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Development of photo-controllable hydrogen sulfide donor applicable in live cells

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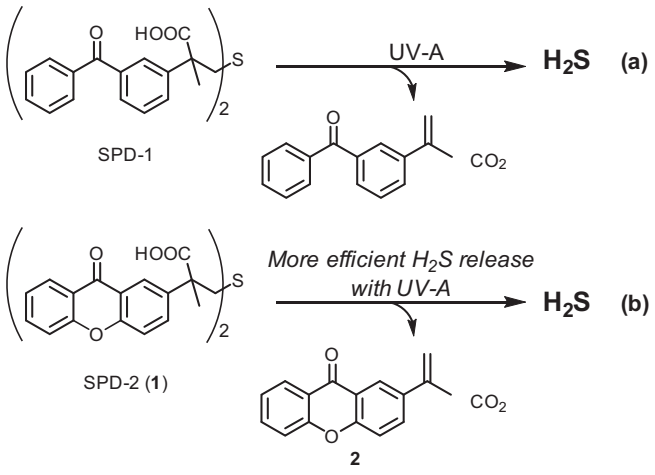
ABSTRACT

Hydrogen sulfide (H_2S) has multiple physiological roles, for example, in vasodilation and inflammation. It is a highly reactive gas under ambient conditions, so controllable H_2S donors are required for studying its biological functions. Here, we describe the design, synthesis and application of a H_2S donor (SPD-2) that utilizes xanthone photochemistry to control H_2S release. H_2S generation from SPD-2 was completely dependent on UVA-irradiation (325–385 nm), as confirmed by methylene blue assay and by the use of a H_2S-selective fluorescent probe. SPD-2 was confirmed to provide controlled H_2S delivery in live cells, and should be suitable for various biological applications.

Hydrogen sulfide (H_2S), like nitric oxide (NO) and carbon monoxide (CO), is a gaseous physiological signal transmitter[1] that has roles in multiple processes, including vascular smooth muscle relaxation,[2] neurotransmission[3] and regulation of inflammation.[4] It is biologically synthesized from L-cysteine and/or L-homocysteine by cystathionine-β-synthase (CBS),[5] cystathionine-γ-lyase (CSE)[6] and 3-mercaptopyruvate sulfur transferase (3MST) coupled with cysteine aminotransferase (CAT).[7] Recently, a novel H_2S biosynthetic pathway from D-cysteine, involving 3-MST and D-amino acid oxidase (DAO), has also been reported.[8] These enzymes have been demonstrated to influence a wide range of physiological and pathological processes.[9,10]

In biological research on H_2S, inorganic sulfide salts, such as Na_2S and NaSH have been widely used as H_2S sources. However, these sources have substantial disadvantages for examination of the physiological functions of H_2S. For example, H_2S generation is very fast, and precise control of the release rate and dosage is not feasible. These compounds cannot mimic biological H_2S release, which is thought to be relatively slow and continuous. Therefore, controllable H_2S donors are required for detailed investigation of the physiological functions of H_2S and for potential therapeutic applications. We focused on photocontrollable H_2S donors, since photolysis-induced H_2S release has the potential to allow precise control of the location, timing and dosage of release.

Some photolysis-inducing H_2S donors, based on geminal-dithiol structure, have already been reported.[11] However, it is difficult to control the rate of H_2S release from these donors because the formation of H_2S depends on hydrolysis of geminal dithiol in aqueous media. Recently, we have reported a H_2S derivative doubly protected with a photolabile protecting group (PPG) as a photo-activatable H_2S donor,[12] based on ketoprofenate PPG[13] which has good light-responsiveness (SPD-1, Scheme 1a). Ketoprofenate



Scheme 1. Structures and photoreactions of SPD-1 and SPD-2.

