Acanthamoeba Actin. Isolation and Properties*

Robert R. Weihing and Edward D. Korn

ABSTRACT: Actin has been isolated from the soil amoeba *Acanthamoeba castellanii* (Neff strain) and shown to resemble rabbit muscle actin in several ways: it is a globular 3S monomer at low ionic strength and a fibrous 30S polymer at high ionic strength; the fibers form viscous complexes with muscle myosin which are dissociated by ATP; the fibrous amoeba actin activates Mg-ATPase of rabbit heavy meromyosin at low ionic strength. The amino acid analysis of *Acanthamoeba* actin, including the presence of 3-methylhistidine, resembles that of various muscle actins and the electrophoretic migration in urea-containing polyacrylamide gels of reduced carboxymethylated amoeba actin is identical with that of similarly treated rabbit muscle actin. The molecular weight (39,500, measured by short-column equilibrium ultracentrifugation in guanidine hydrochloride and 2-mercaptoethanol) of amoeba actin is slightly smaller than that of muscle actin (46,000), and the amoeba actin contains a second unusual amino acid, ϵ-*N*-dimethyllysine, which is not present in muscle actin.

In order to understand the molecular bases of cell movement and cytoplasmic streaming a number of investigators have searched for proteins similar to those of muscle in cells and organisms which lack organized contractile tissues. Highly purified proteins whose chemical and physical properties closely resemble muscle actin have been prepared from the plasmodium of the slime mold *Physarum polycephalum* (Hatano and Oosawa, 1966a,b; Adelman and Taylor, 1969a,b; Nachmias *et al.*, 1970) and the amoebas of *Dictyostelium discoideum* (Woolley, 1970). Less completely characterized as actins are proteins prepared from sea urchin eggs (Hatano *et al.*, 1969; Miki-Noumura and Oosawa, 1969; Miki-Noumura, 1969; Miki-Noumura and Kondo, 1970) and from leukocytes (Senda *et al.*, 1969). Actin has also been indirectly identified *in situ* in several glycerinated nonmuscle cells as thin filaments that form ATP-dissociable complexes with rabbit muscle heavy meromyosin (HMM).[1] Ishikawa *et al.* (1969) first identified HMM binding filaments in embryonic fibroblasts, chondrocytes, epidermal cells, and intestinal epithelial cells. Thin filaments of thrombocytes (Shepro *et al.*, 1969) and of motile extracts of *Amoeba proteus* (Pollard and Korn, 1970) also form ATP-dissociable complexes with rabbit muscle heavy meromyosin.

In preliminary reports (Weihing and Korn, 1969a,b, 1970) we have described briefly the purification and the salient properties of a protein from *Acanthamoeba castellanii* (Neff strain) identified as actin by the criteria defined by Barany *et al.* (1958): the ability to form fibrous polymers; the ability of the fibrous protein to form ATP-dissociable complexes with muscle myosin; the ability of the fibrous protein to activate the Mg-ATPase of myosin. We have also demonstrated that the thin filaments in the cytoplasm of *A. castellanii* (Bowers and Korn, 1968) form characteristic arrowhead complexes *in situ* when glycerinated amoebas are reacted with muscle heavy meromyosin (Pollard *et al.*, 1970). In this paper we describe in detail the methods developed for the isolation of highly purified amoeba actin, and we present a more complete characterization of the protein.

Methods

Large-Scale Culture of A. castellanii. Amoebas were routinely grown in rotating cultures in Neff's medium (Neff *et al.*, 1958) and cell concentrations were estimated as previously described (Weisman and Korn, 1966). To obtain the quantity of cells required for the isolation of actin, however, it was necessary to culture the amoebas in carboys and fermentation tanks. These large quantities of amoebas frequently became contaminated by bacteria or yeast until the following procedures were adopted.

Carboys, containing 15 l. of Neff's medium and sufficient antifoam (Dow Corning, Antifoam AF) to coat lightly about half the neck of the carboy, were sterilized at 130°, 15 psi for 90 min with an aeration and sampling assembly clamped in place. When the carboys had cooled to room temperature (between 22 and 25°) the aeration assembly was removed just far enough to allow inoculation with two 5- to 7-day-old, 1-l. cultures. The amoebas were kept suspended during the growth period (5–7 days) by slow stirring with a magnetic stirrer, and the carboys were continually oxygenated with pure O_2 at a flow rate of 85 cc/min. Throughout the growth period samples were withdrawn through the sampling tube. In nine experiments the average doubling time was about 35 hr for the first 100 hr of growth and about 70 hr for the next 100 hr of growth. The average yield was 1×10^{10} cells which contained 6% cysts.

Amoebas were also grown in Neff's medium in a 300-l. fermenter (Stainless and Steel Products Co., St. Paul, Minn.). Sufficient antifoam was present in the inocula so that additional antifoam was not necessary. Sterilization was at 130° for 60–90 min followed by rapid cooling to the desired temperature. The fermenter was inoculated with the contents of two or three carboys of 5- to 7-day cultures and cells were grown at 25 or 28° for up to 5 days. The cells were kept in suspension by operating the stirrer at the slowest speed possible, and oxygenation was maintained at a rate of about 1700 cc/min. The cultures were sampled daily through a steam-heated sampling valve. In six experiments doubling times varied between 24 and 64 hr. Approximately 2×10^{11} cells were obtained which contained about 2% cysts.

Amoebas were collected from the carboys by low-speed, batch centrifugation and from fermenter cultures by continuous low-speed, refrigerated centrifugation using the 1357A

* From the National Heart and Lung Institute, Laboratory of Biochemistry, Section on Cellular Biochemistry and Ultrastructure, Bethesda, Maryland. *Received August 24, 1970.*

[1] Abbreviations used are: HMM, heavy meromyosin; EGTA, ethylene glycol bis(β-amino ethyl ether)-*N,N'*-tetraacetic acid.

solid basket rotor of the International PR-2 centrifuge while oxygenation was maintained and the fermenter was cooled. Cells were extracted immediately or were stored frozen at −15° for no more than 10 days. Actin could not be identified in cells stored frozen for over 10 days.

Muscle Proteins. Myosin was prepared by the second method of Kielley and Bradley (1956), HMM by the method of Eisenberg and Moos (1968), and actin by the method of Rees and Young (1967) from back and hind leg muscles of New Zealand white rabbits.

Viscosity. Measurements were made at room temperature (24–26°) using Ostwald-type viscometers with a capacity of 3 ml and an outflow time of about 80 sec for water. Viscosities were calculated as follows: relative viscosity = η_R = flow time for protein solution/flow time for buffer alone; specific viscosity = $\eta_S = \eta_R - 1$. Since the graph of specific viscosity *vs.* concentration of actin solutions was linear and passed through or close to the origin, as expected from studies with muscle actin (Maruyama and Gergely, 1962; Nagy and Jencks, 1965), the viscosity data of Table I are expressed as reduced viscosity calculated from the formula η_S/c, where c is the protein concentration in grams per deciliter.

Flow Birefringence. Flashes of light were observed when 0.8 ml of a birefringent solution (light path 0.6 cm) was stirred while held between crossed polaroids.

Analytical Ultracentrifugation. A Spinco Model E ultracentrifuge equipped with electronic speed control was used. Chamber temperature was kept at 20°. In sedimentation velocity analyses the displacements of peaks were measured using a Nikon microcomparator equipped with a plate projector, and uncorrected sedimentation coefficients were calculated in the usual way (Schachman, 1957).

Equilibrium ultracentrifugation was carried out at several speeds by the meniscus depletion method of Yphantis (1964). After several days dialysis of the protein against 5 M guanidine hydrochloride (Mann, Ultra Pure) containing about 1 mM 2-mercaptoethanol (product of Eastman), 0.15 ml of protein solution, and solvent were separately layered over 0.030 ml of FC-43 oil in the two compartments of a double-sector cell with a filled-epon centerpiece. The concentration of guanidine hydrochloride was estimated from the refractive index of the solvent (measured with a Bausch and Lomb, Abbe-type, refractometer) using data of Kielley and Harrington (1960). The protein distribution was measured using the photoelectric scanner set at 292 nm to minimize interference by ATP present in the medium. The slopes of plots of ln c *vs.* x^2 were used to calculate the molecular weight using the formula $M = (2RT/\omega^2(1 - \bar{v}\rho)(\mathrm{d} \ln C/\mathrm{d}X^2)$. The partial specific volume of amoeba actin ($\bar{v}$ = 0.74 ml/g) was calculated by the method of Cohn and Edsall (1943) from the amino acid composition shown in Table III. Partial specific volumes for 3-methylhistidine and ϵ-*N*-dimethyllysine were calculated as described by these authors.

