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A simple highly selective and sensitive hydroquinone-based two-photon fluorescent probe for imaging peroxynitrite in live cells

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ABSTRACT

Exploiting specific and sensitive techniques for tracking peroxynitrite ($ONOO^-$) in the biological systems is of great significance to understand its diverse pathophysiology. Herein, we have constructed a simple, 4-hydroxynaphthalimide-derived two-photon fluorescent probe **TPHQ** for imaging $ONOO^-$ in live cells. Probe **TPHQ** with the recognition receptor of hydroquinone moiety exhibited excellent selectivity towards $ONOO^-$ over various bio-related analytes including H_2O_2 and OCl^-. Additionally, probe **TPHQ** could rapidly monitor $ONOO^-$ with high sensitivity (DL = 16 nM). With probe **TPHQ**, the fluctuations of $ONOO^-$ levels in live cells were successfully tracked by the one-photon and two-photon fluorescence microscopy.

1. Introduction

Peroxynitrite ($ONOO^-$) is formed from the immediate combination of nitric oxide (NO) and superoxide ($O_2^{\bullet -}$) in the biological systems [1,2]. Excessive generation of $ONOO^-$ can cause serious damage to critical cell components, such as proteins, nucleic acids, lipids, and so on [3,4]. Recent evidences have revealed that $ONOO^-$ is associated with Alzheimer's disease, arthritis, cancer, autoimmune and inflammatory diseases, and other disorders [5,6]. On the other hand, growing studies have demonstrated that $ONOO^-$ plays a positive role in signal transduction and antimicrobial activities [7–9]. Consequently, exploiting specific and sensitive techniques for tracking peroxynitrite ($ONOO^-$) in the biological systems is of great significance to understand its diverse pathophysiology.

Fluorescent probe has received considerable attention owing to its preeminent sensitivity, technical simplicity, non-invasion, high temporal and spatial resolutions [10–12]. Up to now, manifold fluorescent probes have been constructed to map $ONOO^-$ in the living systems [13–32]. Yang et al. have developed a novel fluorescent probe containing 4-hydroxyaniline moiety as the recognition receptor of $ONOO^-$ [15], but this probe was prone to be interfered by reactive oxygen species (ROS) including hydroxyl radical ($^{\bullet}OH$) and hypochlorous acid (HOCl). Kim et al. have exploited a fluorescent probe with the recognition unit of boronic acid pinacol ester for $ONOO^-$ [25], yet this recognition moiety of boronate has been proven to detect relevant ROS (*i.e.* H_2O_2, OCl^-) [33–35]. In addition, Wang et al. have developed a novel ketoamide-based fluorescent probe for monitoring $ONOO^-$ *in vivo* [26], but the recognition moiety of ketoamide could be used to design fluorescent probe for detecting H_2O_2 [36]. Therefore, the development of specific fluorescent probes for the detection of $ONOO^-$ without the interference of ROS still remains challenging.

On the other hand, available fluorescent probes are mainly employed short wavelength one-photon excitation to detect intracellular $ONOO^-$ levels. By contrast, two-photon fluorescent probe with the excitation of two near-infrared photons (700–900 nm) has unique inherent superiorities, such as reduced photo-damage, depressed self-absorption, enhanced tissue penetration, and improved detection sensitivity [37–40]. So, constructing two-photon fluorescent probes for imaging $ONOO^-$ in live cells is also imperative.

