MX 367 Diffraction of RC-PSII (photosystem II reaction center) and mexicain crystals

Beamline	Allocated Shifts	Start Date	Finish Date	Local Contact
BM16	2	02 February 2005	03 February 2005	Dr. Ana LABRADOR (e-mail: ana@esrf.fr)
ID14 1	1	02 February 2005	02 February 2005	Tadeusz MUZIOL (e-mail: tadeusz.muziol@embl-grenoble.fr)

Crystals of RC-PSII grown by counter-diffusion techniques in three different media have been test at ID14-1. Diffraction experiments were done at room temperature in the same capillary where the crystals have been grown avoiding crystal manipulation and at 100K using already known cryo-condition, after extracting the crystals from the capillaries. All crystal shows a thin needle shape and clear surface degradation. Sixteen crystals were tested for diffraction but only some of them, cryo-cooled, showed diffraction spots at 25-30 Å. Several images were collected for those crystals and the analysis is ongoing to extract the unit cell dimension.

Further efforts are ongoing to obtain better quality crystals and a new proposal will be submitted during the next opening application period.

Since no crystals of mexicain were available for the time frame, two new systems were included in the proposal.

Mutant forms of HIV-1 PR bear mutations typically arise in patients treated with antiviral drug nelfinavir. These mutations cause a reduction in nelfinavir susceptibility and thus contribute to resistance. Crystals of four different mutants bearing combination of mutations D30N, L90M, A71V and N88D in complex with nelfinavir were grown using hanging drop method. Drops contained 2.5mg/ml protease, 100mM Acetate pH 4.5-5, 0.5-1M Sodium Chloride and 3fold excess of inhibitor. Needle shaped crystals were cryocooled in liquid nitrogen using 20-25% glycerol in crystallization buffer as cryoprotecting solution.

Six crystals were tested for diffraction and two of them were used for collecting complete datasets to resolution 1.6 Å and 1.8 Å for D30N/N88D and L90M/A71V mutant complex, respectively. Both mutant complex crystallized in space group P61 with one dimer per asymmetric unit. Structures were solved by molecular replacement using protein coordinates from Protein Data Bank structure 1nh0. Structures were refined with two inhibitor molecules per dimer bound in two opposite orientations with 50% relative occupancy. Data collection statistics and refinement statistics are summarized in Table 1.

Table 1.

Data collection and refinement statistics of HIV protease mutant complexes		
Mutations	**D30N/N88D**	**D30N/A71V**
Source	ID14-1	BM16
Wavelength (Å)	0.93	0.98
Temperature (K)	100K	100K
Space group	P61	P61
a=b, c (Å)	62.22, 81.46	62.40, 83.10
alpha=beta, gama (º)	90, 120	90, 120
Complexes per asymmetric unit	1	1
Resolution (Å)	1.9	1.85

No. of observed reflections	294947	293147
Redundancy	7.3	7.2
Completeness (%)	98.5 (98.5)	98.8(96.2)
Rsym(%)	5.3 (19.3)	5.7 (29.4)
Average I/σ(I)	7.2 (2.4)	6.2 (2.5)
R value (%)	18.98	19.2
Rfree value (%)	23.98	25.1
No. of reflections in working set (%)	13217	14749
No. of reflections in test set (%)	699	768
No. of solvent molecules	156	156
Average B factor (Å2)	47.7	33.07
R.m.s.d. bond length (Å)	0.01	0.01
R.m.s.d. bond angles (º)	1.6	1.47
Ramachandran plot		
Most favored regions (%)	94.3	95.6
Allowed regions (%)	5.7	4.4
Gener. allowed regions (%)	0	0
Disallowed regions (%)	0	0

(*values in parentheses refer to last resolution shell*)

Solved structures are part of work focused on biochemical and structural study and of four single and double mutants comprising mutations D30N, A71V, N88D and L90M. Our aim is to investigate structural differences between resistant variants bearing various as well as relative to the wild-type enzyme that could explain observed biochemical and inhibition data. Analysis of structures will help to understand the structural basis of HIV PR resistance and structural impact of emergence of individual mutations as well as their combination. The manuscript is in preparation.

Crystal of the thioredoxin mutants grown in capillaries by counter-diffusion techniques were diffracted at BM16. Since crystallization condition compromised a 40% MPD, crystals were directly cryo-cooled at the nitrogen stream in the capillaries. Crystals for three different mutants were tested for high resolution diffraction. Two data sets were collected for the single mutants E85D and D47E at 2.0 Å and 1.9 Å resolutions, respectively. The structure of both mutants will be solved by molecular replacement. Those results will contribute to our current studies on the physicochemical role of charged residues on the thermodynamic denaturation of thioredoxin.