The cytoskeletal protein talin, which is thought to couple integrins to F-actin, contains three binding sites (VBS1–VBS3) for vinculin, a protein implicated in the negative regulation of cell motility and whose activity is modulated by an intramolecular interaction between the vinculin head (Vh) and vinculin tail (Vt) domains. In the present study we show that recombinant talin polypeptides containing the three VBSs (VBS1, residues 498–636; VBS2, residues 727–965; and VBS3, residues 1943–2157) each bind tightly to the same or overlapping sites within vinculin. A short synthetic talin VBS3 peptide (residues 1944–1969) was sufficient to inhibit binding of a 125I-labelled talin VBS3 polypeptide to vinculin, and NMR spectroscopy confirmed that this peptide forms a 1:1 complex in slow exchange with vinculin. Binding of the 125I-labelled VBS3 polypeptide was markedly temperature dependent, but was not inhibited by 1 M salt or 10% (v/v) 2-methyl-2-propanol. Attempts to further define the talin-binding site within vinculin using a gel-blot assay were unsuccessful, but near maximal talin-binding activity was retained by a construct spanning vinculin residues 1–131 in a yeast two-hybrid assay. Interestingly, the talin VBS3 polypeptide was a potent inhibitor of the Vh–Vt interaction, and the VBS3 synthetic peptide was able to expose the actin-binding site in intact vinculin, which is otherwise masked by the Vh–Vt interaction. The results suggest that under certain conditions, talin may be an effective activator of vinculin.

Key words: actin, α-catenin, focal adhesions, integrins.
into the lipid bilayer [31]. Alternatively, vinculin may act by sequestering PtdIns(4,5)P$_2$ from phosphoinositide 3-kinase preventing synthesis of PtdIns(3,4,5)P$_3$, a pathway implicated in the remodelling of focal adhesions induced by platelet-derived growth factor [32]. Finally, it may be significant that the paxillin-binding site in vinculin is constitutively active [33], whereas most of the other ligand binding sites in vinculin are masked by an intramolecular interaction between the vinculin head (Vh) and Vt [34] which is relieved by PtdIns(4,5)P$_2$ [33,35]. This raises the possibility that vinculin might negatively regulate cell motility by sequestering paxillin, which is implicated in focal adhesion signalling [36]. Interestingly, paxillin and vinculin co-localize in the perinuclear region in serum-starved Swiss 3T3 cells which lack focal adhesions, and the ADP-ribosylation factor-1 GTPase drives paxillin (but not vinculin) out of this region into small focal adhesion-like structures [37].

As part of an effort to further define the role of talin and vinculin in integrin-mediated cell adhesion, we have used the yeast two-hybrid system to map the VBSs in talin to three short peptide sequences 25–30 residues in length and spanning talin residues 607–636, 852–876 and 1944–1969 (referred to as VBS1–3 respectively) [17]. The sites are 59% similar, although only two residues are completely conserved, and are predicted to form amphipathic helices. In the present study, we report the biochemical characterization of these sites and show that each binds to the same site in vinculin with high affinity. We also show that a talin polypeptide containing VBS3 competes with the C-terminal Vt for binding to vinculin residues 1–258, although the binding sites for VBS3 and Vt show distinctive features.

**MATERIALS AND METHODS**

**Expression of recombinant talin and vinculin polypeptides**

The plasmid constructs used for expression of chicken talin and vinculin polypeptides in *Escherichia coli* were generated by PCR using primers that contained unique restriction sites, which allowed the PCR products to be cloned directionally into either the NdeI/BamHI sites of pET-15b (Novagen, Cambridge Bioscience, Cambridge, U.K.) or the EcoRI/BamHI sites of pGEX-2T (Amersham Biosciences, Little Chalfont, Bucks., U.K.). The following Bluescript constructs were used as templates; pBS54A, 23B and GG4A which encode chicken talin residues 102–656, 642–1328 and 1646–2541 respectively [16]; and pBS2.89 kb 23B and GG4A which encode chicken talin residues 102–656, and pBScVin5 which encode chicken vinculin residues 1–881 and 642–1328 respectively [16]. The sites are 59% similar, although only two residues are completely conserved, and are predicted to form amphipathic helices. In the present study, we report the biochemical characterization of these sites and show that each binds to the same site in vinculin with high affinity. We also show that a talin polypeptide containing VBS3 competes with the C-terminal Vt for binding to vinculin residues 1–258, although the binding sites for VBS3 and Vt show distinctive features.

