MX-1061 and MX-1062 on ID29, 25.-26- February 2010.
By Hanna-Kirsti S. Leiros and Kenneth Johnson

Project number 1, the Metallo-β-Lactamases (MBLs) VIM-7 and AIM-1

Metallo-β-lactamases (MBLs) are enzymes responsible for resistance to virtually all β-lactam antibiotics in various Gram-negative bacteria, and the task of this project was to solve high resolution crystal structure of the VIM-7 and AIM-1 from the opportunistic human pathogen *Pseudomonas aeruginosa*.

In total we screened about 40 MBL crystals and collected 21 datasets on native and antibiotic soaked crystals. The antibiotics used for soaking were ceftazidime, cefoxitin and meropenem

All X-ray data were collected at λ=1.240 Å on the high energy side of the Zn K-edge which have a peak at λ 1.2835 Å. Since then we can calculate anomalous difference maps and identify the Zn occupancies of the structures that all have 2 Zn-ions in the active site.

For VIM-7 all structures are in space group $P4_3$ and resolutions from 3.5-1.85 Å were obtained. For the data to 2.7-2.21 Å resolution, an over all R-merge of 6-12% was obtained. For previous structure a problem with an oxidized Cys221 into cysteinesulfonic residue (Osc) as also observed for the related VIM-2 also from *Pseudomonas aeruginosa aeruginosa* (Garcia-Saez et al., 2008). Now we obtained a new structure to 1.85 Å with a reduced normal Cys221 which was a new result for the project. A second structure has replaced the catalytic water with a S-atom, and new conformations for residues around the active site was observed for another structure. But unfortunately no complex with the antibiotic was found.

For the other enzyme AIM-1 also collected at λ=1.240 Å, the space group was $P6_1$ with 3 molecules in the asymmetric unit (AU) or $P6_122$ with only 1 molecule in the AU. All R-merge values were lower than 13% for the data to 1.6-2.5 Å resolution. In one dataset to 2.85 Å with an overall R-merge of 12.4%, parts of the antibiotic meropenem were found in the difference electron density. Even if not the complete antibiotic was defined, the result shows that more soaking experiments to optimize time and antibiotic concentration is worth to pursue with. A new higher resolution data set in $P6_1$ was obtained but unfortunately no complex with the antibiotic was found.

Future experiments for this project is to make an inactive mutant to enhance the probability of obtain a protein – antibiotic complex, and also do additional soaking experiments with AIM-1 and meropenem.

For all experiment the sample changer was used, it was reliable and only 1 sample was lost on the floor. The report in ISPyB was very handy to have available after collecting such a high about of X-ray data sets.

Project number 2
Screening heavy atom derivatives of the shrimp DNAse.

Shrimp deoxyribonuclease (sDNase) is an endonuclease cleaving phosphodiester linkages in DNA to yield oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. DNase from shrimp has a very high specific activity, estimated to be 30 times higher than bovine DNase I, which is widely used in modern biotechnology. In addition, shrimp DNase is heat labile, and it has a particularly strong preference for the hydrolysis of double-stranded DNA (dsDNA). In the presence of magnesium as the only divalent cation, single-stranded DNA (ssDNA) is hydrolyzed at a rate of 1-5% of that of dsDNA. Shrimp DNase can therefore be used to specifically degrade dsDNA, leaving ssDNA essentially intact. The higher catalytic efficiency, together with the reduced thermal stability, has made the enzyme an important component in kits used in molecular biology.

The sDNase consists of about 400 amino acids where the C-terminal part of the sequence resembles other endonucleases with a NUC domain. Nucleases with NUC domains are active towards both DNA (dsDNA and ssDNA) and RNA. The closest structural homologue is of a nuclease from an *Anabaena* Sp., but the sequence identity is only 29% over 130 aligned residues. The N-terminal part of the sDNase sequence also shows low sequence similarity (20% over 140 aligned residues) to the structure of human dead-box Rna-helicase. The structure of sDNAse is expected to provide information about the substrate binding site, and possibly explain the high preference for double stranded DNA. This knowledge may further be exploited by rational redesign to yield an enzyme with even higher specificity for dsDNA and higher catalytic activity.

On a previous trip to the ESRF(Dec. 2009, ID29), several data sets from crystals of native sDNAse and from crystals soaked in various heavy atom solutions were taken. The best native crystal diffracted to 1.9Å. The heavy atom data sets showed very little anomalous signal except for that from a crystal soaked in 200mM zinc sulfate. However, these 2.7Å zinc SAD data were not good enough alone to make an interpretable electron density map for building the sDNAse structure. The goal for this trip was to take a native data set to use with the zinc SAD data and to also screen additional heavy atom-soaked crystals for use in phasing the sDNAse structure.

On this trip, a native data set in the crystal form used for the zinc SAD data was collected to 2.1Å giving a data set with a completeness of 97.3% and an R_{merge} of 5.8%. However, the native data were not isomorphous to the 2.7Å zinc SAD data and were thus not useful in improving the poor phases obtained from the zinc SAD data.

