	Experiment title: Structure of the ABC-ATPase GlcV	**Experiment number**: LS-1793
Beamline: ID14-2 BM30A	**Date of experiment**: from: 08 Sep 2000 to: 10 Sep 2000 from: 01 Dec 2000 to: 03 Dec 2000	**Date of report**: 01-08-01
Shifts: 2 6	**Local contact(s)**: Dr. E. Mitchell Dr Michel Roth	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

G. Verdon* and A.M.W.H. Thunnissen*
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Report (for reviewing purposes only)

The large family of ABC transporters is involved in the specific and active translocation of molecules across cellular membranes. ABC transporters function as importing or exporting machineries and underlie essential processes, like the uptake of nutrients and protection from a wide range of noxious compounds. All ABC transporters have a similar molecular organisation that consists minimally of two membrane domains forming a channel in complex with two cytosolic ABC-ATPases (or ATP-binding cassettes) energizing the translocation process. Eukaryotic systems are in majority built-up from one polypeptide chain, while their prokaryotic counterparts are composed of subunits. Prokaryotic uptake systems use an additional protein to capture the compound to be transported.

In the hyperthermoacidophilic *S. solfataricus*, an ABC transporter is dedicated to glucose uptake and has been partially characterized. This system is built from four gene products: a membrane-anchored glucose-binding protein (GlcS), two sugar permeases (GlcT and GlcU) and the ABC-ATPase, GlcV. Our work aims at identifying the functional and structural basis of the ABC-ATPase catalytic cycle-transport coupling.

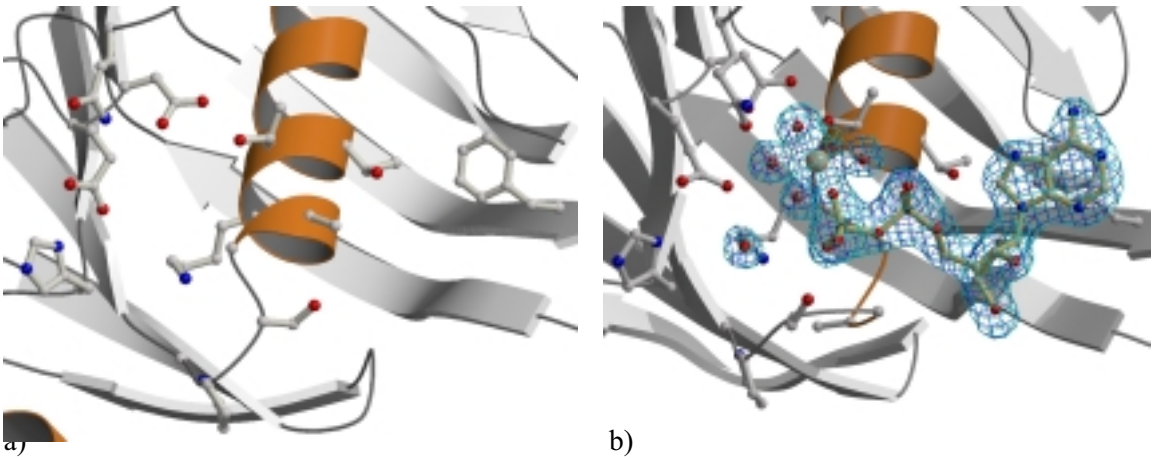
Data collected using the ID14 and BM30A beamlines allowed us to solve the structure of GlcV and currently to investigate its catalytic cycle by means of complexes with various nucleotides.

• Crystal structures of two nucleotide-free states:

In the absence of nucleotide, two related crystal forms of GlcV appear under identical crystallization conditions. Both forms diffract to high resolution and belong to the space group $P2_12_12_1$ (1 and 3 molecules/a.u).
Crystal form A (1 mol/a.u) data extending to 1.65 Å resolution have been collected on ID14-EH2. From the combination with single-wavelength anomalous diffraction data collected on a crystal derivatized with iodide (λ = 1.7 Å; crystal form A, max resolution 2.1 Å, BW7A beamline, EMBL, Hamburg), the crystal structure of GlcV could be solved and has been refined to an R_{fact} of 17 % and an R_{free} of 21 % at 1.65 Å resolution.
Crystal form B (3 mol/ a.u) data collected to 2.1 Å resolution (ID14-EH4, 04/2000) allowed us to solve the structure of GlcV in a distinct conformation (under refinement).

