

functional and interaction studies.

Keywords: kir, potassium channels, membrane proteins

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Structural and Functional Analysis of SHPS-1, a Receptor-type Membrane Protein

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Src homology 2 domain-containing protein tyrosine phosphatase [SHP] substrate 1 (SHPS-1), a receptor-type transmembrane glycoprotein whose cytoplasmic region binds and activates the protein tyrosine phosphatases SHP-1 and SHP-2, and thereby modulates multiple cellular functions. Its extracellular region regulates intercellular communication in the neural and immune systems through its association with CD47 on adjacent cells. Interactions between CD47 and SHPS-1 are implicated in multiple cellular processes, including cell motility [1], neutrophil transmigration, phagocytosis of red blood cells by splenic macrophages [2], and T cell activation. Although the roles of the CD47-SHPS-1 system has been presented, little is known about the cell surface organization of these ligand/receptor complexes and the structural basis for signal transduction. To gain new insights into the physiological and biological roles of the CD47-SHPS-1 system, we determined the crystal structure of the SHPS-1 extracellular domain. The domain adopts a classical immunoglobulin (Ig) fold that was observed to form an antiparallel dimer. A dimeric form of SHPS-1 was observed *in vivo*, and our structural and biophysical data shows that the extracellular domain of SHPS-1 is dimeric in solution, compatible with the view of SHPS-1 acting as a *cis*-dimeric adhesion receptor. Previous investigations showed native CD47 formed *cis*-dimers. These features suggest that both CD47 and SHPS-1 *trans*-interact each other by the formation of *cis*-dimers and offer perceptions into interactions of related Ig superfamily receptors.

[1] Motegi S., et al., *EMBO J.*, 2003, **22**, 2634. [2] Oldenborg P.A., et al., *Science*, 2000, **288**, 2051.

Keywords: SHPS-1, CD47, intercellular communication

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Interaction between Raf Kinase and Raf Kinase Inhibitor Protein

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Human Raf Kinase Inhibitor Protein (hRKIP) has been shown to negatively regulate the Mitogen-Activated Protein Kinase (MAPK) signalling cascade by forming an inhibitory complex with the serine/threonine kinase Raf-1. All existing crystal structures of proteins from the RKIP family feature a highly conserved surface pocket, and it has been postulated that this region forms the primary binding interface with phosphorylated forms of Raf-1. Binding studies using randomised libraries of phosphorylated peptides indicate RKIP preferentially binds peptides containing phosphotyrosine. In this study, we have attempted to introduce phosphoserine, phosphothreonine and phosphotyrosine to crystals of hRKIP, by both co-crystallisation and soaking methods. A stable complex could only be formed with phosphotyrosine. The structure of the hRKIP-phosphotyrosine complex was solved, and confirms phosphotyrosine binds within the conserved pocket. These studies are being extended to study the binding of involving a synthetic 12-mer peptide, incorporating a tyrosine residue known to be phosphorylated in Raf-1 and its adjoining sequence. These experiments aim to provide a model for the interaction of RKIP and Raf-1, aiding our understanding of the molecular control of the MAPK signalling cascade.

Keywords: signal transduction proteins, macromolecular crystallography, ligand-protein interactions

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Structural Studies of Mycobacterial Protein Kinases and Phosphatases

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Mycobacterium tuberculosis pathogenicity relies on the peculiar ability of this microorganism to survive and replicate in human macrophages, establishing persistent infection. To understand the pathogen response to the host's environment, we are studying signaling proteins that are presumed to play an important role in the processes that regulate the complex life cycle of mycobacteria.

Two genes encoding the Ser/Thr kinases PknA and PknB, which have been described as essential by saturation mutagenesis [1], are found in a single conserved operon that also includes the gene *pstP* encoding the only Ser/Thr phosphatase in the mycobacterial genome. The crystal structures of PknB [2] and PstP have recently been determined in our laboratory. Both structures confirm the extraordinary conservation of the protein folds and catalytic mechanisms across the evolutionary distance between eukaryotes and prokaryotes.

We will present a comparative study of these proteins and further characterisation of protein-protein and protein-ligand interactions that could be involved in a putative signaling pathway of *M. tuberculosis*.

[1] Sassetti, et al., *Mol. Microbiol.* 2003, **48**, 77-84. [2] Ortiz-Lombardia, et al., *J. Biol. Chem.*, 2003, **278**, 13094-13100.

Keywords: mycobacteria, signal transduction proteins, protein interactions

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Banana Lectin, a β -prism I Fold Lectin with Two Carbohydrate-binding Sites

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The β -prism I as a lectin fold was first characterised in this laboratory in jacalin from jackfruit seeds. This lectin fold was then thought to be characteristic of the *Moraceae* family. Subsequently, the fold was found in lectins from other families as well. We have now determined the structure of banana lectin, which adopts this fold, complexed with methyl- α -D-mannose. The X-ray analysis has been carried out on a trigonal crystal form at two temperatures. As in the case of other lectins with the same fold, the structure consists of three Greek keys, which form the faces of a pseudo-threefold symmetric prism. In other lectins, the primary carbohydrate-binding site is made up of the loop in one of the Greek keys. In banana lectin, however, two nearly identical binding sites are generated using the loops in two Greek keys. Interestingly, it turns out that while there is no noticeable reflection of threefold symmetry in the sequences of other lectins, some vestiges of this symmetry is seen in the sequence of banana lectin. This may have some evolutionary implications as well.

A comparison of the structures of banana lectin with those of other similar lectins provides insights into the variability in the oligomerization of lectins with β -prism I fold. The plasticity of the subunits in such proteins appears to be related to this variability.

Keywords: lectin crystallography, β -prism I fold, protein-carbohydrate interactions