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Molecular Shape and Self-Association of Vinculin and Metavinculin

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Vinculin, a 130,000-dalton protein localized to adhesion plaques, and metavinculin, a 150,-000 dalton protein closely related to vinculin, have been studied using rotary shadowing and electron microscopy. Both proteins have globular head regions attached to rod-shaped tail domains. Vinculin and metavinculin also both form complexes consisting of four to six individual molecules. These multimers are formed by head-to-head as well as tail-to-tail interactions.

Talin, another protein which has been localized to adhesion plaques and binds to both vinculin and metavinculin, has also been investigated using shadowing techniques. Talin is an elongated, flexible molecule in high ionic strength buffers, as shown here by rotary shadowing and negative stain electron microscopy.

Key words: vinculin, metavinculin, talin, platinum shadowing, self-association

Vinculin is a 130,000-dalton protein originally isolated from smooth muscle and shown by immunofluorescence microscopy to be localized in fibroblast adhesion plaques [Geiger, 1979], as well as in other cellular locations where bundles of actin filaments interact with the plasma membrane [Geiger et al, 1980; Burridge and Feramisco, 1980]. Metavinculin is a related protein of about 150,000 daltons, also isolated from smooth muscle, which shares immunological cross reactivity and similar peptide composition with vinculin [Feramisco et al, 1982; Siliciano and Craig, 1982]. Both proteins bind talin [Otto, 1983; Wilkins et al, 1983; Burridge and Mangeat, 1984], another protein found at many sites of actin-membrane interaction [Burridge and Connell, 1983a,b]. Based on their locations in cells, it has been suggested that these proteins may function in the attachment of actin filament bundles to the plasma membrane. We are interested in understanding the molecular interactions of these proteins, and toward this end we have begun to investigate their ultrastructure by electron microscopy of platinum shadowed molecules. Previous work has shown that vinculin is a globular molecule [Isenberg et al, 1982; Jockusch and Isenberg, 1982]. Here, however, we demonstrate that vinculin has two domains, a globular head region from which an elongated tail region extends.

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Metavinculin demonstrates a similar structure–a globular head domain with a tail attached. Both vinculin and metavinculin self-associate, forming multimeric complexes.

Electron microscopy of talin, which is also found at adhesion plaques, reveals an elongated, flexible molecule approximately 60 nm long, except in low ionic strength buffers, in which talin appears somewhat asymmetrical, yet globular.

MATERIALS AND METHODS

Vinculin was purified according to the method of Feramisco and Burridge [1980], using low ionic strength extractions and ion exchange chromatography on diethylaminoethy (DEAE) cellulose (Whatman DES2, England). Metavinculin was isolated using the techniques described in Feramisco et al [1982] taking fractions from the DEAE cellulose chromatography, performing gel filtration on Sephacryl S-300 (Pharmacia, Piscataway, NJ), then finally running the protein mixture over Bio-Gel HTP (hydroxylapatite), (Bio Rad, Richmond, CA).

Talin was isolated by the method outlined in Burridge and Connell [1983a], and elaborated upon by Molony et al [manuscript in preparation]. The gizzard low ionic strength extractions was precipitated with ammonium sulfate and then resuspended and chromatographed using DEAE cellulose, Sepharose CL-6B (Pharmacia), Bio Gel HTP.

Preparations for electron microscopy were dialyzed into solutions containing glycerol (for shadowing), with ammonium formate substituting for NaCl. Protein concentrations were ~5–25 μg/ml. Negatively stained specimens were prepared on glow-discharged carbon-coated grids, and stained with 1% uranyl acetate. Shadowed specimens were prepared according to Fowler and Erickson [1979], spraying the protein solution onto freshly cleaved mica. Shadowing was performed in a Balzers Electron Gun Evaporation unit with a tilted rotating stage. Platinum and carbon were evaporated onto the specimens at high vacuum. Electron microscopy was performed using a JEOL 100CX electron microscope, with a magnification of 40,000.

RESULTS

Using rotary shadowing, we have obtained high-resolution electron micrographs of vinculin and metavinculin, revealing that both proteins consist of a head and a tail domain.

Vinculin molecules, as shown in Figure 1, do appear globular, but upon closer examination, a small rod-shaped domain is seen extending from the globular region. This tail domain is not usually obvious in negatively stained specimens of isolated vinculin molecules [Milam, in press]; however, rotary shadowing techniques enhance the thickness of the domain to make it more visible (Fig. 1 and Milam [in press]). Measurements of the head and tail dimensions were made. Vinculin heads have diameters of 8 nm (corrected for a 1-nm shell of metal), with tails 19.4 nm long.

