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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

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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.

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Reports on experiments relating to long term projects

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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:** X-ray absorption spectroscopy analysis of the copper binding site in the secreted protein MopE from *Methylococcus capsulatus*	Experiment number:**01-01-773**
Beamline: BM01A	**Date of experiment**: from: 03.10.08 to: 08.10.08	**Date of report**: 17.09.09
Shifts: 15	**Local contact(s)**: Dr. Wouter van BEEK	*Received at ESRF:*

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Report:

Background

Methylococcus 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(1). Other morphological and physiological changes in M. capsulatus are also regulated by the copper-to-biomass ratio, suggesting that the bacterium 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, and M. capsulatus is used as a model organism to study copper homeostasis.

MopE, a protein secreted into the growth medium in large amounts when the bacterium is grown under copper-limited conditions, is believed to act as a copper scavenger(2,3). The crystal structure of M. capsulatus MopE has 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.

Initial studies suggests that the oxidation state of the copper bound to secreted MopE* is copper(I). The X-ray structure of MopE*, however, appears to be a truncated form since about 50 of the N-terminal residues are not visible in the electron density maps. The aim of this experiment is therefore to use X-ray absorption spectroscopy to examine the oxidation state and the geometry of the copper binding site and to establish whether the nature of the binding site in the crystal form is identical to the secreted protein.

Data collection

X-ray absorption data were collected in the fluorescence mode at the copper K-edge. The protein in buffer solution was filled in a Perspex sample holder with kapton windows yielding a sample thickness of 2.5 mm. Spectra were measured with 5 eV steps below the edge, 0.2 eV steps in the edge region, and steps equivalent to 0.04 $Å^{-1}$ increments above the edge (region borders were 8960, 9030, and 9060 eV). Several XAS scans

were collected and summed. All XANES spectra were energy corrected against a copper-foil calibration (8979 eV).
The XAS data were summed and background subtracted, and the EXAFS part of the spectrum extracted to yield $\chi^{exp}{}_i(k)$, using the Athena program.

Results
The XANES analysis of MopE* confirmed that the copper is monovalent in the protein. MopE* exhibited a pre-edge feature at 8982.1 eV attributed to the 1s→4p transition. The position of the feature was shifted to higher energies compared to that seen for copper(I) oxide, 8981.3 eV, and lower energies compared to that reported previously for copper(I) centres in other copper proteins (methane monooxygenase (8983-84 eV) and Cu+-ATPases (8984 eV)). However, the position of the pre-edge feature was comparable to that of the copper(I) model compound, copper(I) diamine in solution (8982.8 eV). In addition, the edge position (8985 eV) was substantially lower than that observed for the copper(II) model compound Cu-tutton (8990-91 eV) clearly supporting the presence of monovalent copper in the MopE* protein. The intensity of the pre-edge feature at 8982 eV was relatively low in MopE*, precluding a linear and two-coordinate copper(I) structure in the protein, thus suggesting either three or four coordinate copper(I) environments in the protein.
The results from EXAFS refinements showed that the copper site in the protein sample could be fitted to 1.6 Cu-N distances at 1.90 Å and 1.1 Cu-O distances at 2.72 Å. This correlated with the pyramidal copper site from crystallographic analysis. However, the bond distances for the first Cu-N (His132) shell were shorter, whereas the bonding to water was longer. The crystal structure of MopE* is of a truncated form of the protein apparently lacking the 46 N-terminal residues, while Mope* in solution lacks 24 residues. The differnces in EXAFS and crystallographic distances could reflect differences caused by by different processing of the polypeptide.
The EXAFS refinements also confirmed a third shell at 2.92 Å, fitted to five carbon atoms. The standard deviations for this shell were high, which could be explained by the low backscattering effect of this element. Also fitted was a fourth shell at 3.28 Å with 1.9 Cu-O distances and a fifth shell at 3.5 Å with 3 C-C distances. It was difficult from EXAFS refinements to distinguish the backscattering from elements with similar numbers of electrons. For this reason, one cannot distinguish between nitrogen and oxygen in the refinements.

A manuscript including the XANES data is in preparation.

Other comments
All data were collected at room temperature. In order to try to reduce thermal motion, and hence get a clearer picture of distances of the copper site, data was tried collected at 4 K using the He cryostat.

The latter experiment was unsuccessful. All data collected indicated metallic copper. In the end data were collected without the protein and the Perspex sample holder. The copper signal was still present. The most probable explanation was therefore that the gold-plated copper sampleholder of the cryostat produces enough scattering, even if the X-rays are not focussed on the cryostat sample holder, to be detected on the fluoresence detector.

During this experiment we experienced technical problems with the cryostat. The cryostat XY-plane was obviously too small to support the weight of the cryostat and we lost the beam, or the beam hit the cryostat. Troubelshooting, and realignment of the cryostat plobably lost us half a day of beamtime.