Biosensors and Bioelectronics 87 (2017) 96–100

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Enhanced response speed and selectivity of fluorescein-based H_2S probe via the cleavage of nitrobenzene sulfonyl ester assisted by ortho aldehyde groups

Jing Lv, Fang Wang, Jian Qiang, Xintong Ren, Yahui Chen, Zhijie Zhang, Yong Wang, Wei Zhang, Xiaoqiang Chen *,[1]

State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemical Engineering, Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University, Nanjing, 210009 China

ARTICLE INFO

Article history:
Received 29 May 2016
Received in revised form
25 July 2016
Accepted 5 August 2016
Available online 5 August 2016

Keywords:
H_2S
Fluorescent probe
Formylated fluorescein
Ortho effect
Thiolysis

ABSTRACT

In this work, we developed three fluorescent probes (**F-1**, **F-2**, and **F-3**) based on fluorescein, mono-formylated fluorescein, and bis-formylated fluorescein for hydrogen sulfide (H_2S) detection. The probe **F-3**, which bears two aldehyde groups, exhibited the fastest response. This fast response is attributed to the ortho effect of the aldehyde group, which enables fast nucleophilic addition of H_2S to an aldehyde group and subsequent intramolecular thiolysis of dinitrophenyl ether. In addition, the aldehyde groups on **F-3** react with biothiols (e.g., cysteine, homocysteine) to form thiazolidine diastereomers, which suppress the fluorescence of fluorescein. The introduction of two aldehyde groups also resulted in high selectivity of **F-3** towards H_2S. Furthermore, good linearity was observed between **F-3** fluorescence intensity at 510 nm and H_2S concentration in the range of 0–10 μM. **F-3** exhibited a detection limit as low as 0.024 μM. Confocal laser scanning micrographs of HeLa cells incubated with **F-3** confirmed that **F-3** is cell-permeable and can successfully detect H_2S in living cells.

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1. Introduction

Hydrogen sulfide (H_2S), a toxic gas that smells like rotten eggs, is an endogenous gas transmitter, similar to nitric oxide (NO) and carbon monoxide (CO) (Wang, 2002). H_2S can cross the cell membrane quickly and exert cytoprotective or cytotoxic effects on various biological targets (Banerjee, 2011; Jiang et al., 2014). Endogenous H_2S is mainly generated from L-cysteine (L-Cys) in reactions catalyzed by cystathionine-β-synthase, cystathionine-γ-lyase, and 3-mercaptopyruvate sulfur transferase (Kabi and Banerjee, 2010). It is hypothesized to be involved in many physiological activities, such as ischemia reperfusion injury, vasodilation, apoptosis, angiogenesis, neuromodulation, inflammation, insulin signaling, and oxygen sensing (Paul and Snyder, 2012; Yang et al., 2008; Lin et al., 2016; Abe and Kimura, 1996). Endogenous H_2S is found in the brain, cardiovascular system, liver, and kidneys. Abnormal levels of H_2S are associated with various diseases, including cirrhosis, heart disease, diabetes, Alzheimer's disease, and Down's syndrome (Yang et al., 2011; Qiu et al., 2012; Zanardo).

Due to the importance of H_2S, rapid and sensitive detection methods are urgently needed. Fluorescence detection methods are a convenient and sensitive approach for H_2S analysis (Johnson, 1998). Recently, fluorescent probes were developed based on mechanisms that include the reduction of an azide or nitro group to amine (Cao et al., 2015; Qiao et al., 2014; Chen et al., 2012; Lina et al., 2013), copper sulfide precipitation (Jacob et al., 2008; Sasakura et al., 2011; Zhang et al., 2011; Gu et al., 2011; Tang et al., 2013a, 2013b, 2013c), and a nucleophilic reaction (Liu et al., 2011; Peng et al., 2012; Liu et al., 2014; Sasakura et al., 2011; Cao et al., 2012; Zheng et al., 2012; Tang et al., 2016). Although many fluorescent probes have been reported, they have a number of drawbacks, including complicated synthesis (Qian et al., 2011, 2012;) and the need for surfactants to promote the reactions (Zheng et al., 2012', 2013; Peng et al., 2014).

Based on the high quantum yield and good photostability of the fluorescein molecule and its formylated derivates (Wang et al., 2013; Kambama et al., 2015), we introduced two dinitrobenzenesulfonate groups to fluorescein and its formylated derivatives to synthesize three probes (**F-1**, **F-2**, and **F-3**) for H_2S detection (Fig. 1a). Based on previous reports, aldehyde group facilitates the thiolysis of nitrobenzene sulfonyl ester (Huang et al., 2014). Thus, the probes **F-2** and **F-3,** derived from mono- and bis-formylated fluoresceins, respectively, were expected to have a

* Corresponding author.
E-mail address: chenxq@njtech.edu.cn (X. Chen).
[1] Present address: Nanjing Tech University, Nanjing 210009, China.

