LS-1796
Marseille BAG

ID14-4
2-4 December 2000

Project	Responsible	B'line	Date	Method	Space Grp	Cell (A)	MW kDa	mol/au	Resol (A)	Rsym (%)	Comp. (%)	Mult.
NiR-Zn	Kieron Brown	ID14-4	02/12/00	MR	P43212	90 90 273	55	1	3.50	9.00	99.00	5.00
CSP2 Br MAD	Valérie Campanacci	ID14-4	02/12/00	MAD	P21	33 54 55 90 93 90	12	2	1.40	5.20	97.00	4.00
XylU-CBM Semet mutant pour MAD	Mirjam Czjzek	ID14-4	02/12/00	MAD	P6522	60.1 60.1 157.5	20	1	2.60	6.08	98.90	6.20
AGX1 + Mgcl2	Caroline Peneff	ID14-4	02/12/00	MR	p21	85.986 70.736 95.603 β=95.033	60	2	2.20	6.70	99.60	3.20
AGX1+ UDPGalNAc	Caroline Peneff	ID14-4	02/12/00	mr	p21	86.108 70.781 96.117 β=95.176	60	2	2.50	7.30	99.80	3.50
AGX2 + MgCl2	Caroline Peneff	ID14-4	02/12/00	mr	p21	85.456 71.372 92.290 β= 92.319	60	2	2.40	8.80	96.00	3.00
Agx2 + UDPGalNAc	Caroline Peneff	ID14-4	02/12/00	mr	p21	85.816 72.344 93.873 β= 93.988	60	2	2.30	7.00	98.90	3.40
AChE-Gallamine complex	Yves Bourne	ID14-4	02/12/00	MR	P212121	79 x 111 x 227	80	2	2.25	5.40	96.40	3.60

ESRF	**Experiment title:** Acetylcholinesterase	**Experiment number**: LS 1796
Beamline: EH4	**Date of experiment**: 2-4 December 2000 from: 8 am to: 7 am	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Elspeth Gordon	*Received at ESRF:*

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The peripheral anionic site of Mouse Acetylcholinesterase

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We used a new crystal form of mouse acetylcholinesterase (AchE) that permits more accurate studies of the peripheral anionic site of this enzyme. This new crystal form diffracts up to 2.2 Å resolution and contains two molecules in the asymmetric unit, with the catalytic gorge entrance being freely solvent accessible in each subunit. This differs from the previous 3 Å resolution structure (1) for which only one molecule out of two possessed a solvent-accessible gorge entrance. In addition, the previous crystal form grew with a high salt concentration compared to a low salt concentration required for the new crystal form, a critical parameter to study ligands directed to the peripheral anionic site. We used a new compound that displays high affinity for this site and performed co-crystallization experiments with AchE using large excess of this compound. Indeed, previous data from similar crystals soaked with similar compounds showed only partial occupancies. Data obtained from these crystals are of excellent quality. For these two structures, rigid-body refinement was then performed on each subunit with CNS using data between 30 Å and 3 Å and gave an R-factor value of 22.1%. For 2% of the reflections against which the two models were not refined, R-free was 21.9%. Refinement of this two structure is underway at 2.25 Å resolution. The final model will be used for a comparative study with other peripheral ligands for which data have been recently collected at ESRF.

References: (1) Bourne, Y., Taylor, P. & Marchot, P. (1999) *J. Biol. Chem*. **274**, 2963-70.

ESRF	**Experiment title: AGX1/2-MgCl2** **AGX1/2-UDPGalNAc**	**Experiment number**: LS 1796
Beamline: ID14-4	**Date of experiment**: from: 02.12.2000 to: 04.12.2000	**Date of report**: 26.02.2001
Shifts: 6	**Local contact(s)**: Elspeth Gordon	*Received at ESRF:*

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

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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. Both AGX1 and AGX2 were crystallised in presence of UDPGalNAc. Solving the structures of these complexes by molecular replacement showed the presence of bound UDPGalNAc in the active site of AGX2 but not in that of AGX1, in which the molecule of UDPGlcNAc already present had not been replaced.

