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, phosphthreonine 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.

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