Regulation of water flow by actin-binding protein-induced actin gelation

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ABSTRACT Actin filaments inhibit osmotically driven water flow (Ito, T., K.S. Zaner, and T.P. Stossel. 1987. *Biophys. J.* 51:745–753). Here we show that the actin gelation protein, actin-binding protein (ABP), impedes both osmotic shrinkage and swelling of an actin filament solution and reduces markedly the concentration of actin filaments required for this inhibition. These effects depend on actin filament immobilization, because the ABP concentration that causes initial impairment of water flow by actin filaments corresponds to the gel point measured viscometrically and because gelsolin, which noncovalently severs actin filaments, solates actin gels and restores water flow in a solution of actin cross-linked by ABP. Since ABP gels actin filaments in the periphery of many eukaryotic cells, such actin networks may contribute to physiological cell volume regulation.

INTRODUCTION

Cells regulate their volumes primarily by adjusting membrane permeability to water and ions (Hoffman and Simonsen, 1989). Another dimension worth considering is that actin filament networks, which invest the periphery of many eukaryotic cells and contribute to cell rigidity (Stossel et al., 1987), also play a role in the reaction of cells to osmotic forces, because polymer gels have well characterized elastic responses to osmotic stress (Tanaka,1981). To test this idea we previously demonstrated that actin filaments (F-actin) retarded osmotically-driven water flow across a semipermeable membrane (Ito et al., 1987).

Two mechanisms appeared to be responsible for this inhibitory effect. One, evident at relatively low actin concentrations, was proportional to the F-actin mass but was independent of the polymer length, varied by reacting actin filaments with the actin filament severing protein gelsolin. These findings suggested that the elastic properties of individual filaments and not interfilament interactions accounted for the osmotic resistance. We proposed that water flow dissipates some energy imposed by an osmotic force but that the residual energy deforms filaments to produce an elastic pressure which decreases the chemical potential difference of water across the membrane, thereby reducing the driving force for water flow. The second mechanism was operative above a critical concentration at which actin filaments predictably become immobilized (Edwards and Evans, 1982), and water flow completely stopped in the setting of the same osmotic stress that only slowed flow at lower F-actin concentrations. Severing the filaments with gelsolin restored flow, suggesting that the basis for this type of water flow inhibition involved immoblization of actin filaments by steric interpenetration.

The cellular actin network is not simply a system of entangled long filaments but rather contains relatively short filaments connected noncovalently in various arrangements by actin cross-linking proteins (Hartwig and Kwiatkowski, 1991). One actin cross-linker, actin-binding protein (ABP), is a high molecular weight homodimer that promotes the perpendicular branching of F-actin, thereby serving as an efficient actin gelation protein (Hartwig and Stossel, 1981; Gorlin et al., 1990). Gels of actin cross-linked by ABP have a high elasticity, reminiscent of covalently cross-linked synthetic polymers (Janmey et al., 1988, 1990). We were therefore interested in examining the effect of ABP on the osmotic behavior of F-actin and report the results of a study of this interaction in this paper.

MATERIALS AND METHODS

We purified actin from rabbit skeletal muscle and gelsolin from human plasma by the methods of Spudich and Watt (1971) and Chaponnier et al. (1986), respectively. To prepare ABP we ground 20 g of freshly obtained human uterus, surgically excised because of leiomyomata, in a Waring blender at ice temperature with four volumes of a solution containing 0.6 M KCl, 10 mM EGTA, 1 mM DTT, 0.5 mM ATP, 10 mM imidazole-HCl, pH 7.5, 0.3 mM PMSF, and 0.1 mg/ml aprotinin. After centrifugation of the homogenate at 140,000 g for 1 h at 4°C, we added one volume of saturated ammonium sulfate solution to the supernatant fluid and dissolved the resulting protein precipitate with the smallest possible volume of a solution identical to the homogenizing fluid except that it contained 1.2 M KI instead of 0.6 M KCl. We applied 10 ml of the solubilized precipitate to a 1.5 × 100 cm column of

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4% Bio-Rad agarose (Bio-gel A-15, 200–400 mesh), the bottom nine-tenths of which was equilibrated with the homogenizing solution and the top tenth with the sample solution. We assayed fractions eluted with homogenizing solution for actin gelation activity according to Yin et al. (1980), pooled 60 ml of active fractions, and dialyzed the protein twice against 3 L of 10 mM sodium phosphate buffer, pH 7.5, containing 0.1 M KCl, 0.5 mM DTT, 0.3 mM PMSF, and 0.5 mM EGTA, clarified the solution by centrifugation, and stored it frozen in lots in liquid nitrogen. ABP retains qualitatively similar actin gelation activity after storage for months, although we have not made a detailed study to compare simultaneously fresh ABP with frozen and thawed ABP.

