2.

The response dynamics of the probe for H_2O_2 indicates that the reaction time is 16 min (Fig. 1D). Take the natural logarithm of the concentration ratio (c_t /c_0), where c_t and c_0 are the concentrations of H_2O_2 at t min and 0 min, respectively. After taking the natural logarithm of the concentration ratio (c_t /c_0)[32], the linear regression equation is used to describe the trend (R^2 = 0.99254). As shown in Fig. 1(D), the reaction kinetic constant is 0.3929 min^{-1}.

3.2. Reaction mechanism

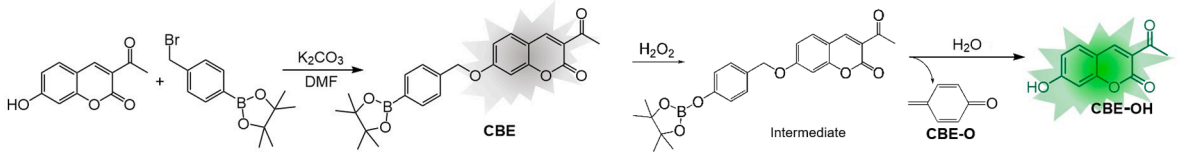
The reaction mechanism was proved by HRMS. The signal of **CBE-O** at 107.0336 is attributed to [**CBE-O** + H]$^+$, and the signal of **CBE-OH** at 203.0338 is attributed to [**CBE-OH**-H]$^+$ (Fig. S2). Combined with the previously published literature [33–42], it can be concluded that H_2O_2 triggers oxidative hydrolysis of the boronic ester and subsequent 1,6-elimination. The boronate group is cleaved to form 5-cyclohexadien-1-one, 4-methylene-, the ether bond is broken, and the hydroxyl group is exposed (Scheme 1). After probe **CBE** reacts with H_2O_2 accompanied by the exposure of hydroxyl group, the absorption band is red-shifted and the fluorescence intensity increases.

3.3. Selectivity and anti-interference compared to other substances

Given the presence of a variety of substances in living cells, it is critical that whether the response of probe **CBE** to H_2O_2 in living cells can be interfered by other substances. Selectivity and competitive selectivity experiments (Fig. 2) show that only H_2O_2 significantly increases the fluorescence intensity over 16 min, while other substances do not induce change after the addition. Under the premise of adding other ions, the response of the probe **CBE** to H_2O_2 is not disturbed. These results indicate that probe **CBE** owns excellent selectivity on H_2O_2 superior to other biorelated analytes.

3.4. Cell imaging study

Judged by pH dependence of the probe for H_2O_2 (Fig. S3), in the pH range of 7.0–10.0, the probe has the best recognition effect on H_2O_2, so the probe is suitable for detection in organism. Due to the good spectral response of probe **CBE** to H_2O_2, the recognition of the probe for H_2O_2 in living cells was also investigated. The photostability of the probe is a critical factor in cell imaging experiments. Next, the photostability of the probe in living cells was firstly studied (Fig. 3A). The I/I_0 of **CBE** in 20 mM PBS solution containing 1% DMSO remains nearly constant after an extended period of excitation, suggesting its high photobleaching resistance and superior photostability. The above property indicates that the probe is suitable for bioimaging, so the cytotoxicity of probe **CBE** for A549 cells was then evaluated. Cell viability is over 80%, even with 200



Scheme 1. Synthetic route of probe **CBE** and its sensing mechanism to H_2O_2.

