Thiol Detection Using DSSQ-1 in iPSC-Derived Neuron Cultures

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Overview

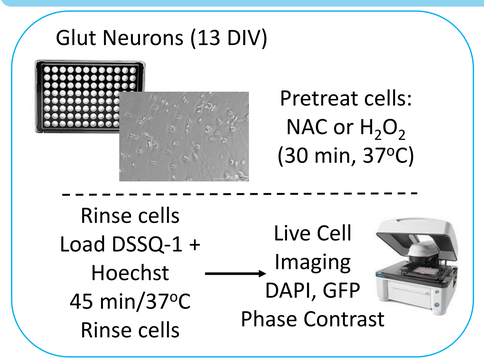
- Neurological disorders associated with inflammation and oxidative stress show reduced glutathione (GSH) levels in the human brain.
- iPSC-derived neurons (BrainXell, Inc) loaded with the thiol-detecting fluorescent probe DSSQ-1 (VIVID Microscopy) = Real-time monitoring of intracellular thiol levels.
- ↑ intracellular [thiol] = ↑ GFP fluorescence
- DSSQ-1 probes used with iPSC-derived cell cultures provides a viable option for high-throughput screening studies.

Introduction

Inflammation and oxidative stress have been implicated in many neurological disorders yet studying the mechanisms of disease can be difficult without the proper tools. Additionally, investigative research in humans has ethical considerations, so providing researchers an alternate model to study neurological function is necessary. Herein, we present an *in vitro* model using BrainXell iPSC-derived neurons, combined with VIVID Microscopy's novel fluorescent dye DSSQ-1, as a method to monitor intracellular thiol levels in real time. DSSQ-1 is a chemical probe, that when reacted with GSH thiol via thiol-disulfide exchange, causes the dye to fluoresce. In neurological diseases such as Parkinson's, amyotrophic lateral sclerosis, and Alzheimer's disease, brain imaging studies have shown that levels of the antioxidant glutathione (GSH) are decreased. This is an important observation since GSH thiol is directly involved in a cell's ability to combat oxidative stress. In healthy cells GSH (thiol) levels increase in their response to combat oxidative stress, but this response can be lacking in diseased brains.

In this study, we show that iPSC-derived cortical glutamatergic neurons loaded with DSSQ-1 can be used to monitor changes in intracellular thiol levels. Exposing glutamatergic neurons to the thiol-containing drug N-Acetyl-L-cysteine (NAC) resulted in a dose dependent increase in fluorescent signal with untreated neurons showing no fluorescence under baseline conditions. Cells preloaded with DSSQ-1 provides both visual and quantitative means to observe changes in a cell's oxidative stress response in a physiologically relevant range of GSH thiol (5-50 mM) as increased thiol concentrations correlates to increased dye fluorescence. The DSSQ-1 dye was easy to load into the neurons and provided a real-time method of observing changes in intracellular thiol levels using standard fluorescent microscopy hardware. DSSQ-1's ability to correlate to intracellular thiol levels makes it a great candidate for use in high-throughput screening of compound libraries to identify modulators of oxidative stress. With BrainXell's ability to create neurological cells from both healthy and custom gene-edited/disease lines, our neurons in conjunction with DSSQ-1 provides researchers a reliable, consistent method to use in translational research without the added variability and risk that comes with *ex vivo* and *in vivo* studies. Having screening tools like this is integral to furthering our understanding of neurological disorders and provides a reliable platform for studying disease mechanisms.

Methods: DSSQ-1 in iPSC-derived Neurons



Cortical glutamatergic neurons (13 DIV) were incubated with NAC or Hydrogen Peroxide (H_2O_2) at 0, 10, 20 or 40 mM in culture medium 100 µL/well; 30min/37°C. Cells were rinsed with HBSS/20 mM HEPES wash buffer; 2x 200uL. Hoechst 5 ug/mL and 25 uM DSSQ-1 was prepped in fresh wash buffer. Buffer was removed from cells and dye mix was added; 100 µL/well. Cells were incubated for 45 min/ 37°C. Wash completed with HBSS/20 mM HEPES buffer; 2x 200uL. Cells imaged using BioTek Lionheart FX imaging system using 20X Olympus objective with GFP, DAPI, and phase contrast filter cubes.

