An Actin-Depolymerizing Protein (Destrin) from Porcine Kidney. Its Action on F-Actin Containing or Lacking Tropomyosin†

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ABSTRACT: An M_r 19 000 protein (destrin) that has the ability to rapidly depolymerize F-actin in a stoichiometric manner was purified from porcine kidney by sequential chromatography on DNase I–agarose, hydroxyapatite, and Sephadex G-75. Its actin-depolymerizing activity is reversibly controlled by changes in KCl concentration but is insensitive to Ca^{2+} concentration. The rate of depolymerization of F-actin by destrin is much faster than that of spontaneous depolymerization induced by dilution and is not markedly decreased by the addition of end-blocking reagents such as cytochalasin B. These results suggest that destrin depolymerizes F-actin by interacting directly with F-actin protomers. Binding of muscle tropomyosin to F-actin slows down the rate of destrin-induced depolymerization of F-actin by about 30-fold. The data suggest that the destrin-induced depolymerization occurs from the ends of F-actin when F-actin is complexed with tropomyosin, but it takes place from the entire length of F-actin in the absence of tropomyosin.

Actin is a major constituent of cytoskeletons in mammalian cells and exhibits a variety of structural forms. In addition, rapid changes in polymeric states of actin are believed to occur during cell cycle and morphological changes in cells.

Many types of actin-binding proteins that regulate the state of actin polymerization and structure have been found and characterized (Weeds, 1982; Craig & Pollard, 1982; Korn, 1982). Recent reports described a class of low molecular weight proteins that can rapidly depolymerize F-actin both in the presence and in the absence of Ca^{2+}, i.e., an echinodermatous actin-depolymerizing protein (M_r 17 000), depactin (Mabuchi, 1983), and an M_r 19 000 protein in mammalian brain (Nishida et al., 1984b). An actin-depolymerizing protein was also found in chick embryo brain, but not purified yet (Bamburg et al., 1980). In this paper, we show that porcine kidney contains a protein that is similar in structure and function to an M_r 19 000 protein found in mammalian brain. This protein is capable of rapidly depolymerizing F-actin as if it destroyed the filaments. Therefore, we have named it destrin. Destrin may be of general importance in mammalian cells.

Effect of tropomyosin binding to F-actin on the depolymerizing action of destrin is also described in this paper. Previous studies showed that a partially purified actin-depolymerizing protein from chick embryo brain does not depolymerize tropomyosin-containing F-actin at all (Bernstein & Bamburg, 1982), but depactin does (Mabuchi, 1982). Here, we demonstrate that binding of muscle tropomyosin to F-actin does not protect the F-actin from depolymerization by destrin but markedly slows down the rate of the depolymerization. Tropomyosin-containing F-actin may be depolymerized by destrin by a monomer-sequestering mechanism, while actin monomers in F-actin that lacks tropomyosin may be directly attacked by destrin.

Experimental Procedures

Purification of Destrin, an M_r 19 000 Protein, from Porcine Kidney. All procedures were performed at 0–4 °C. Porcine kidney (about 600 g) was homogenized with a mixer in 300 mL of a buffer solution containing 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl),[1] 1 mM EGTA, 1 mM ATP, 1 mM DTT, and 1 mM PMSF (pH 7.8) and centrifuged at 20000*g* for 60 min. The supernatant was dialyzed overnight against a buffer solution of 2 mM HEPES, 0.1 mM ATP, and 0.1 mM DTT (pH 7.2) and clarified by centrifugation at 10000*g* for 90 min. To the supernatant solution was added 1 mM each of $MgCl_2$ and $CaCl_2$. Then, the solution was mixed with 60 mL of DNase I–agarose which had been equilibrated with 2 mM HEPES, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ (pH 7.2) (The DNase I–agarose was prepared by utilizing Bio-Rad Affigel 10 as previously described (Nishida et al., 1981)). After the solution was gently stirred for about 1 h, the gels were washed with 10 mM HEPES (pH 7.2) containing 1 mM $MgCl_2$ and $CaCl_2$. Then, destrin-containing fractions were eluted from the DNase I–agarose with a 0.6 M NaCl solution containing 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES (pH 7.2). The eluted fractions were applied to a hydroxyapatite column (1.5 × 7 cm) followed by elution of the adsorbed proteins first with a solution of 10 mM potassium phosphate, 1 mM $MgCl_2$, 0.1 M NaCl, and 0.1 mM DTT (pH 6.8) and then with a linear gradient of 10–150 mM potassium phosphate. Fractions containing destrin (identified by $NaDodSO_4$–polyacrylamide gel electrophoresis) were pooled and concentrated to a volume of about 1 mL by using an Amicon YM-05 membrane. The concentrated sample was gel filtered on a Sephadex G-75 column (1 × 40 cm) as a final purification step. The elution buffer solution consisted of 10 mM PIPES, 100 mM KCl, and 0.1 mM DTT (pH 7.2). In some cases, a chromatography on a phosphocellulose column (1.5 × 4 cm) was carried out before or after the Sephadex G-75 gel filtration (see Results).

Purification of Muscle Proteins. Rabbit skeletal muscle actin was prepared by the method of Spudich & Watt (1971)

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[1] Abbreviations: Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EGTA, ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate; PIPES, piperazine-*N,N'*-bis(2-ethanesulfonic acid); MBS, *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; DNase I, deoxyribonuclease I.

