ESRF	**Experiment title:** Crystal structure of α-L-Arabinofuranosidase from *Bacillus stearothermophilus T6*	**Experiment number:** LS-2020
Beamline: ID29	**Date of experiment:** from: 10-sept-01 8:00 to: 11-sept-01 7:00	**Date of report:** 02-03-02
Shifts: 3	**Local contact(s):** Andy Thompson	*Received at ESRF:*

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Report:

The α-L-Arabinofuranosidase (AF, EC 3.2.1.55) catalyzes the hydrolysis of non reducing terminal α-L-arabinofuranosidic linkages in arabinoxylan, L-arabinan and other L-arabinose containing polysaccharides. AF works in concert with other hemicellulases to degrade the hemicellulose backbone completely. These enzymes are used as tools in basic research elucidating the hemicellulose structure and play an important role for biobleaching in the paper and pulp industry. AF is a complex of four identical subunits with 4×504 amino acids and a molecular weight of 57 kDa per subunit.

α-L-Arabinofuranosidase was overexpressed in *E.coli*, purified to homogeneity and crystallized both as native and Se-Met labelled protein. The crystals belong to the monoclinic space group $P2_1$ with lattice parameters of a=100.8 Å, b=178.1 Å, c=196.2 Å and β=96.1°. The most probable V_M-value is 3.85 Å^3/Da corresponding to two AF-tetramers per asymmetric unit and a solvent content of 68%. A native data set was collected to 2.6Å (100K) resolution at the EMBL Outstation at DESY, Hamburg.

The structure should be solved by SAD/MAD using the Se-Met derivative. The difficulty in this phasing process consists in the large Se-substructure. As each protomer contains twelve Se-Met residues, 96 Se-postions per asymmetric unit have to be located in the course of the MAD experiment.

As the success of the experiment strongly depends on techniques which reduce systematic errors in measurement, Bijvoet pairs were measured on the same diffraction image. About 1000 frames (0.5° per frame) of a peak wavelength data set were measured to 2.8Å resolution. In due to significant crystal decay the measurement could not be continued. Therefore a complete three wavelength MAD data set of another crystal was measured to 2.9Å resolution.

The table shows the data statistics of selenomethionine-AF. The numbers in brackets confer to the highest resolution shell.

	MAD-peak	MAD-inflection	MAD-remote	SAD-peak
wavelength	0.97918Å	0.979338Å	0.915001Å	0.97918Å
resolution	35-2.9Å	35-2.9Å	35-2.9Å	35-2.8Å
reflexes measured	718322	693966	699828	1535731
reflexes unique	187840	186650	186846	227775
completeness	99.8%(99.8%)	99.8%(100%)	99.7%(100%)	99.0%(98.5%)
$I/\sigma(I)$	14.1	14.8	20.5	15.6
	(5.9)	(4.7)	(4.5)	(6.7)
R_{sym}	6.3%	6.3%	7.5%	(8.0%)
	(25.7%)	(31.0%)	(39.3)%	31.2%)

The phasing process is still under way, but up to now neither *SOLVE* nor *SnB* were able to find a significant solution for the substructure that would be one of the largest solved to date.

Due to the large Se-substructure, the low symmetry of the crystals and the resolution of the Se-Met data we probably require supplementary heavy atom data to solve the strucure of α-L-arabinofuranosidase.