ESRF

ESRF	**Experiment title:** Structural studies on the aconitase family of enzymes	**Experiment number**: LS 1745
Beamline: BM 14	**Date of experiment**: From: 8 November 2000 to: 10 November 2000	**Date of report**: 30-8-2001
Shifts: 6	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

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Report:

Like their mammalian cytoplasmic counterparts, the iron regulatory proteins 1 (IRP1s), bacterial aconitases are bifunctional proteins. They function not only as [4Fe-4S] cluster containing enzymes catalysing the interconversion of citrate and isocitrate, but also, in the absence of their iron-sulphur clusters, as RNA stem loop binding post-transcriptional regulators. The family consists of proteins with molecular weights ranging from 68-120 Kda, which, despite belonging to the same family, have considerable architectual variation and low sequence similarities between various members. AcnB has a MW of ~100KDa and is the second of at least three identified aconitases of *E.coli* (Bradbury et al, 1996, Microbiolgy, 142, 389-400). Although the structure of pig mitochondrial aconitase is known, there is only very low sequence homology between *E.coli* AcnB and the pig enzyme, with major insertions and deletions and a different domain organisation. The structure of AcnB would thus represent the first of this type of aconitase structure.

As crystals of seleno-met doped AcnB were available, data had been previously collected on BM30A, in May 2000 during some spare time on allocation LS1590. Data to 3.0Å at three wavelengths were collected, corresponding to the inflection, peak and high energy remote selenium positions, as seen from a fluorescence scan of the crystal. Data collection at the iron edge, from the Fe-S cluster, was also attempted, but no absorption signal could be detected. In this crystal form of AcnB there are 2 monomers in the asymmetric unit, and a total of 46 methionines. Although the seleno-MAD data were of reasonable quality, and the level of selenium incorporation was high (as determined by mass spectrometry) attempts to solve the selenium substructure, using SOLVE or Shake'n'Bake, proved unsuccessful. Currently, a combination of MIR and MAD techniques are being used in an attempt to solve the AcnB structure.

On this LS1745 trip, 6 shifts were allocated on BM14. However, during the first day a problem became apparent with the cooling of the monochromator, making the beam intensity very unstable, rendering data collection impossible. This was fixed by the middle of shift 4. As beam time had been lost 6 new shifts were allocated on the same station – see report 2 for LS1745. With the remaining 2.5 shifts a complete MAD data set was collected on a crystal of an enzyme of unknown structure involved in nucleotide catabolism with possible links to cell signalling and apoptosis and with a potential for use as an antibacterial target. This protein from *C.elegans* crystallises in space group $C222_1$, with a monomer in the asymmetric unit. A fluorescence scan of a mercury doped, back soaked, crystal indicated the presence of bound mercury, and data were collected at the inflection, peak and high energy remote positions for mercury, as determined from the scan. Four Hg sites, corresponding to the four cysteine residues in this protein, were determined using Shake'n'Bake and the phases calculated using MLPHARE. The structure has been refined and a manuscript is in preparation.