Action of a 19K Protein from Porcine Brain on Actin Polymerization: 
A New Functional Class of Actin-Binding Proteins¹

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A 19K protein isolated from porcine brain not only inhibits actin polymerization but depolymerizes actin filaments quickly. The protein reacts stoichiometrically with actin in a 1 : 1 molar ratio. When actin is induced to polymerize with salts in the presence of the brain 19K protein, the lag phase is prolonged, and the extent of polymerization is decreased, but the half-polymerization time is not increased. This can be explained by assuming that the 19K protein severs growing actin filaments and thus causes an increase in the number of filament ends during the polymerization process, thereby accelerating the overall polymerization. Moreover, the low-shear viscosity of actin filaments is reduced much more than the high-shear viscosity by the 19K protein, suggesting that actin filaments become shorter in the presence of the 19K protein than in its absence. Actin filament depolymerization by the 19K protein is much faster than that by brain profilin or than spontaneous depolymerization. This indicates that the 19K protein depolymerizes actin filaments not only by sequestering actin monomers but also by directly attacking the filaments. The number of actin filaments, measured by assaying the nucleating ability, is increased by substoichiometric concentrations of the 19K protein, irrespective of whether the protein is added to actin monomers before polymerization or added to preformed actin filaments. These results suggest that the brain 19K protein not only stabilizes actin monomers but also cuts actin filaments, thereby decreasing the extent of actin polymerization and also changing the filament length. The action on actin of the actin-depolymerizing protein from starfish oocytes resembles that of the brain 19K protein, although the molecular weight of the starfish protein is slightly smaller. The brain 19K protein and starfish protein should be classified into a new functional group of actin-binding proteins.

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Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid); DTT, dithiothreitol; EGTA, ethyleneglycol-bis(2-aminoethyl-ether)-N,N',N"-tetraacetic acid; MES, 2-(N-morpholino)ethanesulfonic acid.
There have been many reports describing the identification, purification, and characterization of actin-binding proteins from a variety of non-muscle cells. These proteins can be classified into essentially three functional groups: actin cross-linking proteins, actin filament capping or severing proteins that regulate the filament size, and actin depolymerizing proteins that stabilize the actin monomer pool (1-3). Profilin (4-6), DNase I (7, 8), vitamin D-binding protein (9) (and serum proteins (10)), and an echinodermatous actin depolymerizing protein (11) are included in the third class. Although detailed analyses of the mechanism of the action of these proteins have not been carried out except for Acanthamoeba profilin (5, 6), it has been thought that the difference in their action on actin is solely due to the difference in the affinity of the proteins for G-actin, and that these proteins do not sever actin filaments or change the distribution of filament length.

In the preceding paper (12), we reported the purification of several G-actin-binding proteins from porcine brain; 26K, 21K, and 19K proteins, and profilin. Here, we characterize the action of the 19K protein on actin polymerization, and demonstrate that the protein not only inhibits actin polymerization but also depolymerizes actin filaments quickly by reacting with actin in a 1:1 molar ratio. Furthermore, we present evidence that the protein severs actin filaments. In addition, we show that a starfish actin depolymerizing protein acts similarly on actin.

**MATERIALS AND METHODS**

*Preparation of Proteins*—A 19K protein and brain actin were isolated from porcine brains as described in the preceding paper (12). Rabbit skeletal muscle actin was prepared by the method of Spudich and Watt (13). Both brain and muscle actins were purified by gel filtration on Sephadex G-100 equilibrated with 2 mM HEPES-0.1 mM CaCl₂-0.2 mM ATP-0.01 % NaN₃ (pH 8.0) (buffer A). An echinodermatous actin depolymerizing protein was isolated from starfish oocytes by the method described previously (14). Fodrin was prepared from porcine brains by a modification of the method of Glenney et al. (15).

*Assays for Actin Polymerization*—Actin polymerization was followed by measuring light scattering (16), absorbance change at 237 nm (17, 18), or high-shear viscosity. Light scattering at 500 nm was measured in a temperature-controlled cuvette chamber using a Hitachi 650-10S fluorescence spectrophotometer. Absorbance change at 237 nm which accompanies the G-F transformation of actin was measured with a Gilford 260 spectrophotometer in a temperature-controlled cuvette chamber. High-shear viscosity was assayed with an Ostwald-type viscometer.

