Evans, G. A., Margolies, D. H., Camarini-Otero, R. D., Ozato, K., & Seidman, J. G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1994–1998.
Goulmy, E., Termijtelan, A., Bradley, B. A., & van Rood, J. J. (1977) *Nature (London) 266*, 544–545.
Goulmy, E., van Leeuwen, A., Blockland, E., van Rood, J. J., & Biddison, W. E. (1982) *J. Exp. Med. 155*, 1567–1572.
Kaufman, J. F., Andersen, R. L., & Strominger, J. L. (1980) *J. Exp. Med. 152*, 37s–53s.
Kessler, S. W. (1975) *J. Immunol. 115*, 1617–1624.
Klein, J. (1978) *Adv. Immunol. 26*, 55–146.
Klein, J. (1979) *Science (Washington, D.C.) 203*, 516–521.
Krangel, M. S., Orr, H. T., & Strominger, J. L. (1979) *Cell (Cambridge, Mass.) 18*, 979–991.
Lopez de Castro, J. A., Orr, H. T., Robb, R. J., Kostyk, T. G., Mann, D. L., & Strominger, J. L. (1979) *Biochemistry 18*, 5704–5711.
Lopez de Castro, J. A., Strominger, J. L., Strong, D. M., & Orr, H. T. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3813–3817.
McMichael, A. J., Ting, A., Zweerink, H. J., & Askonas, B. A. (1977) *Nature (London) 270*, 524–526.
Melief, C. J. M., de Waal, L. P., van der Meulen, M. Y., Melvold, R., & Kohn, H. I. (1980) *J. Exp. Med. 151*, 993–1013.
Melvold, R. W., Kohn, I., & Dunn, G. R. (1982) *Immunogenetics (N.Y.) 15*, 177–185.
Moore, K. W., Sher, B. T., Sun, Y. H., Eakle, K. A., & Hood, L. (1982) *Science (Washington, D.C.) 215*, 679–682.
Nairn, R., Yamaga, K., & Nathenson, S. G. (1980) *Annu. Rev. Genet. 14*, 241–277.
Nathenson, S. G., Uehara, H., Ewenstein, B. M., Kindt, T. J., & Coligan, J. E. (1981) *Annu. Rev. Biochem. 50*, 1025–1052.
Orr, H. T., Lopez de Castro, J. A., Parham, P., Ploegh, H. L., & Strominger, J. L. (1979a) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4395–4399.
Orr, H. T., Lopez de Castro, J. A., Lancet, D., & Strominger, J. L. (1979b) *Biochemistry 18*, 5712–5720.
Parham, P., & Bodmer, W. F. (1978) *Nature (London) 276*, 397–399.
Parham, P., Alpert, B. N., Orr, H. T. & Strominger, J. L. (1977) *J. Biol. Chem. 252*, 7555–7567.
Ploegh, H. L., Orr, H. T., & Strominger, J. L. (1981) *Cell (Cambridge, Mass.) 24*, 287–299.
Robb, R. J., Terhorst, C. T., & Strominger, J. L. (1978) *J. Biol. Chem. 253*, 5319–5324.
Shearer, G. M., & Schmitt-Verhulst, A.-M. (1977) *Adv. Immunol. 25*, 55–91.
Sherman, L. A. (1980) *J. Exp. Med. 151*, 1386–1397.
Terhorst, C., Parham, P., Mann, D. L., & Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 910–914.
Zinkernagel, R. M., & Doherty, P. C. (1979) *Adv. Immunol. 27*, 51–177.

Acumentin, an Actin-Modulating Protein of Rabbit Pulmonary Macrophages†

Frederick S. Southwick,* Noriyuki Tatsumi, and Thomas P. Stossel

ABSTRACT: An actin-modulating protein has been purified from rabbit alveolar macrophages utilizing DEAE-Sepharose and gel filtration chromatography. The purified protein which we have named acumentin is similar in structure and function to a protein found in human granulocytes [Southwick, F. S., & Stossel, T. P. (1981) *J. Biol. Chem. 256*, 3030–3036] and has a Stokes radius of 34 Å and $s_{20,w}$ of 4.02 S, consistent with a globular protein with a native molecular weight of 63 500. Acumentin has a molecular weight of 65 000 as determined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. This protein is present in high concentrations in macrophages, representing about 6% of the total protein in cytoplasmic extracts. Acumentin caps the pointed end of actin filaments labeled with heavy meromyosin [Southwick, F. S., & Hartwig, J. H. (1982) *Nature (London) 297*, 303–307], thereby decreasing the final viscosity of monomeric actin polymerized in its presence without detectably increasing the critical monomer concentration. The activity of this protein is inhibited by KCl concentrations above 0.1 M and is completely inactive at a KCl concentration of 0.3 M. Acumentin's function is equivalent in the presence or absence of $CaCl_2$. The presence of such a calcium-insensitive capping protein in both the human granulocyte and rabbit alveolar macrophage suggests acumentin may be of general importance in constitutively maintaining a shortened actin filament length distribution in the cytoplasm of the nonmuscle cell.

