# LS-1796
# Marseille BAG

# ID14-2
# 5 February 2001

| Project | Responsible | B'line | Date | Method | Space Grp | Cell (A) | MW kDa | mol/au | Resol (A) | Rsym (%) | Comp. (%) | Mult. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AGX1+ UDPGalNAc | Caroline Peneff | ID14-2 | 05/02/01 | MR | p21 | 85.735 70.769 95.384 β=95.26 | 60 | 2 | 2.10 | 6.60 | 97.60 | 2.60 |
| CBM6 complex avec xylose | Mirjam Czjzek | ID14-2 | 05/02/01 | MR | P65 2 2 | 60. 60. 157.5 | 15 | 1 | 2.20 | 12.40 | 97.00 | 3.50 |
| CelE multi-domain cellulase dhurrinase, homologue a rglu avec specificite differente | Mirjam Czjzek | ID14-2 | 05/02/01 | MR | P 21 21 2 | 96.0 118.0 79.0 | 86 | 1 | 1.60 | 6.30 | 98.70 | 2.80 |
| RGlu-e191d en complex avec VAS1 et VAS2 (inhibiteur) | Mirjam Czjzek | ID14-2 | 05/02/01 | test | test | test | | | | | | |
| NOR | Kieron Brown | ID14-2 | 05/02/01 | MR | P 21 21 21 | 93.5 96.2 118.6 | 58 | 2 | 2.20 | 8.40 | 99.50 | 3.20 |
| NiR-Zn | Kieron Brown | ID14-2 | 05/02/01 | test | | | | | | | | |
| BOAR native | Silvia Spinelli | ID14-2 | 05/02/01 | MR | P43212 | 99.5 99.5 275.1 | 60 | 1 | 3.80 | 11.20 | 98.00 | 4.20 |
| CSP2 non ligandee | Valérie Campanacci | ID14-2 | 05/02/01 | MR | p41212 | 70.112 70.112 71.75 | 21 | 1 | 2.13 | 5.60 | 99.60 | 3.90 |
| MT + gpppafg6 | Marie-Pierre Egloff | ID14-2 | 05/02/01 | MR | P3121 | 112.4 112.4 56.7 | 33 | 1 | 3.20 | 8.30 | 98.30 | 2.80 |
| MT+gtpfa6 | Marie-Pierre Egloff | ID14-2 | 05/02/01 | MR | P3121 | 111.6 111.6 56.3 | 33 | 1 | 2.80 | 9.90 | 99.80 | 3.80 |
| MT+gpppafpg4 | Marie-Pierre Egloff | ID14-2 | 05/02/01 | MR | P3121 | 111.3 111.3 56.3 | 33 | 1 | 2.90 | 7.30 | 99.90 | 5.10 |
| complex VHH307-R120 | Silvia Spinelli | ID14-2 | 05/02/01 | MR | P6 | a=b=115.2 c=174.7 | 14 | | 2.39 | 10.00 | 99.80 | 3.50 |

| | | |
|---|---|---|
| ESRF | **Experiment title: Baor salivary lipocalin** | **Experiment number**: LS1796 |
| **Beamline**: ID14-2 | **Date of experiment**: from: 05.02.01 to: 06.02.01 | **Date of report**: Feb 01 |
| **Shifts:** **3** | **Local contact(s)**: Stephanie Monaco | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

Silvia Spinelli , AFMB laboratory, CNRS Marseille

## Report:

## Native salivary lipocalin from BOAR

The salivary protein from boar submaxillary glands belongs to the MUP-like lipocalins.
Crystals of Boar native was frozen to 100 K using 17% glycerol as cryoprotectant.
The space group and cell dimensions were confirmed from a preliminary exposure to be P41212, a=b=70.095 Å , c=71.705 Å.
Two data set were collected at 100 K, one to high resolution with an exposure time of 30 sec per degree and the other to low resolution with 15 sec per degree, see table bellow:

Data collection

| | |
|---|---|
| Total number of observation | 83061 |
| Number of unique reflections | 10956 |
| Overall % data > 1 sigma(I) (last shell) | 99.6 (99.6) |
| Overall R merge (%) (last shell) | 5.6 (29.5) |
| Overall I/sigma(I) (last shell) | 7.3 ( 2.4) |
| Resolution (Å) | 28.7 - 2.1 |

