ESRF	Experiment title: BIMP: Bovine Inositol Monophosphatase	Experiment number: LS-1533
Beamline: ID14-EH3	Date of experiment: from: [redacted] to: 31 August 1999	Date of report: Feb 2000
Shifts: 3	Local contact(s): Mark Van Raaij	*Received at ESRF:*

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Report:

The enzyme inositol monophosphatase is involved in the recycling of inositol from inositol polyphosphate secondary messengers. The enzyme is the only known target of the manic depression drug lithium. Inhibition of the enzyme by lithium ions causes a depletion of inositol and reduced synthesis of phosphatidylinositol leading to attenuation of the production of intracellular secondary messengers in response to external stimuli.

We have crystallised recombinant bovine inositol monophosphatase (BIMP) in collaboration with Dr M. Gore (Southampton). Crystals were flash cooled and brought to ESRF for data collection at 100K on ID14. Here we collected data to 1.6 Å resolution which was the highest achievable resolution on the MARCCD on that beamline at the time of the experiment. This dataset was processed using MOSFLM and the CCP4 suite. It has an R_{merge} of 8.9 % and a completeness of 94.2 %. The data have allowed the structure to be solved by molecular replacement using the program AMORE. The coordinates of the human enzyme were used as a search model. Refinement is in progress and already new information on the metal ion binding and active site geometry is being revealed in extraordinary detail.

The images had strong diffraction spots at the edge of the detector so we are confident that data to atomic resolution can be collected for the native enzyme as well as complexes and mutants. In future, we plan to collect data on complexes of BIMP with lithium and its substrates (inositol and phosphate). We also plan to collect data on several active site mutants to aid mechanistic studies.

During the same experiment we were able to collect data to 3.0 Å resolution on native groEL. Work on this dataset is in progress. During the experiment some beamtime became available on ID14-EH2 which we used to collect data on the aspartic proteinase endothiapepsin complexed with a transition state analogue. We are analysing a number of inhibitor complexes of this enzyme at atomic resolution to complement our neutron Laue studies being conducted at ILL (with Dean Myles). This work is aimed at locating the active site protons in complexes with transition state analogues to improve definition of the catalytic mechanism. The inhibitor analysed in this experiment belongs to the phosphinic acid class. The phosphinic acid moiety is a close analogue of the putative geminal diol intermediate of peptide bond hydrolysis since it possesses two oxygen substituents on the phosphorus atom, unlike most other classes of analogue which only mimic one of the two oxygens of the intermediate. Data were collected using a MARCCD in four passes to circumvent the problem of overloaded reflections. The limiting resolution of the detector was 1.05 Å. Data were processed using MOSFLM and the CCP4 suite and were found to have an R_{merge} of 4.6 % and to be 97.9 % complete. A 2.0 Å structure for this complex had been solved around 10 years ago and this model was refined using SHELX-97 with the new dataset. The resulting map was of excellent quality and the structure is being rebuilt and refined with the atomic resolution data.