MOLECULAR ENGINEERING

Unnatural design

Researchers designed an enzyme to carry out the Diels-Alder reaction, an activity not found in nature.

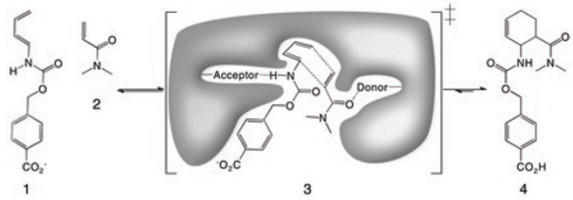
Enzymes have been honed over millions of years of evolution to have unique specificities for catalyzing chemical transformations in the crowded environment of a cell. Over the last few decades, chemists have begun to take advantage of the catalytic properties of enzymes to improve the speed and efficiency of organic reactions. Researchers have used both rational design and directed evolution methods to improve the catalytic activity, stability and selectivity of native enzymes.

Not all organic reactions a chemist might want to use are found in nature, however. Designing an enzyme with a novel activity thus is a huge challenge because there are no existing scaffolds to work from.

But much progress has been recently made in the rational design field. In particular, the Rosetta program from David Baker's laboratory at the University of Washington has proved to be a powerful tool for modeling protein structures. Baker's group has previously used Rosetta to design *de novo* enzymes with bond-breaking activities, but designing an unnatural enzyme with bond-forming activity was an additional challenge. "We have to worry about binding two substrates; they have to be oriented properly relative to one another," explains Baker.

Putting Rosetta to the test, Baker's group selected the Diels-Alder reaction as a model reaction around which to design a *de novo* bond-forming enzyme catalyst. In this reaction a diene, a molecule with two adjacent double bonds, and a dienophile, a molecule with one double bond, undergo an intermolecular cycloaddition reaction to form a cyclohexene product. No enzyme that catalyzes an intermolecular Diels-Alder reaction exists in nature.

To ensure that the two reactants would be in close proximity and in the proper orientation to react, the first step the researchers took was to define the ideal protein active site geometry. They modeled the transition state structure of the reactants, 4-carboxybenzyl *trans*-1,3-butadiene-1-carbamate (the diene) and *N,N*-dimethylacrylamide (the dienophile). They then generated minimal active site models predicted to accommodate this transition state. They also installed a hydrogen bond acceptor residue (glutamine or asparagine) to activate the diene and a hydrogen bond donor residue (serine, threonine or tyrosine) to activate the dienophile, serving to lower the energy barrier of the reaction.



Design of an unnatural enzyme to catalyze the reaction of 4-carboxybenzyl *trans*-1,3-butadiene-1-carbamate (1) and *N,N*-dimethylacrylamide (2) to form a cyclohexene product (4) by stabilizing the transition state (3). Reprinted with permission from the American Association for the Advancement of Science.

Next, they searched a set of 207 protein scaffolds that could host the model active sites. Of approximately 10^6 possible locations in which to situate the active sites, they selected 84 constructs to validate experimentally. Fifty of these were solubly expressed in *Escherichia coli* and could be purified. Using liquid chromatography–mass spectrometry to screen for the formation of the cyclohexene product, they found that two of the constructs, one based on a six-bladed β-propeller scaffold and the other from a ketosteroid isomerase scaffold, had 'Diels-Alderase' activity.

Though this is a breakthrough achievement in protein design, Baker stresses the proof-of-principle nature of this work. "I think one has to recognize that [finding two active enzymes out of 50 constructs] is a pretty low batting average," he says. The catalytic activity of both unnatural enzymes was also much lower than that of a typical native enzyme, though by introducing additional mutations in the active site, the researchers improved the catalytic activities of both Diels-Alderases, demonstrating that these enzymes have potential for use in practical applications.

Further, once a good protein scaffold has been engineered, the active site residues can be easily modified to accommodate substrates with different chemical structures. By mutating a histidine in the binding pocket of the β-propeller scaffold to a smaller asparagine, the researchers altered the specificity to favor a larger dienophile substrate.

Finally, as proof that they had actually made what they had designed using Rosetta, the researchers crystallized an active variant of their β-propeller–based Diels-Alderase. The crystal structure agreed well with the designed model, and most importantly, the side-chain conformations in the active site were close to the predicted orientations.

Still, Baker emphasizes that there is a lot of room for improvement of the computational design methods. "The basic methods need to get better to achieve really active designed catalysts," he says. "But I'm very optimistic about the future of this field; I think there's enormous potential." Perhaps someday researchers will be able to simply push a button to design specific and efficient enzymes to carry out difficult chemical reactions.

Allison Doerr

RESEARCH PAPERS

Siegel, J.B. *et al.* Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. *Science* **329**, 309–313 (2010).