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Interaction of α-actinin and vinculin with actin: Opposite effects on filament network formation

(actin binding proteins/microfilament bundling and crosslinking/viscometry/electron microscopy)

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ABSTRACT **The interaction of actin filaments with two actin-associated proteins, α-actinin and vinculin (M_r 130,000 protein), was studied *in vitro* with viscometry and light and electron microscopy. Vinculin, like α-actinin, binds to F-actin, and the two proteins were found to have different effects on the formation of filament networks: α-actinin crosslinks individual filaments in a manner strongly dependent on temperature and acts as a spacer, whereas vinculin forms actin bundles that display a paracrystalline substructure. In viscometric assays, α-actinin mimics the effect of actin gelation factors, whereas vinculin acts as a gelation inhibitor. These findings imply complementary functions of these proteins in the regulation of cellular mobility.**

Actin filaments are the structural basis of eukaryotic cellular and subcellular motility, such as locomotion, contraction, membrane mobility, and organelle transport. The specificity required to induce and control these different movements probably depends not only on the molecular properties of actin but also on its interaction with other structural proteins. Until now, approximately 20–30 actin-associated proteins have been described, and two of these, α-actinin (1, 2) and vinculin (M_r 130,000 protein; refs. 3 and 4), are closely associated with actin filaments at the stress fiber foci (1–4) in fibroblasts and at junctional complexes of epithelial cells (5). The binding of α-actinin to actin filaments has been studied *in vitro* to some extent (6–9), but no direct evidence has been found for an interaction between vinculin and actin. We demonstrate a specific interaction of purified α-actinin and vinculin with actin filaments, using viscometry and electron and light microscopy. Our results show that both proteins influence the spatial arrangement of actin filaments and suggest an antagonistic function for α-actinin and vinculin in regulating the packing of microfilaments.

MATERIAL AND METHODS

Proteins. Actin was extracted from porcine skeletal muscle. Crude actin was prepared from acetone powder essentially as described (10) and was purified on Sephadex G-150 [Pharmacia, (Uppsala, Sweden)]. α-Actinin was extracted from fresh porcine skeletal muscle and purified as described (8). Vinculin was prepared from frozen chicken gizzard (11). An analysis of these preparations on $NaDodSO_4$/polyacrylamide gels is shown in Fig. 1. Subfragment 1 of myosin (S-1) was a gift from A. Sobieszek. Protein concentrations were determined by the Bio-Rad method.

Viscometry. Under conditions of high shear, viscometry was performed in conventional semimicro Ostwald viscometers (Cannon Instruments) which were calibrated with buffer at 25°C and then filled with 800 μl of the reaction mixture. Viscometry under conditions of low shear was performed with the falling-ball viscometer (12–14): a calibrated microcapillary was set at a fixed angle (56° in this work) and filled with the reaction mixture; then a stainless steel ball was allowed to fall freely through the capillary.

FIG. 1. Analysis of protein preparations on $NaDodSO_4$/polyacrylamide gels. Approximately 25 μg of protein was applied to each slot. Lanes: left, skeletal muscle actin (M_r, 42,000); center, skeletal muscle α-actinin (M_r, 100,000); right, smooth muscle vinculin (M_r, 130,000).

The reaction mixture consisted of G-actin (0.5 mg/ml) and various amounts of α-actinin or vinculin. The storage buffers for the purified proteins were 2 mM Tris·HCl, pH 8.0/0.2 mM ATP/0.5 mM dithiothreitol/0.2 mM $CaCl_2$ (actin)/1 mM $KHCO_3$ (α-actinin)/20 mM Tris·acetate, pH 7.6/15 mM 2-mercaptoethanol/0.1 mM EDTA/20 mM NaCl (vinculin). Polymerization was initiated by either $MgCl_2$ in imidazole buffer (final concentration, 10 mM imidazole, pH 7.5/1 mM ATP/1 mM EGTA/2 mM $MgCl_2$) or KCl (final concentration, 20 mM). Control experiments showed that the storage buffers for α-actinin or vinculin had no effect on the viscosity measured for actin alone, when added in the same amounts as in the test samples.

Aliquots for light and electron microscopy were prepared at 25°C under conditions identical to those used for low-shear viscometry or were taken directly from the sample used for high-shear viscometry.