Additionally, 4 data sets from heavy atom-soaked crystals were obtained. Fluorescence scans were used to locate the absorption edges in order to maximize the potential anomalous signal in the data sets. Three data sets (from Hg- and two Pt- soaked crystals) showed very little anomalous signal as estimated by the anomalous correlation column in the output from XDS processing of the data. A fourth data set from a NaBr-soaked crystal gave data to 2.2Å with anomalous completeness of 99.1% R_{merge} of 8.0%. The data showed a reasonably good anomalous signal to about 3.1Å as indicated by the anomalous correlation column in the output from XDS processing of the data. Potential bromide sites could be found but again the resulting phases were not good enough to build the structure.

All the equipment at the beamline worked very well, especially the sample changer. The data collection for the native sDNAse data were planned using the eDNA software. Future experiments at the ESRF on the shrimp DNAse protein will be attempts to phase the structure using crystals of Se-methionine labeled protein and if necessary further screening of heavy atom-soaked crystals. A sulfur-SAD experiment might be possible as the number of sulfurs to find is reasonable (15 in 1 molecule). However, the orthorhombic setting would require a very long and almost perfect data set for this technique to succeed.

.

Project number 3, Location of the sulfate binding site of the *A. thaliana* epithionitrile-specifier protein.

Plants of the Brassicaceae (Crucifer) family, which include important crops such as oilseed rape as well as the model plant *Arabidopsis thaliana,* contain a group of secondary metabolites called glucosinolates. The nitrile(NSP)- and epithionitrile(ESP)- specifier proteins form a family of helper enzymes that determine the end product formed after hydrolysis of the glucosinolate secondary metabolites by the enzyme myrosinase (EC 3.2.1.147). The myrosinase products are unstable aglycones (thiohydroximate-O-sulfonates) that spontaneously rearrange to form isothiocyanates, but in the presence of various specifier proteins can be differently rearranged to form thiocyanates, nitriles and even epithionitriles if a terminal alkenyl bond is present. ESP products are nitriles and epithionitriles. The exact end product is also affected by the presence of a ferrous iron by an unknown mechanism.

This system, also coined "mustard oil bomb" is an important and complex plant defence system against insect pests and pathogens, and has a beneficial impact on human health. Intact glucosinolates are biologically inactive, but isothiocyanates exert toxic effects on plant pests and pathogens, while having cancer-preventive effects in humans. Nitriles, produced by ESP activity, have a reduced direct toxicity to pests but play their role by attracting natural enemies of these pests. Nitriles are also less beneficial than isothiocyanates in regard to human health. The presence of ESP, by diverting the outcome of glucosinolate hydrolysis, hence plays a pivotal role in determining the biological consequences of this system.

The structure of *A.thaliana* ESP was recently solved (Dec. 2009, ID29) using a crystal of selenomethione-labeled ESP. The structure shows an novel metal-containing active site with a sulfate ion coming from the crystallization conditions bound near the active site metal atom. A higher resolution crystal form of the native protein did not contain bound sulfate. The data sets taken on ESP crystals this trip were an attempt to confirm the sulfate binding site in the higher resolution crystal form.

Thus, four data sets were taken of crystals of native ESP soaked in a solutions containing 100mM sulfate. The best crystal diffracted to 2.0Å giving a data set with overall completeness of 98.1% and R_{merge} of 5.3%. A second best crystal diffracted to 2.1Å giving a data set with a completeness of 97.0% and R_{merge} of 6.0%. The resulting electron density maps from both data sets clearly showed sulfate bound in the same position near the active site iron as was observed for the SeMet phasing data taken in December. This results confirms the sulfate binding site that is likely to be the binding site for the sulfonate group of the substrate and allows a model to be postulated for substrate binding to the active site.

All the equipment at the beamline worked very well. The sample change worked especially well. Data collections were planned using the eDNA software. Future experiments at the ESRF on the *A. thaliana* ESP protein will be attempts to obtain substrate/product-like complexes with the native ESP protein and also structures of mutant proteins to investigate the enzyme mechanism.

In addition

Two X-day data sets were collected on a protein-protein complex of Cod uracil-DNA-glycosylase in complex with proteinaceous inhibitor Ugi, the best to 2.6 Å with and overall R-merge of 14:5%. The complex structure was solved, but also left some big cavities in the AU with unmodeled, and non-ordered protein molecules. This latter problem have not been resolved yet.

Two inhibitor complexes of a protein kinase A in complex with the inhibitors ARC-1039 and ARC-670 were collected to 2.1 and 2.0 Å resolution and R-merge values of < 9.6%. Both these structures are all ready deposited in the PDB bank with PDB IDs: 3AGL and 3AGM

References

Garcia-Saez, I., Docquier, J. D., Rossolini, G. M. & Dideberg, O. (2008). J. Mol. Biol. 375, 604-611.