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Physical Properties and Polymerization Reactions of Native and Inactivated G-Actin

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Actin has been prepared which is tropomyosin-free, and some of its physical properties have been studied in low ionic strength as well as in 0.6 M KI solutions. Native G-actin in 0.5 mM ATP, 2 mM Tris buffer, *p*H 8, has an intrinsic viscosity of about 0.10 dl/g, $s^{\circ}_{20,w}$ of 3.25 Svedberg units, and molecular weight of 57,200. G-actin which has been inactivated by EDTA treatment has an intrinsic viscosity of about 0.075 dl/g, $s^{\circ}_{20,w}$ of 6.6 Svedberg units, and molecular weight of 125,000; when the ionic strength is raised to 0.1, inactivated actin undergoes a transformation into high-molecular-weight aggregates. Native G-actin in 0.6 M KI is gradually inactivated to form high-molecular-weight aggregates, indicating that an ATP solution is to be preferred for performing physical measurements on native G-actin. It is suggested that the large drop in effective volume of the native G-actin molecule which occurs upon its inactivation may be due to the collapse of a flexible, solvent-penetrable domain within the molecule after removal of the Ca-ATP prosthetic group.

The discovery that usual preparations of actin may contain appreciable amounts of tropomyosin (Laki and Cairns, 1959; Laki and Standaert, 1960) casts doubt on the physical measurements made on such preparations and on the interpretations which have been drawn from them. The ability to prepare actin which is free of tropomyosin (Martonosi, 1962a,b; Laki *et al.*, 1962) offers an opportunity to study pure G-actin and its transformation products.

It has long been recognized that G-actin containing bound ATP polymerizes into F-actin upon addition of salt, and that removal of the bound ATP results in a complete loss of this polymerizability (Laki *et al.*, 1950; Straub and Feuer, 1950). G-actin which has been treated in any manner allowing release of bound ATP and loss of ability to form F-actin may be termed inactivated G-actin. Viscosimetry (Martonosi and Gouvea, 1961; Barany *et al.*, 1961; Grubhofer and Weber, 1961; Strohman and Samorodin, 1962) and flow birefringence (Asakura, 1961) have shown that no linear polymerization occurs when inactivated G-actin is treated with salt, but this does not rule out the possibility that other types of aggregates are formed. We shall describe here the use of ultracentrifugal techniques to demonstrate the formation of random aggregates from inactivated G-actin in the presence of salt.

Material and Methods

Actin was extracted from rabbit muscle by the method of Straub (1943) and purified according to Mommaerts (1952a). It was then subjected to a procedure, described by Martonosi (1962b) and by Laki *et al.* (1962), which removes contaminating tropomyosin. The tropomyosin-free F-actin pellet was depolymerized by dialysis against 0.5 mM ATP in 2 mM Tris buffer at *p*H 8.0 for 2–3 days at 3–4°. The G-actin was then clarified by centrifugation for 1 hour at 30,000 rpm.

G-actin was inactivated by ethylenediaminetetraacetate (EDTA) treatment as reported by Maruyama and Martonosi (1961); EDTA releases the bound Ca^{++} and ATP concomitantly from G-actin (Tonomura and Yoshimura, 1961; Maruyama and Gergely, 1961), leading to loss of its polymerizability (Martonosi and Gouvea, 1961; Barany *et al.*, 1961; Grubhofer and Weber, 1961; Strohman and Samorodin, 1962). After removal of free ATP by treatment with Dowex-1 (Asakura, 1961), actin was incubated for 10 minutes at room temperature in the presence of 10 moles EDTA per mole actin in 2 mM Tris buffer *p*H 8.0. The KCl concentration of the inactivated G-actin solution was made 0.1 M by the addition of 3 M KCl.

Viscosity was measured in an Ostwald-type viscosimeter with a water flow-time of 82.5 seconds at 25.0 ± 0.01°. Sedimentation rates were measured at 25° in a Spinco Model E ultracentrifuge equipped with a rotor temperature indicator and control unit.

