Sensors and Actuators B 230 (2016) 773–778

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Construction of a two-photon fluorescent turn-on probe for hydrogen persulfide and polysulfide and its bioimaging application in living mice

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ARTICLE INFO

Article history:
Received 11 December 2015
Received in revised form 21 February 2016
Accepted 25 February 2016
Available online 27 February 2016

Keywords:
Two-photon
Fluorescent probes
Fluorescence sensing
Tissues
Hydrogen persulfide and polysulfide

ABSTRACT

Intense biological studies have indicated that the biological functions associated with hydrogen sulfide may actually be mediated by H_2S_n ($n > 1$). Therefore, it is necessary to construction small-molecule fluorescent probe for the visualization of concentration of H_2S_n ($n > 1$) in living systems. Here, we described a novel two-photon fluorescent turn-on probe, **GCTPOC-H_2S_2**, for specific detection of H_2S_n, and the probe displayed high selectivity and sensitivity to H_2S_n. Furthermore, we demonstrated that the probe **GCTPOC-H_2S_2** could be employed to *in situ* image of H_2S_n in living mice for the first time.

1. Introduction

Reactive sulfur species (RSS) have received a great deal attention due to their interesting biological and physiological functions in biological systems. These reactive sulfur species included cysteine (Cys), glutathione (GSH), homocysteine (Hcy), hydrogen sulfide (H_2S), hydrogen persulfides (H_2S_2), polysulfides (H_4S_4), sulfenic acids and *S*-nitrosothiols [1–3]. Among these RSS molecules, hydrogen sulfide (H_2S) was considered as an important signaling molecule which generated by higher eukaryotic organisms or bacteria. Up or down level of hydrogen sulfide in living biological systems may induce a lot of physiological diseases including Down's syndrome, Alzheimer's disease, liver cirrhosis and diabetes [4]. In contrast, hydrogen persulfide and polysulfide (H_2S_n, $n > 1$) have received less attention in recent years. From a chemistry point, these species (H_2S_n, $n > 1$) could be considered as the oxidized forms of hydrogen sulfide (H_2S) and also belonged to reactive sulfur species family. In a word, H_2S_n ($n > 1$) and H_2S were very likely to coexist in living organisms because they were redox partners [5]. In addition, more and more biological studies indicated that the biological function associated with hydrogen sulfide may actually be mediated based on H_2S_n (n > 1) [6,7]. Therefore, developing new detecting methods for H_2S_n was necessary for the study of biological function of H_2S_n ($n > 1$).

Up to the present, the most commonly used method for H_2S_n detection was the ultraviolet detection method by using the ultraviolet absorption peak [8]. Unfortunately, this method has low sensitivity, complex sample preparation process, and relatively large tissues or cells destruction. Consequently, this method was unsuitable for detecting biological samples. Recently, fluorescence based assays have received great attention due to their high sensitivity, high selectivity, and fluorescent imaging in living biological sample. Given all this, these excellent detection methods have been extensively used in many areas of biological sensing [9–35]. To date, some H_2S_n-induced specific reactions have been used to design hydrogen persulfide and polysulfide (H_2S_n, $n > 1$) fluorescent probes, like 2-fluoro-5-nitro-benzoic ester, benzodithiolone formation, nucleophilic ring-opening reaction [36–42]. Nevertheless, the majority of these fluorescent H_2S_n probes reported were one-photon fluorescent probes, and they displayed a shallow imaging penetration depth. Furtherover, the short excitation wavelength of one-photon microscope (OPM) will result in photobleaching of fluorescent probes and high autoabsorption and autofluorescence of

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http://dx.doi.org/10.1016/j.snb.2016.02.124
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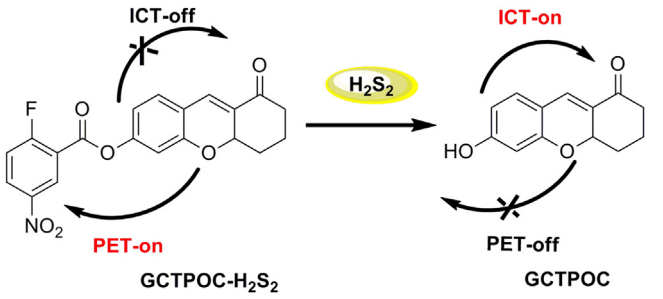

Scheme 1. The possible responsing mechanism of two-photon fluorescent probe **GCTPOC-H_2S_2**.

biomolecules in the biological samples, which greatly limit their biological applications.

As far as we know, two-photon microscope (TPM) was a very promising tool in the study of chemical biology. Two-photon microscope (TPM), which was generated to fluorescence by the two-photon excitation, displayed many advantages over OPM, such as low photodamage to biological samples, high penetration depth, high imaging resolution and three-dimensional imaging of biological samples [43,44]. These advantages were very suitable for imaging in living tissues and living mice. As far as we known, *in situ* imaging of H_2S_n in living mice has not been achieved yet. Therefore, it is of interest to construct novel two-photon fluorescent probes for detection H_2S_n in living biological samples.

