The Actin Filament Severing Protein Actophorin Promotes the Formation of Rigid Bundles of Actin Filaments Crosslinked with α-Actinin

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Abstract. The actin filament severing protein, *Acanthamoeba* actophorin, decreases the viscosity of actin filaments, but increases the stiffness and viscosity of mixtures of actin filaments and the crosslinking protein α-actinin. The explanation of this paradox is that in the presence of both the severing protein and crosslinker the actin filaments aggregate into an interlocking meshwork of bundles large enough to be visualized by light microscopy. The size of these bundles depends on the size of the containing vessel. The actin filaments in these bundles are tightly packed in some areas while in others they are more disperse. The bundles form a continuous reticulum that fills the container, since the filaments from a particular bundle may interdigitate with filaments from other bundles at points where they intersect. The same phenomena are seen when rabbit muscle aldolase rather than α-actinin is used as the crosslinker. We propose that actophorin promotes bundling by shortening the actin filaments enough to allow them to rotate into positions favorable for lateral interactions with each other via α-actinin. The network of bundles is more rigid and less thixotropic than the corresponding network of single actin filaments linked by α-actinin. One explanation may be that α-actinin (or aldolase) normally in rapid equilibria with actin filaments may become trapped between the filaments increasing the effective concentration of the crosslinker.

SINCE the first descriptions of ameboid locomotion a century and a half ago, the concept of a reversible transformation between "sol" and "gel" has been central to hypothesis attempting to explain the phenomenon. Although it was proposed that the protoplasm is a contractile three-dimensional reticulum as early as 1873 (De Bruyn, 1947), only in comparatively recent times has this reticulum been shown to be mainly an actin-based system (Pollard and Ito, 1970). Subsequent research has revealed that cytoplasmic actin filaments are associated with myosin (reviewed by Korn and Hammer, 1988) and a number of other proteins that regulate actin filament assembly and crosslinking (reviewed by Stossel et al., 1985, and Pollard and Cooper, 1986). Because there are multiple crosslinking proteins in these cytoplasmic actin gels, the physiological function of the individual crosslinking proteins by mutation or gene disruption is difficult to demonstrate (Wallraff et al., 1986; Schleicher et al., 1988). Consequently most of our knowledge about cytoplasmic actin gels has come from in vitro reconstitution with actin and purified individual crosslinkers such as α-actinin.

Alpha-actinin is a major actin crosslinking protein in skeletal muscle and nonmuscle cells. Skeletal muscle α-actinins are calcium-insensitive crosslinking proteins. Some, but not all, α-actinins from smooth muscle and nonmuscle cells are inhibited by calcium. *Acanthamoeba* α-actinin is calcium insensitive but is otherwise a typical α-actinin (Pollard, 1981;

Pollard et al., 1986). A quantitative rheological analysis of gels of *Acanthamoeba* α-actinin and actin filaments (Sato et al., 1987) revealed that the crosslinker increases the viscosity and rigidity of the actin filament network more than 40-fold at high rates of deformation. On the other hand, the presence of crosslinker has little or no effect on the viscosity or rigidity at low rates of deformation. The likely explanation for these observations is that crosslinks rearrange rapidly on the sub-second time scale. This complex behavior in vitro may explain in part why the cell cortex (composed largely of actin filaments and crosslinkers like α-actinin) can be rigid in response to rapidly applied external pressure, yet so changeable in shape under steadily applied pressure as during cytokinesis (Bray et al., 1986). In this study we show that filament length at the time of gelation introduces another variable that contributes counter-intuitively to the physical properties of crosslinked actin filament gels. A brief account of some of this work has been published previously (Pollard et al., 1989).

Materials and Methods

Protein Purification

rhodamine according to the method of Tait and Frieden (1982a). Actophorin was purified from *Acanthamoeba castellanii* exactly as described by Cooper et al. (1986). Alpha-actinin was purified from *Acanthamoeba* by the method of Pollard et al. (1986). Rabbit muscle aldolase (type IV) was purchased from Sigma Chemical Co. (St. Louis, MO) and dialyzed before use.

Viscosity Measurement and Rheometry

Low shear rate apparent viscosity was measured with a miniature falling ball device (Maclean-Fletcher and Pollard, 1980) at 25°C. Quantitative physical measurements were made with an R18 Weissenberg rheogoniometer (Sangamo Controls, Ltd., Bognor Regis, Sussex, England) in the small amplitude, forced oscillation mode as described by Sato et al. (1985). Multiple readings were taken at 0.6 Hz to ensure that the sample was not changing during the experiment, which lasted up to 5 h.

Fluorescence Microscopy

Rhodamine-labeled rabbit muscle actin (Rho-actin) was diluted in unlabeled actin to give a final actin concentration 5 μM, 10% labeled. Actin, actophorin, α-actinin, or aldolase were mixed in buffer G (2 mM Tris, pH 7.5, 0.2 mM ATP, 0.5 mM DTT; 0.2 mM CaCl$_2$, 0.02% NaN$_3$). Immediately after the addition of one tenth volume of 10× polymerization buffer KME (0.5 M KCl, 10 mM MgSO$_4$, 10 mM EGTA, 100 mM DTT, 0.1M Tris, pH 8.0), or ME (same as KME without KCl), the samples were taken up by capillary action into "microslides" (Camlab, Cambridge, England) with an internal thickness of 0.05, 0.1, and 0.2 mm. The "Microslides" were then sealed with "Seal-ease" (Clay Adams, Parsippany, NJ), and incubated at room temperature for more than 2 h before photomicrographs were taken.

