	Experiment title: BAG-LEBS-2006-2	Experiment number: MX-554
Beamline: ID29	**Date of experiment**: from: Friday 16/12/2006 at 8:30 Monday Saturday 17/12/2006 at 8:00	**Date of report**: 28/2/06
Shifts: 3	**Local contact(s)**: Dr. A. Royant	*Received at ESRF:*

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Report:

C. Husson, L. Renault (0.5 shift): structural studies of complexes between the methyltransferase Rlma^{II} and RNA and complexes of actin with chimeric proteins of thymosin b4 and ciboulot.

structural studies of complexes between the methyltransferase Rlma^{II} and RNA

We used our 0.5 shift on the beam line ID29 to collect data sets on crystals of the methyltransferase RlmA^{II} (32kDa) in complex with a RNA substrate.

RlmA^{I} and RlmA^{II} are bacterial methyltransferases that modify the N-1 position of 23S ribosomal RNA nucleotides G745 and G748, respectively (Gustafsson *et al*., (1998), *J Bacteriol*; Douthwaite *et al*., (2004), *J Mol Biol*). Methylation of G748 is associated with resistance to tylosin and related 16-membered ring macrolide antibiotics. Our specific aim is to understand, at a molecular level, the structural basis for resistance to macrolide drugs and in particular how resistance enzymes recognize specifically their rRNA target by obtaining a high-resolution structure of RlmA^{II} complexed with its RNA substrate. The structure of

RlmA^{I} was solved (Das *et al.*, (2004), *PNAS*) but no structure of an antibiotic resistance enzyme that targets the ribosomal RNA in complex with its RNA substrate is available yet.

A native and SAD data sets were previously collected but the analysis of the data revealed a presence of pseudo-merohedral twinning. This time, the crystals, which were tested, had been frozen in different ways to try to reduce the twinning. The protein containts zinc. We have collected 4 complete SAD data sets on ID29. Crystals belong to space group P21

Crystal	Resolution range.	Completeness	I/sI	Rmeas %
1	30-2.4 (2.54-2.40)	91.8	8.49 (3.27)	9.2 (33.6)
2	30-2.3 (2.39-2.30)	93.5	7.53 (3.16)	10.3 (30.9)
3	30-2.75 (2.86-2.75)	95.4	10.62 (3.18)	8.3 (33.7)
4	30-2.85 (2.98-2.85)	95.9	9.28 (2.85)	9.3 (36.7)

Taking the pseudo-merohedral twinning into account, the heavy-atom site detection and phasing is underway. Some selenium and Zn sites have already been identified by anomalous difference fourrier from a weak molecular replacement solution but the anomalous signal is weak and the experimental phasing not helpful so far. We are still looking for additional heavy-atom derivatives while we are trying to build and correct part of the model in the poor MR density maps.

structural studies of complexes of actin with chimeric proteins of thymosin β4 and ciboulot.

The conserved WH2 actin-binding module, originally represented by β-thymosins, has been identified in a large number of proteins that all interact with actin and play diverse functions in cell motility. The versatile functions of the WH2 module may be linked to changes in the actin-WH2 interface that would affect actin conformation in a way that would favor or inhibit specific actin-actin interactions, thus accounting for promotion or inhibition of actin self-assembly. It is therefore important to characterize the nature of this change and the evolutionary trends of WH2 domains. The functional evolution of the WH2 domain has thus been approached by engineering a series of chimeras of Ciboulot and Thymosin Ã_4, in order to evaluate the contributions of the different regions of these two proteins containing WH2 domains with opposite functions: promoting actin barbed-end self-assembly and sequestering actin, respectively. Biochemical and dynamic analysis by RMN are combined with structural studies to characterize the molecular determinants controlling the function of promotion or inhibition of actin self-assembly by WH2 domains.

