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Cells of the immune system have inherently high levels of tyrosine phosphorylation. In fact, they express more genes encoding protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) than nearly any other cell type. One PTP expressed exclusively in cells of the immune system is hematopoietic protein tyrosine phosphatase (HePTP). HePTP functions to negatively regulate the activation of T cells, and it does so by dephosphorylating and inactivating its only known substrates, the mitogen-activated protein kinases (MAPKs) Erk1/2 and p38. The importance of this regulation is highlighted by the fact that dysregulation of HePTP is associated with several diseases of the immune system, including acute myelogenous leukemia and non-Hodgkins lymphoma. In order to understand the molecular basis of the HePTP:MAPK interaction, we have generated a series of HePTP substrate-trapping mutants (STMs) in order to selectively populate an HePTP:MAPK peptide dephosphorylation complex. STMs have low catalytic activities yet retain high affinities for their substrates. We first determined the biochemical and biophysical characteristics of these HePTP STMs to identify those STMs most suitable for structural studies. We then crystallized and determined the structures of several HePTP STMs in both their apo forms and in complex with two distinct substrate peptide mimetics, e.g. peptides that correspond to the singly- and dually-phosphorylated activation loop of the MAPK Erk2. Finally, we describe how this biochemical, biophysical and structural data has allowed us to identify, for the first time, the structural features of HePTP that confer such a high degree of specificity for its MAPK substrates.

Keywords: hematopoietic protein tyrosine phosphatase (HePTP), extracellular signal-regulated kinase (Erk), T cell activation

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Structure of *Escherichia coli* tyrosine kinase Etk reveals novel activation mechanism

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While protein tyrosine kinases (PTKs) have been extensively characterized in eukaryotes, far less is known about their emerging counterparts in prokaryotes. The inner-membrane Wzc/Etk protein belongs to the bacterial PTK family, which plays a critical role in regulating the polymerization and transportation of virulence-determining capsular polysaccharide (CPS). The kinase utilizes a unique two-step activation mechanism centering on the intraphosphorylation of a tyrosine residue, although the specific detail remains unknown. Herein we report the first crystal structure of a bacterial PTK, the C-terminal kinase domain of *E. coli* tyrosine kinase (Etk) at 2.5Å resolution. The folding of the Etk kinase domain in bacteria differs markedly from that in eukaryotic PTKs. Based on the structure and supporting mass spectrometric evidence of the PTK observed, a unique activation mechanism is consequently proposed that involves the regulation of the phosphorylation of a single tyrosine residue at position 574 and its specific interaction with a previously unidentified key arginine residue at position 614 (R614) to unblock the active site.

Keywords: protein tyrosine kinases, pathogenic bacterial mechanism, polysaccharides

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Crystallographic analysis of response regulator protein from *Desulfovibrio vulgaris* Hildenborough

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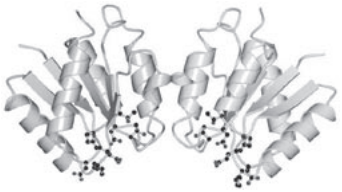
Sulfate-reducing bacteria *Desulfovibrio vulgaris* can generally use hydrogen and organic acids as electron donors for sulfate reduction. Sulfate reduction and hydrogen oxidation are spatially separated in the cytoplasm and periplasm, respectively. It has been proposed that electron transport linking periplasmic hydrogen oxidation to cytoplasmic sulfate reduction is mediated through the high-molecular-mass cytochrome redox protein complex (the Hmc complex). Two genes (rrf1 and rrf2) encoding response regulator proteins with a putative function in the regulation of gene expression are present immediately downstream from the structural genes of the hmc operon. The deletion of the rrf1, 2 genes gives rise to increased hmc operon expression. In order to understand the regulation mechanism of Hmc complex expression, we tried to determine the crystal structure of the response regulator proteins (Rrf1 and Rrf2). We cloned the rrf1and rrf2 genes from *Desulfovibrio vulgaris* Hildenborough and successfully overexpressed, purified and crystallized Rrf1 protein. Here we report the crystal structure of Rrf1 at 2.1 Å resolution.



Keywords: signal transduction, transcription regulation, X-ray crystallography of biological macromolecules

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Crystal structure of human ERK1 kinase mono-phosphorylated at Tyr204

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ERK is a member of MAP kinase family that regulates cell growth and differentiation in response to extracellular stimulation. ERK consists of two major isoforms, ERK1 and ERK2, which have a high degree of amino acid sequence homology and are different from each other in the intravital behavior. In order to investigate *in vivo* function of ERK1, we determined the crystal structure of human ERK1 complexed with 5-iodotubercidin, a potent inhibitor. Purified ERK1 was identified as auto-phosphorylated protein at Tyr204 by Western blot experiments. Crystals of the complex were obtained using a reservoir solution of 30% PEG4000, 0.2 M lithium sulfate,

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

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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

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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

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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