Environmental Management Science Program

Project ID Number 55343

Enzyme Engineering for Biodegradation of Chlorinated Organic Pollutants

Peter G. Schultz
Lawrence Berkeley National Laboratory
1 Cyclotron Road
Berkeley, California 94720
Phone: 510-642-9277
E-mail: pgschultz@lbl.gov

June 1, 1998

Enzyme Engineering for Biodegradation of Chlorinated Organic Pollutants

Peter G. Schultz, Lawrence Berkeley National Laboratory

Research Objective

The generation of biological catalysts for the bioremediation of synthetic products will require in many cases powerful tools for modifying the catalytic and physical properties of natural enzymes. One approach to this problem involves the generation of selective catalysts by exploiting the large chemical diversity of the immune system to generate enzymelike catalytic antibodies. A second strategy involves the development of *in vitro* evolution methods to rapidly evolve protein catalysts with selected functions. We are attempting to combine both approaches to generate catalysts for the bioremediation of halogenated aromatics. Specifically, we are developing general selections and screens for identifying novel catalysts from large libraries of antibody mutants. These methodologies are being initially applied to a well-characterized esterolytic antibody 43C9, but if successful, will provide a general approach for evolving a wide range of catalysts for the dehalogenation of halocarbons.

Research Progress and Implications

Selections allow one to search large libraries of mutants for enhanced function. We are developing a general selection scheme that can be applied to any reaction that can be linked to the release of *p*-aminobenzoic acid, e.g., esterase, amidases, phosphodiesterases, glycosidases, etc. The shikimate pathway produces several small aromatic compounds, including *p*-aminobenzoic acid (PABA). *E. coli* with the chorismate synthase *aroC* mutation require an external source of PABA. A selection scheme based on the release of PABA is general, because PABA can be incorporated into a substrate molecule through either the carboxylic acid or the amine functional groups and with a variety of release mechanisms. As a model system for this selection scheme we chose to use the catalytic antibody 43C9, which hydrolyses the anilide bond in substrate **1**, which was elicited to hapten **2**. Changing the nitro group of the substrate to a carboxylic acid gives a substrate, **3**, which would release PABA upon hydrolysis of the anilide bond.

Figure 1

Using primers designed from a previously published sequence of 43C9, the antibody genes were reverse transcribed from murine messenger RNA and inserted into the expression vector, p4xH. The antibody expressed as a Fab fragment at a level of 1 mg per 1 L shake flask, and was catalytically active. The antibody was subcloned into another expression vector, pFH1, for inducible expression on solid media. Successful expression on minimal plates necessary for PABA selection was confirmed by ELISA. Additionally, HDU83, an *aroC* strain of *E. coli*, was tested with substrate **3** to verify the feasibility of the selection. Substrate **3** concentrations of 20 μ M were required for colony growth, as compared to 20 nM of free PABA required for growth. This 1000 fold difference in minimum concentrations should provide an adequate margin for PABA-based selection. A library of 43C9 was

created by DNA shuffling then ligated back into the expression vector. Two rounds of shuffling have been undertaken, the first simply introduced point mutations into the antibody genes, similar to error-prone PCR. The second (and subsequent) rounds introduce more mutations and also recombine existing mutations to produce more diversity. The library obtained from the second round of shuffling has been transformed into the HDU83 strain and plated onto minimal media containing 20 μ M of substrate **3**.

Planned Activities

We are also developing an automated system for screening libraries of mutants generated by DNA shuffling for enhanced activity. In order that the screen be generally applicable to a wide variety of reactions we are using mass spectrometry to directly detect conversion of substrate to product. Again the antibody 43C9 is being used as a model system. A substrate analogue has been synthesized which is linked to biotin via a positively-charged cleavable linkage

Figure 2

Growth of colonies in 100 μ L cultures is followed by periplasmic lysis to release the antibody into the media. Substrate is added and after a desired reaction time streptavidin magnetic beads are then added followed by separation of reaction product and mass spectrometric analysis by MALDI. We are able to detect 25 pmoles of product. The above system is being automated by integrating a colony picker, robotic arm and 96 well culture plates and multiwell MALDI sample holders. We anticipate that the system under development will allow us to screen in an automated fashion 10,000 mutants/day. The recombination process involved in shuffling should make it possible to rapidly combine additive mutations that lead to enhanced activity. Repetitive rounds of shuffling and screening should allow us to efficiently evolve a large range of catalytic properties.