Si-DMA for Mitochondrial Singlet Oxygen Imaging

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/mt05.pdf

General Information

Singlet oxygen ($^1O_2$) is one of the Reactive Oxygen Species (ROS). $^1O_2$ is known to be a cause of spots and wrinkles of the skin due to its very strong oxidizing potential. In the field of cancer research, $^1O_2$ is of a particular importance because of its key role in photodynamic therapy (PDT), an emerging anticancer treatment using photoirradiation and photosensitizers. Therefore, the monitoring of $^1O_2$ in living cells is highly important for understanding of anti-cancer mechanism of PDT. However, the existing fluorescence probe for the detection of $^1O_2$ cannot be used in living cells because of its cell membrane impermeability.

Majima et. al. synthesized a new far-red fluorescence probe composed of silicon-containing rhodamine and anthracene moieties, namely Si-DMA, as a chromophore and a $^1O_2$ reactive site, respectively. In the presence of $^1O_2$, fluorescence of Si-DMA increases due to endoperoxide formation at the anthracene moiety.[1)] Among seven different ROS, Si-DMA is able to selectively detect $^1O_2$ (Fig. 3). In addition, Si-DMA is able to visualize the generation of $^1O_2$ from protoporphyrin IX in mitochondria with 5-aminolevulinic acid (5-ALA), a precursor of heme (Fig. 4).

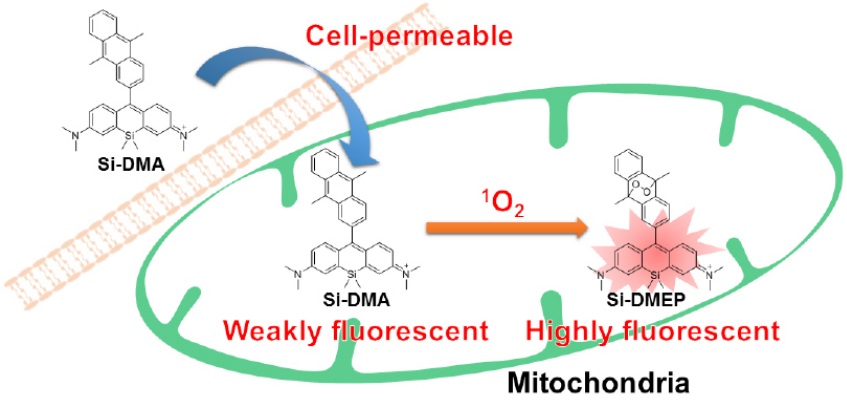

Fig.1 Cell staining mechanism by Si-DMA

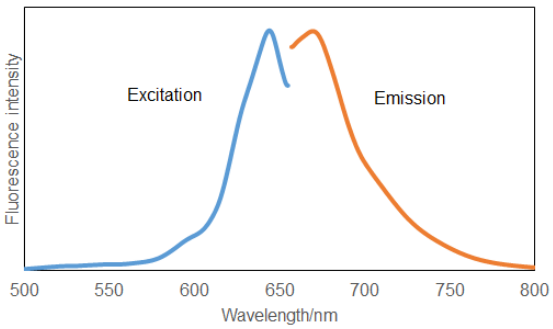

Fig.2 Excitation and Emission spectra of Si-DMA after reaction with $^1O_2$

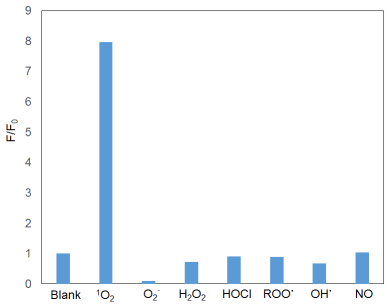

Fig.3 Selectivity of Si-DMA toward various ROS

Kit Contents

Si-DMA 2 µg x 1

Storage Condition

Store at -20°C and protect from light.

Caution:

Si-DMA is sensitive to light. Store unused Si-DMA in the bag at -20°C.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Hanks' HEPES buffer or HBSS
- Micropipettes

Preparation of Solutions

Preparation of 100 µmol/l Si-DMA DMSO stock solution

Add 36 µl of DMSO to a tube of Si-DMA (2 µg) and dissolve it with pipetting.

*Store the Si-DMA DMSO stock solution at -20°C.

Si-DMA DMSO stock solution is stable at -20°C for up to a month.

Preparation of Si-DMA working solution

Dilute the Si-DMA DMSO stock solution with Hanks' HEPES buffer to prepare 25-100 nmol/l Si-DMA working solution.

*Protect the solution from light and use it within the same day, since Si-DMA is not stable in Hanks' HEPES buffer.

Notes

The recommended concentration of Si-DMA working solution for use is 25-100 nmol/l. Once the final concentration of Si-DMA becomes more than 1 µmol/l, Si-DMA may be accumulated to organelle. In addition, the final concentration of Si-DMA becomes more than 5 µmol/l, cytotoxicity may be seen.

General Protocol

Si-DMA staining

1. Prepare cells for the assay.
2. Discard the culture medium and wash the cells with Hanks' HEPES buffer twice.
3. Add an appropriate volume of Si-DMA working solution.
4. Incubate for 45 minutes at 37°C.
5. Discard the supernatant and wash the cells with Hanks' HEPES buffer twice.
6. Add Hanks' HEPES buffer and observe the cells under a fluorescence microscope.