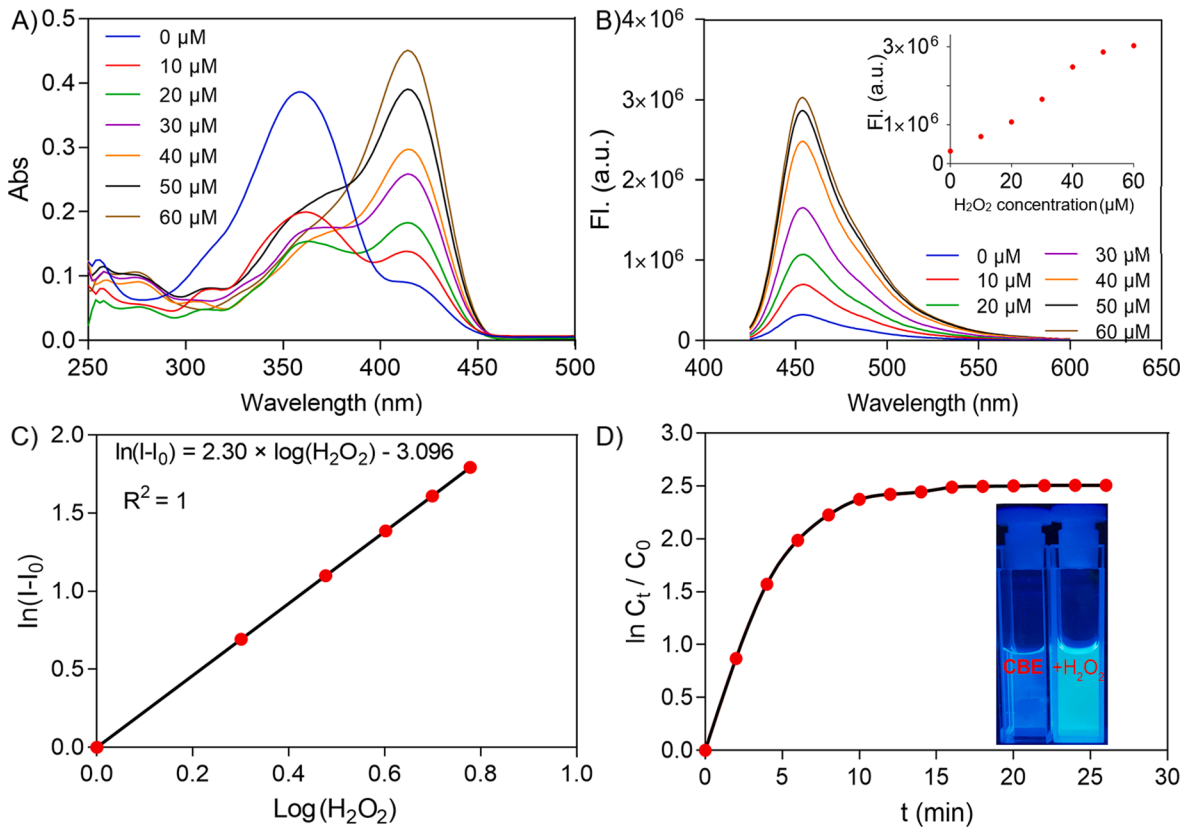


Fig. 1. UV–vis absorption (A) and fluorescence (B) spectral response of probe **CBE** (10 μM) to H_2O_2 in PBS solution containing 1% DMSO. Inset: fluorescence intensity versus H_2O_2 concentration. (C) ln (I-I_0) vs log([H_2O_2]) functional diagram. (D) Reaction kinetic model of probe **CBE** to H_2O_2. Inset: photograph showing the fluorescence of probe **CBE** and its reaction product response seen by the naked eye (under a 365 nm UV lamp).

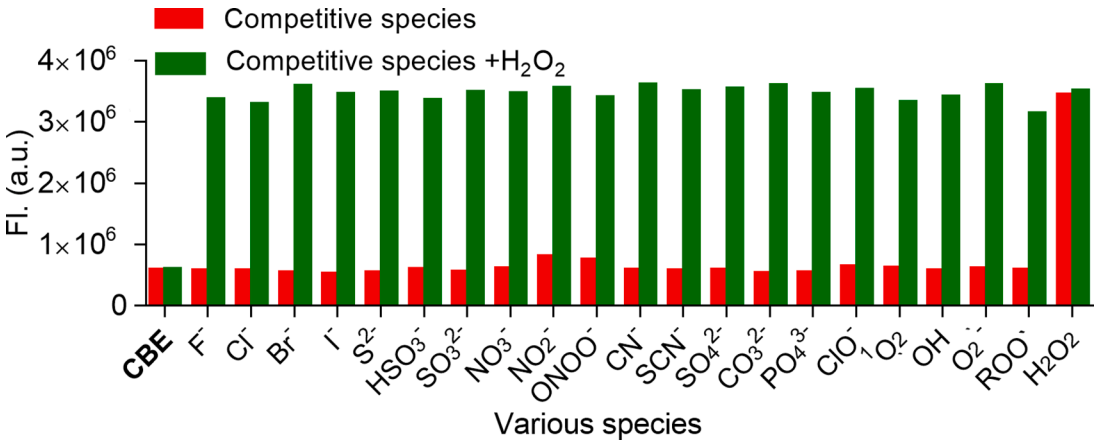


Fig. 2. Selectivity and competitive selectivity of probe **CBE** (10 μM) to H_2O_2 (60 μM) in 20 mM PBS with 1% DMSO.

μM of probe concentration (Fig. S4), which indicates that the probe has low cytotoxicity. The cells were firstly cultivated with the probe (10 μM) for 30 min at room temperature and then washed four times with PBS solution. When only the probe was added to the cells for imaging experiments, no fluorescence is observed. Then the cells were incubated with probe **CBE** and H_2O_2 for 10 min and green fluorescence was observed in the cells (Fig. 3B). This indicates that the probe **CBE** can be used to detect H_2O_2 in biological samples.

Next, the cells were treated with H_2O_2 at different concentrations (Fig. 3C). Cell imaging of the living cells incubated with only the probe shows little fluorescence. And then adding 30 μM H_2O_2, green fluorescent begins to appear. After each test on the basis of original increasing 30 μM H_2O_2 concentration, fluorescence is more and more bright. The results show that probe **CBE** is an enhanced fluorescent probe that could be used for intracellular H_2O_2 recognition.

