ESRF	**Experiment title:** High pressure structures of enzymes from psychropiezophilic bacteria	**Experiment number**: MX142
Beamline: ID30	**Date of experiment**: from: 10-SEP-03 to: 16-SEP-03 **and** from: 21-APR-04 to: 26-APR-04	**Date of report**: 30/08/2004
Shifts: 18+17	**Local contact(s)**: Mohamed Mezouar	*Received at ESRF:*

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Report:

Report on MX142 experiment and exploitation of previous experiments.

In similar previous experiments, we have opened the field of macromolecular crystallography at high hydrostatic pressure up to about 1000-1500 MPa (100 MPa = 1 kbar) through the combination of ultra-short wavelength X-rays from an undulator on the ESRF ID30 beamline, a diamond anvil cell (DAC) and a large image plate detector [1]. Pressure is measured by monitoring the pressure-sensitive laser-excited fluorescence of a ruby chip.

1- Psychropiezophilic enzyme

We have undertaken the study of proteins from Psychropiezophilic bacteria. These proteins are adapted both to low temperature (around 0°C) and high pressure (0.1-100 MPa) encountered in cold deep sea.
Our first priority was the study of alpha-amylase from *Alteromonas Haloplanctis*, a bacteria living in symbiosis with the plancton of Antarctic Ocean. As the strain of *A. Haloplanctis* lives in Antarctic Ocean at various depths, this enzyme may be used to study the influence of pressure on the 3D structure. High resolution information at standard conditions on the native enzyme are available [2].
Despite many problems to stabilize the crystals in the high pressure cell, crystals (space group $C222_1$, cell parameters at 1 bar a = 71.5 Å, b= 139.8 Å, c = 115.3 Å) have been loaded and compressed successfully to pressures between 100 and 200 MPa. It turned out that a region (30%) of the reciprocal space could not be recorded due to the limited opening angle of diamond cells (about 53°) and to the anisotropic shape of these crystals (platelets). The electron density calculated from these truncated data has shown interesting modifications with respect to the 1 bar structure, but more complete data are required for detailed and unambiguous conclusions.
The second priority was Ornithine carbamoyltransferase (OTCase, an enzyme implied in the synthesis of arginine) from *Moritella abyssi* gathered in the Atlantic Ocean at a depth of about 3000 m and a temperature

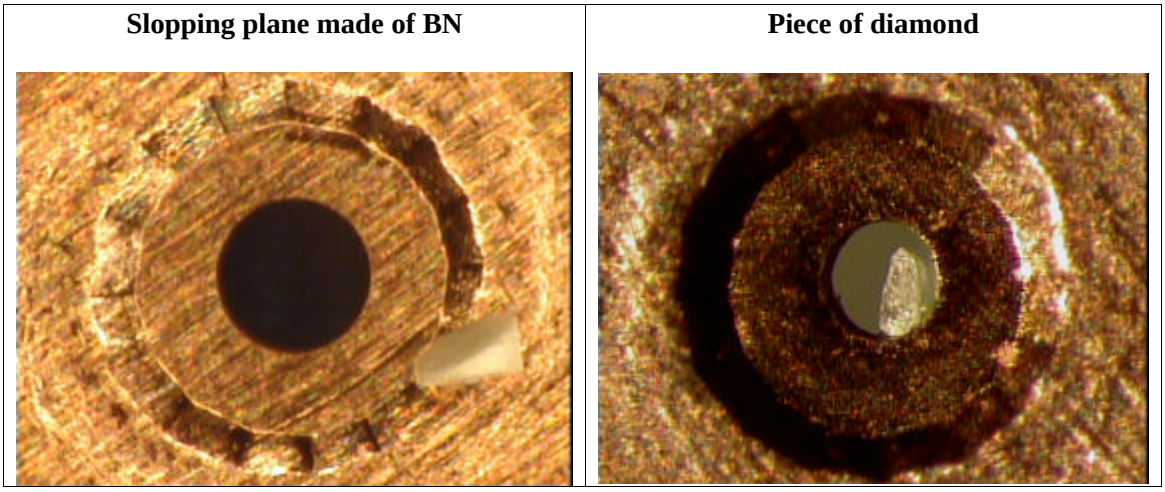
of 2°C. 3D structures of proteins from homologous mesophilic and hyperthermophile systems are available. Crystals of this OTCase are available (space group I23, a = 128.1 Å) and diffract to 4.5 Å resolution using a rotating anode source. It turned out that there were only a few usable crystals in the available crystallization boxes due to a lack of diffraction. The reason is now understood. The lack of diffraction comes from the presence of two oligomers in the crystal packing.

