

Biophysical Characterisation of SH2 Domains: a semisynthetic strategy

Satpal Virdee

Abstract:

Src Homology 2 (SH2) domains are protein modules of ~100 aa and play an integral role in eukaryotic cell signaling. Cell receptors can respond to extracellular messages by the autophosphorylation of intracellular Tyr residues. These phosphorylated Tyr (pTyr) residues are then recognised by SH2 domain-containing signaling proteins. The propagation of extracellular messages by SH2 domains leads to the regulation of essential cellular processes such as cell growth, cell differentiation, cytoskeletal rearrangement and gene activation. Extensive structural and biophysical analysis of the SH2 domain of the Src kinase has been achieved by crystallography/NMR and titration calorimetry respectively revealing the mechanism of the sequence specific binding of pTyr motifs. By adopting a semisynthetic approach we have used expressed protein ligation (EPL) and native chemical ligation (NCL) to construct an SH2 domain from three of its constituent fragments. This will allow the insertion of small synthetic cassettes containing non-standard amino acids into the SH2 domain. Initially we aim to probe the significance of the sidechain length of a Lys residue by inserting Lys derivatives with progressively shorter aliphatic side chains. However, any non-standard groups can be inserted allowing the study of SH2 domains to be conducted with unprecedented flexibility.