protein folded in a $(\beta/\alpha)_5$ manner with a central β-sheet formed from five β-strands and surrounded by sides by two and three α-helices. This fold is similar to other receiver domains, e.g. CheY (*E. coli*) or ethylene receptor ETR1 (*A. thal.*). Major conformational differences between $CKI1_{RD}$ and CheY or ETR1 are located in loops connecting highly conserved secondary structure elements. Financial support of this work by the Ministry of Education, Youth and Sports of the Czech Republic (grants MSM0021622415, LC06034) is gratefully acknowledged.

Keywords: cytokinin signal transduction, two-component regulators, histidine kinases

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Structural basis of type-II membrane protein binding by ERM proteins

Shin-ichi Terawaki[1], Ken Kitano[2], Toshio Hakoshima[2]
[1]University of hyogo, Graduate school of life science, 3-2-1, kouto, kamigori-cho, Ako-gun, Hyogo, 678-1297, Japan, [2]Nara institute of science and technology, 8916-5, takayama, ikoma, nara, 630-0192, Japan, E-mail：terawaki@sci.u-hyogo.ac.jp

ERM (Ezrin/Radixin/Moesin) proteins mediate formation of membrane-associated cytoskeletons by simultaneously binding actin filaments and the C-terminal cytoplasmic tails of adhesion molecules (type I membrane proteins). ERM proteins also bind neutral endopeptidase 24.11 (NEP), a type II membrane protein, even though the N-terminal cytoplasmic tail of NEP possesses the opposite peptide polarity to that of type I membrane proteins. Here, we determined the crystal structure of the radixin FERM (Four point one and ERM) domain complexed with the N-terminal NEP cytoplasmic peptide. In the FERM-NEP complex, the amphipathic region of the peptide forms a β strand followed by a hairpin that bind to a shallow groove of FERM subdomain C. NEP binding is stabilized by β-β interactions and docking of the NEP hairpin into the hydrophobic pocket of subdomain C. While the binding site of NEP on the FERM domain overlaps with the binding site of ICAM-2, NEP lacks the Motif-1 sequence conserved in ICAM-2 and related adhesion molecules. The NEP hairpin, although lacking the typical inter-chain hydrogen bond but is stabilized by hydrogen bonds with the main-chain and side-chains of subdomain C, directs the C-terminal basic region of the NEP peptide away from the groove and towards the membrane. The overlap of the binding sites on subdomain C for NEP and Motif-1 adhesion molecules such as CD44 provides the structural basis for the suppression of cell adhesion through interaction between NEP and ERM proteins.

Keywords: ERM proteins, neutral endopeptidase, FERM domain

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Crystal structure of PIX C-terminus domain and Shank PDZ complex

Hye-Eun Song[1], Bo Ram Kwak[2], Jung Gye Lee[3], Jun Yop An[4], Hyung Seop Youn[5], Soo Hyun Eom[6]
[1]GIST, Life Science, GIST, 1-Oryong-dong, Bukgu, Gwangju, South Korea, Gwangju, Jeonlado, 500-712, Korea (S), [2]GIST, GIST, 1-Oryong-dong, Bukgu, Gwangju, South Korea, Gwangju, Jeonlado, 500-712, Korea (S), [3]GIST, GIST, 1-Oryong-dong, Bukgu, Gwangju, South Korea, Gwangju, Jeonlado, 500-712, Korea (S), [4]GIST, GIST, 1-Oryong-dong, Bukgu, Gwangju, South Korea, Gwangju, Jeonlado, 500-712, Korea (S), [5]GIST, GIST, 1-Oryong-dong, Bukgu, Gwangju, South Korea, Gwangju, Jeonlado, 500-712, Korea (S), [6]GIST, GIST, 1-Oryong-dong, Bukgu, Gwangju, South Korea, Gwangju, Jeonlado, 500-712, Korea (S), E-mail：she@gist.ac.kr

PIX is a guanidine nucleotide exchange factor for Rho family GTPases and it mediates the effects of extracellular signals by interacting with a variety of signaling proteins. The C-terminal region of PIX contains a coiled-coil leucine zipper (LZ) domain essential for self association and a C-terminal PDZ binding motif. Shank is a multidomain scaffolding protein that plays a role in organizing synaptic molecules by binding to various proteins. Many PDZ domains have been shown to have more diverse characteristics in ligand binding modes and organization of PDZs than initially anticipated. However, the PDZ ligands for the structural studies so far have been limited to the short peptides which mimic the C-terminal ends of target proteins. To study the molecular mechanism of PIX - Shank PDZ interaction and multimerization by PIX LZ domain, we solved the crystal structure of PIX C-terminus domain - Shank PDZ complex. This structural study provides a clear picture of how the PDZ domain recognizes an intact C-terminal domain of a target protein.