2- Structural analysis of cowpea mosaic virus

From I23 crystals of CPMV compressed at 330 MPa [3], we have obtained a highly complete 2.8 Å structure factor amplitude set [4] (HP set). On the basis of these data, the structure has been refined and a detailed comparison of the 1 bar and HP structures was performed. We have obtained quantitative information on changes produced by high pressure, including reduction of temperature factors, decrease of the volume of cavities within protein subunits, shortening of hydrogen bond lengths. This is the first study of the high pressure structure of a macromolecular assembly (Girard *et al.*, to be submitted).

3- Technical advances

In order to complete the alpha-amylase data collection, a new DAC has been designed giving an increased useful aperture of about 65°. Extension to 85° aperture is in progress. This development will help to get high completeness from fewer crystals in the case of space groups being less symmetrical than cubic or quadratic. In parallel, we have evaluated the use of low Z (BN, C) splinters (see figure below) to force the crystal to reorient within the pressure cavity. The combination of the new DAC and of the splinters has been successfully used to collect a complete data set from three orthorhombic crystals of urate oxidase from *Aspergillus flavus* (space group I222). Structural refinement is in progress.



References

[1]. Fourme R., Kahn R., Mézouar M., Girard E., Hörentrup C., Prangé T. & Ascone I. High-pressure protein crystallography (HPPX) : instrumentation, methodology and results on lysozyme crystals. (2001) *J. Synchrotron Rad.* **8**, 1149-1156.

[2]. Aghajari N., Feller G., Gerday C. and Haser R. Structures of the psychrophilic *Alteromonas haloplanctis* alpha-amylase give insights into cold adaptation at a molecular level. (1998) *Structure* **6**, 1503-1516.

[3]. Fourme R., Ascone I, Kahn R., Mézouar M., Bouvier P., Girard E., Tianwei Lin. & Johnson J. E. Opening the high-pressure domain beyond 2 kbar to protein and virus crystallography. (2002) *Structure* **10**, 1409-1414 ; *ESRF Highlights* (2002).

[4]. Fourme R., Girard, E., Kahn R., Ascone I., Mezouar M., Dhaussy A.C., Lin.T, Johnson, JE. Using a quasi-parallel beam of ultrashort wavelength for high-pressure virus crystallography: implications for standard macromolecular crystallography. (2003) *Acta Cryst.* D**59**, 1767-1772

Publications

1 - "High-pressure protein crystallography (HPPX): instrumentation, methodology and results on lysozyme crystals"
R. Fourme, R. Kahn, M. Mezouar, E. Girard, C. Höerentrup, Prangé, T. & I. Ascone. (2001) J. Synchrotron Rad., 8, 1149-1156.
2 - "Opening the high-pressure domain beyond 2kbar to protein and virus crystallography"
R. Fourme, I. Ascone, R. Kahn, M. Mezouar, P. Bouvier, E. Girard, J. E. Johnson & T. Lin. (2002) Structure, 10, 1409-1414.
3 - "Protein and virus crystallography under High Hydrostatic Pressure"
R. Fourme, I. Ascone, R. Kahn, M. Mezouar, P. Bouvier, E. Girard, J. E. Johnson & T. Lin. (2002) ESRF Highlights.
4 - "New trends in macromolecular crystallography at high hydrostatic pressure"
R. Fourme, I. Ascone, R. Kahn, E. Girard, M. Mezouar, T. Lin & J. E. Johnson. (2003)
Advances in high pressure bioscience and biotechnology II: Proceedings of the 2nd International Conference on High Pressure Bioscience and Biotechnolog. September 16-19, 2002, Dortmund.
Editor R. Winter, Springer Verlag, pp 161-170.
5 - "Using a quasi-parallel X-ray beam of ultrashort wavelength for high-pressure virus crystallography: implications for standard macromolecular crystallography"
R. Fourme, E. Girard, R. Kahn, I. Ascone, M. Mezouar, A.-C. Dhaussy, T. Lin. & J. E. Johnson (2003) Acta Cryst., D59, 1767-1772.
6 - "State of the art and prospects of macromolecular X-ray crystallography at high hydrostatic pressure"
R. Fourme, E. Girard, R. Kahn, I. Ascone, M. Mezouar, T. Lin. & J. E. Johnson. (2004)
'High Pressure Crystallography', Editors A. Katrusiak & M. F. Mac Millan, Kluwer, Dordrecht, pp 527-542.
7 - "A new dimension in structural biology: fully-fledged high-pressure macromolecular crystallography"
E. Girard, R. Kahn, I. Ascone, M. Mezouar, A.-C. Dhaussy, T. Lin, J. E. Johnson & R. Fourme. (2004)
High Pressure Research, 24, 173-182.
8 - "Crystal structure of a complex macromolecular assembly under high pressure: CpMV at 330 MPa"
E. Girard, R. Kahn, M. Mezouar, A.-C. Dhaussy, T. Lin, J. E. Johnson & R. Fourme. (To be submitted).