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A colorimetric and fluorescence turn-on probe for the highly selective detection of hydrogen peroxide in aqueous solution

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ABSTRACT

A novel colorimetric and fluorescence turn-on probe **TBP-B** for the highly selective detection of hydrogen peroxide (H_2O_2) has been explored. **TBP-B** was non-emissive in aqueous solution and in the aggregate state. After **TBP-B** reacted with H_2O_2 in aqueous solution, the initial benzyl-borate unit in **TBP-B** was cleaved, resulting in the production of AIE-featured luminogen **TBP**, which exhibited an intense orange-red emission. Meanwhile, the color of the solution changed from red to light yellow. The sensing process was completed within 16 min, and the detection limit was as low as 160 nM. Moreover, the sensing mechanism was confirmed by optical spectral studies, NMR spectra and DFT calculations.

1. Introduction

Hydrogen peroxide (H_2O_2), as an important reactive oxygen species (ROS), is produced during the aerobic metabolism process in organisms [1]. H_2O_2 in physiological level is able to regulate fundamental bioactivities, such as immune response, cellular signal transduction, antioxidant defenses stimulation, and so on [2,3]. However, the overproduction or accumulation of H_2O_2 may cause the oxidative damage to cellular proteins, and lead to some neurodegenerative diseases, like Parkinson's and Alzheimer's diseases [4–6]. Besides that, H_2O_2 is also an extremely useful chemical that widely applied in textile and chemical industries, whereas its excessive discharge not only pollute the environment, but also endanger human health [7,8]. Hence, it is greatly significant to develop a simple and efficient method for monitoring H_2O_2 concentration level in biological systems as well as natural environment.

Among various detection technologies for H_2O_2, fluorescence analysis has attracted more attentions owing to its advantages of low cost, simple operation, visualization and high selectivity [9]. Up to now, a plenty of fluorescent probes for H_2O_2 detection have been explored. In these probes, the boronate moiety was utilized as the specific recognition group for H_2O_2 through the chemo specific boronate-to-phenol switch, consequently resulting in the different photophysical properties [10]. In the reaction with boronate, H_2O_2 served as a two-electron electrophilic oxidant as well as a good nucleophile, while most of other ROS operated by one-electron transfers or purely electrophilic oxidation process. Thus this dual-mode reactivity brought the remarkable selectivity for H_2O_2 over other ROS species [1,11]. Even so, some of them generally put up with a long response time (over 30 min) that was harmful for the real-time detection and quantification [12–16]. To overcome this shortcoming, several probes with new frameworks were reported to quickly detect H_2O_2 in a few minutes. But these probes suffered from other various problems with respect to fluorescence turn-off response [17,18], acid or alkaline condition for the detection [19,20], the unsatisfactory detection limit [13,14,21] and in aqueous-organic media containing more than 50% volume fraction of organic solvent [13,14,18,20,22], which to some extent limited their practical applications. On the other hand, most of the reported probes for H_2O_2 outputted the single fluorescence signal. By contrast, the colorimetric/fluorometric dual-channel probes were regarded as preferable owing to the more reliable test results and the easier use directly by naked-eye detection [23]. The comparison of the above H_2O_2 probes (from 2016 to now) was summarized in Table S1. Therefore, the development of a colorimetric and fluorescence turn-on probe for the highly sensitive and rapid detection of H_2O_2 under mild condition is a very meaningful and challenging work.

The aggregation-induced emission (AIE) effect has been widely applied in the design of fluorescence turn-on probes [24]. The reported AIE-probes for H_2O_2 were mainly based on tetraphenylethylene (TPE) skeleton that possessed the AIE characteristic, thus they must be well dissolved in detection media to minimize the background auto-fluorescence until the presence of analytes [15,16,21]. Once the

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environmental factors caused the aggregation, these probes would immediately generate the intense auto-fluorescence, which might lead to the failure for the analytes detection.

