



	<b>Experiment title:</b> 5-Aminolaevulinic Acid Dehydratase	<b>Experiment number:</b> LS-1288
<b>Beamline:</b> BM14	<b>Date of experiment:</b> from: 27/02/99 to: 01/03/99	<b>Date of report:</b> 20/08/99
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Report: This report covers projects LS-1287, LS-1288 and LS-1289 which were blocked together and awarded 2 days of beam time on BM14 in February 1999. The main focus of our experiment was data collection on two complexes of yeast 5-aminolaevulinate dehydratase, both of which diffracted to high resolution. In addition we collected data on MhpC (a C-C hydrolase), a site-directed mutant of methanol dehydrogenase (MDH) and a native dataset on human porphobilinogen deaminase (hPBGD).

5-aminolaevulinate dehydratase (ALAD, porphobilinogen synthase) is a key early enzyme of the porphyrin and corrin biosynthetic pathways which catalyses the condensation of two 5-aminolaevulinic acid (ALA) molecules to form the pyrrole porphobilinogen (PBG). The hereditary deficiency of functional dehydratase in humans is associated with the genetic disease Doss or ALA dehydratase porphyria, a disease with severe neurological symptoms. ALAD is extremely sensitive to inhibition by lead ions which is one of the major manifestations of acute lead poisoning which often leads to neurological disturbances. ALAD structures from several species have been solved showing that the enzyme forms a large homo-octameric structure in which each subunit adopts the TIM-barrel fold. The 20-30 residue N-terminal arm makes extensive inter-subunit interactions allowing formation of an octamer which has all eight active sites exposed on the surface. At the base of each active site are two lysine residues (263 and 210), one of which, Lys 263, forms a Schiff base link to the substrate ALA. Close by is a zinc binding site formed by 3 cysteines and a solvent molecule.

Our objective is to gain an improved understanding of the catalytic mechanism of ALAD by analysis of complexes of yeast ALAD with various inhibitors. The X-ray structure of the complex formed with the inhibitor laevulinic acid has been determined at 2.15 Å resolution using data collected at ESRF (report submitted in 1998). Laevulinic acid binds by forming a Schiff base link with one of the two invariant lysines at the catalytic centre: Lys 263. Our analysis strongly suggest that the interactions found in this complex will also be made by P-side substrate. The structure indicates that the substrate which binds at the enzyme's A-site will interact with the enzyme's zinc ion bound by three cysteines (133, 135 and 143) and a solvent molecule (possibly a hydroxide ion). Inhibitor binding caused a substantial ordering of the active site flap (residues 217 - 235) which was largely invisible in the native electron density map and indicates that this highly conserved yet flexible region has a specific role in substrate binding during catalysis.

The structures of two other complexes of yeast ALAD with succinylacetone (SA) and 4-keto-5-amino-hexanoic acid (KAH) were solved using data collected at BM14 in February 1999. These datasets were collected on a MARCCD and were processed using MOSFLM. The KAH dataset extends to 2.1 Å resolution with an R-merge of 8.7 % (completeness = 99.4 %, multiplicity = 7.0) and the SA dataset extends to 2.0 Å resolution with an R-merge of 10.2 % (completeness = 99.9 %, multiplicity = 4.0). Refinement of both complexes is in progress. The KAH complex currently has an R-factor of 26.7 (R-free = 34.4) and the SA complex has an R-factor of 24.7 (R-free = 36.0).

The KAH molecule binds to the enzyme by forming a Schiff base with Lys 263 and the inhibitor's carboxyl group forms essentially the same interactions as were observed in the laevulinic acid complex. Interestingly the opposite end of the inhibitor molecule binds in a significantly different conformation to that adopted by laevulinic acid such that its amino group makes an H-bond with Lys 210. The SA molecule also binds by forming a Schiff base and interactions similar to those of laevulinic acid are made by the inhibitor's carboxyl group. At the opposite end of the inhibitor, the carbonyl of the 'acetone' moiety interacts by H-bonds with the conserved residues Asp 131 and Ser 179. It has been proposed that this carbonyl group might interact with the active site zinc ion, but the high resolution X-ray structure clearly confirms that this is not the case and the tight binding of the inhibitor must stem from the interactions with the conserved Asp-Ser pair. Succinylacetone binding also causes a substantial structural rearrangement of one of the active site loops, the significance of which is not yet clear. These results and structural studies of other complexes (in progress) will help to piece together a picture of the substrate binding modes and catalytic mechanism.

The other datasets were processed and the corresponding structures are being refined. The dataset collected on the active site directed mutant of MDH has an R-merge of 10.1 % to 2.0 Å resolution. The hPBGD dataset extends to 2.6 Å resolution and has an R-merge of 9.6 %. This dataset has allowed the structure to be solved by molecular replacement using the *E. coli* enzyme as a search model. The native dataset collected on MhpC extended to 2.5 Å with an R-merge of 9.9 %. The structure of this C-C hydrolase is currently being solved by isomorphous replacement.