Studies on the Isolation and Molecular Properties of Homogeneous Globular Actin

EVIDENCE FOR A SINGLE POLYPEPTIDE CHAIN STRUCTURE*

(Received for publication, May 12, 1967)

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SUMMARY

Solutions of globular actin have been isolated from rabbit skeletal muscle and purified by repetitive cycles of the reversible globular → fibrous transformation. Electrophoretic, ultracentrifugal, and gel filtration studies of these preparations revealed the presence of multiple macromolecular components. On the other hand, solutions of G-actin isolated by molecular exclusion chromatography exhibited a high degree of particle homogeneity. An appreciable, yet variable, fraction (15 to 75%) of the total protein isolated from acetone powders by reversible polymerization procedures is not globular actin. The predominant impurity is devoid of both ADP and ATP, and it is not transformed to fibrous actin in the presence of dilute aqueous KCl. Gel filtration chromatography (Sephadex G-200) eliminates this material. In agreement with earlier studies of Adelstein, Godfrey, and Kielly (4), this procedure represents a valuable preparative feature in the isolation of chemically homogeneous, monomeric actin.

In the presence of dilute aqueous solutions of KCl, the chromatographically isolated protein is quantitatively transformed to F-actin. Molecular weight studies indicate that the mass of electrophoretically homogeneous G-actin is close to 46,000 g per mole ($M_w$ = 46,500 and $M_n$ = 45,800). When the reduced S-carboxymethylated protein is transferred to concentrated solutions of guanidine-HCl, the particle weight remains essentially unchanged ($M_w$ = 47,600 and $M_n$ = 47,800). This information implies that globular actin is constructed from a single, covalently linked polypeptide chain.

* This investigation was supported by Research Grant AM 09404 from the National Institutes of Health, United States Public Health Service. A preliminary report of these studies was presented before the 152nd Meeting of the American Chemical Society, New York, New York, 1966.

† King Trust Research Fellow, 1965 to 1966.
all subsequent operations were performed with this solvent at 4°C.)  Acetone powder (20 g) was suspended in 350 ml of solvent and stirred continuously for 30 min. This time period was chosen to maximize the yield, since over a 30-min interval the rate of extraction of G-actin from the acetone powder paralleled the rate of extraction of total protein (see Fig. 1). The muscle residue was separated by centrifugation (10,000 \times g for 4 hours), and the supernatant solution was clarified by positive pressure filtration (nitrogen, 5 to 20 p.s.i.) through a 0.45 \mu m Millipore filter. This crude preparation of G-actin was transformed to filtration (nitrogen, 5 to 20 p.s.i.) through a 0.45 \mu m Millipore residue was separated by centrifugation (10,000 \times g for 2 hours), resuspended in 25 ml of the ATP, pH 7.5; (O---O), extraction solvent: 5 X 10^{-4} M ATP-5 X 10^{-4} M 2-mercaptoethanol, pH 7.5. At this stage, the supernatant solution contained 10 to 15 mg of protein per ml, and 25 ml were applied to a column, 2 X 70 cm, of Sephadex G-250 (Pharmacia) equilibrated at 4°C with 5 X 10^{-4} M ATP, 5 \times 10^{-6} M 2-mercaptoethanol, and 2 \times 10^{-4} M CaCl_2, pH 7.5. (A 1-cm layer of Sephadex G-25 was added to the top of the column to prevent disturbance of resin during application of the sample.) The elution profile was measured by absorbance at \( \lambda = 280 \mu m \). This procedure yielded 150 to 200 mg of G-actin at a concentration of 1.3 to 1.5 mg per ml.

Reduced carboxymethylated actin was prepared as follows. A lyophilized powder of G-actin was dissolved in 5 M guanidine-HCl-0.1 M Tris-1 mm EDTA, pH 8.5, to a final protein concentration of 10 to 20 mg per ml. The solution was gently purified with nitrogen, treated with 1.4 \times 10^{-2} mole of 2-mercaptoethanol per g of protein, and stored in a tightly stoppered vessel at room temperature for 18 hours. Solid iodoacetic acid equal to the molarity of 2-mercaptoethanol was added, and pH 8.5 was maintained with dilute KOH. After 4 hours at room temperature, the solution was dialyzed for 7 days against 5 M guanidine-HCl solvent prior to use. For preparation of salt-free CM-actin,1 guanidine-HCl was removed by gel filtration (Sephadex G-25 was equilibrated with water at 25°C).

Protein Concentration—Extinction coefficients of three separate G-actin preparations (isolated as described above) were determined from optical absorbance (\( \lambda = 280 \mu m \), Zeiss PMQ II spectrophotometer) together with triplicate measurements of protein concentration by micro-Kjeldahl analyses (7). The nitrogen content was taken to be 16.5% by weight, and this value was obtained from amino acid analyses of the same preparations. This procedure yielded a mean extinction coefficient of 1.09 ml per mg-cm.

