Exploring Novel Reactivity for Engineered Myoglobin Biocatalyst: [2,3] Sigmatropic Rearrangement of Allylic Acetals

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Abstract

Biocatalysis is a green, sustainable process in which enzymes, whole-cells or other biological catalysts perform a required synthetic transformation in a highly efficient manner. Recently, the heme protein myoglobin (Mb) has been emerged as a robust biocatalyst. The engineered versions of Mb biocatalyst have shown to perform non-native chemical transformations such as cyclopropanation of activated and unactivated alkenes, hetero-atom insertion (S-H, N-H) reactions etc. in very high yields and in excellent selectivity. Here, we explore the reactivity of engineered Mb variants toward the [2,3] sigmatropic rearrangement of allylic acetals and thioketals to provide a simple, environmentally friendly and sustainable route to synthetically useful multifunctional organic compounds. With the above goal in mind, a feasibility study was performed using acrolein dimethyl acetal and ethyl diazoacetate (EDA) in presence of engineered Mb catalyst, Mb H64V V68A. Along with preforming these reactions under anaerobic, semi-aerobic, and aerobic conditions, this study incorporates additionally diverse experimental and controlled variables such as concentrations of the substrate and enzyme, temperature, pH, time trials, and substrate ratios. Our hope is to explore the prime conditions for this engineered myoglobin biocatalyst and determine conditions that this enzyme is able to function optimally in. Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS) were used to quantify the biocatalysis reaction yields, percent conversions, and reaction rates. Nuclear magnetic resonance spectroscopy (NMR) was used to characterize the standard products and the products obtained from enzyme reactions.

Introduction

Biocatalysis are living organism's way of speeding up internal reactions they have with organic compounds. Biocatalysis is appealing in that it is economical and environmentally green, and it is widely used drug in industries such as pharmaceuticals.[1] An example of biocatalysis are digestive enzymes like pepsin.

Myoglobin carries and stores oxygen in the muscle tissue in all mammals. There are two types of myoglobin in this experiment mutant and wild type to see if we can do the same reaction with the different types.

Ethyl diazoacetate is a diazo compound and is used as a reagent in organic chemistry and it is important component in these reactions.

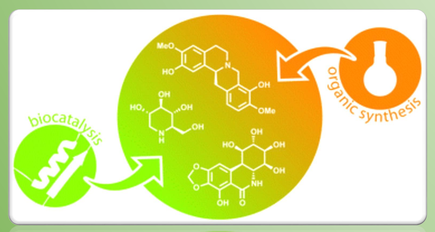


Image from: https://pubs.rsc.org/en/content/articlelanding/2013/ra/c3ra42123f#!divAbstract

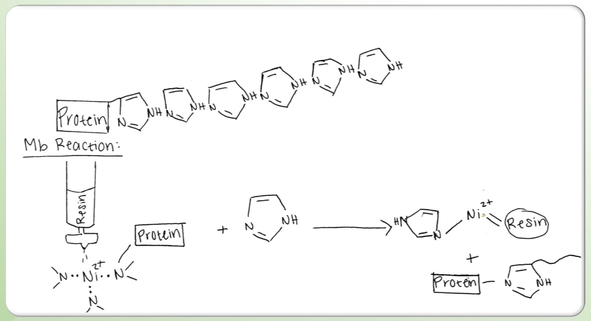
Methods

Reaction conditions:

- **400 µL reaction**
- **2:1 ratio of 400 mM Allylic Alcohol and 200 mM Ethyl Diazoacetate (EDA)**
- **2:1 ratio of 400 mM Allylic Bromide and 200 mM Ethyl Diazoacetate (EDA)**
- **2:1 ratio of 400 mM Acrolein Dimethyl Acetal and 200 mM Ethyl Diazoacetate (EDA)**
- **2:1 ratio of 400 mM Ethyl Acrylate and 200 mM Ethyl Diazoacetate (EDA)**
- **2:1 ratio of 400 mM N,N-Dimethylallylamine and 200 mM Ethyl Diazoacetate (EDA)**
- **92 mM concentration of enzyme**
- **20 µL of Internal standard (1,3-benzodioxole)**
- **Dichloromethane solvent used for extraction**
- **10 mM Sodium dithionite reductant**
- **Reactions were performed in anaerobic conditions**

General reaction procdure:

