Multiplicity in ligand binding: the SH3 domains of CD2AP

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Adaptor molecules are non-catalytic polypeptides that contain one or more domains able to bind to other protein or non-protein ligands. These molecules selectively control de spatial and temporal assembly of multi-protein complexes that transmit intracellular signals involved in regulation of cell growth, differentiation, migration and survival. The importance of such signalling networks is well understood for signal transduction induced by receptor tyrosine kinases (RTKs). Several lines of evidence support a role of CMS/CIN85 (Cas ligand with Multiple SH3 domains/Cbl-interacting protein of 85 kDa) in the regulation of Cbl-directed RTK down regulation. CMS, also known as CD2AP (CD2 associated protein), contains three SH3 domains, a proline-rich region and a coiled-coiled domain, and high sequence identity with CIN85, and hence, it has been assumed to belong to the same family of ubiquitously expressed adaptor molecules and elicit similar biological functions.

Among others, CD2, nephrine, podocine and c-Cbl have so far been identified as natural targets for CD2AP. Intermolecular interactions known until now are mostly mediated via the three N-terminal SH3 domains, named A, B and C. These three SH3 domains share higher similarity among themselves than to any other SH3 domains, suggesting that they may have overlapping specificities in binding. We recently solved the structure of the third SH3 domain of CD2AP by NMR [1].

This presentation is mainly focused on the logical combination of multiple biophysical techniques to study the interactions of the three CD2AP SH3 domains and their natural targets. NMR plays a key role due to the detailed structural information it can provide, addressing the molecular determinants of the protein-protein interaction between the different regions of CD2AP and the other proteins it interacts to. We developed a new method for fast structure determination of weak complexes using Residual Dipolar Couplings (RDCs) in combination with chemical shift perturbation based ambiguous interaction restraints. We used this approach to determine the structure of the complex between CD2AP SH3-C and a new target molecule, Ubiquitin. While interaction between the SH3 domains and CD2 involves an atypical proline-rich region in CD2 in a non 1:1 stoichiometry, the interaction between the SH3 domain and Ubiquitin (1:1 stoichiometry) is driven by other type of tertiary interactions and provides an example of the diversity in targeting by these SH3 domains.

[1] Ortega-Roldan, J.L., Romero-Romero, M.L., Ora, A., AB, E., López-Mayorga, O., Azuaga, A.I. & van Nuland, N.A.J. *J. Biomol. NMR* **2007**, *39*, 331-336.

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