Vinculin multimers were seen at low concentrations at physiological ionic strength. However, at 0.5 M NaCl or similar ionic conditions, all vinculin molecules appeared to form multimers. These multimers resemble clusters of balloons, or parachutes, as seen in Figure 2. Vinculin multimers usually consists of four to six molecules joined head-to-head and tail-to-tail. The tail association appears to be the

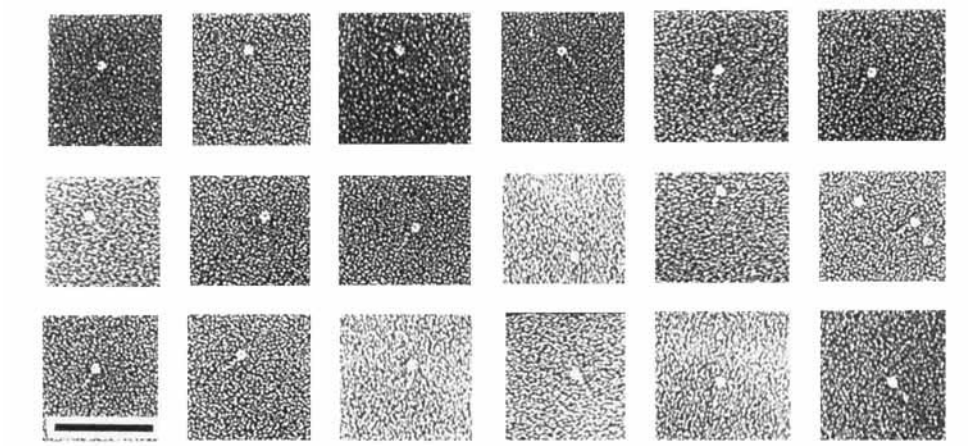

Fig. 1. Rotary-shadowed vinculin molecules demonstrate both a head and a tail domain. The heads average 8 nm in diameter, and the tails are 19.4 nm long. Bar = 100 nm.

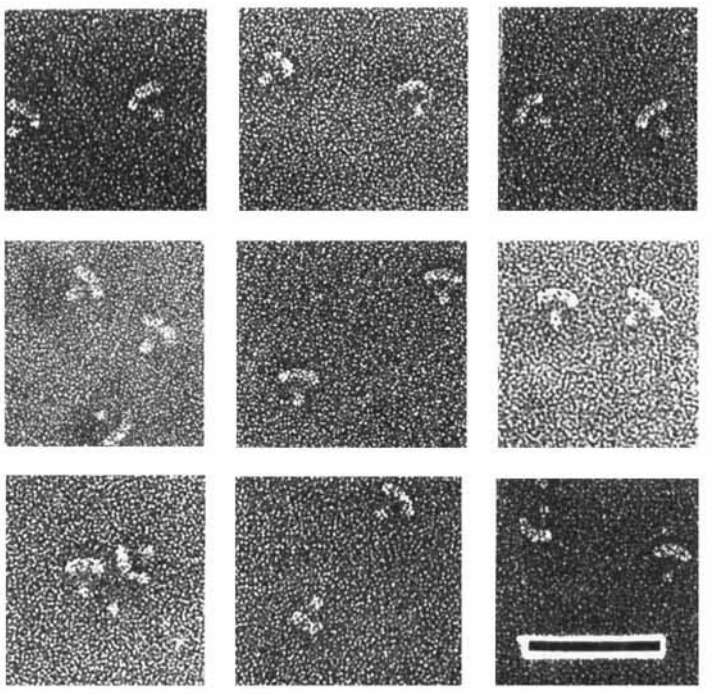

Fig. 2. Vinculin molecules form multimers in high salt buffer conditions. These "parachutes" appear to consist of four to six individual vinculin molecules joined head-to-head and tail-to-tail. Bar = 100 nm.

strongest, since several cases where the tails are bound and the heads are free have been observed. The tail association is also quite extensive, forming a dense region 5–8 nm long at the end of the tail domain.