Gel Filtration. Ascending flow filtration on Sephadex G-200 was carried out under constant hydrostatic pressure maintained with a Marriot bottle. The fluid volume outside the gel particles (V_0) and the total fluid volume (V_t) were estimated from the elution volumes of Blue Dextran and NaCl, respectively. These parameters plus V_e, the elution volume of actin were used to calculate K_D, the partition coefficient between the liquid inside and outside the gel phase according to the formula $K_D = (V_e - V_0)/(V_t - V_0)$.

Electron Microscopy. Actin was negatively stained with potassium phosphotungstate or uranyl acetate and photographed with a Siemens 1A Elmiskop at an instrumental magnification of 30,000–60,000 (Pollard *et al.*, 1970).

Amino Acid Analysis. Proteins were dialyzed against several changes of distilled water, lyophilized, suspended in 6 N HCl in sealed, evacuated tubes flushed three times with N_2, and hydrolyzed for 22, 48, or 72 hr at 105°. The hydrolysates were dried overnight under reduced pressure over NaOH and taken up in water. The pH was adjusted to between 12 and 13 with NaOH and the hydrolysates were dried overnight as before to remove excess ammonia. The hydrolysates were taken up in water, and the pH was adjusted to between 2 and 3 with HCl and suitable aliquots were then analyzed. Half-cystine was estimated in one analysis as cysteic acid after performic acid oxidation by the method of Hirs (1956). In two analyses half-cystine was estimated as *S*-carboxymethylcysteine. The actin was dissolved in 5 M guanidine hydrochloride containing 0.2 M ammonium bicarbonate adjusted to pH 8 with ammonia, and reduced with 2-mercaptoethanol and carboxymethylated with iodoacetamide by the method of Craven *et al.* (1965) as modified by Adelstein and Kuehl (1970).

Neutral and acidic amino acids were determined by the accelerated procedure of Spackman (1964). The systems used for separation of basic and methylated amino acids have been described (Weihing and Korn, 1969a, 1970). Recently, system B of Kuehl and Adelstein (1969) which was previously modified by lowering the flow rate (Weihing and Korn, 1970) was modified a second time by lowering the pH of the buffer from 5.84 to 5.70. This improved the resolution of NH_3 and 3-methylhistidine without affecting the resolution of the methylated lysines. The following retention times for standard amino acids are now observed: ornithine, 197 min; lysine, 207 min; ϵ-*N*-monomethyllysine, 235 min; ϵ-*N*-dimethyllysine, 251 min; ϵ-*N*-trimethyllysine, 261 min; histidine, 293 min; NH_3, 317 min; and 3-methylhistidine, 335 min. Tryptophan was measured spectroscopically in phosphate-buffered guanidine solutions of amoeba actin as described by Edelhoch (1967).

Polyacrylamide Gel Electrophoresis. The alkaline system of Davis (1964) as formulated in 1968 by Canal Industrial Corp. with 4, 5, or 6% separating gels containing 8.5 M urea was used. Reduced, carboxymethylated actin was dissolved in electrode buffer containing 8.5 M urea and 30% glycerol, carefully layered over the stacking gel, and subjected to electrophoresis for about 1.5 hr at constant current of 2.5 mA/tube. Gels were fixed for several hours in the 5% trichloroacetic acid–5% sulfosalicylic acid solution recommended by Chrambach *et al.* (1967) for urea-containing gels, stained 1–2 hr with 0.05% coomassie brilliant blue in 10% acetic acid, and destained in 10% acetic acid.

ATPase. Measurements were kindly performed by Dr. Evan Eisenberg using the pH-Stat method of Eisenberg and Moos (1967).

Protein Analysis. The method of Lowry *et al.* (1951) was used with rabbit skeletal muscle actin from which free ATP had been removed with Dowex 1 (Eisenberg and Moos, 1967) as the standard. The concentration of muscle actin was calculated from the absorbance at 280 nm using the extinction coefficient 1.097 ml/(mg cm) (Rees and Young, 1967). Occasionally the protein concentration of amoeba actin was estimated from the absorbance at 292 nm (to minimize the absorbance from ATP which could not be removed) using the factor 0.5 ml/(mg cm) calculated from the extinction coefficient of muscle actin at 280 nm and the ratio of the absorbance of amoeba actin at 292 and 280 nm

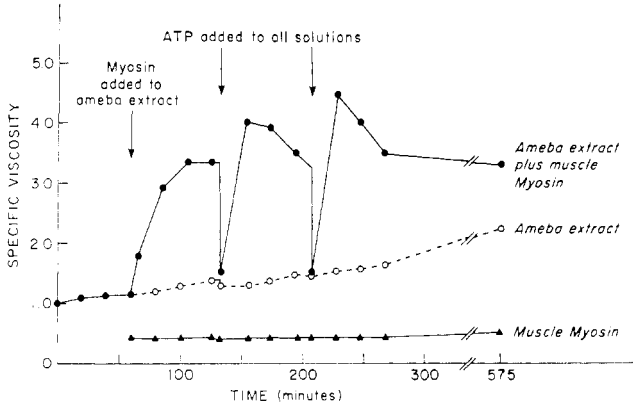

FIGURE 1: Viscosity changes of a crude extract of amoebas upon addition of muscle myosin. The amoeba extract, prepared as described in Results, contained 19 mg of protein/ml of 0.93 M KCl. Myosin (4.7 mg in 0.5 ml of 0.5 M KCl) was added to one of two 3-ml aliquots of amoeba extract and to a separate 3-ml aliquot of 0.93 M KCl. ATP (0.01 ml of 0.1 M, final concentration about 0.3 mM) was added to all three solutions at the time indicated. The measurements of flow time used to calculate the viscosity (see Methods) were begun at the indicated time points.

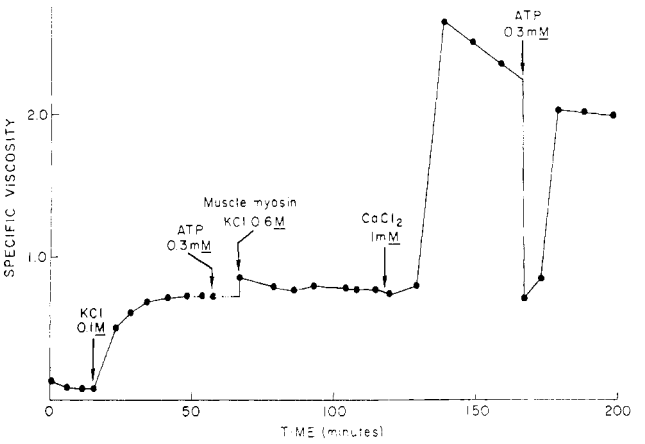

FIGURE 2: Viscosity changes of partially purified amoeba actin. Amoeba actin (10.3 mg) purified through precipitation with acetic acid–acetate was dissolved in 3 ml of 5×10^{-4} M ATP–3×10^{-3} M cysteine (pH 8.1). At the time points indicated 0.1 ml of 3 M KCl (final concentration 0.1 M), 0.01 ml of 0.1 M ATP (final concentration about 0.3 mM), 1.75 mg of muscle myosin in 0.1 ml of 0.5 M KCl plus 0.116 mg of solid KCl (final concentration 0.6 M KCl), and 0.01 ml of 0.3 M $CaCl_2$ (final concentration about 1 mM) were added. Measurements of flow time were begun at the indicated time points.

measured from the spectra used for calculation of tryptophan content (see above).

Measurement of Radioactivity. Proteins (0.1 ml of aqueous solution) were dissolved in 15 ml of 0.4% 2,5-diphenyloxazole in toluene and 0.5 ml of NCS solubilizer (product of Amersham-Searle) and radioactivity was measured using a Beckman LS-250 liquid scintillation spectrometer. The radioactivity of amino acids recovered from the amino acid analyzer was measured as described previously (Weihing and Korn, 1970).

Special Procedures. Water, which was deionized in an apparatus supplied by Hydro Service and Supplies, Inc., Durham, N. C., was used throughout. Dialysis tubing was boiled 0.5 hr in 5% sodium bicarbonate, rinsed, and stored in water at 0–4° (Rees and Young, 1967).

Results

Identification of Actin-Like Activity in Amoeba Extracts. Frozen amoebas were disrupted by thawing, and fresh amoebas by three to four strokes of a tight-fitting Dounce homogenizer. Broken cells were extracted for 1 hr at 0° with two volumes of 1.4 M KCl (final concentration 0.93 M) adjusted to pH 7.5 with 1.0 N NaOH. The extracts were centrifuged at 9000*g* (Sorvall Centrifuge RC-2 B, Rotor SS-34, 8700 rpm) for 15 min and the supernatants were tested for actin-like activity by measuring the change in viscosity caused by addition of purified muscle myosin (Figure 1). In active extracts the rise in viscosity caused by added myosin was greater than the sum of the viscosities of the amoeba extract and myosin measured separately. The viscosity decreased immediately upon the addition of ATP to a value close to that of the sum of the separate viscosities of amoeba extract and myosin. After several minutes, the viscosity increased again, presumably due to the hydrolysis of ATP by myosin and amoeba ATPases. Upon subsequent additions of ATP the cycle was repeated. These results suggested that the extracts contain an actin-like protein (amoeba actin) capable of forming an ATP-dissociable complex with muscle myosin.