Partial Specific Volume The apparent partial specific volume of freshly prepared G-actin was determined according to the equation

\[
\phi' = 1/\rho(1 - dp/dc)
\]

where \( \phi' \) is the apparent partial specific volume of the macromolecule in a multicomponent system (8), \( \rho \) is solvent density, and \( p \) and \( c \) are the density and concentration of the protein solution, respectively. Solution densities were measured at 20 \pm 0.01°C with a magnetic float densitometer\(^2\) calibrated with National Bureau of Standards sucrose solutions (9). Protein concentrations were determined both spectrophotometrically and by micro-Kjeldahl analyses.

Sedimentation Analyses—The Spinco model E analytical ultracentrifuge was used for sedimentation velocity and sedimentation equilibrium experiments. The Rayleigh interference optical system of the instrument (aligned by the method of Richards and Schachman (10)) was employed for sedimentation equilibrium studies, and the camera lens was focused at the midplane of 12-mm double sector aluminum-filled Epon centerpiece cells equipped with sapphire windows.

The molecular weight of native G-actin was measured by the high speed equilibrium method (11). Columns of solution 3 mm long were employed and perfluorotributylamine (FC-43, Minnesota Mining and Manufacturing Company) was added to both solution and reference channels. To minimize unequal

1 The abbreviation used is: CM-actin, reduced carboxymethylated actin.

2We are grateful to Dr. Donald Kupke, Department of Biochemistry, and to Dr. J. P. Senter, Department of Physics, University of Virginia, for their assistance in obtaining these data.
redistribution of low molecular weight buffer components between solution and solvent sectors, care was taken to match the column heights exactly. Rotor velocities varied from 28,000 to 32,000 rpm at temperatures in the range 4-7°. Solvent blanks were run, without disassembling the cell, to correct for any inequalities in the optical system (11). Photographs (Eastman, spectroscopic plates, type II G) were taken at frequent intervals to establish conditions for sedimentation-diffusion equilibrium (approximately 15 hours).

After vertical and horizontal alignment of the photographic plate, interference patterns were measured with a Nikon model 6 microcomparator. Values of fringe displacement were taken at 0.1-mm intervals (plate coordinate), and five fringes were read at each radial coordinate. The average of these values was used to compute solute concentration (c,) as a function of r. Point weight average and point number average molecular weights were evaluated from Equations 2 and 3:

$$M_w(r) = \frac{2RT}{(1 - \phi'p)\omega^2} \frac{d\ln c}{d(r^2)}$$

$$M_n(r) = \frac{c_r}{2RT \int_{r_o}^r c_r d(r^2)}$$

where R, T, \(\omega\), and p have their usual significance. Values of \(M_w(r)\) were calculated from the slope of the least squares line fitted to each set of five adjacent points (11). The integral in Equation 3 was estimated by trapezoidal summation. A PDP-1 digital computer was used for all data reduction, and \(M_w\) and \(M_n\) at infinite dilution of protein were obtained from plots of reciprocal apparent molecular weight as a function of c_r.

The mass of reduced carboxymethylated actin in 5 M guanidine-0.1 M Tris-1 mM EDTA, pH 8.5, was measured by both high speed (30,000 to 40,000 rpm) and low speed (10,000 to 14,000 rpm) sedimentation equilibrium methods. Concentrations of aqueous solutions of guanidine-HCl were measured refractometrically (Abbe refractometer) (12). For the low speed studies, 1-mm columns of solution were layered over FC-43, and rotor velocities were selected such that the equilibrium solute concentration at the midpoint of the liquid column approached the initial concentration, c_o, to within 1% (13). The apparent weight average molecular weight over the entire solution was calculated from Equation 4

$$\bar{M}_{app} = \frac{2RT}{(1 - \phi'p)\omega^2} \frac{c_b - c_a}{c_o}$$

where \(c_b\) and \(c_a\) represent the solute concentration at the base and meniscus of the liquid column, respectively. The initial concentration, \(c_o\), was determined from double sector synthetic boundary experiments. Values of \(\bar{M}_{app}\) were calculated as a function of protein concentration in the range 4 to 15 mg per ml, and plots of 1/\(\bar{M}_{app}\), against c were used to obtain the particle weight at infinite dilution.

Sedimentation velocity studies were performed at 25° with a rotor velocity of 59,780 rpm.

The ultracentrifuge was also used to follow the transformation of dilute solutions of G-actin to F-actin. For this purpose, the absorption optical system of the instrument was employed together with a split beam photoelectric scanner and monochro-

mator (Heckman). The optical system was aligned by the methods of Schachman et al., and the camera lens was focused upon an air liquid meniscus with off axis illumination (14). Radially oriented masks (4 x 20 mm) were mounted upon both collimating and condensing lenses to minimize stray light arising from the various optical components. For studies with G-actin, light of wave length 290 m\(\mu\) was selected to reduce the absorbance of the nucleotide-containing solvent. Under these conditions, optical density was proportional to protein concentration in the range 0 to 1 O.D. units.

