ESRF

ESRF	**Experiment title:** Crystal structure determination of MTase dam	**Experiment number**: MX-182
Beamline: ID14-4	**Date of experiment**: from: 17-nov-03 to: 18-nov-03	**Date of report**: 28-aug-04
Shifts: 3	**Local contact(s)**: ?	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Project: Kinetic crystallography in combination with fluorescence spectroscopy

The enzyme H-ras p21is a small guanine binding protein which is involved in signal transduction. It switches between an active, GTP-bound state to an inactive, GDP-bound state. The hydrolysis of GTP to GDP involves conformational changes of the protein that occur on the timescale of several minutes. Since H-ras p21 was one of the first proteins investigated by time-resolved and kinetic crystallography we use it as a model system for the current project. The aim of this project is to develop a fluorescence spectrometer which is capable to detect in a time-resolved manner fluorescence changes on protein crystals. With this method, it should be possible to detect the point at which the protein crystal reached a certain intermediate state that shall be investigated. The structure of this state can then be investigated by X-ray diffraction.

To introduce a fluorescence sensitive probe to the protein, we introduced a cysteine in one of the regions of the protein that is involved in the conformational changes (Y32C) and labelled it with IANBD, a small fluorescence label which is highly sensitive in its fluorescence properties to environmental changes. We used the isomeric pure forms of R-caged GTP and S-caged GTP to obtain crystals with diffraction power of 1.25A and higher. The crystallization conditions for the modified protein were optimized in order to obtain small (< 100μm) crystals with high diffracting power, which is important to guarantee a homogenous release of the caged GTP.

The light source of our experimental setup is a He-Cd-Laser (Laser-Class 3b) that delivers monochromatic light at two different wavelengths. One of these (325 nm) can be used to release the caged group, while the other (441 nm) is suitable to excite the fluorophore. We plan to attach our fluorescence spectrometer on a suitable synchrotron beamline and perform the fluorescence experiments correlated with X-ray diffraction. For manipulation of the crystals at room-temperature we use a humidifier that delivers a humid air stream. It is capable to conserve and in some cases even enhance the diffraction properties of the

crystals at room temperature, so that all kinetic experiments can be performed without removing the protein crystal from the beam.

During this beamtime we collected two data sets from the two stable p21-complexes: with S-caged GTP (before reaction initiation) and with GDP (end-point of hydrolysis reaction). These were no kinetic crystallography experiments since these complexes could be crystallized as such.

Crystal / data set	**ph7**	**ph16**
	Fluorescence label, GDP	**Fluorescence label, S-caged GTP**
Size	0.2 x 0.1 x 0.1 mm³	0.2 x 0.1 x 0.1 mm³
Spacegroup	H32	P4(1)
Cell dimensions	94,7, 94,7, 129,10	69.1, 69.1, 35.5
Wavelength (Å)	1.006	0.92021
Resolution (Å)	2.2	1.24
Completeness (%)	98.5	96.8
<I/σ>	20.65	18.7
R_{merge} (%)	6.6	4.9
Exposure time (sec/°)	2 sec / 1 °	1 sec / 0.5 °
Status	Structure refined	Structure refined
R_{work} / R_{free} (%)	17.9 / 23.9	19.0 / 21.4

Project: Crystal structure determination of the human PAPS synthetases 1

To add sulfate to biomolecules, organisms synthesize the high energy sulfate ester 3'-phospho-adenosine-5'-phosphosulfate (PAPS). In humans this reaction is catalyzed by the bifunctional PAPS synthetases 1 and 2. In the first step ATP sulfurylase replaces the beta-phosphate of ATP by sulfate and releases pyrophosphate. In the second step the newly formed adenosine-phosphosulfate (APS) is phosphorylated by APS kinase. Phosphorylation of the 3' position of the ribose yields the final product PAPS. Until today bacterial, fungal and yeast ATP sulfurylases and a fungal APS kinase have been characterized by crystallography. In contrast to the human bifunctional protein these enzymes exist as single polypeptides. Homology modeling of the human enzyme predicts three to four separate domains.

We obtained crystals of human PAPS synthetase 1 (hPAPSS-1) and collected a native data set at SLS, Villigen. At ESRF could collect two mercury data sets (MX-102) which allowed us in combination with molecular replacement to determine the phases for this project. The crystals used so far did not contain any of the ligands or substrates for the protein. Therefore, we soaked the crystals with different substrates or inhibitors and collected different data sets.