Electron Microscopy

Samples containing α-actinin (0.5 μM), actin (5 μM) with and without actophorin (3 μM) were copolymerized in a volume of 50 μl on a porcelain tray in a moistened chamber. After 1 h at room temperature, the samples were overlain with fixative containing 1% gluteraldehyde, 2 mg/ml tannic acid, 100 mM sodium phosphate, pH 7.0, 50 mM KCl, and 5 mM MgSO$_4$ (Maupin and Pollard, 1983), for 30 min. The samples were then washed with the above buffer without gluteraldehyde and fixed again with 0.1% OsO$_4$ in the same buffer for 2 min. Samples were then dehydrated with an ethanol series and embedded in Epon. Sections 50–70 nm thick were viewed in a Zeiss 10A electron microscope at 80 kV.

Results

Falling Ball Viscometry

Although actophorin reduces the apparent viscosity of actin filaments alone (Fig. 1 *A*), when included in mixtures of actin and α-actinin, it can increase the apparent viscosity (Fig. 1 *C*) beyond that of actin and α-actinin (Fig. 1 *B*). This effect is biphasic and depends on the concentrations of all three proteins. Low concentrations of actophorin produce maximum viscosity, while high concentrations reduce the apparent viscosity to less than that of the actin and α-actinin alone.

Actophorin causes a similar biphasic increase (although to a lesser extent) in the apparent viscosity of actin filaments when aldolase is used as the crosslinker instead of α-actinin (Fig. 2 *B*). These experiments were done in a buffer without KCl that inhibits crosslinking by aldolase (Griffith and Pollard, 1982).

Quantitative Rheology

The dynamic viscosity and elasticity measure the resistance of a material to deformation by an oscillating force. The dynamic viscosity is the dependence of that resistance on the rate of deformation and is a characteristic of fluids. The dynamic elasticity is the dependence on the magnitude of deformation, a characteristic of solids, and a measure of "stiffness"
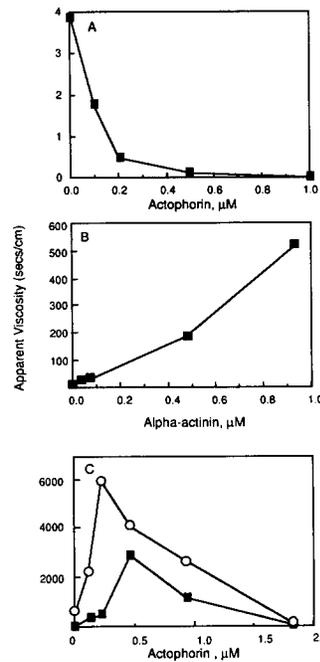

Figure 1. Falling ball viscometry of rabbit muscle actin gels polymerized in the presence of α-actinin and actophorin. Polymerization was initiated by the addition of salts to 50 mM KCl, 1 mM MgCl, 1 mM EGTA at 25°C. (*A*) 10 μM actin and various amounts of actophorin. (*B*) 10 μM actin and various amounts of α-actinin. (*C*) 5 μM actin various amounts of actophorin and α-actinin at 0.47 μM (■) and 0.94 μM (○).

or "rigidity". Viscoelastic materials, such as polymer solutions, have both characteristics, which vary with the frequency of deformation. The data cannot be compared directly to the falling ball results, which are performed at undefined shear rates, and serve as a qualitative measure of viscosity (Maclean-Fletcher and Pollard, 1980; Janmey et al., 1988).

Measurements with a cone and plate rheometer confirm and extend the impressions from the falling ball experiments: the severing protein actophorin can dramatically increase both the dynamic viscosity and the dynamic elastic modulus of mixtures of actin filaments and a cross-linking protein. As reported previously (Sato et al., 1987), α-actinin increases the elasticity (Fig. 3 *A*) and viscosity (Fig. 3 *C*) of actin filaments at high but not low rates of deformation. As expected for a severing protein, actophorin reduces the viscosity (Fig. 3 *D*) and elasticity (Fig. 3 *B*) of actin filaments at all frequencies tested. Paradoxically, mixtures of actin with α-actinin have much higher dynamic viscosity (Fig. 3 *D*) and dynamic elasticity (Fig. 3 *B*) with actophorin than in its absence (Fig. 3, *D* and *B*). The magnitude of the
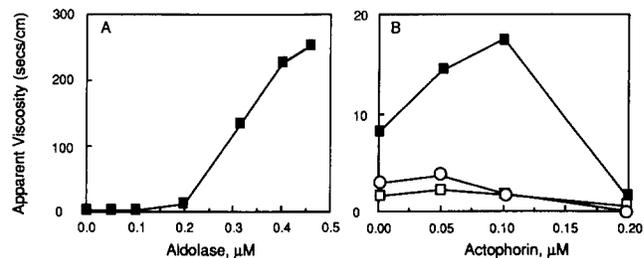