A consistent observation, which has not been explained, is that after the addition of ATP the viscosity recovers to a value significantly higher than that measured before the addition of ATP. Similar behavior was reported by Loewy (1952), who studied the ATP sensitivity of concentrated KCl extracts of the slime mold from which both actin and myosin were subsequently isolated (Hatano and Tazawa, 1968; Adelman and Taylor, 1969a,b; Hatano and Ohnuma, 1970).

Purification of Actin. Ameoba actin was purified by the following modification of methods developed for the isolation of actin from slime mold (Hatano and Oosawa, 1966a,b) and muscle (Szent-Györgyi, 1945; Mommaerts, 1952; Adelstein *et al.*, 1963). All operations were carried out at 0°. The results of a typical purification are summarized in Table I. The criteria of purification are the increases of reduced viscosity and in content of 3-methylhistidine and ϵ-*N*-dimethyllysine.

PRECIPITATION BY MUSCLE MYOSIN. Myosin was added to the 9000*g* supernatant fraction (1 g of myosin/10 g of supernatant protein) and an ameoba actin–muscle myosin complex was precipitated by dialysis overnight against 20–30 volumes of 0.05 M KCl. This precipitate was then washed twice with a volume of 0.05 M KCl equal to the volume of the original 9000*g* supernatant, once with two-thirds that volume of 0.4% $NaHCO_3$, and once with a volume of water equal to that of the $NaHCO_3$. After the water wash the precipitate had swollen to two to three times its original volume.

ACETONE POWDER. The washed actin–myosin precipitate was suspended in water and added rapidly to nine to ten volumes of acetone previously chilled to −20°. The suspension was stirred rapidly sometimes with a Waring Blendor operated at low speed. The precipitate was washed quickly with cold acetone, then with room-temperature acetone, and then air-dried at room temperature. During drying the powder was continually turned over with a spatula to prevent the formation of hard, caked aggregates. The dry powder was stored over NaOH in a vacuum desiccator at 2° for as long as 6 weeks. Actin was extracted from the acetone powder

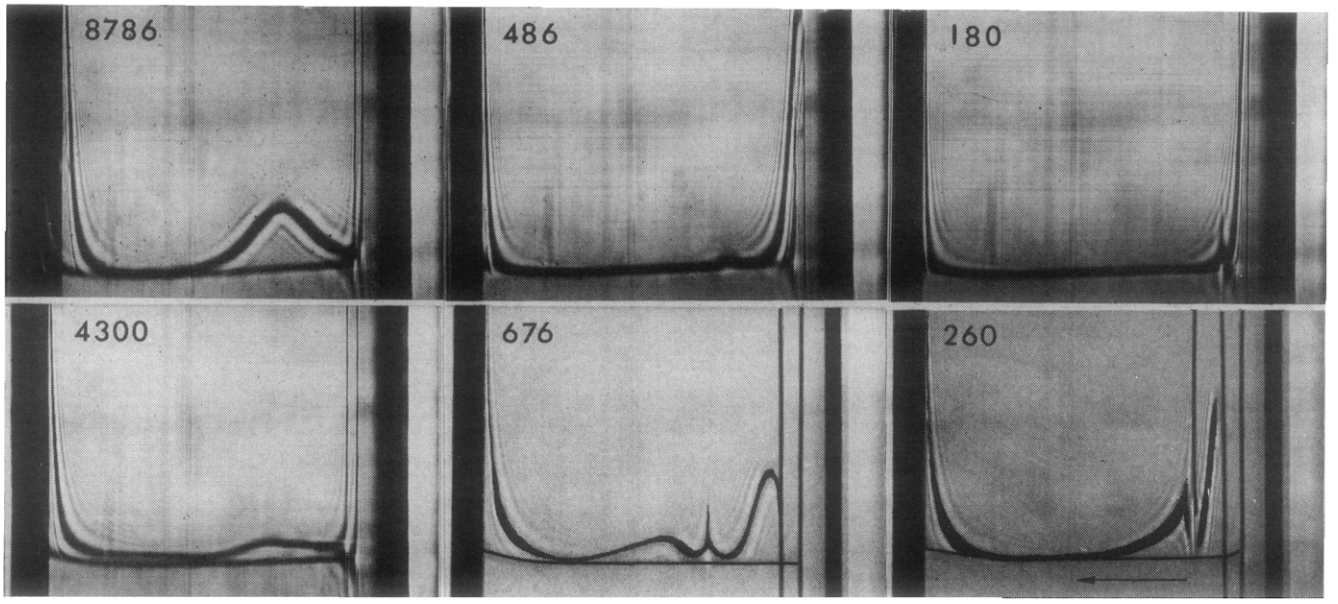

FIGURE 3: Ultracentrifugal evidence for the polymerization of partially purified actin. Actin purified through precipitation with acetic acid–acetate was dissolved in ATP–cysteine in the presence (lower patterns) and absence (upper patterns) of 0.1 M KCl. The protein concentration was 4.4 mg/ml in the absence of KCl and 4.2 mg/ml in the presence of KCl. The rotor speed was 44,000 rpm. The bar angle was 40°. The arrow indicates the direction of sedimentation. Time (in seconds) after reaching speed is shown in the upper left corner of each frame.

TABLE I: Purification of Amoeba Actin.[a]

Fraction	Protein (% of Whole Cells)	Viscosity (dl/g)	Moles/39,500 g 3-MeHis	Moles/39,500 g Me_2Lys
Whole cells	100		0.17	0.14
0.93 M KCl extract	69 (3) (59–78)		0.10	0.18
Acetone powder extract	2.3 (9) (1.4–3.4)	1.4	0.59	0.51
Acetic acid–acetate precipitate	0.9 (9) (0.55–1.5)	1.4	1.0	0.90
105,000*g* pellet	0.36 (9) (0.28–0.43)	2.9	1.0	1.0
Sephadex G-actin	0.14 (9) (0.048–0.31)	3.9	0.78	0.76
Repolymerized[b] F-actin	0.056 (2)		0.95	1.0

[a] Protein values are the average for the indicated numbers of analyses. The range of values is also shown. The viscosity, 3-MeHis and Me_2Lys values are for fractions from one fermenter preparation except for the analyses of whole cells and KCl extract which are each for different preparations. Viscosities were measured after the fractions were adjusted to 0.1 M KCl. [b] Amoeba F-actin was prepared as described previously (Weihing and Korn, 1970) from two separate preparations of amoeba G-actin eluted from Sephadex G-200.

with a solution of 0.5 mM ATP and 3 mM cysteine,[2] pH 8.1 (60 ml/g of powder). The insoluble residue was removed by centrifugation at 10,000*g* for 15 min.

At this stage of purification actin could be detected by a small increase in viscosity upon addition of 0.1 M KCl. The reduced viscosity (Table I) for the crude amoeba F-actin was about one-tenth that of purified muscle actin (Maruyama and Gergely, 1962; Nagy and Jencks, 1965). The contents of 3-methylhistidine and ϵ-*N*-dimethyllysine (an amino acid present in amoeba, but not in muscle, actin) were about five times that of the initial extract and one-half the final maximum value (Table I).

[2] This solution is referred to as ATP–cysteine in the remainder of the text.