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and further purified by gel filtration on Sephadex G-100 equilibrated and eluted with a buffer solution containing 0.1 mM $CaCl_2$, 0.2 mM ATP, 0.05 mM DTT, 0.01% NaN_3, and 2 mM HEPES (pH 7.8). Actin concentration was determined by UV absorption measurement based on $A_{290}^{1\%} = 6.5$. Tropomyosin was isolated from rabbit skeletal muscle as described previously (Wakabayashi et al., 1975).

Purification of 88K Protein/Actin Complex. The 88K protein/actin complex that caps the barbed end of actin filaments (Nishida et al., 1983) was purified from porcine brain as described previously (Nishida et al., 1981).

Isolation of Spectrin/Actin/Band 4.1 Complex. Dr. Matsuzaki prepared the spectrin/actin/band 4.1 complex and kindly provided us with it. Details of the isolation procedure will be reported elsewhere (F. Matsuzaki, personal communication). Briefly, the Triton shell was prepared from bovine erythrocyte ghosts, and subsequently the Triton shell was solubilized into the spectrin/actin/band 4.1 complex.

Assays for Actin Polymerization. High shear viscosity was measured with an Ostwald-type viscometer as previously described (Nishida et al., 1984b). The absorbance change at 237 nm which represents the G–F transformation of actin (Higashi & Oosawa, 1965; Nishida & Sakai, 1983) was measured with a Gilford 260 spectrophotometer in a temperature-controlled cuvette chamber. The concentration of monomeric actin was determined by the method of the DNase I inhibition assay as described by Blikstad et al. (1978). The fluorescence assay using *N*-pyrenyliodoacetamide-labeled actin was carried out by a modified method (Brenner & Korn, 1983) of Kouyama & Mihashi (1981) as previously described (Nishida et al., 1984b). The excitation and emission wavelengths were 365 and 407 nm, respectively.

Chemical Cross-Linking of Destrin and Actin. Destrin and actin were cross-linked with *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester (MBS) or with a zero-length cross-linker, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC). The reaction mixtures contained 4.2 μM actin, 4.6 μM destrin, and 0.15 mM MBS or 30 mM EDC in 10 mM PIPES, 0.05 mM DTT, 0.02 mM ATP, and various concentrations of KCl (pH 7.2). The cross-linking reaction was allowed to proceed for 2 h at 20 °C. Then, the reaction mixture was electrophoresed on 14% acrylamide gels in the presence of $NaDodSO_4$.

Gel Electrophoresis. $NaDodSO_4$–polyacrylamide gel electrophoresis was carried out by the method of Laemmli (1970) with 14% polyacrylamide slab gels. Gels were stained with Coomassie blue, and the intensity of the stained band was determined by scanning the gels with a densitometer.

Others. The pelleting assay and the light scattering assay were carried out as previously described (Nishida et al., 1984a). Low shear viscosity was assayed in a falling ball device (MacLean-Fletcher & Pollard, 1980a). Protein concentration was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Results

Purification of Destrin from Porcine Kidney. Figure 1 shows the $NaDodSO_4$–polyacrylamide gels of destrin fractions at each step in the purification. Destrin was recovered in the 0.6 M NaCl eluate from the DNase I–agarose (Figure 1A). On hydroxyapatite destrin was eluted at a phosphate concentration of 10 mM, just after an elution of a protein having a molecular weight of 26000 (Figure 1B). Finally, destrin was purified to near homogeneity by gel filtration on Sephadex G-75 (Figure 1C). It was eluted as a symmetrical peak at an elution position corresponding to the Stokes radius of 1.8 nm.

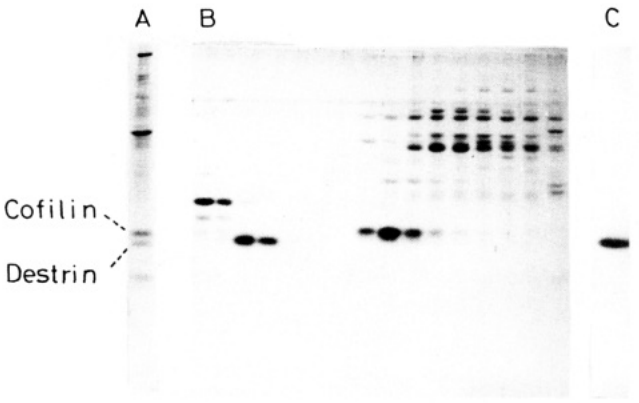

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoretic analysis of the destrin fractions at each step in the purification. (A) 0.6 M NaCl eluate from the DNase I–agarose column; (B) hydroxyapatite column fractions (lanes 1–6, fractions eluting with 10 mM phosphate; lanes 7–16, fractions eluting with a linear gradient of 10–150 mM phosphate); (C) purified destrin after Sephadex G-75 chromatography.

Every fraction from the Sephadex G-75 column was assayed for its activity to depolymerize F-actin by means of the DNase I inhibition assay or the viscosity measurement. The result indicated that the elution of the activity was precisely superimposed on that of destrin which was identified by $NaDodSO_4$–polyacrylamide gel electrophoresis (data not shown). The apparent molecular weight of destrin determined in the $NaDodSO_4$–polyacrylamide gel was 19000. Therefore, destrin seems to be a monomeric and globular protein.

