PROTEIN CRYSTALLOGRAPHY AND ENGINEERING

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The group works at the interface between chemistry and biology. Our major interest is in working out how proteins function and how they might be modified for new and useful purposes. Directed evolution is used to produce mutant proteins that frequently have interesting properties that can be utilised in industrial and environmental applications. These mutants can also be analysed using a variety of techniques, including X-ray crystallography, to further understand the detailed mechanics of protein function.

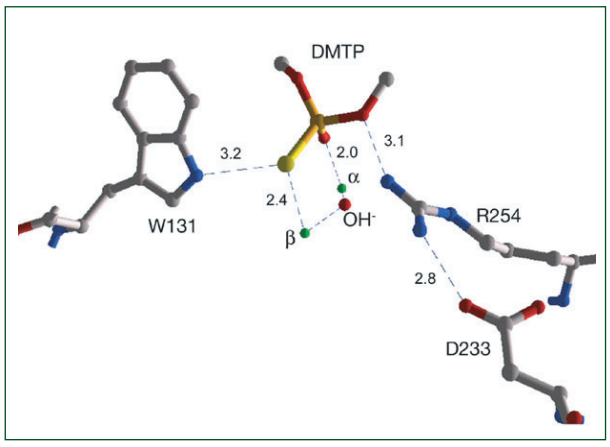
In the past year, we have developed a technique to evolve the proteins to be more soluble. We have also used directed evolution to enhance the expression of an organophosphate degrading enzyme from *Agrobacterium radiobacter* (OPDA). Other papers have focused on the mode action of OPDA – the structure of the protein in the presence of its reaction product has been determined. We have also published the results of a long-term study that involves the evolution and structure analysis of mutant forms of the enzyme dienelactone hydrolase (DLH). The results of this study show how mutations change the way DLH interacts with inhibitors and substrates.

Improving Protein Solubility

Structure function studies are frequently limited by the availability of large quantities of soluble protein. We have developed a procedure for selecting soluble variants of an insoluble protein. The procedure utilises the enzyme dihydrofolate reductase (DHFR) that is inhibited by the antibiotic trimethoprim (TMP). In our procedure, a mutant library of an insoluble target protein is fused to that of dihydrofolate reductase. Variants of the target protein that have enhanced solubility can be selected on the basis of their ability to overcome the normally lethal effects of TMP. This technique can be used to enhance the solubility of proteins for structural genomics studies. *(With J-W Liu)*

Enzyme Engineering with an Organophosphate Degrading Enzyme

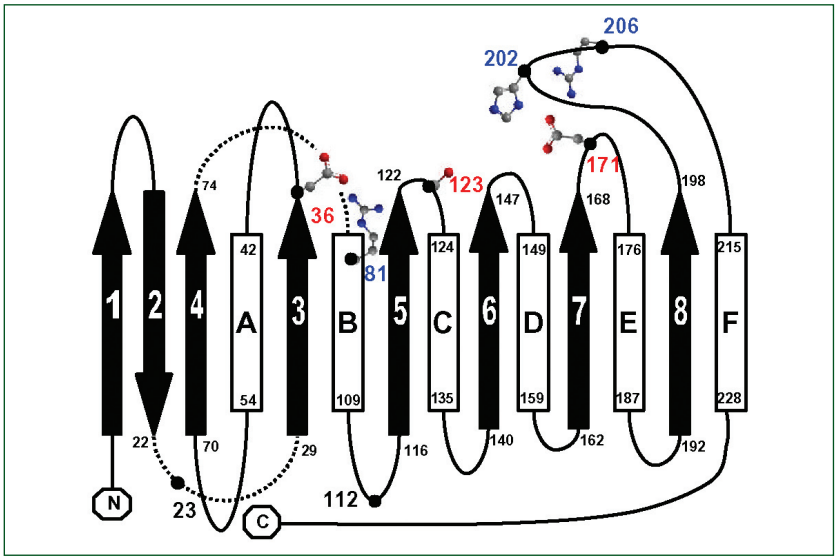
OPDA is a bacterial enzyme that shows considerable utility in bioremediation. The protein was initially discovered in the laboratory of John Oakeshott in the Division of Entomology, CSIRO. In the past years we have obtained the structure of OPDA while more recently our attention has been directed towards evolving the enzyme so that it is more efficiently expressed in *Escherichia coli*. We have also been involved in studies to better understand the mechanism of the protein and to this end we have solved the structure of the protein in the presence of its reaction product. *(With C Jackson, P D Carr, J-W Liu, S Yu-McLoughlin)*



Dimethyl monophosphate (DMMP) in the active site of OPDA. The diagram shows the active site residues that interact with the reaction product.

The Structure of Evolved Form of Dienelactone Hydrolase

Dienelactone hydrolase (DLH) is a bacterial enzyme involved in the degradation of aromatic compounds. It is the smallest member of the α/β hydrolase fold class of proteins. In previous years, the structure of DLH was obtained, its active site identified, and a catalytic mechanism proposed. More recently, we have altered the enzymatic activity of DLH with directed evolution. We have examined the structure of DLH in the presence and absence of inhibitors so that we can determine how the mutant forms of the enzyme interact with substrates. *(With H-K Kim, J-W Liu, P D Carr)*



Schematic diagram of DLH with secondary structure elements represented by arrows (β strands) and rectangles (α helices). The side-chains of key catalytic residues are shown in atomic detail.

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