A fluorescence assay was also used to monitor the G-F transformation of actin. Modification of muscle actin with N-pyrenyl iodoacetamide was carried out by the improved method (19) of Kouyama and Mihashi (20). This pyrene-labeled actin was gel-filtered on Sephadex G-100 as above before use. The protein concentration of the pyrene-labeled actin was determined by the method of Lowry et al. (21), using unmodified actin as a standard. The actin contained 1.0 mol of pyrene/mol of actin. Since the fluorescence of pyrene-labeled actin increases markedly when it polymerizes, the fluorescence measurement is a very sensitive assay method for actin polymerization. Many experiments have demonstrated the validity of this method (19, 20, and our unpublished data). Fluorescence was measured in a temperature-controlled cuvette chamber using a Hitachi 650-10S fluorescence spectrophotometer. The excitation and emission wavelengths were 365 nm and 407 nm, respectively. In every assay, monomeric actin was added last to the solution to initiate actin polymerization (at zero time), and then each measurement was begun.

*Others*—Low-shear viscosity was assayed in a falling ball device (22). Polyacrylamide gel electrophoresis in the presence of SDS was carried out by the method of Laemmli (23). Protein concentration was determined by the method of Lowry et al. (21) using bovine serum albumin as a standard.

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Our viscometer is a spiral glass capillary, which has a long total length and a small diameter. Therefore, the shear rate is rather low when the sample falls, as compared with usual Ostwald-type viscometers. This makes the specific viscosity of actin filaments two- to three-fold higher than usual values. On the other hand, when the sample is drawn up, the actin filaments are subjected to a higher shear force than usual and are cut more frequently. Thus, in our viscometer, actin attains the steady-state faster than in usual viscometers.

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ard. Actin concentration was determined by UV absorption measurement based on $A_{280}^{1%} = 6.5$.

RESULTS

Stoichiometric Action of Brain 19K Protein on Actin—Figure 1 shows the effect of brain 19K protein on the time course of brain actin polymerization in KCl + MgCl$_2$, measured by light scattering (the intensity is proportional to the mass concentration of actin filaments and is insensitive to filament length). The 19K protein inhibited the extent of actin polymerization in the steady state in a concentration-dependent manner such that the amount of actin filaments is inversely proportional to the protein concentration (Fig. 1, inset). From the result shown in Fig. 1 and the value of the critical concentration (0.1 μM actin) under these conditions, it was calculated that 1 mol of the 19K protein produced 0.8 mol of unpolymerized actins, which indicates a 1:1 stoichiometry between the protein and actin.

The effect of the 19K protein on F-actin viscosity is shown in Figs. 2 and 3B, which demonstrate that the protein decreased the viscosity of F-actin very quickly both in KCl + MgCl$_2$ (Fig. 2) and in KCl alone (Fig. 3). Figure 2 shows further that the activity of the protein was heat-labile and did not require Ca$^{2+}$. Addition of the protein to F-actin induced a rapid decrease in viscosity within 3 min and then the viscosity increased gradually and attained a new steady-state level. The steady-state viscosity was inversely proportional to the concentration of the 19K protein (Figs. 2B and 3B inset). From these results and the critical actin concentrations under these conditions (0.1 μM actin in KCl + MgCl$_2$ and 0.7 μM actin in KCl alone), it was suggested in both cases that 1 mol of the 19K protein depolymerized about 0.8 mol of actin monomer present in F-actin. The same stoichiometry was obtained when the 19K protein was added to actin monomers before polymerization (Fig. 3A).

4 The critical actin concentration for polymerization was determined by polymerizing various concentrations of actin to the steady state and plotting the extent of the absorbance change at 237 nm or the light scattering intensity at 500 nm against actin concentration, then extrapolating this line to zero F-actin concentration.

Concomitant with the decrease in the viscosity, sedimentable actin filaments were also reduced in the presence of the 19K protein, as shown in Fig. 4. The SDS-gels in Fig. 4 further demonstrate that the 19K protein remained in the supernatant.