Keywords: shank, PDZ, PIX

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A novel trimeric and coiled-coil structure of a core domain of stomatin from *Pyrococcus horikoshii*

Hideshi Yokoyama[1], Satoshi Fujii[1], Ikuo Matsui[2]
[1]University of Shizuoka, School of Pharmaceutical Sciences, 52-1 Yada, Suruga-ku, Shizuoka, Shizuoka, 422-8526, Japan, [2]Biological Information Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan, E-mail：h-yokoya@u-shizuoka-ken.ac.jp

Stomatin is a major integral membrane protein of human erythrocytes, the absence of which is associated with a form of hemolytic anemia known as hereditary stomatocytosis. It is reported that stomatin modulates the gating of acid-sensing ion channels in mammalian neurons. Stomatin is thought to act as an oligomeric scaffolding protein or as an active signaling component involved in vesicle trafficking. However, the precise function and structure of stomatin has not been elucidated. An open reading frame, PH1511, from the hyperthermophilic archaeon *Pyrococcus horikoshii* encodes p-stomatin, a prokaryotic stomatin[1)]. We determined the first crystal structure of a stomatin-ortholog, the core domain of the p-stomatin PH1511p (residues 56-234, designated as $PhSto^{CD}$) at 3.2 Å resolution[2)]. $PhSto^{CD}$ forms a novel homotrimeric structure. Three α/β domains form a triangle of about 50 Å on each side, and three α-helical segments about 60 Å in length extend from the apexes of the triangle. The α/β domain of $PhSto^{CD}$ is partly similar in structure to the band-7 domain of mouse flotillin-2. While the α/β domain is relatively rigid, the α-helical segment shows a conformational flexibility, adapting to the neighboring environment. One α-helical segment forms an anti-parallel coiled coil with another α-helical segment from a symmetry-related molecule. The α-helical segment shows a heptad repeat pattern, and mainly hydrophobic residues form a coiled-coil interface. The determined structure shows a novel trimeric fold of p-stomatin, and the coiled-coil fold observed in the crystal probably contributes to the self-association.

1) Yokoyama, H. & Matsui, I. (2005). J. Biol. Chem. 280. 6588-6594.

2) Yokoyama, H., Fujii, S. & Matsui, I. (2008). J. Mol. Biol. 376. 868-878.

Keywords: stomatin, coiled coil, flotillin

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Crystal structure of reelin in complex with its receptor

Norihisa Yasui, Terukazu Nogi, Junichi Takagi
Institute for Protein Research, 3-2 Yamadaoka, Suita, Osaka, 565-0871, Japan, E-mail：yasui@protein.osaka-u.ac.jp

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

Nao Miyano[1], Takayoshi Kinoshita[1], Ryoko Nakai[2], Koichi Yokota[2], Hiroshi Ishiguro[2], Toshiji Tada[1]
[1]Osaka Prefecture University, Graduate School of Science, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, [2]Carna Biosciences, KIBC 511, 5-5-2, Minatojima-Minamimachi, Chuo-ku, Kobe, 650-0047, Japan, E-mail：miyano06@b.s.osakafu-u.ac.jp

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

XiaoYan Yang[1], Takayoshi Kinoshita[1], Nao Miyano[1], Tetsuko Nakaniwa[1], Koichi Yokota[2], Hiroshi Ishiguro[2], Toshiji Tada[1]
[1]Osaka Prefecture University, Graduate School of Science, 1-1,Gakuen-cho,Naka-ku, Sakai, Osaka, 599-8531, Japan, [2]Carna Biosciences Inc. , KIBC 511, 5-2-2, Minatojima-Minatomachi ,Chuo-ku, Kobe 650-0047, Hyogo, Japan, E-mail：yang07@b.s.osakafu-u.ac.jp

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

Hiroki Tanaka, Terukazu Nogi, Junichi Takagi
Institute for Protein Research, Osaka University / Japan, 3-2, Yamadaoka, Suita, Osaka, 565-0871, Japan, E-mail：htnk@protein.osaka-u.ac.jp

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