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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:** Investigation of the oxidation state of copper in the MopE protein using online monitoring of the UV/VIS spectrum.	**Experiment number**: MX731
Beamline:	**Date of experiment**: from: 15.11.2007 to: 16.11.2007	**Date of report**: 04.03.08
Shifts:	**Local contact(s)**: Dr. Martin WEIK	*Received at ESRF:*

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Report:

Background:

MopE from *Methylococcus capsulatus* (Bath), a methane oxidizing bacterium, is a protein secreted into the growth medium in large amounts when the bacterium is grown under copper-limited conditions, and it is believed to act as a copper scavenger. Several major morphological and physiological changes in *M. capsulatus* are regulatied by copper, suggesting that the bacterium may have evolved a wide range of proteins to deal with processes such as copper transport, copper detoxification and other regulatory responses to copper. Copper is an essential micronutrient for all living organisms but is toxic at elevated levels. It is therefore important for the cell to keep a tight homeostatic control of free copper ions.

The crystal structure of *M. capsulatus* MopE has previously been solved to 1.35Å, revealing a copper-binding site with a geometry not described before, including an oxidized tryptophan and two histidines. The oxidation of the tryptophan is essential for copper binding since MopE recombinantly produced in *E. coli* does not carry the modification (demonstrated by the crystal structure), nor does it bind copper; copper is not seen in the crystal structure, nor is it detected in ICP analysis. The presence of an oxidised tryptophan in the wild type enzyme, and absence in the *E. coli* produced enzyme were verified by mass spectrometry.

The aim of this experiment was to try to identify the oxidation state of the copper in MopE using online monitoring of the UV/VIS spectrum.

Results

MopE had been crystallised in the presense and absence of Cu^{2+} to yeld crystals of about 400 x 300 x 200 μm^3. The crystals were soaked in reducing (50 mM $Na_2S_2O_4$) and oxidizing (20 mM H_2O_2) conditions prior to flash cooling in liquid nitrogen.

UV/VIS

Untreated and oxidized/reduced crystals were subjcected to recording of the UV/VIS spectrum in the range 190 – 1100 nm before and after collection of a complete data set (ca 200 frames, 3 sec. exposure). The

spectrums were without characteristics and did not display significant difference before and after data collection. The signals below 330 nm and above 800 nm were noisy and impossible to interpret.
One crystal was subjected to the full X-ray beam (1.20869 x 10^{11} photons/sec) for about 800 seconds to check for radiation damage. The spectrum did not change over time with the exception of a rise in absorption around 500-600 nm which is characteristic for glycerol.

X-ray
X-ray data were collected on untreated, oxidixed and reduced MopE crystallized in the presence and absence of added Cu^{2+}. A total of 6 data sets were collected to 1.4 Å, with the exception of one crystal which diffracted to 1.6 Å. The crystals belonged to the orthorhombic space group *I*222. The data were of good quality with R_{merge} in the range 2.5 – 3.9 %, I/σI in the range 9 – 20, completeness in the range 94 – 98% and multiplicity in the range 3.9 – 4.5. Unfortunately, the X-ray data revealed no significant difference in the distances between the copper ions and the neigbouring ligands.

Discussion
The UV/VIS spectrum may be used to distiguish between oxidation states in copper. Cu(I) is invisible in the UV/VIS spectrum while Cu(II) may absorb in the range 330 – 650 nm. The exact wavelength and the degree of absorption will depend on the environment of the ion. For example, sulphur containing ligands will cause a strong absorption around 600 nm, whereas nitrogen containing ligands may cause a weak absorption plateau in the range 600 – 650 nm.
The idea behind the experiment was to subject MopE containing copper to oxidizing and reducing conditions and to compare the spectrums. These spectrums should thereafter be compared to the untreated crystals. Knowing that MopE contains only nitrogen containing ligands, no strong absorption was anticipated, but weak signals were expected at 600 – 650 nm from the crystals soaked in H_2O_2.
In this experiment, none of the crystals displayed any characteristic signatures at all. There are a couple of possible explanations for this:

1) The crystals used for the experiments were all relatively big. It was only possible to obtain a spectrum when exposing only the tip of the crystals. Exposing the main part of the crystal caused absorption of all light resulting in no spectrum at all. When exposing only the tip, the light passes through a lot of cryo solution, and a potential weak signal from the protein may have been absorbed by the solution.
2) The absorption is too weak to be detected even if all cryo solution was removed.
3) A potential problem could be related to the hardware: We could not get a proper signal of the protein at 280 nm, only noise.

Since no useful UV/VIS spectrums were obtained, X-ray data were collected on untreated, oxidized and reduced crystals. Given the difference in ionic radius of Cu(I) and Cu(II), 0.96 and 0.72 Å, respectively, and the high resolution of the X-ray data, one could expect to see differences in copper-ligand distances in the crystals subjected to oxidizing and reducing conditions. However, no significant differences could be observed.
One could speculate whether the copper in MopE is inaccessible to the oxidizing or reducing agents, but this should not be the case sice the copper binding site is open to the solvent. The soaking time could, however, have been too short or the concentation of the oxidizing/reducing agent was too low. Another possibility for the lack of differences in binding distances could of course be that the conformation of the amino acids coordinating the copper ion is unaffected by the oxidation state of copper.

Conclusion
Neiter the recording of the UV/VIS spectrums nor the X-ray data collected in this experiment enabeled us to establish the oxidation state of copper in MopE. The most likely explanation for this is that the inherent biophysical properties of the crystals and/or the protein turn out to be incompatible with the methods tried here. Hence other experiments will have to be performed in order to to identify the oxidation state of copper in MopE.

Other comments

1) Several problems related to MXCUBE were encountered. Some of the problems appeared to occur when eg. the sample changer had been operated from the hutch and not the console room, but not always.
2) “Hardware Repository Server” error and “Vial jam” in the sample changer. Both problems were solved because of clear documentation on the web-pages
3) The sample backlight affected the UV/VIS detectors and had to be turned off when aligning the crystal prior to the UV/VIS scans. This we and the local contact learned first after several scans. Some of the spectrums had to be re-run.