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Keywords: stomatin, coiled coil, flotillin

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Crystal structure of reelin in complex with its receptor

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Reelin is a large secreted glycoprotein that plays important roles in brain development. It acts on migrating neurons and regulates their correct cell positioning in brain structure. The response of neurons requires binding of reelin to its receptors, apolipoprotein E receptor 2 (ApoER2) and very-low density lipoprotein receptor. Reelin consists of a signal sequence, an F-spondin-like domain, a unique region and eight tandem repeats of 350-390 amino acid residues, named reelin repeat. Extracellular region of reelin receptors also have multidomain architecture conserved in low-density lipoprotein receptor (LDLR) gene family proteins: they consist of seven or eight LDLR class A (LA) modules, cluster of three epidermal growth factor (EGF) modules and a YWTD β-propeller domain. Several biochemical studies examining the interaction between reelin and its receptors have established the followings. (1) The reelin fragment composed of the fifth and sixth reelin repeats (R5-6) binds to receptors. (2) The first LA module (LA1) of ApoER2 is required for the binding to reelin. (3) Lys2360 and Lys2467 on reelin constitute the receptor-binding site. In order to understand the mechanisms on the recognition of reelin by its receptors in depth, we determined the crystal structure of a complex between R5-6 and LA1 of ApoER2 at 2.6Å resolution. It revealed that Lys2467 of reelin is recognized by the conserved Trp residue and Ca^{2+}-coordinating acidic residues from LA1. This recognition mode is in fact identical to that employed by LDLR in ligand binding. Lys2360 seems to play an additional but essential role in the recognition by the electrostatic interaction with acidic residues on LA1. Thus, the present study provides structural basis for the initial event during the reelin signaling.

Keywords: receptor-ligand interactions, protein-receptor interactions, protein structural analysis

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Characterization and crystallographic analysis of human Lyn tyrosine kinase domain

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Lyn tyrosine kinase is expressed in haematopoietic tissues and plays a critical role in the signal transduction of immune system. Its excess activity is involved in cancer and inflammatory diseases. The three-dimensional structure of Lyn kinase domain will provide a new insight into understanding the function of enzyme and help to design novel inhibitors. Lyn kinase domain His-tagged at C-terminal was expressed in Sf9 insect cells and purified using affinity and anion-exchange chromatographic techniques. The anion-exchange chromatography yielded four major peaks. They were all assigned as homologous Lyn kinase domain having distinguishable phosphorylation manner by SDS-PAGE, Native-PAGE and Western blot. Although the characterized protein samples were separately examined for crystallization screening, only mono-phosphorylated protein was crystallized. Diffraction data were collected at PF and processed using the program HKL2000. The crystal structure was solved by the molecular replacement method using a Lyn homology model derived from Fyn kinase domain. Structural refinement and model modification are currently in progress.

Keywords: Lyn, Src, crystallization

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Preliminary X-ray analysis of human Frk kinase domain

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Frk is a member of Src family kinases and expressed especially in epithelial tissue. Although developmental expression patterns and functional overexpression have associated this kinase with growth suppression and differentiation, the physiological function remains largely unknown. Excess Frk activity is involved in type I diabetes via beta-cell destruction and numerous human cancers. We aimed to perform X-ray crystallography on Frk to elucidate enzyme function. For the purpose of mass-production of protein, we tried to express the C-terminal His-tagged Frk kinase domain using E. coli. ATA codon corresponding to the second amino acid residue of Ile was mutated to ATT which is the high-frequency codon in E. coli. The expressed Frk kinase domain was highly purified by Ni-NTA affinity and anion-exchange chromatographic techniques. Small crystals were obtained with initial screening using the purified sample. Optimization of crystallization condition for X-ray crystallography is currently in progress.

Keywords: Frk, Src, crystallization

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Crystal structure of synaptic adhesion protein neurexin and neuroligin

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Neurexin (NX) and neuroligin (NL) are membrane spanning adhesion molecules expressed on neurons. They interact with each other at synapse, and then this interaction is believed to recruit neurotransmitter releasing machinery. Thus NX and NL play essential role for synapse formation via their trans-synaptic interaction. Extracellular segment of NL contain a single acetylcholinesterase-like domain. NX has two gene products, α-NXs and β-NXs. α-NX longer form ectodomain contains three repeating units comprised of two laminin G (LG) domains intervened by an epidermal growth

factor (EGF)-like module, whereas β-NX has a single LG domain. Recently several groups have reported crystal structure of β-NX and NL complex revealing a unique binding with 2:2 stoichiometry. However these crystal structures do not provide significant insights into the synaptic signal transduction triggered by β-NX/NL interaction. We determined the crystal structure of β-NX/NL complex at 3.5Å resolution in different crystal form, possibly mimicry their molecular clustering of synaptic cleft. We also report the crystal structure of a single repeat segment of α- NX at 2.3Å resolution (i.e. LG-EGF-LG segment). Comparison of these structures stolongly suggested that α- and β-NX have the different clustering mechanism mediated by the interaction with their ligands NL.

