Evaluation of Biocatalysis in Non-Aqueous Solvent Media Using Engineered Myoglobin Nanoconstructs

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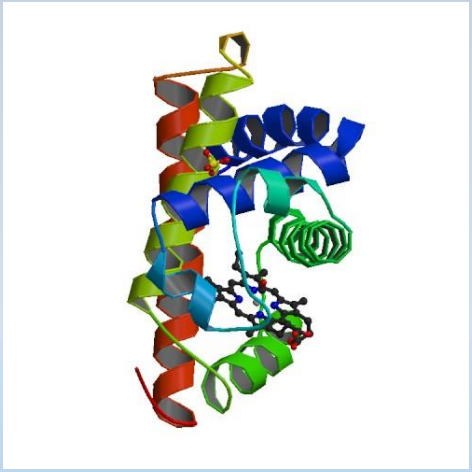
Abstract

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. Currently, the majority of biocatalytic reactions use aqueous buffer solutions so that enzymes/proteins can exist in their biologically active form. However, there are a few disadvantages to using these aqueous solvents such as low solubility of organic compounds in aqueous medium, low turnover numbers, and solvent reagent incompatibility. In this experiment, we will evaluate the effect of non- aqueous solvent media (ionic liquids and deep eutectic solvents) in enzyme/protein mediated organic transformations. In order to improve the efficiency of biocatalysis in non-aqueous media and to ensure the miscibility of the catalyst and the solvent, the engineered catalyst (Mb H64V V68A) was transformed to protein-polymer nanoconstructs.



Methods

- Myoglobin cells were engineered by mutating the active site (Mb H64V V68) with E.coli cells
- Enzyme was eluted through a resin column with four different buffers
- The buffer was exchanged for the buffer used in reactions
- Absorbance was measured in order to get concentration of the enzyme
- Protein cationization was performed and supernatant was dialyzed
- Glycolic acid was added and the supernatant dialyzed
- The protein was lyophilized and stored in a desiccator under vacuum to make the protein melts
- Spectroscopy such as mass spectroscopy, gas chromatography, and nuclear magnetic resonance can be performed on the protein melts
- Organic reactions can then be performed to determine the catalytic activity of the nanoconjugates in those reactions
- Different non-aqueous solvents can then be tested in these reactions

Future Works

We were in the process of completing our myoglobin melts, so no reactions were able to be performed and no data was able to be collected. Hopefully, in the future we will be able to complete our protein melts. Many different organic reactions should be performed with the protein melts and catalytic activity should be determined. Reactions will also be tested with non-aqueous solvent media such as ionic liquids. If catalytic activity of the myoglobin melts with non-aqueous solvent media is determined, then it could show promise for the production of drugs that require non-aqueous solvents for proper delivery and function to be produced using biocatalysis.

References

Perriman, A.W.; Brogan, A.P.S.; Colfen, H.; Tsoureas, N.; Owen, G.R.; Mann, S. Reversible Dioxygen Binding in Solvent-free Liquid Myoglobin. *Nature Chem.* **2010,** 2, 622-626.