Polyphosphoinositides Inhibit the Interaction of Vinculin with Actin Filaments*

(Received for publication, February 23, 1999, and in revised form, April 7, 1999)

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Binding of vinculin to adhesion plaque proteins is restricted by an intramolecular association of vinculin’s head and tail regions. Results of previous work suggest that polyphosphoinositides disrupt this interaction and thereby promote binding of vinculin to both talin and actin. However, data presented here show that phosphatidylinositol 4,5-bisphosphate (PI4,5P$_2$) inhibits the interaction of purified tail domain with F-actin. Upon re-examining the effect of PI4,5P$_2$ on the actin and talin-binding activities of intact vinculin, we find that when the experimental design controls for the effect of magnesium on aggregation of PI4,5P$_2$ micelles, polyphosphoinositides promote interactions with the talin-binding domain, but block interactions of the actin-binding domain. In contrast, if vinculin is trapped in an open conformation by a peptide specific for the talin-binding domain of vinculin, actin binding is allowed. These results demonstrate that activation of the actin-binding activity of vinculin requires steps other than or in addition to the binding of PI4,5P$_2$.

Vinculin, a 117-kDa component of microfilament-associated cell junctions (1), is a modular protein composed of a 95-kDa N-terminal, globular head domain connected by a short proline-rich region to a 30-kDa tail domain (reviewed in Ref. 2). Vinculin has an essential role in embryogenesis (3, 4) and regulatory roles in adhesion, spreading, and motility of cells in culture (5–10). Vinculin probably functions by interacting with particular structural and regulatory proteins found at focal contacts and zonulae adherens (11). Biochemical experiments with purified proteins show that the binding sites on vinculin for talin (12), F-actin (13), acidic phospholipids (14), and vaso-dilator-stimulated phosphoprotein (15), and sites for protein kinase C-mediated phosphorylation (16, 17), are blocked by the intramolecular association (K$_d$ ~ 50 nM) of the head (V$_h$) and tail (V$_t$) regions (12). Therefore, regulation of the head-tail interaction to expose cystic ligand binding and regulatory sites is hypothesized to be critical for recruitment of vinculin to sites of cell adhesion and/or for vinculin-dependent assembly of focal adhesion complexes (13). Elucidation of the factors that regulate the head-tail interaction is central to understanding how events at the cell surface are expressed ultimately in the activities of molecules directly responsible for the functions of focal adhesion plaques.

Evidence from in vitro experiments shows that acidic phospholipids in general (16), or specifically PI4,5P$_2$ (18) block the interaction of purified head and tail domains and act on intact vinculin to expose the binding sites for talin on V$_h$ (18) and for F-actin on V$_t$ (16, 18). This observation provides a mechanistic link between cell surface receptors that modulate the synthesis of PI4,5P$_2$, a signaling molecule (19) and vinculin, a structural molecule involved in assembling a focal adhesion plaque and mediating anchorage to the actin cytoskeleton.

Here we provide new information that modifies the proposed role of PI4,5P$_2$ in activation of vinculin. In particular, we report that occupancy of the lipid-binding sites in purified tail domain prevents occupancy of the actin-binding sites. The effect of PI4,5P$_2$ on the interaction of purified V$_t$ with F-actin had not been examined in previous studies. Therefore, to evaluate the apparent inconsistency of our result with the proposed role of PI4,5P$_2$ on the actin binding activity of vinculin, we have used a novel peptide probe of vinculin conformation to re-assess the effects of acidic phospholipids, including polyphosphoinositides, on the actin- and talin-binding activities of vinculin. We find that when the experiments are controlled for the effect of MgCl$_2$ on the aggregation of PI4,5P$_2$ micelles, the results support a modified model in which the role of PI4,5P$_2$ is to expose the talin-binding site on vinculin while blocking the actin-binding site.