Microscopy. To examine the effect of α-actinin and vinculin on actin filaments, it was essential to avoid artificial bundling, which is often seen in routine preparations of negatively stained F-actin. We used a newly developed technique that minimizes this effect. Samples were diluted to 50–100 μg of actin per ml immediately before being spread onto carbon-coated, glow-discharged Formvar grids, which had been pretreated with 0.02% cytochrome *c* in 0.1% isoamyl alcohol. Adsorption of proteins was followed by posttreatment with the same solution, rinsing with H_2O, and staining with 0.75% uranyl formate (pH 4.7). To

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Abbreviation: S-1, subfragment 1 of myosin.

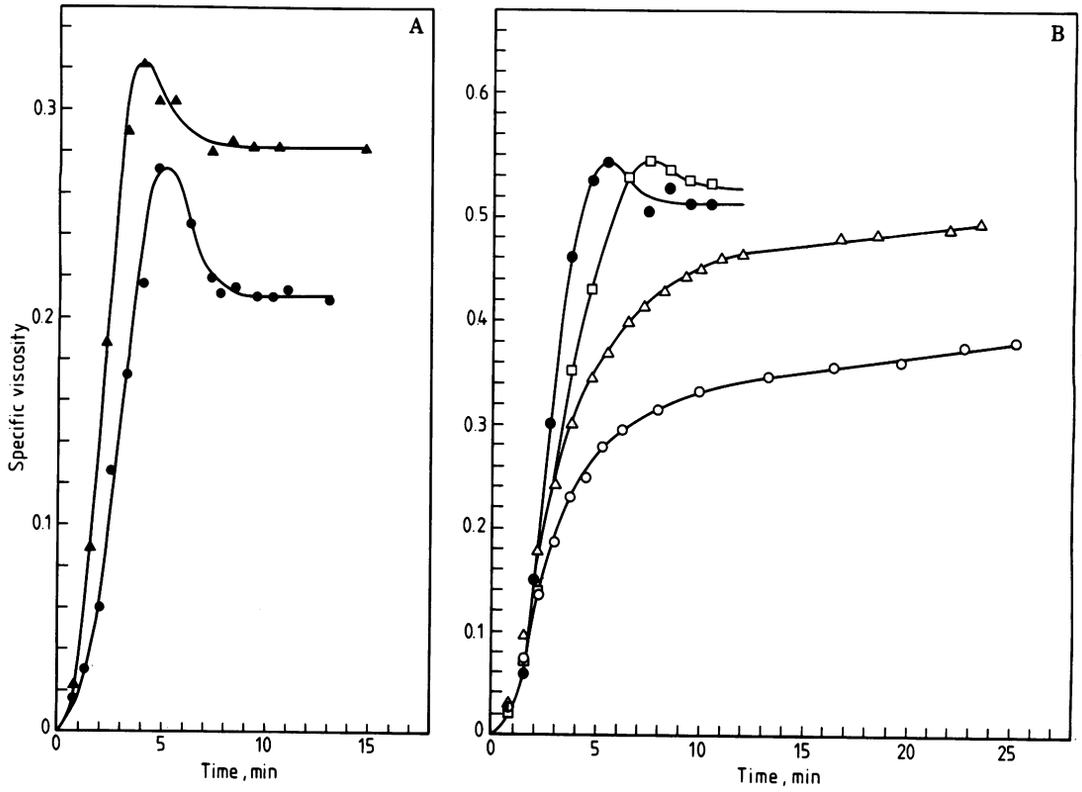

FIG. 2. Viscosity changes in gel-filtered monomeric actin (0.5 mg/ml) polymerized with 2 mM $MgCl_2$ in the presence of α-actinin (*A*) or vinculin (*B*) as measured in an Ostwald viscometer at 25°C. The difference in the final steady-state level between *a* and *b* is a consequence of using two different actin preparations. ●, Actin control (0.5 mg/ml); ▲, addition of α-actinin (50 μg/ml). Addition of vinculin: □, 25 μg/ml; △, 75 μg/ml; ○, 150 μg/ml.

study the possible effect of vinculin on the growth of actin filaments, actin (gel-filtered to remove unbound ATP) was allowed to polymerize onto S1-decorated filament fragments (15) in the presence or absence of vinculin at 25°C. Specimens were examined in a ZEISS EM10 electron microscope.

