ESRF	**Experiment title:** Uppsala (II) BAG, LS-1665 (T. Alwyn Jones BAG) Cellulases; EG3, EG5, EG2	**Experiment number**: LS 1665
Beamline: ID 14:EH4	**Date of experiment**: from: 28 Apr 2000 to: 30 Apr 2000	**Date of report**:
Shifts: 6 (EG3: 1.5)	**Local contact(s)**: Julien Lescar	*Received at ESRF:*

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Report:

Protein Name EG3: Endoglucanase 3 from *T. reesei*

Brief background and outline of project

(Cel12A, GH family 12). EG3 is a minor component in the cellulase system, but may nevertheless play an important role. It differs from the other cellulases in that it does not contain the additional cellulose binding module and linker typical for many cellulases. It is also smaller than other fungal cellolytic enzymes and subsequently appears to have a larger ability to penetrate the cellulose substrate. We have recently solved the structure and now aim at obtaining ligand complexes with wild-type and mutated enzymes for understanding catalytic mechanism, substrate binding and specificity. Very large crystals have been obtained that diffract to very high resolution (0.9-1.0 A) and which could hopefully yield structures at atomic resolution.

Datasets collected on ID14:EH4

Two datasets were collected at ID14:EH4 on protein crystals from two new T. reesei EG3 apo catalytic inactive mutants. Booth datasets were of high quality and fairly high completeness. The space-group of these datasets were the same as previously datasets P21, but the cell parameters were new and the datasts had to bee solved by molecular replacement methods. Bothe these catalytic inactive mutants structures have now been solved and refined. Nither of these structure did contain a ligand in the active site but thise structures will be used in a

manuscript, in progress right now, where we try to explain the catalytic mechanism of the enzyme.

Data statistics for the datasets:

Dataset1:

Ligand: No
Mutation: A
Resolution: 40-1.7A
Space group: P21
Cell: a:62.49, b:77.55, c:83.41, ? : 90, ? :98.46, ?:90
Completeness: 98%

Dataset 2:

Ligand: No
Mutation: B
Resolution: 30-1.2A
Space group: P21
Cell: a:43.1, b:103.8, c:45.33, ? : 90, ? :118.4, ?:90
Completeness: 90%

Protein Name EG5: Catalytic module of Endoglucanase 5 from *T. reesei*
Brief background and outline of project

Trichoderma reesei is a soft-rot fungus capable of degrading cellulosic material. This fungus produce two kind of cellulases, cellobiohydrolases (CBH) and endoglucanases (EG) which role is to break up the cellulose microfibrils. Two CBH's (CBH 1 and CBH 2) and five EG's (EG 1, EG 2, EG 3, EG 4 and EG 5) have been so far reported. The structures of CBH 1, CBH
2, EG 1 and EG 3 are already solved. Cel45A, (GH family 45), is a minor component and it is interesting however, because it has one of the smallest cellulase catalytic modules (~160aa) found in a bi-modular cellulase (i.e. with CBD and linker attached to the catalytic module).

Datasets collected on ID14:EH4

Small crystals (<100um) of the catalytic module of EG5 has been used at ID 14-4, and a single dataset to 2.2 A has been collected. EG5 is homologous to the enzyme BX1 (from the common
blue mussel found in the North Sea) and we expect to be able to solve the structure by molecular replacement.

Data statistics for the dataset:

Ligand: No
Resolution: 35.8-2.2 A
Space group: P1
Cell: a: 37.578, b: 45.263, c: 48.153, ? : 72.12, ? : 70.94, ?: 86.56

Completeness: 97%

Protein Name EG2: Catalytic module of Endoglucanase 2 from *T. reesei*
Brief background and outline of project

(Cel5A, GH family 5). Efficient cellulose degrading organisms secrete a mixture of synergistically acting cellulases with different specificities. For a more detailed picture of how these enzymes cooperate we beleive that it is essential to know the structures of all the enzymes involved in the process. Therefore one of our goals is to determine the structure of those cellulases of Trichoderma, for which it is unknown. EG2 is the second most abundant of the endo-glucanases, constituting 5-10% of secreted proteins under cellulase inducing conditions. Small crystals (<100um) of the catalytic module of EG2 diffracted to 1.8 A at ID 14-4, but were disordered. We hope to solve the structure by molecular replacement using known GH family 5 structures as search models.