Figure 2. Falling ball viscometry of rabbit muscle actin gels polymerized in the presence of rabbit muscle aldolase and actophorin. Polymerization was initiated by the addition of salts to 1 mM MgCl, 1 mM EGTA at 25°C. (*A*) 5 μM actin various amounts of aldolase. (*B*) 5 μM actin various amounts of actophorin and aldolase at 0.2 μM (■), 0.1 μM (○) and 0.05 μM (□).
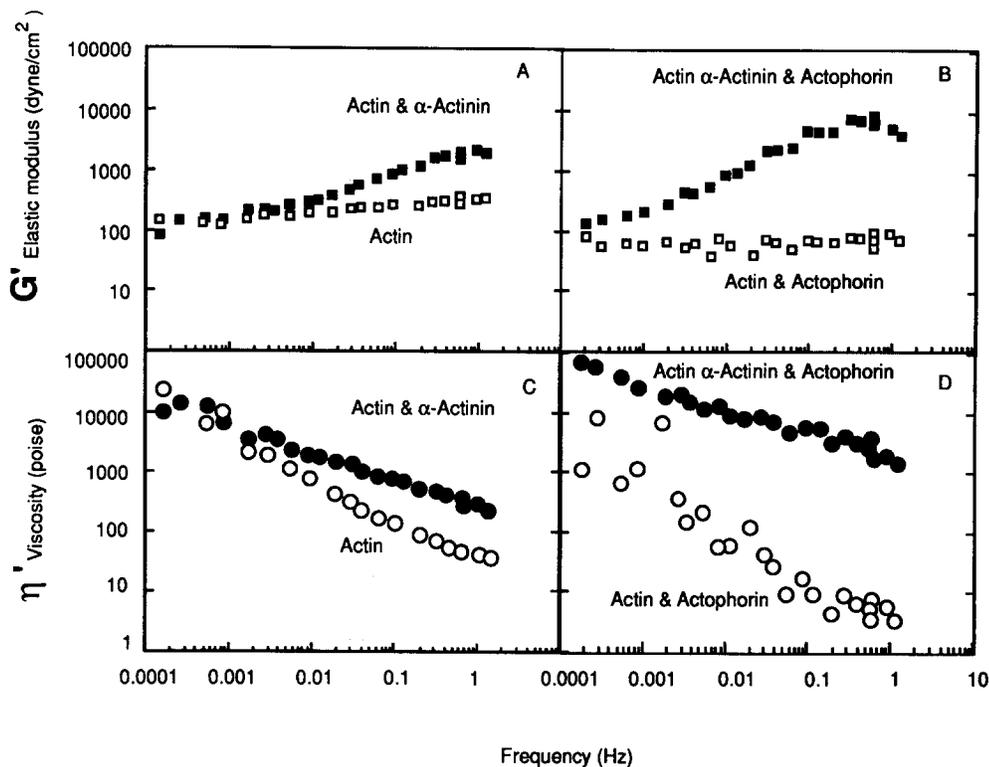
Figure 3. Quantitative rheometric measurement of the dynamic viscosity (o, ●) and elastic modulus (□, ■), as a function of the deformation frequency. The proteins were mixed and polymerization was initiated by the addition of salts to 50 mM KCl, 1 mM MgCl, 1 mM EGTA. (*A*) 10 μM actin (□), or 10 μM actin and 2 μM α-actinin (■). (*B*) 10 μM actin and 5 μM actophorin (□), or 10 μM actin, 2 μM α-actinin, 5 μM actophorin (■). (*C*) 10 μM actin (o), or 10 μM actin and 2 μM α-actinin (●). (*D*) 10 μM actin and 5 μM actophorin (o), or 10 μM actin, 2 μM α-actinin, 5 μM actophorin (●). The slopes of the viscosity curves by linear least squares fit to all the data are: actin, −0.74; actin and α-actinin, −0.46; actin and actophorin, −0.81; actin, actophorin, and α-actinin, −0.39.

difference between the samples with and without actophorin depends on the rate of deformation. At low rates of deformation the values approach those for actin alone. At high frequencies both the viscosity and elasticity of mixtures of the three proteins are 5–10 times higher than for actin and α-actinin, greater than 10 times higher than actin alone and

100 times higher than actin and actophorin. The slope of the viscosity curves without α-actinin were all ~−0.8, consistent with a model of rigid semi-dilute rods (Jain and Cohen, 1981; Doi and Edwards, 1986). The slope of the curves with α-actinin was −0.5, consistent with the model presented in Sato et al. (1987).
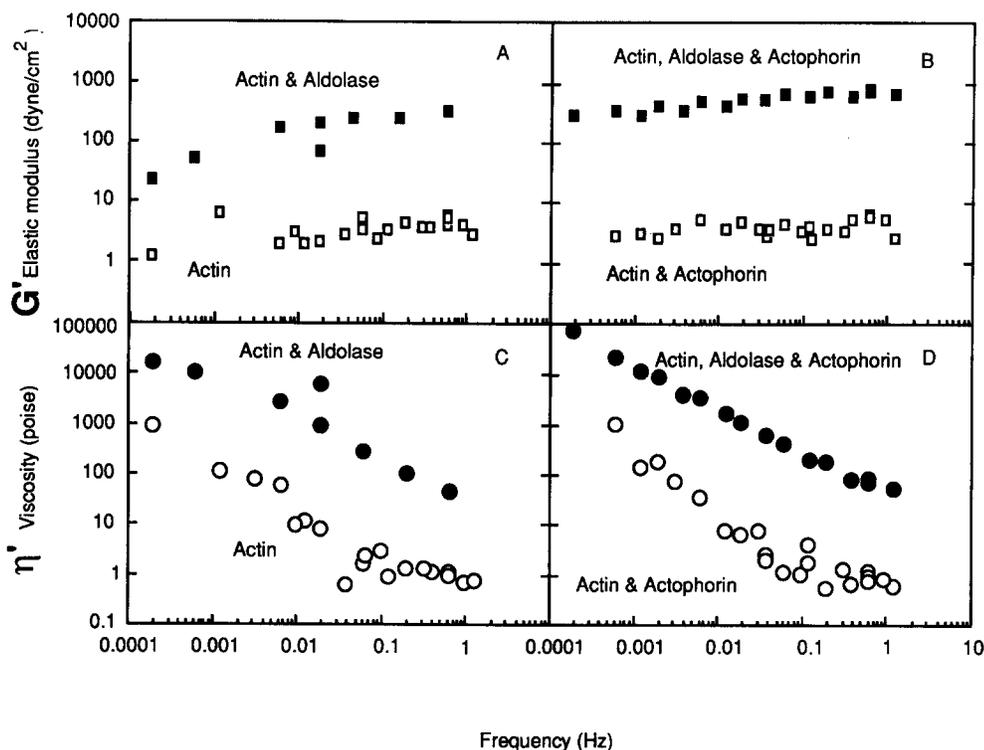