**Electrophoresis Studies**—For polyacrylamide gel electrophoresis studies, 7.5% and 15% gels (5 mm diameter) at pH 9.5 were prepared as described by Davis (15). The electrophore buffer contained 2.88 g of glycine and 0.6 g of Tris in 1 liter of water. For studies of G-actin, deionized and recrystallized urea was added to all solutions and to the electrophore buffer. The electrophoretic properties of G-actin were also examined at pH 7.6 (solvent, 5 x 10^-4 M ATP-1 x 10^-4 Tris-HCl). To minimize changes in pH and ionic concentrations at these low ionic strengths, buffer was rapidly recirculated from one electrode compartment to the other with a high flow peristaltic pump. In all cases, temperature was maintained at 4° and gels were prerun before introduction of the sample to eliminate products of the gel-polymerization reaction. (These gels could be stored in electrode buffer at 4° for 3 weeks without noticeable deterioration.) From 10 to 50 \(\mu\)l of sample (75 to 150 \(\mu\)g of protein) were added to each gel column with a constriction-type micropipet. Initially, the current was set at 1 ma per column. After 10 min, circulation of buffer was begun, and the current was increased to 2.5 ma. Gels were stained with 1% Amido Schwarz in 7.5% acetic acid and destained electrophoretically (16).

**Viscosity**—Solution viscosities were measured at 20 ± 0.1° with a 2 ml Ostwald type viscometer (outflow time = 104 sec for water).

**Amino Acid Analyses**—Samples (3 to 5 mg) of lyophilized, salt-free reduced carboxymethylated actin were hydrolyzed with 6 n HCl (Mallinekrodt) in sealed, evacuated (50 \(\mu\)l of Hg) tubes at 110° for 24 hours. HCl was rapidly removed by rotary evaporation (17), and the hydrolysate was analyzed with a Beckman model 120 R amino acid analyzer.

**Nucleotide Analyses**—Solvent ATP was removed from G-actin solutions by treatment with Dowex 1-acetate (x8,200 to 400 mesh) (18). The mixture was gently stirred for 10 min at 0°, and the resin was removed by centrifugation. Protein-bound nucleotide was liberated by treatment of this solution with 7% perchloric acid for 20 min at 0°, and the precipitated protein was collected by centrifugation. Optical density measurements (\(\lambda = 267 m\mu\)), together with a molar extinction coefficient of 14.7 x 10^4 m^-1 cm^-1 (19), were used to calculate nucleotide concentrations.

**Inorganic Phosphate Analyses**—Solutions of G-actin were polymerized by the addition of 0.1 M KCl and 1 nma MgCl\(_2\). After 2 hours at 25°, F-actin was removed by centrifugation (144,000 x g for 2 hours). Inorganic phosphate concentrations were determined in duplicate with three to five different dilutions of the supernatant solution (20).

**RESULTS**

**Chemical Homogeneity of G-Actin**—The properties of several preparations of G-actin isolated and purified by reversible
FIG. 2. Sedimentation velocity studies of actin before and after gel filtration chromatography. Solutions of G-actin isolated by reversible polymerization and by gel filtration were transformed to F-actin and examined with the ultracentrifuge. Solvent: 5 × 10⁻⁴ M ATP-0.1 M KCl-1 mM MgCl₂, pH 7.5. Rotor temperature and velocity, 4° and 60,000 rpm, respectively. Upper, preparation isolated by reversible polymerization. Photographs and scanner traces were taken 20 min after reaching full speed; protein concentration = 1.9 mg per ml. Lower, preparation isolated by gel filtration. Conditions are identical to those above. For the scanner traces (λ = 290 mp), the monochromator and photomultiplier slit widths were set at 2.0 mm and 0.1 mm, respectively. Direction of sedimentation is from left to right and each calibration step is equivalent to 0.20 O.D. unit. For the schlieren and interference optical studies, F-actin solutions were examined with double sector synthetic boundary cells.

polymerization methods have been examined. In initial studies, these solutions were transformed to F-actin by the addition of 0.1 M KCl-1 mM MgCl₂ and were examined with the analytical ultracentrifuge. Fig. 2 (upper) presents schlieren and interference photographs and photoelectric scanner traces which were taken 20 min after the rotor had attained a speed of 60,000 rpm. At the end of this time period, the rapidly moving F-actin boundary has migrated to the base of the cell and is no longer visible. Yet both refractometric and ultraviolet absorption optical systems reveal an appreciable fraction of at least two components which have not been converted to the more rapidly sedimenting polymer characteristic of F-actin.

The presence of these components may be best appreciated through an examination of the ultraviolet scanner traces. The concentration of the more slowly migrating material was independent of time of polymerization, and in all cases it comprised at least 15% of the total protein. (As shown below, this species does not represent an incompletely polymerized or low molecular weight form of F-actin.) These preparations of G-actin were also studied by polyacrylamide gel electrophoresis and by Sephadex chromatography. As shown in Fig. 3, the electrophoretic patterns exhibit multiple components, and several chromatographic fractions were also identified (see Fig. 4). Thus both the sedimentation characteristics of these prepa-
rations (in the F-actin form) and their electrophoretic and gel filtration properties (in the G-actin form) reflect polydispersity. Of the four chromatographic fractions, Fraction III represents G-actin (see below). Area analysis of elution profiles similar to that shown in Fig. 4 indicated that the shaded zone (labeled Fractions I and II) comprises approximately 15% of the total protein.