In this paper, we report a simple 4-hydroxynaphthalimide-derived two-photon fluorescent probe **TPHQ** employing hydroquinone moiety as new recognition unit of $ONOO^-$ (Scheme 1). Probe **TPHQ** with preeminent features including excellent sensitivity, rapid response, and high specificity towards $ONOO^-$ over

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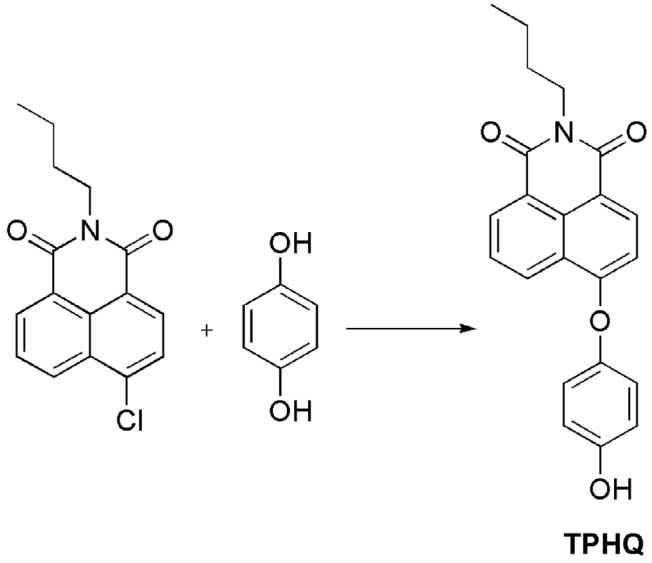

Scheme 1. The synthesis of probe **TPHQ**.

other various bio-related analytes including ROS was successfully applied to the monitoring of the changes of $ONOO^-$ levels in live cells by the one-photon and two-photon fluorescence microscopy.

2. Experimental section

2.1. Materials and general methods

All reagents and solvents were provided by businesses and were of the highest grade. High resolution mass spectra (HRMS) were performed on LC–MS 2010A (Shimadzu) instrument. Absorption spectra were taken on UV-3101PC spectrophotometer. Fluorescence spectra were taken on Horiba FluoroMax-4 spectrophotometer (λ_{ex} = 450 nm). The ^1H NMR and ^{13}C NMR spectra were taken on Bruker AV-400 NMR spectrometer. The one-photon fluorescence imaging of $ONOO^-$ in live cells were measured on Leica TCS SP5. The two-photon fluorescence imaging of $ONOO^-$ in live cells were obtained with Zeiss LSM 880 NLO Confocal Laser Scanning Microscope. Ti: sapphire laser was used to excite the specimen at 800 nm and transmissivity was 6%.

2.2. Synthesis of probe TPHQ

4-Chloro-1,8-naphthalimide (273 mg, 1 mmol) was completely dissolved in 15 mL acetonitrile solution followed by hydroquinone (330 mg, 3 mmol) and cesium carbonate (977.4 mg, 3 mmol). The above solution was then stirred at 90 °C for 24 h. The reacted solution was subjected to suction filtration to remove residual cesium carbonate. The solvent in the collected filtrate was evaporated and the product was purified by silica gel column chromatography (CH_2Cl_2 as eluent) to obtain pure probe (513 mg, 85%). ^1H NMR (400 MHz, $CDCl_3$) δ (*10^{-6}): 0.97(t, *J* = 8.0 Hz, 3H), 1.40-1.49 (m, 2H), 1.68-1.75 (m, 2H), 4.18(t, *J* = 8.0 Hz, 2H), 6.38(s, 1H), 6.82(d, *J* = 8.0 Hz, 1H), 6.98(d, *J* = 12.0 Hz, 2H), 7.06(d, *J* = 12.0 Hz, 2H), 7.78(t, *J* = 8.0 Hz, 1H), 8.42(d, *J* = 8.0 Hz, 1H), 8.65(d, *J* = 8.0 Hz, 1H), 8.72(d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (*10^{-6}): 13.88, 20.41, 30.25, 40.30, 109.51, 115.89, 116.96, 122.30, 122.44, 123.61, 126.43, 128.77, 129.63, 132.01, 133.13, 147.53, 153.90, 160.96, 164.14, 164.62. HRMS (ESI): Calcd for $C_{22}H_{20}NO_4$ $[M+H]^+$ 362.1; Found, 362.1.

