Interaction of the N- and C-terminal Domains of Vinculin

CHARACTERIZATION AND MAPPING STUDIES

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The vinculin head to tail intramolecular self-association controls its binding sites for other components of focal adhesions. To study this interaction, the head and tail domains were expressed, purified, and assayed for various characteristics of complex formation. Analytical centrifugation demonstrated a strong interaction in solution and formation of a complex more asymmetric than either of the individual domains. A survey of binding conditions using a solid-phase binding assay revealed characteristics of both electrostatic and hydrophobic forces involved in the binding. In addition, circular dichroism of the individual domains and the complex demonstrated that conformational changes likely occur in both domains during association. The interaction sites were more closely mapped on the protein sequence by deletion mutagenesis. Amino acids 181–226, a basic region within the acidic head domain, were identified as a binding site for the vinculin tail, and residues 1009–1066 were identified as sufficient for binding the head. Moreover, mutation of an acidic patch in the tail (residues 1013-1015) almost completely eliminated its ability to interact with the head domain further supporting the significance of ionic interactions in the binding. Our data indicate that the interaction between the head and tail domains of vinculin occurs through oppositely charged contact sites and results in conformational changes in both domains.

Focal adhesions are dynamic complexes of structural and signaling proteins located at sites of very close contact between the extracellular matrix and the plasma membrane. These complexes function to link filamentous actin to the extracellular matrix via integrin receptors and a number of cytoplasmic proteins. Vinculin, a soluble 116-kDa protein, is a component of focal adhesions and also plays a role in cadherin-mediated cell-cell adhesion (1). Although the specific role of vinculin in focal adhesions is unknown, genetic approaches have been used to demonstrate the importance of vinculin in both cell culture and animal model systems (2, 3).

Vinculin possesses no known enzymatic activity and is believed to play its crucial role through its ability to associate with a large number of other cytoskeletal and signaling proteins. Vinculin has been shown to associate with talin, α-actinin, paxillin, F-actin, and members of the VASP/Ena and ponsin/ArgBP52/vinexin families (4–7). Interactions such as these may play an important role in vinculin function as its association with focal adhesions has been shown to rely on the presence of protein components in these structures (8). By binding to both structural and signaling proteins and bringing them to proximity, vinculin may act as an adaptor protein and may play an important structural role through cross-linking actin filaments.

Structurally, vinculin is composed of two domains: a large 90-kDa globular head and a 30-kDa elongated tail connected by a proline-rich hinge region. A strong interaction ($K_d \approx 50 \text{ nM}$) between the vinculin head and tail domains has been observed (9), and inactivation of binding sites for other proteins on both the head and the tail domains has been demonstrated to occur during this self-association. For example, both the binding of the head domain to talin and the binding of the tail to actin filaments are inhibited by the head-tail interaction (10, 11).

These observations have led to a model for the vinculin activation in which cytosolic vinculin exists in an inactive, “closed” state, and factors that inhibit the head-tail binding induce vinculin to adopt an active, “open” conformation allowing its recruitment into focal adhesions.

The binding sites involved in this intramolecular interaction have been investigated previously using deletion mutagenesis. The deletion of residues 167–207 resulted in an inability of the head domain to bind the tail in vitro (12) suggesting that this small region either was structurally important for activity or contained the binding site. C-terminal truncation mutants of the vinculin head domain have been used to localize the tail-binding site to the N-terminal 258 amino acids (12), but no attempts have been made to more clearly define the boundaries of this site.

Similar strategies have been used to identify the head-binding site within the vinculin tail domain. C-terminal deletions of the vinculin tail were used to define the C-terminal boundary of the site to between residues 1028 and 1036 (9), but the extent of the binding region is not clear. Interestingly, the C-terminal region of the vinculin tail has also been implicated in binding to actin, phospholipids, and paxillin and is one of two sites responsible for focal adhesion targeting of vinculin (13–16).

Bakolitsa et al. (17) recently solved the crystal structure of the isolated tail domain and demonstrated that it adopts an apolipoprotein E-like four-helix bundle with a C-terminal arm. Many of the tail-binding sites lie within helix 5 and the terminal arm of the vinculin tail.

