



	Experiment title: Protein function in the light of conformational changes caused by Ca ²⁺ binding: Physarum polycephalum myosin RD and drug binding by calmodulin	Experiment number: LS 1885
Beamline: ID 29	Date of experiment: from: 20 / 04 / 2001 to: 21 / 04 / 2001	Date of report: 01 / 03 / 2002
Shifts: 3	Local contact(s): G. Leonard	<i>Received at ESRF:</i>
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Report:

The experiment was a continuation of our previous project (LS 1756). Our target proteins were Physarum myosin II regulatory domain, chymotrypsin and calmodulin. Due to problems with crystal quality of these proteins it was necessary to spend major part of beam time allocated with secondary projects. All data collections were carried out at cryogenic temperature.

Chymotrypsin.

Diffraction images from all the crystals tested showed randomly oriented satellite crystals diffracting only in the low resolution range. (Crystallisation was carried out by batch method and crystals were soaked in the cryo solution. Attempts were made to reproduce crystals at pH 7.0 in hanging drops, with no success so far.) Two datasets were collected from a crystal of gamma-chymotrypsin to 1.4 Å resolution (cell dimensions: a=42.8 Å, b=78.0 Å, c=67.2 Å, β=108.6°, space group P2₁, completeness: 95.3 % to 1.52 Å, R_{merge}=0.092, two molecules per asymmetric unit). The refinement is in progress.

Complement protease C1r. Autoactivation of C1r is the first enzymatic event in the classical pathway of complement activation. Our aim was to study the structural aspects of the unique autoactivation and the very narrow substrate specificity of this enzyme.

As the CCP1 module of the protein is relatively small and it has low sequence similarity with the possible models of molecular replacement, we collected derivative datasets. During data collection several derivative crystals proved to be of low quality (high mosaicity and significant radiation damage), so experiments were carried out on a single wavelength. A

native (resolution limit 2.3 Å, cell dimensions: a=73.96 Å, b=159.45 Å, c=90.33 Å, $\beta=90.6^\circ$, space group P2₁, completeness: 96.4%, R_{merge}=0.084 to resolution 2.4 Å) and two derivative datasets (Hg and Os) were collected. Due to crystal damage the completeness and redundancy of the native dataset is lower than we expected. Both derivatives proved to be weak, however the phase problem could be solved using molecular replacement. Refinement/model building is on the way. For further improving the model better quality dataset is needed. We are working on producing better crystals.

Test: dUTPase of Mason-Pfizer monkey virus. The enzyme family of dUTPases plays a unique preventive role in DNA repair by excluding uracil from DNA. The enzyme requires Mg²⁺ for optimal activity. Ca²⁺ has an inhibitory effect on the enzyme activity. A crystal (approximate dimensions of 0.05x0.05x0.1mm³) of dUTPase from a beta (Type D oncogenic) retrovirus was tested. The diffraction limit was 3.7 Å, unit cell: a=b=60.83 Å, c=64.03 Å, trigonal/hexagonal.