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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF. This double-page report will be reduced by ESRF to a one page, A4 format, and will be published in the Annex to the ESRF Annual Report.

Should you wish to make more general comments on the experiment, enclose these on a separate sheet, and send both the Report and comments to the User Office.

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- type your report, in English.
- make sure the report does not exceed the space available; tables and figures may be included if you wish.
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- bear in mind that the report will be reduced to 71% of its original size. A type-face such as "Times", 14 points, with a 1.5 line spacing between lines for the text produces a report which can be read easily.

Note that requests for further beam time must always be accompanied by a report on previous measurements.

Postal address: User Office, ESRF, B.P. 220, F-38043 GRENOBLE Cedex, France.
Telephone: (33) 76 88 25 52; Fax: (33) 76 88 20 20; e-mail: useroff@esrf.fr

ESRF	**Experiment title:** Phosphor K-edge absorption measurements on Threonine Synthase	**Experiment number**: SC1024
Beamline: ID1	**Date of experiment**: from: 4-12-2002 to: 6-12-2002	**Date of report**: 11-02-2003
Shifts: 7	**Local contact(s)**: Dr Peter Boesecke	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Valérie Biou LEBS, CNRS Gif surYvette

*Jean-Marie Bois, EMBL Grenoble

*Peter Boesecke, ESRF

*Richard Kahn, IBS Grenoble

*Corine Mas, IBS Grenoble

*Lionel Nauton, IBS Grenoble

*Heinrich Stuhrmann, IBS Grenoble

Report:

Background

Threonine synthase (TS) is an allosteric enzyme that catalyses the last step to yield aminoacid threonine. It is activated by S-adenosyl methionine (SAM). The reaction is made through the use of cofactor pyridoxal phosphate (PLP), bound through a Schiff base to lysine 163 of the enzyme in its quiescent state. We have solved the structure of apo-TS, therefore lacking its PLP (Thomazeau et al, 2001). Using the "cryo-bench" spectrophotometer installed at the ESRF, we determined soaking conditions of crystals with PLP. The electron density map collected on a soaked crystal revealed the presence of PLP bound to the enzyme. However, the PLP position is not the same as that seen in the other 5 structures of enzymes from the same family, for example in threonine deaminase (TD). As shown on figure 1, even though it is covalently bound to lysine 163, the PLP cofactor is rotated about 150 degrees away from the usual position. This brings the phosphate in a completely different chemical environment, close to a histidine side chain in particular. This result is potentially very exciting, as it could reveal an inactive position of the PLP, waiting to be triggered by activator S-adenosyl methionine. This is indeed what we observed using phosphor NMR (paper in preparation).

During the present experiment, we carried on absorption and diffraction measurements at the phosphor K-edge.

A special experimental box containing helium gas and a liquid helium cryostream, was designed and built (Carpentier et al, 2002). It allowed the installation of an imaging plate detector in a cylindrical position (see figure 1). It was installed at ID1 prior to the experiment start.

References

Carpentier, P., Boesecke, P., Bois, J.-M., Chesne, M.-L., Fanchon, E., Kahn, R., Stuhrmann, H., Vicat, J. *Acta Physica Polinica A*, (2002), 101, 603-612.

Thomazeau, K., Curien, G., Dumas, R. and Biou, V. Crystal structure of threonine synthase from A. thaliana *Protein Science* (2001), 10, 638-648