- **Mutant Mb enzyme was added to a crimp glass vial and capped**
- **Sodium dithionite and Kpi (pH 7) buffer was placed in a separate degassing vial**
- **Solutions were degassed with pure Argon for 2 minutes before cannulating the reductant into the enzyme solution**
- **Various volumes (50 µL, 20 µL, and 2-10 µL) of 200 mM EDA and 400mM of Allylic Alcohol, Allylic Bromide, Acrolein Dimethyl Acetal, Ethyl Acrylate, or N,N-Dimethylallylamine were added to reaction mixture**
- **Vial was then placed on a stirring plate overnight to allow for reaction to occur**
- **For work up: 20 µL Internal standard was added to reaction mixture which was extracted with 400 µL of dichloromethane and placed in a micro centrifuge vile to be spun down before retrieving the organic material**
- **Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS) were used to quantify yields.**



Data and Results

Table 1: Gas chromatography results for 2:1(50 µL Allylic Alcohol: 50 µL EDA) reaction performed overnight

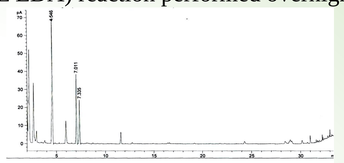


Table 2: Gas chromatography results for 2:1 (20 µL Allylic Alcohol: 20 µL EDA) reaction performed overnight

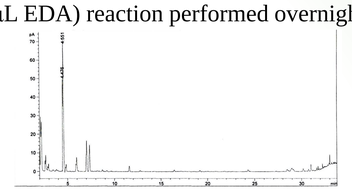


Table 3: Gas chromatography results for 2:1 (10 µL Allylic Alcohol: 10 µL EDA) reaction performed overnight

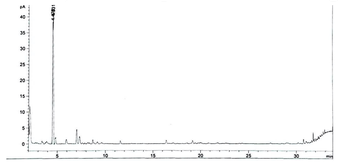


Table 4: Gas chromatography results for 2:1 (50 µL Allylic Bromide: 50 µL EDA) reaction performed overnight

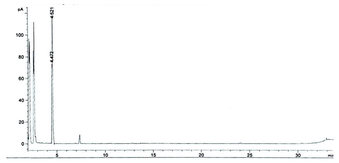
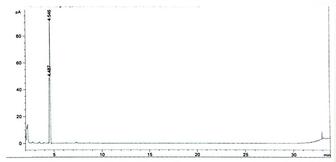


Table 5: Gas chromatography results for 2:1 (10 µL Allylic Bromide: 10 µL EDA) reaction performed overnight



12 other samples were tested using Gas chromatography but proved to be inconclusive as they did not show a separation of components within the reaction mixtures. These samples consisted of 400 mM Acrolein:200 mM Ethyl Diazoacetate (EDA), 400 mM Ethyl Acrylate:200 mM Ethyl Diazoacetate (EDA), and 400 mM N,N-Dimethylallylamine:200 mM Ethyl Diazoacetate (EDA). Each of the previously stated 400 µL reactions were tested using the same 2:1 ratios with volumes of 50 µL, 20 µL, and 2-10 µL. Reactions with Allylic Iodide will also be tested in the future under the same conditions expressed previously.

Conclusion

Gas chromatography (GC) was preformed on all 20 tested samples. The next step would be to preform Gas chromatography-Mass spectrometry (GC-MS) on those samples that showed clear separation of components to quantify analytes. Moving forward, the synthesis of iodonium ylide from dimethyl malonate will be preformed to hopefully produce larger scaled, higher isolated yields from simpler, greener filtration from lower amounts of engineered Mb biocatalyst.[2]

Mb structure from: https://www.researchgate.net/figure/Structure-of-myoglobin-X-ray-diffraction-structure-of-protein-crystals-from-sperm-whales_fig1_259873579

References

(1) Truppo, M.D. Biocatalysis in the Pharmaceutical Industry: The Need for Speed. *ACS Med Chem Lett.* 2017, 8(5), 476-480.
(2) Goudreau, S.R.; Marcoux, D.; Charette A.B. Synthesis of dimethyl 2-phenylcyclopropane-1,1-dicarboxylate using an iodonium ylide derived from dimethyl malonate. *Organic Syntheses.* 2010, 87, 115-125.

Gas chromatography was performed using an Agilent 7890B GC system and a RESTEK Rt®- bDEXse chiral column.