ESRF	**Experiment title:** Structural Studies on the glycoside hydrolase GlvA	**Experiment number:** LS-1071
Beamline: ID14-4	**Date of experiment:** From: 15/12/98 to: 17/12/98	**Date of report:** 27-08-99
Shifts: BAG	**Local contact(s):** Sean McSweeney	*Received at ESRF:* - 1 SEP. 1999

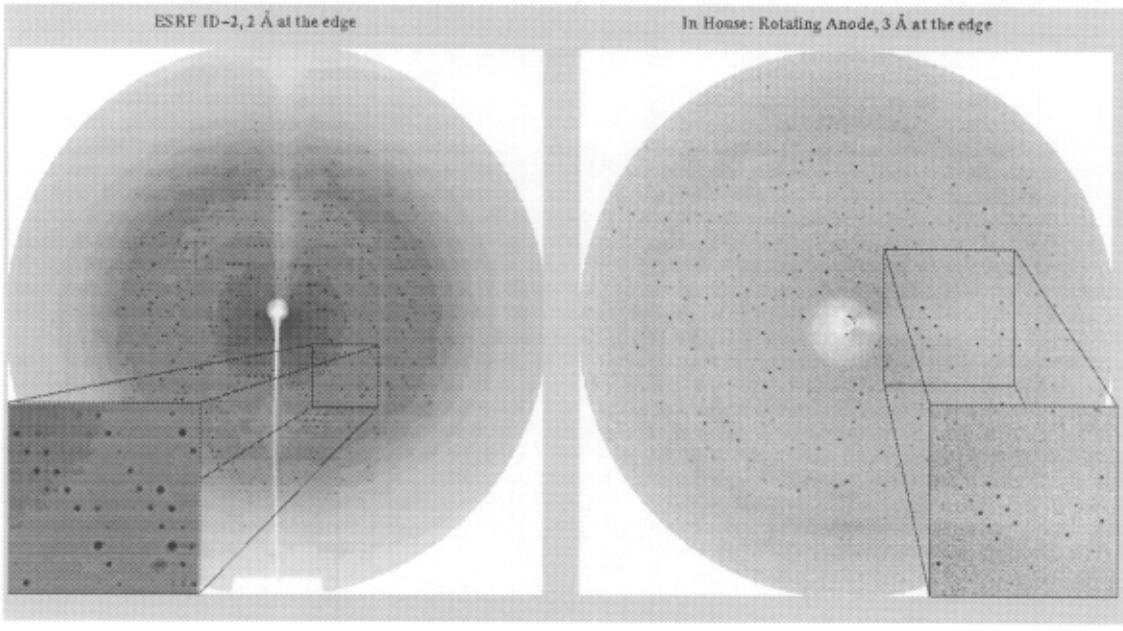
Names and affiliations of applicants (* indicates experimentalists):
Annabelle Varrot*, Gideon Davies

Structural Biology Laboratory, Chemistry Department, University of York, York YO10 5DD, United Kingdom

Report:

The 6-phospho-α-glucosidase GlvA is the last enzyme involved in the phosphoenolpyruvate dependent maltose transferase system in the spore forming organism *B. subtilis*. It has been assigned to family 4 of glycoside hydrolases, for which neither the folding nor the mechanism is known. Its assignment is questioned because as other family 4 members it presents unusual characteristics for a glycoside hydrolase since it requires the presence of NAD(H) and divalent metal ions for catalysis.

Crystals were obtained from a solution containing 100 mM HEPES pH 7.5, 1.15 M tri-sodium citrate and 20 mM $MnSO_4$. 10% (v/v) glycerol was added to the previous solution as cryoprotectant. Native X-ray diffraction data were collected to 2.2 Å resolution from a single crystal flash-frozen at 100K on beamline ID14-4, using an ADSC charge-coupled device (CCD) detector. GlvA crystals belong to the I222 space group with cell dimensions a = 83.26, b = 102.56, c = 145.31 Å.



The first attempt to solve the structure of GlvA was by multiple isomorphous replacement (MIR) using heavy metal derivatives. Several heavy metals were soaked or cocrystallised at different concentrations and for different lengths of time with GlvA crystals and taken beamline ID2B. GlvA crystals diffract very weakly in house (3.5 Å) and synchrotron radiation is needed to get high resolution (beyond 2 Å) and significant diffraction. Synchrotron radiation reveals also the presence of I222/P222 pseudo-symmetry for the crystals soaked or cocrystallised with heavy metals that we cannot detect in house. Data collected in house indexes all in space group I222 but data collected at the ESRF only indexes in P222 with the same unit cell found for I222, indicating that the heavy metals disrupt the body centering. However, data collected until now do not reveal the presence of heavy metal bound to the protein. Mercury derivatives seemed to be a good candidate due to the fact that GlvA contains one free cystein. Recently, at BM14 at ESRF Grenoble, a scan on the crystals cocrystallised with mercury did not give any mercury signal assuring us that the mercury did not bind the cysteine, which is probably buried and not accessible. Previous data collected in P222 space group did not reveal any pic in the patterson map as well. A new approach is being developed, where we will try to solve the structure by multiple anomalous dispersion (MAD) using selenomethionine protein crystals.