EXPERIMENTAL PROCEDURES

Protein Purification—Chicken smooth muscle vinculin and its 95-kDa head fragment were purified (14, 20, 21) and stored at 4 °C in Tris-HCl, pH 7.5, 1 mM EDTA, 0.5 mM NaN$_3$, 0.5 mM bME, 2 mM MgCl$_2$, and 3 mM NaCl. Vh was dialyzed and G-actin was stored in buffer A (2 mM Tris-HCl, pH 7.5, 0.2 mM CaCl$_2$, 0.2 mM ATP, and 0.5 mM bME).

GST- and 6-His-tagged Fusion Proteins—GST/V884–1066 was expressed as a 6-histidine (His)-tagged fusion protein. Construction of 6-His-tagged Fusion Proteins—GST/V884–1066 was constructed and purified as has been described (12) and stored at 4 °C in TREN. For experiments requiring untagged vinculin tail fragment, V884–1066 was expressed as a 6-histidine (His)-tagged fusion protein.

* This work was supported by research Grant GM41605 from the National Institutes of Health and by a grant from the Muscular Dystrophy Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: PI4,5P$_2$, phosphatidylinositol 4,5-bisphosphate; BSA, bovine serum albumin; GST, glutathione S-transferase; bME, β-mercaptoethanol; PI, phosphatidylinositol; PI3P, phosphatidylinositol 3-phosphate; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; Sa, streptavidin; SaAP, SA-conjugated alkaline phosphatase; PAGE, polyacrylamide gel electrophoresis; V$_h$, head domain of vinculin; V$_t$, tail domain of vinculin; PBS, phosphate-buffered saline.
amplification of the appropriate region of the chick embryo vinculin cDNA (24) using in-frame primers. The forward and reverse primers contained NdeI and XhoI sites, respectively. The amplified product was ligated into pCR2.1 vector (Invitrogen, San Diego, CA) according to the manufacturer's instructions, except that reagents were transformed into competent Escherichia coli DH5α cells. Recombinant plasmid was digested with NdeI and XhoI to yield the insert fragment containing the polymerase chain reaction-amplified coding region and flanking vector sequence. The fragments were gel-purified using the Qiagen gel extraction kit (Chatsworth, CA) and ligated into the NdeI and XhoI sites of the pET-15b vector (Novagen, Madison, WI). As described in the manufacturer's instructions (pET System Manual; Novagen), 6-His/V884–1066 was expressed, purified, and then cleaved with thrombin to remove the 6-His tag. V884–1066 was dialyzed against TEAN containing 0.5% βME and 2 × PIC I and II and quantified spectrophotometrically (E$_{280}$ = 18,350) at 280 nm.

**Peptide Synthesis**—The pVR peptide (KKRSTGGFDVYDLYRRVS-SALTITLYVATRPK) with and without biotin added to the N terminus was synthesized using an Applied Biosystems 430 peptide synthesizer, purified by high pressure liquid chromatography, and sequenced by automated Edman degradation at the Biosynthesis and Sequencing Facility, Johns Hopkins University School of Medicine (Dept. Biological Chemistry), Baltimore, MD. Biotinylated pVR was characterized by mass spectrometry at the JHU School of Medicine (Dept. Pharmacology) Spectrometry Center, Baltimore, MD.

**Phospholipid Vesicles**—Phosphatidylincholine, PI, and phosphatidylserine were purchased as chloroform stocks from Avanti Polar Lipids (Alabaster, AL) and stored at −20 °C. PI4P and PI4,5P2 were obtained as lyophilized powders from Sigma and were resuspended in distilled H$_2$O, 0.5% βME, and stored (4 °C) for use in assays. PI3P, PI3,4P$_2$, and PI3,4,5P$_3$ were purchased from Matreya, Inc. (Pleasant Gap, PA) as vacuum-dried powders and then resuspended in chloroform for storage at −20°C.