Herein, we report a novel colorimetric/fluorometric dual-channel probe **TBP-B**, which displays the high selectivity for H_2O_2 detection in PBS buffer solution (pH = 7.4). The quaternarized pyridine unit in **TBP-B** improves the water solubility. **TBP-B** emits scarcely both in aqueous solution and in the aggregation state, thus it thoroughly eliminates the inference of background auto-fluorescence. The presence of H_2O_2 triggers the oxidation reaction of the boronate moiety in pyridine ring and the followed 1,6-rearrangement elimination reaction [12,16,25], consequently resulting in the release of AIE-featured luminogen (**TBP**), which exhibits an intense emission at 606 nm in aqueous solution. Meanwhile, the color change of solution from light red to yellow can be clearly seen by the naked eyes. The sensing process is completed within 16 min, and the detection limit is as low as 160 nM.

2. Experimental section

2.1. Materials and instruments

Unless otherwise stated, reagents and solvents were purchased from commercial suppliers and used without further purification. Double distilled water was used throughout all the experiments. The preparation of the various ROS/RNS was described as follows: (a) ClO^- (NaClO), H_2O_2 and TBHP (*tert*-butylhydroperoxide): these chemicals were purchased directly from the company, and then diluted with PBS (10 mM, pH = 7.4) to make the 10 mM stock solutions; (b) ·OH: To a solution of H_2O_2 (10 mM, 1.0 mL) in PBS (10 mM, pH 7.4) was added $FeSO_4$ solution (10 mM, 1.0 mL) at room temperature to get the 5 mM stock solution; (c) *t*-BuO· (*tert*-butoxy radical): The solution of TBHP (10 mM, 1.0 mL) in PBS (10 mM, pH 7.4) was added $FeSO_4$ solution (10 mM, 1.0 mL) at room temperature to make the 5 mM stock solution; (d) NO: The solution of the H_2SO_4 (3.6 M) was added dropwise into a stirred solution of $NaNO_2$ (7.3 M). The produced gas was allowed to pass through a solution of NaOH (2 M) first and then deionized water to get the 2 mM stock solution; (e) $^{\cdot}O_2^-$: KO_2 was dissolved in dry DMSO to make the saturated solution; (f) $^1O_2$: The solution of $NaMoO_4$ (10 mM) and H_2O_2 (10 mM) was prepared in PBS (10 mM, pH = 7.4) respectively, and mixed the equal aliquots of these solutions to afford the 5 mM stock solution of $^1O_2$; (g) $ONOO^-$: The stirred solution of $NaNO_2$ (0.6 M, 10 mL) and H_2O_2 (0.7 M, 10 mL) in deionized H_2O was added HCl (0.6 M, 10 mL) at 0 °C, immediately followed by the rapid addition of NaOH (1.5 M, 20 mL). Excess hydrogen peroxide was removed by MnO_2. The concentration of ONOO- was determined by UV analysis with the extinction coefficient at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$), and solution was stored at −20 °C for use. The preparation of these ROS/RNS species also could be referred to the reported literatures [26,27]. Moreover, Cys and GSH was prepared in PBS (10 mM, pH = 7.4) to get the 5 mM stock solution.

NMR spectra were obtained on a Bruker Ascend 400 MHz Nuclear Magnetic Resonance Spectrometer. The ^1H NMR (400 MHz) chemical shifts were measured relative to $CDCl_3$ as the internal reference ($CDCl_3$: δ = 7.26 ppm). The ^{13}C NMR (100 MHz) chemical shifts were given using $CDCl_3$ as the internal standard ($CDCl_3$: δ = 77.16 ppm). High-resolution mass spectra (HRMS) were obtained with a Bruker-Q-TOF mass spectrometer. Melting points were taken on with SGW X-4 instrument. Absorption spectra were recorded on TU-1901 spectrometer, which was produced in Beijing Puxi Tongyong Ltd. The fluorescence spectra were tested in a quartz cells with the HITACHI F-2500 fluorescence spectrometer. The pH measurements were performed by Sartorius PB-10 m. Dynamic light scattering (DLS) experiments were investigated with ZEN3600 Malvern particle sizer.

2.2. Synthesis of compound 1

Compound **1** was synthesized according to the previous method [28]. After the purification by a silica gel column, the NMR data of pure compound **1** was described as follows: ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.89 (d, J = 7.6 Hz, 1H, -ArH), 7.80 (d, J = 8.4 Hz, 2H, -ArH), 7.54 (d, J = 7.6 Hz, 1H, -ArH), 7.28–7.32 (m, 4H, -ArH), 7.18–7.20 (m, 6H, -ArH), 7.08 (t, J = 7.4 Hz, 2H, -ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 154.1, 153.2, 148.5, 147.4, 133.7, 132.5, 130.0, 129.9, 129.5, 127.5, 125.2, 123.6, 122.7.