Monomeric actin purified from either muscle or nonmuscle cells in concentrations above a critical level of about 30 μg/mL polymerizes into filaments in the presence of neutral salts. When the polymerization reaction is at equilibrium, the solution contains actin filaments of a very broad exponential length distribution which are in a dynamic equilibrium with actin monomers. As determined by a variety of techniques, the apparent degree of actin assembly is maximal, that is, the average length of actin filaments is longest and the equilibrium monomer concentration lowest when purified actin is incubated at 25–37 °C in a solution containing 0.1 M KCl and 1 mM $MgCl_2$ (Oosawa & Kasai, 1971; Kawamura & Maruyama, 1970). Therefore, it is unexpected that actin in concentrations as high as 2 mg/mL in cytoplasmic extracts of diverse cells

† From the Hematology-Oncology and Infectious Disease Units, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114. *Received March 16, 1982.* Support by grants from the U.S. Public Health Service (HL19429, CA 09032), the Muscular Dystrophy Association, and TOA Electronics Co., Ltd.

0006-2960/82/0421-6321$01.25/0

appears either to be primarily in a monomeric state or else to form relatively short filaments under solvent conditions optimal for the assembly of purified actins into long filaments (Bray & Thomas, 1976; Gordon et al., 1977; Blikstad et al., 1978). This observation has suggested that one or more inhibitors of actin assembly exist in nonmuscle cell cytoplasmic extracts, and several such inhibitors have been isolated (Carlsson et al., 1977; Yin & Stossel, 1979; Bretscher & Weber, 1980; Hasegawa et al., 1980; Southwick & Stossel, 1981). These factors, which have different mechanisms of action, may be important for cytoplasmic structure and movement, because the average length of actin polymers can determine the consistency of actin filament solutions (Stossel et al., 1982).

The cytoplasm of rabbit lung macrophage contains a calcium-binding protein, gelsolin, which shortens actin filaments either when assembled in its presence or when mixed with it after assembly. Gelsolin is active in shortening actin filaments only in the presence of calcium concentration above the submicromolar level (Yin & Stossel, 1979, 1980; Yin et al., 1980). In the presence of sufficient Ca^{2+} gelsolin is bound to one end of the shortened actin filaments (Yin et al., 1981). This end is the end that is "barbed" with respect to the orientation of the arrowhead appearance conferred by the binding of heavy meromyosin molecules onto actin filaments (Huxley, 1963). In this paper we show that the macrophage cytoplasm also contains a protein that shortens actin filaments during assembly irrespective of the calcium concentrations. This protein is similar in structure and function to protein first purified from human granulocytes (Southwick & Stossel, 1981). Elsewhere we show that this protein binds to the ends of actin filaments to which the heavy meromyosin arrowheads "point", that is, the end opposite the gelsolin binding site. Therefore, we have named this protein acumentin from the latin *acumen* meaning point (Southwick & Hartwig, 1982).

Materials and Methods

Purification of Acumentin. Lung macrophages obtained from New Zealand white rabbits as previously described (Hartwig & Stossel, 1975) were washed twice with ice-cold 0.15 M NaCl solution by centrifugation at 250*g* at 4 °C for 10 min. The packed cells were then suspended in an equal volume of ice-cold 0.15 M NaCl solution followed by the addition of 0.05 volume of 0.1 M diisopropyl fluorophosphate (Sigma Chemical Co., St. Louis, MO) maintained as a stock solution of 0.1 M in propylene glycol (Amrein & Stossel, 1980). After standing in ice for 5 min, the cells were washed with an equal volume of deionized water by centrifugation at 250*g* for 10 min. The packed macrophages were suspended in 2 volumes of the homogenizing buffer containing 0.34 M sucrose, 5 mM dithiothreitol, 2 mM ATP, 5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), and 20 mM imidazole hydrochloride, pH 7.5, and broken in the cold (4 °C) by 100 stokes with a tight-fitting pestle in a glass Dounce homogenizer. The homogenates were centrifuged at 12000*g* at 4 °C for 1 h. The supernatant fluid, designated S_1, was adjusted to pH 7.5 by addition of 0.1 M NaOH. The S_1 solution was made 0.6 M with potassium chloride, incubated at 25 °C for 2 h, and centrifuged at 100000*g* for 2 h to remove actin filaments. This supernatant fluid was then dialyzed overnight in 400 volumes of ice-cold solution containing 0.75 mM β-mercaptoethanol, 0.5 mM ATP, 0.1 mM $MgCl_2$, 1 mM EGTA, and 10 mM imidazole, pH 7.5 (buffer A). This actin-depleted macrophage extract was required to assess inhibition of actin assembly in the initial starting preparation (see Results). The extract was applied to a 2.5 × 10 cm column containing DEAE-Sepharose CL-6B anion-exchange resin (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) equilibrated with 500 mL of buffer A. After the sample was applied, the column was percolated with 50 mL of buffer A, followed by 300 mL of 0.1 M KCl in buffer A, followed, in turn, by a 500-mL linear 0.1–0.4 M KCl gradient in buffer A. Eluted fractions with activity decreasing the final viscosity of actin were pooled, dialyzed against 0.1 M KCl in buffer A, and concentrated to a volume of 2–3 mL in a nitrogen pressure concentrator using an Amicon PM-10 ultrafiltration membrane (Amicon Corp., Lexington, MA; lot AE 01248 C). The concentrated sample was gel filtered on a Sephadex G-200 column (1.2 × 95 cm) equilibrated and eluted with 0.1 M KCl in buffer A. On several occasions, to enhance purity, this step was followed by hydroxylapatite chromatography using a 1 × 8 cm column eluted with a 120-mL linear gradient of 0.05–0.4 M potassium phosphate buffer, pH 6.5.

