ESRF	**Experiment title**: Crystallographic studies of the haem- and flavoproteins myoglobin and nrdI in combination with online microspectrophotometry	**Experiment number**: MX-765
Beamline: ID14-2	**Dates of experiments**: From: 13-APR-08 08:00 to: 14-JUN-08 16:00	**Date of report**: 29-AUG-08
Shifts: 4	**Local contact(s)**: Dr. Martin WEIK	*Received at UNIL:*

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Report:

Our studies are focus around the haem protein myoglobin and the flavoprotein nrdI. These proteins are good candidates to be studied with microspectrophotometry since they have characteristic absorptions in the 350-700 nm range.

1. Myoglobin

The main function of myoglobin is oxygen storage in heart and skeletal muscle, but myoglobin exhibit peroxidase-activity during oxidative stress. The main goal of this project has been to investigate the peroxidase reaction cycle in myoglobin (Mb) by trapping intermediates in the cycle. Two of the intermediates have been determined with data from ESRF, the so-called compound II equivalent and the compound 0 equivalent as well as the resting state. Several of these states experience some radiation damage of the metal site as investigated by microspectrophotometry previously with an off-line microspectrophotometer at BM01.

This time with the use of online microscpectrophotometry, we could exactly determine the dose dependence of the different radiation-induced changes. The time/dose for inducing the changes in the different states varied considerable. The resting ferric state was without attentuators reduced to an aqua ferrous state within a few seconds at ID14-2, while the radiation-induced changes in Mb compound II took considerable longer time. This can be seen from the time/dose dependent spectra in Figure 1 and 2, and clearly show the need for online microscpetrophotometry studies for each of the different states of metalloproteins.

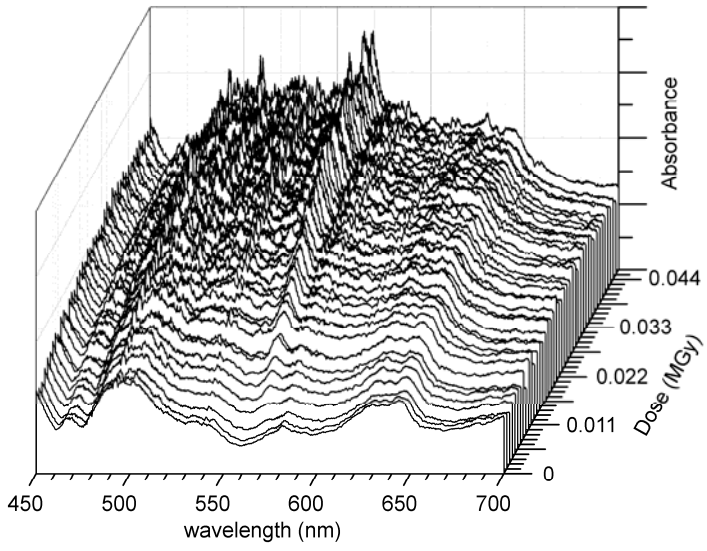

Figure 1: *Radiation-induced changes of resting ferric metMb to aqua ferrous Mb with increased X-ray dose.*

Initial experiments with and without attenuators seemed to indicate that the radiation-induced changes of these redox-states were only dose dependent and not dose-rate dependent. The experiments also made it possible to estimate which dose could be used to collect mostly unredecued dataset of the different states. The collection of a series of mostly unreduced datasets of compound II was started. Slowly annealing of the different states after X-ray exposure was also investigated.

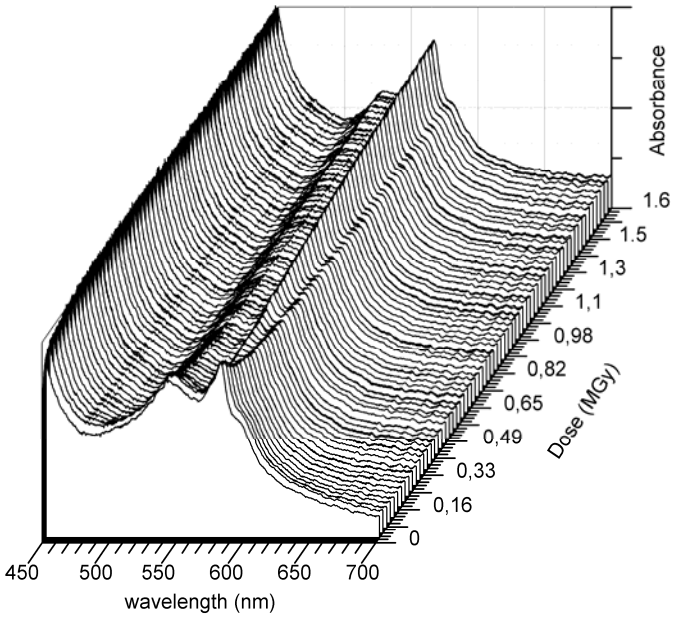

Figure 2: *Radiation-induced changes of Mb compound II with increasing dose.*

2. Ribonucleotide Reductase proteins

The enzyme Ribonucleotide Reductase (RNR) converts the four ribonucleotides to their corresponding deoxyribonucleotides that are necessary for DNA synthesis. The Ribonucleotide Reductase system in *Bacillus cereus* consists of at least 4 proteins; NrdE, NrdF, NrdH, and NrdI. We have performed studies of the flavine co-factor in NrdI. When the oxidized flavin mononucleotide (FMN) cofactor in NrdI is reduced to the semiquinone form the protein undergoes a structural change. In addition to determine the ~1.1 Å 3D- structure of NrdI we have conducted a spectroscopic investigation of the flavin cofactor in the protein crystal. Both the oxidized and semiquinone forms of FMN in NrdI single crystals have been characterized with light absorption and Raman spectroscopy prior to and after data collection. Our goal is to relate the spectroscopy to the structural changes of the flavin during data collection, and also get an improved understanding of the electron transfer mechanism in NrdI.

Unfortunately the NrdI crystals brought to the ESRF did not exhibit satisfying light absorption spectra. The crystals seemed to be too thick, absorbing all light passed through them. We will search for different crystal forms or use smaller crystals in our further experiments. However, we collected datasets of crystals that had been annealed above the glass transition temperature after the first dataset. We are currently analyzing these results.

Related Publications in this periode using ESRF data:

Hersleth, H.-P., Hsiao, Y.-W., Ryde, U., Görbitz, C. H. & Andersson, K. K. (2008) The crystal structure of peroxymyoglobin generated through cryoradiolytic reduction of myoglobin compound III during data collection. *Biochem. J.* **412**, 257-264.

Hersleth, H.-P., Varnier, A., Harbitz, E, Røhr, Å. K., Schmidt, P. P., Sørlie,, M., Cederkvist, F. H., Marchal, S., Gorren, A. C. F., Mayer, B., Uchida, T., Schünemann, V., Kitagawa, T., Trautwein, A. X., Shimizu, T., Lange, R., Görbitz, C. H. & Andersson, K. K. (2008) Reactive Complexes in Myoglobin and Nitric Oxide Synthase. *Inorg. Chim. Acta* **361**, 831-846.