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Discussion Letter

Structural model of vinculin: correlation of amino acid sequence with electron-microscopical shape

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The cytoskeleton-associated protein vinculin exhibits the shape of a 'balloon on a string' when examined by rotary shadowing electron microscopy. Recently, the complete primary structure of chicken vinculin was determined which leads to the questions as to whether the globule might be either hollow or disc shaped, or whether the electron micrographs resemble those of vinculin dimers. Based on a hydrodynamical theory, evidence is presented for the assumption that vinculin as a monomer consists of a compact spherical head 6 nm in diameter connected by a proline-rich domain to a rod-shaped tail about 20 nm in length.

Cytoskeleton; Electron microscopy; Protein structure; Hydrodynamics; Cell adhesion

Vinculin is a ubiquitous, cytoskeleton-associated protein especially localized within focal contacts and the adhering junctions of epithelia (for references see [1]). Together with talin, which can bind to vinculin and the fibronectin receptor, it participates in a linkage between the extracellular matrix and the cytoskeleton (cf. [2]). Recently the complete cDNA-derived amino acid sequence of chicken embryonic vinculin has been published [3] which exhibits 99% identity with the partial sequence covering 88% of the total sequence of chicken embryo fibroblast vinculin [4,5].

In their attempt to correlate the primary and predicted secondary structure with the appearance of avian vinculin as a 'balloon on a string' in the electron microscope [6,7], Coutu and Craig [3] reject the assumption that these pictures reflect a vinculin monomer consisting of a globule connected to a rod and suggest one of the following models: If the electron micrographs represent a monomeric molecule, the head should be either hollow or disc-shaped. Alternatively, they propose that the shape of the particles reflects a dimer of vinculin stabilized by parallel helices in the tail. Here, I will argue for a model of vinculin which is consistent with its shape in electron micrographs, its sedimentation coefficient and the amino acid sequence.

The model is based on Kirkwood's theory on irreversible processes in solutions of macromolecules as worked out by Bloomfield and co-workers [8]. The validity of this theory for the interpretation of data obtained by electron microscopy has been especially demonstrated for several molecules of the extracellular matrix (for references see [9]). The molecule is modelled by n spheres of radii r. A theoretical sedimentation coefficient can be calculated from

$$s_{20,w} = \frac{M(1-v_2\varrho)\left(\sum_{i=1}^{n} r_i^3 + \sum_{i=1}^{n}\sum_{\substack{j=1 \\ i\neq j}}^{n} r_i^2 r_j^2 \langle R_{ij}^{-1}\rangle\right)}{6\pi\eta N_A\left(\sum_{i=1}^{n} r_i^2\right)^2} \quad (1)$$

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where $\langle R_{ij} \rangle$ is the distance between the centers of spheres i and j, N_A is Avogadro's number, M is the molar mass, v_2 is the partial specific volume, η and ϱ are the viscosity and density of the solution, respectively. The relation of the molar mass to the radius is given for a compact spherical molecule by

$$r = \frac{1}{2}\left(\frac{6Mv_2}{\pi N_A}\right)^{1/3} \quad (2)$$

and for a cylindrical molecule of length L by

$$r_{cyl} = \left(\frac{Mv_2}{L\pi N_A}\right)^{1/2} \quad (3)$$

To use eqn 1 a cylindrical part of a molecule has to be modelled by N non-overlapping spheres of radius r in a manner such that the molar masses are equivalent, i.e. $r = r_{cyl}(3/2)^{1/2}$, and $2Nr \cong L$.

Chicken embryo vinculin consists of 1066 amino acid residues accounting for a molar mass of 116989 Da. Partial proteolytic digestion with *S. aureus* V8 protease results in an approx. 100 kDa fragment, which appears globular on electron micrographs and retains talin-binding activity, and an approx. 30 kDa fragment [6]. Two cleavage sites were located to be at His_{851} and Leu_{858} [5] which are near the middle of the proline-rich stretch between residues 837 and 878. In accordance with Coutu and Craig [3], therefore, an amino-terminal spherical head for residues 1–857 and a carboxy-terminal cylindrical tail for residues 858–1066 will be assumed (see fig.1).

