

Experiment title:
**Polarized XANES of hemoprotein
single crystals**

Experiment
number:
LS-714

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J.L. Hazemann

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Names and affiliations of applicants (*indicates experimentalists):

S. Della Longa, Univ. L' Aquila (Italy)
A. Arcovito, Univ. Roma (Italy)
E. Finocchiaro, Univ. Roma (Italy)

Preliminary Report

In the last run session, we have demonstrated the feasibility of the present project. As a first step, we have refined the experimental protocol to carry out polarized X-ray absorption measurement at low temperature.

Myoglobin single crystals ($0.5 \times 0.8 \times 0.5 \text{ mm}^3$) in a cryoprotected mother liquor were placed in quartz capillary tubes, and mounted on a goniometric head. They were pre-oriented on a precession camera for X-ray diffraction placed at the Institute de Biologie Structurale (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 (perpendicular to two polarization axes of the oriented protein crystal). Therefore by using sagittally focussed X-rays, Fe K-edge XANES angular resolved spectra have been acquired between 200K and 10K with a multielement Canberra fluorescence detector.

Damaging effects have been investigated at the measuring conditions, in the case of ferric equometmyoglobin ($\text{Mb}=\text{H}_2\text{O}$) and carboxymyoglobin (MbCO): The Fe-heme site is highly damaged at room temperature in less than one hour. Moreover, in the case of $\text{Mb}=\text{H}_2\text{O}$, still at very low temperature the 10^{11} photon/s

flux on the 0.4*0.4 mm spot produces thermally activated electrons [1] in the mother liquor solvent that can efficiently reduce the ferric iron site, the overall process taking some hours. The undesired effect can be avoided by slightly displacing progressively the sample position. This is limited by the crystal dimensions, as well as the I/O stability vs. energy, that depend in turn by the sagittal focus position. On the other hand, no alteration at the Fe site level has been observed at very low temperature (10K-200K) in the case of MbCO crystals and solutions for the duration of the experiment.

The experimental spectra depicted in Fig. 1 show some preliminar data on the XANES angular dichroism of a MbCO crystal (taken from an old batch of native sperm whale Mb, having $P2_1$ symmetry) at low temperature ($\approx 40K$) that can be reproduced [2] and interpreted in the frame of the multiple scattering approach, by using our G4XANES package [3].

REFERENCES

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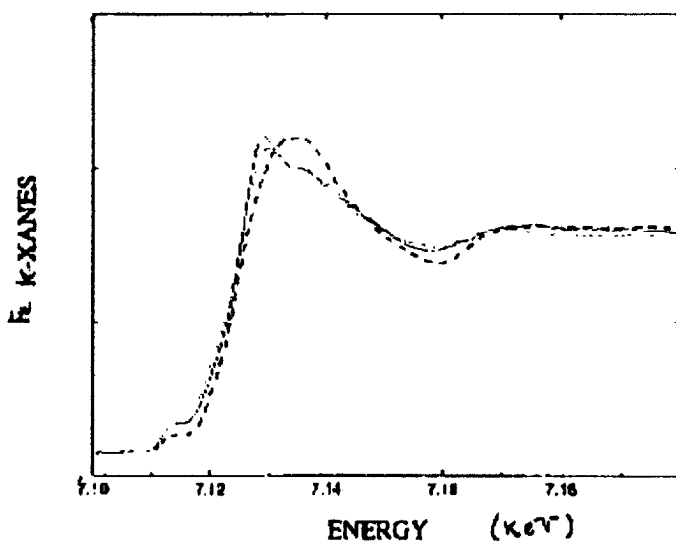


Fig. 1 Angular resolved XANES spectra of MbCO at about 40K. The dashed curve is the spectrum with polarization angle $\alpha \approx 85$ degrees, (α : angle between the photon polarization vector and the beam normal). The dotted and solid curve correspond to spectra with $\alpha \approx 60$ and $\alpha \approx 40$ degrees, respectively.