Figure 4. Quantitative rheometric measurement of the dynamic viscosity (o, ●) and elastic modulus (□, ■), as a function of the deformation frequency. The proteins were mixed and polymerization was initiated by the addition of salt to 1 mM MgCl and 1 mM EGTA. (*A*) 10 μM actin (□), or 10 μM actin, and 5 μM aldolase (■). (*B*) 10 μM actin and 0.5 μM actophorin (□), or 10 μM actin, 5 μM aldolase, and 0.5 μM actophorin (■). (*C*) 10 μM actin (o), or 10 μM actin and 5 μM aldolase (●). (*D*) 10 μM actin and 0.5 μM actophorin (o), or 10 μM actin, 5 μM aldolase, and 0.5 μM actophorin (●). The slopes of the viscosity curves by linear least squares fit to all the data are: actin, −0.83; actin and aldolase, −0.76; actin and actophorin, −0.88; actin, actophorin, and aldolase, −0.80.
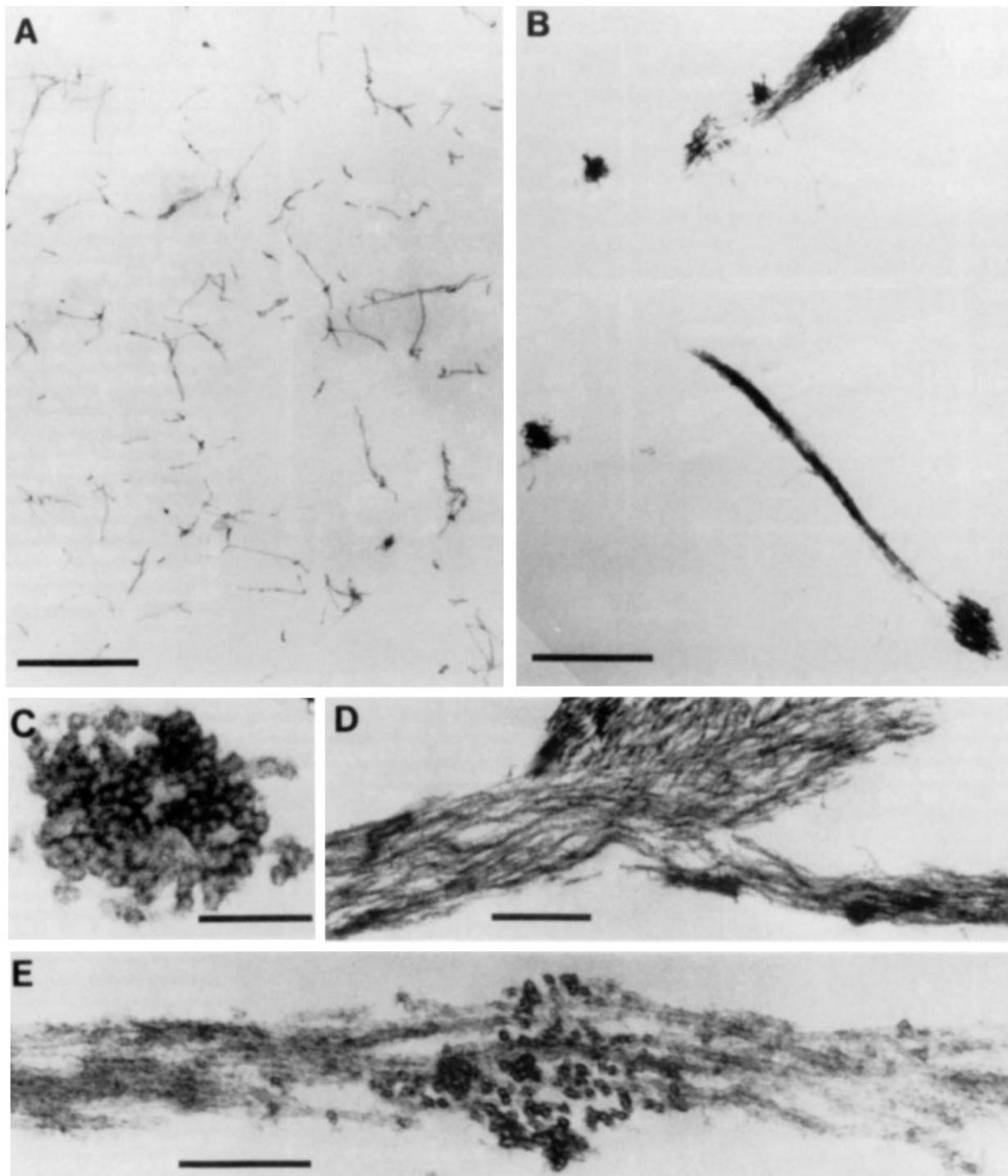
Figure 5. Electron micrographs of thin sections showing the effect of actophorin on gels of actin filaments and α-actinin. Gels were polymerized by the addition of salts as described in Materials and Methods. (*A*) 5 μM actin and 0.5 μM α-actinin showing a random network of filaments. (*B-E*) 5 μM actin, 0.5 μM α-actinin, and 3 μM actophorin. (*C*) High power cross section of a bundle of actin filaments, showing areas of densely packed filaments and less dense areas. (*D*) A junction of three bundles showing individual filaments clearly shared between bundles. (*E*) An intersection between two bundles. The bundle cut in cross section shows "donut" images in the actin filaments coated with tannic acid. Bars: (*A, B,* and *D*) 1 μM; (*C*) 100 nM; (*E*) 250 nM.