For light microscopy, a Zeiss photomicroscope equipped with Nomarski interference optics was used.

RESULTS

Kinetics of Protein Interaction. Two ionic conditions for actin polymerization were employed. One induces rapid polymerization (2 mM $MgCl_2$) and the other favors slow polymerization (20 mM KCl). With Mg-induced rapid polymerization at 25°C, viscosity of the actin control solution (0.5 mg/ml) reached a peak at about 4 min and the final level (steady state) within 8 min. Upon the addition of α-actinin (50 μg/ml), the peak was reached within 3 min, and the steady-state level remained approximately 25% higher than in the control (Fig. 2*A*).

These results confirmed earlier observations that α-actinin binds to F-actin and forms crosslinks that are, at least in part, resistant to the high-shear forces of an Ostwald viscometer (6, 7, 9). Addition of vinculin to actin under identical conditions had the opposite effect: increasing ratios of vinculin/actin caused an increase in the time required to reach a maximum and the final steady-state level (Fig. 2*B*). These data suggest a direct interaction between vinculin and F-actin. This interaction could be demonstrated more dramatically under conditions of slow polymerization (20 mM KCl), where the addition of vinculin at a very low concentration (25 μg/ml) reduced the increase in viscosity by a factor of 10 as compared to the control (Fig. 3). Under conditions of both fast and slow polymerization, the initial time course of actin polymerization (nucleation phase) was not altered by the addition of vinculin (Figs. 2*B* and 3). This suggested that vinculin had no effect on filament nucleation.

Low-Shear Viscometry. The temperature and concentration dependence of the interaction between actin and α-actinin or vinculin was analyzed with the falling-ball viscometer. The temperature dependence of the time taken by the actin control to reach steady-state levels was taken into account by allowing for different polymerization times at various temperatures (Figs.

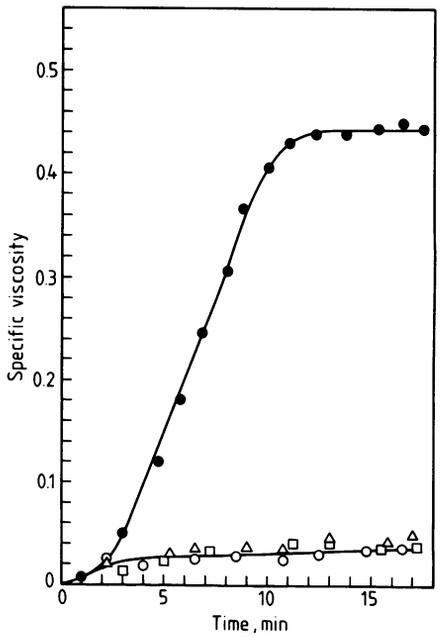

FIG. 3. Viscosity changes of actin (0.5 mg/ml) polymerized with 20 mM KCl, measured in an Ostwald viscosimeter at 25°C in the absence (●) or presence of vinculin: 25 μg/ml (□); 75 μg/ml (△); 150 μg/ml (○).