PRECIPITATION WITH ACETIC ACID–ACETATE. The actin was concentrated by precipitation with one-tenth volume of a solution of 1 M acetic acid and 1 M sodium acetate. The precipitate was suspended in ATP–cysteine (10 ml/g of acetone powder) and dissolved by dialysis overnight against the same solution. At this stage of purification addition of 0.1 M KCl produces a pronounced increase in viscosity (Figure 2) which is accompanied by the appearance of flow birefringence, the conversion of a 3S component into a 30S component (Figure 3), and the appearance of actin-like filaments detectable by negative-staining electron microscopy (Figure 4a,b). These filaments have been shown to form arrowhead-shaped complexes with rabbit muscle heavy meromyosin (Pollard *et al.*, 1970), thus confirming their identification as actin. The F-actin formed by polymerization in 0.1 M KCl forms highly viscous, ATP-sensitive complexes with added

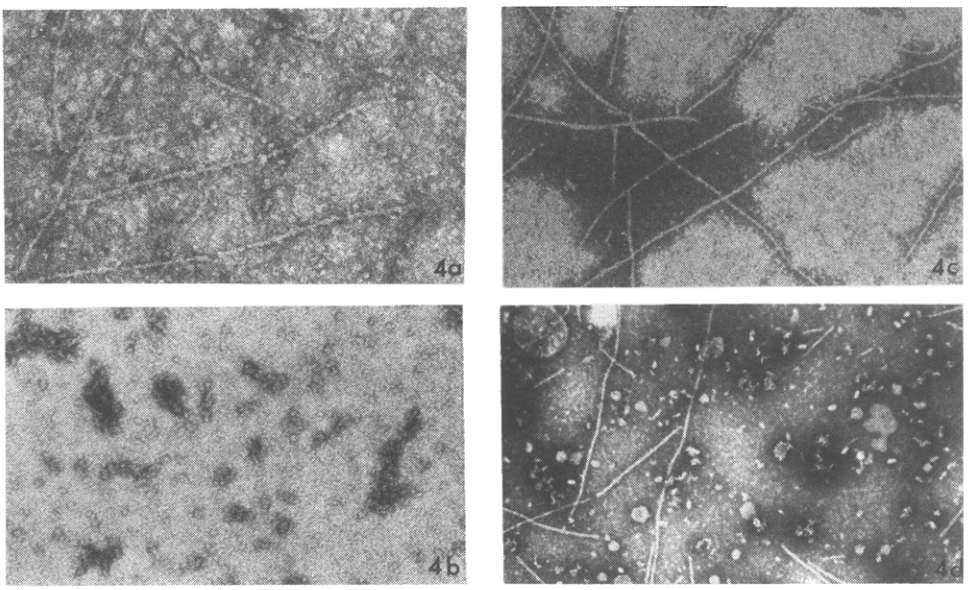

FIGURE 4: Negative-staining electron microscopy of *Acanthamoeba* actin at an intermediate step and at the final step of purification. The actin was negatively stained with uranyl acetate for parts a and b and with potassium phosphotungstate for parts c and d, using procedure described in Methods. Final magnification is 66,150×. (a) F-actin filaments prepared from *Acanthamoeba* actin precipitated with acetic acid–acetate and redissolved in ATP–cysteine. The actin formed filaments after the addition of 0.1 M KCl. Amorphous aggregates of unknown composition are also present. (b) G-actin from *Acanthamoeba*. The actin was precipitated with acetic acid–acetate and redissolved in ATP–cysteine. Filaments are not present in the absence of 0.1 M KCl, but the amorphous aggregates seen in part a are noted. (c) F-actin filaments prepared from amoeba actin eluted from Sephadex G-200. The protein eluted from Sephadex in the peak included at $K_D = 0.5$ (Figure 5) was polymerized with 0.1 M KCl. Filaments are not seen in the absence of KCl. Aggregated material is no longer present. (d) Material eluted from Sephadex G-200 in the void volume (Figure 5). This fraction includes undepolymerized F-actin filaments and amorphous aggregates of unknown composition.

rabbit myosin (Figure 2). The contents of 3-methylhistidine and ε-*N*-dimethyllysine have reached their maxima at this step (Table I).

POLYMERIZATION TO F-ACTIN. Actin was polymerized by the addition of 3 M KCl to a final concentration of 0.1 M and allowing the solution to stand at room temperature for 2–3 hr. F-actin was sedimented by centrifugation at 105,000*g* for 3 hr (Spinco 40 rotor, 40,000 rpm) to form clear pellets with a small, variable amount of brown precipitate in their centers. In later preparations, the brown precipitate was removed by centrifugation at 105,000*g* for 30 min before polymerization. The purity of the F-actin was increased by this step as judged by the increase of reduced viscosity (Table I) but the contents of 3-methylhistidine and ε-*N*-dimethyllysine did not change. It is possible that this step serves to remove denatured actin.

The F-actin pellets were suspended in the ATP–cysteine solution by gentle hand-homogenization, and the suspension was dialyzed for 2–3 days against the ATP–cysteine solution. Depolymerization to G-actin was assumed to be complete when flow birefringence was no longer detectable.

GEL FILTRATION. G-actin was always eluted from Sephadex G-200 as a symmetrical peak with K_D about 0.5 (Figure 5). Muscle actin (Adelstein *et al.*, 1963) and slime mold actin (Adelman and Taylor, 1969a) show nearly identical elution positions. Amoeba actin has never displayed the asymmetrical Sephadex–elution pattern found for muscle actin (Adelstein *et al.*, 1963; Rees and Young, 1967). No significance can presently be attributed to this difference because the unusual behavior of muscle actin is still unexplained.

The included peak was identified as actin by its development of flow birefringence in 0.1 M KCl, the presence of long filaments in such solutions (Figure 4c) which can interact with heavy meromyosin (Pollard *et al.*, 1970), and the high contents of 3-methylhistidine and ε-*N*-dimethyllysine (Tables I and III). Properties of this actin are described in detail below. That additional purification was accomplished by this step is indicated by the increase in reduced viscosity (Table I).

The material eluted in the void volume (Figure 5) was shown by negative-staining electron microscopy to be a mixture of undepolymerized F-actin and amorphous aggregates (Figure 4d) which did not exhibit flow birefringence upon the addition of KCl, and which contained relatively low amounts of 3-methylhistidine and ε-*N*-dimethyllysine (Weihing and Korn, 1970).

Proof That the Actin is Derived from the Amoebas. The low

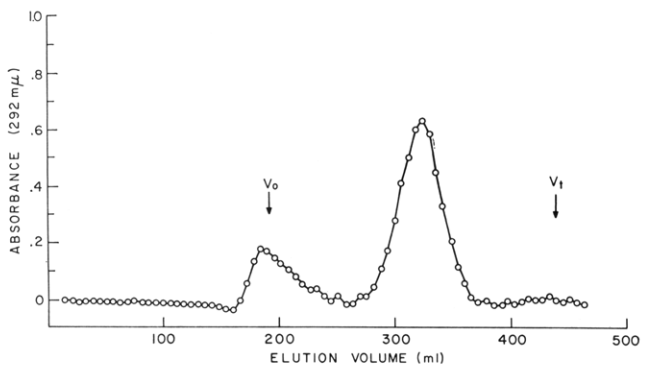

FIGURE 5: Gel filtration of amoeba actin on Sephadex G-200. Amoeba actin (108 mg) purified through centrifugation at 105,000*g* was applied in 25 ml of ATP–cysteine to a column of Sephadex G-200 (2.5 × 90 cm). The protein was eluted with ATP–cysteine. Protein was measured by absorbance at 292 nm. The second peak is G-actin.

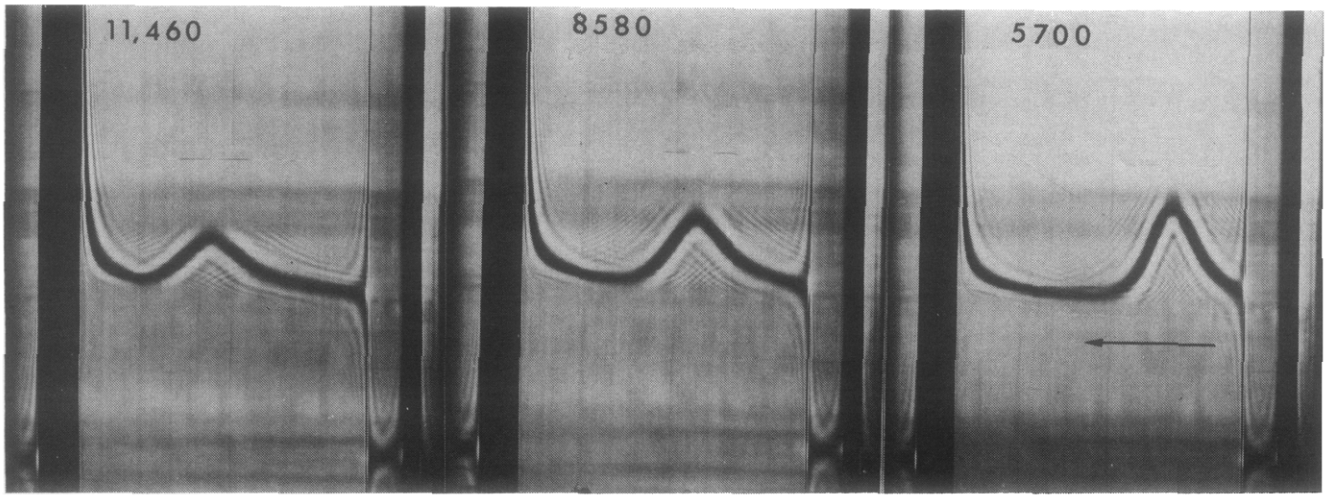

FIGURE 6: Sedimentation velocity analysis of purified amoeba G-actin. Amoeba actin eluted from Sephadex was first concentrated by dialyzing against dry Sephadex G-200. The final protein concentration was 4.0 mg/ml. The rotor speed was 56,000 rpm. The bar angle was 35°. The arrow indicates direction of sedimentation. Time (in seconds) after reaching top speed is indicated in the upper left corner of each frame.

