ESRF	**Experiment title:** Threonine synthase : looking for PLP cofactor Tests on cdsp34 protein (30 kDa, present in potato chloroplasts)	**Experiment number**: LS1655
Beamline: ID14-EH3	**Date of experiment**: from: 12-04-2000 to: 14-04-2000	**Date of report**: 24-05-2000
Shifts: 6	**Local contact(s)**:Stéphanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Valérie Biou IBS
*Karine Thomazeau IBS
Renaud Dumas Aventis Lyon
*Claudine Cohen-Addad IBS
Ron Duggleby Aventis Lyon

Report:

We solved the structure of Arabidopsis threonine synthase using MAD method. However, the PLP cofactor (which is bound through a Schiff base to an aminoacid of the protein) does not show up in the electron density map. We have proof that it is bound to the protein, and we have proof of degradations due to X-ray damage.

In order to try the best we could, we soaked the crystals with PLP and $NaBH_4$ which performs a covalent bond with PLP.

In addition, one crystal was soaked with a substrate analogue. After this, we did a trial with free radical scavengers, mannitol or thiourea.

Three data sets were collected to 2.4Å resolution, neither of which showed the presence of ordered PLP in the active site.

Those crystals are P1 space group, and a minimum of 180° data is needed to get a complete set. Electron density maps calculated on partial data show some full aminoacid side chains (in particular in the active site), which are broken or only partial when more data is added. However, the PLP never shows up even in partial maps. Mass spectrometry measurements on crystals are in progress.

Tests

Crystal tests of cdsp34 protein (30 kDa, present in potato chloroplasts). The crystals, frozen at 100K, diffracted at 19 Å resolution on ID14-EH3 (tests done on april 13th 2000).