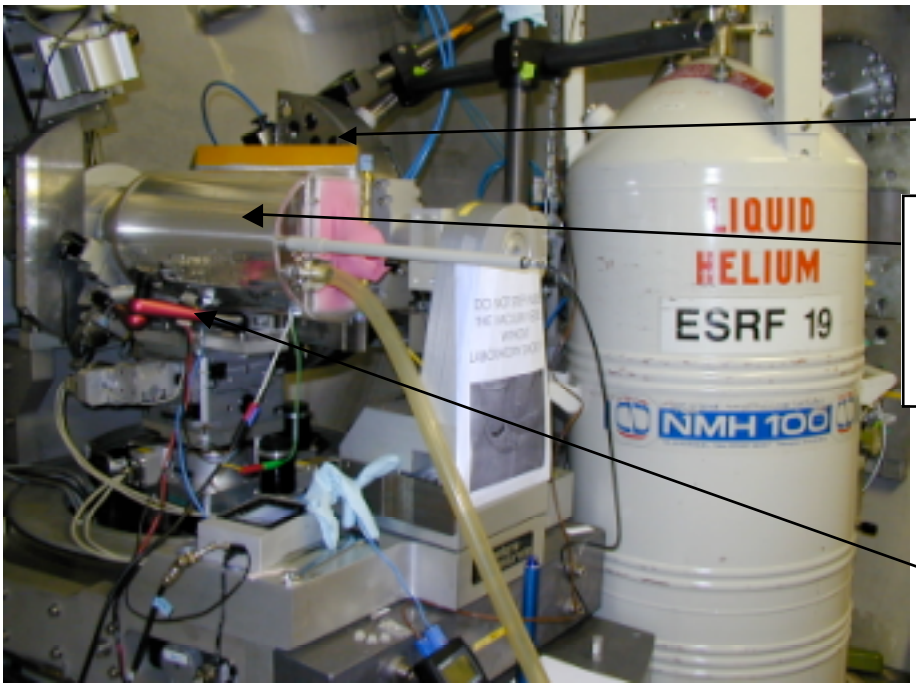

Figure 1: helium box mounted in ID1 experimental hutch.

Experiments performed

A) X-ray absoption near the K-edge of phosphorus (λ_k = 5.76 Å, E_k = 2152 keV)

A photo diode was used to measure the primary beam attenuation.
The samples for transmission measurements need to be thin: the penetration depth of 5.7 Å photons in water solutions (and organic material) is 20 μm.
Both proteins studied have about 1 P atom per 50 kD protein. At 40% hydration (as it is the case for the crystal of threonine synthase) a **change of the transmission by 1% is expected** at the peak absorption.
Protein solutions had to be dried. A drop of the solution was deposited on a 6 μm Mylar foil and left to dry for a couple of hours.

Samples measured:

$(NH_4)_2HPO_4$, embedded in cellulose acetate, thickness of film: 37 μm , 1 scan	
Pyrodoxal phosphate (PLP)	several samples,1 scan each
Threonine synthase (TS) containing PLP	20 scans out of 21 were successful
Cystathionine-ß-lyase (CBL) containing PLP	4 scans out of 7 were successful

The absorption spectra are shown on figure 2. All spectra show an absorption peak at 2153 eV. The $(NH_4)_2HPO_4$ gives a much more intense spectrum due to its higher concentration.
Although the spectrum of PLP in threonine synthase appears to be different from that of PLP in Cystathionine-ß-lyase at energies above the edge, this observation would need to be confirmed by more accurate data.

B) Diffraction measurements near the K-edge of phosphorus:

The influence of absorption is also visible in multi-wavelengths diffraction experiments. For the purpose of an EXAFS (here the term XANES would be more appropriate) measurement, the anomalous dispersion would have to be taken at a reasonably large number of wavelengths. The absorption spectrum would correspond to the average dispersion of the diffracted intensity.
Crystals of threonine synthase available showed no sign of diffraction. The last three hours of beam time were used for **test experiments of X-ray diffraction from tetragonal lysozyme at the wavelength of 5.76 Å.**
Diffraction peaks are observed to 7 Å resolution only (image not shown).