For the preparation of phosphatidylincholine, PI, and phosphatidylserine small unilamellar vesicles, chloroform stocks of these phospholipids were dried under N$_2$ and then swollen at 5 mg/ml in distilled H$_2$O, 0.5% βME, and then sonicated (30 min, 25 °C) in a Branson bath sonicator at the highest power setting. Small unilamellar vesicles containing both PI and PI4,5P$_2$, at the desired ratios were prepared by adding the required amount of PI4,5P$_2$ (in distilled H$_2$O, 0.5% βME) to a known amount of dried PI and then sonicating as described. PI3P, PI3,4P$_2$, and PI3,4,5P$_3$ were dried under N$_2$ and resuspended in H$_2$O, 0.5% βME before sonication (30 min, 25 °C). Likewise, solutions of PI4P and PI4,5P$_2$ were sonicated (30 min, 25 °C) before use in assays. Phospholipid concentrations were determined by inorganic phosphate analysis using the Ames method (25).

**Actin Polymerization**—Unless indicated otherwise, actin polymerization was induced by the addition of 100 mM KCl and 2 mM MgCl$_2$ from a concentrated stock solution as described previously (13). For F-actin binding assays containing lipid, the MgCl$_2$ concentration was reduced to 2 mM to eliminate background sedimentation of vinculin and vinculin tail binding to F-actin. For experiments in low magnesium, actin polymerization was initiated by adding 100 mM KCl and 0.1 mM MgCl$_2$ sonicated (30 s, 25 °C) F-actin seed (polymerized 2 h, 25 °C) to the reaction mix containing 3.2 mM Tris-HCl pH 7.1, 0.2 mM MgCl$_2$, 100 mM KCl, 0.2 mM EGTA, 0.02 mM EDTA, and 0.5 mM βME, purified V884–1066 (2 μM) was incubated (2 h at 25 °C) with a 5 μM F-actin in the presence or absence of 100 μM pure PI4,5P$_2$ micelles (as indicated above each lane) and at either 2 mM or 2 μM MgCl$_2$ (as noted above each gel). After incubation, the reaction mixes (100 μM) were sedimented at 95,000 × g in a Beckman Airfuge and equal volumes of pellet (P) and supernatant (S) fractions were resolved by SDS-PAGE. In the presence of pure PI4,5P$_2$ micelles there is a marked inhibition of V$_t$ binding to F-actin (compare lanes 1 and 2 with lanes 3 and 6). At 2 mM MgCl$_2$ V$_t$ sediments to the same level in the absence of F-actin (lanes 11 and 12) as it does when F-actin is present (lanes 9 and 10) in the reaction mix; however, in the absence of both PI4,5P$_2$ and F-actin, V$_t$ remains in the supernatant (lanes 13 and 14). Reduction of the MgCl$_2$ concentration to 2 mM eliminates the background sedimentation of V$_t$ (lanes 7 and 8). As described in A, V$_t$ was assayed for F-actin binding activity at 2 μM MgCl$_2$ and over a range of lipid (pure PI4,5P$_2$, micelles, PI4,5P$_2$/Triton mixed micelles, or phosphatidylcholine/small unilamellar vesicle) concentrations. The relative amounts of V$_t$ in the pellet and supernatant fractions were quantified by densitometric analysis of Coomassie Blue stained gels.

**Isolation and Analysis of Complexes with Biotinylated pVR**—Complexes with biotinylated pVR were isolated by incubating (0.5 h, 25°C, with inversion) assay mixtures with streptavidin(Sa)-agarose beads (Novagen) followed by low speed (2,000 rpm × 4 min) centrifugation. Equal volumes of the pellet and supernatant fractions were subjected to SDS-PAGE (10% polyacrylamide gel) analysis (28) and the amount of target protein bound to Sa-agarose+pVR complexes was determined by Coomassie Blue staining of the gels. For relative quantitation, stained gels were dried, scanned, and analyzed densitometrically with the NIH Image program.