Prolonged high speed preparative ultracentrifugation has been widely used in the isolation and purification of G-actin. The utility of this procedure stems from the fact that F-actin exhibits a very large sedimentation coefficient, and, consequently, this particle may be readily separated from low molecular weight components by differential centrifugation. In agreement with previously reported results (3, 21), 95 to 98% of the total protein isolated by reversible polymerization methods is sedimentable in the presence of neutral salts (105,000 × g for 2 hours). Yet the ultracentrifuge patterns presented in Fig. 2 (upper) reveal the presence of components which sediment more slowly than F-actin. These findings suggested that the preparative ultracentrifugation procedure does not permit separation of the multiple components observed during analytical ultracentrifugation.

Several lines of evidence indicate that the more slowly sedimenting species represent ionic strength-dependent aggregates of chromatographic Fraction II, i.e. aggregates which exhibit none of the characteristic molecular properties of F-actin. Fractions throughout the gel filtration profile (see Fig. 4) were treated with 0.1 M KCl-1 mM MgCl₂. After 2 hours at room temperature, aliquots of these fractions were centrifuged (14,000 × g for 2 hours), and the concentration of protein remaining in the supernatant solutions was measured. As shown in Fig. 4, both Fractions II and III were largely sedimented from solution. Thus Fraction II, like G-actin, is converted to a relatively high molecular weight component in the presence of neutral salt. Since the G → F transformation is characteristically paralleled by a large increase in viscosity, solutions of both Fractions II and III were adjusted to the same protein concentration (0.5 mg per ml), and the viscosity of each fraction was measured before and after addition of 0.1 M KCl to high molecular weight species in the presence of 0.1 M KCl-1 mM MgCl₂. The protein concentration of each fraction was measured following centrifugation (2 hours, 144,000 × g), and these values were used to compute the relative amount of polymerizable protein.

Fig. 4. Sephadex G-200 chromatography of G-actin prepared by reversible polymerization. Ten milliliters of solution (protein concentration, 13 mg per ml) were applied to a column, 2 × 45 cm, of Sephadex G-200 equilibrated at 4° with 5 × 10⁻⁴ M ATP, pH 7.5. Protein concentrations of the effluent fractions were measured both by optical density at λ = 280 mp (— — — —) and by the Lowry method (— — — —). The upper section of the figure illustrates the degree to which selected chromatographic fractions were transformed to high molecular weight species in the presence of 0.1 M KCl-1 mM MgCl₂. The protein concentration of each fraction was measured following centrifugation (2 hours, 144,000 × g), and these values were used to compute the relative amount of polymerizable protein.

Fig. 3. Polyacrylamide gel electrophoretic patterns of G-actin solutions at various stages of purification. The solvent conditions given below refer to the solutions used during isolation of the protein. A, G-actin prepared by reversible polymerization; solvent: 5 × 10⁻⁴ M ATP, pH 7.5. B and C, G-actin prepared by reversible polymerization; solvent: 5 × 10⁻⁴ M ATP-5 × 10⁻⁴ M 2-mercaptoethanol-2 × 10⁻⁴ M CaCl₂, pH 7.5. D and E, chromatographically isolated G-actin; solvent for D, 5 × 10⁻⁴ M ATP-5 × 10⁻⁴ M 2-mercaptoethanol-2 × 10⁻⁴ M CaCl₂, pH 7.5; solvent for E, 5 × 10⁻⁴ M ATP, pH 7.5. Temperature, 4°; 7.5% cross-linked gels; current: 2.5 ma per sample for 1 hour; 80 to 120 μg of protein were applied to each gel. Electrophoresis was performed at pH 9.5 (gels A through D) and pH 7.6 (gel E).
The sedimentation behavior of these preparations was consistent with the conclusion that higher molecular weight components were progressively removed from solution with increasing rotor velocity. Thus we estimate that the mass of the major component of Fraction II lies within the range 100,000 to 125,000 g per mole.