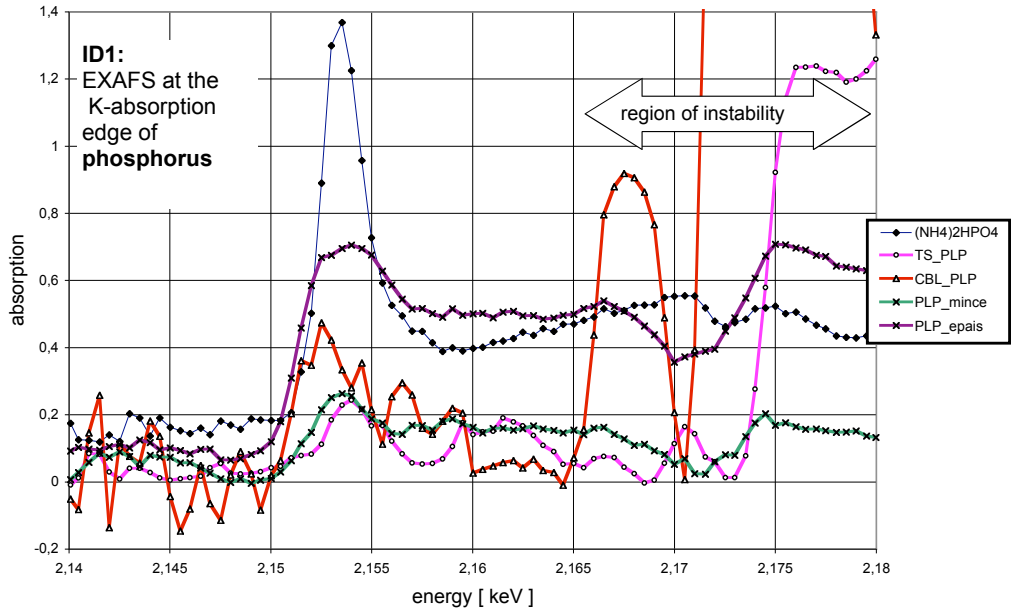

Figure 2 Absorption spectra near the Phosphor K-edge. TS-PLP, threonine synthase; CBL-PLP, cystathionine ß-lyase; PLP_mince; thin film of PLP alone; PLP_epais, thicker film of PLP alone.

Clean X-ray absorption spectra were obtained in relatively narrow energy range, which was fortunately centred at the K-edge of phosphorus. At slightly larger energies, i.e. about 10 eV above the edge, artefacts were introduced. Considerable time has been invested by one of us (P.B.) to find the reason for this unexpected behaviour of the X-ray optics (double mirror – double monochromator). *The production of a monochromatic, 'constant –exit' beam at low X-ray energies will need further systematic studies*. All foreseen samples were not measured due to a lack of time. Also, a fluorescence measurement may help alleviate transmission problems.

A few **X-ray diffraction** test pictures were taken at a temperature of T = 40K. The intensity was lower than expected. A considerable gain should have come from the use of the image plates designed for the use of low energy radiation (LE-IP). Their protective layer of 5 μm cellulose acetate is much lower than that from ordinary Ips with 70 μm protective layer thickness.

Conclusions

The helium box remained operational throughout the beam time. The leaks were negligibly small. In particular the large thin Mylar window of 6 μm thickness was not damaged.
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The helium pressure of 2 mb above atmospheric pressure remained stable. The liquid helium consumption was low, about 1 l per hour.
The sensitivity of image plates designed for low energies needs to by studied over a broad range of wavelengths, in the same way as this has already been done with 'ordinary' image plates (MI384).
The following modifications and additions to the helium box are presently made:

1) intermediate chamber for the transfer of the sample into the helium-filled box.
2) X-Y alignment of the sample in a way which avoids opening the helium box.
3) deviation of the cold helium gas flow by a very thin walled internal cylinder of 8 cm diameter,
4) retractable fluorescent screen inside the helium box, for direct observation of the crystal in the beam. (this works perfectly well with soft X-rays)

These are low-cost modifications which will be used in May 2003. They are essential for diffraction experiments and spectroscopic measurements with soft X-rays, in particular at low temperatures. The system will work in such a way that no air may penetrate the helium-filled box during the experiment, including sample exchange. In future, there should be no problems due to the beam line including the helium box with the image plate. For protein crystallography with soft X-rays the preparation of the crystal and their mounting will decide on the success.