SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo - Studies of proteins in the Oslo region	**Experiment number**: 01-02-633
Beamline: BM01A	**Dates of experiments**: From: 10-JUL-04 08:00 to: 12-JUL-04 08:00	**Date of report**: 26-AUG-04
Shifts: 6	**Local contact(s)**: Dr. Philip Pattison	*Received at UNIL:*

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1. Crystal structures of thermostable tetrameric malate dehydrogenases

We have cloned and expressed several mutants of MDH from *Chloroflexus aurantiacus* that show substantially higher thermostability than the wild-type ca-MDH. In several of the mutants, negatively charged residues have been replaced by either neutral or positively charged residues thus modifying the ionic network in the native ca-MDH tetramer. Using beamline SNBL at ESRF we have obtained diffraction data for both single and double mutants of ca-MDH, with and without bound metal ions:

The present crystal was that of a double mutant (e154q-t187c) in which we have mutated a Thr into a Cys residue in order to form a covalent disulfide bridge accross the dimer-dimer interface in the MDH tetramer. We have shown that this subunit likning substantially increases the thermostability of the enzyme. This was done both by estimating apparent melting temperature (Tm) using CD-spectroscopy and with meassuring the half-life of loss of enzymatic activity upon incubation at different temperatures:

Dataset	Completeness	R(sym)	Resolution	I/sI
mdh	> 98 %		~ 2.28 Å	

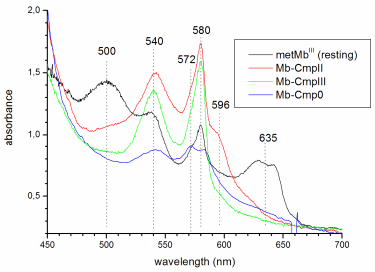
2. Studies of the Dinuclear and a Trinuclear Metal Binding site in R2 of Ribonucleotide reductase (RNR) from mouse.

A novel trinuclear metal cluster has been observed in a crystal soaked in ferrous iron, ascorbate, and methanol. The metal atoms are coordinated by two cys-residues, a backbone oxygen, two water molecules, and an atom that appears to be sulfur bridge. We are currently exploring various soaking conditions in order to characterize the trinuclear cluster further. It is not yet clear whether the trinucelar cluster is an artefact from the soaking conditions or if it has any biological function.. Summary of data collection:

Dataset	Completeness	R(sym)	Resolution	I/sI
R2_meoh	98.7 %	0.14	2.7 Å	8.8

3. 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.



Two datasets (se table below) were obtained, one for the reduced form of myoglobin, and the other for studies of radiation damage (reduction of metal centre by radicals produced by the high-energy X-ray beam). Both datasets were checked before and after datacollection by microspectrophotometry. In addition some shorter exposures of crystal were carried out in combination with microspectrophotometry to investigate the impact of shorter exposure time on the different crystals.

Dataset	Completeness	R(sym)	Resolution	I/sI
mb7s	97.3% (95.7%)	0.035 (0.18)	1.25Å	16.9 (4.1)
mb7t	84.1% (72%)	0.054 (0.26)	1.50Å	13.6 (3.9)

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*. In press

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