SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo - Studies of proteins in the Oslo region	**Experiment number**: 01-02-696
Beamline: BM01A	**Dates of experiments**: From: 26-NOV-04 08:00 to: 29-NOV-04 08:00	**Date of report**: 28-FEB-05
Shifts: 9	**Local contact(s)**: Mogens CHRISTENSEN	*Received at UNIL:*

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1. Crystal structures of nitrogen oxides synthase

Nitrogen oxide synthase (NOS) is the protein responsible for the generation of NO through the conversion of the amino acid L-argine to L-citrulline in two reaction cycles. We have now collected data for the bovine endothelial NOS oxygenase domain (beNOSox) expressed in *E.coli*. Three different datasets were collected. In addition a crystal soaked with biopterin showed heavy radiation damage by loss of resolution so only half a dataset was collected. The 1.9 Å resolution structure of beNOSox at pH 7 collected showed the binding of L-Arg through cocrystallization. Microspectrophotometry was used to check the state of the beNOSox crystals before and after data collection. A reduction by the synchrotron radiation were observed.

Dataset	Completeness	R(sym)	Resolution	I/sI
nos01	98.2% (93.1%)	0.058 (0.191)	2.30 Å	12.9 (5.4)
nos02	99.6% (99.2%)	0.078 (0.431)	1.90 Å	13.3 (3.4)
nos03	aborted			
nos04	97.6% (98,9%)	0.090 (0.381)	2.10 Å	11.2 (3.0)

Values in parentheses are for the highest resolution bin

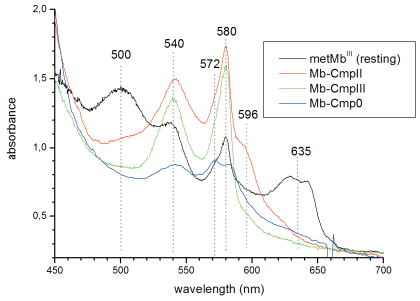
2. Studies of the Tri and Dinuclear Metal Binding site in R2 of Ribonucleotide reductase (RNR) from mouse.

Different oxidation states as well as soaking of different ligands have previously been investigated. A dataset was this time collected at pH 5.0 to investigate the metal occupancy of the novel trinuclear site and the normal dinuclear metal binding site in R2 of Ribonucleotide reductase.

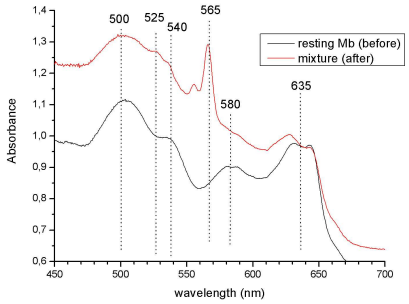
Dataset	Completeness	R(sym)	Resolution	I/sI
R2_ferric	96.8 %		2.7 Å	

3. Crystal structures of myoglobin

The main goal of this project has been to investigate the peroxidase reaction cycle in myoglobin by trapping intermediates in the cycle. Two of the intermediates have been determined, the compound II and the compound 0 equivalent. The introduction of a microspectrophotometer at SNBL has shown that the reaction site of compound II is not significantly reduced during datacolletion, while compound 0 is actually generated from another compound by the synchrotron radiation. The resting form also get reduced by the synchrotron radiation.



Several short datasets (se table below) were obtained to try to overcome the effect of radiation damage (reduction of metal centre by radicals produced by the high-energy X-ray beam). The datasets were checked before and after datacollection by microspectrophotometry. By using 1/20 of the exposure time for a normal myoglobin dataset, a mixture of the normal resting form and the radiation reduced form (peaks at 525 and 565 nm) were observed by microspectrophotometry as seen in the figure to the right. The structure of this mixture showed a longer Fe-O distance than the fully radiation reduced form.



Dataset	Completeness	R(sym)	Resolution	I/sI
mb7u	86.4% (80.6%)	0.058 (0.27)	2.0Å	11.6 (3.4)
mb7v	75.3% (70.7%)	0.15 (0.41)	2.0Å	4.5 (1.8)
mb7w	88.3% (75.3%)	0.062 (0.26)	1.9Å	10.6 (3.3)
mb7y	77.1%(74.6%)	0.087(0.45)	2.0Å	7.3(1.8)

Values in parentheses are for the highest resolution bin

Related Publications in this periode using SNBL data:

[1] Strand, K.R., Karlsen, S., Kolberg, M., Røhr, Å.K., Görbitz, C.H. & Andersson K.K. (2004) *J. Biol. Chem.* **279**, 46794-46801.

[2] Nilsson, K., Hersleth, H.-P., Rod, T.H., Andersson, K.K. & Ryde, U. (2004) *Biophys. J.* **87**, 3437-3447