N-terminal SH3 domain of Nckα recognizes a novel non-canonical PxxPxxDY motif present in CD3ε-derived peptides

C.M. Santiveri[1], A. Borroto[2], L. Simón[1], M. Rico[1], B. Alarcón[2], & M.A. Jiménez[1]

[1]Instituto de Química Física Rocasolano, CSIC, Madrid 28006, Spain.
[2]Centro de Biología Molecular, CSIC, Madrid, Spain

Nck is a multi-domain adapter protein consisting of three consecutive SH3 domains and a C-terminal SH2 domain whose biological function is to link cell surface receptors to the actin cytoskeleton. While the SH2 domain binds to different receptor protein-tyrosine kinases, Nck associates with downstream signaling molecules via its SH3 domains. Using a yeast two-hybrid approach, we showed that the N-terminal SH3 domain (SH3.1) of Nck specifically recognizes a proline-rich sequence contained exclusively in the cytoplasmic tail of CD3ε, a subunit of the T cell antigen receptor complex [1].

To get further insight into the Nck-CD3ε interaction, we have solved the NMR structure of the SH3.1 domain of human Nckα and examined its interaction with two CD3ε-derived peptides, a 17-residue wild-type peptide, CD3ε(150-166), and a shorter peptide, 085, obtained through a PEPSCAN analysis and able to inhibit the SH3.1-CD3ε interaction at concentrations 300-fold lower than the wild-type peptide. Nckα SH3.1 shows the characteristic SH3 fold consisting of two antiparallel β-sheets tightly packed against each other. According to chemical shift perturbation data, both peptides that comprise the non-canonical PxxDY motif recognize the same region in the Nckα SH3.1 domain. This region corresponds to the canonical binding site for proline-rich ligands, but additional favorable interactions are spotted in the case of peptide 085. The Nckα SH3.1 binding site was compared with the binding sites of other SH3 domains able to recognize CD3ε, namely, the first SH3 domains of the isoform Nckβ and Eps8 proteins, as well as with those of the second and third Nck SH3 domains unable to bind CD3ε. This analysis together with the NMR data indicate that the target Nck SH3.1 sequence is the new non-canonical PxxPxxDY motif that binds to the SH3 binding site adopting a class II ligand orientation. A positively charged residue (K/R) at position -2 relative to the WW sequence at the beginning of strand β3 is crucial for PxxDY recognition.

[1] Gil**,** D**;** Schamel, W.W., Montoya**,** M., Sánchez-Madrid**,** F., Alarcón, B., *Cell*, **2002,** 109, 901-912.