yield of amoeba actin protein (about 0.14% of the protein present in whole cells) and the relatively large amounts of muscle myosin used in its isolation (about 10% of the protein present in the extracts) raised the possibility of contamination of the ameoba actin by muscle actin that might have been present in the myosin despite its high purity. The myosin used was free of actin as judged by the lack of effect of ATP on the viscosity of the myosin (Figure 1). Nonetheless, efforts were made to isolate actin from the muscle myosin by the procedures used to prepare amoeba actin. In two experiments, in which 150 and 300 mg of myosin were used, 1.4 and 1.6 mg of protein, respectively, were recovered in the fraction precipitated by 0.1 M acetic acid–0.1 M acetate. This could account for only 5–10% of the amoeba protein recovered at this stage of purification. Moreover, the protein derived from myosin alone had the low ratio of 3-methylhistidine:histidine of 1:20. Even if all that 3-methylhistidine were present in contaminating muscle actin (3-methylhistidine:histidine = 1:8) then only about 30% by weight of the protein would be muscle actin. This small amount of actin could account for less than 5% of the protein obtained as amoeba actin at the final purification step. It was not possible to continue the purification procedure on the small amount of protein obtained from the myosin.

Further evidence that the amoeba actin was not significantly contaminated by muscle actin is the presence of methyllysines in the amoeba actin (Weihing and Korn, 1970; Table III) which are not present in rabbit muscle actin (Kuehl and Adelstein, 1969; Hardy *et al.*, 1970).

As final proof that the purified actin was derived from the amoebas and not from a contaminant of the muscle myosin, actin was isolated from amoebas grown in medium containing [^{3}H]histidine or [^{3}H]lysine. The specific activities of the total proteins and of purified actin from amoebas grown on [^{3}H]-histidine were 130,000 and 79,000 cpm per mg, respectively, and from amoebas grown on [^{3}H]lysine, 1,030,000 and 680,000 cpm per mg, respectively. The similarity of the specific radioactivity of the total protein and of the actin is the anticipated result if the histidine and lysine contents of actin are similar to those of the amoeba extract, and no nonradioactive proteins derived from muscle myosin are left in the purified actin. The purified amoeba actin would have exactly the same specific activity as the total amoeba protein only if the histidine and lysine contents of actin and the total amoeba protein were identical and if the protein compositions of the carrier and radioactive fractions were identical.

Purity of Amoeba Actin. ANALYTICAL ULTRACENTRIFUGATION. In two preparations sedimentation velocity ultracentrifugation analysis of the purified actin revealed a single symmetrical major boundary with a sedimentation coefficient of 2.8 S (Figure 6). A small rapidly sedimenting component was also noted as the rotor accelerated.

The homogeneity and molecular weight of two different preparations of amoeba actin purified by Sephadex G-200 gel filtration were evaluated by equilibrium centrifugation in 5 M guanidine hydrochloride containing 1 mM 2-mercaptoethanol. After centrifugation to equilibrium at several speeds, linear plots of log C *vs.* X^2 were obtained indicating that the actin was monodisperse (Figure 7).

Molecular weights were calculated from the slopes of the log C *vs.* X^2 plots as described in Methods. The data (Table II) seem to indicate a slight concentration dependence of molecular weight. This was also noted for rabbit actin by Rees and Young (1967). Extrapolation of the data to zero protein concentration leads to a calculated molecular weight of 41,500. If this correction is not applied the average molecular weight in the two experiments would be 39,500 (stand-

TABLE II: Apparent Molecular Weight of Amoeba Actin Measured in Guanidine Hydrochloride.[a]

Initial Protein Concn[b] (mg/ml)	Rotor Speed (rpm)	App Mol Wt
0.34	36,000	41,700
	30,000	40,100
	22,000	39,600
1.2	36,000	38,200
	22,000	37,900

[a] The experimental details are described in Methods. [b] The initial protein concentration was estimated by the scanner at 292 nm using the factor 0.5 ml/(mg cm).

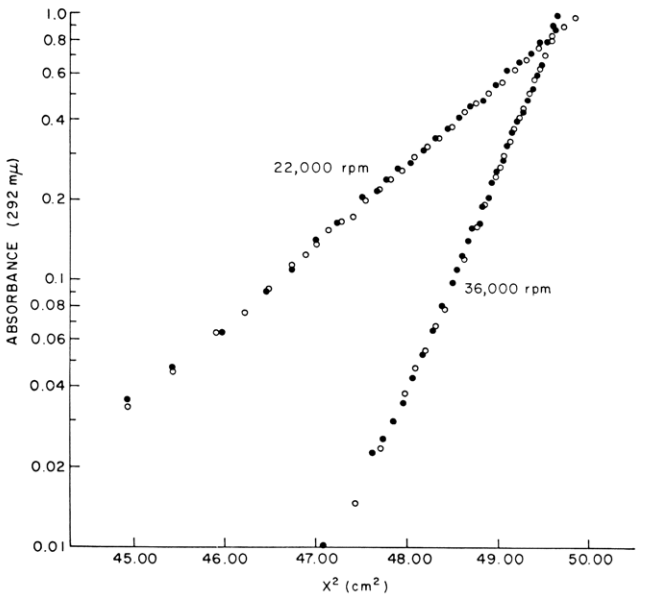

FIGURE 7: Sedimentation equilibrium ultracentrifugation of purified amoeba G-actin. Amoeba actin purified by gel filtration on Sephadex G-200 was prepared and centrifuged as described in Methods at the speeds indicated in the figure until equilibrium was reached. The closed and open circles refer to separate measurements made 12 hr after reaching equilibrium.

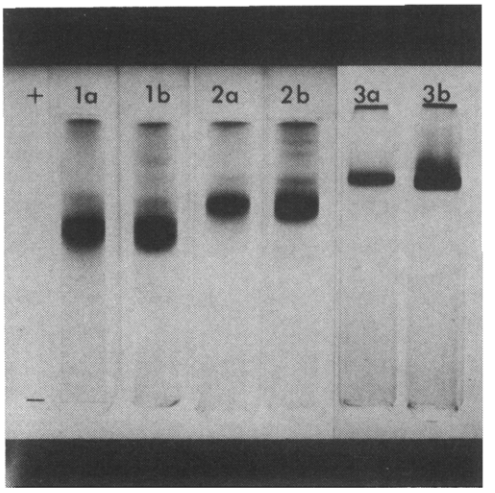

FIGURE 8: Polyacrylamide gel electrophoresis of reduced carboxymethylated actin from *Acanthamoeba* and from rabbit muscle. The designations 1, 2, and 3 refer to 4, 5, and 6% gels, respectively. The designation a and b refer to amoeba actin and muscle actin respectively. About 30 μg of protein from one preparation of amoeba and muscle actin were applied to gels 1 and 2 and about 100 μg of separate actin preparations to gels 3. Experimental procedures are described in Methods.

ard deviation 1400). The average molecular weight was used for normalization of the results of amino acid analyses (see below) because it was felt that too few values were available to justify using the extrapolated value.

DISC GEL ELECTROPHORESIS. When native actin was electrophoresed in the absence of urea a significant fraction of the protein failed to enter the stacking gel, and two or three major bands appeared in the separating gel. When electrophoresis was carried out in 8.5 M urea using reduced, carboxymethylated actin most of the protein from two separate preparations of both muscle and amoeba actin migrated as a single major band (Figure 8) with identical mobilities indicating that the net charges on the two actins are similar. Minor bands were also present in one of the preparations of both amoeba and muscle actin. These results differ slightly from those of Rees and Young (1967) who studied muscle actin prepared as we have prepared it, but who found no minor bands in carboxymethylated muscle actin electrophoresed in 8.5 M urea. It is not known whether the difference is the result of our using a dye of somewhat higher sensitivity than that used by Rees and Young (1967), thereby disclosing impurities of low concentration, or whether our actins were incompletely reduced and alkylated and therefore contained low concentrations of disulfide oligomers. If, however, the results of velocity and equilibrium ultracentrifugation are considered together with these results it is clear that the amoeba actin is as pure as muscle actin prepared in an essentially identical manner. The amoeba actin is, therefore, judged to be at least 90% pure.

