ESRF	**Experiment title:** **Studies on the D405N mutant of the cellobiohydrolase Cel6A from *Humicola insolens***	**Experiment number**: LS-1532
Beamline: ID14-4	**Date of experiment**: from: 9/9/99 to: 12/9/99	**Date of report**: 28/08/00
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Report:

The cellobiohydrolase Cel6A from *Humicola insolens* belongs to the family 6 of glycoside hydrolases. It has a distorted $(\beta\alpha)_8$ barrel fold with its active-site located within an enclosed tunnel formed by two extended loops (Varrot *et al.*, 1999a). This tunnel topography typical for cellobiohydrolase enzymes permits processive hydrolysis of cellulose, where after an initial attack, they perform multiple attacks from one end before the release of their substrate. Cel6A hydrolyses the α-1,4-bonds of cellulose with inversion of the configuration of the anomeric carbon. This requires the presence of two catalytic carboxylate groups: a proton donor to protonate the glycosidic bond and promote leaving-group departure and a catalytic base to activate the hydrolytic water molecule for nucleophilic attack at the anomeric centre. Asp^{405} is the proposed base and in order to confirm its role, it has been mutated to an asparagine. Several structures of the Cel6A D405N mutant in complex with carbohydrates have been determined.

The protein was incubated for at least an hour with 1mM of substrate prior to co-crystallisation. Crystals with 1-methyl-β-D-cellobiosyl-4-S-β-D-cellobioside (IG4) were obtained from a solution containing 100 mM sodium Hepes buffer, pH 7.5, 100 mM magnesium acetate and 20% (w/v) PEG5000MME. X-ray data were collected to 1.7 Å resolution from a single crystal flash-frozen at 100K at European Synchrotron Radiation Facility (ESRF), beamline ID14-4, using an ADSC QUAD-4 charge-coupled device (CCD) detector. The crystals belong to space group $P2_12_12_1$ with cell dimensions a = 57.5, b = 59.2, c = 97.0 Å. Crystals with cellohexaose were obtained from a solution containing 100 mM sodium Hepes buffer, pH 7.5, 200 mM calcium acetate and 21% (w/v) Peg5000MME. 20 % (v/v) glycerol was included in the harvesting solution prior to crystal mounting. Data were collected from a single crystal to 1.7 Å resolution at beamline ID14-2 (λ=0.933) using a MarCCD detector. The crystals belong to space group $P2_1$ with cell dimensions a = 49.4, b = 70.2, c = 51.7 Å and β = 113.5°.

The Cel6A D405N IG4 structure was solved by molecular replacement using the coordinates of the native structure (PDB code 1 BVW, Varrot *et al.*, 1999a) as a search model. Electron density was clear for the four glucose units of the substrate which occupy the +1 to +4 subsites. They all present a classical $^{4}C_{1}$ chair conformation. The substrate binds in a non-productive way meaning that its orientation differs by 180° from the orientation observed for productive binding. Such binding does not give rise to major conformational changes but only to subtle reorientation of the tryptophane aromatic rings such that the stacking interactions are optimised. Hydrogen bonding interactions between the protein and the sugar moieties are mainly mediated through water molecules whilst they are direct for productive binding.

The structure of the Cel6A D405N cellohexaose complex was solved by molecular replacement using the coordinates of the A molecule in the wild-type complex with glucose and cellotetraose (PDB code 2BVW, Varrot *et al.*, 1999b) as a search model. The surface loops forming the lid of the tunnel have changed conformation such that the tunnel is more enclosed as is also observed in the Cel6A glucose/cellotetraose complex. The difference map reveals only density for three glucose units in the active site, which occupy the +1 to +3 subsites. The cellohexaose has therefore been cleaved confirming kinetic results on the fact that the mutation of Asp^{405} did not give rise to an inactive enzyme. Further investigations on additional mutants for this residue are underway.

References

Varrot, A., Schülein, M. & Davies, G. J. (1999a). Structure of the Humicola insolens cellobiohydrolase II (Cel6A) at 1.92 Å resolution. Biochem J 337, 297-304.

Varrot, A., Schulein, M. & Davies, G. J. (1999b). Structural changes of the active-site tunnel of Humicola insolens cellobiohydrolase Cel6A upon oligosaccharide binding. Biochemistry, 38, pp 8884-8891