We measured the apparent viscosity of actin solutions as the time required for a stainless steel ball to fall 2 cm in a micro-capillary tube (MacLean-Fletcher and Pollard, 1980). We used the same procedure as previously described to measure volume flow (Ito et al., 1987) with minor modifications. We dissolved freshly thawed ABP, G-actin, and gelsolin at appropriate molar ratios in 1.5 ml of buffer G (0.2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM ATP, 2 mM Tris-HCl, pH 7.5, 20 mg/ml 40 kD dextran, 5 μg/ml leupeptin, and 5 μg/ml aprotinin) and introduced the protein solution into the inner compartment of the Zimm-Myerson osmometer described in Ito et al. (1987). It was then placed in the temperature-controlled outer compartment of the device which was separated from the inner compartment by a Schleicher & Schuell (Keene, NH) cellulose membrane with a nominal protein retention of 20 kD and which contained 500 ml of buffer F (100mM KCl, 2 mM $MgCl_2$, 0.2 mM ATP, 1 mM EGTA, 10 mM imidazole-HCl, pH 7.5) with or without 20 mg/ml dextran and left standing for at least 4 h at room temperature. During this time magnesium and potassium ions entered the inner compartment and induced actin polymerization. We then imposed a positive or negative osmotic stress on the inner compartment by adjusting the dextran concentration in the outer compartment and noted changes in volume flow by observing the meniscus height over time inside a 25-cm long capillary in continuity with the inner compartment.

We represent volume flow (J_v) by the expression

$$J_v = L_p P_f,$$

where P_f is the osmotic stress and L_p the filtration coefficient of the membrane. The osmotic stress, $P_f = \Delta P_h - RT\Delta C$, where ΔP_h is the hydrostatic pressure difference, ΔC is the dextran concentration difference between the compartments, R the gas constant, and T the absolute temperature. A positive value of P_f designates shrinkage of the actin solution, a negative value indicates swelling. P_f intensities obtainable by this experimental setup ranged from 65 to −26 cm H_2O. L_p has a value characteristic of the membrane and was 4.7 $cm^5/dyn\ s$.

RESULTS

Fig. 1 shows that negative and positive osmotic stresses induced swelling and shrinkage, respectively, of a 2 mg/ml actin solution and that assembly of actin in the presence of a substoichiometric ABP concentration inhibited these changes. Fig. 2 demonstrates the concentration dependence of actin polymerized in the presence or absence of ABP on water flow expressed as the parameter ϵ, representing the inhibitory effect of actin on water flow which is derived from the expression

$$J_v = L_p(1 - \epsilon)P_f,$$

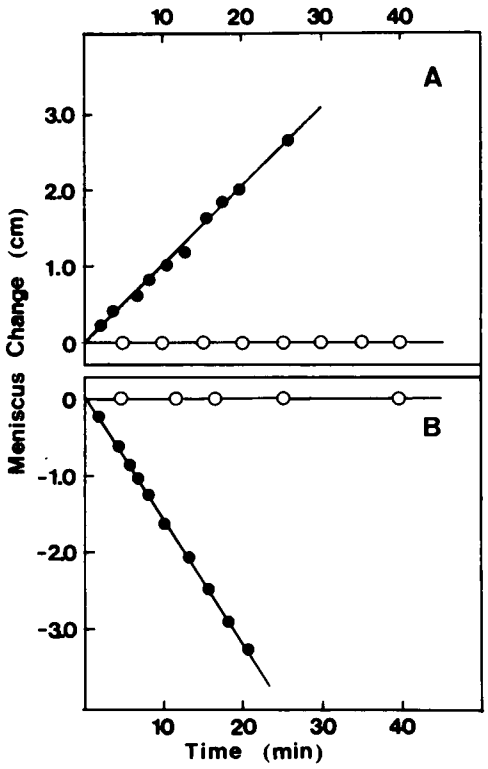

FIGURE 1 Inhibition of volume flow of F-actin solutions by ABP. G-actin, 2 mg/ml, was polymerized in the absence (*closed circles*) or presence (*open circles*) of 0.3 mg/ml (*A*), or 0.5 mg/ml (*B*) of ABP in the osmometer, different osmotic stresses were applied (−26 cm H_2O in *A*, + 64 cm H_2O in *B*), and the change in height of the capillary meniscus was plotted over time.

and which becomes unity when water flow ceases. ABP reduced markedly the concentration of F-actin required for complete obstruction of water flow. In the case of F-actin alone at sufficiently high concentrations to stop water flow, an osmotic stress greater than 20 cm of H_2O restores flow (Ito et al., 1987), but the highest osmotic stress applicable with the experimental system, 65 cm H_2O, did not produce flow in a solution of 2 mg F-actin polymerized in the presence of ABP. Fig. 3 correlates the ABP concentrations required for gelation and alteration of ϵ by a fixed concentration of actin, and shows that abrupt increases in both viscosity of F-actin and the ϵ value occurred at an ABP/actin molar ratio of ~1:700. These results strongly support the role of actin network formation in the cessation of water flow.