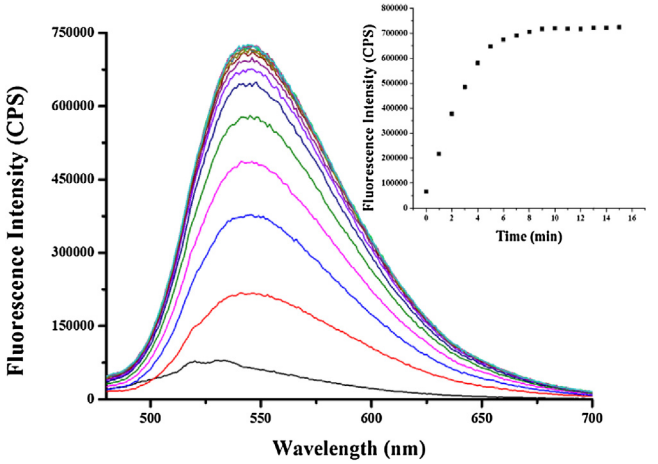

Fig. 1. Time-course of probe **TPHQ** (5 μM) for detecting $ONOO^-$ (10 μM). λ_{ex} = 450 nm, slit widths: $W_{ex} = W_{em}$ = 4 nm.

3. Results and discussion

3.1. Rational design of probe TPHQ

Bearing the above-mentioned considerations in mind, a simple specific two-photon fluorescent probe was constructed to track $ONOO^-$ levels in live cells. Previous reports have demonstrated that the specificity of probe is greatly dependent on the unique reaction of probe and target. Recent studies have revealed that the close coupling of 4-hydroxyaniline group and fluorophore could be used to detect $ONOO^-$ [14,15,17,19]. Hence, we envisaged that the hydroquinone moiety might be an alternative response unit of $ONOO^-$ over ROS. On the other hand, 4-hydroxy-1,8-naphthalimide fluorophore with the excellent photophysical properties was firstly adopted to design fluorescent probe by us [41], and has been extensively used to develop fluorescent probes for detecting various analytes [42–45]. Very recently, we and other groups have developed 4-hydroxynaphthalimide-derived fluorescent probes for two-photon imaging of targets in the biological systems [40,46,47]. Accordingly, we prepared the combination of 4-hydroxynaphthalimide and hydroquinone as a highly selective two-photon fluorescent probe (**TPHQ**) for the detection of $ONOO^-$.

3.2. Fluorescence response of probe TPHQ towards $ONOO^-$

The characteristic spectra of probe **TPHQ** for the determination of $ONOO^-$ were measured in a mixed solution containing ethanol and water (1: 9, v/v, 10 mM PBS, pH = 7.4). In the absence of $ONOO^-$, the probe solution exhibited the negligible fluorescence intensity at 550 nm. With the addition of $ONOO^-$ to the probe solution, the fast and considerable fluorescence enhancement was observed at 550 nm. The time-dependent studies of the fluorescence changes in the presence of $ONOO^-$ indicated that the response of probe **TPHQ** towards $ONOO^-$ is very rapidly (~6 min) (Fig. 1), suggesting that probe **TPHQ** could provide a rapid assay of $ONOO^-$. We then performed the fluorescence titration investigation to determine the sensitivity of probe **TPHQ** for monitoring $ONOO^-$. As shown in Fig. 2, the fluorescence titration of probe **TPHQ** with the increasing concentration of $ONOO^-$ displayed the continuous increase of fluorescence emission peak at 550 nm. Additionally, the fluorescence intensities at 550 nm have an excellent linear relationship with the concentrations of $ONOO^-$ in the range of 0.4–5.0 μM (linear equation: $y = 96686 \times [ONOO^-]$ (μM) −11191, R^2 = 0.9976). Based on 3σ/k, the detection limit (DL) was determined to be as low as 16 nM, indicating the high sensitivity of probe **TPHQ** for detecting