In some cases when the purity of destrin was not good at the hydroxyapatite step, a phosphocellulose chromatography was utilized before or after Sephadex G-75 gel filtration. Destrin was eluted from the column at an NaCl concentration of 50–100 mM in 10 mM PIPES–0.1 mM DTT (pH 6.5). It should be noted that the elution of the actin-depolymerizing activity as assessed by the DNase I inhibition assay was, again, superimposed on the elution of destrin on the phosphocellulose chromatography. These results strongly indicated that the actin-depolymerizing activity resides in the M_r 19000 protein, destrin.

Action of Destrin on F-Actin. Figure 2A shows the effect of destrin on the viscosity of F-actin in a KCl plus $MgCl_2$ medium. Addition of destrin to F-actin induced a rapid drop in viscosity within 2 min, and then the viscosity gradually increased a little and attained a new steady-state level. The steady-state viscosity level was inversely proportional to the concentration of destrin added (Figure 2B, inset). When aliquots of these actin samples with varying amount of destrin were centrifuged at 100000*g* for 45 min, it was found that sedimentable F-actin was reduced with increasing the concentration of destrin added and that destrin did not sediment with F-actin (data not shown).

The DNase I inhibition assay revealed that destrin increased the concentration of monomeric actin in a dose-dependent manner when added to F-actin (Figure 3). Since control experiments revealed that destrin alone does not affect the DNase I activity and that a mixture of G-actin and destrin inhibits the DNase I activity the same as G-actin alone (not shown), we can quantitatively analyze the data such as shown in Figure 3. Repeated experiments showed that about 0.65–0.80 mol of monomeric actin is produced by 1 mol of destrin. Moreover, it should be noted that about the same amount of monomeric actin was detected irrespective of whether the DNase I inhibition assay was carried out 2 min after the addition of destrin to F-actin (Figure 3B) or at the steady state (Figure 3A), indicating the rapidity of destrin-

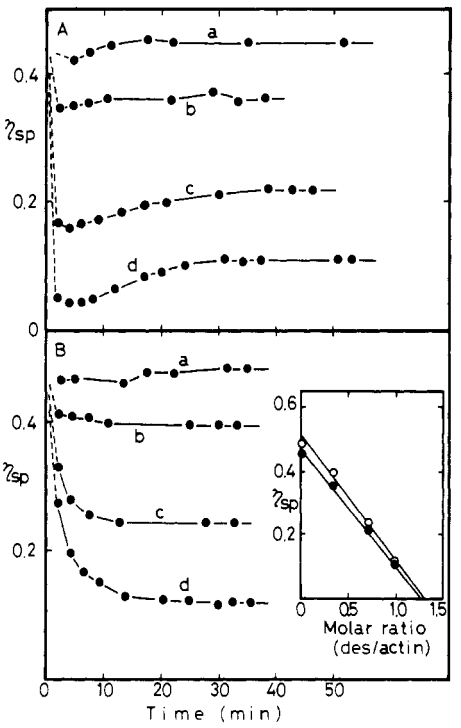

FIGURE 2: Effect of destrin on F-actin lacking (A) or containing (B) 1 μM tropomyosin, assayed with an Ostwald-type viscometer. F-Actin with or without tropomyosin was mixed with destrin at zero time, and the viscosity was followed at 20 °C. The assay conditions were 2 mM $MgCl_2$, 80 mM KCl, 0.01 mM $CaCl_2$, 0.08 mM DTT, 0.02 mM ATP, and 8 mM PIPES, pH 7.2. (Curve a) Actin (3.2 μM) alone; (curve b) actin plus 1.0 μM destrin; (curve c) actin plus 2.2 μM destrin; (curve d) actin plus 3.2 μM destrin. The inset shows the dependence of the steady-state viscosity on molar ratio of destrin to actin [(●) from the data shown in (A); (○) from the data shown in (B)]. des = destrin.

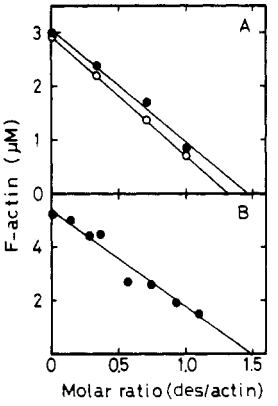

FIGURE 3: Actin-depolymerizing activity of destrin as measured by the DNase I inhibition assay. (A) F-Actin with (○) or without (●) tropomyosin was mixed with increasing concentrations of destrin. The conditions were the same as described in Figure 2. After 3 h of incubation at 20 °C, the concentration of monomeric actin was determined by the DNase I inhibition assay. The data are expressed as the F-actin concentration (=total concentration of actin minus action monomer concentration). (B) Tropomyosin-free F-actin (5.3 μM) was mixed with various concentrations of destrin, and after 2 min of incubation at 20 °C, the concentration of monomeric actin was determined. The data are expressed as in (A).

induced depolymerization of F-actin. All these assays indicate that destrin rapidly depolymerizes F-actin in a stoichiometric manner.