**RESULTS**

**Effect of Polyphosphoinositides and Other Acidic Phospholipids on Binding of Vt to Actin Filaments**—PI4,5P$_2$ presented in micellar form blocks the interaction of purified vinculin tail (V884–1066) with F-actin (Fig. 1A, lanes 1–8). Although the standard buffer for actin polymerization includes 2 mM MgCl$_2$, experiments with micelles of pure PI4,5P$_2$ were done in 0.002 mM Mg$^{2+}$ because 2 mM Mg$^{2+}$ causes mixtures of V$_t$ and PI4,5P$_2$ to sediment in the absence of actin (Fig. 1A, lanes
9–12). Reduction of the MgCl₂ concentration from 2 mM to 2 μM eliminated this problem (compare Fig. 1A, lanes 11–14 and lanes 4, 7, and 8).

Presentation of P14,5P₂ in the form of mixed micelles with Triton X-100 also inhibited interaction of V₃ with F-actin (Fig. 1B). Inhibition of the interaction of Vᵣ with actin by P14,5P₂ presented either in micellar form or as mixed micelles with Triton X-100, is dependent on the concentration of lipid (Fig. 1B). Phosphatidylcholine at up to 100 μM has no effect on F-actin binding by vinculin tail (Fig. 1B). Analysis of other phospholipids revealed that P14,5P₂/Triton X-100 mixed micelles (>200 μM), PI₃,4P₂ (30 μM), PI₃,4,5P₃ (30 μM), and phosphatidylethanolamine (400 μM) inhibited completely the binding of purified vinculin tail to F-actin (data not shown).

Effect of P14,5P₂ on the Actin-binding Activity of Vinculin—Because inhibition of the binding of Vᵣ to actin by P14,5P₂ is not obviously consistent with a role of P14,5P₂ in exposing a functional actin-binding site on vinculin, we wanted to confirm the effects of acidic phospholipids on the conformational state of vinculin by assaying for co-sedimentation of vinculin with F-actin in the presence of either P14,5P₂/Triton X-100 mixed micelles (18) or pure P14,5P₂ micelles (16). When the assay was controlled for the effect of MgCl₂ on the aggregation and sedimentation of P14,5P₂, there was no co-sedimentation of vinculin with F-actin in the presence of micelles composed of pure P14,5P₂ (Fig. 2, A versus B). Divalent cations induce aggregation of P14,5P₂ micelles (29), thus proteins bound to P14,5P₂ micelles co-sediment with cation-induced P14,5P₂ aggregates. Although the Mg²⁺ concentration does not affect the sedimentation of mixed micelles of P14,5P₂ and Triton X-100, when these mixed micelles were examined at more than one concentration, they also failed to induce dose-dependent co-sedimentation of vinculin with F-actin even when the P14,5P₂ concentration was raised to 100 μM (Fig. 2C).

Characterization of Peptide pVR as a Probe of the Open Conformation of Vinculin—As an independent means of assessing the effect of acidic phospholipids on exposure of the actin- and talin-binding sites of vinculin, we have used a peptide (KKKSTGGFGDDVVYDARRVSSALTTLVATRPK) isolated from a phage-displayed random peptide library based on binding of phage to immobilized vinculin (30). This peptide, called pVR, inhibits binding of talin, but not α-actinin or paxillin, to immobilized vinculin, suggesting that the peptide binds at or near the talin-binding domain of vinculin. Control peptides bearing the same net positive charge as pVR but having distinct amino acid sequences do not bind to vinculin (30). Previous work (30) demonstrated that although pVR binds to vinculin that has been adsorbed to poly styrene, polyvinylidene difluoride, or nitrocellulose (Fig. 3B), it does not bind to vinculin in solution (Fig. 3A). The pVR binding site, located in the first 258 amino acid residues of the N-terminal head domain of vinculin, is made accessible for solution-based assays by removing the C-terminal tail domain of vinculin, residues 857–1066 (Fig. 3A) (30), suggesting that the intramolecular head-tail interaction of vinculin blocks the binding site for pVR. That pVR is a specific probe for the open conformation of native vinculin is further supported by the finding that addition of purified vinculin tail domain to the reaction mix inhibits binding of pVR to vinculin head fragment in a dose-dependent fashion (Kᵣ ≈ 0.4 μM) (Fig. 4A). This experiment shows that pVR binding occurs specifically to a site on Vᵣ that is masked by V₃ in the closed conformation of vinculin rather than to a new site created by unfolding of Vᵣ after proteolytic removal of V₃. Conversely, pVR is able to compete binding of Vᵣ to V₃ with Kᵣ = 0.3 μM (Fig. 4B) indicating that Vᵣ and pVR interact at or near the same site on V₃. Collectively, these observations validate the use of pVR as a probe to identify the open conformation of full-length vinculin in vitro.

