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Sensitive determination of endogenous hydroxyl radical in live cell by a BODIPY based fluorescent probe

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ABSTRACT

The sensitive and selective fluorescence probe for hydroxyl radical analysis is of significance because hydroxyl radical plays key roles in many physiological and pathological processes. In this work, a novel organic fluorescence molecular probe OHP for hydroxyl radical is synthesized by a two-step route. The probe employs 4-bora-3a,4a-diaza-s-indacene (difluoroboron dipyrromethene, BODIPY) as the fluorophore and possesses relatively high fluorescence quantum yields (77.14%). Hydroxyl radical can rapidly react with the probe and quench the fluorescence in a good linear relationship (R^2=0.9967). The limit of detection is determined to be as low as 11 nM. In addition, it has been demonstrated that the probe has a good stability against pH and light illumination, low cytotoxicity and high biocompatibility. Cell culture experimental results show that the probe OHP is sensitive and selective for imaging and tracking endogenous hydroxyl radical in live cells.

1. Introduction

Reactive oxygen species (ROS) have been of widespread attention on account of its important roles in the physiology and pathology [1–4]. These small molecules usually relate to mediating redox modifications of biological molecules and probably implicate in aging and various kinds of pathological types, such as cancer, diabetes, neurodegenerative diseases, cardiovascular and inflammatory diseases [5–9]. As one of the most reactive oxygen species, hydroxyl radical can immediately react with biomolecules in living cells, such as DNA bases, amino acids, organic acids, phospholipids and sugars, resulting in cell damage and even apoptosis [10–12]. Since hydroxyl radical is short-lived and reactive, its real-time sensitive and selective detection methods are still in high demand. Currently, there are some methods for the detection of hydroxyl radical, including electron spin resonance [13–16], nuclear magnetic resonance [17], HPLC method [18], UV–vis spectroscopy and spectrofluorometry [4,19], electrochemical method [20] and chemiluminescence [21]. Compared with those conventional methods, fluorescence method possesses the advantages of simplicity, sensitivity and the visible changes of fluorescence color potential for visual detection of the target analyte [22–30]. It is also considered to be a promising way to provide localized information at the target site and real-time monitoring in biological systems with high sensitivity and generally minimal cell disruption [31–38].

In the past decades, organic molecules [39–42], semiconductor quantum dots [43,44], metal nanoclusters [45] and other fluorescent nanoparticles [46] were widely used as fluorescence probes for hydroxyl radicals. Among organic fluorescent molecules, the ones based on fluorophore-linked nitroxides are one of the most successful. But the excitation energy required for the nitroxide-based probes is high [42], which is expected to be damage in living system and high background interference from biological matrices. Quantum dots have advantages of high fluorescence quantum yields, narrow emission and tunable emission spectra, but their applications are limited by the presence of heavy metal atoms with greater toxicity [43,44]. Noble metal nanoclusters have low toxicity and good biocompatibility, but studies have shown that gold nanoclusters (AuNCs) lack the selectivity for the detection of hydroxyl radical. Therefore, it still remains a challenge to develop a novel fluorescent probe based on other fluorophore for selective and sensitive detection of hydroxyl radical, especially in living

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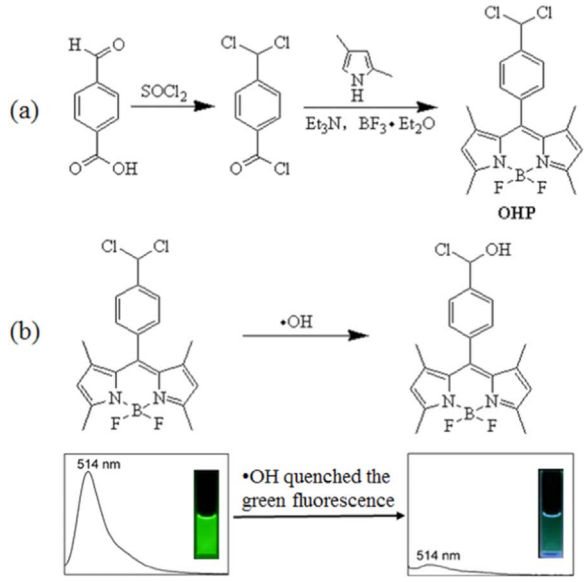

Scheme 1.. (a) Synthetic procedure of the fluorescence probe OHP; (b) Reaction mechanism of OHP with •OH.

cells.

To develop a selective and sensitive fluorescence probe for hydroxyl radical in living cells, some important properties need to be taken into account. The first and foremost is the selectivity of the probe to target analyte without interferences from other species. The second one is the sensitivity of the probe which requires high fluorescence quantum yield. A probe with bright fluorescence also allows smaller amount application, hence reducing the toxicity to the cells. In recent years, 4-bora-3a,4a-diaza-s-indacene (difluoroboron dipyrromethene, BODIPY) dyes have received increasing attention due to their specific properties like chemical robustness, stability under physiological conditions, relatively high fluorescence quantum yields, enable easily tuning of spectroscopic and photophysical properties by suitable substituents [38,47,48]. These unique advantages enable it to become one of the most promising fluorophere.

In this article, a novel organic fluorescence probe (OHP, Scheme 1) based on BODIPY was designed and synthesized for the determination of hydroxyl radical. It is worth mentioned that the probe was synthesized through a two-step route and can be readily purified by crystallization. The fluorescence quantum yield of the probe was estimated to be 77.14%, and experimental results show that the fluorescence of OHP can be effectively quenched through a non-redox mechanism and produce a non-fluorescent molecule. Because of the specific recognition for hydroxyl radical, the designed probe shows high selectivity. Furthermore, the probe has a maximum emission at 514 nm in the visible region, and thus it is expected to be used for simple and selective detection of hydroxyl radical. Importantly, the probe exhibits good stability, low cytotoxicity, good biocompatibility and cell permeability, which specifically and sensitively responses to hydroxyl radical in vitro and vivo.