A useful two-photon fluorescent platform **GCTPOC** has been developed by our group lately [45]. **GCTPOC** dye displayed strong two-photon fluorescence and considerable two-photon cross-section. Further studies showed that **GCTPOC** had tunable two-photon cross-section [45–47]. So **GCTPOC** can be used as a fluorescent platform to construction fluorescent probes based on its tunable two-photon cross-section which induced by intramolecular charge transfer magnism (ICT) efficiency [45–47]. In this work, a novel two-photon fluorescent probe **GCTPOC-H_2S_2** was designed and synthesized based on the **GCTPOC** platform and 2-fluoro-5-nitro-benzoic ester (Scheme 1). From the optical point of view, the 2-fluoro-5-nitro-benzoic moiety is a notorious fluorescence quencher [37,40,42]. The probe **GCTPOC-H_2S_2** displayed high sensitivity and selectivity towards H_2S_n, which maked it suitable for *in situ* imaging of H_2S_n in living mice.

2. Experimental

2.1. Materials and instruments

The detailed experimental materials and instruments in this work were displayed in Supporting information.

2.2. Cytotoxicity assays

The toxicity of probe **GCTPOC-H_2S_2** towards living MCF-7 cells was performed by the standard MTT assays. The detailed experiment methods were presented in supporting information.

2.3. Preparation of liver slices of living mice for Z-scanning confocal imaging

Kunming mice were bought from Xiangya Hospital, the weight of the mice is about 18–25 g, and liver slices were cut to 400 μm thickness. The detailed experiment methods were presented in supporting information.

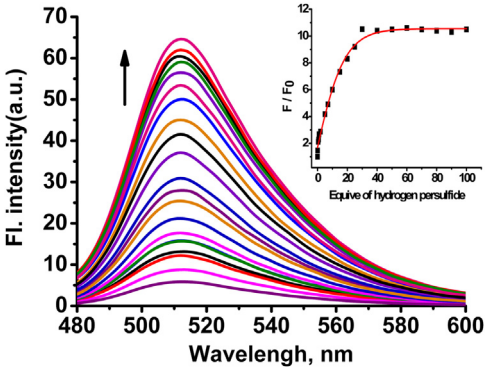

Fig. 1. Fluorescence response of **GCTPOC-H_2S_2** (5.0 μM) in the presence of increasing of Na_2S_2. Inset: fluorescence intensity ratio of **GCTPOC-H_2S_2** (5.0 μM) at 512 nm as a function of Na_2S_2. All of data are obtained after the reaction was conducted for 30 min. λ_{ex} = 450 nm.

3. Result and discussion

The probe **GCTPOC-H_2S_2** was readily synthesized in one step by the esterification reaction of the **GCTPOC** with acrylic acid, and the detailed synthesis steps and characterization data of ^{1}H NMR, ^{13}C NMR, MS (EI), and HRMS(EI) were displayed in supporting information (Scheme S1). Next we first detected the responses of probe **GCTPOC-H_2S_2** to H_2S_n. In our experiments, H_2S_2 was always used as the representative model compound of H_2S_n, which comed mainly from freshly prepared solutions of Na_2S_2. The fluorescence titration of H_2S_2 to **GCTPOC-H_2S_2** probe (5 μM) was conducted in the PBS (25 mM, pH 7.4).

As shown in Fig. 1, free probe **GCTPOC-H_2S_2** showed almost no fluorescence when excited at 450 nm. However, a large fluorescence increase at around 512 nm was observed when addition of different concentrations of H_2S_2 to the solution of **GCTPOC-H_2S_2** (Fig. 1 and inset of Fig. 1). The two-photon cross-section of the probe **GCTPOC-H_2S_2** was negligible (about 0.01GM). However, the reaction product of probe **GCTPOC-H_2S** showed large two-photon cross-sections (about 500GM). These results were in agreement with the one-photon spectrum in Fig. 1. The detection limit for **GCTPOC-H_2S_2** was 1.52×10^{-7} M (S/N = 3) by calculation formula in supporting information (Fig. S1), showing that **GCTPOC-H_2S_2** was highly sensitive to hydrogen polysulfides in the physiological pH conditions. In view of the excellent performance of the probe, we thinked that the probe **GCTPOC-H_2S_2** may be used for the detection of H_2S_2 in biological system.

To get more insight into the likely response mechanism, we decided to use NMR spectroscopy and mass spectrometry to study

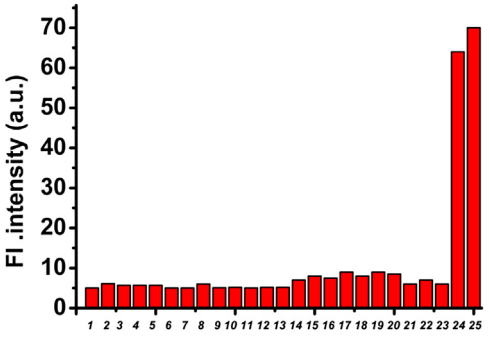

Fig. 2. Fluorescence response of probe **GCTPOC-H_2S_2** (5 μM) to various analytes (100 μM) in PBS (pH 7.4, 25 mM). 1. blank, 2. Cl^-, 3. Br^-, 4. I^-, 5. AcO^-, 6. N_3^-, 7. CN^-, 8. CO_3^{2-}, 9. NO_2^-, 10. K^+, 11. Ca^{2+}, 12. Zn^{2+}, 13. Mg^{2+}, 14. H_2O_2, 15. HClO, 16. GSH, 17. Hcy, 18. Cys, 19. NaSH, 20. S_8, 21. $S_2O_3^{2-}$, 22. SO_3^{2-}, 23. SO_4^{2-}, 24. Na_2S_2, 25. Na_4S_4. All of data are obtained after the reaction was conducted for 30 min. λ_{ex} = 450 nm.