Amino Acid Composition of Amoeba Actin. The amino acid composition of five separate preparations of amoeba actin eluted from Sephadex is similar to that of actin from other species including the presence of the unusual amino acid 3-methylhistidine (Table III) that is also known to be present in actin from rabbits (Asatoor and Armstrong, 1967) and other vertebrates (Johnson *et al.*, 1967). In addition, amoeba actin, but not rabbit muscle actin (Kuehl and Adelstein, 1969; Hardy *et al.*, 1970), contains 1 mole/mole of protein of the unusual amino acid ε-*N*-dimethyllysine and about 0.2 mole/mole of protein of ε-*N*-monomethyllysine. Additional experimental evidence that the ε-*N*-dimethyllysine (as well as 3-methylhistidine) is a component of the amoeba actin has been presented elsewhere (Weihing and Korn, 1970). To summarize briefly: (1) the content of methylated amino acids of the leading and trailing halves of the amoeba G-actin eluted from Sephadex were identical, providing evidence that the composition of the G-actin peak is not a combination of the composition of actin plus some contaminant containing ε-*N*-dimethyllysine. (2) The content of methylated amino acids of amoeba F-actin prepared from the Sephadex purified G-actin was identical with that of the protein which did not sediment, providing further evidence that the methylated amino acids are components of the actin (see also Table I).

Identification of Methylated Amino Acids. 3-Methylhistidine has been identified by its elution time in three systems of ion-exchange chromatography (Weihing and Korn, 1969a, 1970). In all analyses the ratio of A_{440}/A_{570} of the ninhydrin reaction product was close to that observed for authentic 3-methylhistidine. Additional identification was obtained by recovery of radioactive 3-methylhistidine from the radioactive actin purified from amoebas grown in the presence of [^{3}H]histidine. Of the total radioactivity recovered from hydrolysates of the radioactive actin, 98% was recovered in histidine and 3-methylhistidine. Assuming equal specific radioactivity for the two amino acids, then their calculated specific activity (calculated from the specific activity, the molecular weight, and the amino acid composition of actin) was 445,000 cpm/μmole which is somewhat higher than the observed values of 325,000 cpm/μmole for histidine and 234,000 cpm/μmole for 3-methylhistidine. Nonetheless, considering all the possible sources of error (Weihing and Korn, 1970) these data provide additional evidence for the identification of 3-methylhistidine and for its synthesis by the amoebas.

ε-*N*-Dimethyllysine has been identified primarily in a system of ion-exchange chromatography (50-cm column of AA-15

TABLE III: Amino Acid Composition of Acanthamoeba Actin Compared to Rabbit Actin.[a]

Residue	Acanthamoeba (Moles/39,500 g) (Mean ±SEM)	Rabbit[e] (Moles/ 45,000 g)
Lys	18.2 ± 0.3 (7)	21.0
MeLys	0.24 ± 0.05 (5)	0
Me_2Lys	0.78 ± 0.03 (5)	0
Me_3Lys	Trace (5)	0
His[a]	6.3 ± 0.4 (5)	8.1
3-MeHis	0.71 ± 0.04 (7)	0.97
Arg	17.3 ± 0.3 (5)	20.7
Asp	29.8 ± 0.6 (6)	38.6
Thr	25.7 ± 0.4 (5)[b]	27.9
Ser	22.2 ± 0.6 (5)[b]	23.3
Glu	39.4 ± 0.8 (7)	44.5
Pro	18.9 ± 0.5 (6)	20.9
Gly	32.0 ± 1.8 (5)	31.5
Ala	29.8 ± 0.5 (7)	32.3
$^1/_2$-Cys	4.9 ± 0.2 (3)[c]	5.3
Val	20.1 ± 0.9 (7)	21.8
Met	14.8 ± 1.3 (7)[d]	16.9
Ile	25.2 ± 0.5 (5)	28.6
Leu	32.8 ± 0.5 (5)	29.2
Tyr	13.7 ± 0.7 (3)	17.0
Phe	11.8 ± 0.4 (7)	13.7
Trp	5.0 (1)	4.3

[a] Amoeba G-actin eluted from Sephadex as described in the text was analyzed as described in Methods. One or two values in the series of measurements of His, Arg, Asp, Pro, Gly, Ile, Leu, and Tyr were not used because the mean computed without them was more than ±2 std dev different from the deleted value(s). The number of values averaged is shown in parentheses. [b] These values did not decrease uniformly with increasing time of hydrolysis, and therefore the largest values were averaged. [c] $^1/_2$-Cys was determined as cysteic acid or *S*-carboxymethylcysteine as described in Methods. [d] In one experiment methionine was determined as methionine sulfone after performic acid oxidation giving a value of 11.2 moles/39,500 g of protein. Methionine was otherwise determined directly. [e] Data of Adelstein and Kuehl (1970) for rabbit muscle actin.

TABLE IV: Activation of Heavy Meromyosin ATPase by *Acanthamoeba* Actin.[a]

Actin			ATPase (μmole of	
Source	Concn (mg/ml)	Concn of KCl (mM)	P_i/min mg)	Activation Factor
	0	10.7	0.03	
Rabbit	0.2	10.7	0.65	21.6
Amoeba	0.2	10.7	0.16	5.3
Amoeba	0.2	7.7	0.20	6.7
Amoeba	0.2	12.7	0.14	4.6
Amoeba	0.4	12.7	0.21	7.0

[a] All samples contained 4 mM $MgCl_2$, 2 mM ATP, 0.1 mM $CaCl_2$, and 2 mM imidazole buffer (pH 7), actin, and KCl as indicated in a final volume of 7.5 ml. The concentration of HMM in the absence of actin was 1.85 mg/ml. In the presence of rabbit actin the concentration of HMM was 0.28 mg/ml; in the presence of amoeba actin the concentration of HMM was 0.55 mg/ml. The amoeba actin was purified through gel filtration, concentrated by dialysis against dry Sephadex G-200, polymerized by addition of sufficient 3 M KCl to make the solution 0.1 M in KCl, and then dialyzed against 0.1 M KCl, 2 mM imidazole (pH 7), 0.5 mM ATP, and 0.5 mM 2-mercaptoethanol.

or UR-30 resin, 0.35 M citrate, pH 5.70, 28°) which separates all three ϵ-*N*-methyl derivatives of lysine (Methods) and by isotope incorporation experiments showing that amoebas grown in radioactive lysine synthesize radioactive actin containing lysine and ϵ-*N*-dimethyllysine of similar, high specific activity (Weihing and Korn, 1970). As an additional check on the identification of ϵ-*N*-dimethyllysine, the effluent from the amino acid analyzer was collected during the expected time of elution of the methylated lysines and desalted by the method of Dreze *et al.* (1954). The residue was chromatographed on paper in the phenol–cresol system of Stewart (1963) as modified by DeLange *et al.* (1969) which in our hands separated the relevant amino acids with the following R_F's: lysine, 0.22; histidine and ϵ-*N*-monomethyllysine, 0.50; ϵ-*N*-dimethyllysine, 0.77; ϵ-*N*-trimethyllysine, 0.92. The paper chromatogram of the desalted effluent contained a ninhydrin-reactive spot with the R_F of ϵ-*N*-dimethyllysine in addition to two darker spots with the R_F's of lysine and histidine.

The presence of lysine and histidine in the methyllysine effluent was the consequence of purposely overloading the ion-exchange resin with actin hydrolysate in order to collect sufficient methyllysines for paper chromatography. Kuehl and Adelstein (1969) reported identical observations in similar experiments.

A reddish, ninhydrin-negative substance with R_F of 0.46 was also present in the actin unknown. This is thought to represent impurities eluted from the ion-exchange resin during the desalting procedure.

Activation of Rabbit HMM ATPase by Amoeba Actin. The ATPase activity of rabbit muscle HMM is activated by rabbit F-actin at low ionic strength in the presence of Mg^{2+}. It was therefore of interest to look for similar properties in the amoeba F-actin. Amoeba F-actin causes a fivefold activation of the HMM ATPase (lines 1 and 3, Table IV) as compared to a 22-fold activation of the HMM ATPase (lines 1 and 2) by rabbit F-actin at the same ionic strength. The degree of activation by amoeba F-actin decreases as the ionic strength is raised (lines 3–5). Doubling the amoeba actin concentration increases the activation (lines 5 and 6). All these effects are also observed with rabbit F-actin (Leadbeater and Perry, 1963; Eisenberg and Moos, 1968), and the amoeba F-actin is therefore very similar to rabbit actin in this expression of its biological activity.

