A novel SH-sensing fluorescent probe to monitor the redox status of intracellular compartments

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BACKGROUND

Sensing of intracellular redox state is important for detecting the effect of disease and therapeutic agents. However, currently reliable assays that can simultaneously provide information about the redox state of intracellular compartments is not available. Such an assay will improve the detection of abnormal metabolic state and evaluate the impact of therapeutics. Here we report the results of interaction of DSSQ1 (fluorescein-**D**onor tethered *via* a disulfide (S-S) to a para-methyl red **Q**uencher), a novel sulfhydryl (SH)-sensing fluorescence probe with cultured mammalian fibroblasts. Binding of DSSQ1 with free SH-groups in the cytoplasm and other intracellular compartments is accompanied with release of FRET inhibited fluorescein and generation of characteristic green fluorescence, thus allowing monitoring of both intracellular localization and the level of free DSSQ1-positive thiols.

OBJECTIVE

To study dose- and time-dependence of intracellular distribution of a novel SH-sensing fluorescent probe DSSQ1 (courtesy of Dr. Daniel Sem, Concordia University) in cultured NIH/3T3 mouse fibroblasts. Objective of this study is to identify the intracellular compartments accumulating DSSQ1, and verify SH-status sensing capacity of DSSQ1 in cultured mammalian fibroblasts.

STUDY DESIGN

- Cultured NIH/3T3 fibroblasts (passage 3) were plated on glass-bottom MatTek dishes (6,000 cells/cm^2) and incubated in O_2/CO_2 incubators for 24 hours.
- Cells were loaded with DSSQ1 (20 μM), nuclear fluorescent dye Hoechst (1 μg/ml) and TMRM (200 nM), mitochondrial membrane potential sensitive dye in HBSS for 30 min.
- Images of fluorescently labeled fibroblasts were acquired using FV1200 MPE Confocal System (Olympus, USA) and analyzed with Olympus FluoView Software.
- Different doses of DSSQ1 (0-20 μM) were loaded and time-dependent changes in the fluorescence of released FITC monitored.
- Statistical analysis was performed by Student *t*-test or analysis of variance using $P<0.05$ as the criterion of significance.
- Experiments were designed by EH, DS and AJ. Cell culturing, confocal imaging, image acquisition, post-imaging processing and formatting images and figures done by FY, ZG, UN and EH.

RESULTS

Principles of intracellular sensing of free SH-groups using DSSQ1. Two small molecules featuring characteristic electron ***Donor*** and electron ***Acceptor*** properties are dithio-tethered into a single molecule. One of the tethered molecules has fluorogenic properties and disulfide tethering of these molecules causes **FRET** (**F**luorescent **R**esonance **E**nergy **T**ransfer) or quenching effect. Upon cleavage of the disulfide bond, suppression of fluorescence is released and increased fluorescence could be used to monitor efficiency of SH-sensor(Fig. 1).

