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Experiment Report Form

	Experiment title: Crystal structure determination of Triticum aestivum xylanase inhibitor (TAXI) in complex with a xylanase from B. subtilis	**Experiment number**: MX-178
Beamline: BM14	**Date of experiment**: from: 1/12/2003 to: 2/12/2003	**Date of report**: 24/2/2004
Shifts: 3	**Local contact(s)**: Gavin Fox	*Received at ESRF:*

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Report:

Cereal grains contain three groups of important biopolymers: starch, proteins and non-starch polysaccharides. Starch and most of the proteins occur in the endosperm and serve as reserve material for the plant during germination and the initial stages of growth. The non-starch polysaccharides which are, to a large extent, part of the cell walls include mainly arabinoxylan and β-glucan. These components are hydrolysed by xylanolytic and β-glucanolytic enzymes respectively [1]. The degradation of the cell wall polysaccharides during germination improves the accessibility of starch and protein for amylases and proteases [2].

Nowadays the use of endoxylanases in the cereal processing industry is often more based on empirism than on scientific understanding. Recently, a proteinaceous xylanase inhibitor has been discovered in *Triticum aestivum* L. [3]. Determining the structure of Triticum aestivum Xylanase Inhibitor (TAXI) in complex with two different Family 11 xylanases, from *Aspergillus niger* and *Bacillus subtilis* respectively, would be very helpful to get a better insight in the arabinoxylan-endoxylanase-inhibitor system. Untill recently, no structural information was available for any member of the TAXI family, nor any knowledge on the underlying inhibition mechanism could be obtained. Therefore, the elucidation of the structure of TAXI-I in complex

with different glycoside hydrolase family 11 xylanases, not only provides a first model of the TAXI family, it also procures detailed inside into the inhibition mode.

TAXI in complex with *B. subitilis* xylanase has also been crystallized using the hanging drop vapor diffusion method in a condition containing 0.22 M ammonium sulpate, 0.1 M acetate buffer pH 4.6 and 25 % polyethylene glycol 4000. Although these crystals were very small we could collect a full data set on the BM14 beam line at the ESRF (Grenoble, France). The crystals belong to the monoclinic spacegroup C_2 and have cell parameters a = 107.89 Å, b = 95.33 Å, c = 66.31 Å, $\alpha = \gamma = 90.0^{\circ}$, $\beta = 122.24^{\circ}$. Data could be collected up to a resolution of 2.5 Å. Further statistics are summarized in table I.

Previously we collected a SAD dataset on a TAXI (gold) derivative crystal on the BW7A beam line at DESY (Hamburg, Germany), to a resolution of 1.75 Å. A first electron density map was calculated with SHARP [4] and showed clear density for one molecule of TAXI in the asymmetric unit. A 70 % complete model was built in this experimental map and docked into the known sequence using the warpNtrace mode of ARP/wARP [5]. Further building and refinement of the structure resulted in a model, which could be used in solving the structures of TAXI in complex, using Molecular Replacement.

Table 1: Data collection and reduction statistics (TAXI- B. subtilis *xylanase)*

Resolution limit (Å)	2.50	(2.64-2.50)
Total observations	51556	
Unique reflections	20136	(2965)
Completeness of all data (%)	98.0	(98.0)
mean I / σ	8.5	(3.8)
R_{sym} –value (%)	5.8	(20.1)

Values between parentheses indicate data in the highest resolution shell.

References

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