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Highly specific and ratiometric fluorescent probe for ozone assay in indoor air and living cells

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ABSTRACT

Ozone (O_3) is widely used as oxidant in both industrial and household applications. However, long-term exposure to high concentration of O_3 harms lung function and irritates the respiratory system. Therefore, accurate detection of ozone is in great need for both environmental and biological studies for human health. This paper describes a highly specific fluorescent probe for ratiometric detection of O_3 concentrations with a detection limit of 39 nM and detection range up to 24.2 μM, which makes it feasible for quantitative detection of O_3 in air environment and living cells. Besides fluorescent detection, the probe also undergoes a color change from colorless to yellow, which also allows for convenient visual detection of O_3.

1. Introduction

Ozone (O_3) has been widely used in the treatment of environmental pollutant in air, disinfection of drinking water and medical tools, preparation of pharmaceuticals and synthetic lubricants, and other applications [1,2]. However, despite its powerful usage in different applications, there are a large number of evidences showing that O_3 damages lung function and irritates the respiratory system [3,4], which brings a big concern for human health. Studies indicated that long-term exposure to O_3 is related with asthma, bronchitis, heart attack and other cardiopulmonary problems [5,6]. Moreover, current studies indicate that O_3 may be naturally produced in the human immune system as a means of destroying foreign bodies [7–9]. Therefore, quantitative detection of O_3 concentration is a critical topic for environmental evaluation and biological studies.

Fluorescent probes with high sensitivity, excellent specificity and operational simplicity are particularly suitable for qualitative or quantitative analysis of environmental and biological samples [10–12]. Indigo carmine is a classically sensitive fluorescent probe for O_3 detection in air, solution and organisms. However, it suffers from poor selectivity because it yields the same emissive oxidative product in the presence of superoxide ($O_2^{-\cdot}$) as well as O_3 [13], which makes the detection of O_3 hard to be differentiated from $O_2^{-\cdot}$. In order to enhance the specificity for O_3 detection, Koide and Ma groups developed two novel but-3-enyl-based fluorescent probes, which show excellent selectivity for O_3 over other reactive oxygen or nitrogen species (ROS/RNS) in both biological and atmospheric samples [13,14]. However, since these probes are constructed based on fluorophores with single emission channel (e.g. fluorescein or resorufin), they respond to O_3 levels only via fluorescent intensity fluctuation in a single channel. There are significant drawbacks for utilizing single channel detection: it will be more likely to prone to uncertainties due to the factors such as variability in probe distribution, instability in the excitation source, and effects of the microenvironment [15,16]. In contrast, ratiometric probes can solve these problems by the self-calibration mechanism using two emission channels. To our knowledge, none of the existing O_3 fluorescent probes published so far has the capacity to utilize the ratiometric detection scheme [17–19].

To address this issue, here in this study, we developed a specific fluorescent probe, **1**, utilizing ratiometric detection scheme for the sensitive determination of O_3 for the application in atmospheric and biological studies. The reaction mechanism of probe **1** toward

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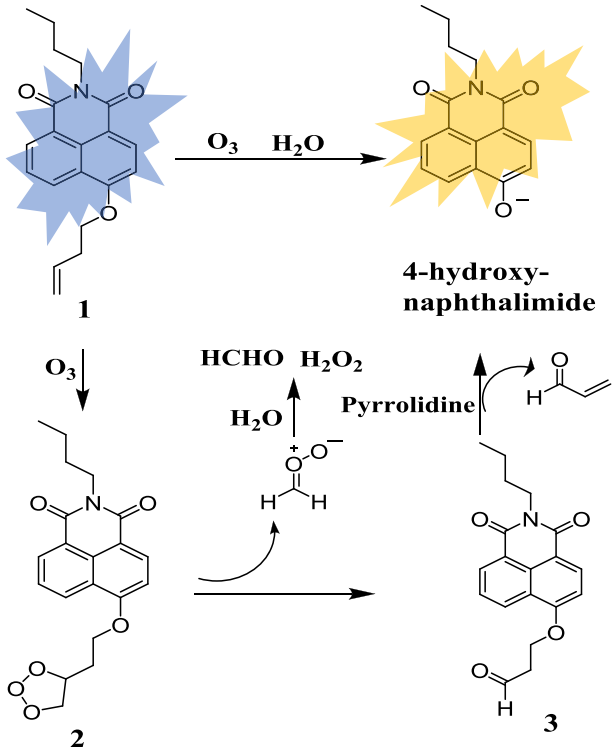


Scheme 1. Proposed reaction mechanism of probe **1** with O_3.

O_3 was shown in Scheme 1. The 4-hydroxy-naphthalimide moiety, containing both an electron donor and an acceptor has been frequently applied in the design of ratiometric fluorescent probes [20–23], due to its excellent internal charge transfer (ICT) structure and desirable photophysical properties, such as a large Stokes shift and insensitivity to pH [24]. In our previous work, we found that replacement of the hydroxyl anion group of 4-hydroxy-naphthalimide with the weaker electron-donating ability of an alkoxy caused a 70–100 nm blue shift [25]. Based on this mechanism, we anticipated that probe **1** anchoring a but-3-enyl group would emit much shorter-wavelength fluorescence relative to 4-hydroxy-naphthalimide. Upon reaction with O_3, probe **1** undergoes β-elimination to yield 4-hydroxy-naphthalimide, accompanied with a large red shift, as shown in Scheme 1. Taking advantage of the known unique additive reaction of a but-3-enyl group by O_3, the O_3 sensitive probe was constructed by linking a but-3-enyl group to 4-hydroxy-naphthalimide fluorophore via a nucleophilic substitution reaction (see synthetic methods).