Discussion

The *Acanthamoeba* actin described in this report possesses the three "characteristic properties" (Barany *et al.*, 1961) of rabbit muscle actin. First, the globular 3S monomers of

amoeba actin can be polymerized to a 30S polymer. The appearance of flow birefringence upon polymerization shows that the polymer is fibrous rather than globular and the fibers have been identified by negative-staining electron microscopy. Second, amoeba actin and muscle myosin combine at high ionic strength to form a hybrid actomyosin as judged by the ATP-reversible increase in viscosity that occurs when the two proteins are mixed. The ultrastructural correlate of this behavior is the formation of ATP-dissociable hybrid arrowhead complexes of amoeba actin and muscle HMM which can be visualized by negative-staining electron microscopy (Pollard *et al.*, 1970). The ultrastructure of the amoeba actin fibers and of the hybrid complexes of amoeba actin and rabbit muscle HMM cannot be distinguished from the corresponding proteins of muscle (Pollard *et al.*, 1970). Third, the amoeba actin activates the ATPase activity of rabbit muscle HMM at low ionic strength in the presence of Mg^{2+}. Additional similarities between amoeba and muscle actins include their amino acid compositions (including the presence of 3-methylhistidine) and their electrophoretic migrations in polyacrylamide gels. Clearly, *A. castellanii* contains actin that is structurally and functionally very similar to muscle actin. But there are differences between the two proteins.

Methylated Amino Acids. While the occurrence of 3-methylhistidine in the amoeba actin was expected because it is a component of other actins (Asatoor and Armstrong, 1967; Johnson *et al.*, 1967), the presence of ε-*N*-dimethyllysine was not anticipated. Since this is the first actin in which ε-*N*-dimethyllysine has been found, it is necessary to consider the possibility that it is actually a component of a contaminating protein. For several reasons this seems highly unlikely.

During the purification procedure, which depends mainly on properties which are presumably specific for actin, the content of ε-*N*-dimethyllysine and 3-methylhistidine reach their final values at the third of five steps (Table I), whereas ε-*N*-trimethyllysine which is also present in the early fractions is removed during the final purification on Sephadex (Weihing and Korn, 1970). ε-*N*-Dimethyllysine then, purifies with 3-methylhistidine, an undoubted component of actin, and not with ε-*N*-trimethyllysine. The highly purified G-actin prepared by Sephadex chromatography can be repolymerized to F-actin with no loss of ε-*N*-dimethyllysine (Table I and Weihing and Korn, 1970).

The only other proteins known to contain ε-*N*-dimethyllysine are certain histones, of which the F-3 histone of kidney has the highest concentration, 5 moles/10^5 g (Hempel *et al.*, 1968), and lobster myosin which contains 0.62 mole/10^5 g (Kuehl and Adelstein, 1970). It is possible, therefore, that amoeba histones and amoeba myosin (if it exists) also contain ε-*N*-dimethyllysine. Neither of these proteins, however, should contaminate the purified actin since myosin is normally denatured by treatment with acetone and neither protein should cochromatograph with actin on Sephadex G-200. Moreover, even a protein as rich in ε-*N*-dimethyllysine as F-3 histone would have to comprise 50% of the purified amoeba actin in order to account for all of the ε-*N*-dimethyllysine. The presence of such a high percentage of a second protein is entirely incompatible with the physical data which suggest near homogeneity for the amoeba actin. Furthermore, as discussed in more detail elsewhere (Weihing and Korn, 1970), if the amoeba actin were only 50% pure its amino acid composition could not resemble so closely that of actins from other sources.

Activation of HMM ATPase. The amoeba actin was less efficient than muscle actin in activating HMM ATPase (Table IV). Part of this difference may be attributed to incomplete polymerization of the actin since only F-actin activates the ATPase. If one corrects the observed activation factor of 5.3 (Table IV) for the estimated yield of F-actin from G-actin (about 35%)[3] a calculated activation factor of 15 is obtained. This compares to an activation factor of 21.6 for muscle actin (Table IV). The remaining difference may reflect a different K_M or V_{max} for the hybrid amoeba actin–muscle HMM system compared to the homologous muscle actin–muscle HMM system, but more detailed kinetic experiments are necessary to resolve this question.

Actin Content of Amoebas. One might attempt to estimate the amount of actin in the amoebas from their content of 3-methylhistidine. The data in Table I show that the 3-methylhistidine content of highly purified actin is only five times greater than in the proteins of the whole cell. If actin were the only protein that contained 3-methylhistidine then about 20% of the total amoeba proteins would be actin. We isolate less than 0.2% of the amoeba proteins as purified actin. Even if one assumes relatively large losses during the purification procedure, it seems most likely that amoeba proteins other than actin contain 3-methylhistidine.

Comparison of Protozoal and Muscle Actins. The width of the F-actin filament and the spacing of the half-pitch of the F-actin double helix revealed by negative-staining electron microscopy are very similar for actins from phylogenetically distant species (Table V). The implied similarities of protein conformation and active sites are supported by the fact that the two protozoal actins are able to form complexes with muscle myosin and HMM (Table V). These complexes are very similar to the one formed between muscle F-actin and HMM in that they (1) are dissociated by ATP (Hatano and Oosawa, 1966a; Adelman and Taylor, 1969b; Pollard *et al.*, 1970; this report); (2) the Mg-ATPase of HMM is stimulated at low ionic strength (Table V), but the stimulation decreases at higher ionic strengths (Adelman and Taylor, 1969b; this report); and (3) muscle tropomyosin renders the *Acanthamoeba* F-actin rabbit HMM ATPase sensitive to EGTA (Eisenberg and Weihing, 1970). The spacings of the HMM attached to the F-actin filaments are also identical (Table V). These observations, and the similarities of their electrophoretic mobilities in polyacrylamide gels and of their amino acid compositions (Hatano and Oosawa, 1966b; this report) make it reasonable to suppose that the several actins contain regions of similar or identical amino acid sequence. The presence of regions of identical amino acid sequence in the actins of mammals, birds, reptiles, amphibia, fish, and molluscs is suggested by the peptide maps obtained by Carsten and Katz (1964).

One distinctive difference among the actins appears to be the presence of *Acanthamoeba* actin of ε-*N*-dimethyllysine, an amino acid which is definitely not present in rabbit muscle actin (Kuehl and Adelstein, 1969; Hardy *et al.*, 1970). The significance of this amino acid is unknown as is also the role of 3-methylhistidine, which is present in all vertebrate actins and in actins prepared from *Acanthamoeba* and *D. discoideum* (Woolley, 1970). *Acanthamoeba* contains substantial amounts

[3] The yield of F-actin from purified G-actin was estimated from the observation that the reduced viscosity of purified amoeba F-actin was only 30% of the value for purified muscle actin (Maruyama and Gergely, 1962; Nagy and Jencks, 1965) and that 40% of the protein sedimented as F-actin after prolonged high-speed centrifugation (Table I, and Weihing and Korn, 1970).

TABLE V: Comparison of Protozoal Actins to Rabbit Actin.[a]

Actin Source	Width of F-actin Filaments (Å)	Half-Pitch of F-actin Double Helix (Å)	Spacings of HMM Complexes (Å)	Activation of Rabbit Myosin or HMM ATPase	App Monomer Mol Wt	Presence of 3-MeHis
Acanthamoeba[b] *castellanii*	58 ± 11	370	370	Yes	39,500	Yes
Physarum polycephalum	75[c] 50–60[d]	350–420[c] 354–364[d]	360[d]	Yes[e]	37,000–44,000[e] 57,000[f]	?
Rabbit muscle	60–70[g]	350–370[g] 380[k]	350–370[g]	Yes[h]	46,000[i]	Yes[j]

[a] Summary of data compiled from various sources. [b] This report: Pollard *et al.* (1970). [c] Hatano and Tazawa (1968). [d] Nachmias *et al.* (1970). [e] Adelman and Taylor (1969b). [f] Hatano and Oosawa (1966b). [g] Huxley (1963). [h] Leadbeater and Perry (1963). [i] Rees and Young (1967). [j] Asatoor and Armstrong (1967) and Johnson *et al.* (1967). [k] Hanson (1967).

of ε-*N*-dimethyllysine (Table I) as well as ε-*N*-monomethyllysine and ε-*N*-trimethyllysine (R. R. Weihing and E. D. Korn, unpublished observations) and all three are probably present in cell proteins other than actin. This suggests that ε-*N*-dimethyllysine may not be of specific functional importance in amoeba actin.

The best values for the molecular weights of protozoal actins seem to center about 40,000 which is 10% less than the most recently reported value for muscle actin (Table V). The one discrepant value for actin from *Physarum polycephalum* (Hatano and Oosawa, 1966b) was obtained with material whose purification did not include gel filtration. This procedure would be expected to remove aggregated protein which, if present, would increase the apparent molecular weight. If these differences in molecular weight prove to be real (and, at least superficially, they seem to be contradictory to the similarities in amino acid composition) they may indicate that the protozoal actins lack some properties of muscle actin (such as interaction with Z-band material, for example) or they may explain the difference in the extent of activation of muscle HMM by *Acanthamoeba* actin.

