ESRF

ESRF	**Experiment title: Recombinant β-glucosidase 1 of maize in complex with inhibitors and natural substrate/ LPHase, family 6 of glycosyl hydrolases**	**Experiment number:**
Beamline: ID14-EH2	**Date of experiment:** from: 16.11.99 to: 17.11.99	**Date of report:**
Shifts: 1	**Local contact(s):**	*Received at ESRF:*

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Report:

Continuation of the experiment β-glucosidase 1 of maize in complex with inhibitors and natural substrate.

We have obtained a complex of the inactive, mutant protein with the natural substratre DIMBOA-glucoside by soaking experiments. The crystal of the mutant protein in complex with DIMBOA-glc belongs to space group $P2_12_12_1$ with unit cell parameters a=93.1Å, b=95.3Å and c=119.7Å. A data set has been to 2.1Å resolution.

The density in the active site clearly shows that the complex is formed, the active sites are however not all occupied. Furthermore, the need for multiple conformations of the glucoside moiety on the catalytic pathway leeds to weak density of the flexible part of the glucosidic ring. The aglycone moiety was unambigously assigned for DIMBOA and dhurrin and the structural refinement is under way. The structural interpretation of the complexes helped localize residues involved in substrate specificity in the aglycone binding pocket.

Refinement data of maize β-glucosidase and its complex

Protein	Space group	Resolution	R and R_{free}	Conditions	Remarks
E191D rglu	$P2_12_12_1$	27 - 2.2Å	20% / 24%	synchrotron	finished
E191D-rglu +				synchrotron	

dimboa-glc	$P2_12_12_1$	30 - 2.1Å	22% / 27%	soaking	not finished

LPHase, family 64 of the glycosyl hydrolases

This glycosyl hydrolase (family 64) produces β-D linked pentaose, which is of industrial interest as primary material in organic synthesis. No 3-D structure of any enzyme of this family is known to date. The crystals have been obtained recently, but show only a very low resolution diffraction pattern in the laboratory. No space group could be determined. Several test images have been taken on ID14-EH2 of crystals of the LPHase, but the crystals were severely twinned and/or mosaic. We therefore intend to try to get higher quality crystals to continue the MIR structure determination.

CelE from *Clostridium cellulolyticum*, family 9 of the glycosyl hydrolases

The family 9 glycosyl enzymes have a high diversity in substrate specificity. They are modular proteins, mainly cellulases and besides the family 9 catalytic domain often have one or more carbohydrate binding modules, which are different for different enzymes, associated in the primary sequence. In order to have a view of how the CBD of CelE influences the catalytic activity of the entire enzyme a structural basis to interpretate the enzymatic experiments is of need.

We recently obtained crystals and determined the conditions for cryo-cooling the crystals. They belong to space group P222 with the unit cell parameters a=93.5Å, b=96.2Å, c=118.6Å. Since the structure of two family 9 enzymes are already available, we hoped to solve the structure by molecular replacement.

A native data set was collected to 1.9Å resolution at ID14-EH2 (98% complete, Rsym 6%). The molecular replacement failed to give a clear structural solution and the production of a seleno-methionine substituted enzyme is under way.