Technical Brief

Outer Membrane Permeabilization *via* Perfringolysin O Treatment

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Perfringolysin O treatment provides a window of opportunity to investigate biochemical reactions in live cells with intact intracellular compartments, including mitochondria.

Introduction

Perfringolysin O creates 25-30 nm diameter pores in membranes, permitting the diffusion of large biomolecules or membrane-impermeable reagents across the outer membrane of live cells.[1] Modified perfringolysin O with a C459A mutation (PFO^{C459A}) has increased cholesterol content required for pore formation and greater selectivity for the outer membrane.[2] This treatment may be used in lieu of laborious sub-cellular compartment isolation procedures to instead interrogate biochemistry in whole cells. PFO^{C459A} treatment is useful in mitochondrial and metabolic studies for the evaluation of drug mechanism of action, metabolic shifts to various stimuli, comparison of healthy and disease states, or manufacturing effects on cell phenotype. Following PFO^{C459A} treatment, measurement of mitochondrial oxygen consumption can probe effects on cellular metabolism.

Methods

Titrate PFO^{C459A} (Item No. 42617) to optimize permeabilization prior to biochemical assays. 0.1-100 nM is a good titration range and treatments of 1-10 nM are ideal for most conditions, with the optimal amount varying by experimental cell type and density. Evaluate *via* flow cytometry to identify a dose that permeabilizes cells to the DNA intercalator DAPI, or in metabolic flux assays, shown here using Cayman's Extracellular Oxygen Consumption Assay Kit (Item No. 502880) and reagents in **Table 1**, to find the minimum dose that maximizes oxidative flux with addition of ADP and an ideal ETC substrate such as succinate.

Table 1 - Complex-specific substrates and inhibitors. The treatments listed show the substrates and upstream complex-specific inhibitors used to isolate and measure active complex turnover initiated from each complex through complex IV (measured oxygen consumption rate).

ETC Complex	Active State Reagents (+ ADP)	Inhibited State Reagents
Complex I	α-Ketoglutarate	Rotenone
Complex II	Succinate + Rotenone	Malonate
Complex III	Glycerol-3-phosphate + Rotenone + Malonate	Antimycin A
Complex V	Succinate	Oligomycin

Results

The maximum oxygen consumption rate was obtained for human hepatocyte model Huh7 cells in mitochondrial assay solution when permeabilized with 1 nM PFO^{C459A} (**Figure 1A**). With the optimal PFO^{C459A} treatment, isolated metabolic flux contributions from each complex were measured through oxygen consumption by complex IV (**Figure 1B**). The consumption of native complex-specific substrates, while inhibiting potential upstream contributions, allowed for measurement of complex-specific turnover in coupled mitochondria.

Conclusions

PFO^{C459A} demonstrates utility in experiments that require outer membrane permeability, especially for interrogation of function for intracellular compartments in their native cellular environment.

References

1. Verherstraeten, S., Goossens, E., Valgaeren, B., *et al.* Perfringolysin O: The underrated *Clostridium perfringens* toxin? *Toxins (Basel)* **7(5)**, 1702-1721 (2015).

2. Johnson, B.B., Moe, P.C., Wang, D., *et al.* Modifications in perfringolysin O domain 4 alter the cholesterol concentration threshold required for binding. *Biochemistry* **51(16)**, 3373-3382 (2012).

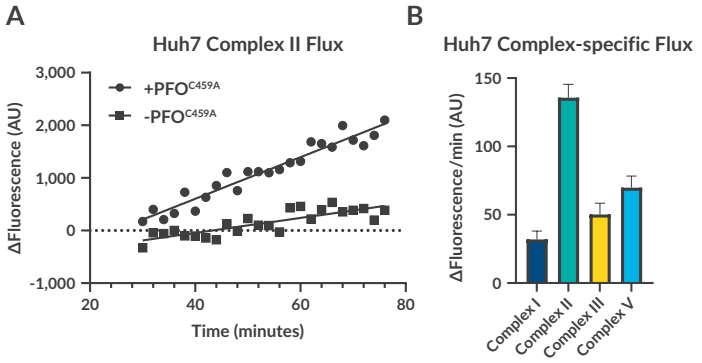

Figure 1 - Complex-specific metabolic flux in human hepatocyte model Huh7 cells. A) Huh7 cells incubated with PFO^{C459A} and complex II substrates show increased oxygen consumption relative to unpermeabilized cells. **B)** Differences in relative oxygen consumption rates were measured for PFO^{C459A}-treated Huh7 cells actively consuming native complex-specific substrates *versus* inhibited controls.