To investigate the vinculin intramolecular association, we have separately expressed recombinant polypeptides of the vinculin tail domain and the N-terminal 266 amino acids of the vinculin head region. We have used these constructs to examine their shapes individually and in a complex by analytical centrifugation and circular dichroism spectroscopy. Further, we developed a solid-phase binding assay to characterize their interaction quantitatively and map regions of the polypeptides sufficient to support the interaction. In this paper, we demon-

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straté the head-tail binding to be strongly ionic in character and propose that it is the result of the interaction between two oppositely charged faces of amphiphilic helices: a basic region (residues 182–226) within the acidic head domain and an acidic region (residues 1009–1066) within the highly basic tail. In agreement with this, we find that residues Asp-1013, Glu-1014, and Glu-1015 in the tail domain are necessary for interaction with the vinculin head domain.

**EXPERIMENTAL PROCEDURES**

**Recombinant Proteins—** Recombinant DNA techniques were from Sambrook et al. (18). Vinculin domains were amplified from a cDNA pool synthesized from the reverse transcription of total human RNA. Initially, DNA encoding vinculin residues 5–266 was amplified using primers 1 and 2 (Table 1). This PCR product was cloned into the XbaI and SalI sites of the pGEX-KG vector (19). Subsequently, this plasmid was used as a template in a PCR reaction using primers 3 and 2 to add the five N-terminal residues. This product was cloned into the PCRBlunt (Invitrogen, Carlsbad, CA) vector and subsequently into the Ncol and SalI sites in the pGEX-KG vector creating construct pKG-V-(1–266).

Truncations of pKG-V-(1–266) were created using the internal HindIII and SacI restriction endonuclease sites. Construct pKG-V-(1–86) was created by digestion of pKG-V-(1–266) with HindIII and religation. pKG-V-(1–181) was created by digestion with SacI and religation. N-terminal truncations were constructed after digestion of pKG-V-(1–266) with either HindIII or SacI, isolation of the cleaved fragment, and re-insertion into the pGEX-KG vector to create pKG-V-(877–1066) and pKG-V-(182–266), respectively. pKG-V-(182–266) was created by the digestion of pKG-V-(877–1066) with HindIII and religation.

To construct vectors to express regions of the vinculin C-terminal tail domain, cDNA encoding amino acid residues 833–1066 was amplified using primers 4 and 5 (Table 1). This PCR product was digested using XhoI and Xhol and cloned into pGEX-KG. This construct was amplified using primers 6 and 7 to clone two additional regions of the vinculin tail domain using primers 8 and 4. These two PCR products were cloned into a TA vector (Invitrogen) prior to cloning into the XbaI and Xhol sites of pGEX-KG thereby creating constructs pKG-V-(1009–1066) and pKG-V-(1009–1066).

Site-directed mutagenesis of residues Asp-1013, Glu-1014, and Glu-1015 was performed using a PCR protocol (20). The resulting PCR product was cloned, sequenced, and placed into pGEX-KG.

Constructs were expressed in BL21(DE3) or RR1 strains of *Escherichia coli*. Cultures were shaken at 37 °C until an OD600 of 1.0–1.5 was reached when they were then transferred to room temperature, and 0.1 mM isopropyl-1-thio-

**RESULTS**

Further purification of V-(1–266) and V-(877–1066) was performed on ion-exchange columns. V-(1–266) was diluted 5-fold in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.0) plus protease inhibitors, applied to a DEAE-Sepharose column (Amersham Pharmacia Biotech), and eluted with a linear 50–250 mM NaCl gradient. V-(877–1066) was diluted 5-fold, applied to an S-Sepharose column (Amersham Pharmacia Biotech), and eluted using a 50–200 mM NaCl gradient.

**Analytical Ultracentrifugation—** Ultracentrifugation was performed in a Beckman model XL-A analytical ultracentrifuge at 20 °C. Sample buffer contained 20 mM Tris-HCl, pH 7.5, 75 mM NaCl, 3 mM MgCl₂, 1 mM EGTA, and 1 mM dithiothreitol. Scans were taken at 280 nm. Sedimentation equilibrium experiments were performed at 30,000 rpm with protein concentrations of 20, 30, and 40 μM. In experiments containing two components, each component was at an equal concentration. Equilibrium was determined by coincidence of scans taken ≥2 h apart. Sedimentation velocity experiments were performed at 60,000 rpm with scans taken at 10-min intervals. Data were analyzed by software provided by Beckman Instruments. Partial specific volumes were calculated by standard methods. In both one- and two-component experiments, data were fitted to a model of a single ideal interacting species.