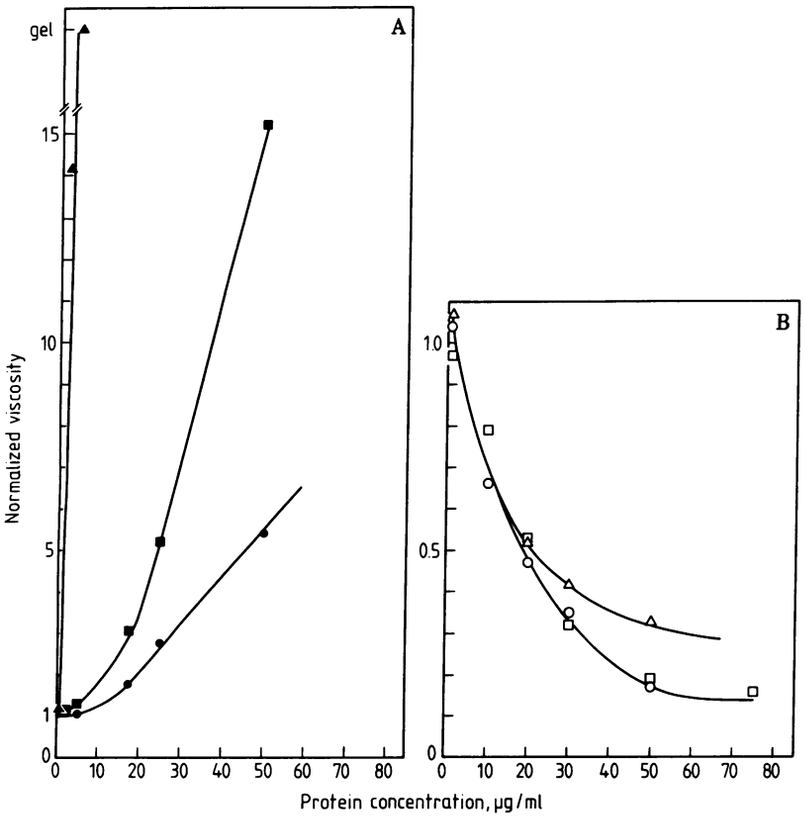

FIG. 4. Viscosity of actin (0.5 mg/ml) polymerized by addition of 2 mM $MgCl_2$ with either α-actinin (*A*) or vinculin (*B*), as measured in a falling-ball viscometer at 4°C (▲, △), 15°C (see Fig. 5), 25°C (■, □), and 30°C (●, ○). The apparent viscosities of the actin controls were different after reaching steady state for the different temperatures: 34.9 cP at 4°C (2-hr polymerization), 26.65 cP at 15°C (30-min polymerization), 17.8 cP at 25°C (10-min polymerization), 14.22 cP at 30°C (10-min polymerization). To enable a comparison between the different temperatures, the apparent viscosities of the actin controls were equated to 1, and the apparent viscosities of the reaction mixtures were normalized by dividing them by the apparent viscosity of actin alone at the same temperature ("normalized" viscosities).

4 and 5). Addition of α-actinin to the reaction mixture increased the viscosity, showing a very strong temperature dependence. At 4°C the increase in viscosity was about 14 times higher than at 25°C (Figs. 4*A* and 5). This temperature dependence had been reported in sedimentation and high-shear viscosity studies (7, 9). The mode of concentration dependence showed a cooperative effect at all temperatures, which is characteristic for all crosslinking proteins or gelation factors (12, 16, 17). Addition of vinculin to the actin solution decreased the viscosity in a concentration-dependent manner (Fig. 4*B*), confirming the kinetic studies under conditions of high shear (Fig. 2*B*). This finding could either reflect a decrease in the rate of polymerization [as in the case of cytochalasin (18, 19) or of actin capping protein (14)] or reflect a parallel alignment (bundling) of actin filaments (which would decrease the degree of network formation), or both. In contrast to the results obtained with α-actinin, the interaction of vinculin with actin showed practically no dependence on the temperature between 4°C and 30°C (Figs. 4*B* and 5).

Morphology. The mode of interaction between actin and α-actinin or vinculin was analyzed microscopically. The $MgCl_2$-induced polymerization of actin (0.5 mg/ml) in the presence of α-actinin (50 μg/ml at 25°C yielded a network with single actin filaments crosslinked sideways by α-actinin, with varying crossover angles, as seen in negatively stained preparations (Fig. 6). The α-actinin molecules acted very efficiently as spacers, so that no filament bundles were observed. In contrast to this, the polymerization of actin in the presence of vinculin (75 μg/ml) resulted in an extensive formation of large bundles (up to several 100 μm in length), which were easily visible in the light microscope (Fig. 7*A*). Low magnification overviews of this effect in KCl-polymerized samples at 25°C are shown (Fig. 7 *B* and *C*). Examination of the same specimens at higher magnification revealed a substructure (Fig. 7*D*) that was very reminiscent of Mg-induced actin paracrystals (20). To determine whether, in addition to its role as a bundling factor, vinculin had any effect on the growth of actin filaments, the mode of actin polymerization onto S1-decorated short fragments was studied (15). In the presence (Fig. 8) of vinculin, the decorated nuclei grew in both directions but faster at the "barbed" than at the "pointed" end (15, 21) and were indistinguishable from control samples without vinculin (data not shown). Thus, the possibility is excluded that vinculin binds to the end of actin filaments and prevents actin monomer addition.