These results seem to indicate that the 19K protein depolymerizes F-actin. However, the reductions in the viscosity and the sedimentability do not necessarily represent net depolymerization of F-actin. They might result from the fragmentation of actin filaments (see the next section). To investigate whether the 19K protein can induce the net depolymerization, we adopted the fluorescence assay using pyrene-labeled actin. As shown in Fig. 5, addition of the 19K protein to F-actin induced a rapid decrease in the fluorescence initially, followed by a fluctuation of the fluorescence intensity especially when larger amounts of the 19K protein were added, and eventually a new steady-state level was reached (Fig. 5A). The steady-state fluorescence intensity was inversely proportional to the concentration of the 19K protein added (Fig. 5B, inset), indicating...
Fig. 2. Effect of the brain 19K protein on actin filaments in a KCl+MgCl₂ medium. (A) Muscle F-actin (which had been polymerized to the steady state in 2 mM MgCl₂ at an actin concentration of 9.3 μM) was mixed with various amounts of the 19K protein at zero time, and the viscosity was followed at 30°C. Final conditions; F-actin (1.6 μM) plus 0 (a), 0.23 (b), 0.46 (c), 0.89 (d), or 1.3 μM (e) 19K protein in the medium described in Fig. 1. (f) and (g) were assayed in the presence of 1 mM EGTA. (f), +0.95 μM 19K protein; (g), +heat-treated (100°C, 3 min) 19K protein (0.95 μM). (B) A plot of the final steady-state viscosity vs. the concentration of the 19K protein.

that the 19K protein depolymerizes F-actin in a stoichiometric manner. It was calculated that about 0.7-0.8 mol of actin was depolymerized by 1 mol of the 19K protein. That the decrease in the fluorescence results from net depolymerization is supported by the following considerations. (i) The 19K protein does not bind to F-actin, as described before (Fig. 4). This excludes the possibility that the 19K protein causes changes in the fluorescence intensity of F-actin by binding to it without inducing depolymerization. (ii) The 19K protein does not change the fluorescence intensity of the pyrene-labeled G-actin, i.e., the fluorescence intensity of the actin below its critical concentration for polymerization was not altered by the presence of the 19K protein (data not shown). This indicates that the change in the fluorescence intensity does not result from the binding of the 19K protein to G-actin but is due to the G-F transformation of actin itself.

Thus, the fluorescence assay clearly proves the depolymerizing action of the 19K protein. It should be noted here that the initial rate of the 19K-induced depolymerization is roughly proportional to the concentration of the 19K protein, as shown in Fig. 5B. This and the rapidly of the depolymerization suggest that the 19K protein depolymerizes F-actin by directly attacking actin monomers in the filament, since if the depolymerization occurred only by a monomer-sequestering mechanism, the initial rate of the depolymerization should not depend on the 19K protein concentration and should be much slower (see "DISCUSSION").

All these results suggest that the 19K protein increases unpolymerized actin, irrespective of whether the protein is added to actin monomers before polymerization or added to F-actin, by reacting stoichiometrically with actin in a 1:1 molar ratio with high affinity.

Regulation of Actin Filament Length by Brain 19K Protein—A remarkable feature of the effect of this protein on actin polymerization is that the protein did not increase the half-polymerization time, although it prolonged the lag time; i.e. the half-polymerization time was 18.2 min in the ab-
Fig. 3. Effect of the brain 19K protein on actin polymerization (A) and on F-actin (B) in a KCl medium, assayed with an Ostwald-type viscometer. (A) Muscle actin (3.6 μM) was polymerized at 30°C in 8 mM PIPES-80 mM KCl-0.01 mM CaCl2-0.08 mM DTT-0.02 mM ATP (pH 7.3) in the absence (a) or presence (0.92 μM, b; 1.8 μM, c) of the 19K protein. (B) Muscle F-actin (which had been polymerized to the steady state in 100 mM KCl) was mixed with the brain 19K protein at zero time, and the viscosity was followed at 30°C in a medium containing 10 mM PIPES-100 mM KCl-0.01 mM CaCl2-0.1 mM DTT-0.02 mM ATP (pH 7.3). (a) F-actin (2.9 μM) alone, (b) +1.5 μM 19K protein. The plots of the steady-state viscosity values vs. the concentration of the 19K protein are shown in the inset of (B) (C, for A; O, for B).

