ESRF	Experiment title: Polarized XANES of hemoprotein single crystals	Experiment number: LS-865
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Report

In the last run session, we have carried out the present project in the case of carboxymyoglobin (MbCO). We have refined the experimental protocol to carry out polarized X-ray absorption measurement at low temperature. MbCO single crystals (0.5*0.8*0.5 mm^3) in a cryoprotected mother liquor were placed in quartz capillary tubes, and mounted on a goniometric head. They were pre-oriented on a X-ray diffractometer placed at the Institute de Biologie Structure1 (IBS). then put inside a cryostat at He atmosphere, on the focal point of the X-ray Absorption Hutch of BM32. The goniometric head inside the cryostat can rotate more than 90 degrees around a vertical axis. By using dynamically focussed X-rays, Fe K-edge XANES angular resolved spectra have been acquired between 200K and 10K with a 24 element Canberra fluorescence detector. At 20K, XANES angular resolved spectra of the cryogenic photoproduct of MbCO (Mb*) have been acquired as well. Fig. 1A shows the XANES angular dichroism observed in MbCO, changing the orientation of the crystal axis a and c with respect to the polarization vector E. The **El/a*** spectrum (corresponding to an angle of **22°** between **E** and the heme normal) and the **E//c** spectrum (i.e. **E** near parallel to the average heme plane) are reported. In Fig. IB, the XANES angular dichroism of Mb* is shown as well. The dramatic spectral changes observed along the heme normal are consequent to the rupture of the Fe-CO bond after absorption of a quantum of light. Owing to the recent improvements in dynamical focussing of the X-ray optics, and the use of the 24 element fluorescence detector, we succed to collect **preliminar polarized EXAFS data on small crystals** (0.2*0.2*0.2 mm^3) with a statistic of about 80000 c/s. A first analysis of these data will allow to solve (i.e. to separately measure with very high resolution) the Fe-N, and the Fe-His distance changes after low temperature photolysis.

Damaging effects have been investigated at the measuring conditions, in the case of ferric aquometmyoglobin (Mb^+H_2O) and carboxymyoglobin (MbCO): in the case of Mb^+H_2O, still at very low temperature the 10^{11} photon/s flux on the 0.4*0.4 mm spot produces thermally activated electrons in the mother liquor solvent that can efficiently migrate to the porphirin and reduce the ferric iron, the overall process taking some hours. The effect can be used to study the modifications of the protein metal site after 'in situ' photoreduction. No changes at the Fe site level have been observed at l0K-200K in the case of MbCO crystals and solutions for the duration of the experiment.

These low temperatureXANES data can be reproduced [l] and interpreted in the frame of the multiple scattering approach, by using our G4XANES package [2]. Further polarized EXAFS data should verify if the the CO molecule inside the hydrophobic myoglobin pocket lies at a distance of 3.5-3.9 Å from the iron [3].

REFERENCES

[1] Bianconi A et al. Nature 318:685-687 (1985).

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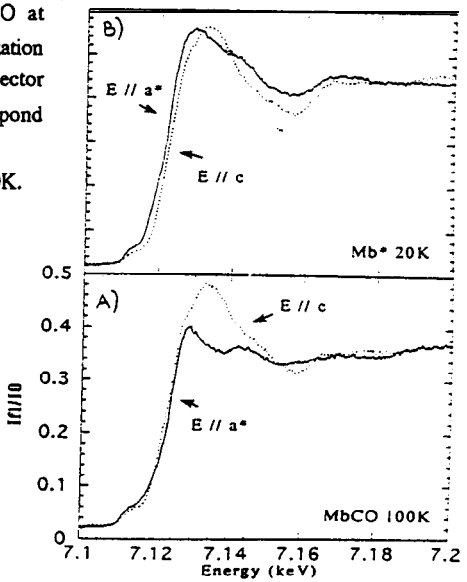

Fig.1 A) Angular resolved XANES spectra of MbCO at T=100K. The E//a* curve corresponds to a polarization angle α (i.e. angle between the photon polarization vector and the heme normal) of 23°. The E//c curve correspond to α=86°, i.e. E ≈ parallel to the heme plane.
B) Angular resolved XANES spectra of Mb* at T=20K.