	Experiment title: Recombinant β-glucosidase 1 of maize in complex with inhibitors and natural substrate	**Experiment number**:
Beamline: ID14-EH2	**Date of experiment**: from: 30.09.99 to: 1.10.99	**Date of report**:
Shifts: 1	**Local contact(s)**:S. Watazuki	*Received at ESRF:*

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Report:

β-glucosidases catalyze the hydrolysis of aryl and alkyl β-D-glucosides as well as β-linked oligosaccharides. In plants, there are two types of β-glucosidases: β-O-glucosidases and myrosinases (β-S-glucosidases). The main role attributed to these enzymes is their implication in defense mechanisms against their pests based on storing and releasing toxic chemicals.

Much progress has been made in understanding the mechanism of catalysis by glycosidases in general and defining the identity and roles of specific amino acids within the active site that are involved in catalysis. However, there is virtually no information as to how β-glucosidases recognize and interact with their substrates, specifically the aglycone moiety, which is the basis of tremendous diversity in β-glucosidase substrates and is responsible for subtle substrate specificity differences. The major goal of our research is to understand the mechanism of substrate specificity of β-glucosidases and related enzymes on the aglycone side of the substrate. This is crucial to engineering enzymes that hydrolyze substrates of interest with desired catalytic efficiency in transgenic organisms or in biotechnological applications.

We have cocrystallized the inactive, mutant protein with the inhibitor DIMBOA, the aglycone-moiety of the natural substrate, and dhurrin. The crystals of the mutant protein as well as the cocrystallized complexes belong to space group $P2_12_12_1$ with unit cell parameters a=93.1Å, b=95.3Å and c=119.7Å. Three data sets

have been collected, the enzyme/dhurrin complex to 2.0Å resolution, the enzyme/DIMBOA complex and the inactive mutant β-glucosidase E191D to 2.2Å resolution.

The density in the active site clearly shows that the complexes are 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 complexes

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 + dhurrin	$P2_12_12_1$	30 - 2.0Å	21% / 25%	synchrotron cocrystall.	not finished
E191D-rglu + dimboa	$P2_12_12_1$	30 - 2.2Å	22% / 27%	synchrotron cocrystall.	not finished