EUROPEAN SYNCHROTRON RADIATION FACILITY

EXPERIMETAL REPORT

Experiment title:		**Experiment number**:
High resolution diffraction of PurE-Ligand complex. Crystal quality evaluation of glucose isomerase grown under microgravity condition in the PCDF on board of the ISS.		MX-1017
Beamline: Bm16	**Date of experiment**: from: 19/09 to: 22/09	**Date of report**: 15/03/2010
Shifts: 9	**Local contact(s)**: Dr Mickael CHERRIER	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Jose a. Gavira (Main Proposer). LEC-IACT (CSIC-U.Granada)

Dr. Estela Pineda (PurE and MCPS). LEC-IACT (CSIC-U.Granada)

Dr Klaas Decanniere (Glucose isomerase). ULB

Report:

Glucose Isomerase:

Crystals of glucose isomerase under microgravity condition in the presence (reactor 2) or not (reactor 1) of impurity were collected under identical condition (distance, DOSE, etc.). All crystals were cryo protected with 20% glycerol in an isotonic solution with the final reactor composition. A total of 36 crystals from reactor 1 and 19 from reactor 2 were used to collect 90 frames with 1 degree oscillation angle. Statistical analysis of those two groups of data sets is been done. From each reactor one crystal was selected to collect a high-resolution data set. We collected those two data sets in to passes, the first to a maxima resolutions better than 1.0 Å (180 frames at 1 degree oscillation angle) and a low resolution pass (180 frames at 1 degree oscillation angle). The structural model in been solved by molecular replacement. Findings regarding structural comparison of these two structures with previously deposited at the PDB will be published as a separate study.

All crystals belong to the orthorhombic space group I 222 with similar unit cell dimensions.

PurE:

Crystals of PurE grown by counter-diffusion techniques in three different media in the presence of different ligands. Different strategies were tested for data collection e.g. room temperature (RT) data collection, extraction of the crystals from the capillary and cryo-protection with different agents and equilibration with the cyo-protectan into the capillaries. In all cases crystals did not diffracted at resolution higher than 3 Å and shown a high degree of internal disorder even those collected at RT.

Further efforts are ongoing to obtain better quality crystals and we are searching for the crystallization of a different polymorph.

MCPS-Malate

Crystal of the protein MCPS were already collected at BM16 under the CRG proposal 16-01-727 in presence of succinate, malic acid and citrate. Only those grown in the presence of succinate and malic acid diffracted to a resolution of 2.4 Å. With any of them we were able to find a MR solution. Crystals belong to the monoclinic space group C2 with unit cell dimensions of approximately 226, 46, 51 Å and angles of 90, 96 and 90.

Selenomethionine crystals of MCPS in complex with malic acid were obtained under similar crystallization condition and data collection was attempted from cryo-protected crystals at BM16 by tuning the wavelength to the pick, inflexion and remote of selenium. Selenomethionine crystals were isomorphs with the previous diffracted one in presence of both succinic and malic acids. The phases were obtained by SAD using data collected at the peak. Structural refinement is undergoing with current R and Rfree values of 0.23 and 0.27% respectively. From this solution we have been able to solve the structure of the succinate complex by MR and the refinement is also ongoing with R and Rfree values of 24 and 31% respectively.

During the time of proposal MX-1016 we tested more than 15 different malic acid crystals from which we were able to collected two complete datasets; one native and the other at the Se-methionine peak. The native dataset was collected diffracting at 2 Å resolution aproximatly.

We lack data of the protein without ligand at higer resolution and in complex with its co-factor citrate. Crystal grown in the presence of citrate shows a different crystal form while crystallization of the native protein remains evasive. We are currently working on improving the crystal of the citrate complex and searching for new crystallization conditions for the full-length protein.