• Structure of the catalytic product complex:

Data have been collected on BM30 A (12/2000) for a complex of GlcV with ADP + Mg^{2+} corresponding to the catalytic product state. After molecular replacement, rigid body refinement and simulated annealing, ADP and Mg^{2+} could be clearly identified in the active site of the three molecules present in the asymmetric unit. The structure has been refined to an R_{fact} of 21 % and an R_{free} of 26 % at 2.1 Å resolution.



a) b)

Fig. : a) nucleotide-free active site, b) catalytic product bound in the active site

Beamline: ID14-EH2	**Date of experiment** 8-10 September 2000	**Date of report**: 8 August 2001
Shifts: 2	**Local contact(s)**: Dr. Edward Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Martin Weik*, Lucy Vandeputte-Rutten*, Piet Gros and Jan Kroon (deceased 3 May 2001)
Dept. of Crystal and Structural Chemistry, Utrecht University, The Netherlands

Raimond B.G. Ravelli, ESRF Grenoble, France.

Joel L. Sussman, Weizmann Institute, Rehovot, Israel.

Report:

The dynamical coupling between a protein and its surrounding solvent is an important, yet open issue. We have used temperature-dependent protein crystallography to study structural alterations that arise in the enzyme Torpedo californica acetylcholinesterase (*Tc*AChE) upon X-ray irradiation at two temperatures: below and above the glass transition of the crystal solvent. Five complete data sets were collected sequentially at 100 and 155 K, each on a single *Tc*AChE crystal. A buried disulfide bond, a buried cysteine and solvent exposed methionine residues show drastically increased radiation damage at 155 K, in comparison to 100 K. Additionally, the irradiation-induced unit cell volume increase is linear at 100 K, but not at 155 K, which is attributed to the increased solvent mobility at 155 K. Most importantly, we observe conformational changes in the catalytic triad at the active site at 155 K but not at 100 K. These changes lead to an inactive catalytic triad conformation and represent, therefore, the observation of radiation-inactivation of an enzyme at the atomic level. Our results show that at 155 K the protein has acquired – at least locally - sufficient conformational flexibility to adapt to irradiation-induced alterations in the conformational energy landscape. The increased protein flexibility may be a direct consequence of the solvent glass transition, which expresses as dynamical changes in the enzymes environment. Our results reveal the importance of protein and solvent dynamics in specific radiation damage to biological macromolecules, which in turn can serve as a tool to study protein flexibility and its relation to changes in its environment. This work is accepted for publication in *Protein Science*.

Weik, M., Ravelli, R.B.G., Silman, I., Sussman, J.L., Gros, P., and Kroon, J. (2001) Specific protein dynamics near the solvent glass transition assayed by radiation-induced structural changes. *Protein Sci. in press.*

ESRF	**Experiment title: The outer membrane protease OmpT from *E. coli* in complex with a substrate analogue.**	**Experiment number**: LS1793
Beamline: ID14-2 ID14-3 ID29	**Date of experiment**: from: 8 September 2000 to: 10 September 2000; from: 3 February 2001 to: 5 February 2001; from: 3 February 2001 to: 5 February 2001	**Date of report**: 8-8-2001
Shifts: 1/4 1/2 1/2	**Local contact(s)**: Dr. Edward Mitchell; Dr. Steffi Artz; Dr. Vivian Stojanoff, Dr. Andy Thompson, Dr. Bill Shepherd	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

L.Vandeputte-Rutten*, M. Weik*, A. Schouten*, R.A. Kramer[1], N. Dekker[1], M. Egmond[1], J. Kroon and P. Gros.

Department of Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands.[1] Department of Enzymology and Protein engineering, Centre for Biomembranes and Lipid enzymology, Institute of Biomebranes, Utrecht University, The Netherlands.

