Experiment title: Multiwavelength Anomalous Dispersion Data Collection for 3D Structure Analysis of 5–Aminolaevulinate Dehydratase	Experiment number: LS-400

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Report:

MAD Data Collection on aPlatinum Co- Crystal of 5-Aminolaevulinate Dehydratase

The enzyme 5-aminolaevulinate dehydratase (ALAD) catalyses one step in the biosynthesis of tetrapyrroles such as haem and chlorophyll. Tetrapyrroles or porphyrins are ubiquitous biological molecules and form numerous essential components of respiration and photosynthesis. Genetic deficiencies in the porphyrin biosynthetic enzymes give rise to hereditary porphyria which have severe neurological symptoms.

ALAD catalyses the dimerisation of two 5-aminolaevulinic acid residues with the elimination of two molecules of water. Four molecules of the resulting pyrrole, porphobilinogen, are then condensed in a reaction catalysed by porphobilinogen deaminase, the 3D structure of which has been solved by our group using high resolution SRS (Daresbury, UK) data (Louie *et al,* 1992). One 5-aminolaevulinate molecule forms the propionic acid (P) side of porphobilinogen with its amino group being incorporated into the pyrrole ring, whereas the other ALA molecule forms the acetic acid (A) side of the product retaining a free amino group (see Fig 1) which is essential for the polymerisation catalysed by the deaminase. Catalysis proceeds by formation of a Schiff base link between the 4-keto-group of substrate (see Fig 1) and an invariant lysine residue (258 in *E coli ALAD;* Gibbs and Jordan, 1986).

Figure 1. The condensation of two 5-aminolaevulinate residues catalysed by 5-ALA dehydratase (* indicates the carbonyl group of the substrate linked to the catalytic lysine (Lys 247) via a Schiff base).

5 - aminolaevulinic acid   Porphobilinogen
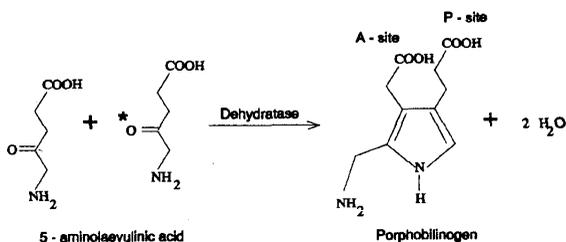
We have reproducibly grown large single crystals (0.5 x 0.5 x 0.3mm^3) of *E coli ALAD* using vapour diffusion. Isomorphous crystals have also been grown in the presence of laevulinic acid, a covalent inhibitor. We have characterised the crystals as belonging to space group 1422 with unit cell dimensions of a=b=130.7Å, c=142.4Å with two subunits (approx 35kDa each) in the asymmetric unit. This is particularly interesting in view of the biochemical experiments which have shown that the dimer is the smallest oligomer possessing catalytic activity (Jordan, 1991). The crystals are rather unstable at the copper Kα wavelength (1.54Å) but survive indefinitely at l00K, which has allowed a 2Å native dataset to be collected at SRS Daresbury.

Recently we have obtained good crystals of the ALAD enzyme from yeast which have the same space group (1422) but a different unit cell (a=b=103.7 & c=167.6Å) and have the advantage of having only one subunit per asymmetric unit. A native dataset has been obtained to a resolution of 2.3Å using cryocooling at SRS (Daresbury UK) and 3 Å data have also been collected from a lead co-crystal. The latter have allowed two lead sites to be identified with reasonable phasing statistics (phasing power = 1.9, Cullis-R=0.7, FOM=0.6). This has enabled us to calculate a SIRAS map which has a good solvent boundary and has allowed the overall organisation of the octamer to be visualized for the first time by X-ray analysis. Several putative elements of secondary structure have been identified so far.

Small co-crystals of the yeast enzyme were also obtained in the presence of platinum tetrachloride and MAD data were collected from one of these on beamline 19 at ESRF. The crystal was frozen at l00K and under these conditions it had a unit cell of a=b= 102.64 Å, c= 169.69 Å with the same space group as the native and lead datasets (1422). The initial XANES scan showed a promising edge for platinum and 95 degrees of data were subsequently collected at three wavelengths at the edge namely 1.074 Å, 1.0738 Å and 1.0162 Å referred to subsequently as 11, 12 and 13. The 11 data was processed to 2.8 Å resolution and had an R-merge of 13.5 %, a multiplicity of 7.2 and completeness of 98.3 %. The 12 data were processed to the same resolution and had an R-merge of 15.6 %, a multiplicity of 7.4 and a completeness of 80.7 %. The remote or 13 data were of poorer quality due to some fading of the diffraction and icing. Consequently this dataset was only processable to 3.5 Å resolution and had the following statistics: R-merge = 18.9 %, multiplicity = 10.0 and completeness = 96.1. Anomalous and dispersive difference Pattersons were calculated using' the 11 and 12 data but subsequent vector searching (VECSUM in CCP4) failed to reveal promising sites. Eventually a low occupancy site was identified in a cross-phased Fourier (phases from the lead data) but this gave poor overall phasing statistics : phasing power = 1.2, figure of merit = 0.29, Cullis-R = 0.9. Incorporation of the Pt data in phasing the protein together with the lead data did not improve the phasing statistics or the interpretability of the map. Consequently we are proposing to obtain better phases by collection of MAD data on selenomethionine-ALAD crystals which have recently obtained.

References

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Jordan P M (1991) in 'New Comprehensive Biochemistry' Ed. Neuberger A and Van Deenen L L N, Elsevier, pp 1-65.

Louie G *et al,* (1992) *Nature* **359,33-39**.