Crystal / data set	**ADP**	**ATPγS**
type	ADP-soak	ATPγS-soak
Size	0.1 x 0.2 x 0.1 mm³	0.25 x 0.1 x 0.1 mm³
Spacegroup	P2(1)	P2(1)
Cell dimensions	78.2, 82.5, 133.1, ß=105°	78.2, 82.5, 133.1, ß=105°
Wavelength (Å)	1.005	1.005
Resolution (Å)	2.0	2.1
Completeness (%)	86	98
<I/σ>	16	17
R_{merge} (%)	7.6	7.5
Exposure time (sec/°)	1 sec / 0.5 °	1 sec / 0.5 °
Status	refined	refined
R_{work} / R_{free} (%)	18.0 / 21.0	17.0 / 21.0

Project: Proteins of the Sox gene cluster from *Paracoccus pantotrophus*

Oxidation of reduced inorganic sulfur compounds (Sox) like hydrogen sulfide, sulfur or thiosulfate to sulfuric acid represents the oxidative half of the global sulfur cycle and is mainly perfomed by specialized prokaryotes. The periplasmic Sox enzyme system that oxidizes thiosulfate to sulfuric acid has been studied in detail from *Paracoccus versutus* and *Paracoccus pantotrophus*. The sox gene cluster in *P. pantotrophus* consists of 15 genes. The seven genes soxXYZABCD code for proteins which are crucial in the oxidation of reduced sulfur compounds in vitro. The seven essential proteins build four protein complexes SoxXA, SoxYZ, SoxB and SoxCD that oxidizes thiosulfate in a reaction yielding eight electrons as summarized in Fig.1.

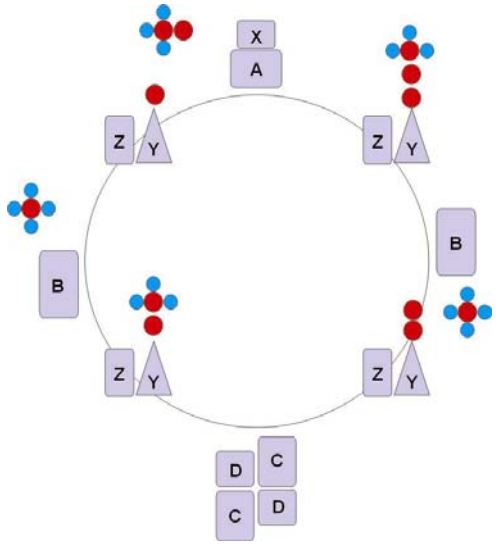

Fig.1. Proposed reaction cycle of the Sox proteins

These proteins are quite different in their type and their proposed catalytical reaction. The protein ensemble includes di-heme and mono-heme *c*-type cytochromes, molybdenum- and manganese containing proteins and proteins which have no prostetic group or metal ion. The central protein is the SoxYZ complex. Thiosulfate is believed to be covalently bound to the thiol of a conserved cysteine of the SoxY subunit by the *c*-type cytochrome complex SoxXA yielding cysteine thiosulfonate. The precise mechanism of this transfer reaction is unknown. The dimanganese SoxB protein interacts with SoxYZ and is proposed to release the sulfone from cysteine thiosulfonate to yield cysteine persulfide. The heterotetrameric molybdoprotein cytochrome complex $Sox(CD)_2$ oxidizes the outer sulfane of cysteine persulfide to cysteine-S-sulfonate and again sulfate is released by the action of SoxB. According to this model SoxXA delivers two and $Sox(CD)_2$ six electrons per molecule of thiosulfate oxidized to sulfate. In this project we are interested in the structural analysis of the different Sox protein complexes and their interactions to study in detail the reaction mechanism.

During this beam time we have analysed different SoxCD crystals. This project is very problematic since (1) the protein has to be expressed in *P. pantotrophus* (low yield), (2) no Se-Met incorporation is possible and (3) the crystals are twinned. For the structure determination we perform different post-translational chemical modifications of the protein in order to change the crystallization conditions and the crystal packing. Using different mercury compounds and the option of X-ray fluorescence scans we could confirm Hg-binding in the crystals. However, no significant anomalous signal could be derived from these crystals so far.

Crystal / data set	**scd147**
type	Hg-soak
Size	0.2 x 0.05 x 0.03 mm³
Spacegroup	P3
Cell dimensions	122, 122, 79
Wavelength (Å)	1.00636
Resolution (Å)	1.8
Completeness (%)	98.2
$\langle I/\sigma \rangle$	16.8
R_{merge} (%)	7.6
Exposure time (sec/°)	3 sec / 0.5 °
Twinning ratio	35 %
Status	No phases
R_{work} / R_{free} (%)	

ESRF	**Experiment title:** Crystal structure determination of MTase dam	**Experiment number**: MX-182
Beamline: ID14-1	**Date of experiment**: from: 09-feb-04 to: 10-feb-04	**Date of report**: 28-aug-04
Shifts: 3	**Local contact(s)**: Dr. Carlo Petosa	*Received at ESRF:*

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Reports

Project: Kinetic crystallography in combination with fluorescence spectroscopy

The enzyme H-ras p21is a small guanine binding protein which is involved in signal transduction. It switches between an active, GTP-bound state to an inactive, GDP-bound state. The hydrolysis of GTP to GDP involves conformational changes of the protein that occur on the timescale of several minutes. Since H-ras p21 was one of the first proteins investigated by time-resolved and kinetic crystallography we use it as a model system for the current project. The aim of this project is to develop a fluorescence spectrometer which is capable to detect in a time-resolved manner fluorescence changes on protein crystals. With this method, it should be possible to detect the point at which the protein crystal reached a certain intermediate state that shall be investigated. The structure of this state can then be investigated by X-ray diffraction.

