Polarized XANES of hemoprotein single crystals

LS-1003

BM32 **Sep 17-22, 1998**

12 **J.L. Hazemann** **Feb 23, 1999**

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Report

In the last run session, we have refined the experimental protocol to carry out polarized EXAFS measurement at low temperature on MbCO and Mb+H_2O. Single crystals in cryoprotected mother liquor were placed in quartz capillary tubes, mounted on a goniometric head, pre-oriented on a X-ray diffractometer placed at the Institute de Biologie Structurel (IBS), then put inside a cryostat at He atmosphere, on the focal point of the X-ray Absorption Hutch of BM32. Fe K-edge XANES and EXAFS angular resolved spectra have been acquired between 10K and 240K with a 30 element Canberra fluorescence detector. At 20K, EXAFS angular resolved spectra of the cryogenic photoproduct of MbCO (Mb*) have been acquired as well. In Fig. 1 the dichroic XANES changes from MbCO to Mb* are shown [1]. Fig. 2 shows the angular dichroism of the $\chi(k)$ of Mb* up to 12.5 $Å^{-1}$, with respect to the polarization vector **E** parallel to axis **a*** and **c**. The spectra have been acquired on small crystals (0.2*0.2*0.2 mm^3) summing over four spectra with a statistic of about 80000 c/s. Similar EXAFS spectra have been acquired for MbCO. Dramatic spectral changes observed along the heme normal follow the rupture of the Fe-CO bond after absorption of a quantum of light. XANES simulations and EXAFS analysis are in progress in our laboratory to solve (i.e. to separately measure with very high resolution) the Fe-N_p and the Fe-His distance changes after low temperature photolysis, and to look if there is any spectral feature in Mb* assignable to the 3.5-4.0 Å far away CO molecule. Moreover photoreduction effects have been eventually found and investigated in

solution and on the crystals 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 is under study the look at modifications of the metal site after 'in situ' photoreduction. No changes at the Fe site level have been observed at 10K-200K in the case of MbCO crystals and solutions for the duration of the experiment.

REFERENCES

[1] Della Longa S et al. Communication presented to the 6th Int. Conf. on "Biophys. and Synch. Rad.", Chicago, Aug 4-8, 1998.

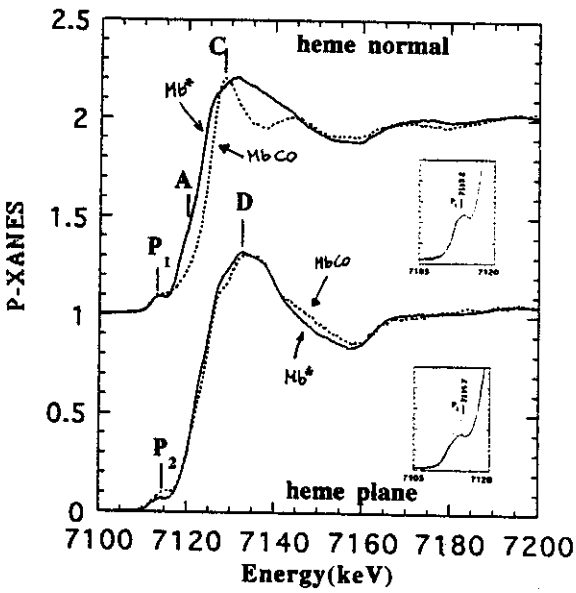

Fig.1 A) Angular resolved XANES spectra of MbCO (dotted curve(and Mb* (solid curve) at T=20K. The **E//a*** upper curves corresponds to a polarization angle α (i.e. angle between the photon polarization vector and the heme normal) of 23^o. The **E//c** lower curves correspond to $\alpha=86^o$, i.e. E ≈ parallel to the heme plane.

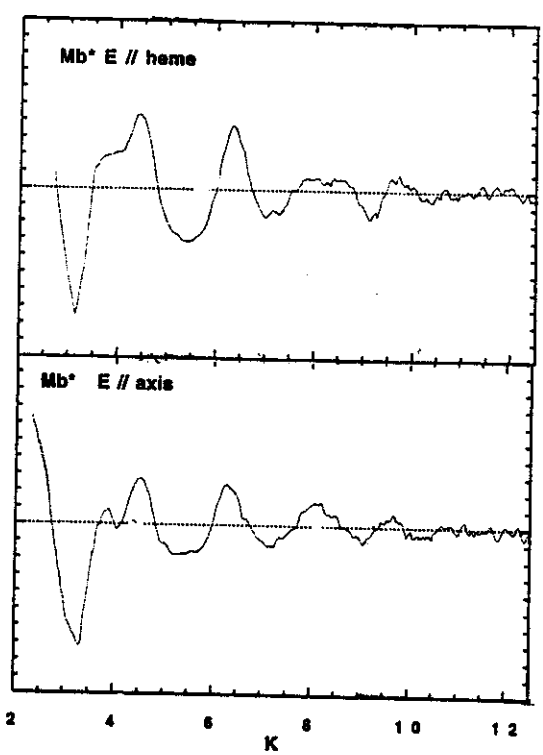

Fig.2 Angular resolved $\chi(k)$ of Mb* at T=20K. Upper curve: **E//c** polarization. Lower curve: **E//a*** polarization.