References

Aoyama, K. (2021). Glutathione in the Brain. *International Journal of Molecular Sciences*, *22*(9), 5010. https://doi.org/10.3390/ijms22095010

Pullela, P. K., Chiku, T., Carvan, M. J., 3rd, & Sem, D. S. (2006). Fluorescence-based detection of thiols in vitro and in vivo using dithiol probes. *Analytical biochemistry*, *352*(2), 265–273. https://doi.org/10.1016/j.ab.2006.01.047

Background Chemistry of DSSQ-1

DSSQ-1 probe chemical structure

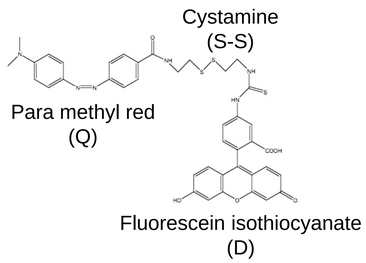


Figure 1. DSSQ-1 chemical structure. DSSQ synthesis involved tethering a fluorescein donor (D) to a para methyl red quencher (Q) using an aliphatic dithiol cystamine linker (S-S).

Thiol detection: DSSQ-1 mechanism of action

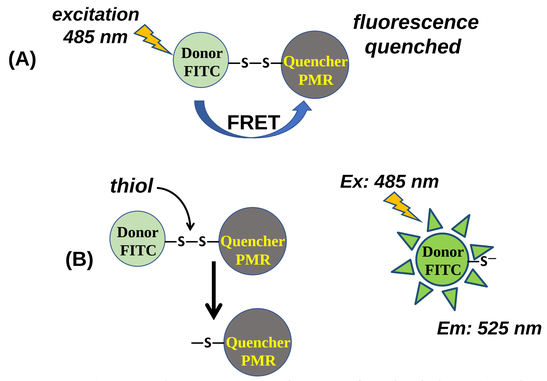


Figure 2. Proposed reaction mechanism for thiol detection by DSSQ-1. (A) Disulfide linker brings donor and quencher into close proximity allowing fluorescence resonance energy transfer (FRET), quenching fluorescence in low-reducing environments. **(B)** Reductive cleavage of the disulfide linker by thiols releases the quencher allowing higher probe fluorescence.

***In vitro* DSSQ-1 reactivity with GSH**

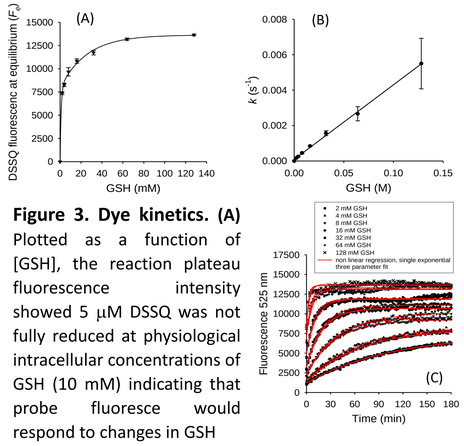


Figure 3. Dye kinetics. (A) Plotted as a function of [GSH], the reaction plateau fluorescence intensity showed 5 µM DSSQ was not fully reduced at physiological intracellular concentrations of GSH (10 mM) indicating that probe fluoresce would respond to changes in GSH levels in the physiologically relevant range of 5-50 nM, pH 7.0. **(B)** Reaction rate constants plotted as a function of [GSH] show k was pseudo first order in [GSH]. **(C)** DSSQ (5 µM) fluorescence intensity observed in the presence of GSH (2–128 mM). Reaction progress curves were fitted (red line) to $F = F_0 + (F_{eq} - F_0)(1 - e^{-kt})$ yielding rate constants k, and equilibrium fluorescence intensity values for each GSH concentration.

