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The Structural flexibility of the shank1 PDZ domain is important for its Binding to different ligands

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Introduction

The PDZ domain of the shank protein interacts with numerous cell membrane receptors and cytosolic proteins via the loosely defined binding motif X-(Ser/Thr)-X-Φ-COOH (Φ represents hydrophobic residues) at the carboxyl terminus of its target protein. We have determined the crystal structure of the shank1 PDZ in complex with the βPIX C-terminal pentapeptide (642–646, DETNL) at 2.3 A resolution and modeled shank1 PDZ binding to selected pentapeptide ligands. The resulting structures revealed a large hydrophobic pocket within the PDZ domain that can accommodate a variety of ligand residues at the P(0) position. A H-bond between His735 and Ser/Thr at the P(-2) position is invariant throughout the model structures. In addition, we identified multiple PDZ domain residues that are able to form H-bonds and salt bridges with an incoming target protein. Overall, our study provides a new level of understanding of the specificity and structural plasticity of the shank PDZ domain

Experiment

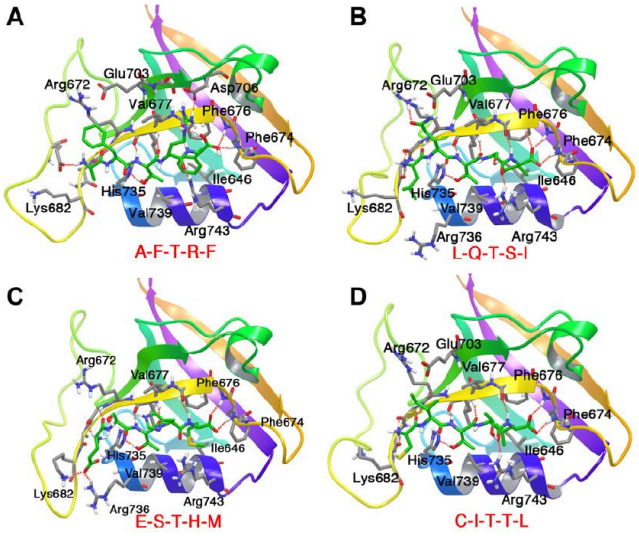
Pentapeptide DETNL, which has the same amino acid sequence as the bPIX C-terminus, was synthesized by Anygen Co.(Korea). The Shank1 PDZ:bPIX complex was then prepared by mixing 10 mg/ml of the protein with the peptide stock at a 1:3 M ratio. Crystals of the shank1 PDZ: βPIX complex were grown by vapor diffusion using 1λ of ~0.8 mM complex and an equal volume of reservoir solution containing 100 mM sodium acetate (pH 5.5–6.0),

0.8 M lithium sulfate and 0.7 M ammonium sulfate. Successful flash freezing was achieved using Paratone N oil (Hampton Research). A diffraction dataset with Bragg spacings of 2.3 A was collected at beam line NW AR12 at the Photon Factory (Tsukuba,Japan) using the X ray beam at a single wavelength (1.000 A).The data set was indexed and processed using the program HKL2000

Results

Comparison of shank1 PDZ–interacting peptides and switch residues in a shank1 PDZ–peptide complex model.

Interaction mode of the receptor tyrosine kinase C terminal pentapeptide (AFTRF) (A), the somatostatin receptor type 2 C-terminal pentapeptide (LQTSI) (B), the Na^+/H^+ exchanger 3 C-terminal pentapeptide (ESTHM) (C), and the CaV1.3 L-type calcium channel C-terminal pentapeptide (CITTL) (D) with the rat shank1 PDZ. The bound peptides and the key PDZ residues involved in the interactions are shown in stick models. The PDZ domains are drawn as ribbon diagrams. H-bonds are represented as red dotted lines. The amino acid sequences of the bound peptides are written in red



References

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