The 3-D structure of the Boar native was determined by molecular replacement, using horse allergen (PDBCODE: 1EW3) as starting model. The model is presently been refined with CNS, slow cooling and energy minimisation, using all data between 25.8 and 2.13 A.
The final R work is 25.4% (R free 28.2%).
A publication of this work is presently in preparation.

| ESRF | **Experiment title: Dengue capping enzyme : soaks with different cap analogs and RNA oligonucleotides** | **Experiment number**: LS1796 |
|---|---|---|
| **Beamline**: ID14-2 | **Date of experiment**: from:05 02 2001 to: 06 02 2001 | **Date of report**: 01 03 2001 |
| **Shifts:** 3 | **Local contact(s)**: Stéphanie Monaco | *Received at ESRF:* |

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Egloff Marie-Pierre *

Benarroch Delphine

Selisko Barbara

Romette Jean-Louis

Canard Bruno

## Report:

## Please refer to the previous reports (06-08 10 2000 and 04-06 11 2000) for general introduction on this project and description of the previous work.

We have obtained biochemical evidence of RNA binding to CEF. Therefore CEF crystals were this time submitted to soaks with some short RNA oligonucleotides (4 to 6 bases ) in the presence or absence of the GTP analog or the GpppA cap analog. The following data sets have been collected:

| | Space group | Resolution (A) | cell | Rmerge (%) | completeness | redundancy |
|---|---|---|---|---|---|---|
| gpppafg6 | P3121 | 3.2 | 112.4 112.4 56.7 | 8.3 | 98.3 | 2.8 |
| gtpfa6 | P3121 | 2.8 | 111.6 111.6 56.3 | 9.9 | 99.8 | 3.8 |
| gpppafpg4 | P3121 | 2.9 | 111.3 111.3 56.3 | 7.3 | 99.9 | 5.1 |

Data are currently under analysis.

## This work has not been published yet and should be kept strictly confidential.

| | | |
|---|---|---|
| ESRF | **Experiment title: AGX1-UDPGalNAc** | **Experiment number**: LS 1796 |
| **Beamline**: ID14-2 | **Date of experiment**: from: 05.02.2001 to: 06.02.2001 | **Date of report**: 26.02.2001 |
| **Shifts:** 3 | **Local contact(s)**: Stephanie Monaco | *Received at ESRF:* |


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# Crystal structures of the human UDPGlcNAc (UDPGalNAc) pyrophosphorylases AGX1 and AGX2


**Caroline Peneff[1], Véronique Zamboni[1], Florence Fassy[2] and Yves Bourne[1].**

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UDPGlcNAc is a ubiquitous and essential metabolite in eukaryotes as well as in prokaryotes. AGX1 and AGX2 are the human enzymes responsible for UDPGlcNAc biosynthesis from UTP and GlcNAc-1P in presence of Mg2+. These enzymes are isoforms of the same gene and differ by the insertion in AGX2 of an additional 17-residue segment near the C-terminus. Substrate specificity studies showed that these two enzymes have both UDPGlcNAc and UDPGalNAc synthesis activity at a ratio of 1:3 for AGX1 and 8:1 for AGX2, suggesting that the 17-residue insert is responsible for a change in the enzyme activity[1].

To better understand the catalytic mechanism of these enzymes as well as the 17 residue-induced substrate specificity change, we have solved the structures of AGX1 and AGX2 complexed with UDPGlcNAc. The structure of AGX1-UDPGlcNAc was solved by Se-Met MAD at beamline BM14 and refined it at 1.9 Å resolution, whilst that of AGX2-UDPGlcNAc was obtained by molecular replacement using the AGX1 model and data collected on beamline ID14-3. (in first part of the year 2000).

These structures reveal an AGX fold composed of 3 domains: a large central domain reminiscent of the Rossmann fold, as found in other nucleotidyltransferases, flanked by two smaller extra domains, the N-terminal domain which defines a new fold and the C-terminal domain which is mostly involved in dimerisation of AGX1. Our results provide new features in the architecture of the UDPGlcNAc binding site located at the dimer interface of AGX1. Yet, a better understanding of the reported substrate specificity change between the two enzymes further required the structures of these enzymes complexed with UDPGalNAc. Crystallisation of AGX2 in presence of UDPGalNAc allowed the obtention of the structure from a data set collected during a previous shift. However, a different strategy was employed for AGX1 which required the dissociation of the dimer. Data were collected on one of the crystals obtained

and the structure solution showed that UDPGalNAc had partly replaced UDPGlcNAc already present in the active site of AGX1.