**Purification of native talin, vinculin and actin**

Talin [39] and vinculin [40] were purified from turkey gizzard smooth muscle. Actin was isolated from rabbit skeletal muscle as described by Spudich and Watt [41], and was further purified by gel-filtration using a Superdex-200 column (Amersham Biosciences).

**Iodination of proteins**

Aliquots of protein (100 µl; 40 µg) in 100 mM potassium phosphate buffer (pH 7.5) containing 150 mM NaCl were labelled (for 30 min) to a specific radioactivity of approx. 3 × 10$^6$ c.p.m./µg by the addition of 100 µCi of [125I]NaI (ICN Biomedicals, Irvine, CA, U.S.A.) in the presence of N-chloro-benzensulphonamide immobilized on polystyrene beads (Pierce). Labelled protein was purified on a PD10 de-salting column (Amersham Biosciences) using the same buffer containing 0.25%, gelatin and 0.01%, sodium azide.

**Biotinylation of polypeptides**

A His-tagged talin polypeptide spanning residues 1943–2157 (VBS3) was biotinylated as follows; 35 µg/ml sulphasuccinimidyldi-6-(biotinamido)-hexanoate (Pierce) was added to 0.75 mg/ml VBS3 in PBS [160 mM Na$_2$HPO$_4$, 30 mM KH$_2$PO$_4$ (pH 7.4), 140 mM NaCl and 3 mM KCl] and incubated at 4°C for 30 min. To stop the reaction 100 mM Tris/HCl (pH 8.0) was then added, and the labelled VBS3 was dialysed into 50 mM Tris/HCl (pH 8.0)/150 mM NaCl.

**Binding of labelled talin polypeptides to vinculin fusion proteins**

Binding of $^{125}$I-labelled or biotinylated talin polypeptides to a GST–vinculin$^{23-258}$ fusion protein was determined using either a pull-down or a gel-blot assay. In the pull-down assay, up to 170 pmol of GST–vinculin$^{23-258}$ was immobilized on GSH–agarose beads and mixed with the appropriate $^{125}$I-labelled talin polypeptide in 500 µl of buffer P [60 mM imidazole, 0.8 mM Tris/HCl (pH 7.5), 190 mM NaCl, 2 mM EGTA, 5 mM dithiothreitol and 0.2%, haemoglobin] for various time periods. The beads were then washed twice with the same buffer, and the amount of bound ligand was determined using a γ-radiation counter. The free ligand concentration in the assay was calculated from the concentration of ligand in the first wash. By the nature of this assay, only sites with slow kinetics ($k_{off} < 0.01$ s$^{-1}$) will be detected. The amount of GST–vinculin bound to the beads was quantified by Western-blot analysis using an anti-GST antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) followed by scanning densitometry. The effective concentration of the vinculin on the beads was not accurately known, but the values quoted refer to the values if the amount was distributed homogeneously in the assay volume. In order to maximize the fraction of labelled talin bound, the concentration of vinculin was greater than the $K_d$ for talin binding in most assays. As a result the observed IC$_{50}$ value for a competing peptide equals $K_d + ([vinculin]/2)$, and is dominated by the latter term for high affinity interactions (i.e. $K_d < 350$ nM).

In the gel-blot assay, *E. coli* expressing the GST–vinculin fusion proteins were lysed and the proteins were resolved by SDS/PAGE and then transferred on to nitrocellulose. The blot was blocked with 25 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1% (v/v) Tween 20 and 4% (v/v) BSA for 2 h at 20°C, and then overlaid with 10 ng/ml biotinylated VBS3 in blocking buffer for 10 h at 4°C. The blot was washed with the same buffer for 1 h at 20°C, overlaid with streptavidin–horseradish peroxidase (Amersham Biosciences) diluted 1:5000 in blocking buffer, and bound VBS3 was detected using an ECL® kit.

