A Mutational Path to New Function in a PDZ Domain

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A fundamental problem in biology is to understand the ability of proteins to rapidly adapt to new functions. Here, we structurally analyze a cooperative two-step mutational path involving a "generalist" intermediate that switches the class specificity of PSD95^{PDZ3}. The structures provide a clear rationale for the strong energetic coupling between the two adaptive mutations and we are able to attribute a simple structural mechanism to explain the non-specific but high-affinity substrate binding in the generalist intermediate. Ongoing experiments are focused on comprehensively mapping the binding specificity of PSD95^{PDZ3} along the path of mutation to new specificity.

I. Introduction

Proteins in nature are somehow robust to mutation and yet able to adapt to new functions with a small number of sequence changes [1,2]. Recent work has argued that these properties are a consequence of a conserved architecture in which a sparse, cooperative network of amino acids (the "protein sector") underlies function [2]. Indeed, in PSD95^{pdz3}, just two mutations within the sector suffice to completely switch the phenotype of the protein from its native class I specificity (recognizing peptides with a X-T/S/-X-ϕ-COOH sequence where X is any residue and ϕ is any hydrophobic residue [8]) to class II specificity (recognizing X-ϕ-X-ϕ-COOH peptides). In addition, this work revealed the existence of a generalist intermediate – a single mutant that shows high-affinity but non-specific binding to ligands from both classes. Such generalist intermediates have been argued to be a basic characteristic of adaptive mutational paths in evolution [3-6]. These findings motive our current studies: a) how can a single mutation create a generalist phenotype and b) what is the structural basis for the cooperativity of mutations in the path of adaptation?

To address these questions, we solved high-resolution atomic structures of wild-type, each single mutant, and the double mutant of PSD95^{PDZ3} unbound and bound to both a native class I ligand (N-TKNYKQTSV-COOH [7]) and a non-native class II variant (N-TKNYKQFSV-COOH (named $T_{-2}F$)). The structures explain the likely origin of the generalist phenotype and the nature of cooperativity in the adaptive path. The results may have broad implications for all PDZ domains and reinforces the sector model for function in proteins.

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II. Structural Results

The two specificity-switching mutations in PSD95^{pdz3} are H372A, directly contacting the peptide ligand, and G330T, a mutation far from the binding pocket that causes the generalist phenotype. Structural analysis shows that G330T stabilizes a novel conformation of a surface loop that permits two alternate conformations of the side chain at position 372 – the primary specificity determinant on the PDZ domain. Thus, G330T creates a plastic binding pocket, capable of sterochemically accommodating both class I and class II ligands. In addition, we see clear evidence of structural coupling between G330T, H372A, and the $T_{-2}F$ mutation on ligand that can explain the complex three-way cooperativity of these perturbations in determining ligand binding.

III. Future Experiments and Conclusions

The suggestion that G330T represents a "generalist" intermediate between class I and class II specificity demands a more comprehensive study. Applying a high-throughput, quantitative binding assay based on next-generation sequencing [2], we characterized the specificity of wild-type, G330T, H372A, and H372A/G330T-PSD95^{PDZ3} proteins over a library of 160,000 PDZ ligands (representing randomization of the C-terminal four amino acids of the target peptide). Consistent with its class I designation, wild-type PSD95^{PDZ3} is specific for N-TKNYKQ(T/S)SV-COOH. Interestingly, G330T shows a specific expansion of binding preference at the -2 position to accept aromatic and bulky hydrophobic amino acids. We are further analyzing these data to ultimately provide a quantitative definition of what it means to be a 'generalist' or 'class-specific' protein.

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