Analytica Chimica Acta 853 (2015) 548–554

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

A two-photon fluorescent probe with a large turn-on signal for imaging hydrogen sulfide in living tissues

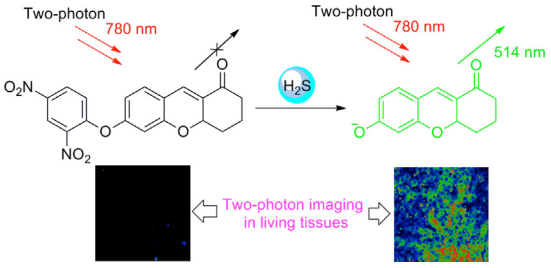
Kaibo Zheng [b], Weiying Lin [a,b,*], Li Tan [b], Dan Cheng [b]

[a] Institute of Fluorescent Probes for Biological Imaging, University of Jinan, Jinan, Shandong 250022, PR China
[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, PR China

HIGHLIGHTS

- A two-photon fluorescent probe for sensing H_2S was developed.
- The probe shows a large turn on signal (120-fold enhancement).
- The probe is suitable for fluorescence imaging of H_2S in living cells and tissues.
- The probe was capable of detecting H_2S up to 170 μm depth in live tissues.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 September 2014
Received in revised form 10 October 2014
Accepted 15 October 2014
Available online 18 October 2014

Keywords:
Two-photon fluorescent probe
H_2S
Bioimaging
Tissues

ABSTRACT

A two-photon fluorescence turn-on H_2S probe GCTPOC–H_2S based on a two-photon platform with a large cross-section, GCTPOC, and a sensitive H_2S recognition site, dinitrophenyl ether was constructed. The probe GCTPOC–H_2S exhibits desirable properties such as high sensitivity, high selectivity, functioning well at physiological pH and low cytotoxicity. In particular, the probe shows a 120-fold enhancement in the presence of Na_2S (500 μM), which is larger than the reported two-photon fluorescent H_2S probes. The large fluorescence enhancement of the two-photon probe GCTPOC–H_2S renders it attractive for imaging H_2S in living tissues with deep tissue penetration. Significantly, we have demonstrated that the probe GCTPOC–H_2S is suitable for fluorescence imaging of H_2S in living tissues with deep penetration by using two-photon microscopy. The further application of the two-photon probe for the investigation of biological functions and pathological roles of H_2S in living systems is under progress.

1. Introduction

Hydrogen sulfide (H_2S), a newly recognized gaseous signaling compound along with two well-known gasotransmitters: carbon monoxide (CO) and nitric oxide (NO) [1,2], is endogenously produced by enzymes such as cystathionine β-synthase (CBS), cysathionine γ-lyase, and 3-mercaptopyruvate sulfurtansferase [3,4]. Although traditionally considered as a noxious gas with an unpleasant smell of rotten egg, numerous investigations have demonstrated that H_2S gets involved in many physiological processes, such as vasodilation, neuromodulation, apoptosis, and regulation of inflammation [5–8]. However, abnormal level of H_2S in cells will cause many types of diseases including Alzheimer's disease [9], Down's syndrome [10], diabetes [11], and liver cirrhosis [12]. Thus, it is of great significance to develop efficient methods for the detection of H_2S in biosystems.

* Corresponding author at: Institute of Fluorescent Probes for Biological Imaging, University of Jinan, Jinan, Shandong, 250022, PR China. Tel.: +86 531 82769031; fax: +86 531 82769031.
E-mail addresses: weiyinglin2013@163.com, weiyinglin@hnu.edu.cn (W. Lin).

http://dx.doi.org/10.1016/j.aca.2014.10.024
0003-2670/

So far, several analytical techniques including colorimetric [13,14] and electrochemical methods [15,16], chromatography [17,18] and sulfide precipitation [19] have been developed for the detection of H_2S. Although these methods are sensitive toward H_2S, they require complicated sample preparation and destruction of tissues or cells; and therefore, are not suitable for living biosystems. In recent years, fluorescent probes, as an excellent detection tools, have attracted increasing attention for high selectivity, high sensitivity, as well as real-time imaging, and they have been widely applied in the detection of anions, cations, and biological molecules [20–27]. The molecular fluorescent probes which have been developed for detecting H_2S are based on the H_2S-induced specific reactions, such as the thiolysis of dinitrophenyl ether [28], nucleophilic reaction [29–32], and reduction of azide, nitro or hydroxyl amine groups to amines [33–38]. However, most of these probes are based on one-photon microscopy (OPM), which only show shallow penetration depth. Moreover, the short excitation wavelength may lead to photobleaching of probes and damage in cells and tissues, and thus, limit their biological applications.

