Report on the project CH 1523

Phosphorus K edge absorption measurements on threonine synthase.

The results of the experiments done at ID01 on 3 to 5 December 2003 have been published:
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X-ray spectroscopy and X-ray diffraction at wavelengths near the K-absorption edge of phosphorus
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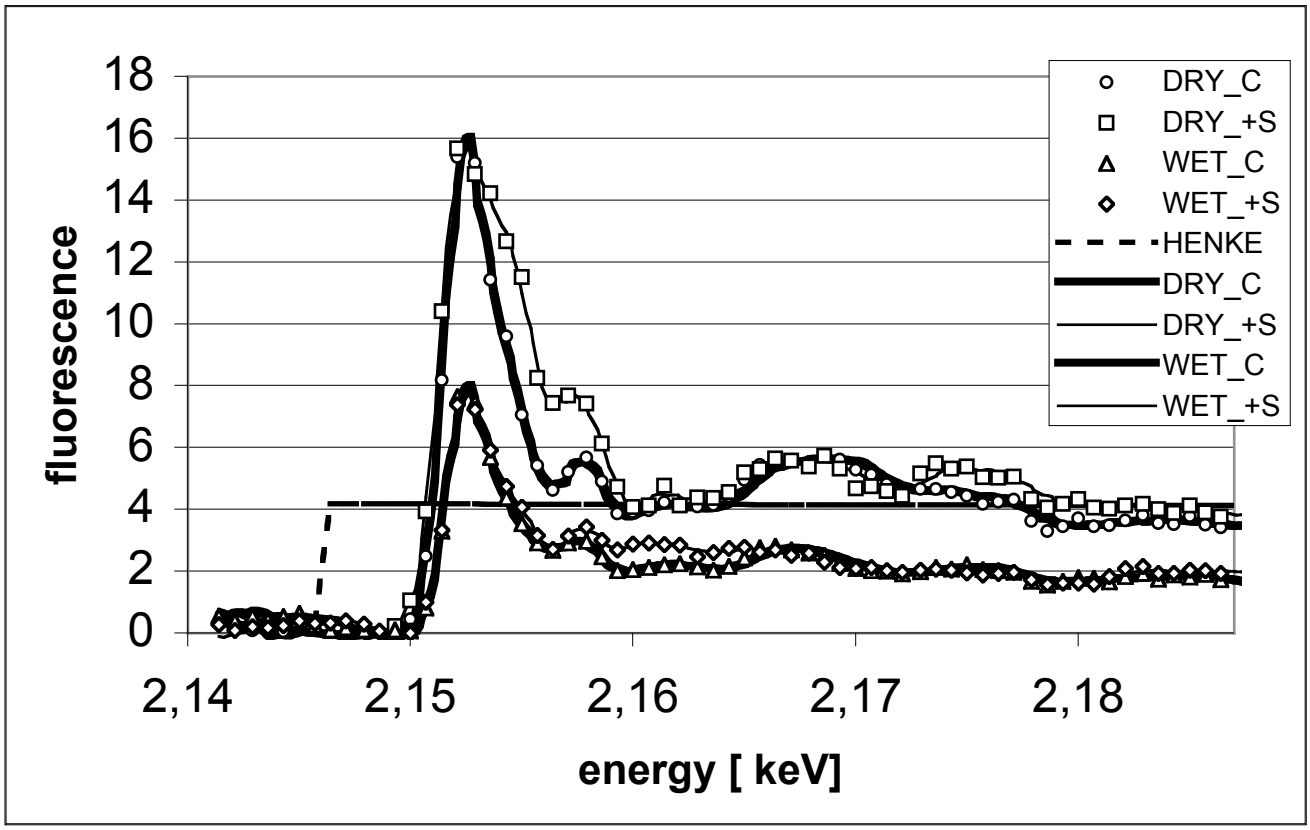
Abstract
Phosphorus is an abundant element in living organisms. It is traceable by its x-ray absorption spectrum showing a strong white line at its K edge, comparable to that observed with the L_{III} edges of rare earth ions. With purple membrane the variation of the imaginary part of anomalous dispersion of phosphorus is found to be close to 20 anomalous electron units. Experiments of anomalous diffraction at wavelengths near the K absorption edge of phosphorus confirm this result. The spatial distribution of lipids derived from anomalous diffraction agrees with earlier results from neutron diffraction.
Test experiments on single crystals of the ADP-ATP carrier protein using 5.76 Å photons gave a first low resolution diffraction pattern. Various techniques of crystal mounting were tried. In addition, fluorescence measurements on a solution of threonine synthase appear to hint out to a change of the phosphate environment of the cofactor upon activator binding.

A new cylindrical helium box with its new sample transfer line had been built and tested well in advance of the start of the experiment on 3 December 2003. The support of the fluorescence detector that had to fit into the helium box turned out to be a last minute action.
In a first step the transmission spectrum of test samples (ammonium dihyhrogenphosphate and purple membrane) was measured at wavelengths near the K-edge of phosphorus. Much better results were obtained by measuring the fluorescence.
In the following days the beam time was shared between diffraction experiments during day time and spectroscopic experiments on threonine synthase doped with PLP and PLP+SAM during night as the latter apparently needed no surveillance.

The diffracted intensity was collected on cylindrically bent image plates that were read out off-line. Data from purple membrane at two different wavelengths could be obtained in good quality within a couple of hours. The numerous tests with crystals of ADP/ATP carrier protein showed that crystals mounted on a plastic foil did diffract to 7 Å resolution while those mounted on loops - most of them had been prepared in advance that way - did not diffract 5.76 Å photons. Neither did the crystals of threonine synthase which were all

mounted on loops. This is probably due to the amount of solvent included with the crystal.
The fluorescence spectra from wet samples of threonine synthase (TS) with pyridoxal phosphate (PLP) were collected in a repetitive mode during night. During the first night the sample temperature had changed incidentally from 100 K to room temperature, leading to a dehydration of the sample. The dry sample gave a much more intense and hence more detailed spectrum than the wet sample. It was decided to study the sample and in particular also the sample (TS+PLP +SAM) both in the wet and in the dry state. **The differences between the fluorescence spectra from samples with and without SAM are clearly visible**.



Bold lines : TS + PLP, thin lines TS+PLP+SAM, The lower pair of curves was obtained at T=100 K, while the upper ones were measured at ambient temperature.

Pioneering experiments with soft X-rays may suffer from occasional incidents. A more serious one has been the accidental damage of the thin plastic window. The formation of frost or humidity made the window very vulnerable during the exchange of the image plate. Thanks to the efficient technical assistance from the staff of EMBL and of ID1 of ESRF the interruptions could be kept to an absolute minimum.