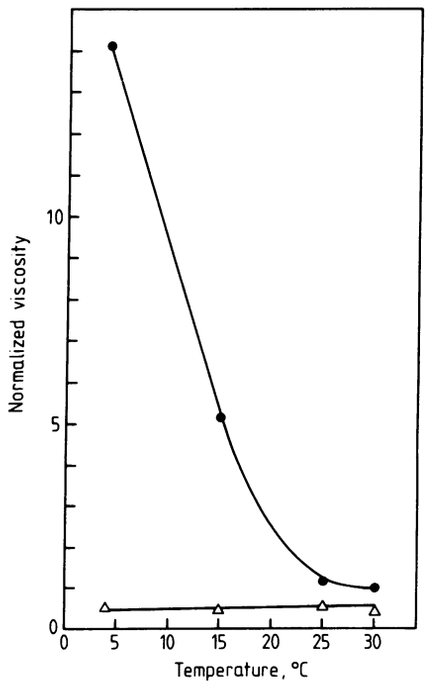

FIG. 5. Temperature dependence of the effect of 2.5 μg of α-actinin (●) or 20 μg of vinculin (△) per ml on the apparent viscosity of Mg-polymerized actin (0.5 mg/ml) as measured in the falling-ball viscometer. Normalized viscosity is explained in Fig. 4.

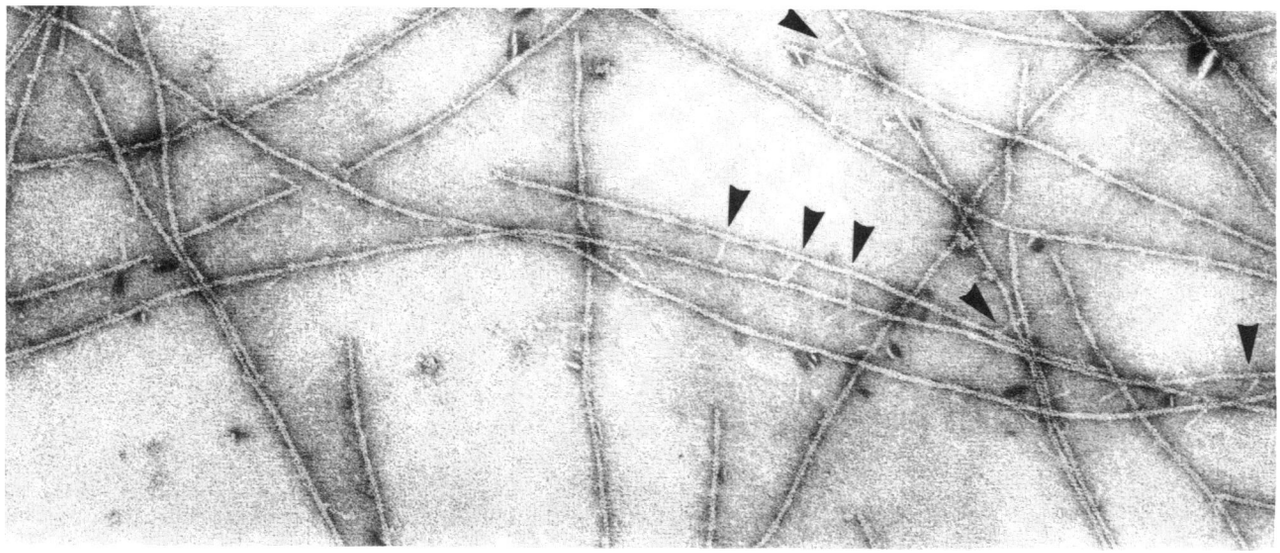

FIG. 6. Negatively stained preparation of actin filaments polymerized by the addition of 2 mM $MgCl_2$, in the presence of α-actinin (50 μg/ml) at 25°C shows the extensive network formation due to crosslinking of individual filaments with α-actinin (arrowheads). (×121,100.)