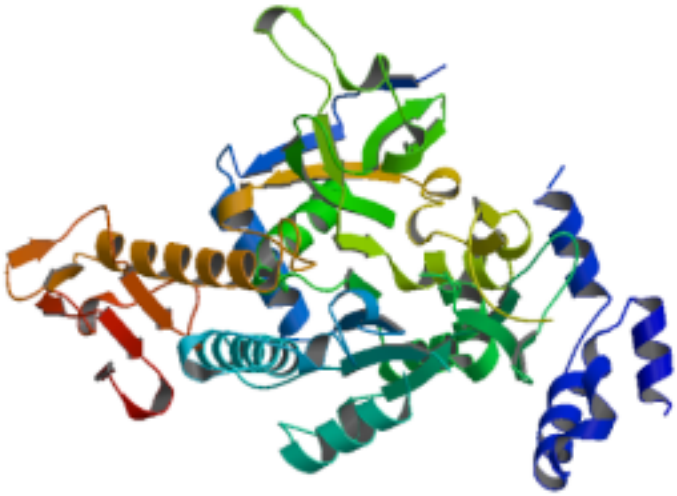

Figure1: Ribbon diagram of the AGX1 structure.

**References**

1 – A. Wang-Gillam, I. Pastuszak & A. D. Elbein. (1998), *J. Biol Chem* **273** (42), 27055-57.

**Publication arisen from this work:**

Peneff C., Zamboni V., Fassy F. and Bourne Y., Crystal structures of the human UDPGlcNAc (UDPGalNAc) pyrophosphorylases AGX1 and AGX2, *in preparation*.

| ESRF | **Experiment title:** Protein Crystallography at AFMB CNRS Marseille | **Experiment number**: LS1796 |
|---|---|---|
| **Beamline**: ID14 EH2 | **Date of experiment**: from: 5 fev 2001 to: 6 fev 2001 | **Date of report**: Feb 01 |
| **Shifts:** 3 | **Local contact(s)**: Stefanie Monaco | *Received at ESRF:* |

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Julie Allouch, CNRS-AFMB Marseille

## Report: CBM module (family 6) from the xylanase U from *C. thermocellum*

Many glycoside hydrolase enzymes are composed of a catalytic domain coupled with one or several "carbohydrate binding modules" ( further called CBM's). These modules, such as the catalytic domains, can be grouped in families, having the same structural fold. Within a family the specificity may vary but the members are marked by highly conserved residues, responsible for the binding sites. The CBM from xylanase U, 133 amino acids long, has been classified in family 6 of the CBM's and we recently determined the structure by MAD on a seleno-methionine, mutant protein. To get high resolution information of the residues involved in substrate binding, the 3D structure of the complex will be of great help. A first temptative of getting a binding module/substrate complex by soaking experiments was performed and a data set of CBM6 crystals soaked for several hours with soluble xylupentaose was collected on ID14 EH2. Unfortunately, it seems that the binding sites are involved in crystal packing contacts and no substrate molecules could be identified in the 2.2 Å density maps. In future experiments we will try to acheive complex structures by co-crystallisation of the CBM6 with xylopentaose and shorter substrate molecules.

**Table 1. Data collection statistics**

| | **CBM6** |
|---|---|
| Space group | $P6_522$ |
| Resolution (Å) | 2.2 |
| No. observations | 30081 |
| No. unique | 8678 |
| $R_{sym}$ (%) | 12.4 (38.2) |
| $I/\sigma(I)$ | 5.2 (1.9) |
| Redundancy | 3.5 |
| Completeness (%) | 97.0 (97.2) |

## CelE from Clostridium cellulolyticum, a family 9 glycosyl hydrolase

Family 9 cellulases display a high variability in substrate specificity. This variability is caused by the presence of one or several variable CBD modules associated with the family 9 catalytic domain within the primary sequence. Cellulase E (82000 Daltons, ~850 aa 1molecule per AU), from *C. cellulolyticum* is composed of a catlytic domain of family 9, a immunoglobulin-like domain and a CBD of family 4. In order to understand the synergy of the independently structured modules a 3-D structural model of the entire enzyme is of great interest. The molecular replacement with representative 3-D structures of family 9 enzymes failed up to date, since the data collected so far were not complete enough. We modified or cryocooling conditions in order to get crystals displaying less mosaicity and collected a hihgly complete data set at 1.6 Å resolution on ID14

EH2. The data revealed that the crystals belonged to space group $P2_12_12$ (unit cell : a=96.6Å, b=110.6Å c=79.8Å). A contrasted but rather weak solution (corr.coef. 15.1, R-factor 50.3 in the resolution range 11-4.5 Å) was obtained by molecular replacement with the catalytic domain of CelD from C.thermocellum. The structure solving procedure is still under way.