The molecular weights were determined in a second Spinco Model E ultracentrifuge equipped with the rotor temperature indicator and control unit and with the Rayleigh interference optical system. With the exception of the actin treated with KI for 3 days, which was examined at 27.0°, all determinations were carried out at 4.0°. The sedimentation equilibrium experiments utilized the short-column technique with interference

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optics as described by Richards and Schachman (1959). Column heights were usually 1.3 mm. The four-place An-J (Spinco) equilibrium rotor permitted running three samples simultaneously. Two 12-mm wedge centerpiece cells were used for the more concentrated solutions, and a 30-mm cell was used for the most dilute solution. The initial concentration of the most concentrated solution in each experiment was determined by a synthetic boundary run in the ultracentrifuge. The concentrations of the more dilute samples were determined with a Zeiss Laboratory Interferometer. The interferometer was also used to adjust the 1,3-butanediol concentration in the buffer for the reference channels of the double-sector cells. The photographic plates were measured with a Nikon Model 6 comparator, and graphs of *lnc vs.* x^2 (where x = radius) were constructed. If the linearity of the plots indicated homogeneity, the values of $dlnc/dx^2$ were calculated, and the apparent molecular weight was calculated with the formula[1]

$$M_{app} = 2RT\,(dlnc/dx^2)/(1 - \bar{v}\rho)\omega^2$$

If the material was nonhomogeneous, the weight average molecular weights were calculated from the formula

$$M_w = 2RT(c_b - c_m)/c_0(x_b^2 - x_m^2)(1 - \bar{v}\rho)\omega^2$$

Where time was a significant factor, or where nonhomogeneity was anticipated, the Archibald technique was used. The method used here, described in detail in another paper (Lewis, M.S., in preparation), uses only the interference optical system. The method is similar to that suggested by Richards and Schachman (1959) except that the concentration gradients at the meniscus and bottom are obtained by measuring the limiting slopes of the fringes directly with the screen protractor of the Nikon Model 6 comparator. The apparent molecular weights at the meniscus and bottom were calculated from the formulas

$$M_m = RT(dc/dx)_m/(1 - \bar{v}\rho)\omega^2 x_m c_m$$

and

$$M_b = RT(dc/dx)_b/(1 - \bar{v}\rho)\omega^2 x_b c_b$$

and their average was taken as the apparent weight average molecular weight.

Results

Preliminary Observations.—When actin prepared by the method of Straub is incubated at room temperature over an extended period, its polymerizability into F-actin is gradually lost (Fig. 1). When a small amount of ammonium sulfate is added to an aliquot of the incubation mixture, turbidity develops. In the course of time, as the polymerizability is lost the turbidity is augmented (Fig. 1). This suggests that the inactivated G-actin is readily precipitated by low concentrations of ammonium sulfate.

During an investigation on the binding of C^{14} EDTA to actin prepared by the method of Mommaerts (Maruyama and Gergely, 1961), EDTA-treated G-actin in 0.1 M KCl was spun for 3 hours at 30,000 rpm in the Spinco Model L ultracentrifuge. Under these conditions a thick precipitate was always observed at the bottom of the tube, whereas with native G-actin a negligible amount of precipitate is encountered. This

[1] In the formulas in this section, R = gas constant, T = absolute temperature, $\bar{v}$ = partial specific volume = 0.716 (Kay, 1960), ρ = solution density, ω = angular velocity (rad./sec.), c_0 = initial concentration, c_m and c_b = concentration at meniscus and bottom, respectively, and x_m and x_b = radius at meniscus and bottom respectively.

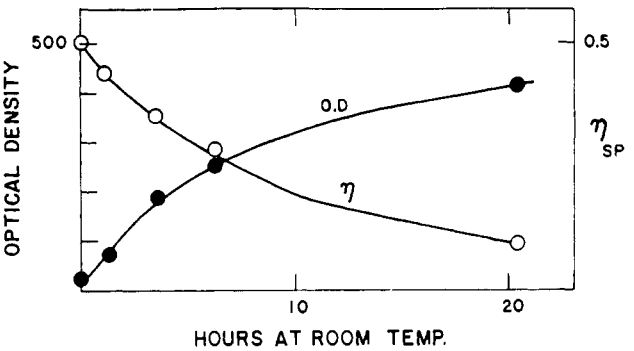

FIG. 1.—Changes in viscosity and turbidity of actin during incubation at room temperature. Crude G-actin, 3 mg/ml, was incubated at room temperature and *p*H 7.5. O–O, relative viscosity of sample polymerized in 0.1 M KCl; ●–●, optical density at 580 mμ of sample in 5% saturation ammonium sulfate.