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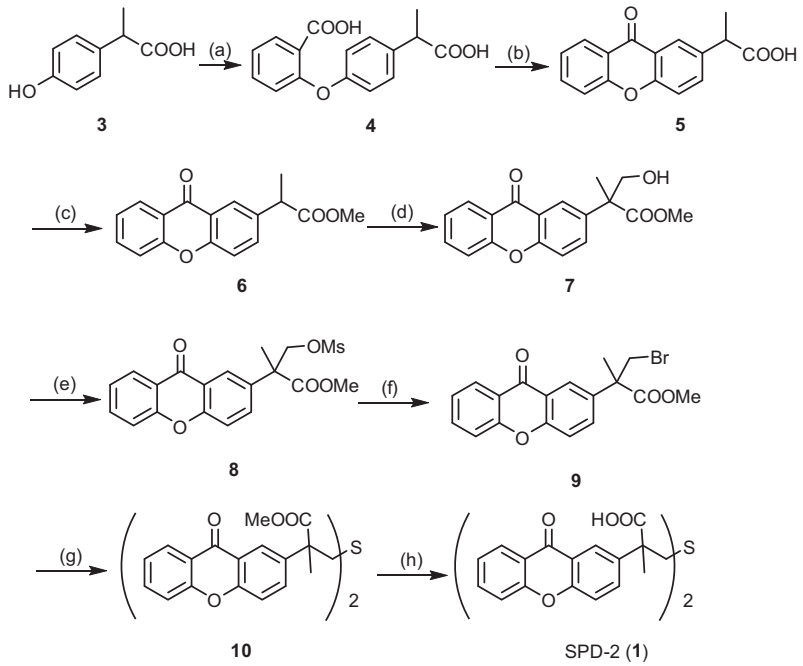

Scheme 2. Synthesis of SPD-2. Reagents and conditions: (a) 2-iodobenzoic acid, Cs_2CO_3, CuCl, TDA-1, dioxane; (b) H_2SO_4, 85 °C, 2 steps 52%; (c) H_2SO_4, MeOH, 96%; (d) paraformaldehyde, K_2CO_3, DMSO, 56%; (e) MsCl, NEt_3, CH_2Cl_2, quant.; (f) LiBr, MeCN, 36%; (g) sodium sulfide nonahydrate, toluene, H_2O, $C_{16}H_{33}(C_4H_9)_3P^+Br^-$, 46%; (h) LiOH, THF, H_2O, MeOH, 84%. TDA-1 = tris[2-(2-methoxyethoxy)ethyl]amine, DMSO = dimethylsulfoxide, MsCl = methanesulfonyl chloride, THF = tetrahydrofuran.

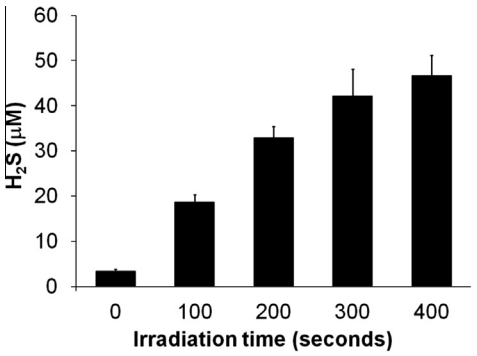

Figure 1. Release of H_2S from SPD-2 (100 μM) in PBS, pH 7.4, with 1% DMSO as detected by the methylene blue method. Formation of methylene blue after irradiation of SPD-2 for various times was determined from the absorbance at 671 nm. Photoirradiation was performed at 325–385 nm and the light intensity was set at 2.0 mW/cm^2. The data represent the average of three independent experiments with standard deviations.

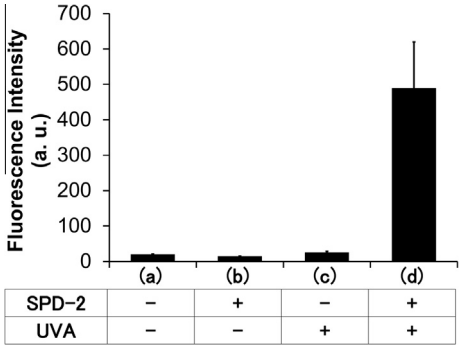

Figure 2. Fluorescence measurement for detection of H_2S generation using HSip-1. The concentration of SPD-2 was 100 μM and that of HSip-1 was 1 μM in phosphate buffer, pH 7.4, containing 1% DMSO. Fluorescence emission was measured at 516 nm with excitation at 491 nm. Measurements were made after incubation of HSip-1: (a) without SPD-2 or irradiation; (b) with SPD-2 but without irradiation; (c) without SPD-2 after irradiation for 300 s; (d) with SPD-2 after irradiation for 300 seconds. Photoirradiation was performed at 325–385 nm and the light intensity was set at 2.0 mW/cm^2. The data represent the average of three independent experiments with standard deviations.

