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Report:

Fructose- 1.6-bisphosphate aldolases catalyse the interconversion of fructose-1.6-bisphosphate to a ketose (glyceraldehyde-3-phosphate) and an aldose (dihydroxyacetone phosphate). The enzymes participate in glycolysis and gluconeogenesis. The aldol condensation is an important reaction in synthetic chemistry and our studies on aldolases are designed to help understand the structure and mechanism with a view to engineering modified aldolases capable of carrying out difficult organic reactions. An ability to regulate stereochemistry would be particularly valuable. Our studies have concentrated on a hexagonal crystal form of the metal dependent bacterial aldolase from E. *coli*. This structure was the first to be determined using MAD methods on BM14 (formally BM19). The structure is that of an $(\alpha/\beta)_8$ barrel with the active centre located in the depression at the C-terminal end of the barrel. Zinc liganding residues and others implicated in catalysis by site-directed mutagensis or, subsequently confirmed using this technique in Dr. A. Berry's laboratory (Univ. Leeds) have been identified. These observations were published in Structure (Cooper et al., 1996 4, pg1303). The emphasis of the project is now to determine accurate structures of enzymes modified by site-directed mutagenesis, with substrate or analogues to dissect the contributions to specificity and reactivity.

We have been able to record three datasets of particular importance to this project. Wild type enzyme has been complexed with phosphoglycolohydroxamate (which mimics the substrate dihydroxyacetone phosphate). The complex gives a new crystal form, trigonal in space group $P3_121$ with a long cell edge of 290A and a functional dimer of molecular mass 78-kDa in the asymmetric unit. Data to 2.0Å, about 80% complete, redundancy of 5, **R** merge 7% have been used to solve and refine this complex. Rfactor is 18%, Rfree is 23%. This provides a picture of a substrate analouge in the enzyme active site and the protein-ligand interactions relevant to catalysis. This model may form the basis for decisions to alter the specificity. Our original structure was at a resolution of around 2.6-2.8A. ESRF data allows us to extend this now to 2.0Å with the complex.

Residue Asp109 is critical to catalysis. The inactivated mutant D109A enzyme has been co-crystallised in the presence of substrate and a very complete data set to 2.3Å recorded. The Rmerge is 7% for a redundancy of 3. Full refinement indicates the perturbation to the enzyme structure by the D109A modification and unfortunately that the substrate is not ordered in the active site. This residue may influence both binding of substrate and the chemical reaction during catalysis.

The original structrue determination was at 2.6Åish! There were many difference density peaks on the surface of the molecule that could represent zinc atoms that were essential to the crystal growth process. Crystals of the native enzyme grown in the presence of cadmium were used to collect 80% of the data to 2.1Å resolution, redundancy of 3, Rmerge 6%. A combination of more ordered crystals and the quality of the ESRF X-ray source have enabled us to obtain this much improved data set. This structure is being refined to help identify transition metal binding sites in the enzyme and to improve the accuracy of the native uncomplexed structure for comparative purposes.

Mr David Hall, a post-graduate student funded by the Engineering and Physical Sciences Research Council (UK) has participated in this project. The results generated will form a large part of his thesis and the training-experience benefited him greatly. I would strongly recommend that all post-graduates working in structural biology should have the opportunity that he has had.

In summary, the award of ESRF beamtime have enabled us to record high quality data that have been used to improve the accuracy of our aldolase structure and provided a model for the mechanism of this type of enzyme. Three publications are currently in preparation.