In addition, In order to get further insight into the catalytic mechanism and most precisely to determine the role of the divalent cation indispensable for the reaction, we crystallised AGX1 and AGX2 in presence of $MgCl_2$ and diffraction data were collected. Unfortunately no extra electron density was observed in the active site suggesting that the magnesium cation was not present.

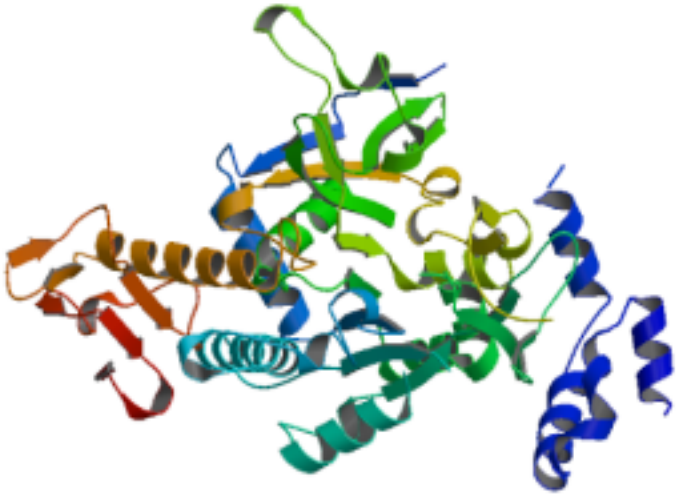

Figure1: Ribbon diagram of the AGX1 structure.

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:** **Crystallographic Studies of the Recombinant Nitrite Reductase Zn Protoporphyrin IX from *Pseudomonas aeruginosa***	**Experiment number**: LS 1796
Beamline: ID14-4	**Date of experiment**: from: 2/12/00 to: 4/12/00	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Elspeth Gordon	*Received at ESRF:*

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Report:

The dissimilatory nitrite reductase catalyses *in vivo*, the reduction of nitrite to nitric oxide according to the reaction $NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O$. Nitrite reductase is a homodimer of 120 kDa, carrying one *c* heme and one d_1 heme per monomer. The *c* heme is the electron acceptor pole and is reduced by cytochrome c551. The *d1* heme is the catalytic site. The crystal structure of nitrite reductase from *Pseudomonas aeruginosa* has previously been solved in both the oxidised and reduced redox states (Nurizzo *et al*. 1997, 1998) and shows that a conformational change in the loop 56-61, and an associated displacement of the N-terminal arom carrying the Ty 10, the 6th axial ligand of the d1 heme iron.

The catalytic cycle of nitrite reductase from *Pseudomonas aeruginosa* involves the electron transfer between the two prosthetic groups of the enzyme, the c-heme and the d_1-heme. The recombinant enzyme from *Pseudomonas putida* has been reconstituted with Zn protoporphyrin IX in place of the d_1 heme by F. Cutruzzolà (Universita di Roma, Italy). The interest of this enzyme is that the mid-point potential of the two hemes is altered and therefore electron transfer disrupted.

Crystals of this enzyme form were obtained and a medium resolution X-ray diffraction data set collected on ID14-2 (ESRF). This 3.5 Å resolution dataset enabled a molecular replacement solution to be obtained and indicate that, as with the mutant enzyme forms, the c-heme domain of the Zn protoporphyrin IX enzyme has undergone a 60° rigid-body rotation. However, the results from this preliminary refinement are questionable because at present there is no evidence of the heme in the crystal structure.

