



**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

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*Received at UNIL:*

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**Investigation of myoglobin:** The myoglobin project was this time further developed. We have previous let myoglobin react with hydrogen peroxide and other organic peroxides at different pH-values. We have in these studies trapped the intermediate called compound II, and looked on the pH dependence.

This time two data sets were collected. One dataset was on a crystal soaked with cyanide. Cyanide bind strongly to the iron in the haem group, and this was done to try to get a better understanding of the reaction site.

The other dataset was on a crystal which had been soaked in hydrogen peroxide. Last time we used 0.72 Å wavelength to try to avoid possible reduction of the oxidized reaction centre. This time we measured light absorption on the crystal after irradiation to check for radiation damage. (We used the microphotospectrometer in connection with ID9.) Both the light absorption spectrum and the crystal structure showed that the peroxospecie called compound 0 had been trapped **this is the the first observation of this species in myoglobin.**

We also irradiated a highly oxidized myoglobin crystals for 15 min. to study the light absorption spectrum after short irradiation time compared to after long irradiation time.

	Resolution (Å)	R <sub>sym</sub> (%)	Completeness (%)	I/σ(I)	Redundancy
CN-	25-1.30 Å	5.1	98.2	12.1	3.5
Cmp0	25-1.35 Å	4.6	98.4	9.4	4.0

#### Related SNBL Publications/Presentations in this periode:

- Lectures – *1<sup>st</sup> Protein Structure and Spectroscopy Subgroup of MedCoast Scandicavia Meeting, Oslo, 14<sup>th</sup>-15<sup>th</sup> January 2003*
  - Hersleth, H.-P. – Structural studies of the myoglobin-peroxide reaction
  - Dalhus, B. – Structural basis for protein thermostability; analysis of interactions within a protein oligomer and engeneering towards higher thermostability
  - Strand, K. R. – Spectroscopic and structural studies of di-nuclear cluster in mouse ribonucleotide reductase R2 protein
  - Andersson, K. K. – HF-EPR and other spectroscopic studies of metalloproteins.
  - Kolberg, M. – Single crystal EPR studies of ribonucleotide reductase R2 from *E. coli*

Strand K.R., S. Karlsen, F.H. Cederquist, A. L. Barra, C.H. Gørbitz and K. K. Andersson (2003) X-ray and spectroscopical studies of dinuclear sites in ribonucleotide reductase R2 from mouse show new carboxylate and tyrosyl radical shifts. *J. Inorg. Biochem.* (in press)