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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