SNBL	**Experiment title**: (360) Studies of the haem containing proteins: myoglobin, haemoglobin, nitrogen oxide synthase and cytochrome c with main interest on different reactive intermediates. 361 Studies of the Dinuclear Metal Binding site in R2 of Ribonucleotide reductase (RNR) from mouse	**Experiment number**: 01-02-360 **01-02-361**
Beamline: BM01	**Date of experiment**: from: 31-JAN-03 07:00 to: 04-FEB-03 07:00	**Date of report**: 30-Mar-03
Shifts: 12	**Local contact(s)**: Jon Are BEUKES	*Received at UNIL:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. K. Kristoffer Andersson, Department of Biochemistry, Univ. of Oslo, Norway

Prof. Carl Henrik Gørbitz, Department of Chemistry, Univ. of Oslo, Norway

Dr. Bjørn Dalhus, Department of Chemistry, Univ. of Oslo, Norway*

Cand.scient Hans-Petter Hersleth, Department of Chemistry, Univ. of Oslo, Norway*

Cand.scient Åsmund K. Røhr, Department of Biochemistry, Univ. of Oslo, Norway*

Lars Male Hartviksen, Department of Chemistry, Univ. of Oslo, Norway

Ane Berg Tomter, Department of Biochemistry, Univ. of Oslo, Norway

1. Structural basis for protein thermostability - crystal structures of thermostable tetrameric malate dehydrogenases

Dalhus, B.[1], Bjørk, A.[2], Mantzillas, D.[3], Eijsink, V.[4] & Sirevåg, R.[2]

[1] Department of Chemistry, University of Oslo, PO Box 1033, Blindern, N-0315 Oslo, Norway.
[2] Department of Biology, University of Oslo, PO Box 1066, Blindern, N-0316 Oslo, Norway.
[3] Department of Biochemistry, University of Oslo, PO Box 1041, Blindern, N-0316 Oslo, Norway.
[4] Department of Chemistry and Biotechnology, Agricultural University of Norway, PO Box 5040, N-1432 Ås, Norway

Enzymes from thermophilic organisms are often in a higher oligomeric state than their mesophilic conterparts. The relative thermal stability of tetrameric malate dehydrogenase (MDH) from *Chloroflexus aurantiacus* (*ca*-MDH), *Chlorobium tepidum* (*ct*-MDH) and *Chlorobium vibrioforme* (*cv*-MDH) can be explained by comparison of molecular interactions across the oligomeric interfaces, as revealed in the crystal structures of these proteins. Using station BM01A at SNBL/ESRF we have obtained diffraction data for the double-mutant E165Q-D166N, processing is difficult due to mosaicity.

This particular mutant has proven to be a difficult one to obtain diffraction data of high quality, as most crystals turn out to have a high mosaicity, resulting in low completeness during data processing. This time two datasets were collected, and finally we were able to collect a complete dataset to about 1.9 Å with >99.5% completeness. The other crystal diffracts to better than 1.6 Å, unfortunately, processing is difficult due to the aforementioned mosaicity issue. The effect of the various mutations on the inter-subunit interactions will be analysed in order to rationalize the increased thermostability of each replacement.

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

Around 20 different R2 proteins were tried, but they all diffracted over 3 Å so no data set was obtained.

Other proteins : **Three dimensional structure determination of enterocin A immunity protein (EntA-im)**

Dalhus, B.[1], Johnsen, L.[2] & Nissen-Meyer, J.[2]

[1] Department of Chemistry, University of Oslo, PO Box 1033, Blindern, N-0315 Oslo, Norway.

[2] Department of Biochemistry, University of Oslo, PO Box 1041, Blindern, N-0316 Oslo, Norway

We have obtained diffraction data for two crystals of enterocin A immunity protein. Datset #1 (with a crystal diffracting to about 1.75Å) was only 75% complete (unfortunate crystal mounting and high mosaicity). Sample #2 did not diffract that good (but still to better than 2Å). The enterocin A immunity protein crystals belong to the monoclinic crystal system with unit cell dimensions a = 116.59, b = 42.37 and c = 66.19 Å and with $\beta = 111.27°$. The symmetry and systematic absences in the diffraction pattern is consistent with space group C2. The presence of two molecules in the asymmetric unit with a molecular weight around 12.2 kDa gives a crystal volume per protein mass (V_m) of about 3.1 $Å^3$ Da^{-1} and a solvent content around 60 % by volume. A paper describing the current experiment is in preparation. (Dalhus, Johnsen & Nissen-Meyer, Acta D)

Related Publications in this periode using SNBL data:

-Hogbom M, Galander M, Andersson M, Kolberg M, Hofbauer W, Lassmann G, Nordlund P, Lendzian F. (2003) Displacement of the tyrosyl radical cofactor in ribonucleotide reductase obtained by single-crystal high-field EPR and 1.4-A x-ray data. *Proc Natl Acad Sci U S A* **100,** 3209-14

-Karlsen, S., K.R. Strand, F.H. Cederquist, A. L. Barra, C.H. Gørbitz and K. K. Andersson (2003) Crystallographic and spectroscopicalstudies of ribonucleotide reductase from mouse show new carboxylate/radical shifts and the radical transfer triggering mechanism. *The 39th Norwegian Biochemical Society Meeting at Geilo*, January 23-26, p. 128