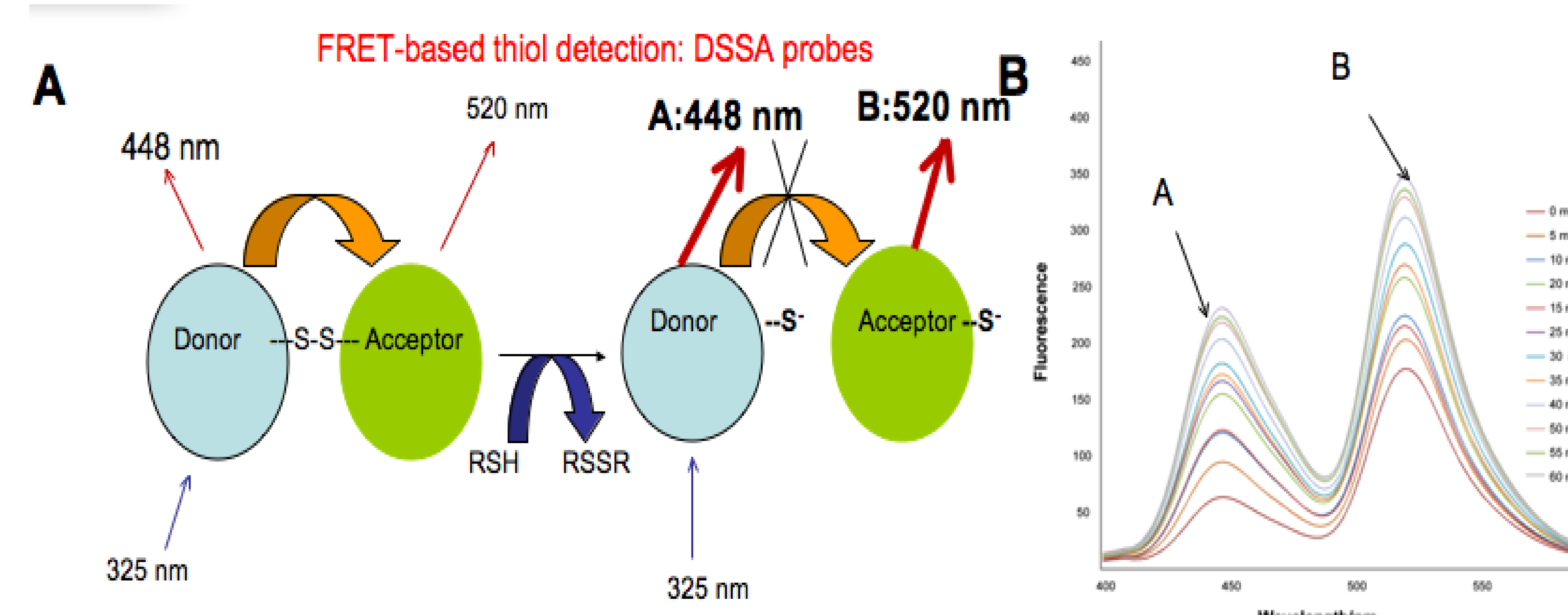

Figure 1. Diagram illustrating the principle of SH-sensing with DSSQ1. Cleavage of (–S-S-) bond in DSSQ1 releases FRET inhibited fluorescence (Green). Shown time-dependence of release of Green fluorescence.

Intracellular localization of DSSQ1. Under the normal conditions the green fluorescence of DSSQ1 was localized to the cytosol, lysosomes, nuclear membrane and within mitochondria of cultured NIH/3T3 fibroblasts (Fig. 2, ***Control***). Co-localization of DSSQ1 (***Green*** fluorescence) with mitochondria (TMRM, ***Red*** fluorescence) produces Orange fluorescence when these two dyes overlap in space (Fig. 2, ***Combined***).