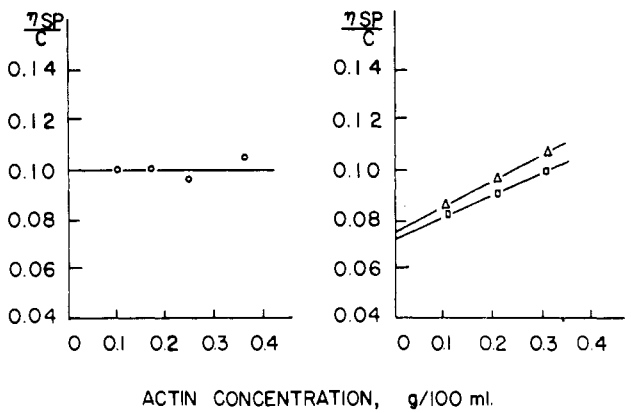

FIG. 2.—Concentration dependence of the reduced viscosity of G-actin. All solutions are 3 mM Tris, *p*H 8.0, at 25°. O–O, native G-actin in 0.5 mM ATP; △–△, inactivated G-actin in 0.5 mM EDTA; □–□, inactivated G-actin in 0.5 mM EDTA and 0.1 M KCl.

observation also suggests the formation of a readily precipitated aggregate upon the addition of salt to inactivated actin.

Viscosity.—The viscosity of native G-actin is very low, and the error in measuring it is very high. The concentration dependence of η_{sp}/c is given in Figure 2; an error of 0.1 second in measurement of outflow time can alter the η_{sp}/c by 0.006, 0.009, 0.012, and 0.015 dl/g at the four decreasing concentrations measured. The intrinsic viscosity is about 0.10 dl/g with no concentration dependence. In view of the magnitude of possible error, it can only be stated with certainty that the intrinsic viscosity is $\leq$ 0.104 dl/g, the value of η_{sp}/c at the highest concentration measured.

Upon inactivation by EDTA, the value of η_{sp}/c at the highest measured concentration is the same as that of native G-actin. However, as indicated in Figure 2, there appears to be a slight concentration dependence which extrapolates to an intrinsic viscosity of about 0.076 dl/g. Similar results are obtained upon addition of salt to inactivated actin. The intrinsic viscosities of these solutions of inactivated actin must also be viewed with great caution because of the significant effect of very small error.

In contrast to these very low values, the intrinsic viscosity of a sample of actin prepared by the method of Mommaerts was 0.53 dl/g in ATP solution, 0.56 dl/g upon inactivation by EDTA, and 0.12 dl/g after the KCl concentration was raised to 0.1 M. This actin preparation contained 15% tropomyosin, and the high

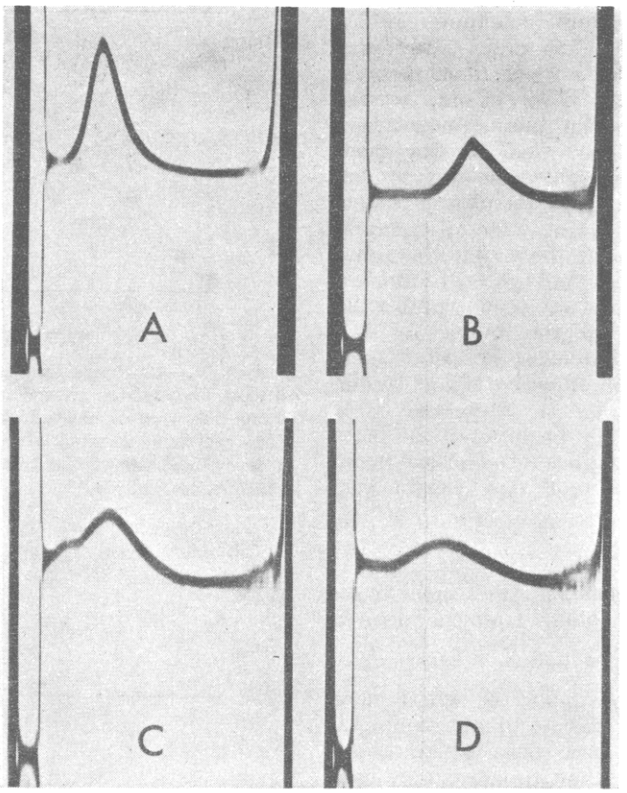

FIG. 3.—Ultracentrifuge diagrams of native and inactivated G-actin. All solutions are 2 mM Tris, *p*H 8.0.