4. Conclusions

In summary, a new fluorescent probe **CBE** based on the framework of coumarin borate with coumarin as a fluorescence group and borate ester as a recognition group was designed and developed. The probe achieves high-sensitivity detection to H_2O_2 in PBS buffer solution with 1% DMSO with a detection limit of 0.17 nM. The probe can specifically recognize H_2O_2 without being interfered by other species, and is accompanied by

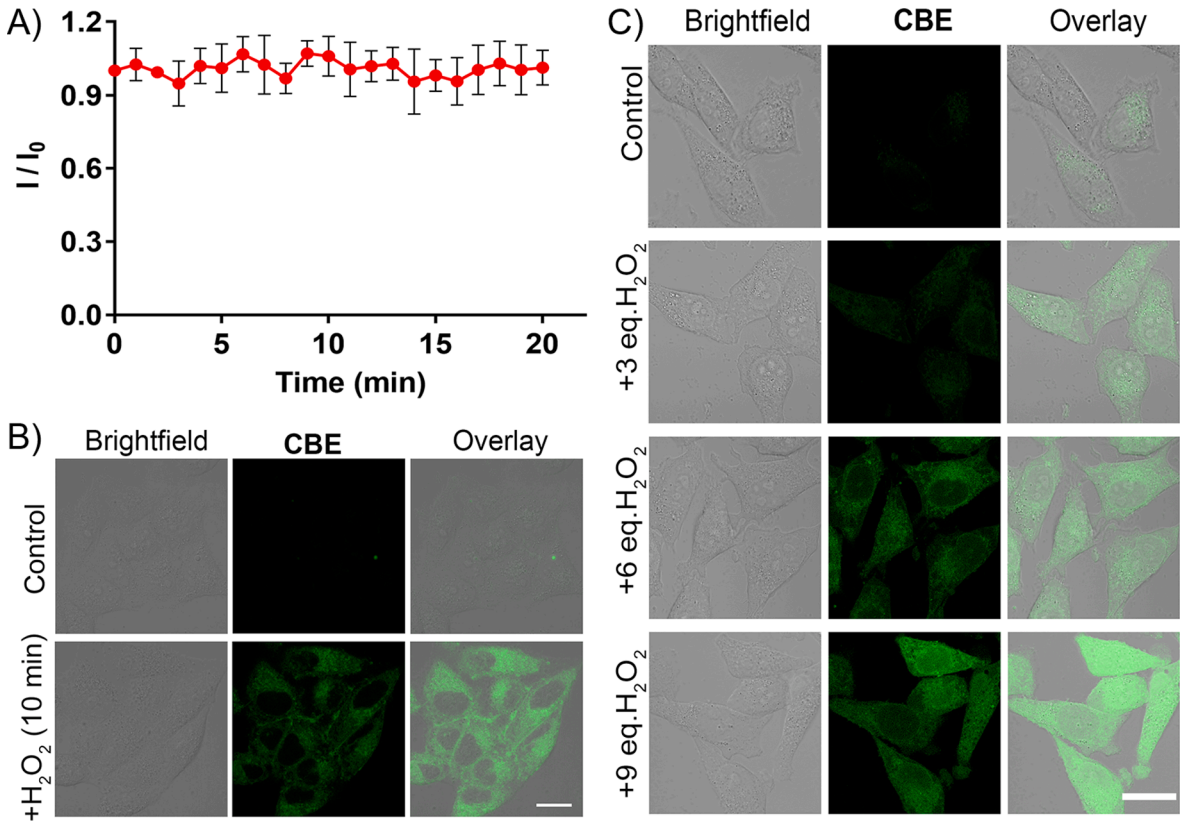


Fig. 3. (A) Photostability of probe **CBE** (10 μM) in 20 mM PBS solution containing 1% DMSO. (B) Fluorescence images for H_2O_2 detection using probe **CBE** before (control) and after 60 μM H_2O_2 for 10 min. Scale bar: 20 μm. (C) Fluorescence images for H_2O_2 detection using probe **CBE** before (control) and after the increasing concentration of H_2O_2 (30 μM; 60 μM; 90 μM). Scale bar: 20 μm.

enhanced blue fluorescence at 454 nm. The absorption peak has 55 nm red shift. At the same time, the reaction between the probe and H_2O_2 confirms to be a good kinetic linear relationship, and the kinetic reaction constant is 0.3929 min^{-1}. The reaction mechanism is that the boronate group is cleaved to form 5-cyclohexadien-1-one, 4-methylene-, the ether bond is broken, thereby forming 7-hydroxycoumarin-3-acetyl. In addition, the probe has excellent anti-photobleaching property and photostability, and can be used to detect H_2O_2 in living cells.

CRediT authorship contribution statement

Shuyue Ma: Investigation, Writing - original draft, Formal analysis. **Kang-Nan Wang:** Conceptualization, Investigation, Methodology, Formal analysis. **Miaomiao Xing:** Investigation, Formal analysis. **Fei Feng:** Investigation, Formal analysis. **Qiling Pan:** Investigation, Formal analysis. **Duxia Cao:** Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.inoche.2020.108414.

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