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

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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

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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

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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.