Metavinculin was observed to have a structure similar to that of vinculin (Fig. 3). The head domain has a diameter of 8.5 nm (corrected for a 1-nm shell of metal), and the tail was 21.5 nm in length. The only difference in the self-association was that the metavinculin multimers were seen even in low ionic strength buffers, although

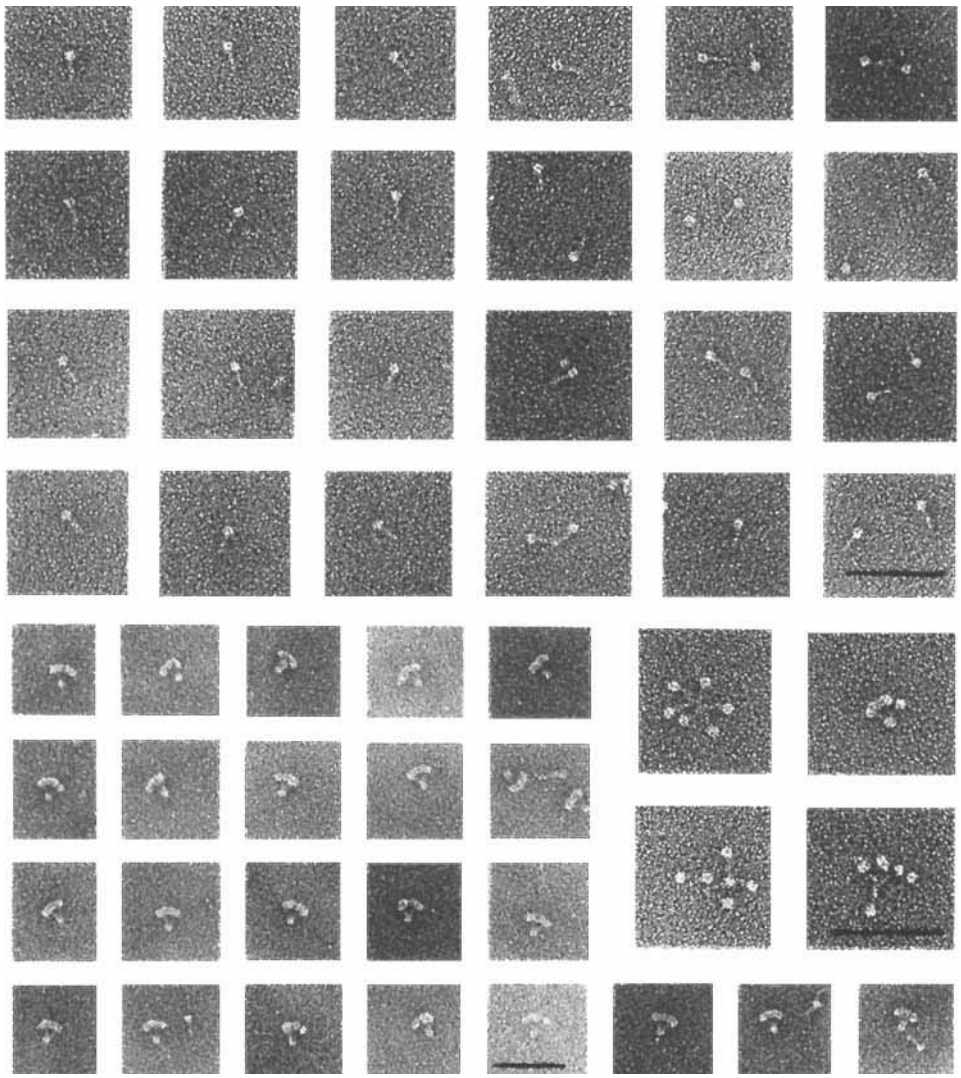

Fig. 3. Metavinculin was purified by the method of Feramisco et al [1982]. Monomeric metavinculin has the same two domains as vinculin—a head (8.5 nm) and a slightly longer tail (21.5 nm). Metavinculin "balloons" also aggregate into tetramers, pentamers, or hexamers that resemble parachutes when associated head-to-head and tail-to-tail. In some cases, the head-to-head associations fall apart, leaving a rosette type of structure as shown above. Bars = 100 nm.

there were also isolated metavinculin molecules present at those conditions. The metavinculin multimers also associated head-to-head and tail-to-tail, with the tail association apparently favored (Fig. 3, inset).

Talin, a 215,000-dalton protein that has been shown to bind to vinculin [Otto, 1983; Wilkins et al, 1983; Burridge and Mangeat, 1984], shows two different conformations, depending on the ionic strength of the buffer. In low ionic strength (0–20 mM NaCl), talin is folded, forming an asymmetric globular molecule. In high ionic strength buffers, hydrodynamic measurements show that talin is a flexible, rod-shaped molecule [Molony et al, manuscript in preparation]. It is probably in this elongated conformation that it interacts with vinculin. Figure 4 shows talin in high ionic strength buffer conditions, prepared both by rotary shadowing (panel A) and negative stain (panel B). These micrographs demonstrate the flexibility of talin molecules.