Several solvent systems were explored to minimize the heterogeneity of G-actin preparations isolated by repeated cycles of reversible polymerization. Among the reagents examined, 5 \times 10^{-4} \text{ M ATP}, pH 7.5, low ionic strength; 5 \times 10^{-4} \text{ M ATP}-0.1 \text{ M KCl}-1 \text{ mM MgCl}_2, pH 7.5, high ionic strength). Temperature, 20 \pm 0.01^\circ C.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>1/100 ml</th>
<th>0.057</th>
<th>0.196</th>
<th>15.2</th>
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</thead>
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<tr>
<td>G-Actin</td>
<td></td>
<td></td>
<td>0.057</td>
<td>0.196</td>
<td>15.2</td>
</tr>
<tr>
<td>Fraction II</td>
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<td>0.061</td>
<td>0.128</td>
<td>0.180</td>
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<table>
<thead>
<tr>
<th>Preparation</th>
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<th>Rotor velocity</th>
<th>M_w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ml</td>
<td>r/min</td>
<td>Meniscus</td>
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<tr>
<td>1</td>
<td>0.52</td>
<td>18,000</td>
<td>106,000</td>
</tr>
<tr>
<td>2</td>
<td>0.32</td>
<td>10,000</td>
<td>104,000</td>
</tr>
<tr>
<td>2</td>
<td>0.32</td>
<td>22,000</td>
<td>94,700</td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
<td>26,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>

Effects of ionic strength upon reduced viscosities of G-actin and chromatographic Fraction II

Viscosities were measured relative to solvent (5 \times 10^{-4} \text{ M ATP}, pH 7.5, low ionic strength; 5 \times 10^{-4} \text{ M ATP}-0.1 \text{ M KCl}-1 \text{ mM MgCl}_2, pH 7.5, high ionic strength). Temperature, 20 \pm 0.01^\circ C.

Physical Properties of Chromatographically Isolated G-actin—All preparations studied exhibited a single electrophoretic component and a single sedimenting boundary, and were quantitatively converted to the fibrous form. Figs. 4 and 5 reveal that the gel filtration elution profiles of G-actin are asymmetrical (see also Reference 4, and Fig. 6 shows that chromatography of electrophoretically homogeneous G-actin yields a single, yet asymmetrical, pattern. A reversibly associating system of the form \text{G-a}_{n} \rightleftharpoons A_{n} would be expected to exhibit a chromatographic picture which is essentially the mirror image of that observed. However, the possibility remained that this asymmetrical profile could reflect a mixture of particles of dissimilar size and similar charge density. Therefore, electrophoretic studies were repeated with smaller pore size polyacrylamide gels (15% cross-linked). Although the particle mobility under these conditions was reduced approximately 2-fold (at constant field strength), only a single component was observed. Thus, in view of the low ionic strength solvent used for gel filtration, it seems likely that these asymmetrical bands arise from interparticle electrostatic repulsive forces, together with charge-dependent exclusion of protein from the gel matrix. Similar features have been observed by Gellotte in studies of the gel filtration behavior of charged cytochrome (22).

![Fig. 5. Comparison of chromatographic elution profiles of reversibly polymerized G-actin isolated in the presence of 5 \times 10^{-4} \text{ M ATP}, pH 7.5 (---), and in the presence of 5 \times 10^{-4} \text{ M ATP}-5 \times 10^{-4} \text{ M 2-mercaptoethanol-5} \times 10^{-4} \text{ M CaCl}_2, pH 7.5 (---). Both G-actin preparations were isolated in parallel from the same acetone powder, and 25 ml of solution (44 mg per mil of protein) were applied to a column, 2 \times 70 \text{ cm}, of Sephadex G-200 equilibrated in each case with the indicated solvent at 4^\circ C.](http://www.jbc.org/)

![Fig. 6. Rechromatography of chromatographically isolated G-actin. Effluent fractions from the G-actin region shown in Fig. 5 were pooled and 10 ml of this solution (protein concentration, 2.3 mg per ml) were applied directly to a column, 1 \times 35 \text{ cm}, of Sephadex G-200. Solvent: 5 \times 10^{-4} \text{ M ATP}-5 \times 10^{-4} \text{ M 2-mercaptoethanol-2} \times 10^{-4} \text{ M CaCl}_2, pH 7.5. Temperature, 4^\circ C. The column void volume (V_0) was measured with Blue Dextran 2000.](http://www.jbc.org/)
tatively transformed to F-actin in the presence of 0.1 mM KCl-
1 mM MgCl₂. Fig. 7 summarizes measurements of the apparent
partial specific volume of the protein dissolved in 5 × 10⁻⁴ M
ATP, pH 7.5. Least squares analysis of these data yields \( \phi' = \)
0.750 ± 0.002 ml per g, a value substantially greater than that
reported by Kay (\( \phi' = 0.716 \) ml per g) for 0.5 M KI solutions
of actin (23). It is now recognized that G-actin is irreversibly
inactivated in the presence of 0.5 M KI, a process accompanied
by time-dependent particle aggregation (24). Mihashi has
reported \( \phi' = 0.732 \) ml per g for G-actin dissolved in 100 mM
MgCl₂-1.4 mM Tris-HCl, pH 8.0, at 20° (25).

