The regulation by vinculin of filamin, α-actinin, and spectrin tetramer-induced actin sol-gel transformation

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1. INTRODUCTION

The cytoplasmic content of eukaryotic cells transforms reversibly from a low viscosity liquid to a rigid gel. This transition corresponds with alterations in cellular events such as cell shape change and motility. Vinculin, a cytoplasmic, M_r 130000, actin binding protein, was first identified and isolated from chicken gizzard smooth muscle [1,2]. Vinculin has since been found in diverse cells and tissues including fibroblasts, smooth muscle cells, intestinal epithelial brush border and chicken cardiac muscle. In each case vinculin has been localized at the ends of the microfilament bundles in close proximity to the plasma membrane [1,3–5]. Vinculin has been demonstrated to decrease the low shear viscosity of F-actin solutions [6,7]; and it has been postulated that this effect is due to shortening of the actin filaments, filament–filament interaction, or formation of actin paracrystalline bundles [6,7]. Vinculin binds with high affinity (K_d = 20 nM) to actin in a ratio of 1 vinculin/1500–2000 actin monomers [7].

A class of fibrous actin binding proteins including filamin, spectrin tetramers and α-actinin have been extensively described [8–10], and demonstrated to exist in diverse eukaryotic nonmuscle cells [10]. These proteins are localized, either associated with, or close to the cytoplasmic surface of the plasma membrane [1,3,5,8–12], in addition to a periodic association of filamin and α-actinin with the microfilaments and Z-line material [11–15]. In in vitro studies, filamin, α-actinin and spectrin tetramers have been demonstrated to crosslink F-actin filaments forming a nonthixotropic gel [16–19]. As these 3 actin binding proteins are all localized in close proximity with vinculin near or on the membrane cytoplasmic surface of various cell types, we decided to ask whether vinculin might play a role in regulating their interaction with actin. The results presented demonstrate that vinculin decreases the critical concentration of spectrin tetramer, filamin, and α-actinin required to induce gelatin of F-actin.

2. MATERIALS AND METHODS

2.1. *Purification of proteins*

Rabbit skeletal muscle actin was prepared from an acetone powder as in [20]. Monomeric actin was purified by gel filtration on Sephacryl-200, and each fraction was measured by low shear viscosity to eliminate components which decrease actin polymerization [21]. Actin was used within 2 days of preparation.

Filamin, α-actinin and vinculin were isolated from chicken gizzard smooth muscle as in [2]. Spectrin tetramers were isolated from fresh human blood as in [22]. Protein 4.1 was isolated from fresh human blood by the method in [23] as

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modified in [24]. All proteins were >95% pure, as judged by SDS–polyacrylamide gel electrophoresis.

2.2. *Low shear viscometry*

Low shear viscosity measurements were performed at 20°C with a falling ball viscometer as originally described in [25]. For non-Newtonian fluids, the observed ball velocity is used to calculate the apparent viscosity given in centipoise (cp) for comparison with Newtonian fluids with the same ball velocity. For these experiments an 80° angle was used.

3. RESULTS

In agreement with [6,7], vinculin decreases the low shear viscosity of F-actin solutions in a concentration-dependent manner (fig.1). This effect has been explained by 3 plausible mechanisms: (i) shortening of actin filaments; (ii) decrease in filament–filament interactions; and (iii) formation of actin paracrystalline bundles [6,7]. Vinculin would therefore appear to have the required characteristics to regulate sol–gel transformation in the cortical region of the cytoplasm immediately subjacent to the plasma membrane.