2. Experimental methods

2.1. Materials and instrumentations

The 4-bromo-1, 8-naphthalimide was prepared according our previous work [25]. All other chemicals used in this paper were obtained from commercial suppliers and used without further purification. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. NMR spectra were recorded on a Bruker Avance III at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR with chemical shifts reported as ppm (in DMSO-d_6, TMS as internal standard). Mass spectra (MS) were measured with Bruker Apex IV FTMS using electrospray ionization (ESI). Absorption spectra were recorded on a Purkinje TU-1901 spectrophotometer. Fluorescence measurements were taken on a Hitachi F-7000 fluorescence spectrometer with a 10 mm quartz cuvette. pH measurements were carried out with a pH acidometer (Mettler Toledo FE-30).

2.2. Synthetic methods

Probe **1** and its ozonation product were prepared according to the route shown in Scheme 2.

2.2.1. Probe *1*

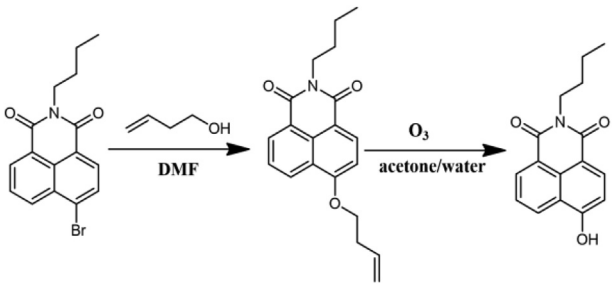
To a solution of 4-bromo-1, 8-naphthalimide (664 mg, 2 mmol) in DMF were added but-3-en-1-ol (0.216 g, 3 mmol), K_2CO_3 (0.414 g, 3 mmol) and 18-crown-6 (53 mg, 0.2 mmol), and the mixture was stirred overnight at 120 °C. After cooling, saturated NH_4Cl aqueous solution was added to neutralize the reaction mixture. Then, the solution was extracted with CH_2Cl_2, and the organic phase was washed with brine, dried over $MgSO_4$, then filtered and the solvent was evaporated. The residue was purified by silica gel column chromatography (petroleum/CH_2Cl_2 = 5/1) to obtain probe **1** as a light yellow powder (357 mg, yield 55.2%). δ_H (400 MHz, DMSO): 8.45 (2H, t, *J* 7.0), 8.38 (1H, d, *J* 8.3), 7.78 (1H, t, *J* 7.8), 7.28 (1H, d, *J* 8.3), 6.00 (1H, tt, *J* 10.2, 6.6), 5.27 (1H, d, *J* 17.3), 5.16 (1H, d, *J* 10.1), 4.36 (2H, t, *J* 6.2), 4.00 (2H, t, *J* 7.3), 2.67 (2H, d, *J* 6.2), 1.67–1.53 (2H, m), 1.34 (2H, dq, *J* 14.6, 7.3), 0.92 (3H, t, *J* 7.3). δ_C (100 MHz, DMSO): 163.97, 163.32, 159.90, 135.15, 133.64, 131.43, 128.97, 128.62, 126.75, 123.22, 122.29, 117.90, 114.55, 107.29, 68.37, 39.64, 33.32, 30.17, 20.29, 14.19. HRMS (ESI) *m/z* found for $C_{20}H_{21}NO_3$ $[M + H]^+$ 324.1596, calculated 324.1594.

2.2.2. The ozonation product of probe *1*

Probe **1** (81 mg, 0.25 mmol) was completely dissolved in 95:5 acetone/water, and cooled to 0 °C in an ice-water bath. Then, a flow of O_3 was bubbled into the sample for 10 min, followed by further reaction was carried out for 30 min at room temperature. Finally, pyrrolidine (83.7 μL, 1 mmol) was added and stirred for 30 min to accelerate the β-elimination of the generated aldehyde **3**. After extraction with ethyl acetate and concentration, the mixture was purified by silica gel column chromatography (petroleum/CH_2Cl_2 = 5/1) to obtain the product as a yellow powder. δ_H (400 MHz, DMSO): 11.85 (1H, s), 8.51 (1H, dd, *J* 8.3, 1.1), 8.45 (1H, dd, *J* 7.3, 1.1), 8.33 (1H, d, *J* 8.2), 7.74 (1H, dd, *J* 8.2, 7.4), 7.14 (1H, d, *J* 8.2), 4.06–3.96 (2H, m), 1.65–1.52 (2H, m), 1.40–1.27 (2H, m), 0.92 (3H, t, *J* 7.4). δ_C (101 MHz, DMSO): 164.11, 163.45, 160.70, 133.98, 131.55, 129.64, 129.32, 126.01, 122.83, 122.27, 113.06, 110.39, 30.22, 20.29, 14.20. HRMS (ESI) *m/z* found for $C_{16}H_{15}NO_3$ $[M + H]^+$ 270.1128, calculated 270.1125.

2.3. General procedures for analysis

Parent stock solution of probe **1** (5.0 mM) was prepared in dimethyl sulfoxide (DMSO). Ozone was obtained by an ozone generator (BEYOK ozone FM-300, Zhejiang, China) and ozone



Scheme 2. The synthesis of probe **1** and its ozonation product.