**Actin co-sedimentation assay**

Vinculin (0.5 µM) was incubated overnight with 5 µM G-actin and a synthetic talin VBS3 peptide (0–500 µM) in buffer A [2 mM Tris/HCl (pH 8.0), 0.2 mM CaCl$_2$, 0.2 mM Na$_2$ATP and 0.5 mM 2-mercaptoethanol]. Actin polymerization was initiated by the addition of 2 mM MgCl$_2$/100 mM KCl and was allowed to proceed for 2 h at 20°C. F-actin was sedimented through a 20% (w/v) sucrose cushion at 440000 g for 1 h and proteins
present in the pellet and the supernatant were analysed by SDS/PAGE followed by staining with Coomassie Brilliant Blue.

**Analysis of the talin-binding site in vinculin using the yeast two-hybrid assay**

Chicken vinculin polypeptides were expressed as fusion proteins with the yeast LexA DNA-binding domain using the vector pBTM116. Each of the three VBSs in chicken talin were expressed as fusion proteins with the GAL4 transactivation domain using the vector pGAD GH (Clontech Laboratories). The sequence of each construct was confirmed by automated DNA sequencing. Plasmids were transformed into the L40 strain of Saccharomyces cerevisiae, and positive interactions were identified using a quantitative β-galactosidase assay as described previously [17].

**RESULTS AND DISCUSSION**

**The three VBSs in talin show similar characteristics**

We have previously shown that there are three non-overlapping VBSs (VBS1–3) in talin that show 59% similarity to each other [17]. To characterize these binding sites in more detail, we initially assayed binding of a recombinant 125I-labelled talin polypeptide (residues 1944–2157) containing VBS3 to a GST–vinculinC,258 fusion protein which contains the talin-binding site. Binding of 125I-labelled VBS3 increased in proportion to the amount of GST–vinculin immobilized on the beads, and there was little binding to GST alone (results not shown). Binding of 125I-labelled VBS3 was saturable (Figure 1A) with an estimated $K_d$ of approx. 39 nM. This value is similar to that reported for the interaction between intact 125I-labelled vinculin and talin [16,34]. Binding could also be readily detected using the unlabelled VBS3 polypeptide and analysis of bound material by SDS/PAGE (Figure 1B), consistent with tight binding.

Binding of 125I-labelled VBS3 to GST–vinculin was progressively inhibited by increasing concentrations of unlabelled VBS3 (either with or without the His-tag) as well as unlabelled VBS1 (residues 498–636) and VBS2 (residues 727–965), whereas a His-tagged talin polypeptide (residues 2270–2541) which contains the C-terminal actin-binding site [11] failed to inhibit binding (results not shown). The results indicate that all three sites in talin interact with the same region in vinculin. If this is correct, then each of the three talin VBS polypeptides should quantitatively inhibit binding of intact 125I-labelled talin to the GST–vinculinC,258 fusion protein, and the results shown in Figure 1(C) confirm that...
Figure 2. Changes in $^1$H NMR spectra of vinculin$_{1-258}$ upon addition of a synthetic talin VBS3 peptide

(A) Changes in $^1$H NMR spectra of vinculin$_{1-258}$ upon addition of a minimal synthetic talin VBS3 peptide (residues 1944–1969; single-letter code: YTKKELIESARKVSEKVSHVLAALQA). Low-field regions of (a) free vinculin$_{1-258}$; (b) an equimolar mixture of vinculin$_{1-258}$ and the minimal VBS3 peptide; and (c) difference spectrum i.e. spectrum (a) subtracted from spectrum (b). Positive resonances correspond to the signals of the complex not present in the spectrum of the free protein. These are either peptide signals or protein signals that change position upon complex formation. The negative signals correspond to the signals of the free protein that change position upon complex formation. The spectrum of the free VBS3 peptide is shown in (d). (B) High-field region of $^1$H NMR spectra of (a) free vinculin$_{1-258}$; (b) equimolar mixture of vinculin$_{1-258}$ and the minimal VBS3 peptide and; (c) equimolar mixture of vinculin$_{1-258}$ and the truncated VBS3 peptide (residues 1955–1969). Please note the change in the vertical scale.

