| SNBL | **Experiment title:**<br>Study of ferryl myoglobin as model for high valent activated oxygen complexes of heme proteins and a with unusual EPR spectrum<br>+ Studies of substrate-bound forms of methane monooxygenase | **Experiment number:**<br>**1-02-311**<br>**1-02-312** |
|---|---|---|
| **Beamline:**<br>BM01A | **Date of experiment:**<br>from: 07-Sept-01 7:00 to: 11-Sept-01 07:00 | **Date of report:**<br>**30-Sept-01** |
| **Shifts:**<br>12 | **Local contact(s):**<br>Dr. Jon Are BEUKES | *Received at UNIL:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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1) We obtained data of myoglobin at different pH and incubation time, see Table of complete sets obtained. The beam was very good for **this group** and overall quality of data good as summarized:

We got three data full (one bad) sets of myoglobin.

| | Resolution Å | $R_{merge}$ % | Completeness % | $R_{work}/R_{free}$ % |
|---|---|---|---|---|
| pH 5.2 - native (good) | 25 -1.35 | 6.1 | 82.5 | |
| pH 5.2 – native (bad) | 1.40 | can not integrate | | |
| pH 6.8 - 5 min with t-butyl-hydrogenperoksid | 25- 1.35 Å – | 4.6% - 96.5% | | |

We did not get any crystals of methane monooxygenase.

**Investigation of malat dehydrogenases (MDH) from Thermophilic bacteria**

Two data set of a single site mutant (T187C) native crystal and a crystal soaked with $Cd^{2+}$ of the MDH-gene from the green gliding thermophilic *Chloroflexus Aurantiacus* were collected to 1.8 and 2.1 Å resolution. Probably, at least one of the dataset should be possible to process. This very interesting mutant with an engineered disulphide bond.

**Investigation of the dinuclear iron site of the R2 subunit of Mouse Ribonucleotide Reductase (RNR)**

5 full data sets were collected during the time allocated for experiments:

Data set 1: Diferrous form of R2

R2 crystals were soaked for about 3 hours in mother liquid containing 5 mM $Fe^{2+}$ and 2 mM ascorbate to prevent oxidation. The crystals were flash-frozen in liquid nitrogen.

Data set 2: Diferric form of R2

R2 crystals were soaked for about 3 hours in mother liquid with 5 mM $Fe^{2+}$ and 2 mM ascorbate to prevent oxidation. Crystals were transferred to cryosolution containing 20 mM $H_2O_2$ and flash-frozen after 30 seconds.

Data set 3: Diferrous form of R2 with azide

R2 crystals were soaked for about 3 hours in mother liquid with 5 mM $Fe^{2+}$ and 2 mM ascorbate to prevent oxidation. Crystals were transferred to cryosolution with 100 mM $NaN_3$ and flash-frozen after 30 seconds.

Data set 4: $Mn^{3+}$ form of R2

R2 crystals were soaked for about 3 hours in mother liquid with 5 mM $Mn^{2+}$ and 2 mM ascorbate to prevent oxidation. Crystals were transferred to cryosolution containing 20 mM $H_2O_2$ and flash frozen after 30 seconds.

Data set 5: $Mn^{2+}$ form of R2 with azide

R2 crystals were soaked for about 3 hours in mother liquid with 5 mM $Mn^{2+}$ and 2 mM ascorbate to prevent oxidation. Crystals were transferred to cryosolution containing 100 mM $NaN_3$ and flash frozen after 30 seconds.

4 of the 5 data sets are now processed and a summary is seen in Table 1. Refinements and map interpretations are in progress. Hopefully, the manganese and iron complexes of R2 may serve as models for the dinuclear iron site of the R2 subunit of RNR in higher organisms and other iron-oxygen proteins.

Refinements and map interpretations are in progress. Hopefully, the manganese and iron complexes of R2 may serve as models for the dinuclear iron site of the R2 subunit of RNR in higher organisms and other iron-oxygen proteins.

Table 1: Data collection statistics (Space group $C222_1$)

| | Data set 1 R2-Fe(II) | Data set 2 R2 – Fe(III) | Data set 3 R2-Fe(II)-azid | Data set 4 R2-Mn(III) | Data set 5 R2-Mn(II)-azid |
|---|---|---|---|---|---|
| Cell dim. (Å) | 76.09<br>107.47<br>91.83 | 75.78<br>106.42<br>91.44 | 76.07<br>107.43<br>91.90 | 76.67<br>107.07<br>91.96 | |
| Resolution (Å) | 2.4 | 1.7 | 2.4 | 2.4 | 2.2 |
| No. of reflection | 13 956 | 36 387 | 13 419 | 14 131 | |
| Completeness | 94.0 % | | 89.2 % | 94.3 % | |
| $R_{merge}$ (%) | 11.9 | 6.1 | 12.6 | 7.3 | |

**Related publications and presentations:**

| | | |
|---|---|---|
| Hersleth, H.-P., B. Dalhus, C. H. Görbitz and K. K. Andersson | An Iron-Hydroxide Moiety in the 1.35 Å Resolution Structure of Hydrogen Peroxide Derived Myoglobin Compound II at pH 5.2 | *J. Biol. Inorg. Chem*. |
| | **Nr./Vol./År:** | **ISSN:** |
| Full paper, in press | (2001) | 0949-8257 |

| | | |
|---|---|---|
| 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 | Structure of the myoglobin intermediate Compound II formed by reaction with hydrogenperoxide | *17th Nordic Structural Chemistry Meeting*, 7th - 10th January 2001, Århus, Denmark, p14 |
| Hersleth, H.-P., B. Dalhus, C. H. Görbitz and K. K. Andersson | Structure of the myoglobin intermediate Compound II formed by reaction with hydrogenperoxide | *Brukermøte norsk synkrotronforskning,* 18.-19. januar 2001, Gardermoen, Norge |

| | | |
|---|---|---|
| Hersleth, H.-P., B. Dalhus, C. H. Görbitz and K. K. Andersson | High Resolution Structures of a Model for Compound II in Peroxidases obtained from hydrogen peroxide incubation with Myoglobin at different pH | *The 37th Norwegian Biochemical Society Meeting at Bieto*, January 18-21, SL5 |
| | | |
| 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) |