Report: For review purposes only

OmpT is protease present in the outer membrane of *E. coli*. It cleaves specifically between two basic residues. OmpT and its homologous (omptins) found in several other Gram-negative bacteria (among which *yersinia pestis* and *salmonella typhimurium*) are involved in pathogenicity of these bacteria. Recently, we solved the crystal structure of OmpT. The article is accepted by EMBO Journal[#]. The structure reveals that OmpT does not belong to any of the classes of proteases known so far. Based on the constellation of active site residues, we propose a novel catalytic mechanism, which involves a His-Asp-water triad at one side and a Asp-Asp couple at the other side of the peptide plane. In order to study OmpT's exact catalytic mechanism, we made attempts to obtain a crystal structure of OmpT in complex with a substrate-analogue. We soaked a OmpT crystal with a peptide (Abz-Ala-Arg-(D)Arg-Ala-Dap(Dnp)-Gly) that inhibits OmpT with a Ki of about 10 μM. The peptide is coloured yellow. The soaked crystals turned yellow, but unfortunately no extra electrondensity was observed in the active site. Since Zn^{2+} also inhibits OmpT, we also collected a dataset with Zn^{2+} soaked in a crystal. The anomalous map clearly shows the Zn^{2+} ions. We are currently refining this

structure. Zn^{2+} induced slight confomational changes. Furthermore, we soaked some other compounds, which were found to have a negative effect on OmpT activty, in OmpT crystal. Unfortunately, no electron density was observed for these compounds as well. Currently, we are making a OmpT mutant wich does not show any activity. We will soak crystals from mutant with a native peptide. Furthermore, we will get more OmpT inhibitor peptides, which will bind in the active site in the crystals. Understanding the exact catalytic mechanism, with the help of a OmpT-substrate crystal structure, will eventually allow us to design very affective OmpT inhibitors, which may be used against omptin-mediated pathogenicity.

[#]Crystal structure of outer membrane protease OmpT from Escherichia coli suggest a novel catalytic site. L.Vandeputte-Rutten, R.A. Kramer[1], N. Dekker[1], M. Egmond[1], J. Kroon and P. Gros. (accepted by EMBO Journal)

	Experiment title: Polygalacturonases of *Aspergillus niger*	**Experiment number**: LS-1793 and LS-1923
Beamline: ID14-3 ID29	**Date of experiment**: from: 25-11-2000 to: 27-11-2000 from: 30-6-2001 to: 2-7-2001	**Date of report**: Aug. 2001
Shifts: 3	**Local contact(s)**: Dr. S. Arzt and dr. R. Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

G. van Pounderoyen* and B.W. Dijkstra
Lab. Of Biophysical Chemistry
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Report (for review purposes only):

Polygalacturonase II

The structure of *A. niger* polygalacturonase II has been solved in our group.
One of our aims is to determine the binding mode of subtrates to this enzyme.
We therefore collected data sets of two different soaks with substrate on ID14-3 (26-11-2000) and on ID29 (1-7-2001). The data characteristics and statistics are as follows:

Space group $P2_1$
Unit cell 49 x 95 x 66Å^3 ; 90.0 95.6 90.0°

Data collection	ID14-3	ID29
Resolution (Å)	1.5	1.7
Completeness (%)	95.1(1.53-1.50Å)	4.8 (1.74-1.70Å)
Completeness overall (%)	97.6	67.3
I/sigma(I)	4.6(1.53-1.50Å)	1.1 (1.74-1.70Å)
I/sigma(I) overall	34.7	16.2
R-merge	0.216 (1.53-1.50Å)	0.379(1.74-1.70Å)
R-merge overall	0.034	0.095
Wilson B-factor (truncate):	12.5	19.6
Wilson scale (truncate):	33.0	13.4
Refinement		
current R-factor	17.8%	20.9%
current R_{free}-factor	21.3%	24.3%

Disappointingly, electron density maps calculated with both these data sets did not show any electron density at the active site region. Therefore the binding mode of the substrate remains undertermined.

Polygalacturonase I

Data of improved crystals of *A. niger* polygalacturonase I have been collected on ID29 (01-7-2001). This crystal diffracted to 2.2 Å instead of 2.8 Å earlier observed. However, the space group before was $P3_1$ or $P3_2$ with a=b=168.3Å, c=64.3Å, but the current improved crystal belongs to a different space group, namely C2, with a=175 Å, b=86 Å, c=66 Å and ß=100°. Since the space group was before $P3_1$ or $P3_2$ only 100 degrees of data was collected. Now the space group turns out to be C2 the data is only 51% complete (40-2.5Å). In scalepack the data do not scale very well (high X^2) and the R-factor is high (13.7% overall). It remains a question if this crystal was not ok or the beamline was not ok, since more data sets collected in the same shift seem to have problems.