Another assay, measurement of pyrene-labeled actin fluorescence, was performed to monitor the time course of destrin-induced depolymerization of F-actin. As shown in Figure 4, addition of destrin to F-actin induced a rapid de-

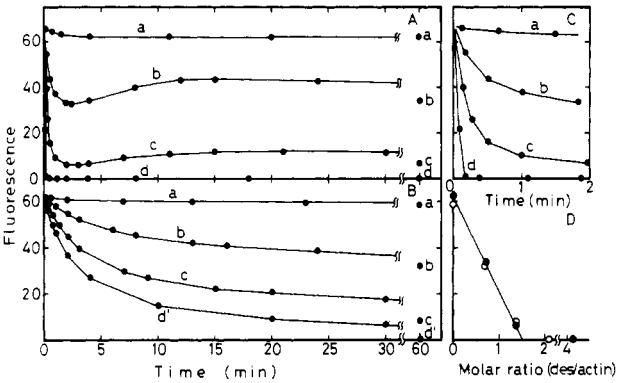

FIGURE 4: Effect of destrin on F-actin lacking (A, C) or containing (B) 0.9 μM tropomyosin, assayed by fluorometry. F-Actin (6% pyrene-labeled actin) with (B) or without (A, C) tropomyosin was mixed with destrin at zero time, and the fluorescence change was monitored at 20 °C. The assay conditions were the same as described in Figure 2. (A, B, C) Curve a, actin (3.0 μM) alone; curve b, actin plus 2.1 μM destrin; curve c, actin plus 3.8 μM destrin; curve d, actin plus 12.3 μM destrin; curve d', actin plus 6.3 μM destrin. (A) and (C) show the same data with different time scales (abscissas). A plot of the steady-state fluorescence intensity vs. molar ratio of destrin to actin is shown in (D) [(●) from the data shown in (A); (○) from the data shown in (B)].

crease in the fluorescence initially followed by a fluctuation of the fluorescence intensity, and eventually a new steady-state level was reached. The steady-state fluorescence intensity was inversely proportional to the concentration of destrin added (Figure 4D). Since destrin does not bind to F-actin as described before, and since the fluorescence intensity of the pyrene-labeled actin below its critical concentration for polymerization is not altered by the presence of destrin (not shown), the decrease in the fluorescence intensity by destrin reflects net depolymerization of F-actin. From the data shown in Figure 4D, it was calculated that about 1.4 mol of destrin was needed to depolymerize 1 mol of actin. This stoichiometry was very similar to that obtained from the DNase I inhibition assay (Figure 3). From these data combined with the critical actin concentration for polymerization under the conditions used (0.15 μM), an apparent dissociation constant for destrin binding to actin can be estimated, as has previously been done for *Acanthamoeba* profilin binding to actin (Tobacman & Korn, 1982). It was calculated to be 0.05–0.10 μM.

Figure 4C shows that the rate of the destrin-induced depolymerization of F-actin depends on the concentration of destrin added. When an excess amount of destrin was added to F-actin (destrin:actin = 4:1), complete depolymerization of F-actin occurred within 10 s (Figure 4C, curve d). Since this rapidity of depolymerization cannot be accounted for by a monomer sequestering mechanism, it seems probable that destrin directly attacks F-actin promoters (see below).

Effect of Tropomyosin. Tropomyosin is associated with a relatively large portion of F-actin in nonmuscle cells (Lazarides, 1976; Matsumura et al., 1983); therefore, it is of interest to examine the effect of tropomyosin binding to F-actin on the action of destrin.

The results of this type of experiments are shown in Figures 2–4. The plots of the steady-state viscosity (Figure 2, inset), the steady-state concentration of monomeric actin (Figure 3A), and the steady-state fluorescence intensity (Figure 4D) vs. molar ratio of destrin to actin were the same irrespective of whether tropomyosin was present or not. Therefore, tropomyosin binding to F-actin does not protect the F-actin from depolymerization by destrin.

However, the rate of depolymerization by destrin was markedly decreased in the presence of tropomyosin. This was