Keywords: structures of biomolecules, cell adhesion, complexes

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Preliminary X-ray analysis of MEK1/ERK2 complex

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The Ras/Raf/MEK (mitogen-activated protein kinase) /ERK (extracellular-signal- regulated kinase) pathway plays a key role in governing cell proliferation, differentiation and survival. The pathway represents an attractive drug target in proliferative diseases. The aim of the present study is to clarify the mode of the interaction between MEK and ERK by analyzing the crystal structure of MEK1/ERK2 complex. A detailed study of the three-dimensional structure of the complex should provide information to design novel and target-specific drugs against proliferative diseases. MEK1 and ERK2 were expressed in *E. coli* with GST tags at their N-termini. The lysate including GST-tagged MEK1 was centrifuged to produce a crude extract, which was then loaded onto a GST-affinity column. By on-column cleavage using precision protease, the desired protein was obtained. Subsequent purification by anion exchange chromatography on a MONO Q column yielded two peaks which were assigned as homologous MEK1. ERK2 was purified by a similar procedure and also split into two peaks on a MONO Q column. For each of four complexes obtained by combining two MEK1 and two ERK2, conditions of crystallization were searched using the commercially available sparse-matrix screening kits. Optimization of crystallization conditions for X-ray crystallography is currently progress.

Keywords: MEK, ERK, crystallization

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Structural biology of a nuclear import of proteins by transportin 1

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The transport of macromolecules between the nucleus and the cytoplasm through nuclear pore complexes (NPCs) is mediated via several transport pathways by transport receptors that are most commonly members of the importin-β family. Transport receptors form complexes with their transport substrates (cargoes) through cognate nuclear localization signals (NLSs) for import substrates or nuclear export signals (NESs) for export substrates, and target substrates to NPC components termed nucleoporins. Transport directionality and interactions between the transport receptor and substrate are regulated by RanGTP and, in the nuclear import system, binding of RanGTP to the receptor in the nucleus is associated with substrate dissociation. Of the several transport pathways, the best characterized is an import pathway mediated by importin-β (karyopherin-β1). Transportin 1 (Trn1) (karyopherin-β2) is a transport receptor that belongs to the importin-β family and has 24% sequence similarity to importin-β. Here we describe four crystal structures of human Trn1 in a substrate-free form as well as in the complex with three NLSs (hnRNP D, JKTBP and TAP, respectively). Our data have revealed that (i) Trn1 has two sites for binding NLSs, one with high affinity (Site A) and one with low affinity (Site B), and NLS interaction at Site B controls overall binding affinity for Trn1, (ii) Trn1 recognizes the NLSs at Site A followed by conformational change at Site B to interact with the NLSs, and finally, (iii) a long flexible loop, characteristic of Trn1, interacts with Site B, thereby displacing transport substrate in the nucleus. These studies provide deep understanding of substrate recognition and dissociation by Trn1 in import pathways.

Keywords: importin, nuclear transport, nuclear pore complex

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Structures of starch binding domain of R. oryzae glucoamylase reveal an amylosic binding model

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Glucoamylase hydrolyzes starch and polysaccharides to β-D-glucose. Rhizopus oryzae glucoamylase (RoGA) consists of two functional domains, an N-terminal starch binding domain (SBD) and a C-terminal catalytic domain, which are connected by an O-glycosylated linker. The SBD of RoGA belongs to the carbohydrate binding modules (CBMs) family 21 (RoGACBM21). The crystal structures of SBD and the complexes with a cyclic carbohydrate, β-cyclodextrin and a linear carbohydrate, maltoheptaose were determined at 1.25, 1.8, and 2.3 Å resolution, respectively. The overall structures of SBD belong to a β-sandwich fold with an immunoglobulin-like architecture. Two carbohydrate binding sites, sites I and II, were determined on the surface of SBD, where site I is a flat and broad hydrophobic binding region created by the aromatic residues, Trp47, Tyr83, and Tyr94; site II is a protruded and narrow binding environment formed by Tyr32 and Phe58. Besides the hydrophobic interaction, two unique polyN loops comprising consecutive asparagines also participate in the sugar binding. To elucidate the mechanism of polysaccharide binding, a number of mutants were constructed and characterized by the quantitative binding isotherm and Scatchard analysis. In addition to sites I and II, a continuous binding surface through Tyr67 and Tyr93 might be essential for long-chain polysaccharide binding. An amylosic binding model for RoGA was proposed.