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

P04.01.29

Acta Cryst. (2008). A**64**, C239

Crystallization of *Clostridium botulinum* serotype D neurotoxin complex

Akifumi Mikami[1], Akihito Yamano[2], Kimiko Hasegawa[3], Keita Miyata[1], Tomoyuki Chikai[1], Tohru Yoneyama[1], Toshihiko Ikeda[4], Koichi Niwa[1], Toshihiro Watanabe[1], Tohru Ohyama[1]
[1]Tokyo University of Agriculture, Department of Food Science and Technology, 196 Yasaka, Abashiri, Hokkaido, 099-2493, Japan, [2]PharmAxess, Inc., 3-9-12 Matsubara, Akishima, Tokyo, 196-8666, Japan, [3]Rigaku Co., 3-9-12 Matsubara, Akishima, Tokyo, 196-8666, Japan, [4]Yokohama College of Pharmacy, 601 Matano, Yokohama, Kanagawa, 245-0066, Japan, E-mail：49070010@cp.bioindustry.nodai.ac.jp

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

P04.01.30

Acta Cryst. (2008). A**64**, C239

Expression and crystallization of *Drosophila* EcR/USP

Saori Kamachi[1], Masami Isogai[1], Asako Yamaguchi[1], Takayoshi Kinoshita[1], Takehiko Ogura[2], Toshiyuki Harada[2], Yoshiaki Nakagawa[2], Toshiji Tada[1]
[1]Osaka Prefecture University, Graduate School of Science, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, [2]Kyoto University, Kitashirakawaoiwake-cho, Sakyo-ku, kyoto, 606-8502, Japan, E-mail：kamachi07@b.s.osakafu-u.ac.jp

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

P04.01.31

Acta Cryst. (2008). A**64**, C239–240

Molecular basis of histone H3K4ME3 recognition by ING4

Ines G. Munoz[1], Alicia Palacios[1,2], David Pantoja-Uceda[1], Irene Luque[3], Francisco J. Blanco[1,2], Guillermo Montoya[1]
[1]Spanish National Cancer Research Centre (CNIO), Macromolecular Crystallography Group, Melchor Fernandez Almagro, 3, MADRID, MADRID, E-28029, Spain, [2]CIC bioGUNE, Bizkaia Technology Park, Building 800, Derio, Bizkaia, E-48160, Spain, [3]Faculty of Science, University of Granada, Granada, E-18071, Spain, E-mail：imunoz@cnio.es

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