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NMR assignment of the C-terminal actin-binding domain of talin

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Abstract Talin is a large dimeric 270 kDa adapter protein which binds the cytoplasmic face of a subset of integrin β -subunits and couples them to the actin cytoskeleton. Here we report the near complete ^{15}N , ^{13}C and ^1H chemical shift assignments for the C-terminal actin-binding domain.

Keywords Talin · Integrin · Actin ·
Cell–matrix adhesion · Actin binding domain

Biological context

Cellular interactions with the extracellular matrix are modulated via dynamic protein complexes associated with the cytoplasmic face of the integrin family of cell adhesion molecules. Talin is one of a number of cytoskeletal proteins including α -actinin, filamin, tensin and ILK implicated in linking members of the integrin family of $\alpha\beta$ -heterodimeric cell adhesion molecules to F-actin (see Critchley 2000 for review). It is large (2541 amino acids) elongated (50–60 nm) protein composed of a globular head (residues 1–400) containing a FERM domain linked to a flexible rod (residues 482–2541) by a short linker sequence. The FERM F3 subdomain contains a binding site for the β -integrin cytoplasmic domain, and recent structural studies have provided a detailed understanding of how F3 recognises both the

NP \times Y motif and membrane proximal sequences within the β -integrin cytodomain (Wegener et al. 2007). The talin rod is made up of a series of amphipathic helical bundles, a number of which contain binding sites for the cytoskeletal protein vinculin which is thought to stabilise focal adhesions, possibly by cross-linking talin to F-actin. The C-terminal region of talin (residues 2300–2541) contains the major binding site for F-actin (Hemmings et al. 1996) that is homologous to that in the yeast protein Slap2 and the Huntingtin interacting protein HIP1, and the related protein Hip1R. This highly conserved domain has been referred to as an I/LWEQ motif (McCann and Craig 1997) or more recently the THATCH (talin-Hip1R/Slap2p actin tethering C-terminal homology) core domain (Brett et al. 2006).

Methods and experiments

Multiple polypeptides corresponding to different C-terminal fragments of talin were made and expressed in *E. coli* BL21 Star (DE3) cultured in M9 minimal media, using ^{15}N -ammonium chloride. The recombinant His-tagged protein was purified by nickel-affinity chromatography and eluted by an imidazole gradient. The His-tag was removed by cleavage with TEV protease prior to further purification by anion-exchange chromatography. NMR samples of [^{15}N , ^{13}C]-labeled C-terminal actin-binding domain were prepared at 1 mM in 20 mM sodium phosphate, 50 mM NaCl, 2 mM DTT, pH 6.0 and 10% v/v D_2O .

Comparison of the NMR spectra of the different C-terminal fragments of talin that contain the actin binding domain led to identification of a minimal region (residues 2300–2482) that incorporates the complete domain. Extension of the sequence at the N- or C-terminus added unstructured regions to the protein, while truncation

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- Hemmings L, Rees DJ, Ohanian V, Bolton SJ, Gilmore AP, Patel B, Priddle H, Trevithick JE, Hynes RO, Critchley DR (1996) Talin contains three actin-binding sites each of which is adjacent to a vinculin-binding site. *J Cell Sci* 109:2715–2726
- McCann RO, Craig SW (1997) The I/LWEQ module: a conserved sequence that signifies F-actin binding in functionally diverse proteins from yeast to mammals. *Proc Natl Acad Sci USA* 94:5679–5684
- Vranken WF, Boucher W, Stevens TJ, Fogh RH, Pajon A, Llinas M, Ulrich EL, Markley JL, Ionides J, Laue ED (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins* 59:687–696
- Wegener KL, Partridge AW, Han J, Pickford AR, Liddington RC, Ginsberg MH, Campbell ID (2007) Structural basis of integrin activation by talin. *Cell* 128:171–182
- Wishart DS, Bigam CG, Yao J, Abildgaard F, Dyson HJ, Oldfield E, Markley JL, Sykes BD (1995) ¹H, ¹³C and ¹⁵N chemical shift referencing in biomolecular NMR. *J Biomol NMR* 6:135–140