and 0.1 M TRIS-HCl buffer, pH 8.5. Diffraction data was collected to 2.4 Å resolution at beamline BL6A of Photon Factory. ERK1 structure was solved by molecular replacement method using the ERK1 homology model as a probe. The structural refinement and model modification are currently in progress. A detailed structure of ERK1 and comparison with that of ERK2 will be reported.

Keywords: kinase, extracellular signal-regulated kinase, active-site structure

P04.12.282

Acta Cryst. (2008). A**64**, C319

Pheromone binding and realease by honey bee PBP is driven by a pH induced domain swapping

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The behavior and the perception of their surroundings by insects are in a large part driven by odorants and pheromones. This is especially true with social insects, such as honey bee, where the queen controls the development and the caste status of the other individuals. Pheromone perception is a complex phenomenon relying on a cascade of recognition events, initiated in antennae by pheromone recognition by a pheromone binding protein (PBP) and finishing with signal transduction at the axon membrane level. With an end to deciphering this initial step, we have determined the structures of the bee antennal PBP (ASP1) in the apo form or in complex with the main component of the queen mandibular pheromonal mixture, 9-keto-2(E)-decenoic acid (9-ODA) and with non pheromonal components. In the apo-protein the C-terminus obstructs the binding site. In contrast, ASP1 complexes have different open conformations, depending on the ligand shape, leading to different volumes of the binding cavity. The binding site integrity depends on the C-terminus (111-119) conformation, which involves the interplay of two factors: the presence of a ligand and a low pH. Ligand binding to ASP1 is favoured by low pH, opposite to what is observed with other PBPs such as those of Bombyx mori or Anopheles gambiae. At pH 7.0, ASP1 dimerizes forming a domain swapped structure, with loose affinity for pheromone. In contrast, Asp35Asn or Asn35Ala mutants are insensitive to pH and form the same monomer at pH 4.0 and pH 7.0. These results illustrate the influence of a unique residue in triggering ligand binding and protein fold, monomer or domain swapped dimer. We propose that this observation can be linked to the function of PBP when located in the lymph or in the vicinity of the SNMP co-receptor.

Keywords: insects behaviour, pheromone binding protein, domain swapping

P04.12.283

Acta Cryst. (2008). A**64**, C319

Structural basis of dynamic polymerization of DIX domains: A revised model of Wnt signaling

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The Wnt signaling pathway controls numerous cell fates in animal development, and is also a major cancer pathway. A key negative cytoplasmic effecter of this pathway is Axin, which promotes phosphorylation of β-catenin and its subsequent degradation. Another key cytoplasmic effector, Dishevelled (Dvl), a positive effector of this pathway, binds to the Wnt transmembrane receptors and interacts with Axin to transduce the Wnt signal. Both Dvl and Axin contain a DIX domain, a functionally important domain whose molecular properties and structure are unknown. We have determined the first crystal structure of the Axin-DIX domain at 2.9 Å resolution. DIX has a ubiquitin-like fold with five β-strands (β1-β5) and one α -helix. DIX interacts with neighboring molecules through a β-bridge between β2 and β4, forming filaments in the crystal by head-to-tail self-interaction through β-bridges. We also demonstrate that the DIX domain of Dvl2 mediates dynamic polymerization, which is essential for the signaling activity of Dvl2 in vivo. The purified DIX domain self-associates in vitro, and polymerizes gradually and reversibly in a concentration-dependent way, ultimately forming fibrils. Our studies point to a new mechanistic principle underlying Wnt signaling - namely signaling by reversible polymerization, whereby the DIX domain mediates the formation of a dynamic interaction platform with a high local concentration of binding sites for transient signaling partners.

Keywords: crystal structure, Wnt signaling, axin

P04.12.284

Acta Cryst. (2008). A**64**, C319–320

Structure of $CKI1_{RD}$, the receiver domain of the histidine kinase CKI1 from *Arabidopsis thaliana*

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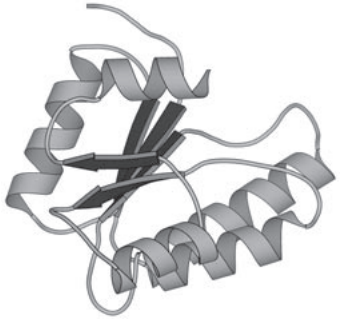
Cytokinins (CKs) are essential phytohormones regulating proper growth and development of plants. CK signal is transduced to the nucleus by a phosphorelay signal transduction pathway via modified two-component system. In *A. thaliana*, signal transduction through cell membrane into the cell is carried out by a family of membrane-associated sensory histidine kinases. One of them, CYTOKININ INDEPENDENT1 (CKI1) constitutively activates the CK signaling pathway and is essential for the female gametophyte development. Here we present crystal structure (2.0 Å resolution) of the receiver domain ($CKI1_{RD}$, residues 979-1120) of CKI1. $CKI1_{RD}$ is a single domain



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

P04.12.285

Acta Cryst. (2008). A**64**, C320

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

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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

P04.12.286

Acta Cryst. (2008). A**64**, C320

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

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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

P04.12.287

Acta Cryst. (2008). A**64**, C320–321

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

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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.