PPG has many advantageous features, such as good water solubility, formation of benign photo-products and a higher release rate than *o*-nitrobenzyl PPG,[14] the most widely used PPG. However, SPD-1 shows maximum absorbance at 260 nm (Fig. S1b), and consequently the efficiency of H_2S release from SPD-1 is insufficient for application in living cells when UV-A (330–380 nm), widely equipped in some microscopies, is utilized as an uncaging light source. Therefore, we set out to design a new photo-induced H_2S donor suitable for cellular application. Recently, xanthone PPGs have been reported as next-generation carbanion-mediated PPGs[15] with excellent absorption in the UVA region (>320 nm). We designed a novel photo-controllable H_2S donor, SPD-2, by utilizing xanthone PPG to protect H_2S directly (Scheme 1b). SPD-2 showed superior photochemical properties to the ketoprofenate-type H_2S donor, and we confirmed that it is available for photo-controlled, site-specific H_2S release in live cells.

SPD-2 was synthesized as shown in Scheme 2. Preparation of the synthetic intermediate was performed as described previously.[15] Xanthone derivative **5** was prepared by Ullmann condensation of *o*-iodobenzoic acid and 2-(4-hydroxyphenyl)propanoic acid (**3**), followed by acid-catalyzed Friedel–Crafts type reaction. After esterification of **5**, hydroxymethylation by treatment with paraformaldehyde and K_2CO_3 gave the desired alcohol **7**. This was mesylated to obtain **8**, which was converted to the brominated derivative **9**. Then, sulfide **10** was synthesized by using sodium sulfide nonahydrate. In this reaction, 2-(prop-1-en-2-yl)-9*H*-xanthen-9-one (Scheme 1b, the proposed photoproduct **2**) was obtained as a by-product, and this was isolated for structural confirmation of the photo-product of SPD-2. Finally, hydrolysis gave the desired product SPD-2. The structure and purity of SPD-2 were confirmed by ^1H NMR, ^{13}C NMR, mass spectrometry and elemental analysis.

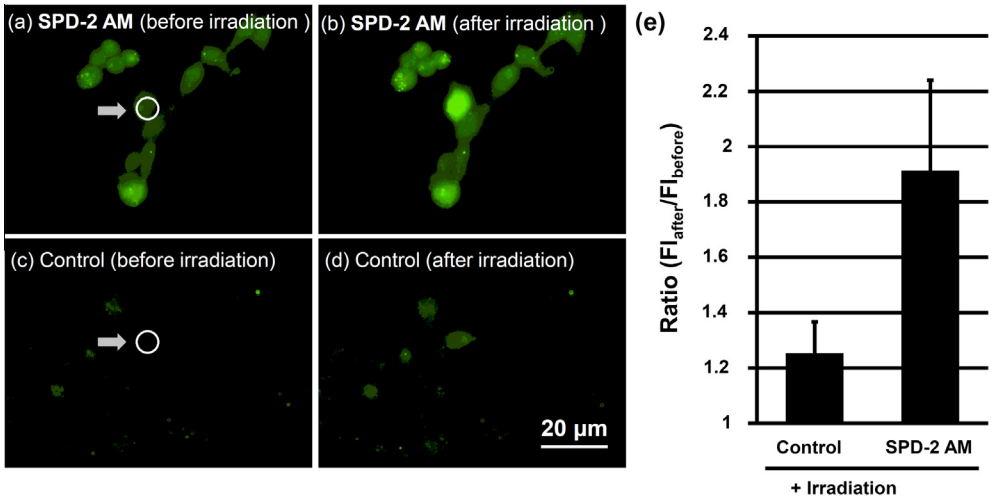

Figure 3. Fluorescence images of cells treated with HSip-1 DA (30 μM) in the presence or absence of SPD-2 AM with UVA photoirradiation. The treated cells were photoirradiated with UVA (330–380 nm) for 5 min. Representative images are shown in panels a–d: (a and b) Fluorescence images of cells treated with SPD-2 AM (a) before and (b) after photoirradiation; (c and d) images of cells without SPD-2 AM (c) before and (d) after photoirradiation. Photoirradiation was performed at the area indicated by the circle. All cells were treated with HSip-1DA. Scale bar = 20 μm. (e) Ratio of average fluorescence intensities inside the indicated circle at 10 and 0 min (FI_{after}/FI_{before}) after 5 min UV-irradiation (330–380 nm) in D-PBS. Data represent means and standard deviation ($n = 3$). $^{*}P < 0.05$ versus control.