A compact sphere of $M = 93649$ Da (residues 1–857) should exhibit a diameter of 6.008 nm (eqn 2). Milam [6] reported a diameter of 8 ± 0.8 nm (corrected for a 1 nm shell of platinum) for the head region of vinculin as determined by rotary shadowing electron microscopy. For thickness measurements, however, this technique is very uncertain. Metal deposition may range from 2.5 nm [10] to 4 nm [11] and also varies within one preparation from droplet to droplet (own experience). Therefore, a computed diameter of 6 nm does not disagree with Milam's measurements. Vinculin visualized by negative staining, which should lead to better thickness determinations, appears almost globular [6,12]. A spherical shape, however, is incompatible with the frictional coefficient of vinculin (see below). Whereas Milam [6]

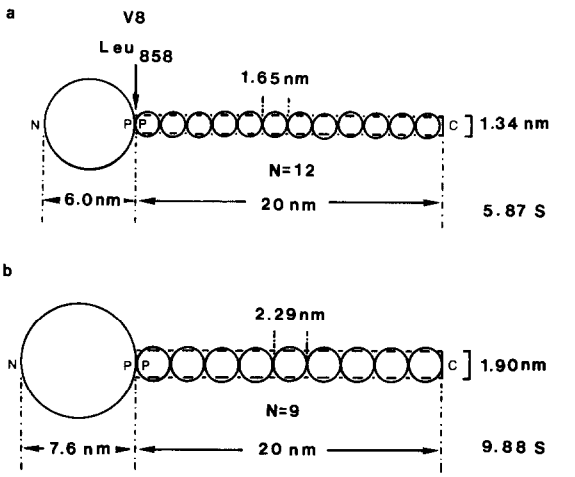

Fig.1. Hydrodynamical model of vinculin assuming a monomeric (a) or dimeric (b) state. 'PP' denotes the putative localization of the proline-rich domain (residues 837–878). Leu_{858} is a cleavage site for *S. aureus* protease V8. All given parameters are computed according to eqns 1–3 assuming amino acid residues 1–857 as being responsible for a spherical head domain and residues 858–1066 as responsible for a cylindrical tail domain, respectively. The dashed line within the rod spheres marks the assumed cylindrical geometry of the tail. N and C denote the amino- and carboxy-terminus, respectively.

only shows a gallery of selected molecules, the overview of Gimona et al. [7] demonstrates that the appearance as a balloon on a string is a rather characteristic shape of vinculin.

For the measurement of extended particles electron microscopy using the rotary shadowing technique is the method of choice. The tail length of vinculin was reported by Milam [6] to be 20 ± 1 nm at both low and physiological ionic strength. If the tail is modelled as a cylinder, the remaining molar mass of 23340 Da (residues 858–1066) leads to a diameter of 1.344 nm (eqn 3). To evaluate a theoretical sedimentation coefficient, this cylinder is divided into 12 linearly aligned spheres 1.651 nm in diameter (cf. fig.1a). With these parameters and assuming a partial specific volume of 0.73 ml/g, and viscosity and density equal to that of water, eqn 1 results in a value of $s_{20,w} = 5.87$ S. This is in excellent agreement with $s_{20,w} = 5.9$ S determined by analytical ultracentrifugation [13]. The values of 6 S determined by centrifugation through a sucrose gradient [6] and 6.4 S determined at varying pH in the pH range 5–8, or changing protein concentration between 0.4 and 4 mg/ml [14] are

also within the range of uncertainty of this model which assumes a rigid rod. Any flexibility within the tail would result in an increase of this value up to a maximum of $s_{20,w} = 8.58$ S which follows for a spherical protein of the same molar mass. The good correspondence of the theoretical value with those reported might indicate a rather inflexible behaviour of the tail region. Indeed, the electron micrographs published [6,7] exhibit the tail as a straight rod. The Perrin factor $F = 1.462$ derived from the theoretical values corresponds to a prolate ellipsoid of revolution with an axial ratio of 8.5.