Purification of Muscle Actin. Actin was purified from rabbit skeletal muscle by the method of Spudich & Watt (1971).

Assay for Acumentin Activity. Samples to be tested were dialyzed against 100 volumes of 0.1 M KCl in buffer A. To 310 μL of each test sample was added 20 μL of 10 mg/mL G-actin (final actin concentration 0.65 mg/mL), and the mixture was incubated at 20 °C for 2 h. Specific viscosities were measured and compared with those of actin incubated in 0.1 M KCl in buffer A.

Viscometry. Viscosity was measured in Cannon-Manning semimicroviscometers (extra low charge, size 100) with buffer flow times of 48–50 s at 25 °C.

Polyacrylamide Gel Electrophoresis. The discontinuous pH 5–15% polyacrylamide gradient slab gel system of Laemmli (1970) was used. Protein samples were boiled for 1 min in 1% sodium dodecyl sulfate, 10% sucrose, and 2% β-mercaptoethanol. The intensity of the Coomassie blue stained polypeptide bands was determined by scanning the gels with a densitometer (E-C gel scanner). For determination of molecular weight, acumentin was subjected to electrophoresis together with four other polypeptides of known molecular weights and the negative logarithm of the relative mobilities plotted vs. the molecular weights as described by Neville (1971).

Isoelectric Focusing. Isoelectric focusing was carried out according to the technique of O'Farrell (1975). Urea was not added to the isoelectric gels.

Stokes Radius Determination. The elution volumes of actin polymerization inhibitor from the Sephadex G-200 column equilibrated with 0.1 M KCl in buffer A were compared with those of standard proteins of known Stokes radii. The void and column volumes were identified with blue dextran and ATP, respectively. The Stokes radius of acumentin was determined by extrapolation of the linear plot of $-\log K_{av}(1/2)$ vs. Stokes radius (Laurent & Killander, 1964).

Analytical Ultracentrifugation. Sedimentation velocity experiments were performed in a Beckman–Spinco Model E analytical ultracentrifuge. Sedimentation studies were done in 0.1 M KCl in buffer A at 20 °C at 42000 rpm, using schlieren optics. The sedimentation coefficient was calculated and correlated to standard conditions according to Schachman (1957).

Amino Acid Analysis. Samples of purified acumentin were dialyzed extensively against distilled water, lyophilized, and hydrolyzed with 5.7 N HCl at 100 °C in an evacuated desiccator for 24 h in the presence of 1:2000 (v/v) mercapto-

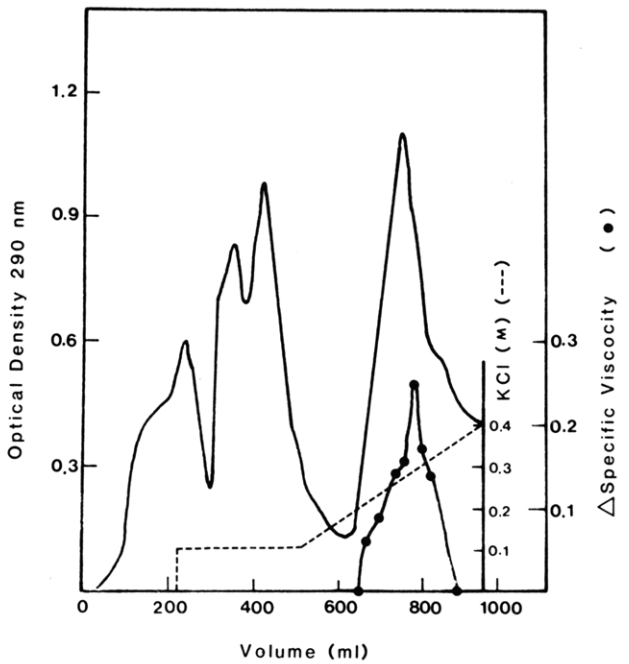

FIGURE 1: Chromatography of S_1 on DEAE-Sepharose. Adherent protein was eluted in 10-mL fractions with 500 mL of a linear 0.1–0.40 M KCl concentrations gradient in buffer A. Absorbance at 290 nm (—), inhibition of actin assembly (●), and potassium concentration of elution buffer (---) are indicated.