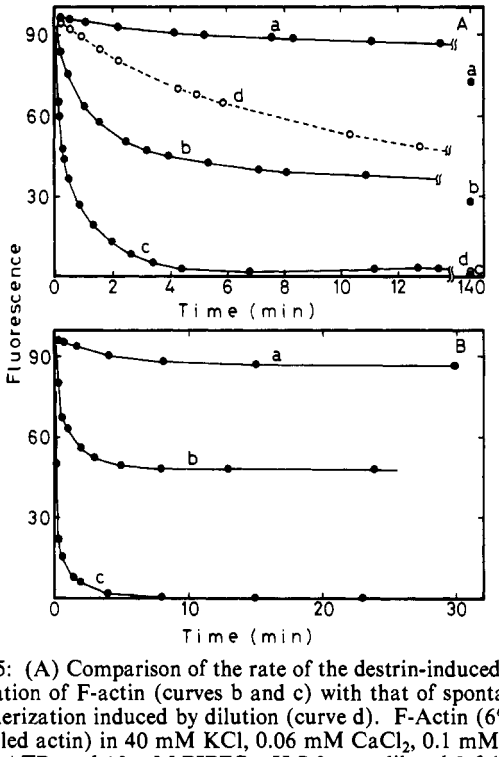

FIGURE 5: (A) Comparison of the rate of the destrin-induced depolymerization of F-actin (curves b and c) with that of spontaneous depolymerization induced by dilution (curve d). F-Actin (6% pyrene-labeled actin) in 40 mM KCl, 0.06 mM $CaCl_2$, 0.1 mM DTT, 0.2 mM ATP, and 10 mM PIPES, pH 7.2, was diluted 8-fold with 2 mM PIPES, pH 7.2, to induce depolymerization (curve d). The same F-actin was diluted 8-fold with a buffer solution of 0.1 M KCl, 0.1 mM DTT, and 10 mM PIPES, pH 7.2, containing various amounts of destrin (curves a–c). The fluorescence change was monitored at 20 °C. Final assay conditions: (curve a) actin (2.2 μM) in 90 mM KCl; (curve b) curve a plus 1.7 μM destrin; curve (c) curve a plus 3.4 μM destrin; (curve d) actin in 5 mM KCl. It is concluded from the data that the destrin-induced depolymerization is much faster than depolymerization upon dilution. When the same F-actin was reacted with destrin (3.4 μM, the same as in curve c) in 5 mM KCl, slightly faster depolymerization than in curve c occurred. This supports the above conclusion. (B) Effect of destrin on F-actin in the presence of cytochalasin B. F-Actin (6% pyrene-labeled actin) with cytochalasin B was mixed with destrin at zero time, and the fluorescence change was monitored at 20 °C. Final conditions were with actin (4.0 μM), cytochalasin B (1.0 μM), and 0 (curve a), 2.0 (curve b), or 6.0 μM destrin (curve c). Other conditions were the same as described in Figure 2. The time required for reaching the half-level of the depolymerization was found to be 5–30 s (curves b and c). These values were not markedly different from those determined in the absence of cytochalasin B (see the text and Figure 4A).

demonstrated by viscometry (Figure 2) and fluorometry (Figure 4). When 3 μM F-actin lacking tropomyosin was induced to depolymerize by the addition of 2.1 or 3.8 μM destrin, the time required for reaching the half-level of the depolymerization was about 20 or 10 s (Figure 4C, curve b or c). This time was found to increase to about 520 or 310 s in the presence of tropomyosin (Figure 4B), demonstrating that binding of tropomyosin to F-actin slows down the rate of destrin-induced depolymerization of F-actin by about 30-fold.

In these experiments, binding of tropomyosin to F-actin was confirmed by both the pelleting assay and the light-scattering assay. About half of added tropomyosin (added tropomyosin:actin = 1:3.5) sedimented with F-actin (as revealed by $NaDodSO_4$–polyacrylamide gel electrophoresis of the pellet and supernatant fractions after centrifugation). Moreover, the light-scattering intensity of F-actin was increased to about 1.5-fold in the presence of tropomyosin, as previously reported by Wegner (1979). These results indicate that nearly saturable amounts of tropomyosin were bound to F-actin under the conditions used.

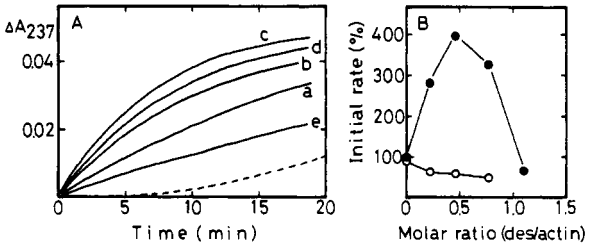

FIGURE 6: Nucleating ability of F-actin partially depolymerized by destrin. (A) An aliquot of F-actin (3.6 μM) which had been partially depolymerized by various concentrations (curve a, 0; curve b, 0.8 μM; curve c, 1.6 μM; curve d, 2.8 μM; curve e, 4.0 μM) of destrin at 25 °C for 5 min was added to actin monomers (4.2 μM) to initiate actin polymerization, and the polymerization kinetics were followed by the A_{237} assay at 25 °C, as shown. The assay conditions were 0.4 mM $MgCl_2$, 15 mM KCl, 0.08 mM $CaCl_2$, 0.08 mM DTT, 0.2 mM ATP, 2 mM PIPES, and 2 mM HEPES, pH 7.5. (---) Actin monomer alone; (curve a) plus F-actin (0.76 μM) without destrin; (curves b–e) plus F-actin with the varying amounts of destrin. (B) The initial rate of the polymerization shown in (A) (corresponding to the number concentration of F-actin) was plotted vs. the molar ratio of destrin to actin (●). The same kind of experiment as (A) was carried out with F-actin (3.6 μM) containing tropomyosin (1.1 μM), and the result is shown in the same figure (○). The nucleating ability of tropomyosin-free F-actin (●) was increased by destrin, while that of tropomyosin-containing one (○) was not.

Mechanism by Which Destrin Depolymerizes F-Actin. When F-actin in KCl was induced to depolymerize by dilution, the time required for half-depolymerization ($t_{1/2}$) was about 850 s (Figure 5A, curve d). On the other hand, the same F-actin was depolymerized by destrin with a $t_{1/2}$ of 16 s (Figure 5A, curve c). Therefore, the destrin-induced depolymerization of F-actin is much faster than spontaneous depolymerization induced by dilution.

Figure 5B shows the effect of destrin on F-actin in the presence of cytochalasin B. Cytochalasin B was previously found to markedly decrease the rate of spontaneous depolymerization of F-actin induced by dilution by capping the barbed end of F-actin (MacLean-Fletcher & Pollard, 1980b; Nishida et al., 1983). Therefore, if destrin depolymerized F-actin solely by sequestering free actin monomers (i.e., by a monomer-sequestering mechanism), cytochalasin B could greatly slow down the destrin-induced depolymerization. This was not the case, since the rate of the destrin-induced depolymerization in the presence of cytochalasin B was similar to that in its absence (cf. Figure 5B with Figure 4A). These results suggest that destrin accelerates depolymerization by directly interacting with F-actin promoters.

