

**Experiment title:**

EXAFS and XANES studies of the haem containing protein:
myoglobin reactive intermediates.

Experiment**number:**

01-01-283

Beamline:

BM01B

Date of experiment:

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Shifts:

12

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We got problem with He-leaks the first day and lost a 1/2 day of collection time, as well as we lost one other 1/2 day of collection time due to a problem in an other beam line.

We started to study a solid pellet of ferric haem model compound to find the iron edge at room temperature. After several trials with different detectors we finally choose a photodiode detector in fluorescence mode. We collected Fe(III) XANES data at 15 K on 10 mM ferric-azide myoglobin complex at pH 6.8 over night to get a reference of a S= 1/2 system, that should be different from a possibly more oxidized compound II. The signal-to-noise ratio was very low preventing us to obtain usable EXAFS data. Next sample was 10 mM compound II myoglobin pH 6.8 in solution that was collected for 14 hours at 15K. The position of the iron-edge was nearly identical to the Fe(III) S= 1/2 from the azide complex (see Figure next page), in addition a small pre-edge peak was observed at 7112,6 eV. The last sample collected for more than 24 hours was compound II myoglobin in crystals under identical conditions as in the solution sample and for the earlier 3D structures. Also this sample had similar Fe-edge to the Fe(III) S= 1/2 from the azide complex and the small pre-edge resembled the edge in solution (see Figure of the three XANES spectra).

Due to the low signal-to-noise ratio it is difficult to draw firm conclusion from the obtained data. The myoglobin compound II data for both in crystal and in solution might be explained by:

-That they both are Fe(III) oxidation state in compound II in myoglobin. We do not have a good model for ferric intermediate $S = 3/2$ state. So we can not give any information if it would be a ferric $3/2$ or $1/2$ spin state that would be spin coupled to a single oxygen-based radical to form the $S=1$ state of compound II in myoglobin.

-That compound II in myoglobin have been photo-reduced by the synchrotron beam from the initially protonated ferryl (Fe(IV)OH) form to a ferric myoglobin.

So at present the new multi-element detector is needed to be able to definitively clarify the oxidation state, and by EXAFS measurements obtain the reaction site distances and compare them to the ones obtained from the 1.35 \AA resolution 3D structure measured at SNBL.