To verify H_2S release from SPD-2 upon photoirradiation, we utilized methylene blue assay, a widely used colorimetric method for H_2S detection.[16] In this study, a 100 μM solution of SPD-2 in pH 7.4 phosphate buffer buffer (containing 1% DMSO as a co-solvent) was prepared. When a solution of SPD-2 was subjected to UV irradiation at 325–385 nm for various durations, H_2S release was clearly irradiation-dependent (Figs. 1, S2). When 100 μM SPD-2 was irradiated for 400 s, the concentration of released H_2S was determined as 47 μM, while SPD-1 released only 15 μM H_2S under the same conditions.[10] This result indicated that SPD-2 is a more efficient H_2S donor than SPD-1, presumably because the maximum absorption range of SPD-2 is well matched to the wavelength of the irradiating light, whereas that of SPD-1 is not (Fig. S1).

For further confirmation of photo-dependent H_2S release, we employed HSip-1, a H_2S-selective fluorescent probe that utilizes azamacrocyclic copper (II) ion complex chemistry to control the fluorescence.[17] As shown in Figure 2, the fluorescence intensity of HSip-1 was dramatically increased upon irradiation in the presence of 100 μM SPD-2 in 100 mM PBS, pH 7.4 containing 1% DMSO as a co-solvent (an aliquot was taken after irradiation for 300 s). Almost no fluorescence increment was observed in the controls (Fig. 2a–c).

We confirmed the formation of the photoproduct **2**, illustrated in Scheme 1b. Photodecomposition of SPD-2 was monitored by HPLC. As shown in Figure S3, the peak of SPD-2 decreased and a new peak around 14 min was observed in the irradiated solution. The absorbance and retention time of the newly formed peak was identical to those of authentic **2**. This result confirmed that photolysis-induced H_2S generation proceeded in the expected manner.

Next, we examined the stability of SPD-2. A solution of 100 μM SPD-2 in pH 7.4 phosphate buffer was incubated for 1 month at 37 °C in the dark. However, no decomposition was detected by means of HPLC (data not shown). This result suggested that SPD-2 is remarkably stable in the dark and has good light-responsiveness as a H_2S photo-donor.

Next, we applied this donor to living cells (Fig. 3). However, SPD-2 probably has poor membrane permeability due to the hydrophilic carboxyl moieties. Therefore, these moieties were acetoxymethylated to improve the membrane permeability. Acetoxymethylated SPD-2 (SPD-2 AM) should permeate cell membrane and be intracellularly hydrolyzed to SPD-2. HEK293 cells were incubated with 100 μM SPD-2 AM and 30 μM HSip-1 DA (membrane-permeable HSip-1) in Dulbecco's modified Eagle's medium (DMEM) for 30 min, then washed with Dulbecco's phosphate-buffered saline (D-PBS), and photo-irradiated for 5 min at the site indicated by the circle in Figure 3a. Upon photoirradiation, fluorescence enhancement was observed (Fig. 3b and e), while little fluorescence increment was observed in cells outside the photoirradiated area. Additionally, SPD-2 did not show strong toxicity at low micromolar level which is anticipated as the active dose of H_2S donors.[18] (Fig. S4). Under the same conditions, SPD-1 showed no fluorescence enhancement (data not shown), probably because its efficiency of H_2S release on irradiation at this wavelength was much lower than that of SPD-2. These results indicated that SPD-2 is suitable for use in live cells.

In conclusion, we have developed a novel photo-controllable H_2S donor, SPD-2, using xanthonate PPG. Generation of H_2S from SPD-2 can be precisely controlled by irradiation at 325–385 nm (UVA). This donor is available both in aqueous solution and in live cells. We expect that SPD-2 will be a useful tool for elucidating a wide range of biological functions of H_2S.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.084.

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