Data Collection Statistics

Beamline	ID14-EH2
Space group	$P4_32_12$
λ (Å)	0.9326
Resolution (Å)	3.5
R_{sym} (%)	8.7 (36.1)
I/σ	6.0 (1.8)
Completeness	99.9 (99.9)
Redundancy	5.6

References

Nurizzo D, Silvestrini MC, Mathieu M.,Cutruzzolla F., Bourgeois D., Fulop V., Hajdu J.,Brunori M., Tegoni M., Cambillau C (1997) Structure, **5**, 1157

Nurizzo D, Cutruzzolla F., Arese M., Bourgeois D., Brunori M., Cambillau C., Tegoni M. (1998) Biochemistry, **37**, 13987

Publications arising from this work

Brown K, Cutruzzolla F., Wilson E.K., Bellelli A., Arese M., Brunori M., Tegoni M., Cambillau C (in preparation)

ESRF	**Experiment title:** Protein Crystallography at AFMB CNRS Marseille	**Experiment number**: LS1796
Beamline: ID14 EH4	**Date of experiment**: from: 2. dec 2000 to: 4. dec 2000	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**:	*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 no structure is known to date. In order to understand the origin of the different specifcities and the role of the binding modules on catalytic activity, the structural model is a precious information that helps interpretate bichemical and enzymatic results. CBM6 from Xylanase U naturally does not contain methionine residues. Four mutants, each introducing a methionine residue, Y40M, R72M, W92M and Y112M, were therefore produced in conditions leading to selenomethionized proteins. Two of the mutants crystallized under the same conditions as the native protein. After testing and analysing the data on ID14 EH2, the crystals of the most suitable mutant protein, CBM6-R72M were chosen for a MAD data collection on ID14 EH4, at the selenium edge. A 30° sweep per wavelength is needed for space group $P6_522$. Complete data sets were collected at three wavelengths, picked up in a flourescence spectra of a crystal, corresponding to the peak (e1; λ=0.9793Å), the inflection point (e2; λ=0.9795Å) and a remote wavelength (e3; λ=0.94646). The data collection statistics are given in Table 1.

Table 1. Data collection statistics

CBM6 R72M	**e1** **peak**	**e2** **inflection**	**e3** **remote**
Wavelength (Å)	0.9793	0.9795	0.9464
Resolution (Å)	2.6	2.6	2.88
No. observations	22264	26065	20733
No. unique reflections	5530	5210	4163
No. anomalous reflections	4037	4064	2935
R_{sym} (%)/ R_{ano}	6.8 / 3.4	5.7 / 2.8	5.6 / 3.0

I/σ(I)	10.0 (5.6)	9.5 (6.4)	10.8 (8.3)
Redundancy	4.0	4.9	5.0
Completeness (%)	98.2 (96.9)	97.9 (97.8)	99.0 (96.4)
Anomalous Completeness (%)	97.3 (95.1)	97.6 (97.1)	98.5 (96.5)

The structure was solved using the programs SHARP and DM, leading to a FOM value of 0.789 at 3.2 Å resolution. The experimental map is shown in Figure 1a,b at 3.2 Å resolution. Clear features delimiting the solvent from the protein are visible (Fig1a) and the secondary structure elements were detectectable (Fig. 1b). A native data set had been collected on ID14 EH2, experiment number LS1657. The refinement of the structural model is under way. Figure 2 shows the ribbon diagram of the structure of CBM6 from Xylanase U, which has the jelly roll fold. The xylan binding site is most probably located on the loops connecting the beta-sheets, involving the aromatic residues highlighted in Figure 2.