This was indeed the case. Although VBS1–3 appear to have similar biochemical properties, sequence comparisons show that only two residues, a leucine and an alanine, are totally conserved across all three sites [17]. The binding activity of each site from human to nematodes identifies a series of totally conserved, predominantly hydrophobic, residues characteristic of each site that we assume defines the protein fold.

A short synthetic talin VBS3 peptide can bind to vinculin

Using a yeast two-hybrid approach, each of the VBSs in talin has been defined to a sequence of approx. 25–30 amino acids [17]. Vinculin residues 1–258 are also predicted to contain a series of amphipathic helices, but the chemical nature of the interaction between the two proteins has not been determined. Talin VBS1 contains three leucine residues (Leu$^{606}$, Leu$^{610}$ and Leu$^{614}$) that align down one side of the helix, which might form a potential leucine zipper [17]. However, in VBS2 one of the leucine residues is replaced by a methionine, and in VBS3 two of the leucine residues are replaced by valine residues. Single point mutations of the conserved leucine residues in VBS1 to alanine did not reduce binding to vinculin residues 1–258, as determined using a yeast two-hybrid assay. While a double leucine (Leu$^{606}$/Leu$^{614}$) mutant showed a marked reduction in binding, the residual binding activity was still quite high. Analysis of binding of the minimal talin VBS3 peptide to GST–vinculin by NMR spectroscopy

Figure 2(A) (spectrum c) with that of the free peptide in Figure 2(A) (spectrum d). However, when vinculin$_{1-258}$ was titrated with the truncated VBS3 peptide, the difference spectrum at all peptide concentrations showed only resonances of the free peptide, and no indication of changes in the protein spectrum (results not shown). From these results we conclude that vinculin$_{1-258}$ binds to the VBS3 peptide, whereas there is no interaction with the truncated peptide. The intensities of the signals in the difference spectrum depend linearly upon the VBS3 peptide concentration until an equimolar ratio of vinculin$_{1-258}$ to VBS3 peptide is reached. After that, a new set of sharp signals appears corresponding to the signals of the free peptide, and no further changes in the broad signals are detected. The resonances of the complex showed no concentration-dependent broadening or chemical shift changes (results not shown). The spectral changes are clearly visible in the high-field region of the spectrum (Figure 2B). Here the spectrum of the complex (Figure 2B, spectrum b) has an additional resonance at 0.32 p.p.m., not observed in the spectrum of the free protein (Figure 2B, spectrum a), that allowed us to monitor complex formation. The intensity of the resonance corresponds to that of a methyl group, and the low chemical shift is most likely the effect of an aromatic ring proximity. All observed spectral changes agree with the formation of a 1:1 peptide–protein complex in slow exchange on the NMR time-scale. The overall changes in the NMR spectra upon complex formation are restricted to a relatively small number of signals, as is evident from the difference spectrum of Figure 2(A) (spectrum c). This indicates that the proton environment change upon peptide binding is restricted to the binding site and that no large conformational changes occur.

Characterization of the nature of the vinculin–talin interaction

Binding of the $^{125}$I-labelled VBS3 talin polypeptide to GST–vinculin$_{1-258}$ immobilized on GSH–agarose beads was slow at 4 °C [time to reach half-maximal binding ($t_{1/2}$) = 131 min], and the amount bound at equilibrium could be reduced to approx. 30 % of maximum by the addition of 600 nM unlabelled VBS2 (Figure 3A). Raising the temperature to 19 and 37 °C markedly increased the rate of binding ($t_{1/2} = 17$ and 13 min respectively). The elution of bound $^{125}$I-labelled VBS3 at 4 °C was also slow ($t_{1/2} = 172$ min), and although raising the temperature to 19 °C did increase the rate of dissociation (Figure 3B), the effect was not as dramatic as that seen for binding. This indicates that as the temperature is increased, the association rate constant increases relative to the dissociation rate constant, i.e. the affinity increases with increasing temperature. The slow time course for $^{125}$I-labelled VBS3 binding to and release from the Vh fragment suggests that the interaction is multistep.