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During this beamtime we tested three different crystals derived from labelled H-Ras p21 (R-caged GTP) flash frozen after 1 min of photolysis (freeze-trapping of the reaction intermediate).

Crystal / data set	ph32
	Fluorescence label, 1 min after reaction initiation
Size	0.2 x 0.1 x 0.1 mm³
Spacegroup	P4(1)
Cell dimensions	69.2, 69.2, 34.5
Wavelength (Å)	1.00
Resolution (Å)	1.8
Completeness (%)	98.2
<I/σ>	19.2
R_{merge} (%)	5.4
Exposure time (sec/°)	1 sec / 1 °
Status	Refinement in progress
R_{work} / R_{free} (%)	

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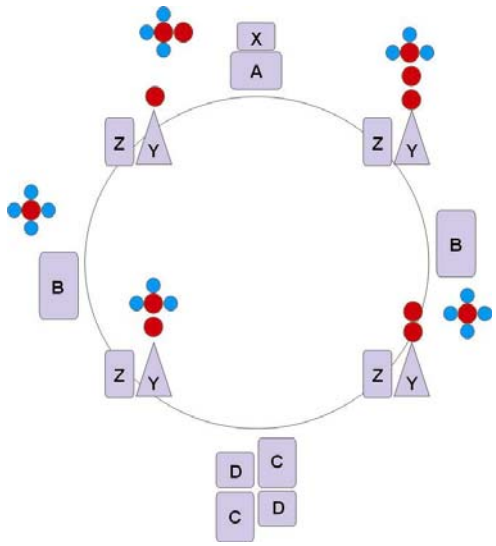

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Within this project we were successful in the crystallization of the SoxB and SoxG protein. We used this beamtime to evaluate the first, very small crystals. However, no useful data set could be collected. The diffraction quality of the crystals is very poor (not better than 7.5 Å).

Crystal / data set	**Scd212**
type	Hg-soak
Size	0.4 x 0.05 x 0.05 mm³
Spacegroup	P3
Cell dimensions	122, 122, 79
Wavelength (Å)	1.00
Resolution (Å)	2.7
Completeness (%)	91.3
<I/σ>	2.3
R_{merge} (%)	14.3
Exposure time (sec/°)	2 sec / 0.5 °
Twinning ratio	20-30 %
Status	No phases
R_{work} / R_{free} (%)	

Project: Crystal structure determination of AF-reductase

Glycogen gives rise to 1,5-anhydro-D-fructose (AF) which is then reduced to 1,5-anhydro-D-mannitol (AM) in bacteria. An enzyme that catalyzes in a NADPH dependend manner the reduction of AF to AM was isolated and purified to homogenity from bacteria. Its molecular mass is 34.8 kDa on the basis of the cDNA sequence. The observed catalytic properties and the amino acid sequence rule out the possibility, that the isolated protein is identical with any known reductase. The unique catalytic potential of the enzyme AF-reductase was demonstrated by preparative conversions of a variety of carbohydrates, and by extensive chemical characterisation of the reaction product. The studies revealed, that this enzyme can be a efficent tool in carbohydrate chemistry. On the basis of these products, strategies will be developed for the convenient production of sugar-derived synthons, rare sugars, fine chemicals and drug combining biotechnical applications and chemical methods.

We have succesfully crystallized the native enzyme. At this beamtime we have collected a first native data set under cryo conditions.

Crystal / data set	**afr8**	**Afr21**
type	native	native
Size	0.1 x 0.05 x 0.05 mm³	0.1 x 0.05 x 0.05 mm³
Spacegroup	P2(1)	P2(1)
Cell dimensions	97.6, 87.2, 154.2, ß=96.8°	97.6, 87.2, 154.2, ß=96.8°
Wavelength (Å)	1.0	1.0
Resolution (Å)	1.9	2.3
Completeness (%)	97.1	85.6
<I/σ>	8.6	5.8
R_{merge} (%)	12.5	17.9
Exposure time (sec/°)	3 sec / 0.5 °	10 sec / 0.5 °
Status	Refinement in progress	
R_{work} / R_{free} (%)		