General Information

It becomes obvious that there are a lots of molecules containing sulfane sulfurs such as persulfides and polysulfides in living body. These molecular species are involved in production, storage and release of hydrogen sulfide, which is recognized as an important physiological mediator. Furthermore, recent studies reveal that persulfides and polysulfides may control intracellular signal transduction through *s*-sulfhydration of proteins.

SSP4 (Sulfane Sulfur Probe 4) is a novel fluorescent probe to detect sulfane sulfurs selectively. SSP4 itself is non-fluorescent, but it emits strong green fluorescence when it reacts with sulfane sulfurs. Thus, SSP4 enables high sensitive fluorescence detection and imaging of sulfane sulfurs.

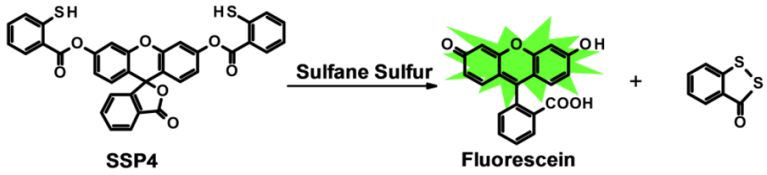


Fig. 1 Reaction of SSP4 with sulfane sulfurs

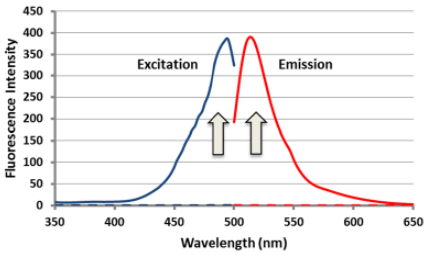


Fig. 2 Excitation and emission spectra of SSP4 after reaction with sulfane sulfurs

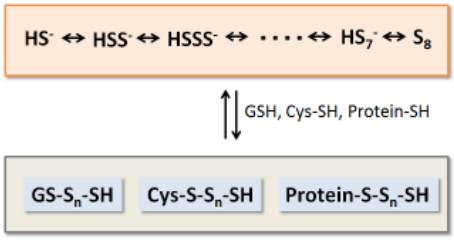


Fig. 3 Chemical species containing sulfane sulfurs

* Sodium trisulfide (Na_2S_3) was added to SSP4 in PBS.
λ_{ex}=482 nm, λ_{em}=515 nm

Contents

SSP4 1 mg x 1

Storage Conditions

Store at ≤ -20 °C and protect from light

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Serum-free medium
- PBS
- Micropipettes

Preparation of Solutions

- 10 mmol/l SSP 4 Stock Solution -

Add 165 µl of DMSO to a tube containing 1 mg of SSP4 and dissolve by pipetting.

*Store at -20 °C and use within two months. Aliquot the solution for longer storage.

- Serum-free Medium containing 0.5 mmol/l CTAB -

Add 1 ml of ddH_2O to 36.4 mg of CTAB in a 1.5 ml-tube, and dissolve by heating to prepare 100 mmol/l CTAB solution. Dilute this solution with a serum-free medium to prepare a serum-free medium containing 0.5 mmol/l CTAB.

* We recommend to use CTAB for introduction of SSP4 into cells.

- SSP4 Working Solution -

Dilute 10 mmol/l SSP4 Stock Solution with a Serum-free Medium containing 0.5 mmol/l CTAB to prepare 5-20 µmol/l SSP4 Working Solution.

*To prepare 20 µmol/l SSP4 Working Solution, add 10 µl of 10 mmol/l SSP4 Stock Solution to 5 ml of a Serum-free Medium containing 0.5 mmol/l CTAB.

General Protocol

-Fluorescence imaging of sulfane sulfurs in cells-

1) Culture cells in a plate or dish applicable for fluorescence imaging.

2) Discard the culture medium and wash the cells with a serum-free medium.

3) Add SSP4 Working Solution to the cells, incubate for 15 minutes in a humidified incubator (*e.g.*, at 37°C, 5% CO_2).

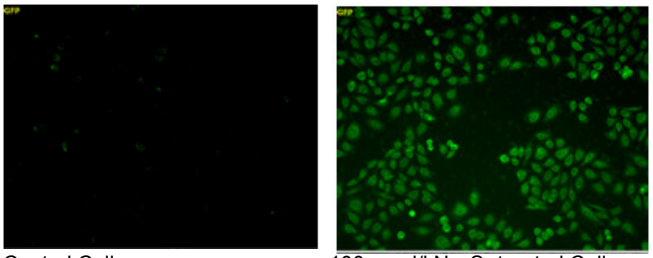
4) Discard the supernatant, wash the cells with PBS twice.