To determine whether the unanticipated effect of actophorin plus α-actinin is specific for α-actinin, we did similar experiments with aldolase. In the buffer without KCl both the viscosity (Fig. 4 *C*) and the elastic moduli (Fig. 4 *A*) for actin alone were less than in Fig. 3. Actophorin had a much less

pronounced effect on actin under these conditions (Fig. 4, *B* and *D*), and there was much greater variability in the measurements because the rheogoniometer was run near its lower limits of sensitivity. Aldolase substantially increased the mechanical parameters of actin at all frequencies, unlike
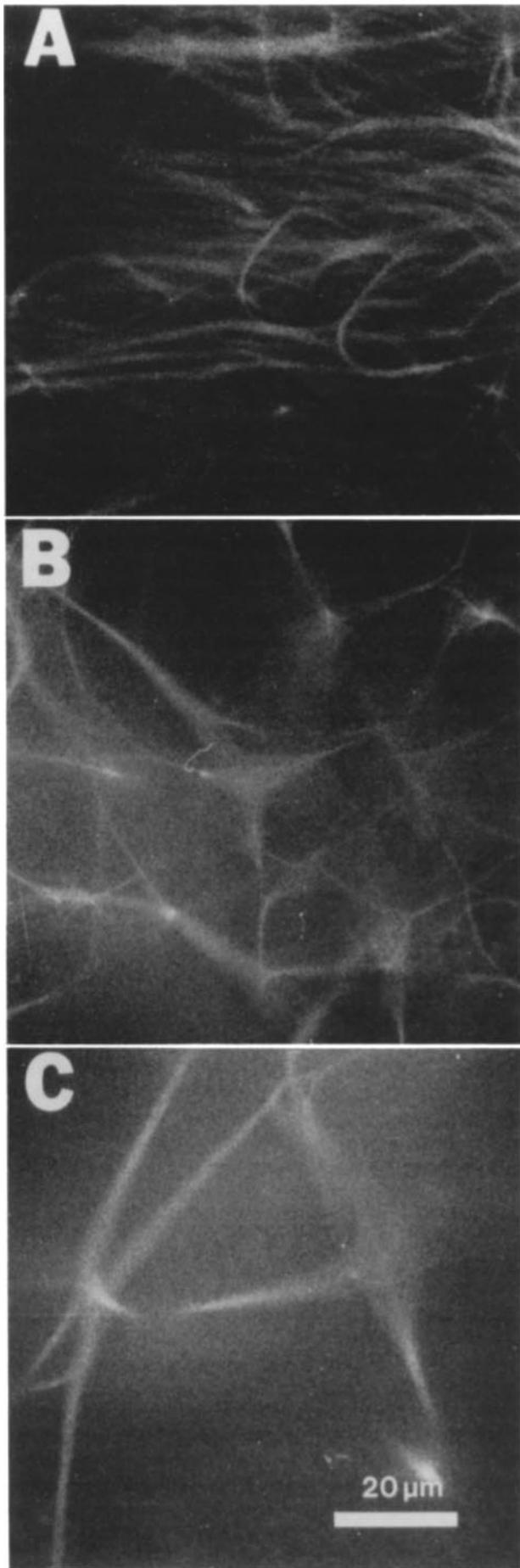
α-actinin, which only increases them at high frequencies (Fig. 3, *A* and *C*). This suggests that aldolase forms less dynamic crosslinks between actin filaments than α-actinin. Mixtures of actophorin, actin, and aldolase have even higher viscosity and rigidity, but the effect of actophorin is smaller with aldolase and actin (Fig. 4) than with α-actinin and actin (Fig. 3).

Structure of Actin Filament Gels

In the absence of actophorin, gels of actin filaments and *Acanthamoeba* α-actinin are homogeneous (Fig. 5 *A*) and optically isotropic (Fig. 18 in Pollard et al., 1982). When the actin is labeled with rhodamine, these gels are homogeneously fluorescent (not shown). The randomly arranged single actin filaments showed no sign of either bundles or other such inhomogeneities (Fig. 5 *A*). Previous experiments with rapidly frozen samples showed that gels of actin filaments and *Acanthamoeba* α-actinin were indistinguishable from actin filaments alone (Pollard et al., 1982).

With concentrations of actophorin that cause the large increase in viscosity and rigidity, the gels of actin and α-actinin are strikingly inhomogeneous, with essentially all of the filaments aggregated into bundles (Fig. 5 *B*). These actin filament bundles are large enough to be seen by light microscopy, where the three-dimensional arrangement of the strands is better appreciated (Fig. 6). Focusing through these networks in the light microscope showed that the strands are connected to each other at foci from which three or four strands radiate.

The length and width of the strands, and the pore size of the network, all depended on the dimensions of the containing vessel. Networks in the 0.05-mm thick tubes (Fig. 6 *A*) were more numerous and smaller than those produced in the larger 0.1-mm tubes (Fig. 6 *B*) which were smaller and more numerous than those produced in the 0.2-mm thick tubes (Fig. 6 *C*). No Brownian motion of these strands was detected at 1,000×. The spaces between the strands appeared empty by fluorescence microscopy (Fig. 6), and few individual filaments were observed by EM (Fig. 5 *B*). These strands were seen to be bundles composed of roughly parallel actin filaments which are arranged very tightly in some areas of the bundles but less tightly in others. No evidence for longitudinal registration between neighboring filaments was observed as has been observed in fascin-containing bundles (Stokes and DeRosier, 1991). Perpendicular sections (Fig. 5 *C*) revealed that some filaments were so close that their tannic acid "halos" were fused. At intersections the filaments from the two bundles interdigitate (Fig. 5, *D* and *E*). EM (Fig. 5 *D*) confirmed the impression from light microscopy that many bundles may radiate from one focus.

At concentrations of actophorin high enough to give low apparent viscosities (Fig. 1), samples of rhodamine actin and α-actinin consisted of a few fluorescent bundles against a high fluorescent background. These bundles were subject to Brownian motion and some were seen to drift.

Figure 6. Fluorescence photomicrograph of an actin gel polymerized in the presence of 5 μM rabbit muscle actin (10% labeled with tetramethylrhodamine) in vessels of various volumes, 0.5 μM α-actinin, and 1 μM actophorin. Width of field is 150 μM. (*A*) Network produced in 0.05 mm thick tube. (*B*) 0.1 mm thick tube. (*C*) 0.2 mm thick tube.

Discussion

We have discovered a paradoxical effect of the actin filament severing protein actophorin: at certain concentrations it increases the rigidity and viscosity of gels composed of crosslinked actin filaments. The result was unanticipated, since actin filament severing proteins, such as actophorin (Cooper et al., 1986) and gelsolin (Janmey et al., 1988; Zaner and Hartwig, 1988) strongly reduce the viscosity of actin filament solutions. Our explanation for the opposite effect on crosslinked actin gels is that the severing protein allows the crosslinker to aggregate the resulting short actin filaments into a meshwork of large bundles which are presumably stiffer than crosslinked networks of single actin filaments. We suggest that this may be one of the major physiological functions of actin filament severing proteins in cells.