2.3. Synthesis of compound TBP

A mixture of compound **1** (229 mg, 0.5 mmol), 4-Pyridineboronic acid pinacol ester (123 mg, 0.6 mmol), $Pd(dppf)Cl_2$ (37 mg, 0.05 mmol) and K_3PO_4 (333 mg, 1.5 mmol) in 1, 4-dioxane/H_2O (4:1, v/v, 2 mL) was refluxed overnight under N_2. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phase was concentrated using rotavapor, and the residue was purified by a silica gel column (PE/EtOAc = 1/1, v/v) to provide **TBP** as an orange solid (148 mg, yield: 65%). m.p. 196.8–198.4 °C. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.77 (d, J = 5.6 Hz, 2H, pyridine H), 7.94 (d, J = 6.0 Hz, 2H, pyridine H), 7.87–7.90 (m, 3H, -ArH), 7.78 (d, J = 7.2 Hz, 1H, -ArH), 7.29–7.32 (m, 4H, -ArH), 7.19–7.23 (m, 6H, -ArH), 7.08 (t, J = 7.4 Hz, 2H, -ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 154.1, 153.6, 150.2, 148.5, 147.3, 144.8, 134.7, 130.2, 130.1, 129.4, 129.3, 129.0, 126.9, 125.1, 123.6, 122.6. HRMS (ESI^+): m/z calcd. for $C_{29}H_{32}N_4NaO_6S$ $[M + Na + 6H_2O]^+$ 587.1935; found: 587.1931.

2.4. Synthesis of probe TBP-B

A mixture of compound **TBP** (92 mg, 0.2 mmol) and 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (89 mg, 0.3 mmol) in toluene (2 mL) was refluxed overnight under N_2. After that, the mixture was concentrated using rotavapor, and the residue was purified by a silica gel column (DCM/MeOH = 7/1, v/v) to provide **TBP-B** as a red solid (86 mg, yield: 57%). m.p. 225.6–228.2 °C. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.51 (d, J = 4.8 Hz, 2H, pyridine H), 8.81 (d, J = 4.8 Hz, 2H, pyridine H), 8.25 (d, J = 7.6 Hz, 1H, -ArH), 7.85 (d, J = 8.8 Hz, 2H, -ArH), 7.77–7.80 (m, 3H, -ArH), 7.64 (d, J = 7.6 Hz, 2H, -ArH), 7.28–7.32 (m, 4H, -ArH), 7.14–7.18 (m, 6H, -ArH), 7.09 (t, J = 7.4 Hz, 2H, -ArH), 6.32 (s, 2H, $-CH_2$), 1.29 (s, 12H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 153.9, 152.9, 152.5, 149.4, 147.1, 144.6, 138.4, 136.0, 135.9, 132.4, 130.6, 129.6, 128.9, 128.8, 126.6, 126.4, 125.6, 124.1, 123.1, 121.8, 84.2, 75.2, 24.9. HRMS (ESI^+): m/z calcd. for $C_{42}H_{38}BN_4O_2S$ $[M\text{-}Br]^+$ 673.2803; found 673.2806.

2.5. Photophysical measurements

The stock solution of probe **TBP-B** (3 mM) was prepared in DMSO, and then was diluted to get the mixed solution of DMSO/H_2O (PBS 10 mM, pH = 7.4) with the specific concentration for the UV–vis absorption spectra and fluorescence spectra measurement. The selectivity experiments of probe **TBP-B** toward H_2O_2 were conducted by adding the same amount of biological thiols and various ROS/RNS into the **TBP-B** assay solution. As for the titration experiments, different concentrations of H_2O_2 were added into the **TBP-B** solution and measured the absorption spectra and fluorescence spectra respectively. In all fluorescence detection experiments, the slit was kept at 5 nm.

2.6. Statement of the detection limit

The detection limit (DL) was confirmed based on the fluorescence titration curve of probe **TBP-B** in the presence of H_2O_2, and was calculated according to the equation "DL = 3σ/k" based on S/N = 3