**Protein Labeling and Solid-phase Binding Assay—** Proteins were labeled using IODO-GEN (Pierce) as described (22); specific activities of 1.5–4.0 × 10⁶ cpm/μg of protein were obtained. A solid-phase binding assay was performed on 96-well polystyrene plates (Fisher). Unlabeled protein was coated on wells at 15 μg/ml in TBS for 2 h, and then the wells were blocked using 3% bovine serum albumin in TBS for 2 h. Labeled protein was added at a minimum concentration of 10 μM in 100 μl. At this concentration, equilibrium was reached after 8 h of incubation; therefore, all further assays were incubated for at least this length of time. Binding was performed in 20 mM MES, pH 6.8, 75 mM NaCl, 3 mM MgCl₂, 1 mM EGTA, and 0.1 mM 2-mercaptoethanol at 20 °C. After 8 h, unbound ligand was aspirated and the wells were washed three times using binding buffer. Wells were then separated, and bound radioactivity was measured in a Wallac model 1470 gamma counter. Nonspecific binding was determined for each assay by determining the amount of radioactivity bound to the wells in the presence of a 100-fold concentration of unlabeled protein over added radioligand. The amount of protein adsorbed to the plate was determined by coating wells with trace amounts of radiolabeled protein in a total concentration of 15 μg/ml. After 2 h, unadsorbed protein was removed, the wells were washed three times with TBS, and radioactivity adsorbed to individual wells was measured.

When binding was performed at variable temperatures, wells containing binding reactions were overlaid with mineral oil, and the plates were wrapped in plastic film. Plates were placed in constant temperature rooms or incubators. All binding data were analyzed using Prism software (GraphPad Software, San Diego, CA). Reaction conditions within each experiment were performed in triplicate. Data are presented as the mean ± S.D.

**Circular Dichroism Spectroscopy—** The far UV circular dichroism spectra of vinculin domains V-(1–266) and V-(877–1066) was determined using a Jasco-J810 spectropolarimeter. Each sample was scanned in the range of 200–260 nm in a quartz cuvette with a path length of 1.0 cm. Spectra were gathered using a Jasco-J810 spectropolarimeter. Each sample was scanned in the range of 200–260 nm in a quartz cuvette with a path length of 1.0 cm. Spectra were analyzed by Prism software (GraphPad Software, San Diego, CA). Reaction conditions within each experiment were performed in triplicate. Data are presented as the mean ± S.D.

**Other Techniques—** Protein determination was performed by the Bradford assay (Sigma) and SDS-polyacrylamide gels using the Laemmlibuffer system (24).

**RESULTS**

To begin our investigation of the interaction between the head and tail domains of vinculin, we cloned and expressed regions of vinculin previously demonstrated to interact (9). These constructs, V-(1–266) and V-(877–1066), were purified in high yield (20–40 mg/liter culture) and in high purity (>95%) (Fig. 1) as assessed by scanning overloaded lanes on an SDS-polyacrylamide gel.
Interplay of Vinculin Domains

**TABLE II**

| Construct | Molecular weight ($M_w$) | Observed molecular weight ($M_O$) | $M_O/M_w$ | $s_{20,w}$ | Frictional ratio |
|-----------|--------------------------|----------------------------------|-----------|-----------|-----------------|
| V-(1–266) | 29,736                   | 32,900 ± 750                     | 1.10      | 2.75      | 1.16            |
| V-(877–1066) | 21,189               | 22,900 ± 1000                    | 1.08      | 2.47      | 1.05            |
| V-(1–266) + V-(877–1066) | 50,925                | 48,100 ± 1600                   | 1.89      | 3.75      | 1.22            |

* Calculated from known sequence.
* Determined using data collected at protein concentration of 20 μM.
* Calculated from observed molecular weight, partial specific volume, and $s_{20,w}$.
* Determined as the sum of calculated molecular weights of V-(1–266) and V-(877–1066).

**Fig. 1.** Vinculin constructs and purification of V-(1–266) and V-(877–1066) domains. A, schematic of the domain structure and deletion mutants used. The head, tail, and proline-rich domain (PRD) are labeled. Numbers indicating amino acid residues encoding each of the vinculin constructs used in this work are listed. B, SDS-polyacrylamide gels of purification steps in the isolation of V-(1–266) and V-(877–1066). Lane 1, lysed cells expressing fusion protein. Lane 2, insoluble fraction of the cell lysate. Lane 3, soluble fraction of the cell lysate. Lane 4, protein eluted from the glutathione-agarose column using thrombin. Lane 5, isolated protein after further purification on an ion-exchange column. MW, molecular weight.