DISCUSSION

The interaction of α-actinin and vinculin with actin can be observed in conventional high-shear viscometry (Figs. 2 and 3) but is much more dramatically demonstrated in low-shear viscometry, where the more delicate filament interactions (such as web formation and T-junctions) are better conserved (12). The effect of α-actinin on actin viscosity was to be expected from earlier studies, which also showed a strong temperature dependence of the amount of α-actinin bound (7, 9). However, our results do not confirm previous suggestions that α-actinin at temperatures above 20°C might bind preferentially to the end of actin filaments. Our data show that even at 30°C, α-actinin increases the viscosity like a typical crosslinking molecule (12, 16, 17) with two identical binding sites. Only the initial slope of the low-shear viscosity curves, not their cooperative character, is changed with temperature (Fig. 4*A*). The electron micrographs confirm this finding and demonstrate the extensive network formation, in which the long rod-like molecules of α-actinin crosslink and space out individual filaments even at 25°C (Fig. 6). Earlier electron microscopic observations on this interaction were performed at an extremely high Mg concentration (0.1 M), and the electron micrographs revealed only interactions of α-actinin molecules with preformed actin bundles (22).

Our finding that vinculin is capable of bundling actin filaments very effectively into paracrystalline structures (Fig. 7) indicate that vinculin interacts directly with actin. This bundling (which occurs in the absence of Ca) results in a dramatic decrease of the viscosity of F-actin solutions, (Figs. 2*B*, 3, and 4*B*), similar to the effect of Mg-induced paracrystal formation on actin viscosity. In contrast to this, bundling factors have been described which increase the viscosity and form a gel with actin filaments (23). To avoid confusion, we propose here to use the term "bundling factor" only for molecules that induce parallel alignment of actin filaments concomitant with a decrease in viscosity, whereas proteins that increase the viscosity of F-actin like villin (a constituent of the microvillus core that crosslinks actin filaments in the presence of Ca; ref. 23) or fascin (17) should be called "crosslinking proteins" or, more generally, "gelation factors." The effect of vinculin on actin viscosity as measured under conditions of low shear is reminiscent of the results obtained with actin capping protein from *Acanthamoeba* (14); however, in contrast to the capping protein, vinculin does not inhibit growth of S1-decorated filament fragments. Thus, vinculin probably binds laterally to actin filaments.

In the cell, both proteins were localized by antibody binding very close to the cytoplasmic side of the plasma membrane (1, 3, 4, 24, 25). With immunoelectron microscopy, vinculin was closer to the specialized membrane of the junctional complexes of intestinal cells than was α-actinin (5). Together with our findings that these two proteins have antagonistic effects on microfilament network formation *in vitro*, this would imply different functions of these proteins near the plasma membrane. We speculate that vinculin may collect the individual filaments into focal contact points, whereas α-actinin may serve to fan them out at a short distance from the membrane and may help to stabilize them against shear forces at areas of high dynamic activity, such as the leading edge of a moving cell.

Note Added in Proof. While this article was in press, we became aware of a report (26) that shows network formation between α-actinin and actin polymerized from profilactin.

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1. Lazarides, E. & Burridge, K. (1975) *Cell* **6**, 289–298.
2. Schollmeyer, J. E., Furcht, L. T., Goll, D. E., Robson, R. M. & Stromer, M. H. (1976) in *Cell Motility*, Cold Spring Harbor Conferences on Cell Proliferation, eds. Goldman, R. D., Pollard, T. D. & Rosenbaum, J. L. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 3, Book A, pp. 499–511.
3. Geiger, B. (1979) *Cell* **18**, 193–205.
4. Burridge, K. & Feramisco, J. R. (1980) *Cell* **19**, 587–595.
5. Geiger, B., Tokuyasu, K. T., Dutton, A. H. & Singer, S. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4127–4131.
6. Maruyama, K. & Ebashi, S. (1965) *J. Biochem.* **58**, 13–19.
7. Holmes, G. R., Goll, D. E. & Suzuki, A. (1971) *Biochim. Biophys. Acta* **253**, 240–253.
8. Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M. & Stromer, M. H. (1976) *J. Biol. Chem.* **251**, 6860–6870.
9. Goll, D. E., Suzuki, A., Temple, J. & Holmes, G. R. (1972) *J. Mol. Biol.* **67**, 469–488.

