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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.

ESRF	**Experiment title:** Structure-function studies of proteins involved in copper homeostasis in methanotroph bacteria containing a copper binding site including an oxidized tryptophan	**Experiment number**: MX-1065
Beamline:	**Date of experiment**: from: 11.12.09, 01.00 to: 11.12.09, 08.00	**Date of report**: 13.04.10
Shifts:	**Local contact(s)**: Dr. Mats Ökvist	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

This experiment was run together with experiments MX-1060 and MX-1063

Aims of the experiment and scientific background

The recent crystal structure of MopE from *Methylococcus capsulatus* (Bath) has revealed a novel copper-binding site including an oxidized tryptophan (kunurenine) and two histidines (1). Recent EPR studies (unpublished) have shown that MopE binds both oxidized and reduced copper, but at different sites. The only protein displaying sequence similarity to MopE is CorA from the methanothroph *Methylomicrobium album* BG8. The structures of MopE and CorA are expected to reveal whether the oxidation of tryptophan is a unique trait of MopE or if the modification could be a more common feature in methane oxidizing bacteria.

M. capsulatus (Bath) is a gram-negative bacterium which is able to use methane as a sole carbon and energy source. The oxidation of methane to methanol is catalyzed by methane monooxygenase, an enzyme which is produced in two forms (sMMO and pMMO) depending on the copper-to-biomass ratio in the growth medium (2). MopE is a protein secreted into the growth medium in large amounts when the bacterium is grown under copper-limited conditions, and is believed to act as a copper scavenger (3,4). The only protein known to date displaying sequence similarity to MopE is CorA from the methanothroph *Methylomicrobium album* BG8 (5). CorA is composed of 204 amino acids, all of which can be aligned to the C-terminal region (aa 56 to 336) of the truncated MopE (2) and with conserved residues around the metal binding site.

Due to a sequence identity less than 20% and unequal lengths of the polypeptide chains, the structure must be solved by experimental phasing.

Methods

X-ray data were collected on three different crystals of CorA. One additional crystal was subjected to X-ray absorption near edge spectroscopy (XANES) to veryfy the presense a copper ion and the oxidation state of the metal.With excellent help of the beamline scientists, Mats Ökvist and David Flot, and Antonia Beteva from BLISS, step size in spec was tried optimized in order to get the best spectrum from the sample.
The UV spectrum was also recorded before and after a fifth crystal of CorA was subjected to heavy doses of X-rays. The help form Antoine Royant is greatly appreciated.

Results

High resolution data were collected on three crystals diffracting to 2.4 – 1.6 Å. The crystals belonged to space group $P2_1$ with cell parameters of 73.8 x 113.2 x 81.5 $Å^3$ and β=104.5. The data were 94 – 98% complete, R_{sym} values around 5% and I/σI of about 10 – 15.

The structure was solved by experimental phasing using SOLVE using the high resolution data collected at ESRF and a low resolution iridium derivative collected at the home source. The structure of CorA displays structural similarities to MopE in the copper binding site and the 8-stranded β-sandwich forming the major secondary structure element, but other parts of CorA are significantly different from the MopE fold.

The XANES analysis verified the presence of copper and the oxidation state was determined

The kynurenine UV signature is at 230 nm, 257 mn and 360 nm. The UV spectrum of X-ray irradiated CorA showed an increase in absorbance at 300 – 450 nm. However, whether the increase is due to modification of the kynurenine or modifications to other parts of the protein cannot be explained by the present data, and more extensive analysis is required.

The structure of CorA is currently being analyzed in more detail, and a manuscript is in preparation.

Other (valid for all experiments; MX1060, MX1063 and MX1065)

Essentially all of the allocated beamtime was spent analysing proteins in the beamtime applications. Only very little time was spent testing other proteins for diffraction. These were crystals of a uracil-dna-glycosylase (UDG) complex, two different kinases and two metallo-β-lactamases (the latter was relevant for experiments MX1061 and MX1062).
The UDG crystal diffracted to about 1.7Å, but peaks were split giving poor statistics and the structure could not be solved.
One of the kinases was p38 from a new species (Atlantic salmon). The crystal diffracted to 2.7 Å, belonged to space group $P2_1$ with cell parameters of 67.78 x 100.93 x 67.89 $Å^3$, b=98.15, and R_{sym} was 5.4%. The structure has been solved, and further analysis of the structure is in progress.. The other kinase was a complex between PKA and two different inhibitors. Both crystals diffracted beyond 2 Å, and belonged to space group $P2_12_12_1$ with cell parameters of about 72 x 75 x 80 $Å^3$. Rsym was about 7-8%. The structures have been solved and the inhibitors are identified. Further analysis is in progress.
Two crystals of metallo-β-lactamases were tested for diffraction, and were found to diffract beyond 2Å. One crystal belonged to space group P43 and the other to P61. The structures have now been solved, and are currently being further refined and analysed.