Results

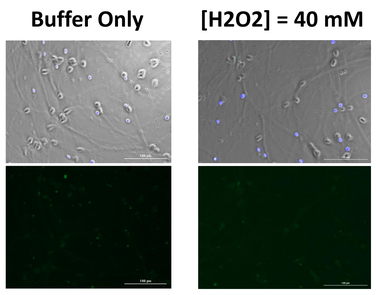


Figure 4. Lack of fluorescence in unchallenged neurons. Healthy cortical glutamatergic neurons were loaded with DSSQ-1 (25 uM) and Hoechst (5 ug/mL) – see methods. GFP fluorescence was not detected under basal conditions, which were monitored for 60 min after cells were loaded with dye. Additionally, GFP fluorescence was not detected in cells pre-exposed to the oxidizing agent hydrogen peroxide.

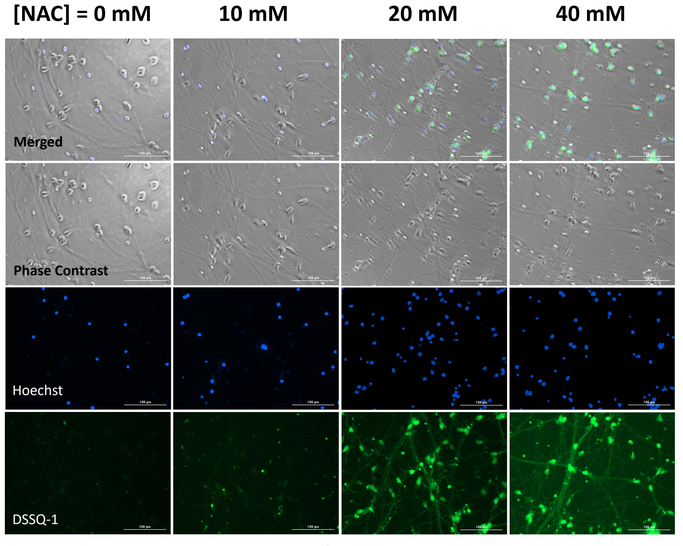


Figure 5

Figure 6

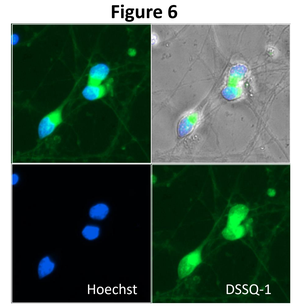


Figure 5. Dose-dependent change in fluorescence in response to thiol uptake. Cortical glutamatergic neurons show a dose dependent increase in DSSQ-1 fluorescence when exposed to increasing levels of N-Acetyl-L-cysteine (NAC) 0-40 mM.

Figure 6. DSSQ-1 localization. A strong GFP signal is seen predominantly in the cytosol and lesser fluorescence in the neurites. It also appeared surrounding the nucleus.

Conclusions

- DSSQ-1 can be successfully loaded into iPSC-derived neurons using standard live-cell imaging buffers at physiologic pH with no apparent signal in healthy neurons under baseline, unstimulated conditions.
- DSSQ-1 was detected in the cytosol and neurites of iPSC-derived neurons. Fluorescence was not detected inside the nucleus, consistent with other cell types evaluated in prior studies using DSSQ-1.
- DSSQ-1 shows a dose-dependent increase in GFP fluorescence in response to an increase in intracellular thiol concentration.
- iPSC-derived neurons (BrainXell, Inc) loaded with the intracellular fluorescent probe DSSQ-1 (VIVID Microscopy) provides an *in vitro* model for monitoring thiol levels and can be used to identify potential targets for therapeutic rescue related to oxidative stress.