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SHORT COMMUNICATION

Molecular interactions between vinculin and phospholipids

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Abstract

The focal adhesion protein vinculin has been implicated in associating with soluble and membranous phospholipids. Detailed investigations over the past ten years describe the intermolecular interactions of the vinculin tail domain with soluble and membrane phospholipids. Previous studies have implied that the tail's unstructured C-terminal region affects the mechanical behavior of cells and that the same region, at the molecular level, has bi-stable behavior sensitive to different protonation states. The aim of this short communication is to discuss whether the C-terminal vinculin tail (Vt) domain interacts favorably with membrane-embedded phospholipids such as PIP_2 and that the region is also an anchor for lipid membranes.

Keywords: actin filaments; focal adhesion proteins; phospholipids; vinculin

The protein structure of vinculin revealed specific intermolecular hydrogen-bonding binding sites for a number of cell adhesion proteins (reviewed by Ziegler et al., 2006). Many of these binding sites on vinculin are cryptic and masked and need to be exposed. In order to break the intramolecular interaction between the N-terminal head and the C-terminal tail domain and to exist in an open (active) state requires Src-mediated phosphorylation on position Y100 and Y1065 (Auernheimer et al., 2015). The head and the tail domain are composed of helical bundles interacting with a variety of focal adhesion proteins, whilst the vinculin tail domain is also believed to associate with the lipid membrane via phosphatidylinositol-4,5-bisphosphate (PIP2) and directly with membrane lipids (Figure 1).

Studying the molecular biophysical behavior and associated molecular interactions of focal adhesion proteins has provided more biological insight into the role of phospholipids that contribute to adhesion turnover, that is, assembly and disassembly. The justification for this research was the need to clarify the contribution, for instance, of vinculin to the binding between phospholipids and its tail domain at the molecular level (Goldmann, 2010). It was elucidated whether the C-terminal region (i) improves the binding between the vinculin tail domain and soluble phospholipids or (ii) acts as an anchor ensuring that the vinculin tail domain interacts with phospholipid membranes. Common phospholipids were used that include phosphatidylinositol-4,5-bisphosphate (PIP_2), dimyristoyl-L-α-phosphatidylcholine (DMPC), and dimyristoyl-L-α-phosphatidylserine (DMPS) in soluble and membranous forms. These studies revealed more insight into the vinculin-lipid interaction that contribute to cell behavior, migration, mechanics, and tumor cell invasion (Goldmann et al., 2013).

Detailed studies over the past 10 years on the molecular and biophysical behavior of vinculin has given additional valuable information about its working mechanism (Goldmann, 2016). Diez et al. (2008) investigated the structural and biochemical properties of the last 21 residues of the vinculin tail domain. Using differential scanning calorimetry (DSC) in the presence of lipid vesicles consisting of DMPC and DMPS and/or DMPG at various molar ratios, they found that these residues insert into lipid vesicle membranes, which was confirmed by molecular dynamics simulations and circular dichroism (CD) spectroscopy. It was, therefore, assumed that these residues adopt an antiparallel β-sheet backbone geometry that ensures association with lipid vesicles.

In a further, more detailed study using cells, Diez et al. (2009) focused on the unstructured C-terminal arm (residues 1052–1066, the so-called lipid anchor) and showed that it influences

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Abbreviations: FAC, focal adhesion complex; FAs, focal adhesions; MV, metavinculin; PIP_2, phosphatidylinositol-4,5-bisphosphate; DMPC, dimyristoyl-L-α-phosphatidylcholine; DMPS, dimyristoyl-L-α-phosphatidylserine; DMPG, dimyristoyl-L-α-phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; DSC, differential scanning calorimetry; CD, circular dichroism; MEFs, mouse embryonic fibroblasts.

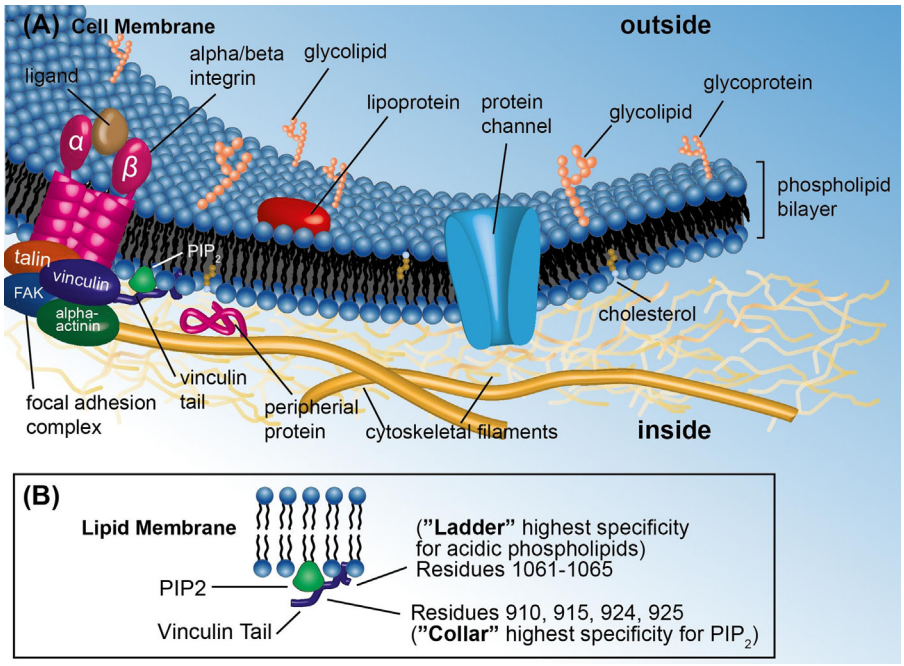

Figure 1 A simplified, schematic representation of the cell membrane consisting of a lipid bilayer embedded with peripheral and integral proteins, which are anchored via focal adhesion proteins to the cytoskeleton (A). The vinculin tail associates with PIP_2 and the charged phospholipid membrane via its basic "collar" and "ladder," respectively (B).

