ESRF	**Experiment title:** Structure determination of pyruvate formate-lyase	**Experiment number:** LS-921
Beamline: ID14 EH3	**Date of experiment:** from: 2.5.1998 to: 4.5.1998	**Date of report:** 17.6.1998
Shifts: 6	**Local contact(s):** Dr. Wilhelm Burmeister	*Received at ESRF:*

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Report:

Pyruvate formate-lyase (PFL, 2 x 85 kDa) catalyses the conversion of pyruvate and coenzyme A into acetyl-CoA and for-mate. It thus catalyses a key step in bacterial anaerobic metabolism, and PFL inhibitors are of potential interest, therefore, as drugs. PFL catalyses proceeds through an acetyl enzyme intermediate with a free radical intermediate. Crystallisation trials with *Escherichia coli* PFL were unsuccesful and therefore we used limited proteolysis to produce a stable, crystallisable N-terminal protein fragment (69.6 kDa) (Leppanen *et al.*, manuscript submitted). The protein fragment was crystallised in sitting drops using vapour diffusion and the crystals belong to the hexagonal spacegroup $P6_1$ or $P6_5$ with cell dimensions a = b = 140.4 Å and c = 215.3 Å. The crystals can be flash-frozen but diffract very weakly, making synchrotron data collection essential. There are most likely four or two molecules per asymmetric unit (V_M of 2.2 or 4.4 respectively), with one local two-fold at $\psi = 8°, \phi = 0°$.

The crystals, mounted on loops, were transported to ESRF in a liquid nitrogen Dewar having been pre-soaked in heavy-atom reagent and flash-frozen. This way, the potential derivatives from the derivative search using our in-house rotating anode source were saved for futher data collection and the diffraction was confirmed.

The best native data set before the present was collected to 3.2 Å (R_{sym} 9.4%) on the X31 beamline at DESY. At ESRF the high flux on the beamline ID14 EH3 with an on-line CCD detector allowed rapid data collection to higher resolution with lower R-factors (Table 1). Data was processed on-line using the programs DENZO and SCALEPACK. In the present status of the beamline, both the native and derivative data were collected using the wavelength of highest intensity (0.933 A). Only for one thiomersal derivative (THIOl, table 1) data was collected also above the mercury L_{III} absorption edge using the wavelength 1.023 Å (TH102). This was not a succesful experiment, because the wavelenghts were not optimised for the mercury edge due to the current, poor tunability and also, because the crystal proved to be poorly derivatized. Earlier derivative search proved mercury compounds to be potential derivatives and, therefore, two more thiomersal and three other mercury derivatives were collected. According to the planned MIR approach other compounds were also tried with the largest differences in intensities in an uranyl acetate derivative.

Isomorphous and anomalous difference Patterson maps calculated with the programs XtalView and FFT (CCP4) for the mercury derivatives show large peaks in the Harker sections. A two site solution for the MEHGl data explains all the major Harker and cross-peaks suggesting two molecules per asymmetric unit with only one major binding site per monomer. The thiomersal Patterson maps have some additional features indicating more binding sites. The signal in anomalous maps is clearly stronger with Harker peaks up to 7 sigma.

Table 1. A summary of selected datasets collected at ID14 EH3.

Data set	Derivative	Resol.(Å)	Mos.(°)	Compl.(%)	R_{sym}(%)	I/σ	R_{iso} (%)
PFL		2.8	0.27	99.3	5.2	22.7	
THIOl	Thiomersal	2.8	0.23	99.5	5.0	22.3	12
TH102	Thiomersal	3.0	0.25	99.3	4.0	31.9	
TH104	Thiomersal	3.0	0.32	99.5	6.3	17.5	20
MEHGl	Me-Hg-Cl	3.0	0.43	98.3	5.9	14.9	20
MEHG2	Me-Hg-acetate	3.0	0.30	97.4	6.6	13.6	20
GOLD1	$KAuCl_4$	4.0	0.80	99.4	6.4	14.6	20
URANl	$UO_2(Ac)_2$	3.1	0.25	99.8	5.1	19.1	12
URAN2	$UO_2(Ac)_2$	3.0	0.49	98.7	5.8	19.4	25

References

Leppanen, V-M., Parast, C.V., Wong, K.K., Kozarich, J.W. & Goldman, A. Purification and crystallisation of a proteolytic fragment of *Esherichia coli* pyruvate formate-lyase. Manuscript submitted.