ENZYME CATALYSTS FOR A BIOTECHNOLOGY-BASED CHEMICAL INDUSTRY

Professor Frances H. Arnold
California Institute of Technology
DOE Contract DE-FG02-93CH10578
Quarterly Progress Report, January 1-April 1, 1998

April 20, 1998

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The goal of this research is to engineer enzymes to be efficient and economically attractive catalysts for the chemical industry. We are attempting to demonstrate generally-applicable approaches to enzyme improvement as well as develop specific catalysts for potential industrial application.

Progress during quarter January 1-April 1 and plans for next quarter.


We have submitted a manuscript for publication that describes our evolution of highly active and thermostable pNB esterases. The summary follows.

The behavior of homologous enzymes evolved for function at different temperatures has given rise to the idea that stability at high temperatures is incompatible with high catalytic activity at low temperatures, through mutually exclusive demands on enzyme flexibility. An alternative explanation for this widely observed trade-off is that one property is lost through random drift while the other evolves under selective pressure. We have used in vitro evolution to probe this relationship between stability and activity in a mesophilic esterase. Six generations of random mutagenesis, recombination and screening stabilized the enzyme significantly (>14 °C increase in melting temperature, T_m) without compromising its catalytic activity at lower temperatures. Although enhancing thermostability does not necessarily come at the cost of activity, the process by which the molecule adapts is important. Single mutations that enhance both properties are very rare. Thus evolution of one property by the accumulation of single amino acid substitutions comes at the cost of another not directly selected for. The in vitro evolution of stable, active enzymes may be best done by recombination of properties evolved separately.

A patent application on the thermostable enzymes is nearly completed.

2. Directed evolution of subtilisin E to enhance thermostability.

Huimin Zhao has completed and successfully defended his thesis on the evolution of thermostable subtilisin E. A final paper is in preparation.


Dr. Alex Volkov has been assembling a genetic system for rapid evaluation and comparison of in vitro recombination methods. This system is necessary, because it remains difficult to perform in vitro recombination by the available methods, including the
DNA shuffling method of Stemmer as well as our new StEP method (published this quarter in Nature Biotechnology). Every new person who learns how to do in vitro recombination or who applies a method to a new gene experiences some difficulty. We also have little quantitative information to compare the efficiencies and performance of available methods. For this reason, Dr. Volkov has prepared a genetic system in which the gene for green fluorescent protein is interrupted by stop codons at varying distances. Only when parent genes containing the wild type sequence recombine will the full length, fluorescent protein be expressed. Using this system we can test how well any given method will recombine mutations that are 50 to 500 bp apart. Using the GFP system, we have found that the StEP method rapidly makes parental sequences with no recombination. Thus we are having to modify the method, originally developed for subtilisin E, so that it will efficiently recombine a wide range of genes.

Collaborations:

We are continuing the research collaboration with ThermoGen, Inc. to evolve thermostable esterases.

Publications:


Important invited lectures:

Science Innovation Topical Lecture,


Invited speaker,
Professor Frances H. Arnold  
California Institute of Technology  
DOE-NREL Grant No. DE-FG02-93CH10578  
Estimation of Funds to be Expensed Next Quarter (3/29/98-6/28/98)

<table>
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<th>Expenses</th>
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