Significance of Actin in Nonmuscle Cells. Actin may be an ubiquitous component of cells. In addition to the ten examples cited earlier, properties attributed to the presence of actin or actomyosin have been described in extracts of blood platelets (Bettex-Galland *et al.*, 1962), thymus nuclei and spinach chloroplasts (Ohnishi *et al.*, 1964; Ohnishi, 1964) and brain (Puszkin *et al.*, 1968) among others (for further review of reports of cellular actin, see Poglazov, 1966). Identifications of actin and myosin in crude extracts by indirect means must, however, be considered with caution. For example, ATP-sensitive viscosity changes may not be specific for complexes of actin and myosin. Puszkin and Berl (1970) have presented evidence that brain microtubule protein may show similar behavior. Isolation of a highly purified protein whose properties can be studied in some detail are necessary to substantiate the tentative identifications of actin and/or myosin in several nonmuscle cells.

All of the studies on actin and myosin (Hatano and Tazawa, 1968; Adelman and Taylor, 1969a,b; Hatano and Ohnuma, 1970) of nonmuscle cells are based on the expectation that these proteins participate in cell locomotion, changes in cell shape, and other cellular and subcellular movements. Very little direct evidence exists to support this hope. The next major step must be to establish the connection between these proteins and the molecular and morphological events of cell motility.

Acknowledgments

We thank Drs. W. W. Kielley, R. S. Adelstein, and W. M. Kuehl for helpful discussions. Dr. E. Shelton and Dr. T. D. Pollard kindly carried out the electron miscroscopy, and Dr. R. Reisfeld carried out polyacrylamide gel electrophoresis at early stages of this work. Miss Paddy L. Wright, Mr. H. Lutterlough, Mr. M. Miles, and Mr. D. Rogerson and his staff provided expert technical help with the amoeba cultures.

References

Adelman, M. R., and Taylor, E. W. (1969a), *Biochemistry 8*, 4964.

Adelman, M. R., and Taylor, E. W. (1969b), *Biochemistry 8*, 4976.

Adelstein, R. S., Godfrey, J. E., and Kielley, W. W. (1963), *Biochem. Biophys. Res. Commun. 12*, 34.

Adelstein, R. S., and Kuehl, W. M. (1970), *Biochemistry 9*, 1355.

Asatoor, A. M., and Armstrong, M. D. (1967), *Biochem. Biophys. Res. Commun. 26*, 168.

Barany, M., Nagy B. Finkelman F., and Chrambach, A. (1961), *J. Biol. Chem. 236*, 2917.

Bettex-Galland, M., Portzehl, H., and Luscher, E. R. (1962), *Nature (London) 193*, 777.

Bowers, B., and Korn, E. D. (1968), *J. Cell Biol. 39*, 95.

Carsten, M. E., and Katz, A. M. (1964), *Biochim. Biophys. Acta 90*, 534.

Chrambach, A., Reisfeld, R. A., Wyckoff, M., and Zaccari, J. (1967), *Anal. Biochem. 20*, 150.

Cohn, E. J., and Edsall, J. T. (1943), Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions, New York, N. Y., Reinhold.

Craven, G. R., Steers, Jr., E., and Anfinsen, C. B. (1965), *J. Biol. Chem. 240*, 2468.

Davis, B. J. (1964), *Ann. N. Y. Acad. Sci. 121*, 404.
Delange, R. J., Glazer, A. N., and Smith, E. L. (1969), *J. Biol. Chem. 244*, 1385.
Dreze, A., Moore, S., and Bigwood, E. J. (1954), *Anal. Chem. 11*, 554.
Edelhoch, H. (1967), *Biochemistry 6*, 1948.
Eisenberg, E., and Moos, C. (1967), *J. Biol. Chem. 242*, 2945.
Eisenberg, E., and Moos, C. (1968), *Biochemistry 7*, 1486.
Eisenberg, E., and Weihing, R. R. (1970), *Nature (London) 228*, 1092.
Hanson, J. (1967), *Nature (London) 213*, 353.
Hardy, M., Harris, I., Perry, S. V., and Stone, D. (1970), *Biochem. J. 117*, 44P.
Hatano, S., Kondo, H., and Miki-Noumura, T. (1969), *Exp. Cell Res. 55*, 275.
Hatano, S., and Ohnuma, J. (1970), *Biochim. Biophys. Acta 205*, 110.
Hatano, S., and Oosawa, F. (1966a), *J. Cell Physiol. 68*, 197.
Hatano, S., and Oosawa, F. (1966b), *Biochim. Biophys. Acta 127*, 488.
Hatano, S., and Tazawa, M. (1968), *Biochim. Biophys. Acta 154*, 507.
Hempel, K., Lange, L. H., and Birkofer, L. (1968), *Naturwissenschaften 55*, 37.
Hirs, C. H. W. (1956), *J. Biol. Chem. 219*, 611.
Huxley, H. E. (1963), *J. Mol. Biol. 7*, 281.
Ishikawa, H., Bischoff, R., and Holtzer, H. (1969), *J. Cell Biol. 43*, 321.
Johnson, P., Harris, C. I., and Perry, S. V. (1967), *Biochem. J. 105*, 361.
Kielley, W. W., and Bradley, L. B. (1956), *J. Biol. Chem. 218*, 653.
Kielley, W. W., and Harrington, W. F. (1960), *Biochim. Biophys. Acta 41*, 401.
Kuehl, W. M., and Adelstein, R. S. (1969), *Biochem. Biophys. Res. Commun. 37*, 59.
Kuehl, W. M., and Adelstein, R. S. (1970), *Biochem. Biophys. Res. Commun. 39*, 956.
Leadbeater, L., and Perry, S. V. (1963), *Biochem. J. 87*, 233.
Loewy, A. G. (1952), *J. Cell. Comp. Physiol. 40*, 127.
Lowry, O. H., Rosebraugh, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Maruyama, K., and Gergely, J. (1962), *J. Biol. Chem. 237*, 1095.
Miki-Noumura, T. (1969), *Develop. Growth Differentiation 11*, 219.
Miki-Noumura, T., and Kondo, H. (1970), *Exp. Cell Res. 61*, 31.
Miki-Noumura, T., and Oosawa, F. (1969), *Exp. Cell Res. 56*, 224.
Mommaerts, W. F. H. M. (1952), *J. Biol. Chem. 198*, 445.
Nachmias, V. T., Huxley, H. E., and Kessler, D. (1970), *J. Mol. Biol. 50*, 83.
Nagy, B., and Jencks, W. P. (1965), *J. Amer. Chem. Soc. 87*, 2480.
Neff, R. J., Neff, R. H., and Taylor, R. E. (1958), *Physiol. Zool. 31*, 73.
Ohnishi, T. (1964), *J. Biochem. (Tokyo) 55*, 494.
Ohnishi, T., Kawamura, H., and Tanaka, Y. (1964), *J. Biochem (Tokyo) 56*, 6.
Poglazov, B. F. (1966), Structure and Functions of Contractile Proteins, New York, N. Y., Academic.
Pollard, T. D., and Korn, E. D. (1970), *J. Cell Biol.* (in press).
Pollard, T. D., Shelton, E., Weihing, R. R., and Korn, E. D. (1970), *J. Mol. Biol. 50*, 91.
Puszkin, S., and Berl, S. (1970), *Nature (London) 225*, 558.
Puszkin, S., Berl, S., Puszkin, E., and Clarke, D. D. (1968), *Science 161*, 170.
Rees, M. K., and Young, M. (1967), *J. Biol. Chem. 242*, 4449.
Schachman, H. K. (1957), *Methods Enzymol. 4*, 32.
Senda, N., Shibata, N., Tatsumi, N., Kondo, K., and Hamada, K. (1969), *Biochim. Biophys. Acta 181*, 191.
Shepro, D., Chao, F. C., and Belamarich, F. A. (1969), *J. Cell Biol. 43*, 129A.
Spackman, D. H. (1964), *in* Serum Proteins and the Dysproteinemias, Sunderman, F. W., and Sunderman, Jr., F. W., Ed., Philadelphia, Pa., Lippincott, p 166.
Stewart, I. (1963), *J. Chromatog. 10*, 404.
Szent-Györgyi, A. (1945), *Acta Physiol. Scand. 9, Suppl.* 25.
Weihing, R. R., and Korn, E. D. (1969a), *Biochem. Biophys. Res. Commun. 35*, 906.
Weihing, R. R., and Korn, E. D. (1969b), *J. Cell Biol. 43*, 155A
Weihing, R. R., and Korn, E. D. (1970), *Nature (London) 227*, 1263.
Weisman, R. A., and Korn, E. D. (1966), *Biochim. Biophys. Acta 116*, 229.
Woolley, D. E. (1970), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29*, 667A.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.