| | | |
|---|---|---|
| | **Experiment title:**<br>Crystal structure determination of P-ATPase Cuat | **Experiment number**:<br>MX-246 |
| **Beamline**:<br>ID14-2 | **Date of experiment**:<br>from: 14-mai-04 to: 15-mai-04 | **Date of report**:<br>28-aug-04 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Dr. Stephanie Monaco | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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**Report:**

## 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 different data sets from crystals soaked with substrates.

| **Crystal / data set** | **Afr68** |
|---|---|
| type | Sugar-soak |
| Size | 0.1 x 0.05 x 0.05 mm³ |
| Spacegroup | P2(1) |
| Cell dimensions | 97.3, 86.8, 153.2, ß=96.9° |
| Wavelength (Å) | 0.933 |
| Resolution (Å) | 2.2 |
| Completeness (%) | 95.2 |
| $\langle I/\sigma \rangle$ | 10 |
| $R_{merge}$ (%) | 8 |
| Status | Refinement in progress |
| $R_{work}$ / $R_{free}$ (%) | |

## 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.

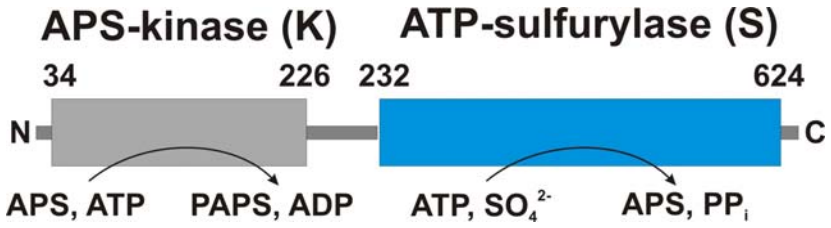


**Fig. 1**. Domain structure of hPAPSS-1.

At this beamtime we collected data sets from the APS- and PAPS-soaked protein crystals. Together with beamtime provided at the SLS, Villigen we could determine different structures of hPAPSS-1 without bound ligands and in complex with different substrates and inhibitors, respectively. The different structures are refined (see Table) and the publication is submitted.

Datacollection of human PAPS synthetase 1 crystals growing in the $P2_1$ monoclinic unit cell: a=78.2 Å, b=82.5 Å, c=133 Å, $\alpha=\gamma=90°$ and $\beta=105°$

| Crystal | native | ADP | ATP$\gamma$S | APS | PAPS | Hg 1 | Hg 2 |
|---|---|---|---|---|---|---|---|
| resolution (Å)[a] | 30-1.75 | 20-2.0 | 20-2.1 | 20-2.05 | 20-1.95 | 20-2.5 | 20-2.5 |
| $\lambda$ (nm) | 0.934 | 1.006 | 0.920 | 0.920 | 1.006 | 1.005 | 1.008 |
| Completness (%)[b] | 96 (88) | 86 (14) | 98 (97) | 98 (90) | 96 (82) | 98 (95) | 94 (86) |
| $R_{symm}$ (%)[c] | 6.9 (58) | 7.6 (40) | 7.5 (36) | 8.3 (74) | 6.6 (36) | 7.7 (29) | 12 (65) |
| $I/\sigma$ | 11 (2.1) | 16 (2.3) | 17 (6.1) | 14 (2.5) | 17 (2.7) | 13 (4.8) | 7.9 (2.0) |
| No. of unique observations | 159267 | 95189 | 94197 | 100470 | 115129 | 101997 | 105045 |
| redundancy | 3.6 | 7.1 | 7.5 | 7.4 | 7.1 | 3.8 | 3.8 |
| Refinement | | | | | | | |
| $R_{work}$ (%)[d] | 18 | 18 | 17 | 20 | 19 | - | - |
| $R_{free}$ (%)[e] | 21 | 21 | 21 | 23 | 22 | - | - |
| Phasing | | | | | | | |
| centric FOM | - | - | - | - | - | 0.25 | |
| acentric FOM | - | - | - | - | - | 0.37 | |

## 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 have collected a high-resolution data set of native CuatB and a medium data set of CuatB crystals soaked with the non-hydrolysable nucleotide AppNHp. The structure refinement of the different froms is in progress. However, we have to deal with two major problems: (1) The phases derived from Se-Met substituted protein are very bad (phasing power 1.4, FOM 0.83 after resolve) and (2) the average B-factor is rather high (48 Å²). Important loop regions of the protein are not well resolved in the electron density. In further experiments we plan to use shrinkage of the crystals in order to improve the diffraction quality and to stabilize the protein packing.

| **Crystal / data set** | **mlb110** | **mlb122** |
|---|---|---|
| type | AppNHp-soak | native |
| Size | 0.2 x 0.05 x 0.02 mm³ | 0.2 x 0.2 x 0.02 mm³ |
| Spacegroup | P2(1)2(1)2 | P2(1)2(1)2 |
| Cell dimensions | 52, 69, 72 | 73, 52, 140, ß=97° |
| Wavelength (Å) | 0.933 | 0.933 |
| Resolution (Å) | 1.9 | 2.3 |
| Completeness (%) | 99.6 | 94.5 |
| $\langle I/\sigma \rangle$ | 9.85 | 7.1 |
| $R_{merge}$ (%) | 7.4 | 8.4 |
| Exposure time (sec/°) | 1 sec / 0.5 ° | 1 sec / 0.5 ° |
| Status | Phases obtained | |
| $R_{work}$ / $R_{free}$ (%) | Refinement in progress | |

## 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 beam time we collected two data sets. The corresponding crystals were flash frozen 10 min after photolytical reaction initiation.

| **Crystal / data set** | **Ph70** | **ph71** |
|---|---|---|
| | **Fluorescence label, S-caged GTP, 10 min** | **Fluorescence label, S-caged GTP, 10 min** |
| Size | 0.2 x 0.1 x 0.1 mm³ | 0.2 x 0.1 x 0.1 mm³ |
| Spacegroup | P4(1) | P4(1) |
| Cell dimensions | 69.1, 69.1, 34.6 | 69.2, 69.2, 34.6 |
| Wavelength (Å) | 0.933 | 0.933 |
| Resolution (Å) | 1.8 | 1.6 |
| Completeness (%) | 97.3 | 97.1 |
| $<I/\sigma>$ | 15.9 | 15.3 |
| $R_{merge}$ (%) | 4.9 | 5.1 |
| Exposure time (sec/°) | 2 sec / 1 ° | 1 sec / 0.5 ° |
| Status | Refinement in progress | Refinement in progress |
| $R_{work}$ / $R_{free}$ (%) | | |

| ESRF | **Experiment title:**<br>Crystal structure determination of P-ATPase Cuat | **Experiment number**:<br>MX-246 |
|---|---|---|
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| **Shifts:**<br>3 | **Local contact(s)**:<br>William Shepard | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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**Report:**

This beamtime was canceled by the applicants due to local duties at the university.