LS-1796
Marseille BAG

ID14-3
6-8 October 2000

Project	Responsible	B'line	Date	Method	Space Grp	Cell (A)	MW kDa	mol/au	Resol (A)	Rsym (%)	Comp. (%)	Mult.
GlmU-GlcN-1-P	Gerlind Sulzenbacher	ID14-3	06/10/00	MR	R 3	92.477 92.477 279.196	51	2	2.10	5.60	99.90	4.30
GlmU-RO	Gerlind Sulzenbacher	ID14-3	06/10/00	MR	R 3	92.351 92.351 279.371	51	2	2.50	2.90	99.70	2.60
AChE complexed to PAS inhibitors	Yves Bourne	ID14-3	06/10/00	MR	P2(1)2(1)2(1)	79 x 111 x 227	80	2	2.25	4.10	99.50	3.40
apha1,3 complex	Louis Gastinel	ID14-3	06/10/00	MR	P4(1)2(1)2	95.6 x 95.6 x 110.7	30	1	2.40	5.00	99.50	6.70
MT+GTPanalog	Marie-Pierre Egloff	ID14-3	06/10/00	MR	P3121	112.2 112.2 56.46	32	1	2.80	4.40	99.00	4.00
MT + GMP	Marie-Pierre Egloff	ID14-3	06/10/00	MR	P3121	112.2 112.2 56.33	32	1	2.80	6.80	97.70	4.40
MT + myco	Marie-Pierre Egloff	ID14-3	06/10/00	MR	P3121	111.85 111.85 55.72	32	1	3.00	17.10	96.50	3.50

ESRF	**Experiment title: GlmU-RO** **GlmU-GlcN-1-P**	**Experiment number**: LS 1796
Beamline: ID14-3	**Date of experiment**: from: 06.10.2000 to: 08.10.2000	**Date of report**: 24.02.2001
Shifts: 6	**Local contact(s)**: Laurence Dumon	*Received at ESRF:*

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Report:

Crystal structure of *S. pneumoniae* GlmU and its complexes

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The bifunctional bacterial enzyme *N*-acetyl-glucosamine-1-phosphate uridyltransferase (GlmU) catalyses acetyltransfer from acetyl-coenzyme A (AcCoA) to glucosamine-1-P (GlcN-1-P) with release of GlcNAc-1-P, and subsequently uridyltransfer from UTP to GlcNAc-1-P in the presence of Mg^{2+}, yielding PPi and the nucleotide-activated precursor sugar UDP-GlcNAc. UDP-GlcNAc is the main precursor for bacterial cell wall biosynthesis, and the absence of a bifunctional enzyme equivalent to GlmU in eukaryotes, makes this enzyme an attractive target for the design of new antibiotics.

We have solved the structure of the full-length enzyme from *Streptococcus pneumoniae* by Se-Met MAD at beamline BM14 (in the year 1999) and collected data for complexes with substrates, reaction intermediate and the final product at beamlines ID14-EH2 and ID14-EH3 (in first part of the year 2000).

The enzyme assembles into a trimeric arrangement, with the LβH acetyltransferase domains packed tightly against each other in a parallel fashion, and the uridyltransferase domains being projected away from the threefold axis by a long α-helical arm. AcCoA binding induces the formation of a long and narrow tunnel, enclosed between two adjacent LβH domains and the interchanged C-terminal region of the third subunit, giving rise to an original active site architecture at the junction of three subunits. Clear difference density could be observed for bound acetyl-CoA, UDP-GlcNAc, UTP and Mg^{2+}, giving insight both into substrate recognition and reaction mechanism.

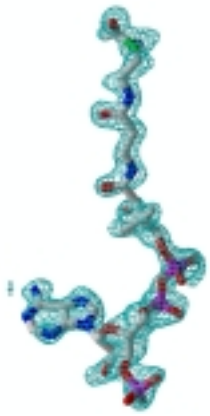

Figure 1: 1.75 Å resolution averaged 2Fo-Fc map calculated prior inclusion of AcCoA into the model (1.0 σ, blue; 3.0 σ, black).

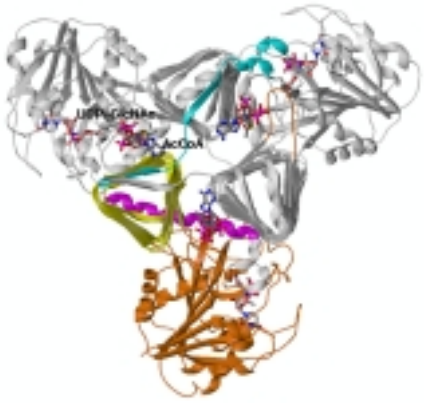

Figure 2: Ribbon diagram of the GlmU trimer with bound AcCoA and UDP-GlcNAc viewed down the LβH axis

GlmU represents an attractive target for the development of new antibiotics. In order to get insight into the exact mode of binding of the natural substrate for the acetyltransferase reaction and of a potential inhibitor developed by our industrial co-workers, we have collected data on crystals soaked in GlcN-1-P and RO on beamline ID14-EH3. Data collection statistics are given in Table 1. Unfortunately no binding for either compounds could be observed.

Table 1. Data collection statistics for GlmU-GlcN-1-P and GlmU-RO on beamline ID14-EH3.

	GlmU-GlcN-1-P	**GlmU-RO**
Method	F_o - F_c	F_o - F_c
Space group	R 3	R 3
N. of molecules / asymmetric unit	2	2
Cell dimensions	a=b=92.477,c=279.196	a=b=92.351,c=279.371
Resolution (Å)	2.1	2.5
R_{merge}	5.6	2.9
I / σ(I)	8.1	16.9
Redundancy	4.3	2.6
Completeness (%)	99.9	99.7

References

Brown, K., Pompeo, F., Dixon S., Mengin-Lecreulx, D., Cambillau, C. & Bourne, Y. (1999). *EMBO J.* **18**, 4096-4107.

