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 $a = b = 129.8$, $c = 44.4$ Å, and $\gamma = 120^{\circ}$. Structure determination of EDGP is now in progress.

Keywords: extracellular dermal glycoprotein, carrot, crystalazation

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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 toxin complexes (TCs) through association with non-toxic non-hemagglutinin (NTNHA; 130 kDa) and three hemagglutinin (HA) subcomponents, HA-70, HA-33 and HA-17 (70, 33 and 17 kDa, respectively). Serotype A-D strains produce M-TC (BoNT/NTNHA complex; 280 kDa) and L-TC (BoNT/NTNHA/HAs complex; 750 kDa), while serotypes E and F strains produce only M-TC. The M-TC is formed first by assembly of a single BoNT and a single NTNHA molecule, and is subsequently converted to the complete L-TC. However, M-TC containing nicked NTNHA at unique site could no longer convert to the L-TC with HA subcomponents. Although the crystal structures of several serotype BoNTs and serotype D HA-33/HA-17 complex[1] have been determined, no structure of BoNT complex forms (M-TC and L-TC) has been demonstrated. In this study, highly purified M-TC was obtained from the supernatant of *C. botulinum* type D strain 4947 through several chromatographic runs. The nicked form of M-TC was prepared by limited trypsin treatment, and was crystallized using the hanging-drop vapor-diffusion technique. The drops consisted of 6 μl protein solution (2.8 mg ml^{-1}) and 4 μl reservoir solution (0.1 M MES pH 6.5, 0.18 M cesium and 15% PEG 6000) derived from condition number 24 of the Hampton Crystal Screen 2. The crystals grew at 293 K and reached dimension of $0.2 \times 0.1 \times 0.05$ mm in 7 days. X-ray data were collected on a Rigaku R-AXIS VII imaging-plate system, using CuKα radiation from a Rigaku FR-E rotating-anode generator. The crystals diffracted to approx. 8Å resolution.

[1]Hasegawa K. et al., J. Biol. Chem. 2007, 282, 24777

Keywords: crystallization, botulinum toxin complex, protein interactions

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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, cleavage 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

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Molecular basis of histone H3K4ME3 recognition by ING4

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The Inhibitors of Growth (ING) family of tumor suppressors consists of five homologous proteins involved in chromatin remodeling. They form part of different acetylation and deacetylation complexes, and are thought to direct them to specific regions of the chromatin, through the recognition of trimethylated-K4 in the histone-3 tail (H3K4me3) by their conserved Plant HomeoDomain (PHD). We have determined the crystal structure of ING4- PHD bound to H3K4me3, which reveals a tight complex stabilized by numerous

interactions. NMR shows that there is a reduction in the backbone mobility on the regions of the PHD that participate in the peptide binding, and binding affinities differ depending on histone tail lengths. Thermodynamic analysis reveals that the discrimination in favor of methylated lysine is entropy driven, contrary to what has been described for chromodomains. The molecular basis of H3K4me3 recognition by ING4 differs from that of ING2, which is consistent with their different affinities for methylated histone tails. These differences suggest a distinct role in transcriptional regulation for these two ING family members due to the antagonistic effect of the complexes that they recruit onto chromatin. Our results illustrate the versatility of PHD fingers as readers of the histone code.

Keywords: ING, inhibitor of growth, PHD, plant homeodomain, histone 3

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A new type of precipitant, metal cyanide complex

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Transition metal salts, such as Fe, Ni, Co, and Cr salt, are generally avoided in protein crystallization because of fear of protein inactivation or denaturation. However, if transition metal is incorporated in crystal structure, they are useful for SAD, or MAD phasing. Here we cast spotlight on metal cyanide complex salts as protein precipitant, because metal cyanide complex has relatively low affinity for protein and chemically stable over a wide range of pH. We tested five metal cyanide complex salts, $K_3Cr(CN)_6$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, $K_3Co(CN)_6$, and $K_2Ni(CN)_4$. Three proteins, lysozyme, proteinase K, and trypsin were tried to crystallize using each salt as a precipitant by batch method. No additional compound added to mother liquid without buffer component (from pH 4 to pH 9). Diffraction data from grown crystals were collected using CuKα radiation. Metal sites were located using anomalous signal by the program *SHELXD*. Phase calculation was performed by SAD method using program *MLPHARE* and automatic model-building by *ARP/wARP* were performed. All tested protein can be crystallized by metal cyanide complexes. The crystal form was isomorphous to that grown with non-metal precipitant except in the case of lysozyme with $K_4Fe(CN)_6$, where we obtained a new crystal form. In the crystal, metal complexes bind to positively-charged surfaces of the protein. In the case of Cr, Fe, and Co salt, location of metal sites, phasing by SAD method, and automatic model-building were accomplished easily and successfully. But, in the case of Ni salt, location of metal sites was difficult, and phasing power on SAD phasing is as low as that of native (non-metal) crystal because of low f" value of Ni at the wavelength of CuKα radiation.

Keywords: new type of precipitant, transition metal complex, SAD method

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Crystallization and crystallographic analysis in a microfluidic chip

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Microfluidic technology opened new possibilities for the crystallization of biological macromolecules. Indeed, microfluidic systems offer a lot of advanges for crystal growth: they enable easy handling of nano-volumes of solutions and, thus, extreme miniaturization and parallelization of crystallization assays. In addition they provide a convection-less environment a priori favorable to the growth of high quality crystals. Pioneer examples implementing free interface diffusion [1] and nano-batch [2] crystallization in microfluidic chips have already demonstrated the value of this technology, especially for high throughput screening applications in structural genomics. We will present the results of a collaborative work initiated in 2004, that is focused on the design of a versatile, low cost and easy-to-use crystallization chip. A novel chip based on the counter-diffusion of solute molecules playing the role of crystallization agents will be described [3]. The chip is made of rigid polymers (e.g. PMMA, COC) that are impermeable to gases and compatible with crystal examination and monitoring in polarized light. Selected materials are also transparent to X-rays, and three-dimensional protein structures can be determined from crystals contained inside this device (image on the left) using X-ray diffraction data collected on a synchrotron source (middle). The outstanding quality of the electron-density maps (right) demonstrates that on-chip crystal analysis is feasible.

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Keywords: biomacromolecule crystallization, microsystems, *in situ* diffraction

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Improving protein crystallization: A large-scale evaluation of protein reductive methylation

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Obtaining diffraction quality crystals is a major bottleneck in protein X-ray crystallography. Chemical modification of proteins can alter their surface properties and crystallization behavior and influence crystallization success. We evaluated how the methylation of lysine residues in proteins may improve crystallization on a set of 370 unique proteins (<30% sequence identity) that failed to produce diffraction quality crystals. Applying well-established methods, the proteins were methylated and screened using standard crystallization procedures. Crystal structures of 26 new proteins were determined, four in both native and methylated states. Crystals of