Number- and weight-average molecular weights of six dif-
ferent preparations of G-actin were determined by the high
speed sedimentation equilibrium method as a function both of
concentration and rotor velocity. The results presented in
Table III reveal that the mean particle weight is close to 46,000

![Fig. 7. Apparent partial specific volume of chromatographi-
tically isolated G-actin. Solution densities were measured as a
function of protein concentration with a magnetic float densitom-
eter. Temperature, 20 ± 0.01°; solvent: 5 × 10⁻⁴ M ATP, pH
7.5. The open circle represents the measured density of the
solvent.](image)

![Fig. 8. High speed sedimentation equilibrium studies of actin
as a function of protein concentration. Native G-actin
(○-○); initial protein concentration, 1.3 mg per ml; solvent:
5 × 10⁻⁴ M ATP, pH 7.5; rotor temperature, 7°; 28,000 rpm. Reduced
carboxymethylated actin (×-×); initial protein concentration,
1.1 mg per ml; solvent: 5 M guanidine-HCl-0.1 M Tris-HCl, pH 8.5;
rotor temperature, 25°; 32,000 rpm.](image)

![Fig. 9. Gel electrophoretic patterns of 8 M urea solutions of
reduced carboxymethylated actin. A lyophilized powder of
chromatographically isolated G-actin was dissolved in 5 M guani-
dine-HCl, reduced and alkylated, and exhaustively dialysed
against 8 M urea. Electrophoresis solvent: 8 M urea, Tris-glycine
system, pH 9.5; temperature, 4°; current, 2.5 ma per gel for the
time indicated in the figure. Approximately 130 µg of protein
were applied to the gel column.](image)

![g per mole. As shown in Fig. 8, the apparent molecular weight
is somewhat dependent upon concentration, and the values
summarized in Table III were obtained by extrapolation of
plots of \( 1/M_{app} \) against \( c \) to \( c = 0 \). It may be noted that plots
of this type are theoretically appropriate when applied to the

![Table III](image)
treatment of uncharged polymers. At present, theoretical analyses of charge-dependent systems at sedimentation equilibrium do not suggest a satisfactory procedure for extrapolation of data at finite protein concentrations to infinite dilution (see Fujita (26) for a discussion of this problem). The necessarily low ionic strength solvent conditions employed may be partly responsible for the nonideal behavior, and for this reason, the data of Table III may be low estimates of the true mass. On the other hand, the molecular weight of G-actin at the base of the centrifuge cell was only about 5% lower than the infinite dilution value (see Fig. 8), even at the highest rotor velocities and protein concentrations. Thus it is unlikely that electrostatic effects play a significant role under the solvent conditions employed.

Properties of Actin in Concentrated Aqueous Guanidine HCl—
The mass of several preparations of reduced carboxymethylated actin dissolved in concentrated solutions of guanidine-HCl has also been measured. To ensure stoichiometric alkylation of sulfhydryl groups, the amino acid composition of each preparation was determined. (Cystine was not detected, and 13.0 \text{-} 

Table IV presents molecular weight values obtained from high speed sedimentation equilibrium analyses of reduced carboxymethylated actin as a function of protein concentration. Rotor velocities varied from 10,000 to 14,000 rpm. Initial protein concentrations are expressed in refractive index units (fringes) obtained from companion synthetic boundary cell experiments. Solvent: 5 mM guanidine-HCl-0.1 mM Tris-HCl-1 \times 10^{-4} \text{M EDTA}, pH 8.5. Rotor temperature, 25°C.

![Fig. 10. Low speed sedimentation equilibrium analyses of reduced carboxymethylated actin as a function of protein concentration. Rotor velocities varied from 10,000 to 14,000 rpm. Initial protein concentrations are expressed in refractive index units (fringes) obtained from companion synthetic boundary cell experiments. Solvent: 5 mM guanidine-HCl-0.1 mM Tris-HCl, pH 8.5. Rotor temperature, 25°C. The two symbols correspond to different actin preparations.](image-url)

### Table IV

**Molecular weight of reduced carboxymethylated actin by high speed equilibrium sedimentation**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Initial concentration (mg/ml)</th>
<th>Rotor velocity (rpm)</th>
<th>$M_a$</th>
<th>$M_o$</th>
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<tbody>
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<td>1</td>
<td>0.68</td>
<td>31,410</td>
<td>52,600</td>
<td>48,100</td>
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<tr>
<td>2</td>
<td>0.90</td>
<td>35,595</td>
<td>46,100</td>
<td>44,800</td>
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<tr>
<td>2</td>
<td>0.90</td>
<td>39,900</td>
<td>43,100</td>
<td>45,000</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>31,410</td>
<td>51,900</td>
<td>49,800</td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>32,000</td>
<td>44,000</td>
<td>46,800</td>
</tr>
<tr>
<td>5</td>
<td>1.03</td>
<td>30,000</td>
<td>47,900</td>
<td>46,300</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>47,600 ± 4,000</td>
<td>47,800 ± 1,900</td>
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</table>

Fig. 10. Low speed sedimentation equilibrium analyses of reduced carboxymethylated actin as a function of protein concentration. Rotor velocities varied from 10,000 to 14,000 rpm. Initial protein concentrations are expressed in refractive index units (fringes) obtained from companion synthetic boundary cell experiments. Solvent: 5 mM guanidine-HCl-0.1 mM Tris-HCl, pH 8.5. Rotor temperature, 25°C. The two symbols correspond to different actin preparations.