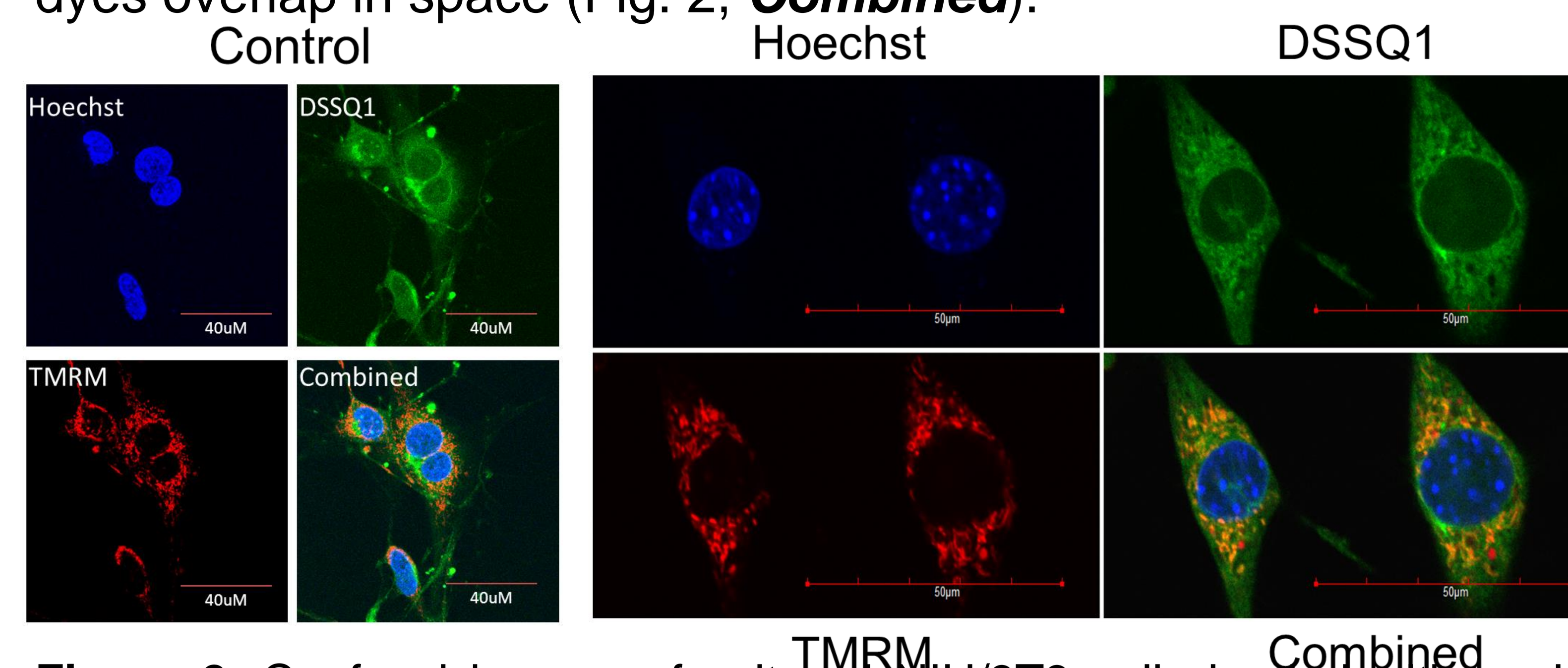

Figure 2. Confocal images of cultured NIH/3T3 cells loaded with nuclei sensitive dye Hoechst (***Blue*** fluorescence), mitochondrial TMRM (***Red*** fluorescence). Incubation of these cells with DSSQ1 generates ***Green*** fluorescence, characteristic of fluorescein conjugated SH-groups.

Effect of low and high redox status of incubation conditions on DSSQ1 uptake and SH-sensing. H_2O_2 in dose-dependent manner (0-100 μM) decreased uptake and processing of intracellular DSSQ1 (Fig. 3, ***left panel***). In contrast, in the presence of N-acetyl cysteine (NAC, 10 mM), reducing agent and precursor of intracellular reduced GSH, uptake and retention of DSSQ1 in the cytosol and intracellular mitochondria and lysosomes is increased (Fig. 3, ***right panel***).