Usage Examples

Fluorescence microscopic detection of $^1O_2$ in HeLa cells treated with 5-aminolevulinic acid (5-ALA)

1. HeLa cells at 2.4×10^5 cells/ml (200 µl) were seeded on a µ-slide 8 well (Ibidi) in DMEM (10% fetal bovine serum, 1% penicilin-streptmycin) and cultured at in a 5% CO_2 incubator overnight at 37 °C.
2. The cells were washed with 200 µl of Hanks' HEPES buffer twice.
3. 5-ALA in Hanks' HEPES buffer (150 µg/ml, 200 µl) was added to the µ-slide, and the cells were cultured in a 5% CO_2 incubator for 4 hours at 37 °C.
4. The cells were washed with 200 µl of Hanks' HEPES buffer twice.
5. Si-DMA working solution (40 nmol/l, 200 µl) was added, and the cells were cultured in a 5% CO_2 incubator for 45 minutes at 37 °C.
6. The cells were washed with 200 µl of Hanks' HEPES buffer twice.
7. Hanks' HEPES buffer (200 µl) were added, and the cells were observed under a fluorescence microscope.

Filter (wavelength/band pass)
Fluorescence imaging: 600/50 nm (Ex), 685/50 nm (Em)

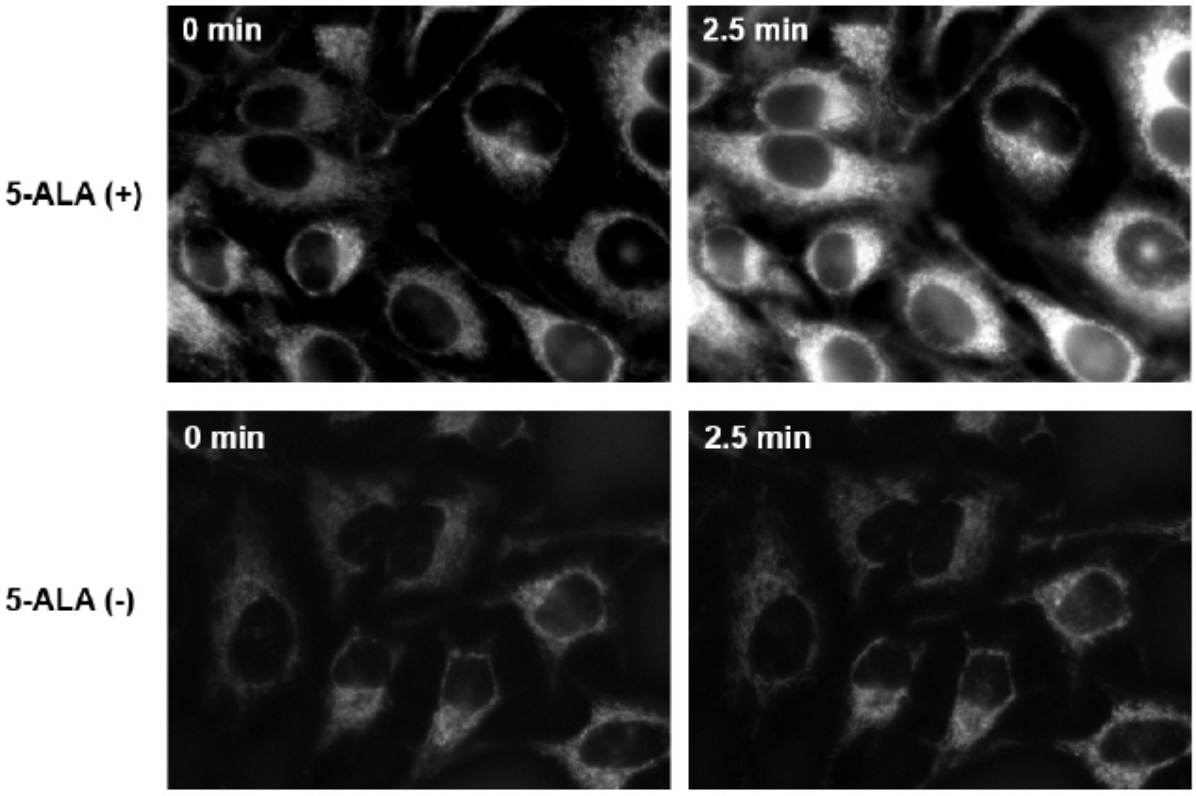

Fig.4 Fluorescence imaging of mitochondrial $^1O_2$ with Si-DMA in HeLa cells treated with 5-ALA.

Fluorescent of Si-DMA in 5-ALA-treated HeLa cells increased after 2.5 minutes irradiation. It was found that Si-DMA was able to visualize in real time the $^1O_2$ generated from protoporphyrin IX in mitochondria.

References

1) S. Kim, T. Tachikawa, M. Fujitsuka, T. Majima, "Far-Red Fluorescence Probe for Monitoring Singlet Oxygen during Photodynamic Therapy", *J. Am. Chem. Soc.*, **2014**, *136* (33), 11707-11715.

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