EUROPEAN SYNCHROTRON RADIATION FACILITY

EXPERIMETAL REPORT

Experiment title:		**Experiment number**:
High resolution studies on the bacterial proteins TodT, TodS, PtxS, McpS (P. Putida) and on the chloroplast enzyme NADPH Thioredoxin Reductase		MX-1016
Beamline: **Id14-4**	**Date of experiment**: from: 25-11-2009 to: 26-11-2009	**Date of report**: 15-03-2010
Shifts: 3	**Local contact(s)**: Ricardo Ferraz Leal	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists):	Estela Pineda Molina (Main Proposer) Laboratory Laboratorio de Estudios Cristalográficos IACT-CSIC. Universidad de GranadaEdificio P. B. López-Neyra. P. T. Ciencias de la Salud. E-18100 Armilla (Granada)	José Antonio Gavira (Co-proposer) Laboratory Laboratorio de Estudios Cristalográficos IACT-CSIC. Universidad de GranadaEdificio P. B. López-Neyra. P. T. Ciencias de la Salud. E-18100 Armilla (Granada)

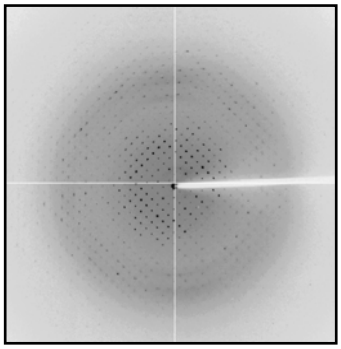
Report:

MCPs

We started our diffraction studies with the Ligand Binding Domain (LBD) of the chemotactic protein MCPs. MCPs crystals grow as thing plates. Many different cryoconditions were tested.We had previously collected native datasets on this protein together with one of its natural ligands, malic acid at BM16 (proposal CRG-1610-727). As there was not a solved structure useful for molecular replacements analysis we produced MCPs crystals derivatized with Se-methionine to be tested at both BM-16 (proposal MX-1017) and Id14-4 (this period). These crystals had grown after incubation of the protein with three ligands: malic acid, succinate and citrate. At BM16, we collected several datasets at the selenium peak and also native ones (these last ones of low quality). At Id14-4 we continued collecting data of the derivatized protein. We tested more than 15 different malic acid crystals. Experiments were performed at 100K. Although most of the crystals were quite anisotropic and unstable at most of the cryo-conditions, two complete datasets were collected (one native and the other at the Se-methionine peak). The native dataset was collected at 2 Å resolution. First processing with malic acid containing crystals showed the following unit cell parameters:

a=226.067 b=46.039 c=50.823
α=90.00 β=95.93 γ=90.00

and space group:
C 1 2 1



Succinate crystals behaved in a similar way and gave the same unit cell parameters.
Citrate crystals could not be tested due to wrong freezing conditions.
With data collected at both BM16 (MX1017 and CRG-1610-727) and Id14-4 (MX-1016) we have solved the structure of MCPs together with malic acid, by SAD, and succinate, by MR. The structures are still under refinement.
Despite these results, we have not solved the structure of MCPs with citrate and without ligand. As the citrate crystals were different in shape and behavior and ITC experiments show citrate binds to MCPs with lower affinity than succinate and malic acid we think citrate could bind in a different way to MCPs. In order to corroborate this hypothesis it would be essential to get well diffracting citrate crystals. Finally, the native protein could also show a different conformation in the absence of ligand. We are working on getting new crystals of both, the native protein without ligand and of MCPs with citrate. Further X-ray experiments with these crystals will be essential to understand how this protein recognizes its different co-factors.

PtxS

During this beam time we were testing also crystals from the bacterial protein PtxS involved in glucose metabolism.
These crystals were obtained by the counter-diffusion method. Capillaries were pre-equilibrated in cryo-buffer (30% glycerol) before crystals were placed into a drop with reservoir solution plus cryo-buffer prior to be frozen in a loop. In other cases crystals were kept in the capillary for diffraction test. We tested more than 10 different crystals at 100K but in all cases we obtained poor diffraction (6-7 Å). Crystals grow as very thin plates and cryo-protection seems to be an important bottleneck in this project. We tested several cryo-conditions (MPD, PEG 400, glucose, sucrose, sodium malonate....) without success. Crystal improvement is still ongoing in the lab in order to get better diffracting crystals.

TodT

Crystals of the two-component system protein TodT were also measured at Id14-4. This protein represents an important project for the lab as there is no structural information about how TodT binds to DNA and promotes the activation of genes involved in toluene metabolism. Thus it represents and important biotechnological tool. We tested very small TodT crystals with hexagonal shape. These crystals appear in very specific crystallization conditions and are very difficult to improve. We brought TodT crystals with and without DNA. Unfortunately crystals size was a limiting factor in order to test diffraction as only very few and weak spots were detected. We are working on protein and crystal and improvement for further diffraction experiments.

0.1mm Capillaries

TodT plus DNA

TodT

NTCR

We tested diffraction for NTCR crystals. Unfortunately, no diffraction was observed. This protein needs several co-factors (including detergents) for its purification and crystallization. Although crystals are quite reproducible more efforts need to be done to get well diffracting crystals.

Xylanase

Xilanase crystals were obtained in different crystallization conditions using the counter-diffusion method including mix of PEGs, Amonium sulphate and Sodium formate. All conditions were improved by fine pH screen but only sodium formate at pH 4.0 produce crystals of sufficient size for x-ray data collection. Crystal cryo-protected in 20% glycerol diffracted X-ray to a maximum resolution of 1.6 A. Several crystals were tested at ID14 but only one present sufficient quality, in terms of mosaicity, for data collection. Crystal belongs to the monoclinic space group C2 with unit cell dimension a=226.470 b=46.060 c=51.090 and angles α=90.00 β=96.08 γ=90.00. The structure is been solved by MR and is under refinement with R and Rfree values 22 and 28% respectively.