

Design of a novel enzyme to catalyze a Diels-Alder reaction

Alexander George*, Jason DeChancie†, and K. N. Houk†

*Department of Chemistry and Biochemistry, Swarthmore College

†Department of Chemistry and Biochemistry, UCLA

Introduction

Enzymes catalyze almost all biological reactions. The ability to custom-design enzymes to catalyze any desired reaction would allow for faster and more specific syntheses, and also holds great therapeutic potential. The Houk lab has pioneered using computational techniques as a means of designing new enzymes.

The Diels-Alder reaction is one of the most important synthetic reactions due to its ability to form carbon-carbon bonds in a single, concerted step. It was chosen as a target for enzyme design because of its synthetic importance and the lack of any known instances of biological Diels-Alder reactions.

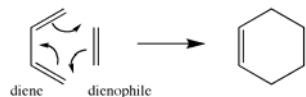


Figure 1. The Diels-Alder reaction of 1,3-butadiene with ethene.

Materials and methods

Novel proteins are designed via the “inside-out” method developed by the Houk lab. The first step is to determine the lowest energy geometry for the transition state and all catalytic enzymes; this “theozyme” is obtained by applying quantum mechanical geometry optimization to all reasonable conformations of the transition state. The theozyme is then inserted into a known protein which is re-engineered to support it, and this new protein is then expressed, purified, and tested for catalytic activity.

All geometry optimizations and energy calculations were performed using Gaussian03 software at the B3LYP/6-31G* level of theory.



Figure 2. Outline of inside-out enzyme design process.

Substrate selection

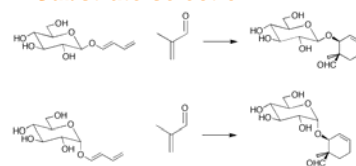


Figure 3. Diels-Alder reactions of β (top) and α (bottom) glucose-dienes with methacrolein.

In selecting a Diels-Alder reaction to target, we required the following attributes:

- Water-soluble substrates
- A carbonyl group on the dienophile for catalysis (see below)
- Substrates that could easily be bound by an enzyme

The reaction of a sugar-modified 1,3-butadiene with methacrolein was chosen because it fulfills all of the stated conditions. The glucose moiety makes the traditionally non-polar diene soluble in water and is readily bound by many proteins. Methacrolein is a desirable dienophile due to its being miscible with water and its possession of a carbonyl group as its electron-withdrawing group.

Mechanism of catalysis

MacMillan has previously shown that proline-derived catalysts effectively catalyze Diels-Alder reactions where the dienophile has a carbonyl electron-withdrawing group. The two combine to form an iminium intermediate, with the positively charged nitrogen serving as an electron sink to activate the dienophile.

We will use lysine as a catalytic residue to form an iminium intermediate with methacrolein. To test the effectiveness of this mechanism of catalysis, we calculated the optimum geometries and transition state energies for a reduced model system, and found iminium catalysis to be more than sufficient.

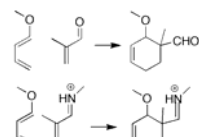


Figure 4. Model Diels-Alder reaction under thermal (top) and iminium catalysis (bottom) conditions.

- ΔG^\ddagger (thermal): 32.3 kcal/mol
- ΔG^\ddagger (iminium catalyzed): 8.8 kcal/mol
- Desired $\Delta\Delta G^\ddagger$: 10 kcal/mol
- Calculated $\Delta\Delta G^\ddagger$: 23.5 kcal/mol

Theozyme optimization

We are currently optimizing the geometries of 10 possible theozymes and the corresponding uncatalyzed reactions. The anomer of the sugar-diene can exist in 3 plausible conformations and the anomer can exist in 2, and each conformation can undergo a Diels-Alder reaction on either its *re* or *si* face.

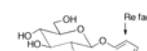


Figure 5. Different dienophile approaches.



Figure 6. One potential theozyme.

Active site design

While iminium intermediates are common in enzymatic catalysis, they almost always proceed immediately to an enamine, which is incapable of undergoing a Diels-Alder reaction. As such, our new active site will have to exclude water and basic residues other than the catalytic lysine in order to prevent enamine formation.

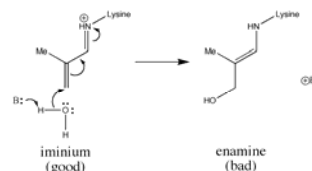


Figure 7. Mechanism of enamine formation.

In addition, in order to form the iminium intermediate, lysine must be present in its nucleophilic (non-protonated) form. In order to prevent lysine from being protonated, our active site will feature a non-polar pocket around the catalytic lysine.

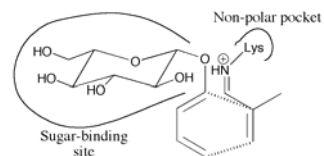


Figure 8. Proposed active site, with transition state.

Conclusions & future work

While there are no known biological catalysts for Diels-Alder reactions, we have found a Diels-Alder reaction that is amenable to enzyme catalysis. We have identified a mechanism of catalysis that sufficiently lowers the activation energy, using nucleophilic lysine to form a more reactive iminium intermediate with the dienophile. Several factors that must be considered in active site design have also been considered, including exclusion of water and protection of a nucleophilic lysine.

The next step will be to identify proteins that bind glucose and redesigning the areas next to their ligand binding sites to accommodate our theozyme. Of particular interest are periplasmic sugar binding proteins, which bind sugars in a pocket deep within their structure. This should serve to exclude solvent, and thus prevent enamine formation.

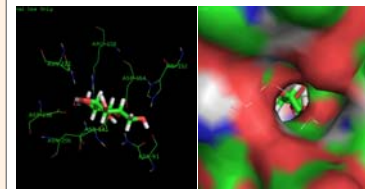


Figure 9. Ligand-binding residues (left) and surface (right) of the glucose binding site of glucose/galactose binding protein.

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Acknowledgments

We would like to thank the California Nanosystems Institute for funding Alex's Summer research, and Collin Purrington for providing the poster template.

For further information

Please contact ageorge1@swarthmore.edu. More information on this and related projects can be obtained at www.chem.ucla.edu/dept/Faculty/houk.html.