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 β 4 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