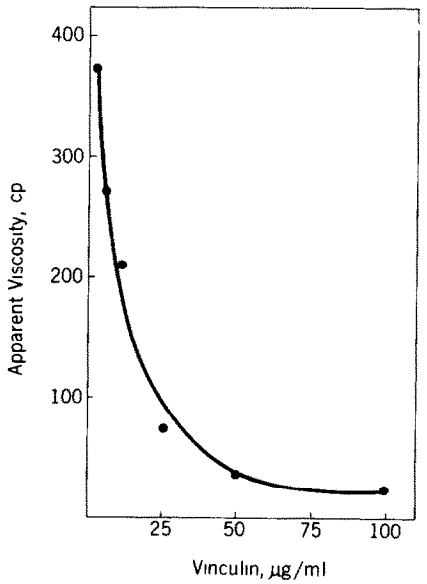
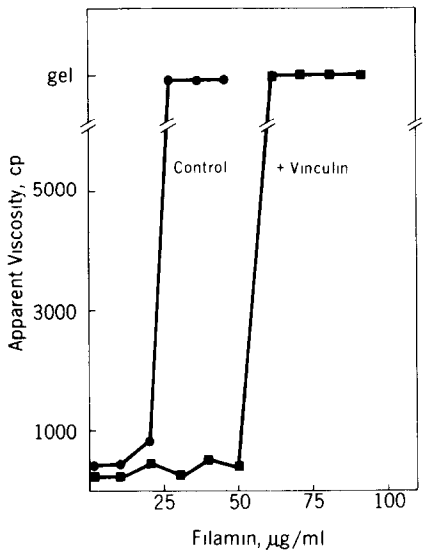


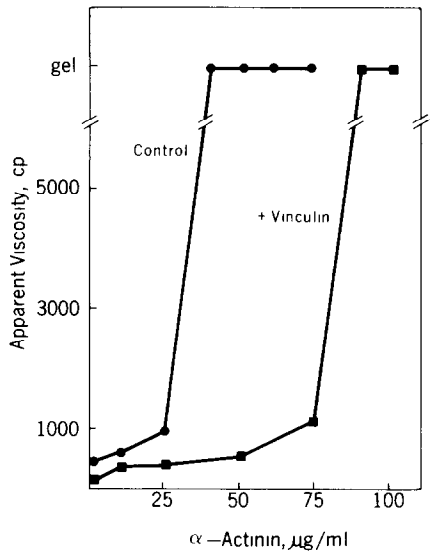
Fig.1. Effect of vinculin on the low shear viscosity of F-actin solutions. Incubations were carried out at 20°C in a total vol. of 0.2 ml polymerizing buffer containing: 0.1 mg F-actin, 2 mM Tris–Cl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.2 mM DTT (pH 7.6) with varying amounts of vinculin. The polymerization was induced by the addition of $MgCl_2$ to a final concentration of 2 mM. The time of incubation at 20°C was 1.5 h.

The sol–gel transition of actin occurs in the presence of actin crosslinking proteins such as filamin, α-actinin and spectrin tetramers when the critical concentration of these crosslinkers is reached [6,19,26]. As demonstrated in fig.2A,B, and C, the critical concentrations for gel formation of actin (0.5 mg/ml, 20°C) are ~20–30 μg/ml for filamin, ~30–40 μg/ml for α-actinin and ~80–90 μg/ml for spectrin tetramers. Gel formation occurred at a molar ratio of ~1 α-actinin dimer/65 actin monomers, 1 spectrin tetramer/125 actin monomers and 1 filamin dimer/265 actin monomers (table 1). Protein 4.1, which apparently strengthens the spectrin tetramer–actin interaction in the erythrocyte membrane skeleton by forming a spectrin–protein 4.1–actin ternary complex, has recently also been detected in non-erythroid eukaryotic cells [10]. Protein 4.1 at a concentration of 15 μg/ml decreased the critical concentration of spectrin tetramer required for gel formation of actin to 25–35 μg/ml (equivalent to 1 spectrin tetramer/360 actin monomers) (fig.2C, table 1). As demonstrated in fig.2 and table 1, the addition of vinculin at a molar ratio of 1 vinculin/75 actin monomers increases the critical concentrations of filamin, α-actinin, and spectrin tetramers ± protein 4.1 required for gel formation of actin, 2–3-fold. Vinculin at 20 μg/ml increases the critical concentrations of crosslinking proteins to 80–90 μg α-actinin/ml, 55–65 μg filamin/ml, 180–190 μg spectrin tetramer/ml and 60–70 μg spectrin tetramer + protein 4.1/ml (fig.2 and table 1). We have demonstrated that vinculin does not compete with filamin, α-actinin, or spectrin tetramers for binding to F-actin by sedimentation analysis (not shown).