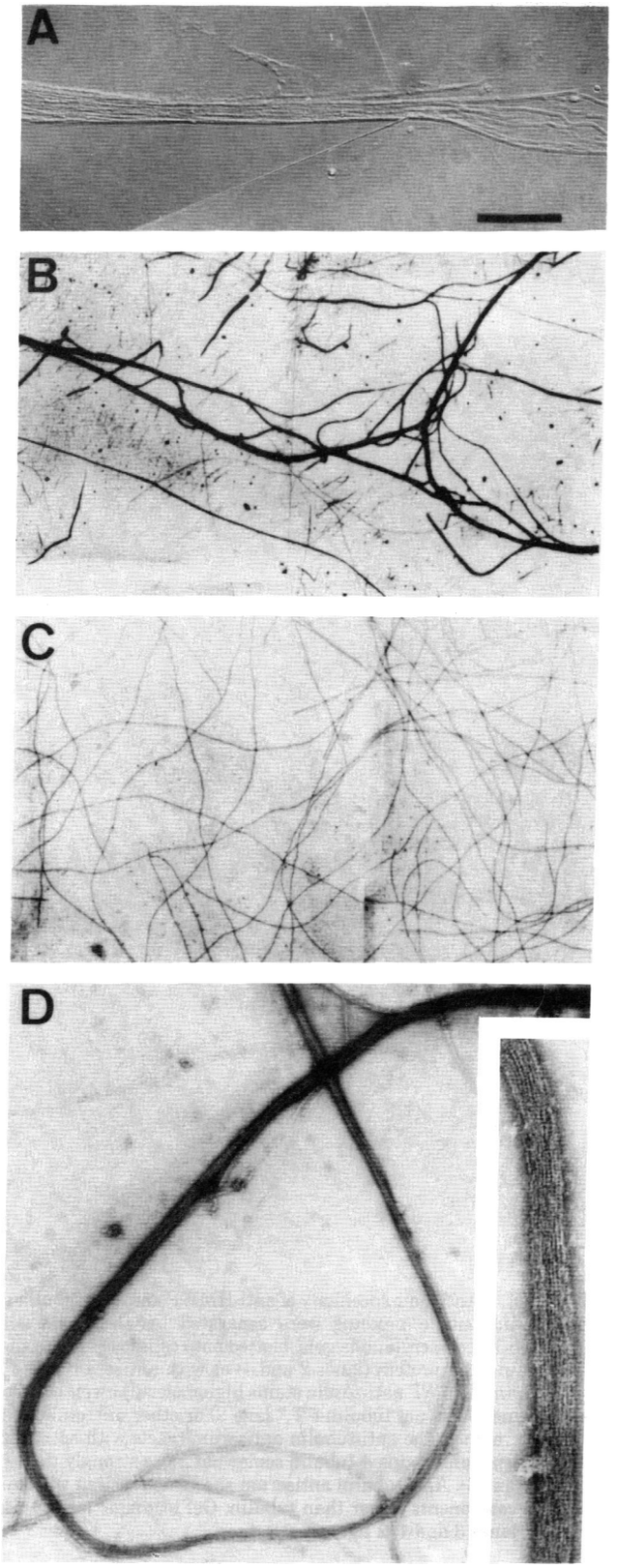

FIG. 7. The effect of vinculin on actin filament bundling. (*a*) Light micrograph of actin (0.5 mg/ml) polymerized in the presence of 20 mM KCl and vinculin (75 μg/ml), as seen in Nomarski interference optics. Bar, 20 μm. (*b*) Identical preparation to *a* seen in negative staining at low electron microscopic magnification (×6350.) (*c*) Control specimen; actin polymerized with 20 mM KCl in the absence of vinculin. (×6350.) (*d*) Higher magnification of a vinculin-induced actin bundle from the preparation in *b*. (×26,650.) (*Inset*) Parallel alignment of actin filaments and a paracrystalline packing. (×102,500.)