Aldolase served as a control actin gelation factor to eliminate the possibility that the viscosity increase was due to an unanticipated interaction between actophorin and α-actin. The results obtained with aldolase were qualitatively similar to those with actophorin and α-actinin. We realize that aldolase alone can bundle but this requires much higher aldolase ratios to actin than we used here (Morton et al., 1977). The binding of aldolase to actin is probably relevant physiologically, since aldolase is known to be associated with the solid phase of cell cytoplasm, probably actin filaments (Pagliaro and Taylor, 1988).

A Proposed Mechanism for the Formation of the "Gel of Bundles"

We propose that actophorin brings about the formation of a "gel of bundles" as a result of its severing activity. In the absence of actophorin, actin filaments grow so rapidly that they quickly become immobilized (Tait and Frieden, 1982b) and are presumably able to make crosslinks with α-actinin only where two microfilaments meet. In this way a homogeneous gel forms. In the presence of actophorin, however, actin filaments are cut as they form (Maciver et al., 1991) and these short filaments are free to diffuse, rotate, and interact laterally with other short filaments via α-actinin, to form bundles of filaments. In these bundles the individual filaments are probably less prone to shortening by actophorin since any severed ends would have a high probability of reannealing, not being free to diffuse from the site. Thermal motion, which is presumed to influence filament severing (Maciver et al., 1991), would also be reduced. These bundles interact with other bundles, or form the platform for further actin polymerization to link the structure into the rigid network of filaments that we observed.

We offer two possible explanations for why a gel of bundles is more rigid than a random network of actin filaments crosslinked by α-actinin. The most likely explanation is that the extent of crosslinking is much higher in the actin filament bundles than in random networks. In bundles, the filaments are much closer together, offering many more sites close enough to be bridged by a crosslinking protein. Furthermore, the aggregation of the actin filaments raises their concentration locally and this should increase the rate of α-actinin binding. This is especially important in the case of *Acanthamoeba* α-actinin which is in such rapid equilibrium with actin filaments that networks resist deformation only at high rates of shear (Sato et al., 1987). In a bundle the crosslinks would still dissociate at their characteristic rate of less than ten per second, but the rate of reassociation would be higher, leading to trapping of α-actinin in the bundles and a higher concentration of crosslinks.

A second factor that may contribute to the rigidity of the gel of bundles is that bundles of crosslinked filaments may respond differently to stress than isometric gels. If a bundle is stressed in a direction perpendicular to its long axis the result is to stretch one side and to compress the other, whereas the same stress on an equivalent mass of isotropic gel may be expected to result in filament displacement, alignment with the direction of the stress (Daniel et al., 1988), and finally rupture.

The notion that crosslinking proteins may become concentrated in bundle arrangements of filaments is supported by the work of Tsukita et al. (1988) who visualized an actin network by fluorescence microscopy using FITC-phalloidin and antibodies to actinogelin. Rhodamine-labeled anti-rabbit IgG showed that actinogelin had indeed become localized to the actin-rich regions. The formation of bundles at high concentrations of α-actinin relative to actin and the consequent entrapment of crosslinker may partially explain the apparent cooperativity in the association of α-actinins with actin filaments (Duhaiman and Bamburg, 1984). At a given actin concentration the formation of bundled filaments rather than isotropic gels does not always produce high viscosity. Brown (1985) observed a reduction in viscosity coinciding with the formation of filament bundles. However, the use of low concentrations of actin (4.1 μM) with relatively high concentrations of gelating factor (0.7 μM) in the absence of a filament severing protein may have led to the formation of unconnected bundles.

Gel vs Bundle Formation

Actin filament crosslinking proteins differ in their tendency to form bundles, it is generally held that crosslinkers which form isotropic gels at low concentrations relative to actin, form bundles at higher concentrations (Pollard and Cooper, 1986; Fig. 4 in Hou et al., 1990). This has been found to be the case for a number of proteins (Schliwa, 1981; Brown, 1985; Hou et al., 1990), however, crosslinkers may differ in their tendency to form bundles due to their affinity for actin filaments and by the geometry of the interaction with the filaments. Actin binding protein (ABP) from macrophages (ABP is similar or identical to filamin) (Hartwig et al., 1980) and 120-kD protein from *Dictyostelium* (Wolosewick and Condeelis, 1986), tend to form perpendicular, rather than lateral branches between filaments and so these crosslinkers do not readily form bundles. Even within the α-actinin group there exists a range of actin filament bundling activity, α-actinin from *Acanthamoeba* and chicken gizzard tending to form bundles at high concentration, while under identical conditions α-actinin from *Dictyostelium* does not (Meyer and Aebi, 1990).

Microheterogeneity has been detected in gels formed under linear shear (Cortese and Frieden, 1988), possibly as a consequence of filaments being bundled by alignment under shear. Interestingly, at concentrations of filamin that induce bundling (filamin:actin 1:50–1:10), these workers found a decrease in translational diffusion coefficients and an increase

in fluorescence anisotropy similar to that induced by shear in the absence of filamin. In the presence of the filament severing protein gelsolin, changes in the fluorescence anisotropy could be detected even at molar ratios of 1:600, suggesting a phenomenon similar to that reported here. Thus, it seems that filament length as well as actin concentration determine whether bundling or gel formation prevails.

Electron micrographs show that the bundles formed in the presence of actophorin and α actinin vary in their order, being close packed in some areas and loose in others. Although we cannot see α actinin in our preparations, we consider the "dynamic" model of α actinin (Meyer and Aebi, 1990) a likely explanation. According to this model, α actinin can bind perpendicularly or in a parallel manner to the actin filaments. This "dynamic" model may explain the time-dependent ordering of filament bundles formed in the presence of fascin (Stokes and DeRosier, 1991).

