Analysis of the binding of an SH3 domain to proline-rich peptides using a chimeric fusion protein

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A complete understanding of the thermodynamic determinants of binding between SH3 domains and proline-rich peptides is crucial to the development of rational strategies for designing ligands for these important domains. Recently we engineered a single-chain chimeric protein by fusing the α-spectrin SH3 domain to the decapeptide APSYSPPPPP (p41)[1]. This chimera mimics the structural and energetic features of the interaction between SH3 domains and proline-rich peptides[2]. Here we show that analysing the unfolding thermodynamics of single-point mutants of this chimeric fusion protein constitutes a very useful approach to deciphering the thermodynamics of SH3-ligand interactions. To this end, we investigated the contribution of each proline residue of the ligand sequence to the SH3-peptide interaction by producing six single Pro-Ala mutants of the chimeric protein and analysing their unfolding thermodynamics by differential scanning calorimetry (DSC) [3].

Structural analyses of the mutant chimeras by circular dichroism, fluorescence and NMR together with NMR-relaxation measurements indicate conformational flexibility at the binding interface, which is strongly affected by the different Pro-Ala mutations. An analysis of the DSC thermograms on the basis of a three-state unfolding model has allowed us to distinguish and separate the thermodynamic magnitudes of the interaction at the binding interface. The model assumes equilibrium between the “unbound” and “bound” states at the SH3-peptide binding interface. The resulting thermodynamic magnitudes classify the different proline residues according to their importance in the interaction as P2 ~ P7 ~ P10 > P9 ~ P6 > P8, which agrees well with Lim’s model for the interaction between SH3 domains and proline-rich peptides. In addition, the thermodynamic signature of the interaction is the same as that usually found for this type of binding, with a strong enthalpy-entropy compensation for all the mutants. This compensation appears to derive from an increase in conformational flexibility concomitant to the weakening of the interactions at the binding interface. We conclude that our approach, based on DSC and site-directed mutagenesis analysis of chimeric fusion proteins, may serve as a suitable tool to analyse the energetic of weak biomolecular interactions such as those involving SH3 domains.

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