Fig. 4. Centrifugation assay for the effect of the brain 19K protein on actin. Actin samples from the experiment shown in Fig. 3 were further incubated at 10°C for 3 to 5 h and then a 0.3 ml aliquot of each sample was centrifuged for 60 min at 100,000 × g and 6°C. Pellet fractions were analyzed by SDS-polyacrylamide gel electrophoresis (15% acrylamide). Slots a, b, and c correspond to samples a, b, and c in the experiment shown in Fig. 3A, and slots d and e correspond to the samples a and b in Fig. 3B, respectively. In slot f, phosphorylase b (94K), bovine serum albumin (68K), actin (42K), carbonic anhydrase (31K), trypsin inhibitor (21.5K), and cytochrome c (12.5K) were electrophoresed as molecular weight markers. Arrows indicate the electrophoretic position of the brain 19K protein. Note that the 19K protein did not sediment with actin filaments.

ends, thereby accelerating polymerization after actin filaments are formed by nucleation. That the protein does not nucleate actin polymerization was clearly seen both in KCl+MgCl2 (Fig. 1) and in KCl alone (Fig. 3A). If this is so, the number concentration of F-actin polymerized in the presence of the 19K protein must be higher than that in the absence of the protein. This was demonstrated by assaying the nucleating ability of the F-actin solution. The F-actin polymerized in the presence of the protein showed 4- to 5-fold higher activity to induce actin elongation than that in the absence of the protein when added to actin monomers (Fig. 6). Since the 19K protein alone did not nucleate actin polymerization, the higher rate of elongation is due to a higher number concentration of F-actin. The number concentration of F-actin showed a biphasic dependence on the 19K protein concentration added (Fig. 6B).
Fig. 5. Effect of the brain 19K protein on F-actin, assayed by fluorimetry. Muscle F-actin (6%, pyrene-labeled actin) which had been polymerized to the steady state in 1 mM MgCl₂ + 100 mM KCl was mixed with various amounts of the 19K protein at zero time, and the fluorescence change was monitored at 20°C in a medium containing 10 mM PIPES-1 mM MgCl₂-100 mM KCl-0.01 mM CaCl₂-0.1 mM DTT-0.02 mM ATP (pH 7.3). Final conditions; F-actin (1.1 μM) plus 0 (a), 0.35 (b), 0.67 (c), or 1.0 μM (d) 19K protein. (A) and (B) show the same data with different time scales (abscissas). A plot of the steady-state level (the fluorescence intensity at 70 min) vs. the concentration of the 19K protein is shown in the inset of (B). The units for the fluorescence intensity are arbitrary.

Fig. 6. The number concentration of F-actin polymerized in the presence or absence of the brain 19K protein. The number concentration of F-actin was estimated by assaying the nucleating ability of each solution. Brain actin (1.3 μM) was polymerized at 20°C for 18 to 20 h in the medium described in Fig. 1 in the absence (a) or presence (b), 0.35; (c), 0.81 μM, (d) of the 19K protein, and then an aliquot of each solution was added to brain actin monomer (final 0.55 μM F-actin (control) and 3.3 μM actin monomer) to initiate actin polymerization in 4 mM PIPES-40 mM KCl-1.1 mM MgCl₂-0.06 mM CaCl₂-0.12 mM ATP (pH 7.3) at 30°C. (A) The polymerization of actin monomer alone (-----) or actin monomer plus F-actin which had been polymerized in the presence (b, c, d) or absence (a) of the 19K protein was followed by the A₂₃₇ assay. (B) The initial rate of the polymerization shown in (A) (corresponding to the number concentration of F-actin) was plotted against the concentration of the 19K protein in each F-actin solution.

The nucleating ability of F-actin partially depolymerized by the 19K protein was also investigated. The result is shown in Fig. 7, which demonstrates that the 19K protein cuts as well as depolymerizes the steady-state actin filaments to produce more filaments. Addition of higher concentrations of the 19K protein, however, decreased the filament number and further increase in the concentration of the added 19K protein resulted in the complete absence of F-actin (data not shown). Therefore, in this case also, the number concentration of F-actin showed a biphasic dependence on the concentration of the 19K protein. This can be well understood in terms of the stoichiometric depolymerizing action of the 19K protein. The experiment indicates further that the protein does not cap the filament ends, since the result means that addition of substoichiometric amounts of the 19K protein does not inhibit the elongation of the actin filament at all.

