	Experiment title: Crystal structure determination of SoxXA	**Experiment number**: MX-102
Beamline: ID29	**Date of experiment**: from: 10-mar-03 to: 11-mar-03	**Date of report**: 28-aug-04
Shifts: 3	**Local contact(s)**: Dr. Edward Mitchell	*Received at ESRF:*

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Report

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 we aimed to determine the phases with MAD using Se-Met substituted crystals.

During this beamtime overall four crystals were tested for diffraction and fluorescence signal. From one crystal a 2.3x redundant data set at the white line could be collected. However, no useful anomalous signal could be derived for phase determination. Further experiments will be needed.

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 have tested four small crystals for diffraction and collected one complete data set of H-ras p21 with fluorescence label and R-caged GTP (see Fig.1).

Crystal / data set	**P68**
Size	0.2 x 0.1 x 0.1 mm³
Spacegroup	P4(1)
Cell dimensions	69.2, 69.2, 35.2
Wavelength (Å)	0.97903
Resolution (Å)	1.7
Completeness (%)	97.3
$\langle I/\sigma \rangle$	5.58
R_{merge} (%)	29.3
Exposure time (sec/°)	0.3 sec / 0.5 °
Status	Structure refined
R_{work} / R_{free} (%)	21.9 / 25.6

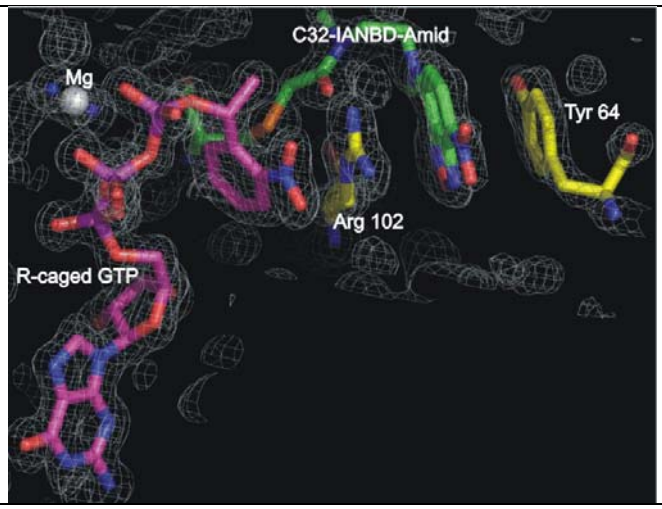

Fig. 1.
H-Ras p21 (residues 1-166, Y32C,C118S, C32-IANBD-amide, R-caged GTP) at 1.25 Å resolution. The fluorophore is displayed in green, the nucleotide in purple. The two aromatic moieties are stacking with the side chain group of residues Arg-102 and Tyr-64 (yellow) of the neighboured molecule

Project: Crystal structure determination of CuatB

The phylogenetically related P-type ATPases are primary active ion pumps, that are used to transport mono- or divalent cations across membranes. One sub-species of the superfamily is the heavy metal-, or CPX-type ATPase, so called due to a specific (presumably metal binding) sequence motif. In principle these enzymes can act in both directions and they are employed in uptake or export of metals such as copper, cobalt, zink, silver or cadmium. Due to their intermediate phosphorylation and to their conformational switching during catalysis these ATPases are called P-type or E1-E2 enzymes. They are integral membrane proteins, which have a distinct domain architecture. The integral membrane part consists of 8 putative transmembrane spans; in addition three hydrophilic domains facing the cytoplasm could be resolved: a metal binding-, a phosphatase- and a nucleotide binding/phosphorylation- domain. The CPX-ATPases are homologous to the sarcoplasmic Ca^{++}-ATPase, whose structure is known, but the overall similarity is low (less than 20 % sequence identity).