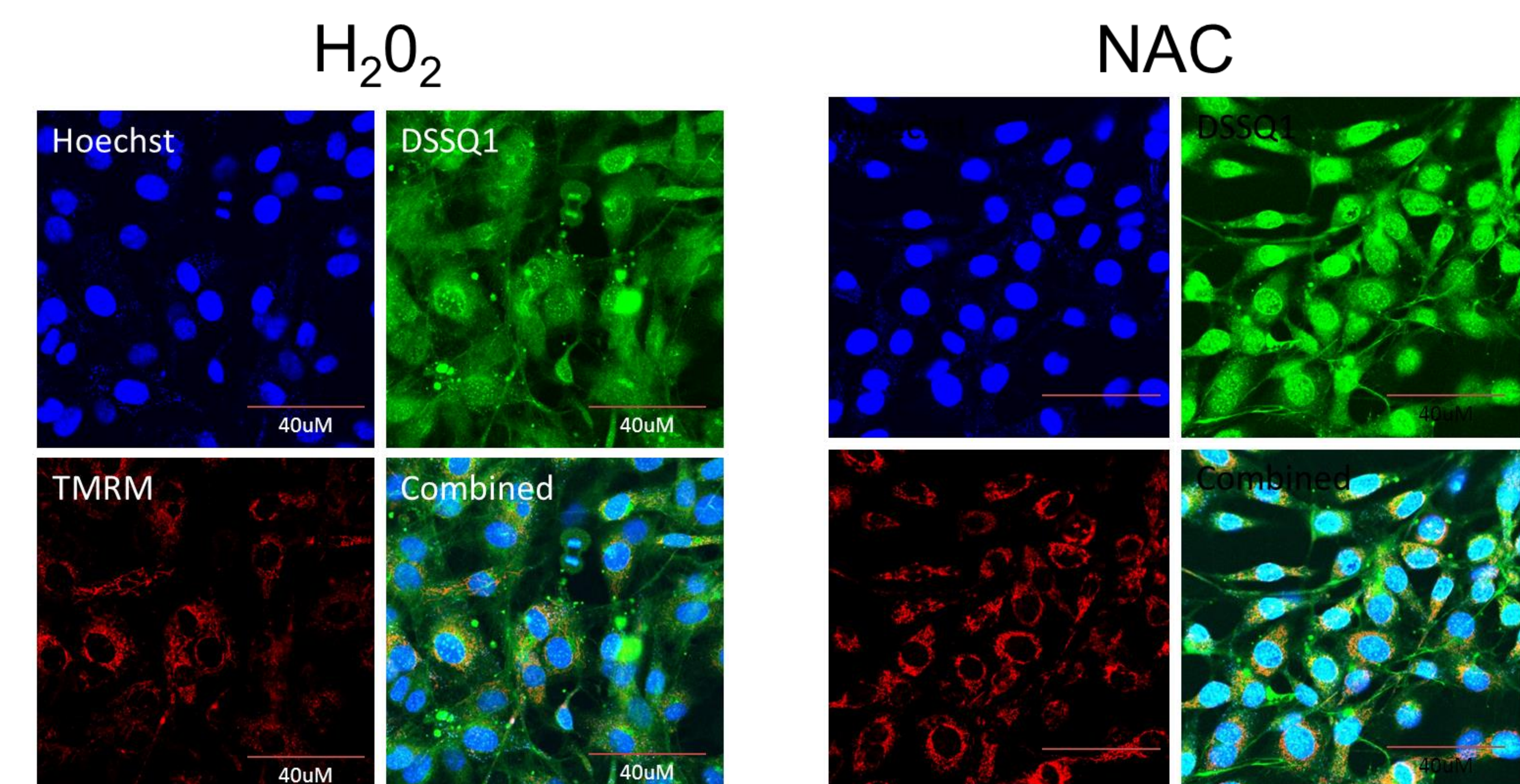

Figure 3. Confocal images of cultured NIH/3T3 cells loaded with nuclei sensitive dye Hoechst (***Blue*** fluorescence), mitochondrial TMRM (***Red*** fluorescence) in the presence of oxidizing H_2O_2 (100 μM, ***left panel***) or reducing NAC (10 mM, ***right panel***). Incubation with DSSQ1 generates ***Green*** fluorescence characteristic of fluorescein conjugated SH-groups.

CONCLUSION

- The chemical structure of the novel SH-sensing fluorescent dye (***DSSQ1***) is a cell-permeable compound which does not demonstrate detectable cytotoxicity in the range of 0-20 μM.
- The ***DSSQ1*** compound is distributed within the cytoplasm, and localizes to lysosomes, mitochondria and nuclear membrane, but is excluded from the nuclei.
- DSSQ1 accumulation and fluorscence is affected by redox status of the incubation medium and could be used to monitor the relative redox status of the cell.

REFERENCES

- The chemical structure of novel SH-sensing fluorescent dye (U.S. Patent No. 7,820,833).

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