**Table 1. Data collection statistics**

| | **CelE** |
|---|---|
| Space group | $P2_12_12$ |
| Resolution (Å) | 1.6 |
| No. observations | 307568 |
| No. unique | 111341 |
| $R_{sym}$ (%) | 6.3 (26.6) |
| I/σ(I) | 6.8 (2.5) |
| Redundancy | 2.8 |
| Completeness (%) | 98.7 (98.4) |

**ZMGlu1 from Maize, the inactive mutant E191D in complex with two synthesized inhibitor molecules**

β-glucosidases constitute a major group among glycoside hydrolases. They have been the focus of much research recently because of their important roles in a variety of fundamental biological and biotechnological processes. For example, plant β-glucosidases have been implicated in defense against pests, phytohormone activation, lignification and cell wall catabolism. They belong to families 1 and 3 of the glycoside hydrolases and hydrolyze either O-linked β-glycosidic bonds, or S-linked β-glycosidic bonds (myrosinase). The mechanism and the site of substrate (i.e., aglycone) recognition and specificity was directly investigated in maize β-glucosidase by x-ray crystallography using crystals of a catalytically inactive mutant (ZMGlu1E191D) in complex with synthesized inhibitor molecules. The inhibitors are glucose units with the C1 position rigidified by a double bond to a nitrogen group (Figure 1). These inhibitor molecules mimic the transition state and give helpfull informations of how the substrate olecules are bound within the catalytic active site.

**Table 1. Data collection statistics**

| | **Inhibitor A** | **Inhibitor B** |
|---|---|---|
| Space group | $P2_12_12_1$ | $P2_12_12_1$ |
| Resolution (Å) | 2.2 | 2.0 |
| No. observations | 194654 | 286955 |
| No. unique | 61510 | 85752 |
| $R_{sym}$ (%) | 8.4 (34) | 8.1 (38) |
| I/σ(I) | 3.1 (1.8) | 6.9 (1.9) |
| Redundancy | 3.2 | 3.3 |
| Completeness (%) | 99.5 (100) | 96.6 (94.0) |

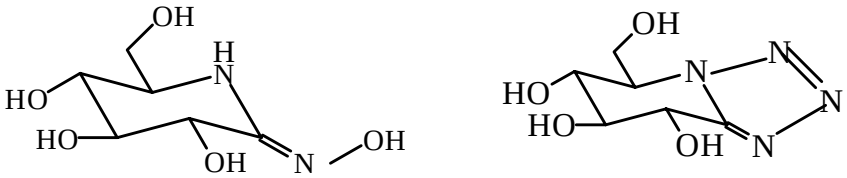


Figure 1a,b: Chemical structure of the inhibitor molecules A (left) and B used in the soaking experiments with ZMGlu1 E191D. The molecules mimic the transition state of the two-step catalytic reaction in β-glucosidases.

| | | |
|---|---|---|
| ESRF | **Experiment title:**<br>Structure of Lama Antibody | **Experiment number**:<br>LS 1796 |
| **Beamline**:<br>ID14-2 | **Date of experiment**:<br>from: 05.02.01 to:06.02.01 | **Date of report**:<br>Feb 01 |
| **Shifts:**<br>**3** | **Local contact(s)**: Stephanie Monaco | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

Silvia Spinelli, AFMB laboratory, CNRS Marseille

## Report:

**Lama antibody**

Crystals of the lama single domain VHH 307 complex with a dye (R120) were frozen to 100 K using 25% glycerol as cryoprotectant.

The space group and cell dimensions were confirmed from a preliminary exposure to be P6, a=b=115.2 Å , c=174.7 Å.

One data set was collected to 100 K, with an exposure time of 32 sec per degree, see table bellow:

Data collection

| | |
|---|---|
| Total number of observation | 512992 |
| Number of unique reflections | 66365 |
| Overall % data > 1 sigma(I) (last shell) | 99.8 (99.8) |
| Overall R merge (%) (last shell) | 10.0 (39.0) |
| Overall I/sigma(I) (last shell) | 6.0 ( 2.0) |
| Resolution (Å) | 20.0 - 2.39 |

The resolution of this structure is currently under way by molecular replacement.