Physiological Relevance of These Findings

We propose that severing of actin filaments could promote the formation of anisotropic crosslinked structures in cells similar to those that we have observed in vitro. Since actin filaments in cells (Small, 1981; Podolski and Steck, 1990) are considerably shorter than the 5 μm-plus length of filaments polymerized in vitro (Pollard, 1983; Lanni and Ware, 1984; Maciver et al., 1991), it was already clear that severing proteins could contribute to this limitation of length. The current results suggest one potentially important consequence of this severing activity.

In cells there is a spectrum of actin filament anisotropy with generally short filaments arrayed in bundles of various size, interspersed with random networks (Small, 1981). In extreme examples such as the brush border, bundled filaments predominate (Mooseker and Tilney, 1975), whereas isotropic gels and very small bundles predominate in newly produced extensions of the leading lamella of fibroblasts (Small, 1981). The dynamics of actin in these lamella are particularly interesting since the isotropic actin gel is transformed over time into an increasingly bundled gel and is concomitantly infiltrated with myosin (DeBiasio et al., 1988). In the fibroblast, a progression of cytoskeletal arrangements is seen from the newly polymerized actin network at the leading edge (Wang, 1985), to the production of "arcs" (Heath, 1983) composed of small bundles of filaments which are continually swept towards the nucleus where an arrangement of larger perinuclear actin bundles known as the cellular geodome is found. As actophorin-like proteins (Mabuchi, 1983; Bamburg et al., 1980) and gelsolin (another actin filament severing protein), have been isolated from a number of vertebrate sources, it is possible that severing activity in concert with crosslinking proteins also found in protruding lamella (Geiger et al., 1984) form an increasingly anisotropic gel by a mechanism similar to that postulated here.

References

Bamburg, J. R., H. E. Harris, and A. G. Weeds. 1980. Partial purification and characterization of an actin depolymerizing factor from brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 121:178–182.

Bray, D., J. Heath, and D. Moss. 1986. The membrane associated 'cortex' of animal cells: its structure and mechanical properties. *J. Cell Sci. Suppl.* 4:71–88.

Brown, S. S. 1985. A Ca^{2+} insensitive actin-crosslinking protein from *Dictyostelium discoideum. Cell Motility.* 5:529–543.

Cooper, J. A., J. D. Blum, R. C. Williams, Jr., and T. D. Pollard. 1986. Purification and characterization of actophorin, a new 15,000-dalton actin binding protein from *Acanthamoeba castellanii. J. Biol. Chem.* 261:477–485.

Cortese, J. D., and C. Frieden. 1988. Microheterogeneity of actin gels formed under controlled linear shear. *J. Cell Biol.* 107:1477–1487.

DeBiasio, R. L., L. L. Wang, G. W. Fisher, and D. L. Taylor. 1988. The dynamic distribution of fluorescent analogues of actin and myosin in protrusions at the leading edge of migrating swiss 3T3 fibroblasts. *J. Cell Biol.* 107:2631–2645.

De Bruyn, P. P. H. 1947. Theories of amoeboid movement. *Quart. Rev. Biol.* 22:1–24.

Doi, M., and S. F. Edwards. 1986. The theory of polymer dynamics. Clarendon Press, Oxford. 391 pp.

Duhaiman, A. S., and J. R. Bamburg. 1984. Isolation of brain α-actinin. Its characterization and a comparison of its properties with those of muscle α-actinins. *Biochemistry.* 23:1600–1608.

Geiger, B., Z. Avnur, G. Rinnerthaler, H. Hinssen, and V. J. Small. 1984. Microfilament-organizing centers in areas of cell contact: cytoskeletal interactions during cell attachment and locomotion. *J. Cell Biol.* 99:83s–91s.

Griffith, L. M., and T. D. Pollard. 1980. Cross-linking of actin filament networks by self-association and actin-binding macromolecules. *J. Biol. Chem.* 257:9135–9142.

Hartwig, J. H., J. Tyler, and T. P. Stossel. 1980. Actin-binding protein promotes the bipolar and perpendicular branching of actin filaments. *J. Cell Biol.* 87:841–848.

Heath, J. P. 1983. Behavior and structure of the leading lamella in moving fibroblasts. I. Occurrence and centripetal movement of arc-shaped microfilament bundles beneath the dorsal cell surface. *J. Cell Sci.* 60:331–354.

Hou, L., L. Luby-Phelps, and F. Lanni. 1990. Brownian motion of inert tracer macromolecules in polymerized and spontaneously bundled mixtures of actin and filamin. *J. Cell Biol.* 110:1645–1654.

Jain, S., and C. Cohen. 1981. Rheology of rodlike macromolecules in semidilute solutions. *Macromolecules.* 14:759–765.

Janmey, P. A., S. Hvidt, J. Peetermans, J. Lamb, J. D. Ferry, and T. P. Stossel. 1988. Viscoelasticity of F-actin and F-actin/gelsolin complexes. *Biochemistry.* 27:8218–8227.

Korn, E. D., and J. A. Hammer III. 1988. Myosins of non-muscle cells. *Annu. Rev. Biophys. & Biophysical Chem.* 17:23–45.

Lanni, F., and B. R. Ware. 1984. Detection and characterization of actin monomers, oligomers, and filaments in solution by measurement of fluorescence photobleaching recovery. *Biophys. J.* 46:97–110.

Mabuchi, I. 1983. An actin-depolymerizing protein (depactin) from starfish oocytes: Properties and interaction with actin. *J. Cell Biol.* 97:1612–1621.

Maciver, S. K., H. G. Zot, and T. D. Pollard. 1991. Characterization of actin filament severing by actophorin from *Acanthamoeba castellanii. J. Cell Biol.* 115:1611–1620.

