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A cell surface specific two-photon fluorescent probe for monitoring intercellular transmission of hydrogen sulfide

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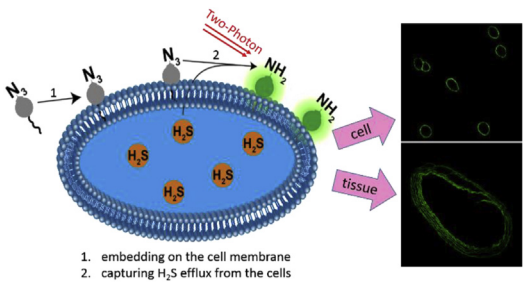
HIGHLIGHTS

- ASNHN-N_3 was designed and synthesized as a cell surface specific probe for H_2S.
- ASNHN-N_3 is a two-photon turn-on fluorescent probe with high sensitivity and selectivity.
- H_2S efflux from living cells was firstly monitored.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrogen sulfide (H_2S) is a new endogenously generated gasotransmitter and has implicated in many physiologies and pathologies closely related to its intracellular and intercellular signaling transduction. Although many fluorescent probes have been exploited to track and quantify H_2S in living systems, none of them could be used for monitoring intercellular transmission of H_2S. Herein, we developed a cell surface specific H_2S probe, 4-azido-6-sulfo-*N*-hexadecyl-1,8-naphthalimide, sodium salt (ASNHN-N_3), trying to investigate the behaviors of extracellular release of H_2S. ASNHN-N_3 is week fluorescent and could react with H_2S at 37 °C in pH 7.4 buffer solutions to form product ASNHN-NH_2 with strong fluorescence ($\Phi = 0.22$). Using ASNHN-N_3 as H_2S probe, excellent linear correlation versus the concentration of NaHS was obtained ranging from 0 to 10 μM and the detection limit was 0.75 μM. With the lipid anchor and the hydrophilic sulfonic group introduced into the 1,8-naphthalimide (a skeleton of two-photon fluorescent probe), the amphiphilic probe is located at the surface of living cells which can record H_2S efflux from the cell diffusing across the plasma membrane in living cells and deep-tissue by using two-photon microscopy. Thus we present a new strategy for further studying the mechanism of signaling molecules in cell communication and signal pathways.

1. Introduction

Hydrogen sulfide (H_2S) which smells like a rotten egg has been identified as biosynthetic gasotransmitter after the other two important endogenous signaling molecules, nitric oxide (NO) and carbon monoxide (CO) [1–3]. As a signaling molecule, H_2S plays crucial physiological roles and has diverse pathophysiological

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functions in biological processes, such as neuromodulation in the brain, anti-inflammation in the immune system, smooth muscle relaxation in the vascular system, and cytoprotective effect [4–6]. In addition, the misregulation of physiological concentrations of H_2S is related to many diseases including liver cirrhosis [7], Down's syndrome [8], Alzheimer's disease [9] and diabetes [10]. H_2S can pass through the cellular membranes easily to participate in cellular signaling transduction, but due to its fast catabolism and extinction, the detailed function of H_2S is not fully understood. Therefore, efficient assay methods for monitoring diffusion and intercellular transmission of H_2S in biological systems is strong needed.

Fluorescent probe is an essential tool in the molecular recognition events in biological systems. A number of fluorescent probes have been exploited to track and quantify H_2S in living systems based on reduction of azide/hydroxyl amine group/nitro to amines [11–22], thiolysis of dinitrophenyl ether [23–25] and azamacrocyclic metal ion complexes [26–31]. Because of lots of advantages including stronger penetration depth, better three-dimensional spatial localization, longer observation times and fewer autofluorescence in cells and tissues [32–35], two-photon probes have also increasingly adopted in H_2S imaging, such as SHS-M_1, SHS-M_2 [16], FS_1 [36], TP-PMVC [37], TAB-2-Cu^{2+} [38] and so on. However, these existing fluorescent probes including the TP fluorescent probes are membrane-permeable, which can only be used for detecting intracellular H_2S in situ.

H_2S is a plasma membrane permeable intracellular and intercellular gasotransmitter [39]. The role of H_2S as intracellular gasotransmitter is closely related to its generation, distribution and concentration in living cells, while its role and mechanism as intercellular gasotransmitter is based on its intercellular transmission. However, the reports for monitoring H_2S in living organism are focused on its generation and distribution in cells. The extracellular release of H_2S from cells is the first step of its intercellular transmission. To the best of our knowledge, such first step is still not visualized to date.

