Integrin connections to the cytoskeleton through talin and vinculin

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Abstract

Integrins are αβ heterodimeric receptors that mediate attachment of cells to the extracellular matrix and therefore play important roles in cell adhesion, migration, proliferation and survival. Among the cytoskeletal proteins that interact directly with the β-chain cytoplasmic domain, talin has emerged as playing a critical role in integrin activation and linkage to the actin cytoskeleton. Talin (2541 amino acids) is an elongated (60 nm) flexible antiparallel dimer, with a small globular head connected to an extended rod. The talin head contains a FERM (4.1/ezrin/radixin/moesin) domain (residues 86–400) with binding sites for several β integrin cytodomains and the talin rod contains a second lower-affinity integrin-binding site, a highly conserved C-terminal actin-binding site and also several binding sites for vinculin. We have determined previously the crystal structures of two domains from the talin rod, spanning residues 482–789. Talin-(482–655), which contains a VBS (vinculin-binding site), folds into a five-helix bundle whereas talin-(656–789) is a four-helix bundle. We have also reported the crystal structure of the N-terminal vinculin head domain in complex with an activated form of talin. In the present paper, we consider how binding sites buried within the folded helical bundles of talin and α-actinin form interactions with vinculin.

Introduction

Cell adhesion contributes to most common human diseases including inflammation, thrombosis, tumour metastasis and infection. Integrins are the principal family of cellular receptors that mediate interactions with the ECM (extracellular matrix) and are involved in a wide variety of physiological processes, including cell proliferation, the suppression of apoptosis, cell migration, embryogenesis and haemostasis [1]. Integrins are non-covalent αβ heterodimers; each subunit has a large N-terminal extracellular domain (>700 residues), a single transmembrane helix and a short (13–70 residues) cytoplasmic domain [2]. Ligand binding to integrins leads to integrin clustering and the subsequent recruitment of actin filaments to the integrin cytoplasmic domains, a process which is facilitated by a series of interacting cytoskeletal proteins including talin, vinculin, α-actinin and filamin [1]. These cell–ECM junctions can be observed in cultured cells and are termed FAs (focal adhesions). Vinculin plays a key role in muscle structure and hereditary mutations in the muscle-specific isoform meta-vinculin result in heart defects (dilated cardiomyopathy) in humans [3]. Tractional forces developed in the cytoskeleton are conveyed to the ECM through the integrins, as are stresses applied to cells from the ECM [4]. Physiological connection of integrins to actin is an essential component of these processes. Increases in adhesion strength are highly correlated with recruitment of talin, vinculin and kinases which ultimately precipitate signalling cascades controlling cell fate [5]. Figure 1 shows some of the key macromolecular interactions which give rise to the formation of FAs.

Which VBSs (vinculin-binding sites) within talin are exposed?

Vinculin does not bind integrins, but is thought to play a key role in FA assembly by indirectly connecting talin and α-actinin to the actin cytoskeleton and recruiting additional proteins such as paxillin and vinexin [6]. A crystal structure of the full-length vinculin molecule shows a circular five-domain (Vd1–Vd5) autoinhibited conformation in which the N-terminal head domains Vd1 and Vd4 form an extensive interaction with the C-terminal tail domain (Vt) [7]. The intramolecular Vd1–Vt interaction regulates vinculin activity by masking the binding sites for talin and α-actinin in the Vd1 domain [7]. We have previously determined the crystal structure of Vd1 in complex with a tVBS (VBS from talin) (Figure 2A) and have shown that binding involves burying the hydrophobic face of an α-helix within the Vd1 cleft and, using SPOT-peptide analysis have also shown that the consensus for Vd1 recognition of the tVBS is LXXAAXXXVAXXVXXLI and that talin contains a large number (~11) of these sites [8].

These studies showing that the VBS motif is largely hydrophobic in combination with crystal structures showing...
Some of the key macromolecular interactions which give rise to the formation of FAs

Integrin $\beta$ subunit peptides bind to talin and $\alpha$-actinin (red arrows). Talin and $\alpha$-actinin bind to vinculin (blue arrows) through short VBS motifs. Collectively, these proteins bind actin through globular helical domains (green arrows). Both talin and $\alpha$-actinin are dimeric (not shown). FERM, 4.1/ezrin/radixin/moesin.

that the tVBS is buried within the core of the helical bundles have proven controversial as a structure which contains a VBS helix will probably undergo a major conformational change/disruption upon binding vinculin [9]. Indeed, mutagenesis shows that the VBS residues contribute to interhelical contacts and the stability of the bundle [10]. The Vd1 binding to native talin purified from turkey gizzard talin could be detected in pull-down and ITC (isothermal titration calorimetry) assays, demonstrating that one or more VBS motifs in talin are available for binding [10]. Furthermore, the finding that Vd1 exposes a cryptic trypsin-cleavage site toward the N-terminal region of the talin rod (between residues 898 and 899) strongly suggests that this region is a hotspot for vinculin binding [10]. These observations correlate well with NMR structural data showing this region lies within a four-helix bundle (talin residues 755–889) which unfolds spontaneously upon addition of Vd1 [11]. It is hypothesized that this helical bundle is a hotspot for the interaction as it has buried hydrophilic residues in the shape of a central ‘belt’ of four threonine residues resulting in a less stable structure. Demonstration of this principle has been made by investigating the correlation between fold stability and Vd1 binding. A quadruple mutant of the talin-(755–889) four-helix bundle replacing the threonine residues with hydrophobic side chains was substantially more stable to thermal and urea denaturation and was also markedly less effective at binding vinculin [10].

