This presentation will discuss crystallization of human α-thrombin-bivariludin complex and bovine trypsin-BPTI complex, aiming to investigate enzyme-inhibitor interactions in terms of hydrogen position and protonation state. Despite neutron diffraction having a great advantage to observe hydrogens, which play important roles in protein activities, the neutron 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-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. Thus, the native crystals were orthorhombic, while the derivatives appeared tetragonal. Attempts to phase the structure are currently underway.

Keywords: potassium channel, calcium signalling, Slo1

**P04.01.27**

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

**Towards the structure of the β 4 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 Ca²⁺ influx. The physiological consequences of this simple regulatory loop are diverse: from vasodilation, to neurosecretion, to neuronal excitability. To help elucidate the relationship between the BK channel and EDGP, 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 β-four 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 Ta3Br4 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: cell cycle and development, protein interactions, ribonuclease

**P04.01.28**


**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
binding protein (LBP), which binds insulin and 4-kDa hormone-like-peptide (leginsulin). LBP shows protein kinase activity in vitro and the activity is stimulated by binding of leginsulin. Carrot EDGP and soybean LBP share about 33% sequence homology. Carrot EDGP binds also insulin and leginsulin from soybean in vitro and localizes in the plasma membrane and middle lamellae of cell walls, EDGP also shows protein kinase activity. However, detail about function of EDGP is still unclear. Thus, we work on structural study of carrot EDGP. The structure will provide to a clue to understand the function of EDGP and pave a way for further analyses, which expected new solution for plant defense systems and in signal transduction. EDGP was purified from culture medium of carrot callus by ion exchange chromatography. Crystals of EDGP were obtained by conventional hanging drop vapor diffusion method. The crystal belongs to hexagonal system with cell dimensions of \( a = b = 129.8 \), \( c = 44.4 \, \text{Å} \), and \( \gamma = 120^\circ \). Structure determination of EDGP is now in progress.

Keywords: extracellular dermal glycoprotein, carrot, crystalization

**P04.01.30**

**Acta Cryst.** (2008). A64, C239

**Expression and crystallization of Drosophila EcR/USP**

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The ecdysteroid hormones regulate the major stage of insect development, especially molting and metamorphosis, by binding to a heterodimer composed of the ecdysone receptor (EcR) and the ultraspiracle protein (USP). Even though all insects use ecdysteroid, 20-hydroxyecdysone, as a natural molting hormone, they exhibit different sensitivity for non-steroidal ecdysteroid agonists. The aim of this work is to clarify the molecular mechanism of the functional complexes and the binding mode of non-steroidal agonists to EcR. Here we report the expression and crystallization of EcR- and USP-ligand binding domains, EcR-LBD and USP-LBD, from *Drosophila melanogaster* toward structure solution by X-ray crystallography. EcR-LBD with an N-terminal GST/His tag (GST/His-EcR-LBD) and USP-LBD with a C-terminal His tag (USP-LBD-His) were expressed in *E. coli*. The expression plasmids for these genes were constructed with two procedures as follows: EcR-LBD and USP-LBD genes were cloned in pET20b and pET41a expression vectors, respectively, and both of EcR-LBD and USP-LBD genes were tandem inserted into pET41a. In the latter procedure, the complex of GST/His-EcR-LBD and USP-LBD-His were expressed in *E. coli* Rosetta2(DE3) pLysS strain. After purification by affinity and anion-exchange columns, clearance of EcR-LBD from GST-His was achieved using a site-specific protease, enterokinase. The search of the crystallization condition is currently in progress.

Keywords: ecdysone receptor, *Drosophila*, crystallization

**P04.01.29**

**Acta Cryst.** (2008). A64, C239

**Crystallization of Clostridium botulinum serotype D neurotoxin complex**

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Clostridium botulinum produces seven immunologically distinct neurotoxin (BoNT, 150 kDa) serotypes, classified as A-G. In culture fluid and naturally contaminated foods, BoNT exists as part of large complexes and the binding mode of non-steroidal agonists to EcR. Here we report the expression and crystallization of EcR- and USP-ligand binding domains, EcR-LBD and USP-LBD, from *Drosophila melanogaster* toward structure solution by X-ray crystallography. EcR-LBD with an N-terminal GST/His tag (GST/His-EcR-LBD) and USP-LBD with a C-terminal His tag (USP-LBD-His) were expressed in *E. coli*. The expression plasmids for these genes were constructed with two procedures as follows: EcR-LBD and USP-LBD genes were cloned in pET20b and pET41a expression vectors, respectively, and both of EcR-LBD and USP-LBD genes were tandem inserted into pET41a. In the latter procedure, the complex of GST/His-EcR-LBD and USP-LBD-His were expressed in *E. coli* Rosetta2(DE3) pLysS strain. After purification by affinity and anion-exchange columns, clearance of EcR-LBD from GST-His was achieved using a site-specific protease, enterokinase. The search of the crystallization condition is currently in progress.

Keywords: ecdysone receptor, *Drosophila*, crystallization