Analytical centrifugation was used to measure the shape and interaction of the domains in solution (Table II). Data in each case were analyzed as ideal single component systems to determine whether the individual domains self-associate over a range of concentrations. The data are presented as a ratio of the observed molecular weight ($M_O$) to the theoretical molecular weight ($M_w$) (Table II). The observed molecular weights for V-(1–266) and V-(877–1066) are 32,900 ± 750 and 22,900 ± 1000, respectively, which indicate that the domains act as monomers over the concentration range tested (20–40 μM). To examine the interaction in solution, an equilibrium mixture of the two proteins was tested using sedimentation equilibrium. Analysis of the data by fitting to a single ideal species gave a molecular weight of 46,100 ± 1600, almost equal to the expected weight of the heterodimer, 50,925. This result indicates that the recombinant domains interact strongly to form a 1:1 complex. We attempted to calculate the $K_d$ by fitting the same data to a dimerization model using the average of the monomeric weights of each component (25), but the near totality of the dimerization precluded determination of a dissociation constant. To further analyze the overall conformation of the domains, sedimentation velocity was used to determine hydrodynamic parameters. The sedimentation coefficients were determined at 60,000 rpm at 20 °C and were corrected to obtain $s_{20,w}$, the sedimentation coefficient in water at 20 °C. The $s_{20,w}$ for V-(1–266) and V-(877–1066) was determined to be 2.75 and 2.47, respectively, and was then used to determine the frictional ratios of each domain (Table II). V-(1–266) and V-(877–1066) have frictional ratios of 1.16 and 1.05, respectively, and thus appear to be globular. The complex of the two domains had a frictional ratio of 1.22, implying that the complex is more asymmetric than either of the two individual domains.

To examine the secondary structure of the expressed domains, circular dichroism was used. V-(1–266) and V-(877–1066) were found to be 66 and 45% α-helical, respectively, by this method (Fig. 2). This fraction of α-helix calculated from the spectra is low for V-(877–1066) in comparison with the determined crystal structure of the vinculin tail, which shows this domain to be ~60% helical (7). When V-(1–266) and V-(877–1066) were mixed the resulting α-helical content was 46%. The complex is clearly less α-helical than the theoretical average of the individual spectra (Fig. 2), indicating that conformational changes occur during the association of the head with the tail domain. Given the magnitude of the change, it is unlikely that a conformational change in either one of the individual domains alone could account for the observed spectra. For example, if a change in V-(877–1066) alone was responsible for the observed change in the secondary structure, it would require a 60% decrease of its α-helical content. Therefore, it is likely that more moderate changes in the structure of both domains occur during their association.

We used a solid-phase binding assay to confirm and extend the results obtained from the analytical centrifugation experiments. A binding isotherm demonstrated specific and saturable binding with a $B_{max}$ of 7.2 ± 0.15 nM, which indicates a stoichiometry of 1.1 ± 0.45 mol of V-(1–266)/mol of V-(877–1066) (Fig. 3). The $K_d$ of a single class of binding sites was determined to be 93 ± 10 nM (inset). The binding was salt-sensitive with over 50% inhibition at 200 mM NaCl (Fig. 4) due to an effect on binding affinity rather than on maximum binding capacity (data not shown). This salt sensitivity was not due to alteration of the conformation of the recombinant domains as the circular dichroism spectra were unaffected over a concentration range of 50–150 mM NaCl (data not shown). The affinity of the interaction was also affected by a temperature variation between 4 and 55 °C (Fig. 5). Lower temperatures led to a greater time to reach equilibrium (3 h at 45 °C versus 24 h at 4 °C) and resulted in an ~20-fold decrease in affinity from 45 to 4 °C (Fig. 5A). Evidently the domains must be quite stable as binding was still observed at 55 °C. The pH optimum of binding was 7.5 (Fig. 5B) with strong declines below pH 7.0 and above pH 8.0. Variation of the concentration of divalent cations (MgCl$_2$, CaCl$_2$, MnCl$_2$) and the addition of EDTA had no effect on the interaction of V-(1–266) with V-(877–1066) (data not shown). These results indicate that the reaction between V-(1–266) and V-(877–1066) is mediated by both electrostatic and hydrophobic forces.