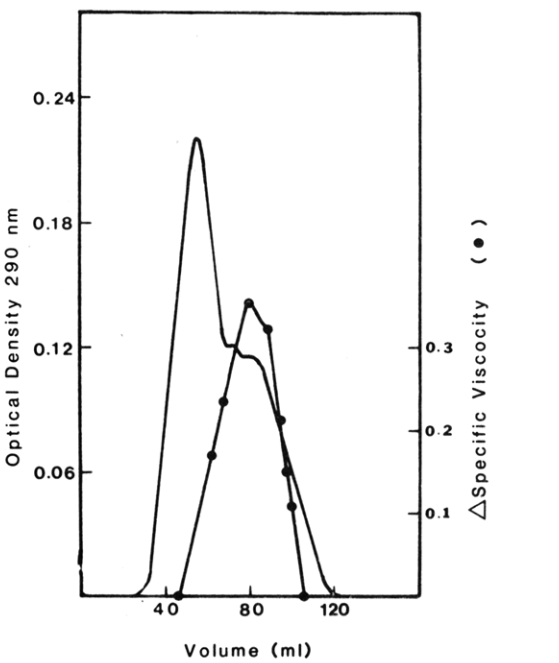

FIGURE 2: Chromatography of DEAE inhibitor peak on Sephadex G-200. Pooled DEAE-Sepharose fractions with inhibitory activity were filtered through a Sephadex G-200 column (1.2 × 95 cm). Samples of 2 mL were collected and assayed for inhibitory activity as described in the text. Absorbance at 290 nm (—) and inhibition of actin assembly (●).

ethanol. Amino acid analysis was performed in a Beckman 121-MB amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA).

Rotary Shadowing. A 50 μg/mL concentration of purified acumentin in 50% glycerol was sprayed onto mica; the mica was dried under vacuum and rotary shadowed with platinum on carbon at a 4.5% angle as described by Tyler et al. (1980).

Results

Purification of Acumentin from Macrophages. The starting material for purification of acumentin consisted of macrophage extract from which actin had been removed by ultracentrifugation as described under Materials and Methods. When actin was not utlracentrifuged from the extract, the solution formed a rigid gel, making evaluation of actin filament formation by viscosity or sedimentation impossible. Purified skeletal muscle monomeric actin (1.1 mg/mL) was added to the actin-depleted macrophage extract (6.5 mg/mL). This extract's effect on the final viscosity of actin proved to be dependent on the potassium chloride concentration of the solution. Incubation for 2 h at 25 °C in ionic conditions which should favor the assembly of purified actin (0.1 M KCl) resulted in a final viscosity of actin in buffer A of 0.82, while the final viscosity of the same concentration of actin polymerized in the presence of extract was 0.36. This marked reduction in the final viscosity of actin polymerized in extract was reversed by raising the KCl concentration to 0.6 M. In this ionic condition the specific viscosities of 1.1 mg/mL of purified actin polymerized in buffer (0.67) and extract (0.65) were nearly the same. Similar results were noted in three of three other experiments. These findings were indicative of the presence of a KCl-sensitive activity in the actin-depleted macrophage extract which reduces the final viscosity of actin.

As shown in Figure 1 an activity inhibiting the final viscosity of actin eluted as a single peak from DEAE ion-exchange chromatography of the actin-depleted macrophage extract. The active fractions were pooled, concentrated, and gel filtered through a Sephadex G-200 column. As shown in Figure 2,

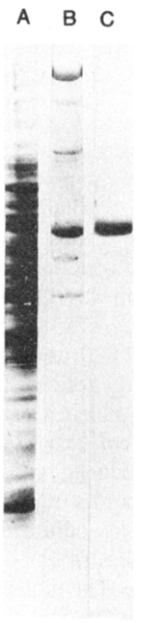

FIGURE 3: Coomassie blue stained polyacrylamide gels of various purification steps. (A) S_2; (B) DEAE peak fraction; (C) G-200 peak fraction after electrophoresis in dodecyl sulfate. The approximate protein loads applied to the gels were (A) 200, (B) 50, and (C) 20 μg.

