Introducing Biocatalysis in an Undergraduate Teaching Laboratory Using A Cyclopropanation Reaction

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Abstract

Biocatalysis is the use of enzymes and proteins to perform chemical transformations. Enzymes and proteins are increasingly used in organic reactions due to excellent chemo-, regio- and stereo-selectivity, environmental sustainability, milder reaction conditions, improved productivity, simplified work-streams and greater economical saving potential. In recent years, there have been a number of studies reported the use of heme containing proteins and enzymes in catalyzing non-natural C-C insertion reactions.[3] The purpose of this project is to design a biocatalysis experiment that we can be incorporated into an undergraduate organic chemistry teaching laboratory.

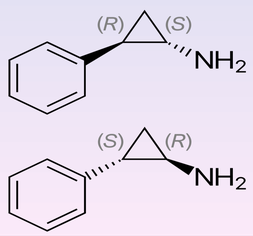
In this study, commercially available wild-type bovine hemoglobin was used as as a non-native biocatalyst to perform a cyclopropanation reaction with commercially available styrene and ethyl diazoacetate as substrates. In order to identify the best reaction conditions, we optimized various conditions such as enzyme concentration, substrate concentration, substrate ratio, pH, and temperature. The catalytic activity of the hemoglobin, percentage conversions, and stereo selectivity of the reaction were determined by chiral gas chromatography and the results were standardized with a calibration curve. Optimized reaction conditions will be tested with a group of 95 undergraduate students. This experiment will be an inexpensive and sustainable way to introduce biocatalysis to an undergraduate teaching laboratory.

Introduction

Biocatalysis afford chemists exquisite control over the outcome of chemical reactions and gives highly favorable products.[1] Biocatalysis is becoming very popular in organic chemistry due to its environment friendly nature and high yields of desired single enantiomeric products.

Metalloporphyrins and diazo compounds can be used to generate reactive metal carbenoids, which is an excellent intermediate for introducing cyclopropane moieties in organic compounds.[1]

Biocatalysis is increasingly being used in the pharmaceutical industry to generate higher yield of an active single enantiomer of a drug candidate or an existing drug. One example is the anti-depressant drug Tranylcypromine, also known as Parnate. The two enantiomers of Tranylcypromine have been used pharmaceutically as a racemic mixture, but the two enantiomers display different biological potencies. To obtain the more desirable enantiomer, a reaction with E.coli cells expressing Mb(H64,V68A) in the presence of styrene and EDA was performed to incorporate the desired stereochemistry and ultimately resulting in one desirable product (99.9% ee and 99.9% de)[2]



Methods

Reaction conditions:

- **400 µL total reaction volume**
- **2:1 ratio of 200 mM Ethyl Diazoacetate (EDA) and 100 mM Styrene**
- **60 µM concentration of enzyme in reaction**
- **20 µL of Internal standard (1,3-benzodioxole)**
- **Dichloromethane solvent used for extraction**
- **10 mM Sodium dithionite reductant (if used)**
- **Reactions were performed in aerobic*, semi-aerobic* and anaerobic conditions**

General reaction procdure:

- **Enzyme was added to a crimp glass vial and capped**
- **Reductant and Kpi (pH 7) buffer was placed in a separate degassing vial**
- **Both solutions were degassed using pure Argon and then reductant was cannulated to the enzyme solution**
- **100 µL each of EDA (0.8 M) and Styrene (0.4 M) were added to the reaction mixture**
- **Vial was then placed on a stirring plate to allow for reaction to occur**
- **For work up: 20 µL Internal standard was added to reaction mixture**
- **Extracted with 400 µL of dichloromethane**
- **Organic extract was analyzed using chiral gas chromatography#**

×**Reactions performed under semi-aerobic conditions were not degassed, and reactions done under aerobic conditions were not degassed or capped.**

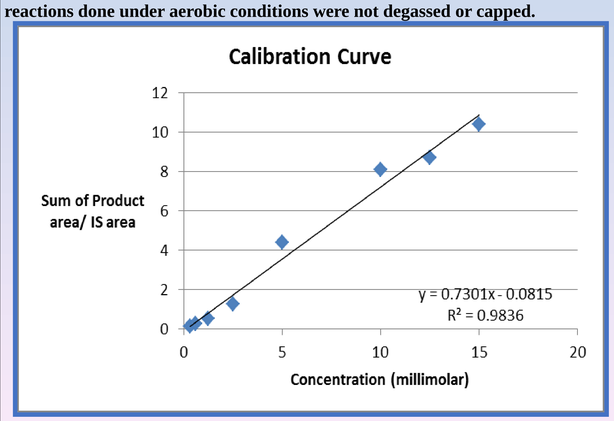
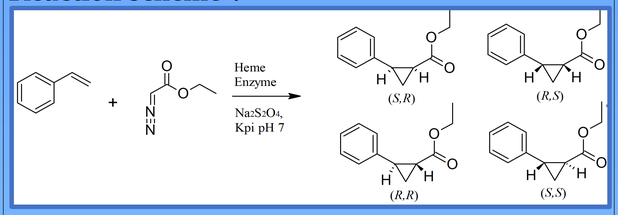