Effect of Polyphosphoinositides on Exposure of the Binding Site for pVR—Using biotinylated pVR to examine the effects of PI₄,5P₂ on the interaction of full-length vinculin with F-actin, as described in Fig. 1A, purified vinculin (1 μM) was incubated (2 h, 25 °C, and 2 μM MgCl₂) with 5 μM F-actin in the presence or absence of either 20 μM or 100 μM PI₄,5P₂ micelles as indicated above each lane. After incubation, the reaction mixtures (100 μl) were sedimented at 95,000 × g in a Beckman Airfuge and equal volumes of pellet (P) and supernatant (S) fractions were resolved by SDS-PAGE. Comparison of lanes 1–4 with 5–12 shows that in the presence of either 20 μM or 100 μM pure PI₄,5P₂ micelles there is no detectable induction of vinculin binding to F-actin (B), effect of 2 mM MgCl₂ on the sedimentation of vinculin in the presence of pure PI₄,5P₂ micelles. As has been described, 1 μM vinculin was assayed for F-actin binding activity at 2 mM MgCl₂, and in the presence or absence of either 20 μM or 100 μM pure PI₄,5P₂ micelles as indicated above each lane. Under these conditions, sedimentation of vinculin is as high in the absence of F-actin (lanes 3, 4, 7, 8, 11, and 12) as it is in the presence of F-actin (lanes 1, 2, 5, 6, 9, and 10). Reduction of the MgCl₂ concentration to 2 mM eliminates this high background sedimentation of vinculin (compare with Fig. 2A). In the absence of both PI₄,5P₂, and F-actin, vinculin does not sediment when the MgCl₂ concentration is 2 mM (Fig. 2B, lanes 3 and 4). C, effect of PI₄,5P₂/Triton mixed micelles on the interaction of vinculin with F-actin. Native vinculin was assayed as described in A for F-actin binding activity in the presence of 0, 10, 50, and 100 μM PI₄,5P₂/Triton X-100 mixed micelles, as indicated above each lane, at 2 mM MgCl₂, PI₄,5P₂/Triton X-100 mixed micelles do not induce vinculin co-sedimentation with F-actin. Note that there is no background sedimentation of vinculin at 2 mM MgCl₂ in the presence of PI₄,5P₂/Triton X-100 mixed micelles (lanes 7, 8, 11, 12, 15, and 16).
we examined the interaction of pVR with vinculin tail in the presence of PI4,5P2. Although vinculin tail contains the high affinity PI4,5P2 binding site (14), pVR does not bind to purified vinculin tail in the presence of PI4,5P2 (Fig. 5C, lanes 13–16). Thus, polyphosphoinositide-induced binding of pVR to vinculin is mediated by the specific interaction of the peptide with its binding site in vinculin head domain.

Among the acidic phospholipids tested, only the polyphosphoinositides (PI3,4P2, PI4,5P2, and PI3,4,5P3) activated vinculin for pVR binding (Fig. 6A) when the assay was performed in at least 100 mM salt. This polyphosphoinositide-induced activation of vinculin is dose-dependent (Fig. 6C) and peaks at about 40 μM lipid. When the concentration of polyphosphoinositide is >40 μM, the level of pVR binding by vinculin decreases due to an apparent interaction between pVR and polyphosphoinositide at high concentrations of phospholipid (data not shown). Vinculin also binds to pVR when PI4,5P2 is presented in mixed vesicles with PI. However, maximal activation occurs only when PI4,5P2 represents at least 50% of the vesicular lipid (Fig. 6D).