The results shown in Figs. 6 and 7 suggest that the 19K protein not only depolymerizes actin filaments but also changes the filament size distribution; i.e., the filaments in the presence of the 19K protein are shorter than in its absence. Con-
The number concentration of F-actin partially depolymerized by the brain 19K protein. The number concentration was estimated by assaying the nucleating ability of each solution. Brain F-actin (which had been polymerized to the steady state in 50 mM KCl+3.5 mM MgCl₂ at an actin concentration of 9.0 μM) was incubated for 6 min at 30°C with various amounts of the 19K protein in a medium of 10 mM PIPES-100 mM KCl-0.5 mM MgCl₂-0.01 mM CaCl₂ (pH 7.3) (when the F-actin concentration was 1.2 μM and the 19K protein concentration was 0 (a), 0.07 (b), 0.49 (c), 1.0 (d), or 1.6 μM (e)). Then, an aliquot of each F-actin solution was added to brain actin monomer (final 1.1 μM F-actin (control) and 3.3 μM actin monomer) to initiate actin polymerization at 30°C in 7 mM PIPES-70 mM KCl-0.3 mM MgCl₂-0.03 mM CaCl₂ (pH 7.3). (A) The polymerization of actin monomer alone (-) or actin monomer plus F-actin which had been incubated with the 19K protein (b, c, d, e) or not (a) was followed by means of the A237 assay. (B) The initial rate of the polymerization shown in (A) (corresponding to the number concentration of F-actin) was plotted against the concentration of the 19K protein of each F-actin solution.

Inconsistent with this suggestion, the low-shear viscosity of actin filaments was decreased by the 19K protein much more strongly than was the high-shear viscosity (Fig. 8A). This is well accounted for by the fact that the low-shear viscosity of actin filaments depends more strongly on the filament size distribution than does the high-shear viscosity (24). Because the overall rate of polymerization is not decreased by the 19K protein (Fig. 1), and because the extent of the decrease in the low-shear viscosity by the 19K protein is quite large as compared with that resulting from mere reduction of the mass concentration of F-actin (data not shown), the results could not be fully accounted for only by a decreased amount of F-actin in the presence of the protein.

Flory’s network theory (25) predicts that proteins which change the filament size distribution will alter the concentration of cross-linker required for gelation. The result obtained in an experiment using actin, the 19K protein and fodrin is shown in Fig. 8B, which demonstrates that the minimum concentration of fodrin required for gelation increased when the F-actin was mixed with a substoichiometric amount of the 19K protein. This also supports the actin filament-cutting activity of the protein.

Action of Starfish Actin-Degradation Protein on Actin—Starfish actin-degradation protein, originally described by Mabuchi (11), was purified by a method similar to that used for the purification of the brain 19K protein (14). The purified protein was nearly homogeneous on an SDS-polyacrylamide gel (Fig. 12, lane c). This
Fig. 9. Effect of the starfish actin depolymerizing protein on the time course of actin polymerization. Muscle actin (5.1 μM) was polymerized at 25°C in 4 mM PIPES-100 mM KCl-2 mM MgCl₂-0.15 mM CaCl₂-0.03 mM EDTA-0.13 mM ATP (pH 6.8) in the absence (a) or presence (0.5 μM, (b); 1.3 μM, (c); 3.0 μM, (d)) of the starfish protein. The time course of the absorbance change at 237 nm was followed.

proteins depolymerized F-actin quickly, and produced a DNase I inhibitor, as reported previously (11, 14). About 1.2 mol of the protein was required to stabilize 1 mol of actin monomer (data not shown).