Diffracting crystals have been obtained with different chimeric proteins having different actin-sequestering activities. A previous data set has been collected at ESRF on july 2006 with the drug Latrunculin inhibiting actin polymerization. We succeeded to obtain small crystals of a more “physiological” chimeric protein/ actin complex without the drug in different conditions. These rod-like crystals were tested on ID29. Most of the crystals suffer of twinning. Four complete data sets on two different different chimeric proteins in complex with actin could be collected on ID29. Crystals are all twinned by pseudo-merohedry with a fraction of twinning of 50% in P21 emulating the orthorhombic symmetry. Two different complexes are currently being refined with the twinning fraction. The C-terminal part of the chimeric proteins regulating most of the sequestering activities seems to be flexible in both complexes. We are trying o obtain the complexes in different conditions to see this important C-terminal part of the chimeric proteins.

ZEEH Jean-Christophe, HANSSON Sebastian (0.5 shift): Complex between the small G protein Arf1D17-GDP and LM11 inhibitor (LM11) of the nucleotide exchange

ARF1 is a small G protein and involved in vesicular transport in cell. In 2003, we obtained the structure of Arf1 complexed with its regulator ARNO (a guanine exchange factor). By in silico screening, we find a new inhibitor of exchange reaction of Arf1 catalysed by ARNO, called LM11.The mechanism of this inhibitor is known and LM11 binds to the complex Arf1/ARNO and Arf1 alone , so we try to obtain a crystal of ARF1 with LM11 by soaking ARF1 in LM11 solution. We want to collect data for molecular replacement since a model for Arf1-GDP is already known.

8 crystals were tested at different angles but some of them were salt and the other were not diffracting to high resolution. This might be because the cryo-protection steps were not optimal. I am working in cryo-protection step to improve the data collection quality.

Sonia Fieulaine (0.5 shift) - Projects: structural study of peptide deformylases (PDF)

NME pathway is responsible of the first methionine cleavage of each newly synthesized protein. NME is ensured by methionine aminopeptidases (MAP) and peptide deformylases (PDF). PDF enzymes are found in bacterial cytoplasm, and eukaryotic organelles. We have started a comparative structural study of the NME pathway, in bacteria, plants and animals. During this beam-time, we have studied eukaryotic mitochondrial PDFs from plants (*Arabidopsis thaliana*) and animals (human).

Three complete data sets were colleted from crystals of free *A. thaliana* PDF1B. The best one (2.0 Å resolution) is currently under refinement.
Crystals of human PDF1A were tested, but they showed very bad diffraction pattern.

Nicolas Leulliot, Jenny Keller (1.5 shift): yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project

1) Virar 79.
Space group $P6_522$
Resolution 2.9 Å.
Completion= 98.6%; Multiplicity = 10
Rsym=11.6%

ORF79 from Acidianus Filamentus Virus 1 (AFV1) is a 11.6 kD protein which has no homologue identified from sequence. Although no functional information is available, sequence analysis reveals a putative Zn binding site (HCA profile). We have collected a 2.9Å resolution MAD dataset at the Zn edge from a crystal grown in the absence of zinc. Unfortunately the low occupancy of the Zn sites did not allowed us to solve the structure. Purification of the protein in the presence of zinc is currently performed.

2) RNA modifying complex.
Space group $P2_1$
Resolution 2.8 Å.
Completion= 99.9%; Multiplicity = 7
Rsym=11.8%

During this session, we have collected a 2.8A resolution SAD dataset from SeMet labelled protein crystals. This allowed us to solve the 70kD binary complex of a RNA modification enzyme. One protein (A) is responsible for the catalytic activity while the second (B) is necessary for in vivo stabilisation of the enzyme and the in vitro activity. To understand how the B protein influences the activity of A, the A protein was also solved alone. We found that the A protein undergoes substantial conformational change upon binding to B in the region of the catalytic site. It is therefore probable that the B protein stabilises an active conformation of the enzyme, and might also serve as a docking platform for the RNA. The structure of this complex is under refinement using a 2.4A resolution dataset previously collected on beam line ID14-EH4.