ESRF	**Experiment title: Study of ferryl myoglobin as model for high valent activated oxygen complexes of heme proteins and a with unusual EPR spectrum**	**Experiment number**: LS-1895
Beamline: 14: 3	**Date of experiment**: from: April 30 01 to: May 1 01	**Date of report**: 30 September 01
Shifts: 3	**Local contact(s)**: **Dr. Joanne E. MCCARTHY**	*Received at ESRF:*

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Report:

We obtained data of myoglobin at different pH and incubation time, see below of the 3 complete sets obtained. The beam was very good for the native pH 6.8 set overall quality of data good as summarized:

myoglobin resting pH 5.2 - 1.40 Å resolution - Rmerge 7.7% - Completeness (observed) 83.8%, **but we could not process this data.**

myoglobin resting pH 6.8 - 1.20 Å resolution - Rmerge 5.9% - Completeness (observed) 90.4% - Rwork 18.1% Rfree 19.6%, complete structure is solved

myoglobin incubated with t-butyhydroperoxide at Ph 6.8 - 1.40 Å resolution - Rmerge 8.3% - Completeness (observed) 86.4%, same structure as with native/resting protein. **The native pH 6.8 will be used in a novel prublication.**

Binding of Co(II) and Mn(II) to R2 from mouse Ribonucleotide Reductase (RNR)

Two full data sets were collected during the time allocated for this experiment. These were on R2 crystals from mouse RNR soaked with Co(II) and Mn(II) for 3 hours. Earier experment with shorter soak times did only have one metal ion binding in the dinuclear site (earlier reported wrongly with site full in report LS 1884). The structures of the two metal complexes of R2-RNR have been satisfactorily refined and selected data for the models are listed in

Table 1. The metal center for the Mn-R2 complex is seen in Figure 1 and the Co complex in Figure 2. A manuscript is in preparation with data from the Co-R2 complex and spectroscopy.

Table 1 *Statistics from data collection and refinement for Co(II)-R2 and Mn(II)-R2 from mouse RNR*

	Co(II)-R2	Mn(II)-R2
Unit cell (Å)	75.1 106.9 91.5 90 90 90	75.8 107.3 92.0 90 90 90
Space group	C2221	C2221
Resolution (Å)	2.4	2.2
No. of reflections	15274	19473
R-factor	21.8	21.1
R-free	29.7	27.8

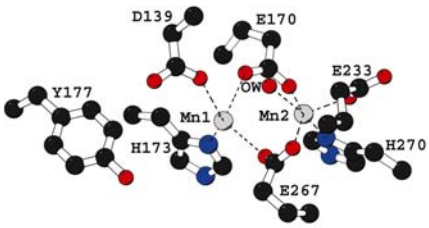

Figure 1 Metal center in Mn(II)-R2 from mouse RNR

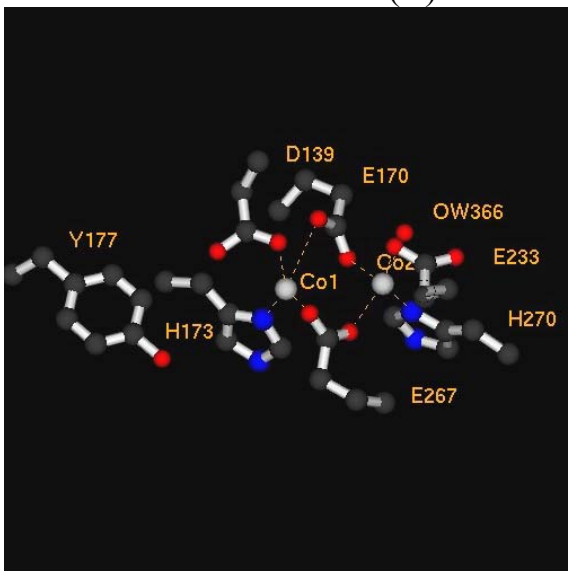

Figure 2. Metal center in Co(II)-R2 from mouse RNR

Investigation of malat dehydrogenases (MDH) from Thermophilic bacteria

Two data set of a single site mutant (T187C) native crystal and a crystal soked with Cd^{2+} of the MDH-gene from the green gliding thermophilic *Chloroflexus Aurantiacus* were collected to 2 Å resolution. Unfortunately, both datasets proved to be impossible to process, propably due to twinning in the enantiomeric space group $P4_32_12$. This very interesting mutant is now crystallized in another space group and data was collected collected at SNBL (see report 01-01-311/312).

Related publication and presentations:

Hersleth, H.-P., B. Dalhus, C. H. Görbitz and K. K. Andersson	Compound II in Peroxidases: New Resonance Forms Suggested by pH Dependent Structures of Myoglobin Intermediates Formed by Oxidation with Peroxides.	*J. Inorg. Biochem*.
page	**Vol./År:**	**ISSN:**
260	260 (2001)	0162-0134

Hersleth, H.-P., B. Dalhus, C. H. Görbitz and K. K. Andersson	The Structure of the Myoglobin Comopound II Intermediate Formed by Reaction with Hydrogen Peroxide	*NorFA Research Training Course: Application of X-ray Synchrotron Radiation In Chemistry, Biology and Physics*, 24th June - 1 July 2001, Sønderborg, Denmark., (poster)

Karlsen, S., K.R. Strand, and K. K. Andersson	Binding of Co(II) and Mn(II) to R2 from mouse Ribonucleotide Reductase (RNR)	*NorFA Research Training Course: Application of X-ray Synchrotron Radiation In Chemistry, Biology and Physics,* 24th June - 1 July 2001, Sønderborg, Denmark, (poster)

Copy of Pubished abstract:

Compound II in Peroxidases: New Resonance Forms Suggested by pH Dependent Structures of Myoglobin Intermediates Formed by Reaction with Peroxides.

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The biological conversions of O_2 and peroxides to water as well as certain incorporations of oxygen atoms into small organic molecules can be catalyzed by metal-ions in different clusters or cofactors. The catalytic cycles of these reactions pass through similar metal-based complexes in which one oxygen- or peroxide-derived oxygen atom is coordinated to an oxidized form of the catalytic metal-center. In haem-based peroxidases or oxygenases the ferryl ($Fe^{IV}O$) form is important in the compound I and compound II complexes, which are two and one oxidation equivalents higher than the ferric (Fe^{III}) form, respectively. In this study we report three high resolution X-ray structures of a compound II model protein, obtained by reacting hydrogen peroxide with ferric myoglobin at pH 5.2 (with 1.35 Å resolution), 6.8 and 8.7. The molecular geometry is virtually unchanged compared to the ferric form, indicating that these reactive intermediates do not undergo large structural changes. The essential Fe···O distance is 1.9 Å at all pH-values. This observation, together with the hydrogen bonding of the distal histidine, suggests that the dominating compound II resonance form is possibly a hydroxyl radical-ferric iron[1] at low pH and a hydroxyl-ferryl iron at high pH, and not an oxo-ferryl form as has been suggested previously. The 1.9 Å Fe···O distance is in agreement with an EXAFS study of compound II in horseradish peroxidase.

Reference:

1. Hersleth, H.-P., Dalhus, B., Görbitz, C.H. and Andersson, K.K. (2001) *J. Biol. Inorg. Chem.* (in press)

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