# Annotated Bibliography: Hydrogen Peroxide And Superoxide Production

## 1. Tabatabaie, T., Potts, J.D., & Floyd, R.A. (1996). Reactive oxygen species-mediated inactivation of pyruvate dehydrogenase. Archives of biochemistry and biophysics, 336 2, 290-6 .

Link: https://reader.elsevier.com/reader/sd/pii/S0003986196905603?token=B3D1700E8648443D02D6E292FBAA78A4F81E06FAA0F7D8BA0FDC8B4DF7F1919DCED1F9C4EC6478EFC4140962074C94C2

The purpose of this study was to monitor the inhibitory effects of reactive oxygen species (ROS) on pyruvate dehydrogenase complex (PDHc) and how it could be related to health concerns. PDHc and lactate dehydrogenase assays were both completed respectively for comparison using a spectrophotometer to monitor NADH production as a measure of enzymatic activity. It was found that protease inhibitors treated with XO/HX inactivated PDHc but lactate dehydrogenase was found to be resistant to inactivation. The data found in this study points towards the involvement of superoxide radicals in ischemia/reperfusion insult that causes conditions such as ischemic brain damage. This connects to my project because this article shows that the presence of NAD+ and O2 have direct relationships with the production of hydrogen peroxide and superoxides which in turn will deactivate dihydrolipoamide dehydrogenase (a subunit of PDHc) which we are wanting to observe. Furthermore, this study demonstrates the specificity of PDHc compared to other enzymes such as lactate dehydrogenase in the capacity for oxidative inactivation and damage due to superoxide presence.

## 2. Yan, L. J., Sumien, N., Thangthaeng, N., & Forster, M. J. (2013). Reversible inactivation of dihydrolipoamide dehydrogenase by mitochondrial hydrogen peroxide. Free radical research, 47(2), 123–133. doi:10.3109/10715762.2012.752078

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3690130/

This study focuses on the monitoring of reduction and oxidation reactions of dihydrolipoamide dehydrogenase (E3) caused by hydrogen peroxide and reactive oxygen species (ROS). The purpose of the study was to clarify the mechanism of E3's oxidative inactivation by ROS and whether it is reversible. Plate reader assays and fluorescence probing were completed to better understand the chemical changes of these reactions. Absorbance changes for E3 catalyzation through NAD+ dependent oxidation of dihydrolipoamide was monitored as well. Mass spectroscopy of cysteine oxidative modification of E3 was performed and it was found that antimycin A concentration had a positive correlation to the presence of superoxides and thus inhibited enzyme activity. Inactivation of the enzyme was shown to be reversible by cysteine and GSH presence. Due to only complex-III ROS and hydrogen peroxide being able to inhibit E3 activity it seems there is a correlation between the two biomolecules. This study relates to

my proposed project because both it and my project focus on observation and data collection relative to the oxidative inhibition of E3 by hydrogen peroxide.

## 3. Eyassu, F., & Angione, C. (2017). Modelling pyruvate dehydrogenase under hypoxia and its role in cancer metabolism. Royal Society open science, 4(10), 170360. doi:10.1098/rsos.170360

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5666243/

The purpose of this study is to construct a model of pyruvate dehydrogenase complex (PDHc) that could be used for hypoxia-based simulations relative to cancer cell metabolism. Flux balance analysis (FBA) integrated with mitochondrial matrix reactions was the primary technique of constructing this model. Hypoxic simulations were completed with a multitude of variables. Through these simulations a large-scale metabolic model of PDHc was constructed. This model is significant because it can be integrated into other PDHc models in order to assist in the compilation of a complete structure of the enzyme complex. It was found that flux output of PDHc decreased proportionally to decreasing oxygen presence. These hypoxic conditions lead to pyruvate dehydrogenase kinase 1 activation which in turn leads to the production of reactive oxygen species. This study relates to my proposed project because the data collected during the hypoxia simulations could be used as a comparison between high NAD+ concentration and lack of oxygen as both lead to inactivation. Furthermore, the data that is present within the constructed model from this paper could be used to integrate more information into the development of our own model or experimentation of dihydrolipoamide dehydrogenase.

## 4. Kareyeva, A. V., Grivennikova, V. G., Cecchini, G., & Vinogradov, A. D. (2011). Molecular identification of the enzyme responsible for the mitochondrial NADH-supported ammonium-dependent hydrogen peroxide production. FEBS letters, 585(2), 385–389. doi:10.1016/j.febslet.2010.12.019

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3022077/

The purpose of this study was to determine the enzyme responsible to produce both hydrogen peroxide and superoxides. Complex I catalyzed superoxide production sees a peak in activity when NADH is low and is strongly inhibited by the presence of NAD+, both of which are present at the E3 site of pyruvate dehydrogenase complex (PDHc). Hydrogen peroxide formation was monitored at a wavelength of 572nm and superoxide production was monitored as well by following a SOD-sensitive cytochrome c reduction at 550nm. Through comparisons of collected data, comparative clone testing, and protein fingerprint information collected from the NCBI database, it was obvious that the protein was dihydrolipoyl dehydrogenase. This article relates to my study because it delves into the direct correlation between the production and presence of superoxides and hydrogen peroxide and their inhibitory effects on E3 activity. Furthermore, as we are also going to be using the cytochrome c method to monitor the activity of superoxides, the data presented in this article could be used for a comparison to see if our data is consistent

with literature.