VBSs in $\alpha$-actinin

$\alpha$-Actinin is a rod-shaped antiparallel dimer of two 100 kDa monomers; this configuration positions its actin-binding CH
together, these results are consistent with the idea that the actin-binding helices are part of a complex of protein-protein interactions that stabilize the dimeric structure of the actin filament.

Why is the talin-(755–889) VBS active? Analogy with NESs (nuclear export sequences)

An NES is a short amino acid sequence with critical hydrophobic residues which targets a protein for export from the cell nucleus to the cytoplasm through the nuclear pore complex. Leucine-rich NESs conform more or less to the consensus LXXXLXXXXLXL [17,18] (Figure 3A). The presence of regularly spaced large hydrophobic amino acids such as leucine or isoleucine seems to be the most important feature of the signal. Interesting similarities exist between structures of coiled-coil domains for proteins containing NESs and the talin helical bundles. The NMR structure of the FABD (F-actin-binding domain) of tyrosine kinase Bcr-Abl reveals a four-helix bundle with the predicted NES located in helix 3 spanning three turns of the helix [19] (Figure 3B). Biochemical studies reveal that the buried NES is non-functional in the intact FABD protein [19]. These data are equivalent to the talin-(482–655) five-helix bundle which contains an inactive VBS which does not bind in vitro or localize to vinculin in FAs [10]. The analogy extends further as mutants which disrupted the helical bundle and showed weak actin binding of FABD resulted in greater nuclear export presumably by exposing the buried NES. Similarly, mutations of leucine to alanine in the VBS1 sequence predicted to decrease the binding to vinculin actually substantially increased binding in context of the talin-(482–655) five-helix bundle as they act to disrupt the restraints of the topology exposing the VBS.

Continuing with the analogy, the STAT (signal transducer and activator of transcription) domain structures also reveal helix–bundle coiled-coil structures with a buried NES [20]. Here, the NES is active in vivo, and Soler-Lopez et al. [20] have compared Dictyostelium and mammalian STAT domain coiled-coil structures and hypothesized that the NES is active in the structure due to buried hydrophilic residues forming intrahelical hydrogen bonds within the core of the helical bundle reducing its stability (Figure 3B). Indeed, experiments in mammalian STAT domains are similar to those described above on talin-(482–655) which show that mutation of leucine residues to alanine from the consensus increases nuclear export in cells. This could be due to weaker binding and increased release from the exportin machinery, but it could also potentially be due to a second mechanism of destabilizing of the coiled-coil domain which is observed in the talin-(482–655) construct. How do these considerations apply to α-actinin? Examination of the four three-helix bundles from the spectrin-like repeats (R1–R4 in Figure 2C) reveals that R1 contains two buried interhelical contacts from tyrosine side chains (residues 286 and 326), R2 contains two buried histidine side chains (residues 444 and 454), R3 contains a single buried histidine side chain (residue 558), and R4 has a partially buried tyrosine side chain (Figure 3B). The buried tyrosine residue of R4 is reminiscent of the talin-(482–655) VBS motif.
Figure 3 | Similarity between VBS and NESs

(A) Consensus sequence for VBS spans five turns of a helix and 16 residues, whereas the NES is shorter at 11 residues and 3.5 turns of a helix. The N-terminal 12 residues from the αVBS2 sequence are aligned with the C-terminus of αVBS1 which shows some similarity in the pattern of hydrophobic side chains. (B) Core stability influencing binding-site exposure. Structures of the FABD of tyrosine kinase Bcr-Abl and Dicysteletum STAT protein coiled-coil domain (Ds-STATc) highlighting buried residues from the NES in cyan. The more stable FABD does not have an active NES, whereas buried hydrophilic side chains in mammalian STAT domains result in a less stable bundle which can expose the NES motif. This is compared with the C-terminal helical bundle from α-actinin (α-actinin-c), which has a partially buried tyrosine residue in the hydrophobic core.

VBS3 structure which has a single tyrosine residue within the core of the protein and binds spontaneously to vinculin in a similar fashion to talin-(755–889), but contains only a single VBS [21]. One difference between the STAT proteins and talin-α-actinin is that the latter have quaternary structures which are likely to further restrict exposure of binding sites.

Summary

Vinculin is a key player in FA assembly [22] and plays a major role in the contractile apparatus of the sarcolemma of cardiac myocytes [23]. The molecular basis of how it acts is poorly understood, but ground is being gained in terms of the structure and biochemical properties of vinculin and its ligands talin and α-actinin which should ultimately contribute to an understanding of its action in vivo [6]. Talin is now well established as being essential for integrin activation and the initial weak link between integrins [24,25] and the actin cytoskeleton, and α-actinin has established a role as a cross-linker of actin filaments providing stiffness to actin filament networks. The activation of vinculin has been shown to require VBS helices combined with F-actin [7,26]. The exposure of VBS helices is likely to involve unfolding of domains in talin or α-actinin and this could potentially occur in response to stimuli such as force or phosphorylation of specific residues [9,27]. One way to address this would be to construct mutant α-actinins which have a disulfide-locked R4 helical bundle to restrict unfolding/exposure of the VBS [28]. Vinculin interactions need to be highly reversible, since residency times in adhesions are low (∼1 s below 1 min), i.e. strong binding must be counteracted by a strong release mechanism allowing refolding/inactivation of helical bundles [28]. This is likely to be driven by the strength of the head–tail interaction in vinculin which is able to induce dissociation of Vd1 from a complex with a ligand such as VBS1 from talin and allow refolding of the respective bundle [29].

References