The three VBSs in talin are each predicted to form short amphipathic helices containing approx. 21 residues [17]. Vinculin residues 1–258 are also predicted to contain a series of amphipathic helices, but the chemical nature of the interaction between the two proteins has not been determined. Talin VBS1 contains three leucine residues (Leu$^{606}$, Leu$^{610}$ and Leu$^{614}$) that align down one side of the helix, which might form a potential leucine zipper [17]. However, in VBS2 one of the leucine residues is replaced by a methionine, and in VBS3 two of the leucine residues are replaced by valine residues. Single point mutations of the conserved leucine residues in VBS1 to alanine did not reduce binding to vinculin residues 1–258, as determined using a yeast two-hybrid assay. While a double leucine (Leu$^{606}$/Leu$^{614}$) mutant showed a marked reduction in binding, the residual binding activity was still quite high. Analysis of binding of the minimal talin VBS3 peptide to GST–vinculin by NMR spectroscopy...
Characterization of the talin–vinculin interaction

Figure 3 125I-labelled talin VBS3 binding to and dissociation from GST–vinculin

(A) 125I-labelled VBS3 (20 nM) was incubated with GST–vinculin,258 immobilized on GSH–agarose beads at 4, 19 or 37 °C for various times, before removing unbound material by washing. The amount of 125I-labelled VBS3 bound was determined using a γ-radiation counter. Binding was also determined in the presence of 600 nM unlabelled VBS2 polypeptide to determine binding specificity. Under these conditions, binding was limited to approx. 30% of the maximum. Each of these results was reproduced on 2–4 separate occasions. (B) 125I-labelled VBS3 was bound to GST–vinculin,258 for 21 h before washing away unbound material. The dissociation of 125I-labelled VBS3 was followed over time in the presence of 5 μM unlabelled VBS3 at both 4 and 19 °C. Each of these results was reproduced on 2–4 separate occasions.

Figure 3 shows binding of 125I-labelled VBS3 to GST–vinculin,258 at concentrations up to 10% (results not shown).

Each of the VBSs in talin is basic (pI values of approx. 9.1), but single point mutations of conserved basic residues (Lys

suggests close contacts between aromatic and aliphatic residues in the complex. However, the alcohol 2-methyl-2-propanol, which is a potent inhibitor of hydrophobic interactions [44], failed to inhibit binding of 125I-labelled VBS3 to GST–vinculin,258 at concentrations up to 10%. (results not shown). Each of the VBSs in talin is basic (pI values of approx. 9.1), but single point mutations of conserved basic residues (Lys

Further characterization of the talin-binding site in the Vh

We have previously mapped the talin-binding site in the Vh to residues 1–258 using both gel-blot and solid-phase assays [45]. However, attempts to further define this site by introducing N-terminal deletions have failed using both in vitro binding [45] and yeast two-hybrid assays [17]. The recently determined structure of the N-terminal region of α-catenin [46], which is homologous to vinculin, provides insights into the possible structure of this region of vinculin. α-Catenin residues 82–262 comprise five helices that form two distinct substructures. Helix 1 runs antiparallel to the first half of the long helix 2, and the C-terminal part of helix 2 forms part of an antiparallel four-helix bundle with helices 3–5. Since the core hydrophobic residues of α-catenin are totally conserved in vinculin, along with the proline residues that introduce a kink into helices 3 and 4, it seems likely that vinculin residues 70–247 will adopt a similar fold. A structure for the complex between α-catenin and its ligand β-catenin has also been solved [46]. This shows that residues 59–82 of α-catenin (equivalent to residues 40–63 of vinculin) are also helical (helix 0), and again the core hydrophobic residues are conserved in vinculin. Helices 0, 1 and the N-terminal part of the long helix 2 pack against a short helix from β-catenin (residues 121–141) involving both polar and apolar contacts, i.e. three helices in α-catenin bind to a short helix in β-catenin. It is interesting that vinculin also interacts with a short peptide sequence in talin that is predicted to be helical [17], and it is tempting to speculate that vinculin residues 40 (predicted start of helix 0) to part way through long helix 2 (predicted to end at residue 147) contain the talin-binding site.