We have expressed and purified distinct fragments of one of the two CPX-type ATPases of the thermoacidophile Sulfolobus solfataricus (named Cuat) in the heterologous expression host Escherichia coli. The 30 kDa nucleotide binding/phosphorylation domain (termed CuatB) could be crystallized with native protein as well as with Se-Met substituted protein.

During this beamtime we wanted to collect complet SAD/MAD data sets from the Se-Met substituted protein. Overall we have tested 16 crystals. The fluorescence scan indicated the incorporation of selene. However, the fluorescence peak was rather small and broad. In addition the diffraction pattern displayed high mosaicity and multiple crystal layers. Therefore, no useful data set could be collected. Further optimization of the crystallization and cryo conditions will be needed.

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 per-fomed 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.

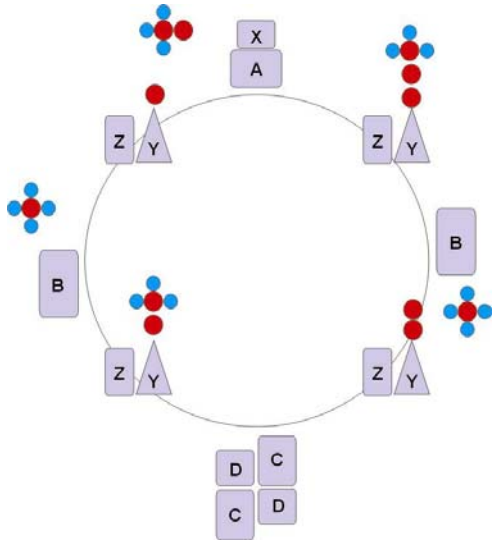

Fig.1. Proposed reaction cycle of the Sox proteins

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.

SoxXA is a protein complex consisting of a mono heme c-type cytochrome SoxX (app. 15 kDa) and a diheme c-type cytochrome SoxA (app. 30 kDa). It crystallizes in the space group P2(1). One complete native data set from SoxXA was collected at SLS, Villigen. The phase determination was performed in part by Fe-MAD experiments (data collected at EMBL-beamline at DESY, Hamburg) and molecular replacement. At this beamtime we tried to obtain better Fe-SAD phases for resolving some structural problems during refinement. Overall four crystals were tested. However, since all crystals displayed multiple lattices no useful data set could be obtained.

A second protein of this gene cluster, which was investigated during this beam time was SoxCD. The hetero tetramer crystallizes in the space group P3. For SoxCD the aim is to solve the structure using MIR phasing. At this beamtime we collected three data sets of SoxCD, one native and two derivatives. Since all crystals displayed severe twinning problems (30 – 50 % merohedral twinning in spacegroup P3) we could not derive useful phases. Further experiments with modified protein and different crystals will be needed.

Crystal / data set	**Scd116**	**Scd75**	**Scd169**
type	Hg-soak	native	Sm-soak
Size	0.3 x 0.05 x 0.05 mm³	0.2 x 0.03 x 0.03 mm³	0.2 x 0.03 x 0.03 mm³
Spacegroup	P3	P3	P3
Cell dimensions	122, 122, 79	122, 122, 79	123, 123, 81
Wavelength (Å)	1.008	0.975	1.5
Resolution (Å)	3.0	3.0	3.5
Completeness (%)	94.8	99.8	85.3
$\langle I/\sigma \rangle$	6.7	5.3	2.1
R_{merge} (%)	10.4	12.3	16.2
Exposure time (sec/°)	2 sec / 0.5 °	2 sec / 0.5 °	2 sec / 0.5 °
Status	No phases	No phases	No phases
R_{work} / R_{free} (%)			

Project: DNA methyltransferases

Many organisms ranging from bacteria to mammals expand the information content of their genome through methylation of specific DNA sequences. DNA methylation has many biological functions, including regulation of gene expression, protection against endogenous restriction endonucleases or directing DNA mismatch repair after replication. The regulatory influence of methylation on DNA has attracted increasing interest over the past years, especially fueled by the different genome sequencing initiatives.