http://dx.doi.org/10.1016/j.bios.2016.08.018
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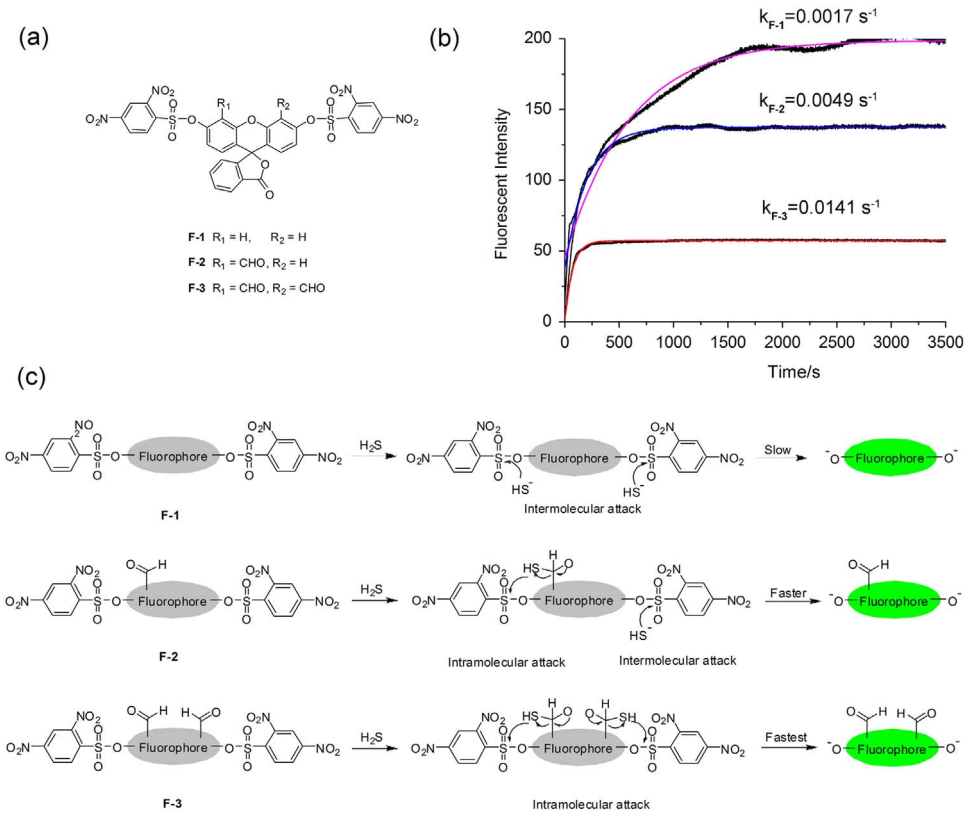

Fig. 1. (a) The chemical structures of the three probes **F-1, F-2,** and **F-3**. (b) The fluorescence change of **F-1**, **F-2**, and **F-3** (10 μM) over time after 50 equivalents of H_2S were added (50 mM HEPES, pH 7.4), Slit: 3 nm/5 nm. (c) The reaction mechanism of the probes with H_2S.

faster response to H_2S than **F-1**, in which no aldehyde group was introduced. Both H_2S and thiols are known to lead to the cleavage of a benzenesulfonate group due to the nucleophilicity of the mercapto group (Yu et al., 2014; Zhai et al., 2015). Thus, nucleophilic reaction-based H_2S probes usually suffer from the interference of thiols (Maeda et al., 2005, 2006; Chen et al., 2010a, 2010b). Among the probes developed in this work, the aldehyde groups in **F-2** and **F-3** can react with biothiols such as Cys and Hcy to form a thiazolidine moiety, which retards further cleavage of the benzenesulfonate group and suppresses the generation of fluorescence. Therefore, the introduction of ortho aldehyde groups in probe **F-3** greatly enhances both response speed and selectivity for the detection of H_2S.

2. Experimental materials and methods

2.1. Materials and instruments

Details for the materials and instruments employed in these studies can be found in the Supplementary Information.

2.2. Synthesis

The three probes were prepared *via* a substitution reaction between hydroxyl groups and 2,4-dinitrobenzene sulfonyl chloride. Experimental procedures and spectral data are detailed in the Supplementary Information.

2.3. Fluorometric analysis

The probes were dissolved in dimethylsulfoxide (DMSO) to prepare a 1.0 mM stock solution. The analytes were dissolved in deionized water to prepare a 10 mM stock solution. After the probe solutions were diluted in HEPES buffer (50 mM, pH 7.4) to the required concentrations for measurements, different analytes were added. All fluorescence measurements were conducted at room temperature.

2.4. Cell culture and fluorescence imaging

HeLa cells, purchased from Nanjing Cobioer Biosciences Company, were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (V/V) Fetal Bovine Serum (FBS, Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2. Cells were transferred to culture dishes and incubated for 20 h. Two groups were studied as follows: (I) HeLa cells were incubated with **F-3** (5 μM) for 30 min (II) HeLa cells were pre-treated with probe **F-3** (5 μM) for 30 min and then exposed to H_2S (8 μM) for another 30 min. Cell imaging was carried out after washing the cells in PBS (pH 7.4). Cells were imaged by confocal scanning microscopy.

3. Results and discussion

3.1. Synthesis of the probes

Mono- and bis-formylated fluoresceins were synthesized according to previous methods (Chen et al., 2010a, 2010b). The synthesis of the three probes is illustrated in Scheme S1. Probe structures were confirmed by ^1H NMR, ^{13}C NMR and HR-MS. (see Supplementary Information).

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