the activity eluted as a single, nearly symmetrical peak. In some preparations, a third purification step, hydroxylapatite chromatography, was utilized. Polyacrylamide gel electrophoresis in dodecyl sulfate of active samples after each purification step (Figure 3) demonstrated that increases in specific activity correlated strongly with enrichment of a M_r 65 000 polypeptide. Yields, total protein, and specific activities of the pooled fractions containing activity at various steps are shown in Table I. Following the last purification step, the 65 000-dalton polypeptide was 85–91% pure as assessed by densitometry of Coomassie blue stained polyacrylamide gels

Table I: Purification of Macrophage Acumentin

fraction	protein: total protein (mg)	protein: protein[a,b] (mg)	protein: yield (%)	activity: specific[c]	activity: total	activity: yield (%)
extract	1012	60.7		0.20	202.4	
DEAE-Sepharose	120	30	49.4	0.50	55.2	27.3
gel filtration	6.8	5.4	8.9	2.91[d]	19.7	9.7

[a] Determined for pooled fractions. [b] Percentage of total protein in 65 000-dalton polypeptides as determined by quantitative densitometry of Coomassie blue stained polyacrylamide gels. Total protein determined by Bio-Rad protin assay (Bradford, 1976). [c] Determined by viscosity: specific viscosity per milligram of protein. [d] The peak fractions contained up to 90% inhibitor by densitometry and had specific activities as high as 4.0.

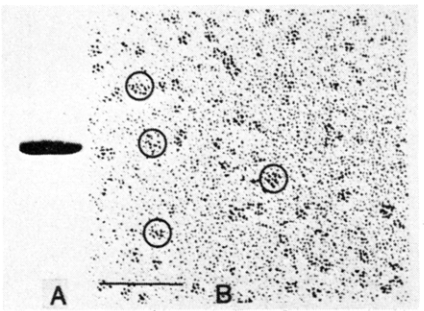

FIGURE 4: (A) Coomassie blue stained polyacrylamide gel of acumentin purified by DEAE ion exchange and G-200 gel filtration after electrophoresis in dodecyl sulfate; protein load approximately 30 μg. (B) Electron micrograph of the same preparation of acumentin after rotary shadow staining. Bar represents 50 nm.

of peak fractions subjected to electrophoresis on polyacrylamide gels in dodecyl sulfate. Of the minor components comprising 15% or less of the nine preparations purified, there was no correlation between their density and activity during various purification steps. Unlike the inhibitor protein purified from granulocytes which contained two polypeptide subunits, only one polypeptide chain was observed in the active fractions. If the protein was kept at 4 °C for prolonged periods, active fractions acquired a 62 000 as well as 65 000 polypeptide without apparent loss of activity. We suspect this lower molecular weight monomer is a proteolytic fragment.

Physical and Chemical Properties of Acumentin. The molecular weight of reduced and denatured macrophage acumentin estimated from its mobility during electrophoresis in polyacrylamide gels in sodium dodecyl sulfate is 65 000 (Figure 4A). The Stokes radius of acumentin was 3.4 nm and the $s_{20,w}$ was 4.02 S. The molecular weight of the native protein calculated from its Stokes radius, partial specific volume, and sedimentation coefficient in physiologic ionic strength was 63 500 (Siegel & Monty, 1966). The low frictional coefficient (1.3) is consistent with a globular protein (Siegel & Monty, 1966). The molecular size and shape were confirmed in the electron microscope. Low-angle platinum-on-carbon shadowed replicas reveal globular particles 6–8 nm in diameter (Figure 4B). The radius calculated from the measurement of these particles in electron micrographs was 3.08 ± 0.32 nm (mean ± SD).

Table II shows the amino acid composition of the purified protein. Acumentin's isoelectric point was 4.7.

Effects of Purified Acumentin on Actin. The maximum viscosity of both control and acumentin-treated actin polymerized by the addition of 0.1 M KCl was reached by 30 min at 25 °C in buffer A. The final viscosity of G-actin assembled in the presence of acumentin was diminished relative to the control actin and remained constant over time. The amount of inhibition was equivalent, whether acumentin was preincubated with actin before addition of KCl or added simultaneously with this cation. Addition of up to 0.3 mg of acumentin to 0.6 mg of preformed F-actin only minimally decreased the viscosity as compared to that of F-actin in buffer alone (data not shown).

Table II: Amino Acid Compositions of Rabbit Alveolar Macrophage Acumentin and Granulocyte Inhibitor Protein

amino acid	residues[a]: macrophage	residues[a]: granulocyte	mol %: macrophage	mol %: granulocyte
aspartic acid	53	65	10.4	13.2
threonine	28	22	5.5	4.5
serine	31	32	6.0	6.2
glutamic acid	57	60	11.2	12.2
proline	19	22	3.7	4.4
glycine	92	39	18.0	8.0
alanine	46	38	8.9	7.9
valine	24	25	4.7	5.1
methionine	9	10	1.7	2.1
isoleucine	22	27	4.2	5.5
leucine	39	53	7.7	10.9
tyrosine	8	14	1.6	2.9
phenylalanine	10	13	1.9	2.6
lysine	55	40	10.7	8.1
histidine	8	8	1.6	1.6
arginine	19	24	3.7	4.9
total	520	492		

[a] Calculated assuming a molecular weight of 63 000.