By contrast, two-photon microscope (TPM) provides an attractive technique to study biomolecules in live cells and tissues. TPM, where fluorescence is triggered by two-photon excitation, shows a variety of advantages over conventional one-photon fluorescence microscopy [39–45]. It can facilitate three-dimensional imaging of living tissues, reduce photodamage to biosamples, increase tissue penetration, and lower background fluorescence. These contribute to the capability of imaging in deep tissues and animals. In spite of these favorable properties, only a few two-photon fluorescent probes have been constructed for imaging H_2S in living tissues [46–51]. However, it is still necessary to develop two-photon fluorescent H_2S probes with a large turn-on signal.

Recently, our group have reported a fluorescent dye GCTPOC [52], which contains a rigid oxygen-bridge and a hydroxyl group at the 2-position (para-position). GCTPOC exhibits excellent two-photon properties with a two-photon cross-section (σ) above 810 GM and a two-photon excitation action cross-section above 270 GM, indicating that the dye is potentially useful for bioimaging applications [52]. GCTPOC has been demonstrated to be an efficient two-photon platform for designing two-photon probes with its tunable two-photon properties at the hydroxyl group. In this work, we developed GCTPOC–H_2S as a new two-photon fluorescent turn-on probe for H_2S based on the GCTPOC two-photon platform [52] and thiolysis of dinitrophenyl ether (Scheme 1). The two-photon probe GCTPOC–H_2S has a large fluorescence enhancement, which renders it attractive for imaging H_2S in living tissues with deep tissue penetration.

2. Experimental

2.1. Materials and instrumentation

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a labtech UV power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; the pH measurements were carried out on a Mettler–Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

2.2. DFT calculations

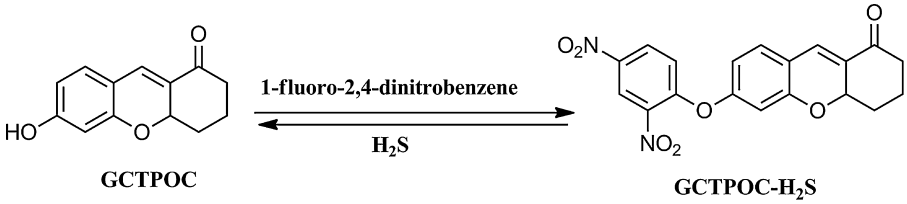
The ground state structures of GCTPOC–H_2S and GCTPOC were optimized using DFT with B3LYP functional and 6–31 G (d) basis set. The initial geometries of the compounds were generated by the GaussView software. The excited state related calculations (UV–vis absorption) were carried out with the time dependent DFT (TDDFT) with the optimized structure of the ground (DFT/6–31 G (d)). The emission of the fluorophores was calculated based on the optimized S_1 excited state geometry. All of these calculations were performed with Gaussian 09 (Revision A.01) [53].

2.3. General procedure for the spectra measurement

The stock solution of the probe GCTPOC–H_2S was prepared at 0.5 mM in DMSO. The solutions of various testing species were prepared from $CaCl_2$, KCl, KI, NaCl, KBr, NaN_3, $Na_2S_2O_3{\cdot}5H_2O$, Na_2SO_3, $NaNO_2$, CH_3COONa, ascorbic acid, GSH, cysteine, H_2O_2, NaClO in the twice-distilled water, and superoxide ($O_2^{\bullet-}$) was added as solid KO_2. The test solution of the probe GCTPOC–H_2S (5.0 μM) in 3 mL 25 mM PBS buffer (pH 7.4) with 20% ethanol was prepared by placing 0.03 mL of the probe GCTPOC–H_2S stock solution and 0.6 mL ethanol in 2.4 mL of the aqueous buffer. The resulting solution was shaken well and incubated with appropriate testing species for 40 min at 37 °C before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 410 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

2.4. Cytotoxicity assays

HeLa cells were cultured in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Immediately, before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of probe GCTPOC–H_2S (99.9% DMEM and 0.1% DMSO). The final concentrations of the probe were kept from 0.5 to 10 μM (n = 3). The cells were then incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h, followed by MTT assays. Untreated assay with DMEM (n = 3) was also conducted under the same conditions.



Scheme 1. Design and synthesis of GCTPOC–H_2S.