Alteration of the C-Terminal Ligand Specificity of the Erbin PDZ Domain by Allosteric Mutational Effects

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Abstract

Modulation of protein binding specificity is important for basic biology and for applied science. Here we explore how binding specificity is conveyed in PDZ (*p*ostsynaptic density protein-95/*d*iscs large/*z*onula occludens-1) domains, small interaction modules that recognize various proteins by binding to an extended C terminus. Our goal was to engineer variants of the Erbin PDZ domain with altered specificity for the most C-terminal position (position 0) where a Val is strongly preferred by the wild-type domain. We constructed a library of PDZ domains by randomizing residues in direct contact with position 0 and in a loop that is close to but does not contact position 0. We used phage display to select for PDZ variants that bind to 19 peptide ligands differing only at position 0. To verify that each obtained PDZ domain exhibited the correct binding specificity, we selected peptide ligands for each domain. Despite intensive efforts, we were only able to evolve Erbin PDZ domain variants with selectivity for the aliphatic C-terminal side chains Val, Ile and Leu. Interestingly, many PDZ domains with these three distinct specificities contained identical amino acids at positions that directly contact position 0 but differed in the loop that does not contact position 0. Computational modeling of the selected PDZ domains shows how slight conformational changes in the loop region propagate to the binding site and result in different binding specificities. Our results demonstrate that second-sphere residues could be crucial in determining protein binding specificity.

Introduction

PDZ (*p*ostsynaptic density protein-95/*d*iscs large/*z*onula occludens-1) domains are among the most abundant protein–protein interaction domains in multicellular organisms. They help to organize signaling complexes and regulate trafficking of receptors and ion channels by acting as protein scaffolds with diverse binding partners. PDZ domains are composed of seven β-strands and one or two α-helices, and they generally recognize protein C termini, which bind along a groove formed by the α2 helix and the β2 strand (Fig. 1). In early studies, two specificity classes were postulated based on two ligand positions, class 1 [X-(T/S)-X-φ_{COOH}] and class 2 (X-φ-X-φ_{COOH}), where "X" is any amino acid and "φ" is a hydrophobe [1,2]. Thus, according to the accepted nomenclature, the initially postulated specificity classes were based on the recognition of ligand position 0 (C-terminal residue) and position −2. However, a later study showed that any of the last seven ligand residues can potentially interact with the PDZ domain, and, accordingly, PDZ domains were grouped into at least 16 specificity classes [3]. This study also suggested that specificities for positions 0 and −2 were mainly affected by mutations at PDZ positions close to the ligand residue, whereas specificities for positions −1 and

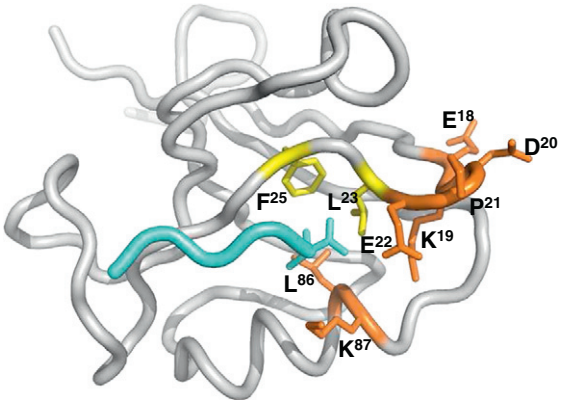

Fig. 1. Erbin-PDZ library design. The Erbin-PDZ and peptide ligand ($TGWETWV_{COOH}$) main chains are shown as gray and cyan tubes, respectively. The C-terminal side chain of the peptide ligand is shown. The Erbin-PDZ residues that were diversified in the library are labeled and shown as sticks colored orange or yellow if they were allowed to vary as all 20 genetically encoded amino acids or as predominantly hydrophobic amino acids, respectively. The figure was generated using PyMOL (http://www.pymol.org/) using NMR structure coordinates (PDB entry 1N7T), and residue numbering corresponds to that in the PDB file.

–3 were affected by mutations throughout the peptide-binding site. Thus, it seemed likely that the structural basis for ligand specificity may be easiest to decipher for sites 0 and –2, given that local effects mediated by direct contacts are easier to rationalize and engineer.

Here we have assessed the range of specificities that can be accommodated within site 0 of a single PDZ domain and we have explored whether specificity can be altered by mutations at positions that are in direct contact with the ligand or by conformational changes caused by mutations at distal positions. We chose the human Erbin PDZ domain (Erbin-PDZ) as the model system, as Erbin-PDZ has been extensively studied structurally and by mutagenesis [4–8]. We constructed a combinatorial phage-displayed library of Erbin-PDZ variants with mutations within and around site 0, and we selected for variants that bind to a set of 19 peptide ligands that differed only at the C terminus and represented all genetically encoded amino acids except proline. Despite extensive efforts, we were only able to evolve Erbin-PDZ variants with selectivity for aliphatic C-terminal side chains, and thus, since wild-type (wt) Erbin-PDZ prefers Val but tolerates Leu and Ile at site 0, only minor changes in specificity were obtained. Surprisingly, we found that changes in specificity appeared to generally arise from conformational changes caused by mutations at positions that do not contact the ligand. Our results suggest that more dramatic changes in PDZ domain site 0 specificity will likely require changes not only at positions that contact the ligand but also at distal positions that influence the main-chain conformation of the domain.

Results

Design and construction of the Erbin-PDZ library

We inspected the structure of Erbin-PDZ in complex with a high-affinity peptide ligand ($TGWETWV_{COOH}$) to identify residues that might influence site 0 specificity and, thus, would be candidates for mutagenesis to alter specificity (Fig. 1). We identified three side chains that make contact with the side chain of the C-terminal Val of the ligand (Leu23, Phe25, Leu86), and we defined this set as the site 0 binding pocket. In addition, we identified five contiguous residues (Glu18–Glu22) that form a loop (loop 18–22) that immediately precedes Leu23, and we reasoned that mutations in this region might alter the conformation of the site 0 pocket through indirect conformational effects. Finally, we identified the solvent-exposed Lys87, which precedes Leu86 in helix α2 and could potentially be recruited for C-terminal ligand recognition if there were substantial main-chain conformational changes in the site 0 pocket region. A phage-displayed combinatorial library of Erbin-PDZ variants was constructed by replacing seven of these nine positions with degenerate codons encoding for all 20 natural amino acids. Positions 23 and 25 were replaced with a degenerate codon encoding mainly for hydrophobic amino acids because these positions reside in the carboxylate binding loop and are conserved as hydrophobes across the PDZ domain family [9].

Binding selections for Erbin-PDZ variants with altered specificities

Phage pools representing the Erbin-PDZ library were cycled through rounds of binding selections with a set of 19 peptides that differed in sequence from a high-affinity Erbin-PDZ ligand ($TGWETWV_{COOH}$) only at the C-terminal position. The 19 peptides together represented sequences with 19 of the 20 natural amino acids at the C terminus. Pro was excluded because the Pro side chain forms a bond with the peptide main chain, disrupting hydrogen bonding interactions between backbone nitrogen on the peptide and the PDZ domain. Because our goal was to select PDZ domains with specificity for a particular amino acid rather than promiscuous domains that could accept a wide variety of C-terminal residues, we performed the selections with each peptide of interest immobilized on a solid support and mixtures of other peptides in solution at high concentration. Under these conditions, we expected that promiscuous domains would bind to the competitor peptides in solution and only domains that were selective for the