EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

<table>
<tr><td> </td><td>Experiment title:
VsNeuD - a possible sialic acid acetyl-transferase
Reference number: 18393</td><td>Experiment number:
MX-743</td></tr>
<tr><td>Beamline:
ID14-1</td><td>Date of experiment:
from: 03.03.08 08:30 to: 03.03.08 24:00</td><td rowspan="2">Date of report:

Received at ESRF:</td></tr>
<tr><td>Shifts:
2</td><td>Local contact(s):
Dr. Juan Sanchez-Weatherby</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
Hanna-Kirsti S. Leiros*, Bjørn Altermark and Ingar Leiros*
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</table>

Report:

Background and summary

Two shifts of beam time were granted in order to get high resolution diffraction data from *Vibrio salmonicida* NeuD crystals. *V. salmonicida* is a gram negative bacterium pathogenic to several fish species. The outermost sugar in the *V. salmonicida* serotype C2 lipopolysaccharide layer consists of the sialic acid like sugar legionaminic acid (Leg5Am7Ac). Based on sequence- and gene-arrangement conservation, the VsNeuD seems to be involved in acetylating one of the precursor molecules in the synthesis of this sugar. The acetyl donor is the cofactor AcetylCoenzyme A. The crystals that were tested were NeuD alone and NeuD co-crystallized with UDP-GlcNAc, UDP, AcetylCoenzyme A and Coenzyme A. The structure of a NeuD homologue called PglD from *Campylobacter jejuni* have recently been published (Rangarajan et al. 2008) and served as molecular replacement model for solving our structure. A total of 13 datasets were collected, however 12 of them contained only the apo enzyme, but in one dataset we were able to identify coenzyme A. The best apo-enzyme structure and the enzyme and cofactor structure is solved to 1.50 and 1.75 Å resolution, respectively.

Results

The ID14-1 is equipped with a sample changer which made the crystal screening much faster. Most datasets were processed on site during data collection. The statistics for the highest resolution dataset from the apo- and Coenzyme A-complexed crystals are presented below:

<u>VsNeuD apo structure</u>

High quality data was collected to 1.50 Å. Space group was $P2_12_12_1$, with unit cell of a=90.82 Å, b=94.82 Å and c=103.01Å. In total 100 degree data was used, and integration and scaling were carried out using XDS , and the structure factors were obtained with TRUNCATE (CCP4i). Overall the data were 99.8% complete,

R-sym was 5.0%, I/σ_I was 14.3, the multiplicity was 4.1 and the overall Wilson B-factor was 17 $Å^2$. The structure was solved using MolRep of CCP4, using PglD (PDBid: 2NPO) as search model. For the refined structure the final R-factors are 18.3% (R-work) and 21.2% (R-free).

VsNeuD-Coenzyme A structure

The structure is solved to 1.75 Å resolution. The spacegroup and unit cell parameters were only slightly different compared to the apo structure. The data set was 99 % complete, R-sym was 5.0%, I/σ_I was 16.6, multiplicity was 4.4 and the overall Wilson B-factor was 23 $Å^2$. The final R-factors are 18.6% (R-work) and 21.6% (R-free).

Structural features

The biological unit is a trimer, as shown in Figure 1, which is also the content of the asymmetric unit. Two of the three co-factor binding sites are occupied, and weak density can be observed at the third site. The substrate binding pockets contain two sulphate ions, and the structure contains two glycerol molecules. Several 2-mercaptoethanol molecules are participating in disulphide bridges with surface cysteines. In the apo structure one cysteine resides near the active site and is bound to a 2-mercaptoethanol molecule. In order to get crystals with bound cofactor we had to purify the protein without reducing agent, add some cofactor and then add the reducing agent. In the VsNeuD-coenzyme A structure the terminal sulfhydryl group of CoA is forming a disulphide bond with the same cystein residue near the active site. This disulfide bond is probably not relevant for the catalysis, and could be an artifact of the protein purification and crystallization in an oxidizing environment.

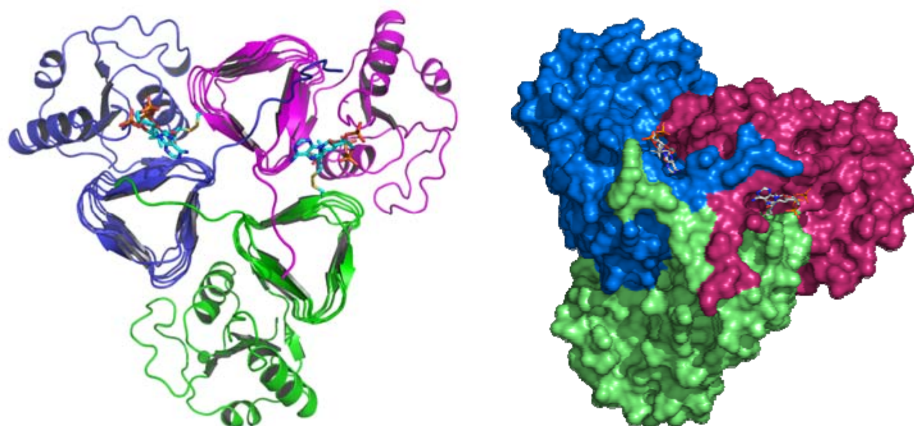

Fig.1. Left: Cartoon representation of trimeric VsNeuD in complex with Coenzyme A. Right: Surface representation of the same structure. Each monomer is coloured separately, and two of the three cofactor binding sites are occupied. The terminal sulfhydryl group of the cofactor is covalently bound to a cysteine residue.

Conclusions

We have solved the structure of VsNeuD to high resolution both in its apo form and in complex with its co-factor. The biologically relevant molecule consists of a trimer with each of the three substrate- and co-factor binding sites situated between two monomers. The carboxy terminal part of the protein monomers folds into a left-handed parallell beta helix. The structures are currently being compared to PglD from *C. jejuni* and the manuscript is in preparation. The NeuD protein is unique to bacteria and is a potential drug target. The structures may be utilized in a search for new and much needed antibiotics.

Additional results

Totally for all projects 46 crystals were tested and 16 complete data sets were collected, so the use of the sample changer greatly improved our scanning to find the best crystal of a given condition.

For one additional project X-ray data were collected on MutT from *Vibrio cholerae* (VcMutT) a 15 kDa protein with 132 residues. MutT is a 8-oxo-dGTP pyrophosphohydrolase that hydrolyses 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate, thus preventing incorporation of 8-oxodGTP into DNA. Two VcMutT crystals were tested, and two data sets to 2.35 Å and 2.45 Å resolution were obtained. All data were integrated with XDS, and statistics from the best data set gave an overall completeness of 99.3%, R-sym of 5.2%, I/σ_I of 18.5, a multiplicity of 5.1 and a Wilson B-factor of 54 $Å^2$.

The structure of MutT from *E.coli* has been determined by NMR (Abeygunawardana et al. 1995) but using this as a model for molecular replacement (MR) was not successful. Further, many other X-ray structures of proteins with 20-27% sequence identity to VcMutT have been tried with the MR programs Molrep, Phaser and BALBES. Still no MR solution has been found. Production of Se-Met protein is now underway and will hopefully help solving this structure.

References

Abeygunawardana, C, Weber, DJ, et al. (1995). *Biochemistry* **34**(46): 14997-5005.

Rangarajan, ES, Ruane, KM, et al. (2008). *Biochemistry* **47**(7): 1827-36.