



	Experiment title: Structure determination of PK	Experiment number: MX579
Beamline: ID23-1	Date of experiment: from: 15/02/2007 to: 16/02/2007	Date of report: 01.09.2008
Shifts: 3	Local contact(s): Dr. Joanne McCarthy	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Yvonne Carius*, Axel Scheidig* Christian-Albrechts-Universität zu Kiel Zoologisches Institut – Abteilung Strukturbioogie Zentrum für Biochemie und Molekularbiologie Am Botanischen Garten 1-9 D-24118 Kiel		

Report:

Heterofermentative degradation of pentoses in lactic acid bacteria takes place via the phosphoketolase pathway. Xylulose 5-phosphate phosphoketolase (EC 4.1.2.9) is the central enzyme of this pathway. In presence of inorganic phosphate, this enzyme catalyses conversion of xylulose 5-phosphate (X5P) into glyceraldehyde 3-phosphate and acetylphosphate. So far, a limited number of molecular data are available for phosphoketolases, particularly for those from lactic acid bacteria (LAB). We have recently succeeded to clone and express in a prokaryotic system and perform a preliminary characterization of X5P phosphoketolase of *Lactococcus lactis* ssp. *lactis* (strain IL1403), one of the most important representatives of LAB in dairy industry. The protein has a molecular weight of 93.3 kDa.

We have native crystals of PK available, which are diffracting on a sealed tube X-ray generator up to 2.8 Angstroms. Furthermore, we expressed and crystallized Se-Met substituted PK (26 selenium atoms within 842 amino acids).

During the provided beam time at ID23-1 we have tested overall 20 different crystals of PK. From four crystals we could collect SAD and MAD data sets, respectively. The data statistics from the different crystals are given below. Currently we are still working on the phase evaluation. At the moment we cannot reproduce these Se-Met crystals and therefore cannot optimize the data collection. In the near future we plan to use MIR-data sets for further optimization of the experimental phases.

Crystal / data set	Se-Met PK 36
Type	peak
Size	0.1 x 0.05 x 0.02 mm ³
Spacegroup	P222
Cell dimensions	a=139.0 Å, b=157.7 Å, c=160.7 Å
Wavelength (Å)	0.97925
Resolution (Å)	3.0
Completeness (%)	99.5
<I/σ>	8.7
R _{merge} (%)	32.4
Exposure time (sec/°)	2 sec / 1.0 °
Status	Phases refinement in progress
R _{work} / R _{free} (%)	Not available

Crystal / data set	Se-Met PK 40			
Type	peak	inflection point	high remote	low remote
Size	0.1 x 0.05 x 0.02 mm ³			
Spacegroup	P222			
Cell dimensions	a=139.8 Å, b=156.1 Å, c=160.0 Å			
Wavelength (Å)	0.97925	0.9797	0.97565	1.0332
Resolution (Å)	3.2	3.2	3.2	3.4
Completeness (%)	98.1	98.2	98.0	99.5
<I/σ>	6.7	5.9	6.6	9.25
R _{merge} (%)	20.1	24.0	21.6	27.7
Exposure time (sec/°)	1	1	1	3
Status	Phases refinement in progress			
R _{work} / R _{free} (%)	Not available			

Crystal / data set	Se-Met PK 41
Type	peak
Size	0.1 x 0.05 x 0.02 mm ³
Spacegroup	P222
Cell dimensions	a=139.3 Å, b=157.9 Å, c=160.7 Å
Wavelength (Å)	0.97925
Resolution (Å)	3.0
Completeness (%)	96.0
<I/σ>	5.19
R _{merge} (%)	17.1
Exposure time (sec/°)	5
Status	Phases refinement in progress
R _{work} / R _{free} (%)	Not available

Crystal / data set	Se-Met PK 53			
Type	peak	inflection point	high remote	low remote
Size	0.1 x 0.05 x 0.02 mm ³			
Spacegroup	P222			
Cell dimensions	a=138.2 Å, b=157.4 Å, c=160.2 Å			
Wavelength (Å)	0.97925	0.97965	0.97565	1.0332
Resolution (Å)	3.4	3.3	3.7	3.5
Completeness (%)	96.6	96.9	96.5	96.2
<I/σ>	3.7	3.3	3.6	3.7
R _{merge} (%)	30.2	35.0	31.0	30.4
Exposure time (sec/°)	1	1	1	5
Status	Phases refinement in progress			
R _{work} / R _{free} (%)	Not available			