To better define the sites of interaction on the molecule, deletions were made separately in both domains, and binding
activities were measured. Within residues 1–266, five deletion mutants were constructed and studied (Fig. 1). Unstable without the GST moiety, these deletions were used as fusions. The GST moiety had no effect on binding of the domains when fused to either V-(1–266) or V-(877–1066) (not shown). The purified deletions of V-(1–266) were iodinated and tested for their ability to bind V-(877–1066). Mutants containing residues 1–86 and 1–181 showed no ability to bind V-(877–1066), but mutants GST-V-(87–226) and GST-V-(182–266) were able to bind to V-(877–1066) with \( K_d \) values of 180 ± 60 nM and 770 ± 120 nM, respectively (Fig. 6). To further delineate this binding region, the sequence common to GST-V-(87–226) and GST-V-(182–266) was expressed and tested for binding. This construct, GST-V-(182–226), bound to V-(877–1066) with a \( K_d \) of 280 ± 40 nM (Fig. 6). Thus, this segment is sufficient to support binding to the tail although with a 3-fold decrease in affinity.

Similarly, we created deletion mutants of the vinculin tail to help map a minimal binding region for the head domain. Constructs GST-V-(877–964) and GST-V-(1009–1066) were designed to collectively include all known binding sites of ligands of the vinculin tail. These proteins were expressed, purified, coated onto wells, and probed with \(^{125}\text{I}-\text{V-(1–266)}\) to determine whether the deletions bind to the vinculin head (Fig. 7A). Of the two constructs, only V-(1009–1066) supported binding to V-(1–266). This interaction occurred with a \( K_d \) of 630 ± 50 nM (Fig. 7A).

The inhibition of binding between V-(1–266) and V-(877–1066) by salt is indicative of an ionic interaction. Because the 182–226 sequence was basic, we suspected that acidic residues within 1009–1036 would be involved, in particular an acidic patch near residue 1013 seen in the crystal structure. To test this possibility, site-directed mutagenesis was used to change three acidic residues (Asp-1013, Glu-1014, and Glu-1015) to alanine within V-(877–1066). The mutations had little effect on the conformation of the domain as measured by circular dichroism (Fig. 7B). The difference in the measured \( \alpha \)-helical content...
Regulation of protein function by intramolecular autoinhibition has been recognized to occur within a diverse group of proteins (26). In these proteins, activity is inhibited in the intramolecularly associated state, and separation of the self-associated domains via binding of an activator, phosphorylation, or proteolysis restores activity. Expression of the separate interacting domains of vinculin facilitated characterization of the binding and better definition of the regions of the polypeptides involved. Our measured $K_D$ of $93 \pm 10 \text{ nM}$ agrees with earlier observations of a tight interaction ($K_D$ estimated at $50 \text{ nM}$) (9) between the separate domains, suggesting an extremely tight interaction within the whole molecule where the domains are physically connected. We have also found that the interaction is sensitive to increases in ionic strength and temperature. These data indicate that both electrostatic and hydrophobic forces play important roles in the interaction of the head and tail domains. Furthermore, the observation that the association is greatly decreased below pH 7 is in agreement with the observation that the head and tail domains of vinculin remain tightly associated after V8 protease cleavage within the proline hinge sequence but can be separated at a low pH (27).

The observed effect of salt concentration on the interaction of the head and tail domains is consistent with its observed effect on whole vinculin. At low and physiological concentrations of salt, vinculin is monomeric as observed using rotary shadowing and electron microscopy, but at high salt concentration vinculin is seen as multimers of four to six molecules (28, 29). Based on our data, high ionic strength might lead to the dissociation of the head-tail interaction allowing intermolecular interactions to occur. In addition, the dissociation of the head and tail that is induced by certain lipids is decreased at low ionic strength (30), possibly due to the higher head-tail binding affinity.