To investigate this possibility, we expressed a series of deletion mutants based on the GST–vinculin,258 construct where the C-terminal boundary was designed according to the α-catenin structure. Talin binding was assayed using biotinylated VBS3 and an SDS gel-blot assay. In agreement with previous results, GST–vinculin,258 retained talin-binding activity in this assay [44], but a deletion which removed residues 215–258 (predicted helix 5) completely abolished binding (Figure 4A). Identical results were obtained when binding was assayed to purified GST–vinculin,211 immobilized in microtitre wells (results not shown). However, we have previously obtained different results using the yeast two-hybrid assay. Thus a LexA fusion protein containing vinculin residues 1–167 supported binding to each of the three VBSs in talin just as well as the 1–258 construct, while deletion of residues 1–57 almost completely destroyed binding [17]. We therefore tested the ability of vinculin residues 1–131 to bind to talin VBS3 in the yeast two-hybrid assay. The results showed that it retained approx. 70% activity of the control vinculin,258 construct (Figure 4B).

The difference in the results obtained using the two assays is illustrated by point mutations in the highly conserved sequence Arg

in vitro (Glu behavied as wild-type) [45], whereas the Glu

mutation, were unable to bind talin in vitro (Glu behavied as wild-type) [45], whereas the Glu

mutation had no major effect on the binding of a vinculin,258 construct to talin VBS1–3 in the yeast two-hybrid assay (Figure 4C). It is interesting to note that there is an identical Arg-Gln-Gln-Glu sequence in all vertebrate α-catenins, which is located at the end of helix 3 and is part of the antiparallel four-helix bundle [46]. Presumably, this region is essential to the overall protein fold, and mutations therein may reduce the affinity of GST–vinculin for talin VBS3 such that binding can no longer be detected in assays which include extensive washing steps. In the yeast two-hybrid assay, the components do not undergo physical separation, and therefore this method is probably better at detecting lower affinity binding interactions. In conclusion, the results from the yeast two-hybrid experiments strongly indicate that the talin-binding site in vinculin is contained within residues 1–131. Whether the interaction is mediated in a similar way to the α-catenin–β-catenin

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Figure 4 Identification of the talin-binding site in vinculin using in vitro binding and yeast two-hybrid assays

(A) The series of GST–vinculin fusion proteins shown were expressed in E. coli, resolved by SDS/PAGE and transferred on to nitrocellulose. The blot was overlaid with 10 ng/ml biotinylated-VBS3 for 10 h at 4°C, washed and then bound VBS3 was detected with streptavidin–horseradish peroxidase and an ECL2 kit. VBS3 bound specifically to GST–vinculin1–258, but failed to interact with any of the truncated polypeptides. This result was reproduced on two separate occasions. (B and C) Talin polypeptides spanning VBS1 (residues 498–636), VBS2 (residues 727–965) and VBS3 (residues 1943–2157) expressed in yeast as GAL4 transactivation domain fusion proteins were tested for binding to vinculin residues 1–258, and mutants thereof expressed as Lex A DNA-binding domain fusion proteins. Interaction between the talin and vinculin polypeptides was monitored by assaying β-galactosidase activity (arbitrary units) in yeast cell lysates. Activity values are shown above each column, and are the means of duplicate determinations. The experiments were repeated at least three times with essentially the same result. Lamin–Lex A and Byr2–GAL4 fusion proteins were used as negative controls, and the interaction between Ras–Lex A and Byr2–GAL4 was used as a positive control (results not shown). Vinc, vinculin.

interaction [46] awaits the determination of the structure of the complex.

Does the Vt bind to the same site in vinculin as talin?

