doi:10.1016/j.jmb.2005.12.076 J. Mol. Biol. (2006) 357, 562–573

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Structure of the α-Actinin–Vinculin Head Domain Complex Determined by Cryo-electron Microscopy

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The vinculin binding site on α-actinin was determined by cryo-electron microscopy of 2D arrays formed on phospholipid monolayers doped with a nickel chelating lipid. Chicken smooth muscle α-actinin was cocrystallized with the β1-integrin cytoplasmic domain and a vinculin fragment containing residues 1–258 (vinculin$_{D1}$). Vinculin$_{D1}$ was located at a single site on α-actinin with 60–70% occupancy. In these arrays, α-actinin lacks molecular 2-fold symmetry and the two ends of the molecule, which contain the calmodulin-like and actin binding domains, are held in distinctly different environments. The vinculin$_{D1}$ difference density has a shape very suggestive of the atomic structure. The atomic model of the complex juxtaposes the α-actinin binding site on vinculin$_{D1}$ with the N-terminal lobe of the calmodulin-like domain on α-actinin. The results show that the interaction between two species with weak affinity can be visualized in a membrane-like environment.

Keywords: cell adhesion; cytoskeleton; actin binding proteins; crystallography; calorimetry

Introduction

Focal contacts and focal adhesions are an important class of structures connecting the cytoskeleton with the extracellular matrix. Cell-matrix adhesions play a diverse role in many cellular processes.[1] About 50 proteins have been localized to focal adhesions.[2] Many of these are signaling proteins while four have been identified as important for structural integrity. The integrins are the major cell-matrix receptors and the cytoplasmic proteins talin, vinculin and α-actinin play the key role in attaching actin filaments to the integrins.

Integrins are heterodimeric transmembrane proteins with the two chains designated α and β. In common with many cell-matrix adhesion proteins, the integrin polypeptide chains have a large extracellular domain, a single transmembrane α-helix and a much smaller cytoplasmic domain. Both α and β chains participate in ligand binding, but the β chains alone define cytoskeletal interactions.[3] A key aspect of integrin function is its ability to coordinate signals from the extracellular environment to the cell interior and from the cell interior to modulate integrin affinity for extracellular matrix molecules.[4] X-ray crystallography[5–8] and electron microscopy (EM)[9,10] have provided detailed structures of integrin extracellular domains and of some of their complexes. Interactions between cytoplasmic domains in inactive integrins have been probed by NMR[11] while X-ray crystallography in combination wtih NMR has determined the structure of the β3-integrin cytoplasmic domain complexed with talin.[12]

α-Actinin, talin and vinculin share in common the ability to bind actin while both α-actinin and talin can bind integrin cytoplasmic domains. Vinculin in turn has high affinity for talin and, although it is thought to have only a supporting role in integrin adhesion, it does have additional binding sites for α-actinin[13] and F-actin.[14] The atomic structure of full-length vinculin has recently been determined[15,16] as have several structures of vinculin$_{D1}$ in complex with peptides derived from talin[17] and

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Abbreviations used: EM, electron microscopy; Cam, calmodulin; ABD, actin binding domain; DSC, differential scanning calorimetry.

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α-actinin.[18] However, vinculin interactions with full-length α-actinin are unclear as are the spatial relationships in the context of complete focal adhesions.

α-Actinin is an antiparallel homodimer, polypeptide chain weight of 110 kDa, that crosslinks actin filaments in a wide variety of structures.[19,20] α-Actinin has three types of domains: the N-terminal actin binding domain (ABD), a C-terminal calmodulin-like domain (Cam), and a central domain consisting of four tandem three-helix bundles (R1–R4), which are homologous to those found in spectrin. X-ray crystal or NMR structures exist for a number of α-actinin domains,[21–24] but no X-ray structure has been published of the complete molecule. Recently, a 3D image obtained by cryo-EM of chicken smooth muscle α-actinin was used to construct an atomic model of the complete molecule.[25]