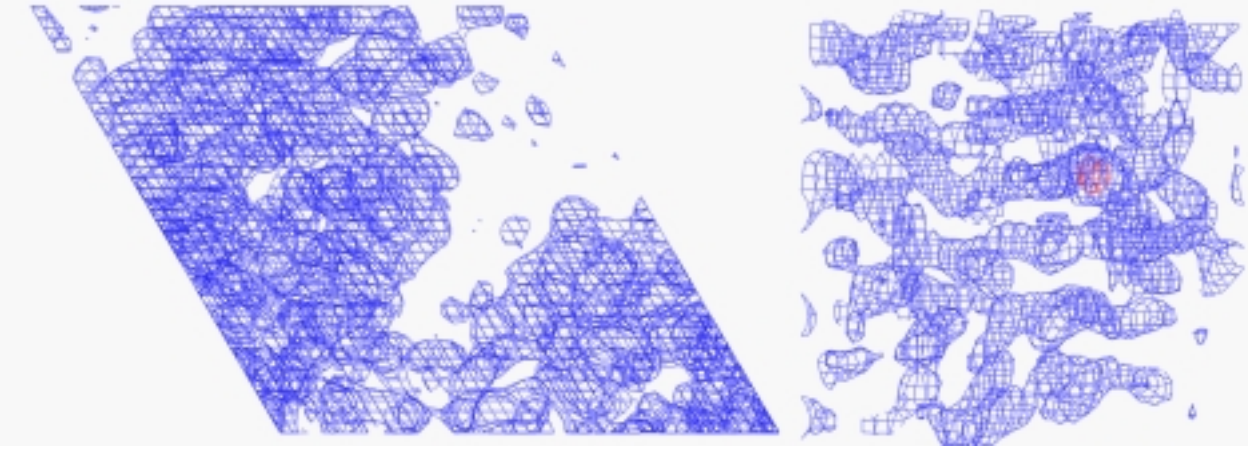

Figure 1a,b: Experimental map at 3.2 Å resolution, contoured at 1σ (blue) and 5σ (red). The red density corresponds to the Selenium of the selenometionine of the mutant R72M.

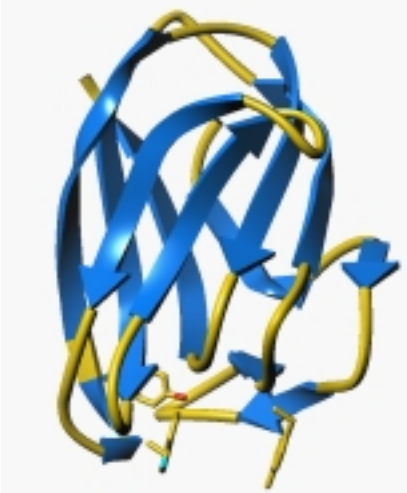

Figure 2: The CBM6 fold belongs to the so called jelly-roll's, formed by two beta-sheets stacked one onto the other. The binding site is most probably located on the bottom, where a tryptophane and a tyrosine align in a parallel manner.

ESRF	**Experiment title:** **CSP/bromo-dodecanol complex**	**Experiment number**: **Ls1796**
Beamline: ID14-EH4	**Date of experiment**: from: 2 Dec 2000 to: 4 Dec 2000	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Elspeth GORDON	*Received at ESRF:*

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Report:

Crystals of MbraCSP complexed with a brominated-pheromone-like compound (bromo-dodecanol) were frozen to 100 K with no cryoprotectant.

The space group and cell dimensions obtained were the followings: $P2_1$, 33.4x54.2x56.4 Å, $\alpha=\gamma=90°$, $\beta=93.9°$.

Three data set were collected to 100 K, with an exposure time of 3 sec per degree, see table bellow.

Data collection			
Wavelength (Å)	0.919647 (*f*'max)	0.920167 (*f*'min)	0.911656 (remote)
Total number of observation	285196	515850	433035
Number of unique reflections	38729	39019	31988
Overall % data > 1 sigma(I) (last shell)	95.7 (95.7)	93.7 (93.7)	96.0 (96.0)
Overall R merge (%) (last shell)	5.7 (28.0)	7.5 (26.6)	6.7 (29.2)
Overall R anomalous (%) (last shell)	5.1 (3.7)	5.0 (20.6)	3.8 (13.9)
Overall I/sigma(I) (last shell)	5.7 (2.6)	4.6 (2.4)	5.7 (2.4)
Resolution (Å)	30.0 - 1.4	30.0 - 1.4	30.0 – 1.5
Redundancy	2.9	3.9	5.4

After indexation and integration, data were analyzed by SOLVE and CNS using the phasing power of bromine. Unfortunately, no site was found, probably due to the mobility of the brominated ligand in the cavity of the protein.