Figure 9 shows the effect of the starfish protein on the time course of muscle actin polymerization in KCl+MgCl₂, measured by the absorbance change assay. This assay cannot quantify the extent of actin polymerization exactly in this case, since the extent of the absorbance change of actin that accompanies the polymerization may differ in the presence and absence of the starfish protein. However, it is reasonable to assume that the time course of the absorbance change reflects the polymerization kinetics. The starfish protein increased the duration of the lag time and did not nucleate actin polymerization (Fig. 9). However, the time required for attaining the steady-state level decreased with increasing the amount of added starfish protein (Fig. 9). The polymerization curve in the presence of the protein showed an overshooting. Since these actions of the starfish protein are very similar to those of the brain 19K protein, it was predicted that the starfish protein acts by cutting the growing actin filaments, as does the brain protein. This was demonstrated by assaying the nucleating ability of the F-actin solution. Irrespective of whether the starfish protein was added to actin monomers before polymerization (Fig. 10) or added to preformed F-actin (Fig. 11), the protein consistently increased the filament number. These results suggest that the starfish protein severs actin filaments, and therefore regulates the filament size, as well as depolymerizes actin filaments and prevents actin monomers from polymerizing.

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Fig. 11. The number concentration of F-actin partially depolymerized by the starfish protein. Muscle F-actin (which had been polymerized to the steady state in 100 mM KCl+1.5 mM MgCl₂ at an actin concentration of 15 μM) was incubated with various amounts of the starfish protein for 50 min at 20°C in a medium of 6 mM PIPES-40 mM KCl-30 mM NaCl-1.2 mM MgCl₂-0.3 mM EGTA-0.1 mM ATP (pH 6.8) (when the final F-actin concentration was 6.3 μM and the concentration of the starfish protein was 0 (a), 1.4 (b), 2.9 (c), or 5.7 μM (d)). Then, an aliquot of each sample was added to actin monomer (final 0.6 μM F-actin and 3.0 μM actin monomer) to initiate actin polymerization as in Fig. 10A. (A) and (B) show the polymerization curves and the plot for the number concentration of F-actin vs. the concentration of the starfish protein, respectively, as described in Fig. 7.

Fig. 12. SDS-polyacrylamide (15%, acrylamide) gel electrophoresis of the brain 19K protein and the starfish actin depolymerizing protein. Slot a, the brain protein (0.6 μg); slot b, the brain protein (0.4 μg) + the starfish protein (1.5 μg); slot c, the starfish protein (1.5 μg).

DISCUSSION

In the present study, we have characterized the action of the brain 19K protein and the starfish actin depolymerizing protein on actin polymerization. The brain protein inhibits actin polymerization and depolymerizes actin filaments quickly by reacting stoichiometrically with actin in a 1:1 molar ratio. This was demonstrated by light scattering, viscosity and fluorescence assays. It should be mentioned that the action of the brain 19K protein on muscle actin was indistinguishable from that on brain actin. The starfish protein also reacts with actin in a 1:1 molar ratio, as originally reported by Mabuchi (11). In addition, both proteins were found to cut actin filaments and thus to alter the filament size distribution. The main evidence is that irrespective of whether the proteins were added to actin before polymerization or added to F-actin, they increased the number concentration of F-actin. The filament number can be determined by assaying the nucleating ability of each F-actin solution, since our previous studies demonstrated that the elongation rate is proportional to the filament number if the actin monomer concentration is fixed (18). Further evidence is that substoichiometric concentrations of the brain protein strongly inhibited the low-shear viscosity of actin filaments. This result is consistent with shorter filament length in the presence of the protein (shorter length would inevitably result from the increase in the filament number caused by the cutting action of the protein). Further evidence for the cutting activity of the protein is that in the presence of the protein, the minimum concentration of fodrin, an actin cross-linker, required for gelation was higher than that in its absence.

It should be noted that these proteins, unlike other severing proteins such as gelsolin and fragmin, do not nucleate actin polymerization or cap the barbed end of actin filaments. Because the severing proteins previously identified (1-3) preferentially bind to one end of actin filaments, for the most part the barbed end, they remain at, and thus cap, the newly formed filament ends after they cleave the filament. Therefore, they do not increase the nucleating ability of the F-actin solution (although they augment the filament number),

In spite of the functional similarities between the starfish and brain proteins, the molecular weights are different (Fig. 12).
but rather inhibit it by capping the barbed end. In contrast, both the brain and starfish proteins seem to cut the filament by depriving the filament of actin monomer. After cutting, they bind to the removed actin monomers, not to the filament end, thereby increasing the nucleating ability of the F-actin solution. Schematic representations of the modes of action of these two groups of severing proteins are shown in Fig. 13.