The final viscosity of a fixed actin concentration (1.1 mg/mL) assembled in the presence of acumentin decreases in direct proportion to the concentration of acumentin added (data not shown). (Samples were incubated for 2 h at 25 °C in buffer A made 0.1 M with 3 M KCl.) The activity of acumentin (defined as the apparent reduction in actin concentration) as a function of acumentin concentration can be described by a straight line with a slope of 1.7; that is, 1 g of acumentin has an effect on final viscosity equivalent to reducing the actin concentration by 1.7 g. When these values are expressed in moles, addition of 1 mol of acumentin is equivalent to removing 3 mol of actin from the solution as measured by viscometry. Acumentin is present in high concentrations in actin-depleted macrophage cytoplasmic extracts, representing 6–7% of the total protein. On the basis of the above stoichiometric analysis, the decrease in specific viscosity of purified actin polymerized in the presence of this extract can be completely accounted for by acumentin. Recent studies of acumentin reveal that this protein reduces the viscosity of actin by nucleating G-actin and capping filament ends, resulting in a shortened filament length distribution (Southwick & Hartwig, 1982). The simplest interpretation of the data, therefore, is that when the average filament length is decreased, acumentin reduces the apparent effective concentration of actin as measured by viscometry.

Figure 5 shows the effect of acumentin on the apparent critical concentration of actin. Although the slope defining viscosity as a function of total actin was decreased, suggesting an inhibition of elongation, the intercept or the critical concentration remained the same as actin polymerized in salt solutions alone.

Figure 6 shows the effect of different KCl concentrations on the final viscosity of actin assembled in the presence of a fixed concentration of acumentin. As the KCl concentration was raised above 0.1 M KCl, the viscosity of the solution increased, nearly approaching that of actin in buffer. The

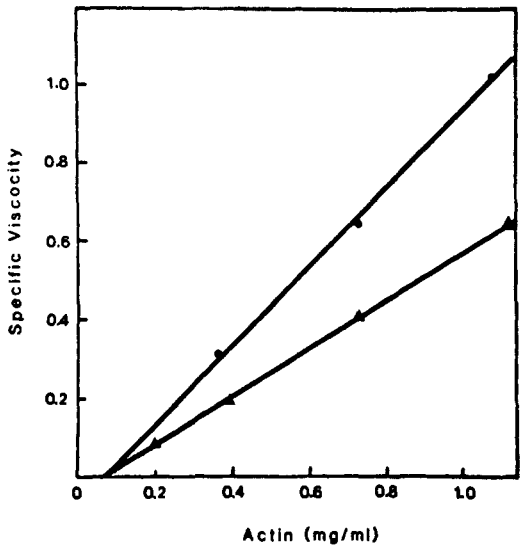

FIGURE 5: Effect of acumentin on the apparent critical monomer concentration of actin. Various concentrations of skeletal muscle G-actin were mixed with buffer A made 0.1 M with 3 M KCl or with a fixed weight ratio of acumentin (3:1, actin to acumentin) in the same buffer. Specific viscosities of the samples were measured after incubation at 25 °C for 2 h. Critical monomer concentration was defined as the x intercept of the line representing specific viscosity as a function of actin concentration. Actin alone (●); actin and acumentin (▲).

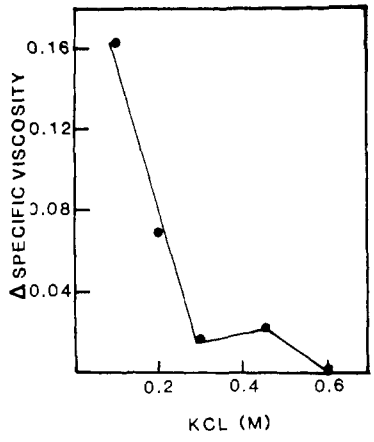

FIGURE 6: Effect of KCl concentration on the viscosity of actin polymerized with purified acumentin. Acumentin (final concentration 0.15 mg/mL) was mixed with KCl solutions of increasing concentration in buffer containing 10 mM imidazole, 0.5 mM ATP, 1 mM EGTA, and 1 mM $MgCl_2$, pH 7.5. Purified skeletal muscle G-actin was then added to each sample (final concentration 0.65 mg/mL) and incubated for 2 h at 25 °C. The specific viscosities of these solutions were compared to that of G-actin incubated in the same buffers. The vertical axis represents the decrease in specific viscosity induced by acumentin at each KCl concentration.

acumentin activity was not reduced when the assay system contained 1 mM $MgCl_2$. Varying the free calcium concentration between 10^{-8} M and 10^{-4} M by means of calcium–EGTA mixtures also did not detectably alter this effect on actin viscosity.