5) Add PBS to the cells, analyze the cells under a fluorescence microscope.

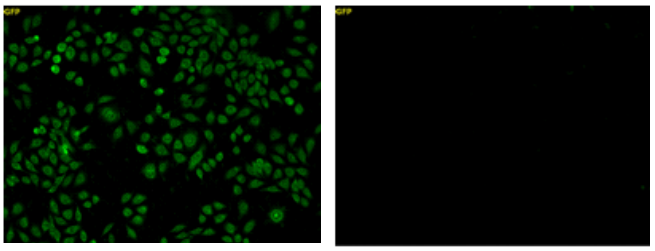
Experimental Example 1

- Detection of sulfane sulfurs in cells treated with a sulfane sulfur donor or sulfane sulfur quencher -

1) CHO cell suspensions prepared with serum-containing DMEM were inoculated in a 96-well black clear bottom plate to prepare 10^4 cells/well, and incubated in a humidified incubator (*e.g.*, at 37°C, 5% CO_2) overnight.
2) The culture medium was discarded, and the cells were washed with a serum-free DMEM.
3) 100 µl of a 100 µmol/l Na_2S_3 or 1 mmol/l NaCN (serum-free DMEM) was added to the each well, and the cells were incubated for 15 minutes in the incubator.
4) The supernatant was discarded, and the cells were washded with a serum-free DMEM twice.
6) 100 µl of a 20 µmol/l SSP4 Working Solution was added to the cells, and the cells were incubated for 15minutes in the incubator.
5) The supernatant was discarded, and the cells were washed with PBS twice.
6) 100 µl of PBS was added to the each wells, and the cells were analyzed under a fluorescence microscope.



Control Cells　100 µmol/l Na_2S_3 treated Cells　(Exporsue time: 1000 msec)
Fluorescence signals were observed in cells treated with 100 µmol/l Na_2S_3 (sulfane sulfur donor).



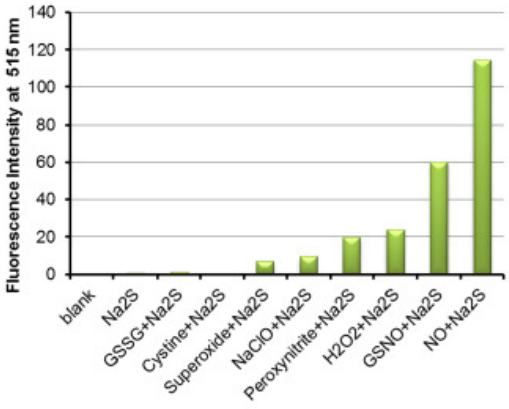
Control Cells　1 mmol/l NaCN treated Cells　(Exporsue time: 2000 msec)
No fluorescence signals were observed in cells treated with 1 mmol/l NaCN (sufane sulfur quencher).

Fig. 4　Fluorescence images of sulfane sulfurs in cells treated with Na_2S_3 or NaCN

Experimental Example 2

-Detection of sulfane sulfurs produced by reactions between hydrogen sulfide and reactive oxygen (or nitrogen) species -

1) 100 µmol/l Na_2S and 100 µmol/l reactive oxygen (or nitrogen) species were mixed in PBS, and incubated at room temperature for 10 minutes.
2) 10 mmol/l SSP4 Stock Solution was added to the reaction mixture (SSP4 final conc.:10 µmol/l) , and incubated at room temperature for 30 minutes.
3) The fluorescence intensities were measured at 515 nm (λ_{ex}=482 nm) with a fluorophotometer.



Chemical species containing sulfane sulfurs were produced by these reactions. Especially, the reaction between hydrogen sulfide (H_2S) and nitric oxide (NO) gave the highest generation efficiency of sulfane sulfurs.

References

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2) T. Ida, T. Sawa, H. Ihara. Y. Tsuchiya, Y. Watanabe, Y. Kumagai, M. Suematsu, H. Motohashi, S. Fujii, T. Matsunaga, M. Yamamoto, K. Ono, N. O. Devarie-Baez, M. Xian, J. M Fukuto, and T. Akaike, "Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling", *Proc. Natl. Acad. Sci. U S A.*, **2014**, *111*, 7606.
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