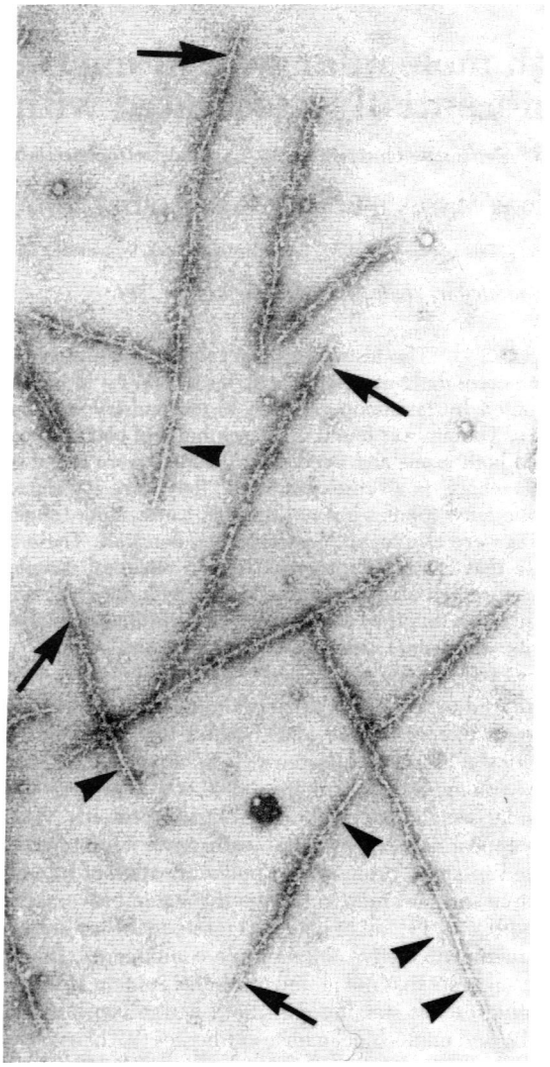

FIG. 8. Negatively stained preparation of actin filaments grown on S1-decorated filament fragments, in the presence of vinculin (75 μg/ml). Growth was faster at the barbed (arrowheads) than at the pointed (arrows) ends. These specimens could not be distinguished from a control without vinculin (not shown). (×80,250.)

10. Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **246**, 4866–4871.
11. Feramisco, J. R. & Burridge, K. (1980) *J. Biol. Chem.* **255**, 1194–1199.
12. Mac Lean-Fletcher, S. D. & Pollard, T. D. (1980) *J. Cell Biol.* **85**, 414–428.
13. Mac Lean-Fletcher, S. & Pollard, T. D. (1980) *Biochem. Biophys. Res. Commun.* **96**, 18–27.
14. Isenberg, G., Aebi, U. & Pollard, T. D. (1980) *Nature (London)* **288**, 455–459.
15. Woodrum, D. T., Rich, S. & Pollard, T. D. (1974) *Biol. Bull.* **147**, 503–504.
16. Brotschi, E. A., Hartwig, J. H. & Stossel, T. P. (1978) *J. Biol. Chem.* **253**, 8988–8993.
17. Bryan, J. & Kane, R. E. (1978) *J. Mol. Biol.* **125**, 207–224.
18. MacLean-Fletcher, S. & Pollard, T. D. (1980) *Cell* **20**, 329–341.
19. Lin, D. C., Tobin, K. D., Grumet, M. & Lin, S. (1980) *J. Cell Biol.* **84**, 455–460.
20. Hanson, J. (1973) *Proc. R. Soc. London Ser. B* **183**, 39–58.
21. Wegner, A. (1976) *J. Mol. Biol.* **108**, 139–150.
22. Podlubnaya, Z. A., Tskhovrebova, L. A., Zaalishvili, M. M. & Stefanenko, G. A. (1975) *J. Mol. Biol.* **92**, 357–359.
23. Bretscher, A. & Weber, K. (1980) *Cell* **20**, 839–847.
24. Geiger, B. & Singer, S. J. (1979) *Cell* **16**, 213–222.
25. Hoessli, D., Rungger-Brandle, E., Jockusch, B. M. & Gabbiani, G. (1980) *J. Cell Biol.* **84**, 305–314.
26. Blikstad, I., Eriksson, S. & Carlsson, L. (1980) *Eur. J. Biochem.* **109**, 317–323.