In addition to the density of inter-filament cross-links, filament length is an important determinant of actin gelation by ABP (Yin et al., 1980; Hartwig and Stossel, 1981). Fig. 4 depicts some effects of gelsolin-induced filament length changes on the viscosity and osmotic properties of actin cross-linked by ABP. We set the

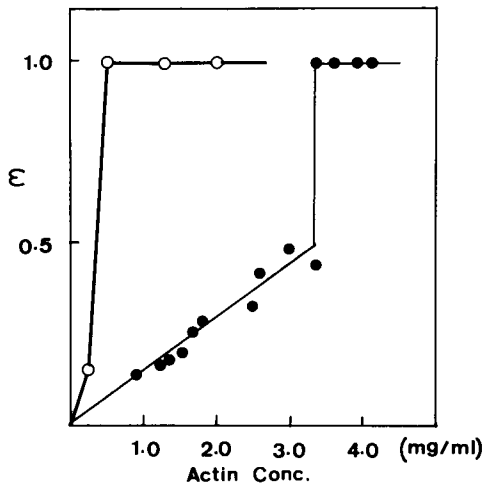

FIGURE 2 Dependence of ϵ, derived from the slope of P_f versus J_v, on different F-actin concentrations polymerized with (*open circles*) or without (*closed circles*) ABP, present at a molar ratio of 1: 72.

average actin filament length at two values by polymerizing actin in the presence of two different gelsolin concentrations and activated gelsolin by addition of calcium (Yin et al., 1980; Janmey and Stossel, 1986). As actin filaments grew from gelsolin nuclei, the ABP dimers present cross-linked them. Fig. 4 *C* shows that the viscosity of actin and ABP polymerized with the lowest gelsolin concentration was high and that this actin solution inhibited osmotic water flow in the presence of ABP, whether or not calcium was added to activate the gelsolin. Fig. 4 *A* demonstrates, however, that addition of calcium-activated gelsolin at this concentration to a preformed actin-ABP network lowered its viscosity and restored water flow. The higher gelsolin concentration prevented ABP from raising the viscosity of actin or inhibiting water flow, and this effect depended on gelsolins being activated by calcium (Fig. 4 *B*).

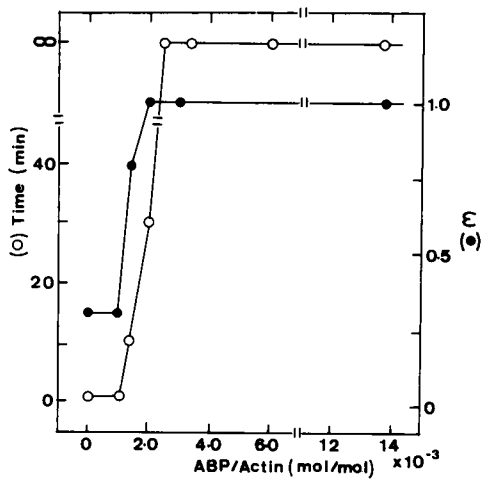

FIGURE 3 Effect of different ABP concentrations on ϵ (*closed circles*) and on the apparent viscosity (*open circles*) of 2 mg/ml F-actin.