Discussion

When skeletal muscle actin is added to actin-depleted macrophage extracts under ionic conditions which should favor purified actin assembly, the final viscosity of the actin solution is much lower than that in buffer alone. Raising the potassium chloride concentration to 0.6 M completely reverses this effect. The KCl-sensitive activity has been purified by using DEAE ion-exchange and gel filtration chromatography. The resulting highly active preparations contain a nearly homogeneous polypeptide of 65 000 daltons. The activity does not correlate with the presence of any other polypeptide band in various purification steps. The protein is very similar to a protein purified from granulocytes as assessed by its behavior during electrophoresis on polyacrylamide gels in dodecyl sulfate, gel filtration, and analytical ultracentrifugation and by its amino acid composition (Southwick & Stossel, 1981). Although the protein purified from granulocytes contained a mixture of 65 000- and 62 000-dalton polypeptides, we suggested that the 62 000-dalton peptide was derived from the 65 000-dalton polypeptide (Southwick & Stossel, 1981). The less active neutral protease activity of macrophages compared to that of granulocytes (Amrein & Stossel, 1980) may account for the absence of this lower molecular weight polypeptide in fresh macrophage inhibitor preparations, although it appeared in aged preparations of the macrophage protein.

Functionally, the proteins purified from granulocytes and macrophages are also very similar. As observed with the actin inhibitor protein from granulocytes, actin assembled in 0.1 M KCl in the presence of the macrophage acumentin has a lower final viscosity relative to control actin. Both proteins only minimally decreased the viscosity of preformed actin filaments, and the effect of both proteins on actin assembly is sensitive to the KCl concentration. KCl concentrations above 0.1 M diminish the proteins' activity.

In previous work we described the granulocyte protein as an "inhibitor of actin polymerization" which implied that it might act by binding to actin monomers or oligomers and prevent them from being assembled into long filaments. Recent studies described elsewhere (Southwick & Hartwig, 1982) have revealed that the macrophage protein actually binds to the "pointed" ends of actin filaments decorated with heavy meromyosin. There it prevents the exchange of actin monomers and inhibits the annealing of actin fragments at that end. Acumentin nucleates actin assembly, an effect which initially produces multiple short filaments. Capping of the pointed ends of these filaments prevents their annealing and maintains a shortened length distribution. Inhibition of annealing may also explain the small diminution in viscosity of preformed actin filaments mixed with acumentin. Both mixing and viscometry can be expected to shear actin filaments, breaking then into shorter fragments which are inhibited from annealing in the presence of acumentin.

Kirschner (1980) has suggested that "capping" of the pointed end of actin filaments would lower the critical monomer concentration. Defining critical concentration as the x intercept of the straight line relating actin viscosity to total actin concentration, we found the critical monomer concentration at which actin monomers began to polymerize was not detectably altered by acumentin. There are several reasons for this discrepancy between theoretical expectations and actual measurements. First, viscometry tends to overestimate the true critical monomer concentration. This measurement fails to differentiate actin monomers from oligomers containing 15–16 actin molecules. For example, the viscosity of F-actin mixed with gelsolin and calcium in a molar ratio of 16:1 is the same as that of buffer alone. On the basis of viscosity, this solution would be interpreted as containing only monomeric actin; however, on the basis of stoichiometric analysis and turbidity studies, this ratio of actin to gelsolin would be expected to produce actin filaments containing approximately 16 monomers (Yin et al., 1980). Second, capping of the pointed end of actin filaments would be expected to result in a very small change in critical monomer concentration, since the exchange rate of actin monomers at this end is estimated to be one-third to one-fifth of the exchange rate observed at

the barbed filament end. On the basis of rate constants for dissociation of actin monomers from filaments determined under ionic conditions used in our experiments, the critical concentrations of actin at the pointed and barbed filament ends are 0.64 and 0.23 μM, respectively (Pollard & Mooseker, 1981). The combined critical concentration of both ends would be 0.31 μM; therefore, capping the pointed end would lower the critical concentration by 0.08 μM, a change which would be undetectable as measured by viscometry. On the other hand, capping of the barbed end of actin filaments would be expected to cause proportionally greater change in the monomer concentration which is measurable by this technique (Yin et al., 1981). Agents which stabilize actin filaments such as phalloidin diminish the dissociation rate constants of monomers at both filament ends (Lengsfeld et al., 1974; Dancker et al., 1975; Wieland, 1975; Faulstich et al., 1977) and would be expected to cause a proportionally greater decrease in monomer concentration than proteins preventing exchange only at the pointed end. This decrease has also have been measured by the viscometric technique (Estes et al., 1981).