MacLean-Fletcher, S. D., and T. D. Pollard. 1980. Viscometric analysis of the gelation of *Acanthamoeba* extracts and purification of two gelation factors. *J. Cell Biol.* 85:414–428.

Maupin, P., and T. D. Pollard. 1983. Improved preservation and staining of Hela cell actin filaments, clatherin-coated membranes, and other cytoplasmic structures by tannic acid-gluteraldehyde-saponin fixation. *J. Cell Biol.* 96:51–62.

Meyer, R. K., and U. Aebi. 1990. Bundling of actin filaments by α actinin depends on its molecular length. *J. Cell Biol.* 110:2013–2024.

Mooseker, M. S., and L. G. Tilney. 1975. The organization of an actin filament-membrane complex: filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. *J. Cell Biol.* 67:725–743.

Morton, D. G., F. M. Clarke, and C. J. Masters. 1977. An electronmicroscopic study of the interaction between fructose diphosphate aldolase and actin-containing filaments. *J. Cell Biol.* 74:1016–1023.

Pagliaro, L., and D. L. Taylor. 1988. Aldolase exists in both the fluid and solid phases of cytoplasm. *J. Cell Biol.* 107:981–991.

Podolski, J. L., and T. L. Steck. 1990. Length distribution of F-actin in *Dictyostelium discoideum. J. Biol. Chem.* 265:1312–1318.

Pollard, T. D. 1981. Purification of a calcium-sensitive actin gelation protein from *Acanthamoeba. J. Biol. Chem.* 256:7666–7670.

Pollard, T. D., and J. A. Cooper. 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu. Rev. Biochem.* 55:987–1035.

Pollard, T. D., and S. Ito. 1970. Cytoplasmic filaments of *Amoeba proteus.* I. The role of filaments in consistency changes and movements. *J. Cell Biol.* 46:267–289.

Pollard, T. D., U. Aebi, J. A. Cooper, M. Elzinga, W. E. Fowler, L. M. Griffith, I. M. Herman, J. Heuser, G. Isenberg, D. P. Kiehart, J. L. S. Maclean-Fletcher, P. Maupin, M. S. Mooseker, M. Runge, P. R. Smith, and P. Tseng. 1982. The mechanism of actin-filament assembly and cross-linking. In Cell and Muscle Motility. Vol 2. Dowben, R. M., and J. W. Shay, editors. Plenum Publishing Corp., New York. 15–44.

Pollard, T. D., K. A. Magnus, S. K. Doberstein, P. Goldschmidt-Clermont, D. A. Kaiser, L. M. Machesky, S. K. Maciver, D. L. Rimm, and D. Wachs-stock. 1989. Structure-function studies of the actin filament system of Acan-thamoeba. In Springer series in Biophysics. Aebi, U., and J. Engel, editors. Vol. 3. Cytoskeletal and Extracellular Proteins. 2nd Intern. Symp. European Biophys. Soc. Gwatt, Switzerland. 271–279.

Sato, M., G. Leimbach, W. H. Schwarz, and T. D. Pollard. 1985. Mechanical properties of actin. J. Biol. Chem. 260:8585–8592.

Sato, M., W. H. Schwarz, and T. D. Pollard. 1987. Dependence of the mechanical properties of actin/α-actinin gels on deformation rate. Nature (Lond.) 325:828–830.

Schleicher, M., A. Noegel, T. Schwarz, E. Wallraff, M. Brink J. Faix, G. Gerisch, and G. Isenberg. 1988. A Dictyostelium mutant with severe defects in α-actinin: its characterization using cDNA probes and monoclonal antibodies. J. Cell Sci. 90:59–71.

Schliwa, M. 1981. Proteins associated with cytoplasmic actin. Cell. 25:587–590.

Small, J. V. 1981. Organization of actin in the leading edge of cultured cells: influence of osmium tetroxide and dehydration on the ultrastructure of actin meshworks. J. Cell Biol. 91:695–705.

Stokes, D. L., and D. J. DeRosier. 1991. Growth conditions control the size and order of actin bundles in vitro. Biophys. J. 59:456–465.

Stossel, T. P., C. Chaponnier, R. M. Ezzel, J. H. Hartwig, P. A. Jamney, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner. 1985. Non-muscle actin binding proteins. Annu. Rev. Cell Biol. 1:353–402.

Tait, J. F., and C. Frieden. 1982a. Polymerization-induced changes in the fluorescence of actin labeled with iodoacetamidotetramethylrhodamine. Arch. Biochem. Biophys. 216:133–141.

Tait, J. F., and C. Frieden. 1982b. Polymerization and gelation of actin studied by fluorescence photobleaching recovery. Biochemistry. 21:3207–3214.

Tsukita, S., N. Mimura, S. Tsukita, K. Khono, T. Ohtaki, T. Oshima, H. Ishikawa, and A. Asano. 1988. Characteristic structures of actin gels induced with hepatic actinogelin or with chicken gizzard alpha-actinin: implication for their function. Cell Motil. Cytoskeleton. 10:451–463.

Wallraff, E., M. Schleicher, M. Modersitzki, D. Rieger, G. Isenberg, and G. Gerisch. 1986. Selection of Dictyostelium mutants defective in cytoskeletal proteins: use of an antibody that binds to the ends of α-actinin rods. EMBO (Eur. Mol. Biol. Organ.) J. 5:61–67.

Wang, Y.-L. 1985. Exchange of actin subunits at the leading edge of living fibroblasts: a possible role of treadmilling. J. Cell Biol. 101:597–602.

Wolosewick, J. J., and J. Condeelis. 1986. Fine structure of gels prepared from an actin binding protein and actin: comparison to cytoplasmic extracts and cortical cytoplasm in amoeboid cells of Dictyostelium discoideum. J. Cell Biochem. 30:227–243.

Zaner, K. S., and J. H. Hartwig. 1988. The effect of filament shortening on the mechanical properties of gel-filtered actin. J. Biol. Chem. 263:4532–4536.