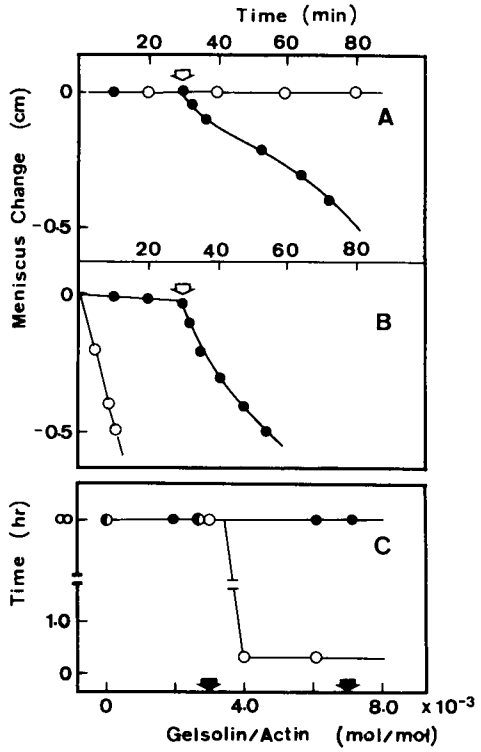

FIGURE 4 Effects of gelsolin and calcium on osmotic stress-induced volume flow and on the apparent viscosity of actin assembled with ABP. In *A* and *B*, 2 mg/ml G-actin were polymerized in the osmometer; the molar ratios of actin/ABP/ gelsolin were 1 : 0.014: 0.003 in *A*, and 1 : 0.014 : 0.007 in *B*, respectively. The solution in the outer compartment of the osmometer was Buffer F. (*closed circles* in *A* and *B*) or Buffer F containing 1 mM $CaCl_2$ (*open circles* in *A* and *B*); all solutions contained 5μg/ml aprotinin and 5 μ/ml leupeptin. After actin polymerization was complete, 500 μl of 1 M $CaCl_2$ added to the outer compartment at the times indicated by the arrows. In *C*, 2 mg/ml G-actin was polymerized in Buffer F with ABP (ABP/actin = 1 : 72) and with gelsolin at the indicated molar ratios in the presence (*open circles*) and absence (*closed circles*) of calcium, and the apparent viscosities were measured. The arrows on the abcissa indicate vlaues of actin/gelsolin, and are 0.003 (*left*) and 0.007 (*right*), respectively.

DISCUSSION

Differently constructed actin networks have, despite the superficial similarity of relatively immobile actin filaments, very different mechanical properties (Janmey et al., 1988), and we show here that they also can have variable osmotic behavior. ABP reduced several-fold the

concentration of F-actin required to stop water flow and increased the osmotic stress required to induce flow. The potency of ABP in inducing actin network formation is attributable to its ability to promote the perpendicular branching of actin filaments, which is an efficient way to recruit a maximal number of rodlike chains into a three-dimensional network using a minimal number of crosslinks (Hartwig and Stossel, 1981).

We purified the ABP protein for the experiments described here from human uterine smooth muscle, and this ABP has functional gelation activity qualitatively similar to that reported for ABP purified from rabbit lung macrophages, human blood platelets, and toad oocytes, but greater than that of chicken gizzard filamin, an ABP-related protein. The lower gelation activity of gizzard filamin (Brotschi et al., 1978) is not simply due to its origin from smooth muscle. The smooth muscle ABP from human uteri forms highly elastic actin gels (Janmey et al., 1990) and yields one-dimensional cyanogen bromide peptide maps more similar to human platelet ABP than to chicken gizzard filamin (Hock et al., 1990). Chicken gizzard filamin may be a unique isoform of ABP with properties different from many other cytoplasmic and smooth muscle ABPs.

The experiments described in Fig. 3 show that inhibition of water flow by F-actin follows the same critical concentration dependence on actin filament cross-link (ABP) density, as does three-dimensional network formation as defined by an abrupt increase in solution viscosity. The experiments depicted in Fig. 4, however, illustrate both the network solvation effect of shortening filament length and importance of kinetic factors in network assembly. The density of ABP combined with actin in the absence of gelsolin was sufficient to induce actin gelation and to inhibit water flow. Polymerization of actin with a low but not high gelsolin concentration shortened the average filament length distribution sufficiently to prevent their being tied into a complete network by the number of ABP molecules available. The concentration of gelsolin that was too low to prevent ABP from inducing an actin network during actin polymerization was capable of destroying a preformed ABP-actin network after addition of calcium, which activated the gelsolin molecules to sever filaments between cross-link points.

The effect of ABP on actin gelation and water flow have certain physiological implications. The structure of actin gels induced by ABP in vitro resembles the architecture of actin in the cell cortex (Hartwig and Shevlin, 1986), although the actin concentration practical for experiments performed in vitro, such as those described here, are much lower than the 10–20-mg/ml value in the cell. The ABP-based actin networks in the cell periphery could therefore constitute a relatively strong buffer against cell swelling or shrinkage by small fluctuations in osmotic pressures that constantly affect cells of mammalian organisms. These fluctuations, in contrast to the more drastic changes accommodated by membrane ion-channel cell controlled volume regulation (Hoffman and Simonsen, 1988), have been estimated in the 10 mOsm range. This report suggests that the ABP-based actin network could resist such stresses without accompaniment of water flow across the cell membrane.

The resistance of the ABP-based actin network to water flow also has implications for mechanisms by which cells extend protrusions from the cell surface. One theory to explain such membrane extension invokes osmotic swelling of an actin gel after severing of actin filaments by gelsolin (Oster and Perelson, 1987). This mechanism requires that water flows from outside the cell across the membrane into the locally expanding region of the disaggregating actin cortex. Unless the regions of the cortex neighboring this swelling domain can resist water flow, however, the osmotic gradient would dissipate before the membrane could expand. Our results indicate that the ABP-based actin gel in the cell cortex could provide this resistance to water flow.

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