TABLE V

**Studies on interaction of nucleotide with G-actin**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Grams of G-actin per mole bound nucleotide</th>
<th>G-Actin in 5 \times 10^{-4} \text{M ATP}</th>
<th>G-Actin treated with Dowex 1-acetate</th>
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<td></td>
<td></td>
<td>G-Acin in 5 \times 10^{-4} \text{M ATP}</td>
<td>G-Acin treated with Dowex 1-acetate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>released/mole bound nucleotide</td>
<td>released/mole bound nucleotide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>polymeric</td>
<td>polymeric</td>
</tr>
<tr>
<td>1</td>
<td>51,700</td>
<td>100</td>
<td>1.32</td>
</tr>
<tr>
<td>2</td>
<td>42,300</td>
<td>100</td>
<td>1.01</td>
</tr>
<tr>
<td>3</td>
<td>48,000</td>
<td>99</td>
<td>1.37</td>
</tr>
<tr>
<td>Mean</td>
<td>47,300 ± 4,700</td>
<td></td>
<td>0.79</td>
</tr>
</tbody>
</table>

Protein-bound nucleotide concentrations were determined by perchloric acid extraction of G-actin solutions previously treated with Dowex 1-acetate. Formation of inorganic phosphate associated with the G \rightarrow F transformation at 25°C was measured colorimetrically 2 hours after addition of 0.1 M KCl-1 mM MgCl₂ to G-actin solutions.

$0.3 \text{ residues of S-carboxymethyl cysteine per } 10^5 \text{ g of protein were recovered}.$ Initially, the velocity sedimentation behavior of solutions of CM-actin in 5 M guanidine-HCl was evaluated with a double sector capillary synthetic boundary cell. For this purpose, a lyophilized preparation of G-actin was reduced and alkylated in the presence of 5 M guanidine-HCl, and examined directly with the ultracentrifuge. (Dialysis against 5 M guanidine-HCl was omitted to prevent loss of any low molecular weight dialysable material.) Both schlieren and absorption optical systems revealed a single sedimenting boundary. In addition, analyses of CM-actin dissolved in 8 M urea indicated a single electrophoretic component (see Fig. 9). Table IV presents molecular weight values obtained from high speed sedimentation equilibrium studies of five preparations of CM-actin dissolved in 5 M guanidine-HCl-0.1 M Tris-HCl-0.1 mM EDTA, pH 8.5. Fig. 8 illustrates that the apparent mass of CM-actin is concentration-dependent, and values of $1/M_{app}$ as a function of $c$ were used to estimate the particle weight at infinite dilution. As shown in Table IV, the number ($M_a$ = 47,000) and weight average ($M_o$ = 47,800) molecular weights are closely similar. When taken together with the electrophoretic and sedimentation velocity studies, these numbers reflect a high degree of particle homogeneity. The low speed sedimentation equilibrium method has also been used to measure the molecular weight of CM-actin under conditions which minimize redistribution of guanidine-HCl, and values of $M_{app}$ were estimated over a wide range of protein concentrations (4 to 14 mg per ml). Least squares analysis of the results presented in Fig. 10 yields $1/M_{app} = 1.93 \times 10^{-5} + 0.39c \left( M_o = 49,000 \text{ g per mole} \right)$. Thus both the low and high speed studies place the molecular weight of CM-actin in 5 M guanidine-HCl close to 48,000 g per mole,³

³The particle weight of CM-actin in 5 M guanidine-HCl probably represents a somewhat high estimate of the true mass, since $q'$ was taken to be 0.750 ml per g. Previous studies have shown that the apparent partial specific volume usually decreases by 2 to 3% when proteins are transferred from dilute aqueous solutions to concentrated guanidine-HCl (12, 27). The thermodynamic partial specific volume ($\bar{\gamma}$) of CM-actin may also be calculated from a knowledge of its amino acid composition, together with the apparent specific volume of each residue (28). Although this procedure neglects the possibility of preferential interaction of the macromolecule with one or more solvent components (H₂O, guanidine-HCl, etc.), we estimate $\bar{\gamma} = 0.73$. On this basis, the molecular weight of CM-actin is close to 45,000 g per mole.
Nucleotide-binding Properties of G-actin—The minimum molecular weight of G-actin based upon the number of moles of nucleotide bound per g of protein was also determined (see "Experimental Procedure"). As shown in Table V, analyses of three preparations yield 1 mole of bound nucleotide per 47,300 ± 4,700 g of protein. Previously reported values for this parameter lie in the range 60,000 to 62,000 g (29, 30). Since chromatographic Fraction II is devoid of bound nucleotide, the higher estimates may reflect the presence of a significant amount of this component. Table V also illustrates that the salt-induced polymerization of chromatographically isolated G-actin is accompanied by liberation of approximately 1 mole of inorganic phosphate per mole of bound nucleotide.

DISCUSSION

In 1951, Mommaerts established that the reversible globular to fibrous transformation of actin is a property which may be effectively utilized to eliminate a large fraction of other components from aqueous extracts of muscle acetone powders (31).