Figure 1:Calibration Curve

Data and Results

Reaction scheme[4]:



Conditions	TTN	%ee (cis)	%ee (trans)	%de
Anaerobic	163	0.66%	3.7%	83.9%
Semi-aerobic	5	10%	6.5%	81.7
Aerobic	6	0%	5.3%	78.7

Table 1: Results for reactions performed overnight

Conditions	TTN	%ee (cis)	%ee (trans)	%de
Semi-aerobic	2	3%	10%	78.1%
Anaerobic	3	0.56%	5.1%	85.6%

Table 2: Results for reactions performed without reductant

Conditions	TTN	%ee (cis)	%ee (trans)	%de
Anaerobic	154	0.29%	4.8%	85.8%
Semi-aerobic	3	10.6%	6.2%	77.06%

Table 3: Results for reactions performed in two-hours

Conditions	TTN	%ee (cis)	%ee (trans)	%de
One-hr	110	16.2%	7.5%	92.7%
30-min	150	1.5%	4.3%	85.9%
15-min	277	0.9%	3.6%	86.2%
10-min	244	1.1%	3.8%	86.2%
5-min	266	5.0%	4.8%	89%

Table 4: Results for reactions performed in varying amounts of time (Anaerobic)

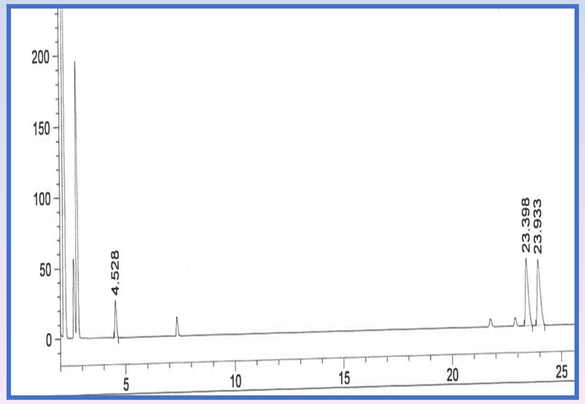
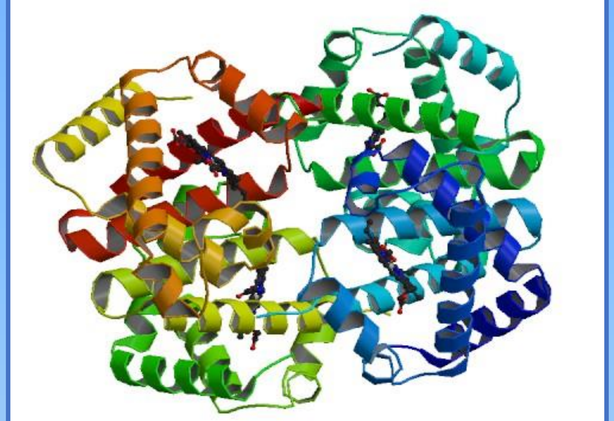


Figure 2: Gas Chromatogram

Conclusion

The reaction conditions that would be ideal for introducing this biocatalysis in an organic undergraduate teaching laboratory was found to be the one with a two hour reaction time, 60 µM catalyst, 2:1 substrate ratio, with a reductant, and under anaerobic conditions. Our data did show that TTN was high for reactions performed in shorter time frame. Duplicate reactions need to be performed for verifying the short reaction time results.



Hb structure from: http://www.rcsb.org/structure/2QSP

Future Work

- **Verify the short reaction time results.**
- **Assess the effect of organic solvents such as THF, DMSO, ethanol and methanol in this reaction.**
- **Perform the reaction with 95 undergraduate students in organic chemistry I laboratory.**

References

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(2) Bajaj, P.; Gopeekrishnan, S.; Tyagi, V.; Fasan, R. *Angew. Chem Int. Ed.* **2016**, *55*, 16110-16114.

(3) Gober, J.; Brustad, E. Non-natural Carbenoid and Nitrenoid Insertion Reactions Catalyzed by Heme Proteins. Current Opinion in Chemical Biology. **2016,** 35, 124-132.

(4) Tinoco, A.; Yang, W.; Bacik, J.; Carminati, D.M.; Moore, E.J.; Ando, N.; Zhang, Y.; Fasan, R. Origin of High Sterocontrol in Olefin Cyclopropanation Catalyzed by an Engineered Carbene Transferase. *ACS Catal*. **2019**, 9, 1514-1524.

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