



	Experiment title: Structure of the 6-phospho-3-hexulose isomerase, YckF, from <i>Bacillus subtilis</i>	Experiment number: LS-1673
Beamline: ID14-1 BM14	Date of experiment: from: 19/07/00 to: 22/07/00 from: 06/05/00 to: 08/05/00	Date of report: 29/08/01
Shifts: 1+3	Local contact(s): Hassan Belrhali (ID14-1) and Gordon Leonard (BM14)	<i>Received at ESRF:</i>

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Report:

6-phospho-3-hexulose isomerase (PHI) catalyses the isomerisation of D-arabino-3-hexulose-6-phosphate to D-fructose-6-phosphate. PHI is a key enzyme in the fixation of carbon, in the form of formaldehyde, by methylotrophic bacteria. This process is part of the ribulose monophosphate (RuMP) pathway, the key function of which is the condensation of formaldehyde with ribulose-5-phosphate by 3-hexulose-6-phosphate synthase (HPS) to form D-arabino-3-hexulose-6-phosphate, which in turn is isomerised by PHI to fructose-6-phosphate. In order to allow illumination of the catalytic mechanism and substrate specificity of this important family of proteins, and related homologues of unknown function from various pathogenic bacteria such as *H. influenzae* and *C. pneumoniae*, the structure YckF, a PHI from *Bacillus subtilis* was determined.

Native and selenomethionyl crystals of YckF were grown from 0.8 M sodium formate, 1 mM EDTA, 25 % (w/v) polyethylene glycol 2000 monomethylether and 10 % (v/v) MPD in 20 mM Na-HEPES buffer, pH 7.4. They belong to space group P6₅22 with unit-cell dimensions a=b=72.4, c=241.2 Å and have two molecules in the asymmetric unit. 120 images of 0.5 degrees were collected for each wavelength of a MAD experiment conducted on beamline D14-4 (May 2000) at the ESRF, Grenoble. Native data, 100 images of 0.5 degrees was collected on beamline ID14-1 (July 2000) at the ESRF, Grenoble. The structure reveals a homotetrameric organisation of subunits, an observation in common with other sugar isomerases. In order to further understand the function of YckF, structures of this protein in complex with both substrate and product are currently being pursued.