DISCUSSION

This study was initiated with the goal of visualizing the interaction of vinculin and talin by electron microscopy. This interaction has not yet been observed using this technique, but we report here several interesting observations on the structures of the molecules involved. We also describe the structure of metavinculin, a protein closely related to vinculin that is found only in muscle tissues [Feramisco et al, 1982; Siliciano and Craig, 1982].

Electron microscopy shows that vinculin and metavinculin have very similar structures: a globular head domain and a rod-shaped tail domain. Both proteins associate into what appear to be tetramers, pentamers, and hexamers, by head-to-head and tail-to-tail associations. Measurements of individual vinculin and metavinculin molecules differ more significantly in the tail domain than in the head domain, with metavinculin's tail being longer by 1–1.5 nm. However, we have not yet

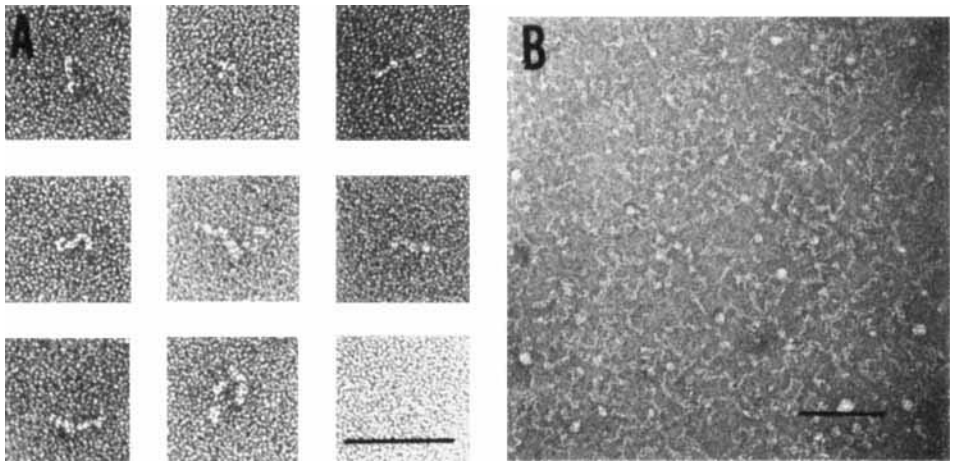

Fig. 4. A) Rotary-shadowed talin molecules in elongated conformation. The average length of talin is 60 nm, corrected for a 1-nm shell of metal. Bar = 100 nm. B) Negatively stained talin in high ionic strength buffer conditions. Under these conditions, talin is elongated rather than globular, as it appears in low ionic strength buffer conditions. Note the flexibility of the molecules. Bar = 100 nm.

determined whether this increased size detected at the electron microscope level is sufficient to account for the increased molecular mass of metavinculin of about 20,000 daltons.

Proteolysis of both vinculin and metavinculin results in a major fragment with an apparent molecular weight of between 90,000 and 100,000 daltons [Feramisco et al, 1982]. Electron microscopy on this major fragment of vinculin indicated that the tail had been removed but that the remaining head domain also appeared to be less compact [Milam, in press]. In future work, it will be important to compare the major fragments of vinculin and metavinculin with the aid of the electron microscope. This should help to determine whether the difference in their molecular weights can be accounted for by the apparently longer tail of metavinculin.

The head domain (90,000-dalton fragment) of vinculin has been shown to contain the binding site for talin [Burridge and Mangeat, 1984]. Metavinculin also binds to talin [Burridge and Mangeat, 1984]. This association takes place at high ionic strength (150–200 mM NaCl), at which concentration talin is in its elongated conformation. Vinculin associates with talin as a nonomer, as shown by sedimentation analysis [Burridge and Mangeat, 1984]. It is not known, however, whether or not it can also associate with talin when vinculin has formed the multimeric "parachutes."

This work illustrates the shapes of three molecules involved in adhesion plaques of fibroblasts and dense plaques of smooth muscle. Future electron microscopic studies should lead to the visualization of the talin-vinculin and the talin-metavinculin interactions, and to a greater understanding of which domains of the proteins are involved in these associations.

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