Using the same assumptions as above, a dimeric molecule is described by a head sphere 7.569 nm in diameter and 9 rod spheres 2.290 nm in diameter (cf. fig.1b). These parameters predict a sedimentation coefficient of $s_{20,w} = 9.88$ S ($F = 1.376$) which differs by 65% from the values determined experimentally.

The validity of the applied theoretical approach might be illustrated by its application to nidogen/entactin, a basement membrane protein of similar size and of dumb-bell shape. From electron micrographs of negatively stained preparations the molar masses of the terminal globules were determined to be 85 and 38 kDa, respectively, and 27 kDa for the connecting strand 17 nm in length has been reported [15]. The theoretical sedimentation coefficient is thus 6.57 S which is comparable with $s_{20,w} = 5.9$ S determined by analytical ultracentrifugation [15]. The molar masses of the three domains correspond well with those analyzed from the amino acid sequence, i.e. 70 and 36 kDa for the terminal globules, 28 kDa for the strand [16].

For the existence of vinculin as a dimer, Coutu and Craig [3] discuss the possibility of whether the approx. 200 carboxy-terminal amino acid residues would form a linearly extended α-helix, which should be stabilized by salt bridges to the adjacent α-helix. Usually such an intended coiled-coil conformation is characterized by a heptad repeat of hydrophobic residues in positions 1 and 4, and charged residues in positions 5 and 7. I have analyzed this part of the sequence quantitatively using the parameters of Parry [17] for coiled-coil structures. Due to Pro_{886} and Pro_{897} an α-helical structure could only start at Met_{898} but should be interrupted especially by Pro_{989}. Furthermore the cysteine residues in positions 950, 972 and 989 are unfavourable for a coiled-coil. In no permutation of residues 898–1062 could I find more than two subsequent heptads with a coiled-coil factor in excess of 2×10^{-8} which seems necessary to predict a coiled-coil confidently.

The 209 carboxy-terminal residues account for a molar mass of $M = 23\,340$ Da which for a 20 nm long rod leads to a ratio $M/L = 1167$ nm^{-1} for a monomer or 2334 nm^{-1} for a dimer. These values are considerably higher than those of single- (750 nm^{-1}) and double-stranded (1500 nm^{-1}) α-helices, respectively. The putative linkage (residues 837–878) between a spherical head and a rod-like tail contains four stretches of two to five consecutive proline residues. In an aqueous environment these could form short polyproline II helices with *trans* peptide bonds which would result in a very extended conformation ($M/L = 311$ nm^{-1}) and could favour the accessibility for several proteases [5] within this region.

The available data resulting from rotary shadowing electron microscopy, ultracentrifugation, proteolytic cleavage and amino acid sequences are in agreement with the proposed model that the chicken vinculin monomer consists of a compact spherical domain about 6 nm in diameter linked by a proline-rich domain to an about 20 nm long rod-like domain. Further structural studies, however, are indeed necessary, especially to elucidate the folding of vinculin (about 50% α-helix content is predicted from circular dichroism measurements [13]) and to localize the sites for phosphorylation, acylation, binding to actin and the insertion into membranes (cf. [1,3,4,18]). It is noteworthy that vinculin from porcine stomach does not exhibit any indications for a rod-like domain [7]. Short amino-terminal sequences of this protein and fragments thereof exhibit only four amino acid substitutions and the deletion of two alanine residues (positions 851–902) [5]. Amino acid analysis revealed a small but significant increase in the amount of glycine and proline when compared with avian vinculin [7]. A variation especially of these amino acids, however, could change the protein structure drastically.

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