**pVR Induces Actin-binding Activity in Vinculin**—The preceding results indicated that pVR could be used to determine whether vinculin in the open conformation can bind F-actin when the actin-binding site is not blocked by PI4,5P2. In solution, the closed conformation of vinculin is favored strongly because of the high affinity of the head-tail interaction and the fact that head and tail interact intramolecularly. However, equilibrium kinetics predicts that vinculin can be trapped kinetically in open form in the presence of a large molar excess of a ligand that inhibits the intramolecular head-tail interaction. pVR blocks the bimolecular interaction between the purified head and tail fragments of vinculin with a Kd of ~0.3 μM (Fig. 4B), consistent with the affinity (Kd ~ 0.1 μM) of pVR for vinculin head (30). When the concentration of pVR is raised to 6,000-fold molar excess over the Kd determined for the bimolecular interaction of head and tail (Kd ~ 50 nM), vinculin becomes competent to bind F-actin (Fig. 7). This effect of pVR on binding of vinculin to actin is specific because 95-kDa head fragment alone does not co-sediment with F-actin at similar concentrations of pVR. Sedimentation of vinculin requires the presence of both pVR and F-actin, and sedimentation of vinculin with actin depends upon the amount of pVR present (Fig. 7).

**DISCUSSION**

We have found that micellar PI4,5P2 enables vinculin to bind pVR, but does not allow binding to F-actin. This result is
consistent with the observation that PI4,5P2 inhibits binding of F-actin to purified Vt. We also show that vinculin does bind F-actin when the intramolecular interaction of head and tail is disrupted by an approximative 6,000-fold molar excess of pVR over the Kd for the bimolecular interaction of the purified head and tail domains. Previous studies have shown that PI4,5P2 and several other acidic phospholipids, inhibit the bimolecular interaction of purified head and tail domains. 

In addition, there is partial overlap of vinculin's F-actin binding regions (residues 884–1012 and 1012–1066) (31, 32) and acidic phospholipid binding regions (residues 916–970 and 1012–1066) (33, 34). Therefore, it is likely that PI4,5P2 inhibits the interaction of vinculin and Vt with F-actin by occluding the actin-binding site.

Altogether, the data are consistent with a model in which polyphosphoinositides present in the form of micelles disrupt the head domain of vinculin by binding to the tail domain. Ligand-binding sites in Vt are unmasked, but the F-actin binding sites in Vt are occluded by the bound phospholipid (Fig. 8).

Based on earlier work (33), the tail domain of open vinculin will be partially buried in the hydrophobic core of the lipid micelles (Fig. 8).

Several other actin-binding proteins are also inhibited by PI4,5P2 (35) and recent structural evidence suggests a common mechanism of PI4,5P2 action. The atomic structure of gelsolin indicates that polyphosphoinositides compete with F-actin for binding to overlapping, solvent-exposed sites on plasma gelsolin (36). Evidence also suggests a competitive mechanism for PI4,5P2 disruption of profilin-actin (37) and destrin-actin (38) complexes.

A possible source of the difference between our results on the effects of micellar PI4,5P2 and those reported previously (16) is
Vinculin assays. We observed that when 2 mM MgCl₂ was a component over a range (30–500 m) to the assay mix caused sedimentation of lanes 1 pure PI₄,₅P₂ micelles to the vinculin and vinculin tail in the absence of F-actin. At millimolar concentrations of divalent cation, PI₄,₅P₂ micelles form binds to the tail domain of vinculin and disrupts the intramolecular interaction with the head domain. As a result, the talin-binding site in Vᵢ becomes accessible, but the actin-binding sites in Vₑ are blocked by the bound PIP₂. pVR displaces the head-tail interaction by binding at or near the talin-binding site on Vₑ leaving Vᵢ free to associate with F-actin.