We then examined whether destrin has the ability to cut F-actin. If destrin cuts F-actin, the number concentration of F-actin is increased by partial depolymerization by destrin. In order to estimate the number of filament ends, the nucleating ability of F-actin was investigated (a nucleation assay). We previously utilized this assay to demonstrate that cofilin and an M_r 19 000 protein from brain increase the number of F-actin (Nishida et al., 1984a,b). As shown in Figure 6A, the F-actin partially depolymerized by destrin induced a faster polymerization of actin than did the original F-actin, when added to actin monomers (cf. curves b–d with curve a). Since destrin alone did not nucleate actin polymerization (see Figure 9), this result suggested that the number concentration of F-actin was increased by destrin. When F-actin was reacted with a higher concentration of destrin, the nucleating ability was decreased (curve e). This can be well understood in terms of the actin-depolymerizing activity of destrin. Thus, destrin has the ability to cut F-actin as well as to depolymerize F-actin. The experiment shown in Figure 6 further indicates that de-

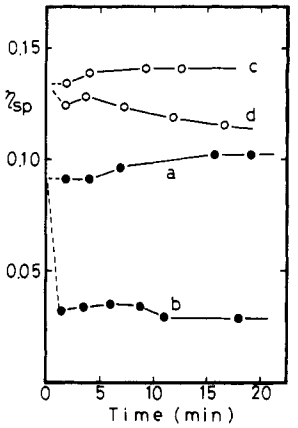

FIGURE 7: Effect of destrin on both-ends-blocked F-actin, assayed by viscometry. Actin with (○) or without (●) tropomyosin was polymerized onto the spectrin/actin/band 4.1 complex and then incubated with the 88K protein/actin complex (see the text). The both-ends-blocked F-actin thus formed was mixed with destrin at zero time, and the viscosity was followed at 20 °C. The solution conditions were the same as described in Figure 2. (Curve a) Actin (2.5 μM) with the spectrin/actin/band 4.1 complex (7 μg/mL) and 88K protein/actin complex (5 μg/ml); (curve b) curve a plus 2.5 μM destrin; (curve c) curve a plus tropomyosin (0.75 μM); (curve d) curve c plus 2.5 μM destrin.

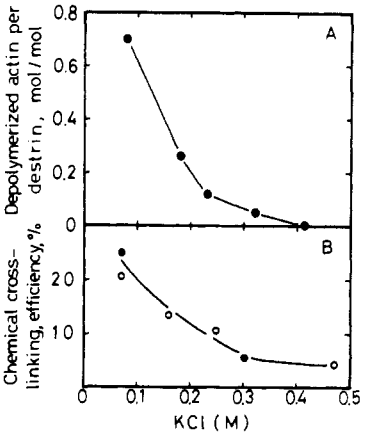

FIGURE 8: (A) Effect of KCl concentration on the actin-depolymerizing activity of destrin. F-Actin (3.8 μM, 6% pyrene-labeled actin) was incubated with or without destrin (3.8 μM) in the presence of various concentrations of KCl at 20 °C for about 3 h, and then the fluorescence intensity of each sample was measured. From this, the ability of destrin to depolymerize F-actin was quatitated. The solution conditions were the same as described in Figure 2 except for the KCl concentration. (B) Effect of KCl concentration on the binding of destrin to actin. Destrin and actin were cross-linked with MBS (●) or EDC (○) in the presence of various concentrations of KCl. The reaction was carried out as described under Experimental Procedures. The reaction product was analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Both cross-linkers generated a cross-linked product with an apparent molecular weight of 62K. The efficiency of the chemical cross-linking was defined as (destrin in the 62K cross-linked product)/(total destrin).

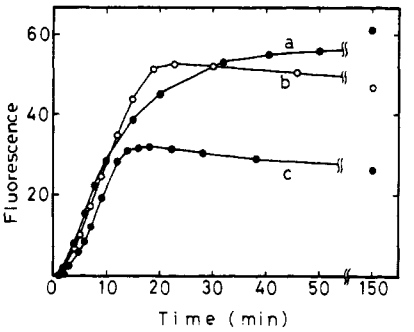

FIGURE 9: Effect of destrin on the time course of actin polymerization. Actin (3.8 μM, 6% pyrene-labeled actin) was polymerized at 25 °C in the absence (curve a) or presence (curve b, 1.1 μM; curve c, 3.3 μM) of destrin, and the increase in the fluorescence intensity was monitored. The assay conditions were the same as described in Figure 2.

strin does not cap the barbed ends of F-actin, since the result means that destrin, even at very low concentrations, did not inhibit the elongation of the added F-actin at all.

Interestingly, tropomyosin-containing F-actin was not cut by destrin. As shown in Figure 6B, the nucleating ability of tropomyosin-containing F-actin was not increased by destrin.

