function of the enzyme consists in catalytic oxidation of the primary alcohol in various oligosaccharides 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_322, 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_322) and one or more symmetry operators arise from twinning of the crystal. Further experiments to produce well diffracting crystals without twinning are in progress. Acknowledgement: This work was supported by GA AV CR, project IAAS0500701 and by GA CR, project 305/07/1073. Collaborative Computational Project, Number 4 (1994). Acta Cryst. D50, 760-763.


Keywords: carbohydrate oxidase, crystallization, X-ray structure analysis

P04.01.24

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

Stephen R Shoulder1, Russell Jarrott1, Begona Heras1, Martin J Scanlon2, Jennifer L Martin1

1University of Queensland, Institute for Molecular Bioscience, 306 Carmody Rd, Building 80, Brisbane, Queensland, 4072, Australia, 2Department of Medicinal Chemistry, Victorian College of Pharmacy, Monash University, Victoria 3052, Australia, E-mail: s.shouldice@imb.uq.edu.au

*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

P04.01.23

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

Masaru Tsunoda1, Kaoru Suzuki1, Tsubasa Sagara1, Atsushi Takahashi1, Junji Inokoshi2, Satoshi Omura2, Takashi Sekiguchi1, Akio Takenaka1, Haruo Tanaka1

1Iwaki Meisei University, Faculty of Pharmacy, 5-5-1 Chuodai-iino, Iwaki, Fukushima, 970-8551, Japan, 2Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo, 108-8641, Japan, 3The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo, 108-8642, Japan, E-mail: masaru@iwakimu.ac.jp

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 domain families. HIV gp120, consisting of highly conserved tandem repeats, is a primary target for HIV infection and is a critical step involved in the entry of HIV to 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

P04.01.25

**Crystallization of serine proteases for neutron single crystal structure determination**

Taro Yamada1, Kenji Kawamura1, Yuki Onishi1, Takuya Ishikawa2, Katsuhiko Kusaka1, Ichiro Tanaka1, Nobuo Niimura1

1Ibaraki University, 4-12-1 Nakanarusawa, Hitachi, Ibaraki, 316-8511, Japan, 2Kyoto University Research Reactor Institute, 2 Asahisho, Nishi, Kumatori, Osaka, 590-0494, Japan, E-mail: taro@mx.ibaraki.ac.jp

Crystallization of serine proteases for neutron single crystal structure determination

Keywords: neutron diffraction, structure determination, protein science
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 mm3 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 mm3 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

**P04.01.26**

**Acta Cryst. (2008). A64, C238**

**Structural basis for the antiproliferative activity of the Tob-hCafl complex**


1Graduate School of Pharmaceutical Sciences, Hokkaido University, Department of Structural Biology, N-21, W-11, Kita-ku, Sapporo, Hokkaido, 001-0021, Japan, 2Kyoto Prefecture University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan, 3Institute of Medical Science, University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108-8639, Japan, E-mail: horiuchi@pharm.hokudai.ac.jp

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 (Cafl), which belongs to the ribonuclease D (RNase D) family of deoxyribonucleases 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 Cafl (hCafl). Tob exhibited a novel fold, whereas hCafl most closely resembled the catalytic domain of yeast Pop2. Interestingly, the association of hCafl 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/Cafl 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

**P04.01.27**

**Acta Cryst. (2008). A64, C238**

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

Oliver B Clarke, Jacqui M Gulbis

The Walter and Eliza Hall Institute of Medical Research, STRUCTURAL BIOLOGY, 1G Royal Pde, Parkville, Victoria, 3050, Australia, E-mail: clarke@wehi.edu.au

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 Ca2+ 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 Ta6Br14 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

**P04.01.28**


**Crystallographic study of extracellular dermal glycoprotein of carrot**

Takuya Yoshizawa, Hiroshi Hashimoto, Toshiyuki Shimizu, Hisashi Hirano, Mamoru Sato

Yokohama City University, Tsurumi Suehirochou 1-7-29, Yokohama, Kanagawa, 230-0045, Japan, E-mail: yoshi30@tsurumi.yokohama-u.ac.jp

Carrot extracellular dermal glycoprotein (EDGP) may play important role in plant defense systems and in signal transduction. Expression of EDGP is induced by both 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 leginsul