4. DISCUSSION

Filamin, α-actinin and vinculin in association with actin have been localized in close proximity to the intercalated discs of cardiac muscle cells, and in the cortical region of the cytoplasm immediately subjacent to the plasma membrane surface of fibroblasts ([1,3,4,11,12], V.E.K., unpublished).





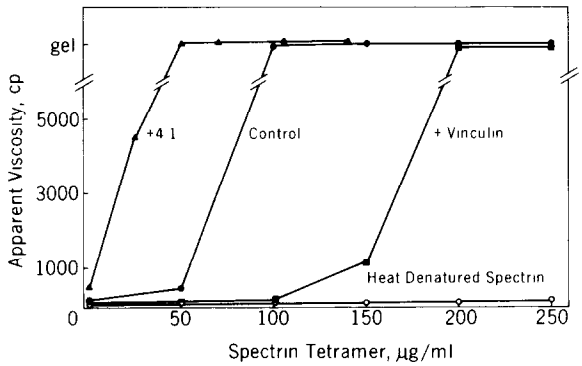


Fig.2. Effect of vinculin upon the actin gelation induced by filamin, α-actinin, and spectrin tetramer. Incubations were carried out as in fig.1, except that vinculin was added at a constant concentration of 20 μg/ml. (A) Crosslinking by filamin; (B) Crosslinking by α-actinin; and (C) Crosslinking by spectrin tetramer ± protein 4.1 (15 μg/ml). In C, (○) is the same as the control except that heat-denatured (60°C, 15 min) spectrin tetramer was substituted in the incubation. All expts were carried out using $MgCl_2$ (2 mM) or KCl (50 mM) to induce actin polymerization; similar results were obtained in either case. The $[Ca^{2+}]$ = 0.2 mM, and [EGTA] = 4 mM yielding a final $[Ca^{2+}] = 5 \times 10^{-8}$ M.

α-Actinin and vinculin have been found to be associated with actin in the dense plaques of smooth muscle cells and the zonula adherens of brush border epithelial cells [5]. Therefore, the regulation of the filamin–actin and α-actinin–actin interactions by vinculin which we have demonstrated in vitro, may have important physiological consequences in these cell types. The attachment of short actin filaments (protofilaments) to the human erythrocyte membrane through interaction with spectrin tetramer and protein 4.1 has been extensively described [8–10]; whether a structurally related analogue of vinculin exists in erythrocytes is not yet known. However, recently we have made the initial identification of a struc-

Table 1

Effect of vinculin upon the critical crosslinking ratio

	Mol G-actin monomer/[a]mol of crosslinking protein
Actin + spectrin tetramer	125
Actin + spectrin tetramer + vinculin	60
Actin + spectrin tetramer + 4.1	360
Actin + spectrin tetramer + 4.1 + vinculin	170
Actin + filamin	230
Actin + filamin + vinculin	90
Actin + α-actinin	65
Actin + α-actinin + vinculin	30

[a] Calculated using the following M_r: spectrin tetramer (920000), filamin (500000), α-actinin (200000) and actin (43000)

turally and functionally related analogue of the spectrin molecule associated with the membranes of various nonerythroid cell types including embryonic chicken cardiac myocytes, mouse fibroblasts, rat hepatoma cells, neuroblastoma cells, and brain tissue [10,27,28]. These membrane-associated spectrin-like proteins have been demonstrated to be associated with actin [10,27,28], in cell types in which actin-associated vinculin is also found in close proximity to the cytoplasmic surface of the plasma membrane. Therefore, our current finding that vinculin regulates the spectrin–actin interaction in vitro may have profound physiological implications in various eukaryotic cells.

A number of agents have been found to affect F-actin polymerization in a manner similar to vinculin. These include cytochalasins, villin from intestinal microvilli, gelsolin from macrophage, and the capping protein from Acanthamoeba [8]. All of these agents increase the critical concentration for actin gelation induced by crosslinking proteins. It has been suggested in [29] that the network theory of gel formation in [30] can be used to explain the effect of various actin-binding agents on the formation of actin gels in the presence of crosslinking proteins. Our results demonstrating a clear effect of vinculin in regulating the gel point of actin–filamin, actin–α-actinin and actin–spectrin tetramer mixtures is consistent with the network theory.

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