All these data suggest that the destrin-induced depolymerization occurs only from the ends of F-actin when F-actin is complexed with tropomyosin, but it takes place from the entire length of F-actin in the absence of tropomyosin. If so, even both-ends-blocked F-actin must be easily depolymerized by destrin, but when it is complexed with tropomyosin, the depolymerization must be very slow. This was experimentally demonstrated (Figure 7). In this experiment, both-ends-blocked F-actin was made by using the spectrin/actin/band 4.1 complex and 88K protein/actin complex. First, actin was polymerized onto spectrin/actin/band 4.1 complex. (Because this polymerization was inhibited by more than 80% by cytochalasin B or 88K protein/actin complex (not shown), at least 80% of F-actin thus formed was blocked at the pointed end.) Then, the barbed end of the F-actin was blocked by the 88K protein/actin complex. As shown in Figure 7, the viscosity of the both-ends-blocked F-actin was quickly decreased by addition of destrin (closed circles), while in the presence of tropomyosin, the viscosity of the F-actin decreased very slowly (open circles).

Effect of KCl Concentration. Figure 8A shows the effect of KCl concentration on the actin-depolymerizing activity of destrin measured by fluorometry. The activity of destrin was decreased with increasing the KCl concentration. One mole of destrin can depolymerize 0.70 mol of actin at 80 mM KCl, while at 410 mM KCl destrin cannot depolymerize F-actin at all.

This KCl sensitivity of the activity of destrin was reversible. When the concentrated KCl solution was added to actin previously incubated with destrin, net polymerization of actin occurred. Conversely, when the KCl-free buffer solution was added to the actin–destrin mixture containing a high concentration of KCl to lower the KCl concentration, net depolymerization was induced (data not shown). Thus, the actin-depolymerizing activity of destrin was reversibly controlled by changes in KCl concentration.

Varying the Mg concentration (0–4 mM) did not affect the depolymerizing activity of destrin. The activity was also insensitive to Ca^{2+} concentration (10^{-8}–10^{-4} M).

In parallel with the KCl sensitivity of the depolymerizing activity of destrin, binding of destrin to actin also showed the KCl dependence. Treatment of actin–destrin mixture with either EDC or MBS under the conditions specified under Experimental Procedures generated a cross-linked product with an apparent molecular weight of 62 000, which was probably a 1:1 complex of actin and destrin. The efficiency of this chemical cross-linking reaction showed dependence on the KCl concentration, as shown in Figure 8B.

Effect of Destrin on Actin Polymerization Kinetics. Figure 9 shows the time course of actin polymerization in the presence or absence of destrin assayed by fluorometry. The lag phase preceding a detectable increase in fluorescence was observed both in the presence and in the absence of destrin, suggesting that destrin, unlike end-blocking proteins, does not nucleate actin polymerization. In the presence of destrin, the maximal

level of fluorescence was attained faster than in its absence, although the extent of polymerization was decreased. This phenomenon can be explained by assuming that destrin accelerates the overall polymerization by cutting growing F-actin to produce more filament ends during the polymerization process.

Discussion

In this study, we purified from porcine kidney an M_r 19 000 protein, called destrin, that is capable of rapidly depolymerizing F-actin in a stoichiometric manner. The Stokes radius of the protein is 1.8 nm, suggesting that destrin is a monomeric and globular protein. Its actin-depolymerizing activity was demonstrated by a variety of assay methods including the viscosity measurement, the pelleting assay, the DNase I inhibition assay, and the fluorescence measurement using the pyrene-labeled actin.

These assays indicated a 1:1 stoichiometry between destrin and actin. A 1:1 complex formation between destrin and actin was also suggested by a chemical cross-linking experiment, which demonstrated the KCl sensitivity of destrin–actin binding. Since the depolymerizing action of destrin also showed the similar KCl dependence, we inferred that the binding of destrin to actin is the cause of the destrin-induced depolymerization of F-actin. That the actin-depolymerizing activity of destrin was reversibly controlled by changes in KCl concentration rules out the possibility that destrin irreversibly inactivates actin by proteolyzing or modifying actin molecule.

The apparent K_D value for destrin–actin binding under physiological ionic conditions (2 mM $MgCl_2$ and 80 mM KCl, pH 7.2) was estimated to be 0.05–0.10 μM in this study. This K_D value is smaller than that for brain profilin–actin binding (1–1.5 μM) or that for cofilin–actin binding (about 0.2 μM) determined recently (Nishida, 1985). Therefore, the affinity of destrin for actin is relatively strong.

One point of this paper is that the destrin-induced depolymerization of F-actin is very rapid. The rate of the depolymerization depends on the concentration of destrin added, and when an excess amount of destrin was reacted with F-actin (destrin:actin = 4:1), complete depolymerization of F-actin occurred within 10 s (Figure 4). This rapidity of depolymerization cannot be explained only by the ability of destrin to interact with monomeric actin and thus reduce the concentration of polymerizable actin pool. Indeed, the destrin-induced depolymerization of F-actin was much faster than spontaneous depolymerization induced by dilution (Figure 5A). Therefore, it is highly probable that destrin induces depolymerization of F-actin by interacting directly with F-actin promoters as well as sequestering free actin monomers. That cytochalasin B did not decrease markedly the rate of destrin-induced depolymerization but did the rate of spontaneous depolymerization (Figure 5B; Nishida et al., 1983) is consistent with the above conclusion.

