Verification of the K_{cat} Value for AAT via COPASI

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Introduction

Although light activated enzymes such as DNA photolayse do exist, the vast majority of enzymes do not require the absorption of light for catalytic activity. In some cases, however, light can initiate biological activity such as the CO dissociation which can be initiated in hemoglobin and myoglobin by blue light absorption. This brings up the question of whether light excitation can generally affect the catalytic activity of chromophoric enzymes by accelerating cofactor-dependent rate-limiting steps.

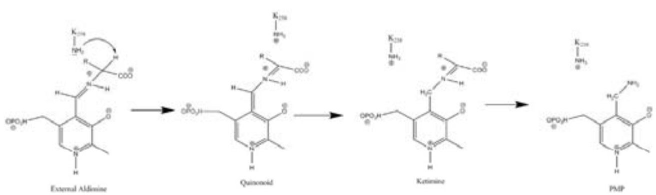
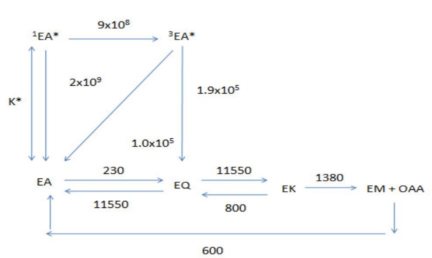


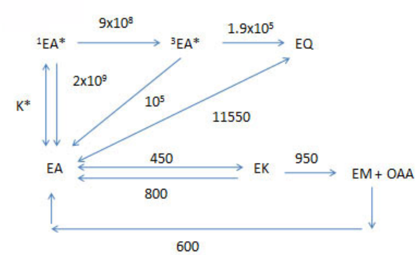
Figure 1: Mechanism of the Transamination Half-Reaction

Pyridoxal 5'-phosphate (PLP; Vitamin B6) and Aspartate Aminotransferase (AAT)

PLP is a chromophoric cofactor required for catalytic activity by a wide variety of enzymes. While PLP enzymes are thermally activated *in vivo*, it has been reported that some PLP enzymes can be activated by UV light. Previous studies with apsartate aminotransferase (AAT) suggest that the carbanionic quinonoid intermediate is photogenerated by UV laser excitation. AAT is central to nitrogen metabolism in all living systems and has a large body of literature. As such, it is a useful prototype for fundamental studies on this class of enzymes, and was used by Melissa Hill in her paper "Light-Enhanced Catalysis by Pyridoxal Phosphate-Dependent Aspartate Aminotransferase" *J. Am. Chem. Soc.*, 2010, 132 (47), pp 16953–16961



Model 1: The quinonoid is on the productive pathway.

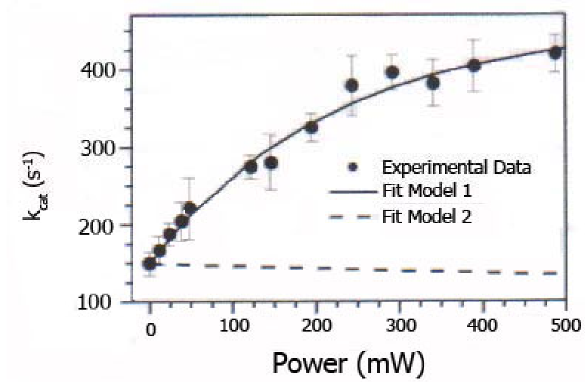


Model 2: The quinonoid is off of the productive pathway

Verification of Experimental K_{cat} values via COPASI

In order to verify Melissa Hill's experimental results, the computer program COPASI will be used. COPASI stands for COmplex PAthway SImulator and is an open source application for solving mathematical models of biological processes. It is based on the GEPASI simulation software that was developed in the early 1990s by Pedro Mendes. COPASI is the result of an international collaboration between the University of Manchester (UK), the University of Heidelberg (Germany), and the Virginia Bioinformatics Institute(USA). Current development efforts are supported by a grant from the National Institute of Health and the German Ministry of Education.

The two models of AAT mechanisms where turned into COPASI files by identifying the species and reactions of the mechanism as well as defining the container in which the reactions would take place. Each reaction was given a rate law using a mass action model which was either reversible or irreversible depending on the reaction.



Graph 1: Power Dependence of the AAT Catalytic Activity

Conclusions

Unfortunately, the complexity of COPASI and the lack of any experienced users prevented the successful verification of the experimental data within the time allotted to this internship. Verification of the K_{cat} value is still ongoing and will be completed in the near future.

This material is based upon work supported by the S.D. Bechtel, Jr. Foundation and by the National Science Foundation under Grant No. 0952013. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the S.D. Bechtel, Jr. Foundation or the National Science Foundation.