ESRF	Experiment title: Screening microcrystals of large macromolecular systems.	Experiment number: LS-2168
Beamline: ID13	**Date of Experiment:** from: **June 13th, 2002** to: **June 15th, 2002**	**Date of Report:** **August 28th 2002**
Shifts: 3	**Local contact(s):** Dr. Manfred Burghammer	*Received at ESRF:*

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Report:

Microcrystal screening of several large macromolecular systems was tried. From two systems progress was very good (light harvesting complex II and the C and N termini of Mucin) and for two systems progress was disappointing (one P-type ATPase and transhydrogenase).

The light harvesting complex II (LHCII) is the most abundant chlorophyll-containing complex in plants. LHCII act as light-harvesting antenna, its pigments absorb light and transfer the energy to chlorophylls in the photosynthetic reaction centers [1]. Plants are continuously subjected to changes in environmental conditions. In particular, plants are exposed to changes in light spectrum and intensity, which lead to imbalance between the excitation rates of the two different photosystems (PSI and PSII). Imbalanced excitation rates cause sub-optimal utilisation of the absorbed light and may lead to oxidative damage. Plants have evolved regulatory mechanisms to adapt to environmental changes [2, 3]. Adaptions to momentary changes are achieved by reversible phosphorylation of the LHCII complex, eventually leading to a redistribution of LHCII between the two photosystems.

The structure of the LHCII complex determined by electron crystallography [4] reveals no information on the N-terminal domain which is involved in phosphorylation and regulation. Our aim is to determine high-resolution structural information of the LHCII complex in different phosphorylation states to obtain information on the regulatory mechanism. We have obtained small LHCII crystals with dimensions of at most 30x30x30 micrometer. These crystals show poor diffraction at most X-ray sources (typically less than 15 Å), however at the ID13 beamline during this experiment the crystals diffracted to better than 4 Å resolution. Due to high mosaicity and multiple crystal lattices, a complete dataset has not yet been obtained. We believe to have overcome these problems by improving quality and cryo-preservation of these crystals.

Mucins are large polymeric glycoproteins that confer mucus its viscous and protective properties. One of these mucins, the gel-forming MUC2, is a normal component of the intestinal mucus. In several respiratory diseases it is also produced in the lungs and results in higher viscosity of the mucus. In Gothenburg, the group of Professor Gunnar Hansson has unique expertise in the assembly process of

mucins [5-7], a process that strongly influences the properties of mucins. As the mucin itself is very large, recombinant techniques have been developed to express the secreted N- and C-terminal multimers (tri- and dimers, respectively) in CHO cells. These cells have been adapted to grow in protein-free media, and a method for their purification has been developed. The approximate sizes the N-terminal trimers and the C-terminal dimers are 700 and 300 kDa, respectively, without glycosylation.

Crystallization trials for both the N- and C-termini were begun in Gothenburg as a collaboration between the molecular biology group of Gunnar Hansson and the X-ray crystallography group of Ute Krengel. These trials were successful and crystals of both domains were obtained, but are small (10-20 micrometer). Crystals diffracted at the microfocus beamline to 3.5 Å resolution, while experiments at MAX-Lab II in Lund only yielded 10-20 Å resolution. Complete X-ray diffraction data have been collected on native crystals of both the N- and C-termius and on various putative heavy atom derivatives of the C-terminal domain of MUC2.

Successful results from two of these projects clearly depends on future ID13 microfocus beamtime because of the extremely small crystals. We are confident that higher resolution structural information will be interesting to a broad medical and scientific community.

References:

[1] Heathcote, P., Fyfe, P.K. and Jones, M.R. (2002) Reaction centres: the structure and evolution of biological solar power. Trends. Biochem. Sci. 27, 79-87.

[2] Allen, J.F., Bennet, J., Steinback, K.E. and Arntzen, C.J. (1981) Chloroplast protien phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature 291, 21-25.

[3] Allen, J.F. and Forsberg, J. (2001) Molcular recognition in thylakoid structure and function. Trends Plant Sci. 6, 317-326.

[4] Kühlbrandt, W., Wang, D.N. and Fujiyoshi, Y. (1994) Atomic model of plant light-harvesting complex by electron microscopy. Nature 376, 614-621.

[5] Asker, N., Axelsson. M.A.B., Olofsson, S.-O. and Hansson, G.C. (1998) Dimerization of the human MUC2 mucin in the endoplasmatic reticulum is followed by a N-glycosylation dependent transfer of the mono- and dimers to the Golgi apparatus. J. Biol. Chem. 273, 18857-18863.

[6] Axelsson, M.A.B., Asker, N., and Hansson, G.C. (1998) O-glycosylated MUC2 monomer and dimer from LS 174T cells are water soluble, whereas larger MUC2 species formed early during biosynthesis are insoluble and contain nonreducible intermolecular bonds. J. Biol. Chem. 273, 18864-18870.

[7] Asker, N. Axelsson, M.A.B., Olofsson, S.-O., and Hansson, G.C. (1998) The human MUC5AC mucin dimerizes in the rough endoplasmatic reticulum, similarly to the MUC2 mucin. Biochem. J. 335, 381-387.