Actin polymerization and depolymerization kinetics in the presence of the brain and starfish proteins also support the dual functions. The proteins prolong the lag time and decrease the extent of polymerization when added to actin monomers before polymerization. This aspect of their action can be well explained by a simple monomer sequestering mechanism which operates in the case of profilin (5, 6). However, unlike profilin they accelerate the overall polymerization. This can be accounted for only by assuming cutting activity of the proteins, as discussed in "RESULTS."

Actin filament depolymerization by these proteins occurs much more rapidly than that by profilin (33). The mechanisms by which the brain 19K and starfish proteins depolymerize F-actin seem to be as follows. (i) Since the proteins sequester free actin monomers, monomers depolymerize from filament ends to restore the steady-state monomer concentration (critical concentration). (ii) They directly attack monomers at the filament ends and remove the monomers by forming a 1:1 complex with them. (iii) They directly remove monomers from the entire length of the filament. The mechanism (i) holds for all monomer sequestering proteins. For example, profilin action is explainable solely by mechanism (i), because the rate of depolymerization by profilin is comparable to that of spontaneous depolymerization induced by decreasing the ionic strength of the medium. The latter was demonstrated to occur only at the filament ends (18). The 19K protein-induced depolymerization cannot be explained solely by the monomer sequestering mechanism, as already mentioned in "RESULTS."

Mechanism (iii) accounts well for the rapidity of the depolymerization by the brain 19K and the starfish proteins, because the increase in the filament ends caused by mechanism (iii) accelerates the depolymerization by mechanisms (i) and (ii). Of course, mechanism (iii) itself depolymerizes F-actin rapidly. Mechanism (iii) is supported by the experimental evidence that these proteins increase the filament number, but do not cap the filament ends, when added to F-actin.

As shown in Figs. 2, 3B, and 5, the brain 19K protein-induced depolymerization of F-actin showed complex kinetics. Moreover, actin polymerization in the presence of the protein often showed an "overshooting." These phenomena

![Fig. 13. Schematic representation of the action of two groups of actin-severing proteins on F-actin. (A) Both the brain 19K protein and the starfish protein cut and depolymerize F-actin by removing actin monomers along the entire filament length by forming a 1:1 complex. This group of proteins is classified as "cutting and depolymerizing proteins." (B) "Capping and severing proteins" (such as gelsolin, villin and fragmin) cut F-actin, and bind to the newly formed filament end. •, actin monomer; ⋄, "cutting and depolymerizing proteins"; ▲, "capping and severing proteins."](J. Biochem.)
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appear to result partly from the fact that the protein has cutting activity as well as depolymerizing activity, but a full explanation is not possible at present.

Our conclusion is that the brain 19K protein and the starfish protein are functionally distinct from previously identified actin-binding proteins. As described in the introduction, actin-binding proteins have been classified into three groups: cross-linking proteins, capping and/or severing proteins, and depolymerizing proteins. However, the appearance of proteins having dual functions (cutting and depolymerizing) requires a modification of this classification. Since previously identified severing proteins such as gelsolin (24, 26), villin (27), and fragmin (28) are all capping proteins, they are classified as “capping and severing proteins,” together with proteins which have capping activity alone and not severing activity, such as β-actinin (29), Acanthamoeba (30), and brain (31) capping proteins, and acumenitin (32). Depolymerizing proteins such as profilin (4-6), vitamin D-binding protein (9), and serum proteins (10) should be called “monomer sequestering proteins,” because the depolymerization kinetics by these proteins are explainable simply by a monomer sequestering mechanism and because there is no evidence that they attack F-actin directly. In this respect, DNase I is an exceptional protein, since there is evidence that it binds to, and attacks, actin filaments directly (7, 8). Finally, the brain 19K protein and the starfish protein are classified as “cutting and depolymerizing proteins.”

The preliminary experiments suggest that proteins of this type exist in other cells.

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