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Characterizing the roGFP2-Orp1 Fluorescent Biosensor for Detecting Oxidative Stress in Mammalian Cells

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Parkinson's disease is a neurodegenerative disease involving the death of neurons in the substantia nigra and loss of the neurotransmitter, dopamine. The disease leads to progressive loss of motor control. Exact causes and mechanisms by which Parkinson's disease proceeds are unknown, however, previous experiments determine oxidative stress in mitochondria as a factor that results in cell death. Strategies have been implemented to generate fluorescent biosensors to monitor reactive oxygen species (ROS) concentrations while simultaneously measuring the spatiotemporal distribution and correlation between the ROS, cellular function and organelle. Orp1, an enzyme found in yeast, is a sensitive oxidizing species and when coupled with fluorescent protein, roGFP2, the pair acts as a fluorescent biosensor for the ROS, hydrogen peroxide. In this study, roGFP2-Orp1 protein was expressed and purified from bacterial cell cultures and hydrogen peroxide oxidation assays were conducted to compare performance against characteristics reported in the literature. roGFP2-Orp1 is a fluorescence excitation ratiometric probe and the biosensor signal is obtained by the ratio of fluorescent intensities measured with 390 nm and 480 nm excitation. Sigmoidal kinetics were observed for biosensor oxidation by hydrogen peroxide. We also observed the RoGFP2-Orp1 is highly susceptible to air oxidation. Finally the mitochondrial targeting mito-roGFP2-Orp1 gene was subcloned into a GW1 plasmid vector for mammalian expression. Future work will entail transfection of mitochondrially-targeted roGFP2-Orp1 into cultured mouse midbrain neurons to enable live-cell imaging of mitochondrial oxidative stress in cellular models of Parkinson's disease.

KEYWORDS

Parkinson's, roGFP2-Orp1, fluorescent, biosensor, oxidative, stress, hydrogen, peroxide