Cell membrane is a biological glycerophospholipid bilayer that separates the interior of cells from the extracellular environment [40]. Thus, fluorescent probe anchored on outer surface of plasma membrane is the best way to monitor the extracellular release of H_2S and avoid the interference of intracellular H_2S. When intracellular H_2S is released out from cells, the concentration of H_2S on the outer surface of cells will be increased and then H_2S will be trapped by the cell surface specific fluorescent probe. Recently, our group has proposed an amphiphilic fluorescent probe DSDMHDAB to visualize NO released out of the cells on the outer surface of plasma membrane [41]. Under this inspiration, we developed a new two-photon cell surface specific probe, 4-azido-6-sulfo-*N*-hexadecyl-1,8-naphthalimide, sodium salt (ASNHN-N_3). ASNHN-N_3 contains a long hydrophobic alkyl chain to embed into the lipophilic region of the cell membrane. Besides, ASNHN-N_3 has a hydrophilic sulfonic group to improve its water solubility to avoid the internalization of the probe into the cytoplasm and keep the response moiety on the outer surface of cell membrane. As expected, we successfully applied ASNHN-N_3 to realize the visualization of H_2S efflux from the cell on the external surface of the membrane in live cells and living liver and offer a panoramic view of vascular tissue for extracellular H_2S imaging by TPM.

2. Experiments

2.1. Reagents

Unless otherwise noted, chemicals were purchased from commercial suppliers and used without further purification. Dry methanol was purified by distillation. 4-amino-1,8-naphthalic anhydride (95%) was purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM) was purchased from Thermo scientific (Waltham, MA, U.S.A.). Fetal bovine serum (FBS) was taken from Tianhang Biological Technology (Zhejiang, China). Penicillin, streptomycin, and trypsin were obtained from Amresco (Solon, Ohio, U.S.A.). Phorbol-12-myristate-13-acetate (PMA) was obtained from Sigma. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Biosharp (Hefei, China). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Sigma-Aldrich. Phosphate-buffered saline (PBS) solution consisted of 8.00 g/L NaCl, 0.20 g/L KCl, 0.20 g/L KH_2PO_4, and 2.78 g/L $Na_2HPO_4{\cdot}12H_2O$, and pH values were adjusted to 7.4 with 1.0 M HCl and 1.0 M NaOH. Sodium phosphate solution buffer was prepared by mixing 0.10 M H_3PO_4 solution with 0.10 M Na_3PO_4 solution and adjusted to the required pH values.

2.2. Apparatus

Water used in this work was purified by a Milli-Q system (Millipore). NMR spectra were recorded on a Varian Mercury VS instrument at 300 MHz (Varian, Palo Alto, CA) using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra was performed using an LTQ-Orbitrap Elite (Thermo-Fisher Scientific, Waltham, MA, U.S.A.) mass spectrometer. UV-vis absorption spectra were recorded using a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Tokyo, Japan) and fluorescence spectra were obtained with a RF-5301PC spectrofluorophotometer (Shimadzu, Tokyo, Japan) with a 1 cm standard quartz cell. The pH was measured on a Mettler-Toledo Delta 320 pH meter. HPLC analysis was performed on a Fortis Xi C18 column (5 μm particle size, 150 × 4.6 mm i.d.) using an Agilent 1100 HPLC system with UV−vis detector. Cytotoxicity was determined on a Thermo Scientific microplate reader. One-photon fluorescence imaging experiments were performed on a Nikon confocal laser scanning microscope (TE2000, Japan). Two-photon fluorescence imaging experiments were obtained by using a ZEISS LSM 710 (Germany).

2.3. Cell culture and cell imaging

HeLa human cervical cells and RAW 264.7 murine macrophages were maintained following protocols provided by the Animal Experiment Center of Wuhan University (China). The cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The cells were detached and seeded in glass-bottomed dishes (NEST) and allowed to settle for 24 h before imaging.

To confirm the localization ability of ASNHN-N_3 to the plasma membrane, the cultured cells were washed with PBS and then loaded with ASNPN-N_3 (20 μM) for 5 min (in culture medium containing 0.2% DMSO) followed by addition of NaHS (200 μM) without removing the excess probe and then co-incubated for 30 min at 37 °C and then subjected to imaging analysis.

To validate the localization ability of ASNHN-N_3 to the plasma membrane, the cultured cells were washed with PBS and then loaded with DiI (5 μM) for 5 min (in culture medium containing 0.2% DMSO). After rinsed with PBS three times, the cells were further incubated with ASNHN-N_3 (20 μM) for 5 min (in culture medium containing 0.2% DMSO). After removed the excess probe by washing cells three times with PBS, the cells were incubated with NaHS (200 μM) for 30 min at 37 °C and then subjected to

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