Acumentin differs in structure and function from other modulators of actin described in the past. β-Actinin, a protein purified from skeletal muscle, is the only other agent thus far suggested to bind to the pointed end of actin filaments. When copolymerized with actin, this protein also shortens actin filament length. However, unlike acumentin, its activity is enhanced by the addition of $MgCl_2$ at concentrations of 1 mM or higher. β-Actinin also differs structurally from acumentin, being composed of 37 000- and 34 000-dalton polypeptides (Maruyama et al., 1977).

The finding of high concentrations of acumentin in cytoplasmic extracts of human granulocytes and now rabbit alveolar macrophages raises the possibility that this protein may be present in other nonmuscle cells. Such a calcium-insensitive capping protein could serve to maintain a short filament length distribution when cytoplasmic calcium concentrations are low.

Acknowledgments

We thank Dr. John Hartwig for providing the electron micrograph of acumentin as well as his analytical assistance in preparing the manuscript.

References

Amrein, P. C., & Stossel, T. P. (1980) *Blood 56*, 442–447.
Blikstad, I., Markey, F., Carlsson, L., Persson, T., & Lindberg, U. (1978) *Cells (Cambridge, Mass.) 15*, 935–943.
Bradford, M. (1976) *Anal. Biochem. 72*, 248.
Bray, D., & Thomas, C. (1976) *J. Mol. Biol. 105*, 527–544.
Bretscher, A., & Weber, K. (1980) *Cell (Cambridge, Mass.) 20*, 839–847.
Carlsson, L., Nystrom, L.-E., Sundkvist, I., Markey, F., & Lindberg, U. (1977) *J. Mol. Biol. 115*, 465–483.
Dancker, P., Low, I., Hasselbach, W., & Wie, L. T. (1975) *Biochim. Biophys. Acta 400*, 407–414.
Estes, J. E., Selden, L. A., & Gershman, L. C. (1981) *Biochemistry 20*, 708–712.
Faulstich, H., Schafer, A. J., & Weckauf, M. (1977) *Physiol. Chem. 358*, 181–184.
Gordon, D. J., Boyer, J. L., & Korn, E. D. (1977) *J. Biol. Chem. 252*, 8300–8309.
Hartwig, J. H., & Stossel, T. P. (1975) *J. Biol. Chem. 250*, 5699–5707.
Hasegawa, T., Takahashi, S., Hayashi, H., & Hatano, S. (1980) *Biochemistry 19*, 2677–2683.
Huxley, H. E. (1963) *J. Mol. Biol. 3*, 281–308.
Kawamura, M., & Maruyama, K. (1970) *J. Biochem. (Tokyo) 67*, 437–457.
Kirschner, M. W. (1980) *J. Cell Biol. 86*, 330–334.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 317–330.
Lengsfeld, A. M., Low, I., Weiland, T., Dancker, P., & Hasselbach, W. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2803–2807.
Maruyama, K., Kimura, S., Ishii, T., Kuro, D. A., Ohashi, K., & Muramatsu, S. (1977) *J. Biochem. (Tokyo) 81*, 215–232.
Neville, D. M., Jr. (1971) *J. Biol. Chem. 246*, 4866–4871.
O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.
Oosawa, F., & Kasai, M. (1971) in *Biological Systems* (Timasheff, S. N., & Fasman, G. D., Eds.) Part A, Chapter 6, pp 261–321, Marcel Dekker, New York.
Pollard, T. D., & Mooseker, M. S. (1981) *J. Cell Biol. 88*, 654–659.
Schachman, H. K. (1957) in *Enzymology*, Chapter 2, Academic Press, New York.
Siegal, L. M., & Monty, K. J. (1966) *Biochim. Biophys. Acta 112*, 346–362.
Southwick, F. S., & Stossel, T. P. (1981) *J. Biol. Chem. 256*, 3030–3036.
Southwick, F. S., & Hartwig, J. H. (1982) *Nature (London) 297*, 303–307.
Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Stossel, T. P., Hartwig, J. H., Yin, H. L., Zaner, K. S., & Stendahl, O. I. (1982) *Cold Spring Harbor Symp. Quant. Biol.*, 569–578.
Tyler, J. M., Anderson, J. M., & Branton, D. (1980) *J. Cell Biol. 85*, 489–495.
Wieland, T. (1977) *Naturwissenschaften 64*, 303–309.
Yin, H. L., & Stossel, T. P. (1979) *Nature (London) 281*, 585–586.
Yin, H. L., & Stossel, T. P. (1980) *J. Biol. Chem. 255*, 9490–9493.
Yin, H. L., Zaner, K. S., & Stossel, T. P. (1980) *J. Biol. Chem. 255*, 9494–9500.
Yin, H. L., Hartwig, J. H., Maruyama, K., & Stossel, T. P. (1981) *J. Biol. Chem. 256*, 9693–9697.