EUROPEAN SYNCHROTRON RADIATION FACILITY

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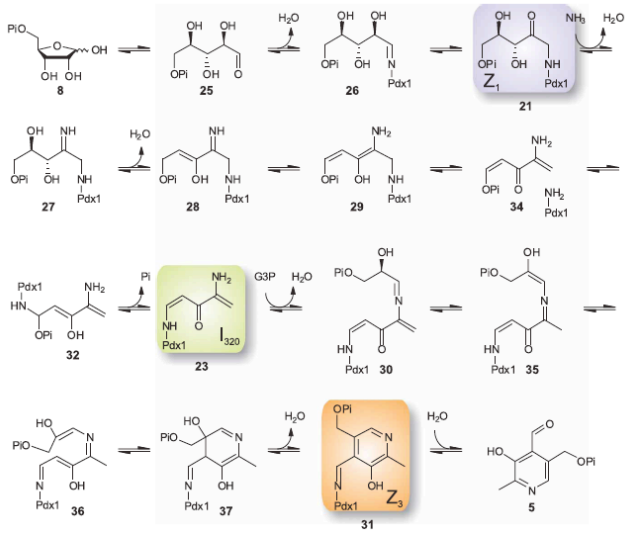
Application for beam time at ESRF – Experimental Method

Aims of the experiment and scientific background

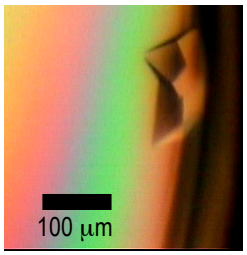
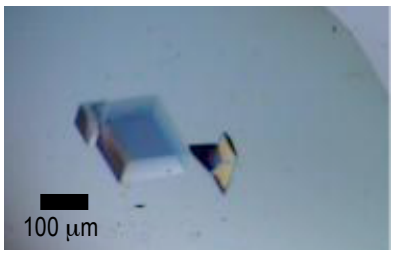
Pyridoxal 5-phosphate (PLP) is a biological active form of Vitamin B6 and is synthesized by the glutamine amidotransferase PLP synthase. The enzyme complex is made up from 24 protein subunits of the two proteins Pdx1 and Pdx2. We have determined several structures, using ESRF beamlines[1-3]. The core of the enzyme complex is formed by the dodecameric Pdx1 subunit. Pdx2 proteins attach to this core to supply ammonia by glutamine hydrolysis. In vitro, ammonia can also be supplied in the absence of Pdx2 in the form of ammonium salts, creating a viable system to study PLP biosynthesis.



The synthesis of PLP by Pdx1 from the carbohydrates ribose 5-phosphate, glyceraldehyde 3-phosphate and ammonia is proposed to occur in more than a dozen reaction steps, involving chromophoric intermeditates[4]. Indeed, the nature of these intermediates is under intense investigation, and several structures of crystallographic complexes are already known[5,6]. Here we propose studying the two chromophoric intermediates 23 and 31 in the scheme (taken from [4]).

Experimental method

We routinely produce Pdx1 crystals from an eukaryotic and from a prokaryotic organism (recombinantly expressed in *E. coli*) that diffract to 2 Å or better, as shown in the table below.

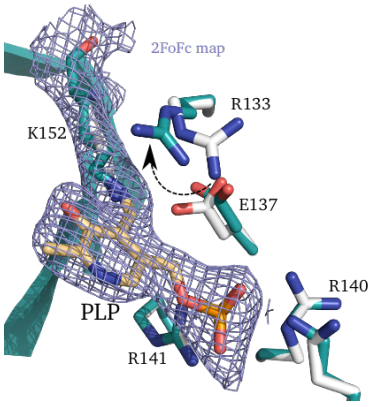
	Planta: *Arabidopsis thaliana* Pdx1.3	Extremophiles: *Thermus thermophilus* PdxS
		
Diffraction Limit	1.8 Å	1.6 Å
Space group	R3	R3

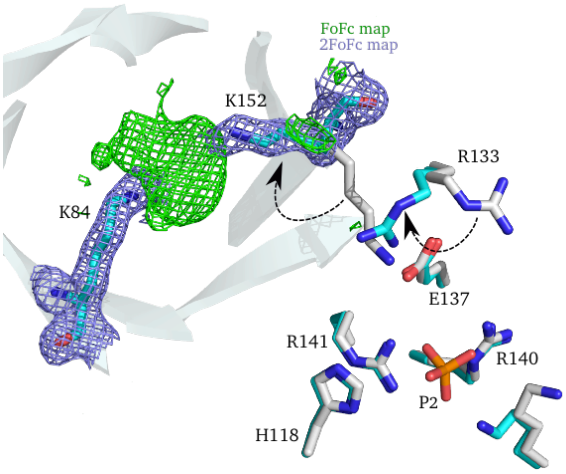
Previous experiments established conditions that are optimal for intermediate formation with regards to substrate concentrations as well as exposure times. To generate the chromophoric intermediate 23, crystals are soaked in substrate solutions of ribose 5-phosphate and ammonium salts for four days, to saturate the complexes. PLP is added to native crystals for minutes to hours to generate the product complex.

The proposed experiment will make use of the microspec to collect online spectra from 240-450 nm. These data are required to describe the nature of the bound chromophoric intermediates. The data will also provide evidence whether the species observed in the crystals are consistent with or differ from intermediates occurring in solution. This knowledge is crucial for unequivocal assignment of the crystallographic data.

Results expected

Previously, we have been able to collect data from crystal complexes of the intermediate 23 (below) and of the PLP adduct 31 (right), in both crystalline systems, at resolutions of 2.3 Å or better. The intermediate 23 is characterised by formation of an absorption maximum at 315 nm[7]. Our unpublished crystallographic data show how transamination through this intermediate leads to PLP formation. Further, soaking product PLP is consistent with formation of a covalent intermediate, in contrast to the free form of PLP detected recently[6].





The proposed experiment we will directly detect the intermediate 23. Further, we will be able to determine the nature of the PLP intermediate (due to the shift in absorption maxima, 415 nm *vs.* 395 nm). Two experimental strategies are formulated:

1.) Screen for presence of chromophoric intermediates, using the microspec, on crystals soaked according to previously established conditions for Arabidopsis Pdx1.3(23) and Pdx1.3(31), as well as *Thermus* PdxS(23) and PdxS(31); collection of complete datasets for best diffracting crystals on the basis of strongest absorbance present; collection of online spectra for these crystals.

2.) Screen for presence of chromophoric intermediates, using the microspec, on crystals soaked under varied conditions; investigation of differences in absorbance spectra; full data collection on the basis of these differences to potentially identify new intermediate species.

The proposed studies are essential to understand the complex reaction pathway of PLP biosynthesis. The data collected will allow unique insights into how the enzyme Pdx1 catalyses key steps of PLP formation.

References

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