As a first step toward identifying specific residues involved in the interaction, deletions were used to further define the interacting regions. In the N-terminal domain, we identified a small peptide (residues 182–226) as a binding site for the vinculin tail. Although this region has a somewhat reduced affinity in comparison with the complete V-(1–266), it must contain the major binding determinants. The location of the site between residues 182 and 226 likely explains the inactivity of the 167–207 deletion mutant (12) due directly to removal of interacting residues rather than structural change. This region likely adopts an $\alpha$-helical conformation as the homologous region in the related protein, $\alpha$-catenin, is also $\alpha$-helical (31). In addition, our circular dichroism measurements point to a high $\alpha$-helical content (Fig. 2). It is interesting that construct V-(1–266) has a theoretical pI of 5.11, whereas the tail-binding region within it, residues 182–226, has a calculated pI of 8.16. Thus, this binding site is a basic island exposed on the surface of an $\alpha$-helix within the acidic head domain. This led us to look for an oppositely charged region in the tail sequence as possible interaction sites. It was unexpected that the shorter deletion mutant V-(182–226) rather than the larger V-(182–266) bound with a higher affinity, and we suspect that the extra 40 residues either affect the folding of the 182–226 region or bind to it in competition with the vinculin tail. There are a number of acidic residues in the 226–266 sequence (calculated pI is 4.8) that could interact with the basic V-(182–226) region. In the intact molecule, constraints of tertiary structure would presumably prevent these effects.

Within the vinculin tail sequence, previous deletion analysis had defined a C-terminal boundary to the head-binding site. Truncations of the tail domain from 1066 back to residues 1043 or 1036 remain competent to bind the head domain, but the tail domain lacking residues 1028–1066 is inactive (12). Our finding that residues 1009–1066 can bind to the head limits the N-terminal boundary of the site to residue 1009, thus mapping the major site of contact to between residues 1009 and 1036. The reduced affinity of the V-(1009–1066) piece ($K_D$ 630 nM) versus the V-(877–1066) (93 nM) for the head indicates that there are sequences outside this area required for high affinity binding or structure determination. It is clear, however, that this region contains a site sufficient to support binding to the
head. In the recently determined crystal structure, this sequence forms a loop and part of helix 5 at one end of the tail. Binding of the head to this loop could result in an elongated complex consistent with the ultracentrifugation data, which shows a higher degree of asymmetry than either individual domain. Another notable feature of this sequence is the preponderance of acidic residues; although the tail domain of vinculin is basic overall with a theoretical pI of 9.15, the calculated pI of residues 1009–1063 is 4.14. To verify our prediction that acidic residues are critical for the interaction, we mutated three consecutive residues (Asp-1013, Glu-1014, and Glu-1015) to alanine and found, as expected, a drastic decrease in binding affinity. Whereas the decreased affinity of V-(1009–1066) for V-(1–266) indicates that other regions of the tail contribute to the binding, the abrogation of binding as a result of these mutations within V-(877–1066) shows that those contributions alone cannot support the interaction. This clearly demonstrates that the region 1009–1036 is the major contact site for V-(1–266), and very likely one or more of the acidic residues mutated are directly involved in the binding. Deletion experiments demonstrating a requirement for residues 1028–1036 (12) are not inconsistent with our results and indicate that 1028–1036 are also necessary either to maintain the structure of the binding site or to make significant contacts. Taken together, these results strongly support ionic interactions between the oppositely charged sequences as major binding determinants.

It is interesting to note that several other vinculin-binding sites are dependent upon sequences near the 182–226 and 1009–1036 binding sites. Talin has been demonstrated to bind the vinculin head (7) and is dependent upon residues 167–207 (32). Further, a lipid binding domain (residues 1016–1066), a paxillin-binding site (requiring residues 978–1066) (15), an actin filament-binding site, and an oligomerization site (13) all have been demonstrated to be near the head-binding site within the tail. The proximity of these sites suggests a direct mechanism for interactions and regulation to occur. A simple model for vinculin regulation postulates that phosphatidylinositol 4,5-bisphosphate “opens” vinculin by somehow causing head-tail dissociation. A lipid-binding site near or coincident with the head-binding site raises the possibility of direct competition. Further experiments are needed to resolve the precise mechanism.

The conformational flexibility of vinculin is largely unexplored but seems considerable. Our circular dichroism data show the first evidence of conformational changes in the head domain, but there is supporting evidence for distinct conformations of the tail domain in the presence of actin or lipid (33). Evidence of conformational change in both of these domains indicates that the head and tail domains may adopt different conformations in the vinculin open and closed states irrespective of ligand binding. The existence of multiple structures suggests that a steric blocking model for vinculin regulation may be too simplistic and that allosteric mechanisms may predominate. Thus, vinculin is expected to show complex behavior in response to its various binding partners and may function in both regulatory and structural roles. The studies described here will provide a basis for further investigation of this complicated protein.

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