focal adhesion turnover and is important for cell migration and adhesion. Using physical methods, for example, magnetic tweezer or traction force microcopy, they characterized the cell mechanical behavior in mouse embryonic fibroblast MEFvin-(−/−) cells transfected with EGFP-linked vinculin deficient in the lipid anchor (vinΔC). Attenuated traction forces were found in these cells that expressed vinculin with point mutations (R1060 and K1061 to Q) in the lipid anchor, which impaired lipid binding. However, traction generation was not diminished in cells that expressed vinculin with impaired lipid binding caused by point mutations in helix 3 (residues 944–978). Mutating the Src phosphorylation site (Y1065 to F) resulted in reduced traction generation (Auernheimer et al., 2015). These observations show that both the lipid binding and Src phosphorylation of vinculin's C-terminus are important for cell mechanical behavior.

Palmer et al. (2009) explored the specificity of vinculin tail (Vt)-lipid binding by conducting lipid co-sedimentation experiments. They found that Vt shows a specific association with PIP_2 compared to phosphatidyl-ethanolamine (PE), PC, PS, or phosphatidylinositol (PI) when mixed in lipid vesicles. Lipid co-sedimentation as well as NMR analyses indicated that the removal of the hydrophobic hairpin in Vt does not alter its structure or PIP_2 association. A further study by the same group using again lipid cosedimentation assays to test Vt binding to PIP_2 in small, uni-lamellar vesicles, they found that neither the mutation nor phosphorylation of Y1065 within Vt affect PIP_2 association (Tolbert et al., 2014) confirming their earlier results.

However, Wirth et al. (2010) characterized the energetics and dynamics of multi-lamellar lipid vesicles (MLVs) consisting of DMPC and DMPS, which associate with vinculin using DSC and CD spectroscopy. Biochemical data from tryptophan quenching and SDS–PAGE experiments supported the calorimetric and CD spectroscopic findings insofar that only vinculin's C-terminus (Vt) inserts into lipid membranes. These in vitro results provided insight into the mechanical behavior of the vinculin tail (Vt) region, which supported data described by Diez et al. (2009) in cells and are contradictory to findings by Palmer et al. (2009) and Tolbert et al. (2014). The contrasting results can probably be explained by the different composition of lipids and charges in the vesicles used (uni-lamellar vs. multi-lamellar).

Dwivedi and Winter (2016) used different biophysical methods to determine the nanoscopic interactions of activated and auto-inhibited states of vinculin with artificial lipid membranes. They found that weak interactions occur between vinculin and lipid membranes and argued that the driving force includes the tethering of the C-terminus to the lipid membrane and hydrophobic helix-membrane interactions. Activated vinculin, however, showed a strong association with membranes through specific interactions with clusters of PIP_2 embedded in lipid membranes. According to these researchers activated vinculin harbors in PIP_2 clusters that may form small oligomeric interaction

platforms for further interaction partners, which is necessary for the proper function of focal adhesion points.

Recently, Chinthalapudi et al. (2014) reported that PIP_2 binding to vinculin alters the vinculin structure to higher order oligomerization, which is necessary for maintaining optimal focal adhesions (FAs), the control of vinculin dynamics and turnover in FAs, and the organization of actin stress fibers, as well as cell migration and spreading. This scenario differs completely in meta-vinculin (MV) (Chinthalapudi et al., 2016), where the symmetric MV dimer bridges two PIP_2 molecules compared to the asymmetric vinculin dimer that bridges only one PIP_2.

Izard and Brown (2016) reviewed the role and mechanism of phospholipids in regulating the structure and function of vinculin and its muscle-specific MV splice variant. They concluded that for a complete understanding of these processes, it is necessary to look at how vinculin/MV regulate cell motility, migration, and wound healing to understand their role in cancer and cardiovascular diseases.

Finally, Thompson et al. (2017) conducted a series of experiments and computer-assisted assays on the lipid association of vinculin. They found that the basic collar of vinculin specifically recognizes PIP_2, while the basic ladder drives association with the lipid bilayer. Vinculin mutants deficient in PIP_2-dependent liposome association in MEF indicated that PIP_2 binding is not required for localization of vinculin to FAs or FA strengthening but is necessary for vinculin activation and turnover at FAs via force transduction. All these studies revealed more insight into the role of vinculin-lipid interaction that contributes to cell behavior, migration, and mechanical forces according to Nagasato et al. (2017).

In conclusion, PIP_2 embedded in lipid membranes is probably responsible for the priming of vinculin, its localization and the turnover of FAs and Src-mediated phosphorylation of vinculin for its activation, Vt membrane insertion, and force transmission. Note, that the model of "combinatorial activation" of vinculin by phosphorylation, protein, -and lipid binding is still unresolved.

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