One of the challenges for structural studies of focal adhesions is the presence of many components that have weak pair-wise affinities. Both α-actinin and vinculin are localized to focal adhesions, but their affinities for each other are relatively weak.[13] Biochemical evidence for the α-actinin–vinculin interaction has come from several sources[26–29] and the vinculin-binding site on α-actinin localized to a region spanning the third α-helix of R4 and the beginning of the Cam domain.[13] The present study was undertaken as a first step in reconstituting focal adhesions using lipid monolayers as an assembly medium. To achieve this goal, we synthesized the β1-integrin cytoplasmic domain (β1-integrin$_{CD}$) with a His$_5$-tag at its N terminus and bound it to a lipid monolayer containing a synthetic lipid with a nickel chelating group. This arrangement places the integrin peptide in its native orientation at the cell membrane and was used to determine the integrin-binding site on α-actinin.[30] The present report builds the structure further by adding the first 258 residues of the vinculin head domain (vinculin$_{D1}$).

Results

Arrays of the ternary complex, α-actinin:vinculin$_{D1}$:-β1-integrin$_{CD}$, were obtained under several sets of conditions. However, arrays did not form unless both the Ni-chelating lipid and the β1-integrin$_{CD}$ were present and array formation was enhanced by addition of ~12% (mol:mol) of the cationic surfactant didodecyldimethyl ammonium to the lipid mixture. The 2D arrays of the ternary complex, cell dimensions $a=263(\pm 2)$ Å, $b=204(\pm 2)$ Å, $c=100$ Å and $\gamma=107(\pm 0.6)°$, two-sided plane group $p2$, were isomorphous at this resolution (~20 Å) with arrays of α-actinin alone.[25,31] The contrast transfer function corrected images were merged with average phase residuals of 22° (Table 1). A total of 105 images from arrays tilted to as high as 70° were merged to the same phase origin as the α-actinin reference map[25] to make the final 3D map.

Table 1. Phase residuals by tilt angle

Tilt angle (deg.)	Phase residuals (deg.) (by tilts)	No. of images	No. of comparisons
0	20.45±2.41	19	3404
30	22.98±4.96	12	1219
45	23.54±2.76	29	2654
60	21.51±5.05	33	859
70	24.14±3.64	12	263

In this unit cell, the individual α-actinin molecules lack 2-fold symmetry.[25] The 2-fold symmetry of the unit cell relates the two α-actinin molecules within, not the two polypeptides that comprise each molecule. One end of each molecule within the unit cell has the ABD arranged around a crystallographic 2-fold axis. This end is referred to as the "paired end".[25] At the other end of the molecule, the ABD is unassociated with the ABD of its crystallographic neighbors and is referred to as the "free end". One advantage of this unit cell is the large spaces on either side of the R1–R4 domain and the free surface on the solvent face of the arrays that facilitate addition of binding partners.

The 3D density map and vinculin$_{D1}$ difference peaks

The 3D reconstruction of the β1-integrin$_{CD}$:-α-actinin:vinculin$_{D1}$ complex shows additional density at the ends of the molecule not seen in the reconstruction of α-actinin alone. A difference map was calculated by subtracting the previously determined α-actinin reference map[25] from the ternary complex. The significant difference peaks were obtained by contouring this map at a 3σ density cutoff (red surface envelopes in Figure 1(a) and (b) and orange surface envelopes in Figure 1(c) and (d)) and superimposing them over the 3D density map of the ternary complex (white envelope in Figure 1(c) and (d)) or the α-actinin reference map (white envelope in Figure 1(c) and (d)). There was no significant difference peak at the location of the β1-integrin$_{CD}$ binding site on α-actinin. To verify the presence of a feature this small at this resolution would require the addition of a gold label to the peptide, as was done earlier to identify the β1-integrin$_{CD}$ binding site.[30]

There are two significant positive peaks in the difference map at the 3σ cutoff. The largest peak is located over the ends of the α-actinin where the Cam-like domain and ABD are located. The smaller peak is located approximately in the center of the R1–R4 domain (Figure 1(a) and (d)) and could be either a second vinculin$_{D1}$ binding site or a local conformational change. This peak is much smaller than the main peak and is too small to obtain a vinculin$_{D1}$ orientation, so we have not pursued it further. If it is due to a secondary vinculin$_{D1}$ site, the occupancy is much lower than the primary site.

The larger difference peak contoured at 3σ has a size and shape that is very suggestive of the vinculin$_{D1}$ structure (Figure 1(c) and (d)). All crystal