the concentration of MgCl₂ used in the buffer for actin-binding assays. We observed that when 2 mM MgCl₂ was a component of the actin polymerization buffer (as in Ref. 16), addition of pure PI₄,₅P₂ micelles to the assay mix caused sedimentation of vinculin and vinculin tail in the absence of F-actin. At millimolar concentrations of divalent cation, PI₄,₅P₂ micelles form large aggregates that pellet at speeds required to sediment F-actin (29). Under these conditions, proteins that bind to PI₄,₅P₂ also sediment. The published data does not exclude the possibility that the MgCl₂ effect on aggregation of PI₄,₅P₂ micelles is a source of the difference between our results and those of Weekes et al. (16).

Our data indicates that the physical packing of polyphosphoinositides in pure micelles is crucial for opening vinculin because mixed micelles of Triton X-100 and PI₄,₅P₂ are unable to cause binding of pVR or F-actin to vinculin. This finding is inconsistent with an earlier report that mixed micelles containing 0.33 molecules of PI₄,₅P₂/Triton micelle (10 mM PI₄,₅P₂) activates vinculin to bind both talin and F-actin (18). In our experiments, there is not a dose-dependent effect of mixed micelles containing from 0.33–3 molecules of PI₄,₅P₂ per micelle on co-sedimentation of actin and vinculin. Although it is possible that we have not reproduced exactly the conditions of the previous report, the absence of data demonstrating a dependence of vinculin co-sedimentation with F-actin on the amount of PI₄,₅P₂ in the Triton micelles (18), together with the results presented here, argues against a role for PI₄,₅P₂ in exposing the actin-binding activity of vinculin. This conclusion is strengthened by the demonstration of dose-dependent inhibition of F-actin binding to purified Vᵢ by PI₄,₅P₂ micelles and by PI₄,₅P₂/Triton mixed micelles.

Using pVR as a probe for the talin-binding conformation of vinculin, we examined the effects of various acidic phospholipids on exposure of the talin-binding site on vinculin. In buffers containing at least 100 mM KCl, only pure micelles of the polyphosphoinositides (PI₃,₄P₂, PI₄,₅P₂, and PI₃,₄,₅P₃) induce a dose-dependent binding of pVR to vinculin. The finding that 3-phosphorylated inositol lipids can induce conformational change in vinculin suggests that intracellular signaling through phosphoinositide 3-kinase (to produce PI₃,₄P₂ and PI₃,₄,₅P₃) along with those signals mediating PI₄,₅P₂ levels, may also be important in vinculin activation.

Evidently, differential exposure of various ligand-binding sites on vinculin can be achieved depending on the mechanism of conformational alteration. For example, PI₄,₅P₂-induced changes in vinculin unmask the talin and pVR-binding sites but block the actin-binding sites, whereas pVR-induced changes occupy the talin-binding site and expose the actin-binding sites (Fig. 8). The implication is that depending on the mechanism of activation, vinculin might be assembled into complexes that differ by virtue of which ligand-binding sites on vinculin are functional. This provides a potential mechanism for building functionally dissimilar protein complexes from the same repertoire of protein components.

Currently it is thought that signal transduction events stimulate the local synthesis of PI₄,₅P₂ at focal adhesions. This newly synthesized PI₄,₅P₂ then recruits and/or opens vinculin to expose the sites for talin and F-actin, thereby contributing to assembly of plaque components and linkage of actin to the plasma membrane (18). However, our results indicate that in order for PI₄,₅P₂-bound vinculin to bind actin, the PI₄,₅P₂ would have to be hydrolyzed or displaced from vinculin. PI₄,₅P₂ hydrolysis could occur by the action of a specific phospholipase or phosphatase, making the induction of vinculin’s actin-binding activity a two step process. Alternatively, vinculin might be activated by yet unknown mechanisms that expose both the talin- and actin-binding activities.

Acknowledgments—We thank Jodie Franklin and Jennifer Senft for synthesis, purification, and sequencing of pVR, and Amina S. Woods for the mass spectrometry of biotinylated pVR.

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