function of the enzyme consists in catalytic oxidation of the primary alcohol in various oligosacharides with the highest activity towards D-cellobiose (Kulys et al., 2001). Crystallization experiments were performed using Hampton Research Crystal Screen and Index solutions. Crystals show variable morphology depending on crystallization conditions and feature varying stability. The measured hexagon-shaped crystal grew with Jeffamine ED2001 as precipitant. X-ray diffraction data were collected at beamline BM14, ESRF in Grenoble using the MARMosaic 225 detector. The crystal diffracted up to 2.7 Å resolution, however, a rapid intensity fall off occurred beyond 3.5 Å resolution. The space group was indicated as $P6_222$, with unit cell parameters $a = b = 55.7$ Å, $c = 610.4$ Å. The crystallographic symmetry was verified with program Pointless (Collaborative Computational Project, Number 4, 1994). Extensive molecular replacement trials with a model of 39% sequence identity (PDB code 1zr6) failed. The size of the molecule, size of the unit cell and high symmetry of the space group inadvertently resulted in significant overlaps of the 'solutions'. The real crystallographic symmetry is lower (subgroup of $P6_222$) and one or more symmetry operators arise from twinning of the crystal. Further experiments to produce well diffracting crystals without twinning are in progress.
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Keywords: carbohydrate oxidase, crystallization, X-ray structure analysis

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Crystal structure of actinohivin; A novel anti-human immunodeficiency virus protein

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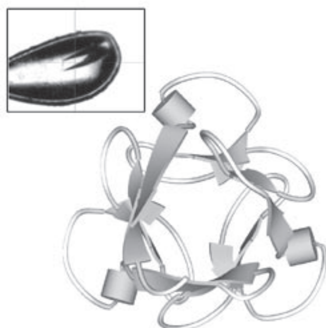
Actionohivin (AH) is a protein found in a culture filtrate of the newly discovered genus actinomycete *Longispora albida*. AH is a potent anti-human immunodeficiency virus (HIV) protein that inhibits viral entry to cells by binding high-mannose-type saccharide chains of HIV gp120. Consisting of highly conserved sequence which has three-tandem repeats, it might belong to carbohydrate binding module (CBM) family 13. To confirm mechanisms of specific binding of sugar to AH and to provide new approach for activity improvement, it was examined by crystallization and X-ray diffraction. Single crystals were obtained for several months. Crystals were soaked in a solution of platinum and were diffracted at maximum resolution of 1.1Å. Single wavelength anomalous dispersion method was used for initial phase determination. The crystal structure of AH has a pseudo threefold axis and consists of three domains which are analogous to each other. Three segments are consistent with highly conserved tandem repeats. The active site, LD and QXW motif, was positioned in central area of each segment.



Keywords: HIV drug design, high-resolution crystal structures, AIDS inhibitors

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Structural and functional analysis of an important *Pseudomonas aeruginosa* redox protein

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Pseudomonas aeruginosa is ubiquitous in soil and water and also occurs regularly on the surfaces of plants and occasionally animals. *P. aeruginosa* is an opportunistic human pathogen. It almost never infects healthy tissues, yet there is hardly a tissue that it cannot infect if defences are compromised in some manner. Therefore, *P. aeruginosa* is often responsible for nosocomial infections. To survive within a host, bacteria produce and secrete a variety of virulence factors. These are typically protein molecules that specifically influence host function and allow the bacterium to thrive. Even though each pathogenic species possesses a specific repertoire of virulence factors, a common feature of these molecules is that they are usually secreted to the surface of the cell or released into the extracellular environment to interact with host components. Often, these virulence factors contain disulfide bonds, which are incorporated to stabilize tertiary structure in a foreign environment. This involves the oxidation of two cysteine residues. It is now well established that the disulfide bond (Dsb) family of proteins catalyzes the formation of disulfide bonds in the bacterial periplasm. The key enzyme, DsbA, catalyzes disulfide bond formation by donation of its active-site disulfide to a folding protein substrate, via a mixed disulfide intermediate. The reduced DsbA produced by this reaction is then re-oxidized by the integral inner membrane protein DsbB. We have determined the 1.6 Å resolution crystal structure of the DsbA enzyme of *P. aeruginosa* (PaDsbA). Additional functional analysis of purified PaDsbA has enabled us to compare PaDsbA to previously characterized oxidoreductases. This information is vital to future studies aimed at combating this important pathogen.