The methyltransferase (MTase) dam from E.coli methylates the adenine at the N6 position within the palindromic sequence GATC. The MTase dam belongs to the alpha-group of MTases and is not part of a restriction-modification system. It has a molecular weight of 32 kDa and uses AdoMet as a cofactor for activity. Dam-dependent DNA methylation has been shown to be involved in DNA mismatch repair, regulation of initiation of replication, regulation of replication and segregation of the chromosome. There are biochemical results indicating a 1:2 ratio of DNA to protein. This is unexpected and would be the first example for a MTase binding with two molecules to the substrate DNA. The structural characterization of the E.coli dam:DNA complex is topic of current research for elucidation of the reaction mechanism and study of specific Mtase inhibitors. Recently we have crystallized dam in complex with specific double-stranded DNA. The binary complex crystallizes as very thin needles (around 80 µm x 10µm x 10µm) in space group P21. For the determination of the phases we have tried different approaches (heavy atom soaks, iodine labeled DNA and Zn-soak). So far no phase information could be derived. For this beamtime we have soaked the crystals with thallium since DNA sometime has specific binding sites for this element. We have tested three crystals anc could collect one data set.

Crystal / data set	**Dam302**
type	Tl-soak
Size	0.1 x 0.02 x 0.02 mm³
Spacegroup	P2(1)
Cell dimensions	35.6, 63.3, 147, ß=92°
Wavelength (Å)	0.934
Resolution (Å)	3.0
Completeness (%)	92.3
$\langle I/\sigma \rangle$	9.81
R_{merge} (%)	8.2
Exposure time (sec/°)	1 sec / 0.5 °
Status	No phases
R_{work} / R_{free} (%)	

ESRF	**Experiment title:** Crystal structure determination of SoxXA	**Experiment number**: MX-102
Beamline: ID14-4	**Date of experiment**: from: 10-mai-03 to: 11-mai-03	**Date of report**: 28-aug-04
Shifts: 3	**Local contact(s)**: Dr. Andrew McCarthy	*Received at ESRF:*

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Report:

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We obtained crystals of human PAPS synthetase 1 (hPAPSS-1) and collected a native data set at SLS, Villigen. At the last beamtime at ID29 we aimed to determine the phases with MAD using Se-Met substituted crystals which was unsuccessful. For this beamtime we tested crystals soaked with different mercury compounds. From two crystals we could collect complete data sets at different wavelengths. The obtained phases were not good enough to trace the molecule. In combination with molecular replacement we were able to obtain a complete model of the protein. Refinement is in progress.

Crystal / data set	**Sh_hg1**	**Sh_gh2**
type	Hg-soak	Hg-soak
Size	0.2 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	1.008
Resolution (Å)	2.5	2.5
Completeness (%)	98	94
$\langle I/\sigma \rangle$	13.1	7.9
R_{merge} (%)	7.7	12.0
Exposure time (sec/°)	1 sec / 0.5 °	1 sec / 0.5 °
Status	Phases obtained	Phases obtained
R_{work} / R_{free} (%)		

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At the last beamtime at ID29 we could not collect any useful data set since the crystals displayed severe lattice disorder. This time we were more successful and could collect one data set for Se-Met substituted crystal. Overall 15 crystals had to be screened to find one which looked promissing.

Crystal / data set	**mlb41**	
type	Se-Met	
Size	0.2 x 0.2 x 0.05 mm³	
Spacegroup	P2(1)2(1)2	
Cell dimensions	52.7, 69.4, 72.0	
Wavelength (Å)	0.979	0.975
Resolution (Å)	2.2	2.3
Completeness (%)	99.5	96.5
$\langle I/\sigma \rangle$	8.8	5.6
R_{merge} (%)	11.1	6.13
Exposure time (sec/°)	0.5 sec / 0.5 °	1 sec / 0.5 °
Status	Phases obtained	
R_{work} / R_{free} (%)	Refinement in progress	