Metal-dependence of 2,4'-dihydroxyacetophenone dioxygenase (DAD)

Austin Herbert, Biology
Dr. Kenneth Roberts, Department of Chemistry and Physics

Research Question: This study seeks to better understand the mechanism of **2,4'-dihydroxyacetophenone dioxygenase (DAD)** by investigating the resting oxidation-state of the iron in the active site in DAD. We hypothesize that the active-site metal of DAD is Fe^{3+} because Fe^{2+} is not stable in the presence of oxygen.

Project Description:

Mechanisms for C-C bond cleavage require strict conditions in synthetic methods. However, biological systems readily employ a plethora of mechanisms to efficiently and effectively facilitate the degradation of chained and aromatic hydrocarbons.[1-7] Unfortunately, many mechanisms of enzymes are inadequately studied. Dioxygenases are a class of dioxygen reducing enzymes which readily activate both oxygen atoms for incorporation into the substrate.[8] **2,4'-dihydroxyacetophenone dioxygenase (DAD)** from betaproteobacterium *Burkholderia* sp. AZ11 is a dioxygenase responsible for the cleavage of 2,4'-dihydroxyacetophenone (DHAP) into 4-hydroxybenzoate and formate.[9] X-ray crystallography shed some light on the metal-containing active-site of DAD, which appears to be used in the C-C bond cleavage.[10-12] Based off of the dark-gray coloration of purified and precipitated DAD protein, prior studies have concluded that the metal in the active site is iron, however, this is based solely on qualitative information.[10] As the DAD mechanism is poorly understood, this study hopes to characterize the mechanism of C-C cleavage done by DAD. To evaluate the metal-dependence of the active-site we will remove and replace the active-site of DAD with comparable metals (examples below). The reconstituted enzyme will be tested for catalytic activity using UV absorbance assays for the oxidation of DHAP. To execute this experiment, the native metal ion will be removed with sodium dithionate and EDTA to yield an apoenzyme (apoDAD), which can be assumedly reconstituted with other metal ions such as Fe^{2+}, Fe^{3+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}. We expect to observe 100% catalytic activity from DAD in the Fe^{3+} state because Fe^{2+} is not stable in the presence of oxygen, oxidizing to Fe^{3+}, and the enzyme does not require reduction to work. Alternatively, the reaction may still occur under aerobic conditions with Fe^{2+} if it's being reduced to Fe^{3+} by the substrate as seen in the aromatic amino acid hydroxylases.[13] Other metals besides iron may facilitate the reaction either as Lewis acids, e.g. Zn^{2+}, Ca^{2+}, and Mg^{2+}, or by performing one-electron switching, e.g. $Cr^{2+/3+}$ or $Cu^{1+/2+}$. Electron paramagnetic resonance (EPR) spectroscopy will be used to further characterize the resting state metal-center in DHAP-bound DAD as well as unbound DAD. Additionally, stopped-flow assays will be used to observe any activity from the Fe^{2+} enzyme, as well as allow us to develop baseline kinetics. Determining the identity of the catalytic metal in DAD will provide insights into the mechanisms involved, i.e. Lewis acid reaction or one-electron chemistry.

Project Outcomes:

Knowing the identity of the metal-center of DAD will facilitate the understanding of the chemical reactions catalyzed by DAD. Also, knowing the metal-center of the DAD active-site will help us experiment and determine the baseline kinetics, and whether or not other metals would work as replacements. The results of these experiments will be submitted as part of a larger study for peer-reviewed publication in ACS *Biochemistry* towards the end of 2019. This project will be my first work as a college graduate and it will challenge me to apply learned knowledge such as: quantitation and methodology of enzyme kinetics and protein characterization. This study will add great value to my future applications to graduate school by continuing my involvement in professional research in biochemistry, and will help me build my network as a young professional by collaborating with biochemists at USC-Columbia.

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CURRENT AND PENDING SUPPORT for Dr. Kenneth M. Roberts

1) RISE 2018 – USC Office of the VPR – 2,4'-Dihydroxyacetophenone Dioxygenase (DAD): Metal-Dependence and Steady-State Kinetics – May –Dec 2019 - $6000