In intact vinculin, an intramolecular interaction between Vh and Vt obscures the binding sites for talin [34] and α-actinin [47] in Vh, and for F-actin in Vt [48]. This suggests that either Vt and talin bind to the same site in Vh, or that Vt sterically inhibits binding of talin. To explore these possibilities, we tested the ability of Vt and VBS3 to compete for binding to GST–vinculin1–258 immobilized on GSH–agarose beads. Unlabelled VBS3 was an effective inhibitor of 125I-labelled Vt binding with an IC50 value of approx. 56 nM (Figure 5A). In contrast, binding of 125I-labeled VBS3 (20 nM) to GST–vinculin1–258 was not inhibited by unlabelled Vt even when used at concentrations as high as 6 μM (Figure 5B). This is surprising given that the Kd value for the interaction between Vt and Vh has been estimated to be between 50 nM [34] and 93 nM [49], and is therefore in the same range as the vinculin–talin interaction [16,34]. Miller et al. [49] have recently shown that the binding of Vt to a GST fusion protein containing vinculin residues 1–266 is markedly reduced by salt concentrations above 100 mM. Since the above experiments were conducted in 190 mM salt, we repeated the experiment in 50 and 220 mM salt. However, Vt (5 μM) again failed to inhibit binding of VBS3 to GST–vinculin1–258 (Figure 5B).

We have previously shown that Vt binds to sequences within vinculin residues 1–258 [35], and recent studies by others indicate
VHS peptide can activate vinculin

The intramolecular interaction between Vh and Vt can be relieved by PtdIns(4,5)P₂ [33,35], exposing the talin and α-actinin binding sites in Vh and the VASP binding site in the proline-rich domain [51]. However, PtdIns(4,5)P₂ appears to mask the F-actin binding site in Vt [52], and, perhaps significantly, the binding sites for PtdIns(4,5)P₂ [31] and F-actin [53,54] in Vt partially overlap. This suggests that vinculin activated by PtdIns(4,5)P₂ on the cytoplasmic face of the plasma membrane may be able to bind talin or α-actinin, but not F-actin. Whether vinculin can be activated by other mechanisms remains to be investigated. The Shigella protein IpaA has been shown to bind with high affinity (Kₐ = 5 nM) to the talin-binding site within Vh exposing the F-actin binding site in Vt [55]. Similarly, a vinculin-binding peptide isolated by phage display, and which shows some similarity to talin VBS3 [56], inhibited binding of Vh to Vt, and exposed the F-actin binding site in Vt [52]. To investigate whether authentic talin VBS3 can also activate vinculin, the effect of a VBS3 synthetic peptide on the ability of vinculin to bind F-actin was assessed using a co-sedimentation assay. Vinculin isolated from turkey gizzard binds poorly to F-actin, and >80% of the protein remained unbound in the supernatant. The addition of increasing concentrations of VBS3 peptide markedly enhanced the amount of vinculin that co-sedimented with F-actin (Figure 6), although the peptide did not cause vinculin to sediment in the absence of F-actin (results not shown). These results support the idea that talin can shift the equilibrium for vinculin from the closed to the open conformation.

However, previous studies have shown that although Vt was an effective inhibitor of talin binding to the Vh (IC₅₀ of approx. 40 nM), talin only partially inhibited (approx. 50%) binding of the 95 kDa Vh to Vt [34]. Moreover, this level of inhibition was only achieved when talin and Vh were preincubated for 16 h. One possibility is that the VBSs in intact talin in solution are not maximally active, and that they are therefore less effective at competing with Vt for binding to Vh. It has been noted that vinculin binds more efficiently to the talin rod than intact talin [57]. Perhaps the VBSs in talin recruited to the cytoplasmic face of integrins are activated such that talin can now displace the Vh–Vt interaction. Interestingly, the integrin-binding site(s) in talin has recently been shown to be activated by PtdIns(4,5)P₂ [58].

REFERENCES

9 Knezic, I., Leisner, T. M. and Lam, S. C.-F. (1996) Direct binding of the platelet integrin αIIbβ3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the coplasosome domains of both αIIb and β3. J. Biol. Chem. 271, 16416–16421