	Experiment title: Structure and Mechanism of Quercetinase	**Experiment number**: LS1793
Beamline:	**Date of experiment**: from: 5 Oct 2000 to: 6 Oct 2000	**Date of report**: 01-08-01
Shifts: 3	**Local contact(s)**: Dr Germaine Sainz	*Received at ESRF:*

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Report (for review purposes only):

Intensities from a crystal of *Aj*2,3QD·KOJ were collected at the beam line BM14 (EMBL/Grenoble) using again a MAR345 detector at 100 K. Integration, scaling and merging was done with the *HKL* suite [Otwinowski, 1997 #157]. Data collection and processing parameters together final refinement statistics are summarized in Table 1. The relatively high R_{symm} value of 13% of the *Aj*2,3QD·KOJ data set is the result of the poor quality of the only crystal which still diffracted after the soaking procedure and on which a full data collection could be performed.

Table 1. Data collections and refinement statistics

data set	*Aj*2,3QD·KOJ
source	BM14
detector	MAR345
T (K)	100
wavelength (Å)	1.033
resolution range (Å)	50.0-2.15
space group	$P2_1$
molecules in the a.u.	4
cell dimensions a (Å) b (Å) c (Å) β (°)	 108.64 55.40 124.43 98.26
# observations	150878
# unique	72101
completeness (%)	89.2 (86.5)
R_{symm}[1](%)	13.0 (37.6)
⟨I⟩/⟨σ(I)⟩	7.03 (3.0)
Wilson *B* value[2](Å^2)	23.1

	Experiment title: Structures of human chitinases	**Experiment number**: LS1793
Beamline:	**Date of experiment**: from: 25 Nov 2000 to: 27 Nov 2000	**Date of report**: 01-08-01
Shifts: 3	**Local contact(s)**: Dr Steffi Artz	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report (for review purposes only):

Chitinases have been found in many organisms in which chitin is a structural component like fungi, arthropods and nematodes. Surprisingly, mammalian proteins homologous to chitinases have also been identified during the last years. They include the enzymes chitotriosidase, the cartilage glycoprotein 39 (HC-gp39), YKL39, YM1, and oviduct glycoproteins. Their exact physiological role is unknown. Since chitin has not been identified in mammals it has been proposed that mammalian chitinase like proteins (mCLP) could share a common ancestor and have developed the ability (adapted) to bind and hydrolyze chitin-related oligosaccharides with different substrate specificity.

The structure of native HCgp39 was solved previously in our laboratory. The structure solved in presence of chitin fragments and chitin-like oligosaccharides will give more insight into the binding preferences of HCgp39 and it will help to identified what is most likely to be the physiological ligand for this protein.
The data set collected at the ESRF beamline ID14b has yielded valuable informations on the binding properties of HCgp39. The structure solved in presence of chitobiose ($GlcNAc_2$) showed that short chitin fragments bind with high affinity to a site located on the surface of the protein and not in the chitin binding groove.

DATA COLLECTION STATISTICS		
Beamtime	28/30-11-00	28/30-11-00
Beamline	ID14-b	ID14-b
Crystal	HC-gp39 + $GlcNAc_2$	Leu-aminopeptidase
Space group	$P4_3$	$P6_322$
Cell parameters	a=b=127.7 Å, c=107.4 Å	a=b=116.0 Å c=136.9 Å
Temperature	100 K	100 K
Resolution	50 – 2.7 Å	50 – 2.75 Å
Completeness (%)	97.9 (99.8)	99.7 (98.8)
Redundancy	4.5	11.5
I/σI	10.4 (2.2)	14.8 (2.85)
R_{merge} (%)	10.0 (53.4)	7.4 (39.9)
REFINEMENT		
R_{factor} (%)	21.7	The structure could not be determined by molecular replacement
R_{free} (%)	26.6	