Keywords: crystallographic structure determination, protein crystallography, protein biochemistry

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Crystallization of serine proteases for neutron single crystal structure determination

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P4

This presentation will discuss crystallization of human α-thrombin-bivariludin complex and bovine trypsin-BPTI complex, aiming neutron single crystal structure determination to investigate enzyme-inhibitor interactions in terms of hydrogen position and protonation state. Despite neutron diffraction has a great advantage to observe hydrogens, which play important roles in protein activities, the diffraction experiment still needs crystals larger than 1 mm^3 because intensity of neutron beam is limited. To grow big crystals, first, a crystallization phase diagram was drawn for each protein-inhibitor complex. Then, based on the phase diagram, proper crystallization condition was tried and refined. Bovine trypsin-BPTI complex crystal larger than 1 mm^3 can be grown constantly using vapor diffusion method, if the crystallization is started from near the nucleation border on the phase diagram. We are refining the crystallization condition with checking a crystal quality with x-ray diffraction experiments. For human α-thrombin-bivariludin complex, to grow big a crystal needs macroseeding because reproducibility of crystallization is low and the same method as used for bovine trypsin-BPTI complex was not applicable. The crystallization method is as follows: A crystal was seeded at unsaturated region on the phase diagram to prevent other crystals from growing. When the growing stopped, new solution of α-thrombin-bivariludin complex was added. So far, this method gave a crystal with 0.4 x 0.6 x 0.9 mm size. Further effort to improve the crystal size is undergoing.

Keywords: crystal growth, serine protease, neutron single crystal structure determination

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Structural basis for the antiproliferative activity of the Tob-hCaf1 complex

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Cell proliferation and differentiation in multicellular organism are regulated precisely by a variety of proteins that control replication, transcription, translation, and signal transduction. Loss of antiproliferative mechanisms causes abnormal cell proliferation and leads to diseases. Thus, in normal cells, cell cycle progression is tightly regulated by several antiproliferative proteins. The Tob/BTG family is a group of antiproliferative proteins containing two highly homologous regions, Box A and Box B. These proteins all associate with CCR4-associated factor 1 (Caf1), which belongs to the ribonuclease D (RNase D) family of deadenylases and is a member of CCR4 complex. To help elucidate the relationship between the antiproliferative activity of Tob and the degradation of the poly (A) tail, we determined the crystal structure of the complex of the N-terminal region of Tob and human Caf1 (hCaf1). Tob exhibited a novel fold, whereas hCaf1 most closely resembled the catalytic domain of yeast Pop2. Interestingly, the association of hCaf1 was mediated by both Box A and Box B of Tob. Taken together with cell growth assays using both wild-type and mutant proteins, structural studies revealed that complex formation is crucial to cell growth inhibition. The Tob/Caf1 complex serves as a scaffold to tether the poly(A) binding protein and the CCR4 deadenylase complex, thus enhancing the deadenylation efficiency of the poly(A) tail and leading to the suppression of cell proliferation.

Keywords: cell cycle and development, protein interactions, ribonuclease

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Towards the structure of the β4 subunit of the human BK channel

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The BK potassium channel is intimately involved in the regulation of calcium signalling pathways, most notably in providing a negative-feedback mechanism to regulate the activity of L-type-voltage-dependent calcium channels (VDCCs), preventing runaway Ca^{2+} influx. The physiological consequences of this simple regulatory loop are diverse; from vasodilation, to neurosecretion, to neuronal excitability, the BK channel has a variety of physiological roles, each of which requires the channel to have different electrophysiological properties. The phenotypic diversity of the BK channel is mediated by association with a class of tissue-specific transmembrane proteins, the BK β-subunits. These proteins have diverse effects on the molecular properties of the channel. To resolve the ambiguities surrounding the structure and function of the β-subunits, we aimed to determine the structure of the ectodomain of one of these proteins, the β4 subunit of the human BK channel. Here, we report the expression, refolding and crystallisation of the β4 subunit ectodomain and discuss progress towards structure determination. We are producing the β4 subunit ectodomain by expression in Escherichia coli followed by purification and refolding of the recombinant protein. Crystals were obtained and a complete native dataset collected. Derivatisation with Ta_6Br_{14} resulted in an unusual shift in the symmetry of the crystals - the native crystals were orthorhombic, while the derivatives appeared tetragonal. Attempts to phase the structure are currently underway.

Keywords: potassium channel, calcium signalling, Slo1

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Crystallographic study of extracellular dermal glycoprotein of carrot

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Carrot extracellular dermal glycoprotein (EDGP) may play important role in plant defense systems and in signal transduction. Expression of EDGP is induced by biotic or abiotic stress. The amino acid sequence alignment shows that EDGP shares significant sequence homology with proteins from legumes, tomato, Arabidopsis, wheat, and cotton. Most of the Cys residues in these proteins are conserved. EDGP has six disulfide bonds all Cys residues are involved in disulfide bond and EDGP has four N-linked glycan chains. The glycans and glycosylation in EDGP are essential for defense systems and EDGP secretion. The protein from soybean is termed as leginsuln