2. Materials and methods

2.1. Materials

4-formylbenzoic acid, 2,4-dimethylpyrrole, boron trifluoride diethyl etherate (BF_3•Et_2O) and fluorescein were purchased from Aladdin Reagent Co. Ltd. Hydrogen peroxide (H_2O_2), tert-Butyl hydroperoxide (TBHP), sodium hypochlorite (NaClO), potassium dioxide (KO_2), bovine serum albumin (BSA), L-Arginine (Arg), L-Tryptophan (Trp), L-Cysteine (Cys), L-Aspartic acid (Asp), L-Lysine (Lys) and lipopolysaccharides (LPS) were obtained from Sigma Aldrich. Thionyl chloride ($SOCl_2$), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), triethylamine (Et_3N), dichloromethane (CH_2Cl_2), toluene, sodium sulfate anhydrous (Na_2SO_4), calcium chloride dihydrate ($CaCl_2$·$2H_2O$), potassium chloride (KCl), magnesium chloride hexahydrate ($MgCl_2$·$6H_2O$), ferric chloride hexahydrate ($FeCl_3$·$6H_2O$), ferrous sulfate heptahydrate ($FeSO_4$·$7H_2O$), zinc chloride ($ZnCl_2$) and copper chloride dihydrate ($CuCl_2$·$2H_2O$) were purchased from Sinopharm Chemical Reagent Co. Ltd. The pH of the solution was adjusted by phosphate buffer. The phosphate buffer was prepared with sodium dihydrogen phosphate dihydrate (NaH_2PO_4·$2H_2O$) and disodium hydrogen phosphate dodecahydrate (Na_2HPO_4·$12H_2O$). The chemicals and solvents were used directly without further purification unless otherwise stated. All the aqueous solutions were prepared with ultrapure water (18.2 MΩ•cm) from Millipore water purification system.

2.2. Instrumentation and methods

Fluorescence spectra and UV–vis absorption spectra were recorded at room temperature on a Perkin-Elmer LS-55 luminescence spectrometer and Shimadzu UV-2550 spectrometer, respectively. Nuclear magnetic resonance (NMR) spectra were obtained from a Varian Mercury-400 NMR spectrometer with TMS as an internal standard operating at 400 MHz for ^1H and at 100 MHz for ^{13}C. Mass spectra were measured by Agilent Technologies 6224 TOF LC/MS mass spectrometer in ESI positive or negative mode. Photographs were taken with a canon 350D digital camera, and the pH values were measured by PHS-3C pH meter. Silica gel-60 (230–400 mesh) was used as the solid phases for column chromatography. Thin-layer chromatography (TLC) was performed with Merck F254 silica gel-60 plates. TLC plates were viewed with UV light. Confocal images were obtained by Laser Confocal Microscopy (OBSERVER Z1, ZEISS) and a 48× oil-immersion objective lens.

2.3. Synthesis of fluorescence probe OHP

To a dried 10 mL round bottomed flask with a magnetic stir bar and equipped with a water cooled condenser, 4-formylbenzoic acid (45 mg, 0.3 mmol) was dissolved in 2 mL $SOCl_2$. The mixture was then heated in an oil bath at 80 °C for 2 h and then subsequently allowed to cool to room temperature. The solution was transferred to a dried 50 mL round bottomed flask and concentrated by evaporation under reduced pressure. Dry CH_2Cl_2 (10 mL) followed by 2,4-dimethylpyrrole (66 μL, 0.63 mmol) was charged to the residue in the flask with a magnetic stir bar and equipped with a water cooled condenser. The mixture was heated in an oil bath at 50 °C for 80 min and subsequently allowed to cool to room temperature. Then the solution was concentrated to 1–2 mL by evaporation under reduced pressure. Toluene (17 mL), triethylamine (167 μL) was charged to the flask and the mixture was allowed to stir at room temperature for 15 min, at which point BF_3•Et_2O (193 μL) was injected dropwise to the flask, and the reaction mixture was heated to 50 °C for 1 h. The mixture was then allowed to cool to room temperature and the solvent was removed by evaporation under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (30 mL) and transferred to a separatory funnel. The organic layer was washed with water (3×20 mL), dried over Na_2SO_4, filtered and finally concentrated to get crude product. The crude product was purified on silica gel chromatography eluted with ethylacetate/petroleum ether (1:20 v/v) to give the desired product. Yield (35.9 mg, 29.5%). HR-MS (*m/z*, ESI) calculated for $C_{20}H_{19}BCl_2F_2N_2$ $[M+H]^+$, 407.1065; found 407.1064. ^1H NMR (400 MHz, DMSO) δ 7.82 (d, *J* =8.2 Hz, 2 H), 7.62 (s, 1 H), 7.49 (d, *J* =8.1 Hz, 2 H), 6.20 (s, 2 H), 2.46 (s, 6 H), 1.34 (s, 6 H). ^{13}C NMR (100 MHz, DMSO) δ 155.68 (s), 143.08 (s), 142.11 (s), 141.21 (s), 136.07 (s), 130.94 (s), 129.05 (s), 127.46 (s), 122.05 (s), 71.83 (s), 14.69 (s), 14.46 (s).