Publication arisen from this work:

Sulzenbacher, G., Gal, L., Peneff, C., Fassy, F.& Bourne, Y. (2000). Crystal structure of Streptococcus pneumoniae N-acetyl-glucosamine-1-phosphate uridyltransferase bound to acetyl-coenzyme A reveals a novel active site architecture. *J. Biol. Chem., in press*.

ESRF	**Experiment title: Dengue capping enzyme : soaks with mycophenolic acid, GMP and a GTP analogue**	**Experiment number**: LS1796
Beamline: ID14-EH3	**Date of experiment**: from: 06 10 2000 to: 08 10 2000	**Date of report**: 01 03 2001
Shifts: 6	**Local contact(s)**: Laurence Dumon	*Received at ESRF:*

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Report:

Dengue has recently been classified by the World Health Organization as a priority in emerging diseases due to increasing epidemics over the past 20 years. Dengue, dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) are prevalent in over 100 countries and threaten the health of more than 2.5 billion people, living in tropical and subtropical regions. The annual incidence is estimated to be in the tens of millions, with an estimated 500 000 hospitalized cases of DHF/DSS, 90% of whom are children under the age of 15 years. The average mortality rate is 5%, with some 24 000 deaths each year. A specific cure for dengue does not exist, the treatment is largely supportive. Vaccination strategies are complicated by the presence of four serotypes DEN-1, 2, 3 and 4 present in all tropical areas around the world.

Dengue belongs to the flavivirus subgroup of the *Flaviviridae* family comprising several other important human pathogens like yellow fever and encephalitis viruses. The other two subgroups consist of pestiviruses (bovine viral diarrhea, classical swine fever) and hepatitis C viruses. Dengue virus contains a single positive-stranded RNA genome which has a type 1 cap at its 5' end and is translated directly into a polyprotein. The polyprotein is subsequently processed by viral and host cell protease activities to produce three structural and seven non-structural proteins. Enzyme activities which are essential for virus replication are associated with the non-structural proteins. In particular, proteins NS3 and NS5 were described to interact *in vivo* and *in vitro*

to constitute the RNA replicase complex. NS3 presents several activities associated with virus replication: NTPase, helicase and RNA binding, and a protease activity within the N-terminal 167 residues.

The cap is a structure which is essential for both mRNA stability and for binding to the ribosome during translation. It is found at the 5'-end of viral and cellular eukaryotic mRNA. mRNA capping results of three chemical reactions: The 5'-triphosphate of the mRNA is first converted to a diphosphate by an RNA triphosphatase. The second reaction is a transfer of a GMP moiety from GTP to the 5'-diphosphate RNA by the guanylyltransferase (capping enzyme) to yield $G^{5'}$-$ppp^{5'}$-N. In a third reaction utilizing S-adenosyl-L-methionine as the methyl donor, the transferred guanosine moiety is methylated by a methyltransferase at its N7 position to yield $^{7Me}G^{5'}$-$ppp^{5'}$-N (cap 0 structure). In some instances, a second methyl transfer reaction methylates the 2'-OH of the first nucleotide 3' to the triphosphate bridge to yield $^{7Me}G^{5'}$-$ppp^{5'}$-$N_{2'0Me}$ (cap 1 structure).

We reasoned that structural insights into viral RNA capping and its inhibition would help the rational design of inhibitors directed against Flaviviruses. NS5 was shown to exhibit RNA-dependent RNA polymerase activity producing double-stranded RNA and sequence analyses localized the polymerase activity to the C-terminal domain of NS5 (Pol-domain). The N-terminal domain is expected, on the basis of sequence comparison studies, to present an S-adenosylmethionine (SAM)-utilizing methyltransferase activity (MTase domain) that has not been demonstrated yet. Based on three dimensional structure prediction and sequence analysis, a thirty-three kDa N-terminal domain of NS5 of the Dengue virus type 2 (New Guinea) was cloned and expressed in a soluble form in *E.* This domain is referred to as CEF (Capping Enzyme of Flavivirus).

This domain has been purified, crystallized and its structure has been solved by the MAD method on BM14. This work has been described in the previous BAG report. In order to get futher insights into the CEF mecanism and to corroborate some biochemical results, we needed to get the crystal structure of CEF in complex with substrates or substrate analogues. Examination of the crystal packing shows some large chanels suitable for the diffusion of small molecules. Three data sets have been collected on three crystals which have been soaked in mycophenolic acid, GMP, and a GTP analogue, respectively. The data collection statistics are given below:

	Space group	Resolution (A)	cell	Rmerge (%)	completeness	redundancy
Mycophenolic acid	P3121	3.0	111.8 111.8 55.7	17.1	96.5	3.5
GMP	P3121	2.8	112.2 112.2 56.3	6.8	97.7	4.4
GTP analogue	P3121	2.7	112.2 112.2 56.46	4.4	99.0	4.0

After molecular replacement and refinement, the Fourier difference maps were inspected. An extra density was found in the case of the GTP analogue. These data were refined between 30 and 2.7 A resolution to an R factor of 21.3 (FreeR of 24.1). Neither GMP nor mycophenolic acid bound to CEF.

These results have not been published yet and should be kept confidential.

ESRF	**Experiment title:** Acetylcholinesterase	**Experiment number**: LS 1796
Beamline: EH3	**Date of experiment**: 6-8 October 2000 from: 8 am to: 7 am	**Date of report**:
Shifts: 6	**Local contact(s)**: Laurence Dumon	*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 and R-free value of 25.4. For 2% of the reflections against which the two models were not refined, R-free was 24.9%. 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.