On the other hand, the inherent instability of G-actin in aqueous solutions of high ionic strength has virtually excluded the use of other potentially powerful methods for the separation and purification of proteins. Recently, Adelstein et al. (4) observed that this difficulty could be avoided by gel filtration chromatography of G-actin at low ionic strength. In the studies outlined above, gel filtration and reversible polymerization methods have been utilized together to prepare electrophoretically homogeneous G-actin of uniform particle size.

It is of interest to consider the solution properties of the predominant non-actin component (Fraction II) which is observed when solutions of actin are studied chromatographically (see Fig. 4). As illustrated by the gel filtration profiles presented in Fig. 4, this component usually comprises about 15% of the total protein isolated from acetone powders by reversible salt-induced polymerization; occasionally, it may represent nearly 75% of the actin preparation. Unlike G-actin, Fraction II is not transformed to fibrous actin in the presence of dilute aqueous KCl (see Figs. 2 and 3 and Table I). On the contrary, this component forms amorphous aggregates which are not separated from native actin during multiple polymerization cycles. This feature stems from the fact that both Fraction II and actin (in the presence of neutral salt) are removed from solution by preparative ultracentrifugation (2 hours, 105,000 x g). These observations illustrate the difficulty in analyzing the purity or "activity" of G-actin solutions solely by velocity sedimentation studies.

In agreement with the initial observation of Feuer et al. (32), several studies have indicated that reducing reagents retard time-dependent inactivation of G-actin (as judged by its progressive failure to polymerize) (2, 33, 34). This feature may be pertinent to the properties of chromatographic Fraction II, since the quantity of this component is minimized by low concentrations of 2-mercaptoethanol and calcium. Fraction II also lacks bound nucleotide, and its mass (mol. wt. = 100,000 to 125,000 g per mole) is approximately twice that of native G-actin. These properties are reminiscent of the findings of Strohman and Samorodin (29), who observed that treatment of G-actin with low concentrations of EDTA quantitatively liberates bound ATP and simultaneously inactivates the protein. In addition, Lewis et al. (24) demonstrated that EDTA converts G-actin to a dimer which forms amorphous high molecular weight aggregates in the presence of 0.1 M KCl. Thus, although we have not examined the origin of Fraction II in detail, this component may represent an inactive nucleotide-free dimer of native G-actin. Moreover, reducing agents such as 2-mercaptoethanol may serve to protect actin from air oxidation of one or more residues which are essential for the structural integrity of a protein-calcium-nucleotide complex (see also 29, 35, 36).

The molecular weight studies presented above place the mass of chromatographically isolated G-actin close to 47,000 g per mole. This value is appreciably lower than those obtained from preparations isolated by reversible polymerization methods (mol wt = 57,000 to 62,000 g per mole; φ' taken to be 0.716 (23, 24)). It is in excellent agreement with the particle weight estimated from measurements of protein-bound nucleotide (mol. wt. = 47,300). It may be noted that a molecular weight of 116,000 g per mole has been reported for G-actin (37). This number may reflect the presence of a considerable amount of the 100,000 to 125,000 molecular weight component (Fraction II). Adelstein et al. (4) have recently reported a particle weight of 47,000 g per mole (φ' taken to be 0.716 ml per g) for chromatographically isolated G-actin dissolved in dilute solutions of ATP.

Several lines of evidence have been presented which favor either a single stranded (38-40) or a double stranded (4, 41) structure for the G-actin molecule. In the present study, both low and high speed sedimentation equilibrium methods have been used to measure the particle weight of S-carboxymethylated actin dissolved in concentrated aqueous guanidine-HCl over a 60-fold range of protein concentration. Under these conditions, the molecular weight approaches 48,000 g per mole, a value in close agreement with that obtained for native G-actin. (Mihashi and Ooi have reported a value of 28,000 g per mole for actin dissolved in concentrated aqueous guanidine-HCl and subsequently dialyzed against 0.03 m KOH (40). This result may have arisen from hydrolytic cleavage of the polypeptide backbone under the strongly alkaline conditions employed.) In addition, the mass of G-actin was quantitatively recovered as a single electrophoretic component (8 M urea) and a single sedimenting boundary (5 m guanidine-HCl). Thus we infer that the molecule is constructed from a single, covalently linked polypeptide chain.5

REFERENCES


5 These numbers are based upon a value for the apparent partial specific volume for G-actin dissolved in 0.5 m KI (23). If we take φ' = 0.750 ml per g, earlier estimates of the mass lie in the range 64,000 to 70,000 g per mole.

4 In a preliminary report, Adelstein et al. (4) presented evidence which suggested that actin is composed of two chains of similar, but not identical, mass (mol. wt. = 28,200 g per mole in concentrated guanidine-HCl). More recent studies by these authors indicate that the mass is closer to 50,000 g per mole under these solvent conditions (personal communication from Dr. R. Adelstein and Dr. W. W. Kielley).
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