Another point of this paper is that binding of tropomyosin to F-actin greatly slows down the rate of destrin-induced depolymerization of F-actin. We infer that the destrin-induced depolymerization occurs from the entire length of F-actin in the absence of tropomyosin, but it takes place only from the ends of F-actin when F-actin is complexed with tropomyosin. Several experiments support this. (i) The number concentration of F-actin was increased by partial depolymerization by destrin in the absence of tropomyosin, but not in the presence of tropomyosin (Figure 6). (ii) Both-ends-blocked F-actin was easily depolymerized by destrin, but when it was complexed with tropomyosin the depolymerization became very slow (Figure 7). These two experimental results imply that destrin has the cutting activity. Moreover, the kinetics of actin polymerization in the presence of destrin (Figure 9) were also explained by assuming the cutting ability of destrin. Recently, it was reported that tropomyosin binding to F-actin inhibits the cutting action of gelsolin (Fattoum et al., 1983) and villin (Bonder & Mooseker, 1983) but does not inhibit the capping activity of villin (Bonder & Mooseker, 1983). Therefore, it is naturally assumed that binding of tropomyosin to F-actin inhibits the cutting action of destrin but permits the depolymerization from the ends of F-actin.

We infer that destrin is capable of taking actin molecules away from the entire region of F-actin, thereby cutting F-actin. Preliminary experiments demonstrated that the increased nucleating activity of the F-actin partially depolymerized by destrin (see Figure 6) persisted with time of incubation and that F-actin polymerized in the presence of destrin also showed a higher nucleating activity than control F-actin [see Nishida et al. (1984b)]. These results imply that at steady state the number concentration of F-actin is higher in the presence of destrin than in its absence. This may be explained by assuming that destrin continuously cuts annealed filaments or that destrin binds to either end of F-actin, thereby inhibiting annealing. Since several observations (see the next paragraph) are not consistent with the latter assumption, we favor the former. Continuous cutting by destrin of spontaneously annealed F-actin may be possible, because the binding of destrin to actin seems to be reversible as suggested by the result that the actin-depolymerizing activity of destrin can be reversibly controlled by changes in KCl concentration.

F-Actin cutting proteins such as fragmin, gelsolin, and villin seem to bind to the barbed end of F-actin. However, destrin does not seem to cap the barbed end, since substoichiometric concentrations of destrin do not inhibit the elongation of F-actin at all (Figure 6). Moreover, destrin does not have the ability to nucleate actin polymerization either in $MgCl_2$ plus KCl (Figure 9) or in KCl alone (data not shown), suggesting that it does not bind to either end of F-actin, because the pointed end-blocking proteins, β-actinin (Maruyama et al., 1977) and acumentin (Southwick & Hartwig, 1982), as well as the barbed end-blocking proteins are known to be capable of nucleating actin polymerization. Our preliminary experiments also revealed that low concentrations of destrin do not inhibit the polymerization of actin nucleated by the barbed end-blocking protein, 88K protein/actin complex (Nishida et al., 1983), again indicating the inability of destrin to bind to the pointed end. Thus, we conclude that destrin does not cap either end of F-actin. However, we cannot rule out the possibility of a low-affinity binding of destrin to either end of F-actin.

Mabuchi purified from starfish oocytes an actin-depolymerizing protein called depactin (Mabuchi, 1983). Depactin was very similar to destrin in function, but its molecular weight was smaller than destrin [see Nishida et al. (1984b)]. Although Mabuchi stated that tropomyosin had little effect on the depolymerizing action of depactin, the data in his paper clearly demonstrated that tropomyosin markedly slows down the rate of depactin-induced depolymerization [see Figure 2 in Mabuchi (1982)]. This correlates well with our result obtained with destrin. In contrast to these results that destrin as well as depactin is capable of depolymerizing tropomyosin-containing F-actin eventually, Bernstein & Bamburg (1982) reported that a large portion of tropomyosin-containing F-actin was not depolymerized at all by a partially purified actin-depolymerizing protein from chick embryo brain even after 3 h of incubation. This phenomenon cannot be easily

explained, since tropomyosin is thought not to alter the G–F equilibrium and since it is thought that the depolymerizing proteins have the ability to bind to actin monomer, then reduce the polymerizable actin pool, and finally depolymerize F-actin even when they cannot directly attack F-actin promoters. When the observed difference is ascribed to the functional difference of our protein and theirs is not clear at present, because the purification and detailed characterization of a chick embryo protein has not been reported.

In summary, all the data presented here are consistent with our simple interpretation that the destrin-induced depolymerization takes place from the entire length of F-actin, but when F-actin is complexed with tropomyosin, it occurs only from the ends of F-actin. However, it should be noted that if destrin randomly bound to F-actin promoters and immediately took them away from F-actin, the rate of the depolymerization of tropomyosin-free F-actin should be much faster than that observed here. Therefore, destrin may take actin molecules away from the ends of F-actin more easily than from the midregion of F-actin. In this context, the suggestion of Wegner (1982) that tropomyosin inhibits spontaneous fragmentation of F-actin is important. The effect of tropomyosin on the destrin action may be partly explainable in terms of this suggestion. At any rate, the elucidation of the precise mechanism by which destrin depolymerizes F-actin needs further experimentation.

We previously isolated an M_r 19 000 protein from porcine brain that is capable of rapidly depolymerizing F-actin (Nishida et al., 1984b). This brain protein is very similar, in structure and function, to destrin described here and therefore may be also called destrin. Destrin may be a ubiquitous protein in mammalian cells.

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