	Protein	Concentration	Solvent	Temperature	Time	$s_{20,w}$
A.	Native G-actin	0.37%	0.5 mM ATP	25.1°	70 min.	2.59 *S*
B.	Inactivated G-actin	0.3%	0.5 mM EDTA	25.0°	66 min.	4.92 *S*
C.	Same, immediately after addition of salt	0.3%	0.5 mM EDTA, 0.1 M KCl	25.6°	13 min.	13.3 *S*
D.	Same, 24 hours after addition of salt	0.3%	0.5 mM EDTA, 0.1 M KCl	25.5°	13 min.	16.9 *S*

intrinsic viscosity in the two solutions at low ionic strength can be ascribed to the polymerized tropomyosin. The lowest previously reported intrinsic vicosity for actin has been 0.14 dl/g in 0.6 ionic strength phosphate-buffered KI (Kay, 1960), a value which is about 40% higher than that found here for tropomyosin-free native G-actin.

After polymerization of G- to F-actin, very large intrinsic viscosities of 5–10 dl/g can be measured. It is apparent that the inactivated actin is not forming elongated asymmetrical aggregates upon addition of salt.

Sedimentation Measurements.—In ATP solution, G-actin exhibits a single peak in the ultracentrifuge (Fig. 3*a*). Not all samples are completely homogeneous, since prolonged ultracentrifugation sometimes reveals spreading of the leading edge of the boundary, which varies in degree from preparation to preparation. Upon inactivation with EDTA, a single peak with about double the sedimentation coefficient of native actin is present (Fig. 3*b*). After the addition of salt to inactivated actin, transformation to rapidly sedimenting material occurs (Fig. 3*c,d*). Initially, two peaks can be observed (Fig. 3*c*); the slower peak corresponds to the single peak present before addition of salt. After 24 hours, the slower peak disappears (Fig. 3*d*). The fast peak broadens, and its sedimentation coefficient increases during storage at 0° from 13.3 *S* initially to 16.9 *S* at 24 hours and to 20.9 *S* at 48 hours. It is evident that heavier and more heterogeneous aggregates are forming with time.

The concentration dependence of sedimentation for actin is shown in Figure 4. In ATP solution two different preparations of native actin had an $s^{\circ}_{20,w}$ value of 3.25 *S;* this is somewhat higher than the value of 3.02 *S* reported by Kay (1960). Actin inactivated by EDTA had an $s^{\circ}_{20,w}$ of 6.6 *S*. Immediately after the addition of salt to inactivated actin, the $s^{\circ}_{20,w}$ of the aggregate was approximately 16 *S;* the time dependence of the sedimentation coefficients which has been previously described suggests that no great significance can be attached to this value.

These results indicate that upon inactivation of actin a limited aggregation occurs, and when salt is added to inactivated actin unlimited aggregation supervenes.

Molecular Weight Determinations.—Figure 5 shows the concentration dependence of the reciprocal of the molecular weight of actin obtained by sedimentation equilibrium or Archibald techniques. In ATP solution, native actin appears to be homogeneous on the basis of the $dlnc/dx^2$ plots, and extrapolation of the reciprocal of molecular weight to infinite dilution gives an M_0 of 57,200. Actin inactivated by EDTA also appears homogeneous, and at infinite dilution it has an M_0 of 125,000. Upon addition of salt to inactivated actin, gross heterogeneity becomes apparent and the extrapolated molecular weight of 547,000 represents a crude average.

The molecular weight of 57,200 for native G-actin is in excellent agreement with the values obtained by Mommaerts (1952b) and by Laki and Standaert (1960).

Contamination with tropomyosin may be responsible for values of 62,000 or greater obtained for various actin preparations in water-ATP medium at neutral *p*H (Kay, 1960; Ooi, 1961).

The sedimentation studies revealed that limited aggregation of inactivated actin occurs; the molecular weight determinations show that this proceeds to dimer formation and no further. The heterogeneity of the aggregation products formed upon addition of salt to inactivated actin, which was suggested by the velocity sedimentation diagrams, is supported by the Archibald molecular weight determinations.

Molecular Weight Determinations in KI Solutions.—Since G-actin does not polymerize to F-actin in concentrated KI solutions (Szent-Gyorgyi, 1951), the molecular weight was studied as a function of time in a solution of 0.6 M KI, 0.5 mM ATP, and 0.04 M sodium ascorbate, *p*H 7.0. All samples were kept at 0° until the time of centrifugation. Figure 6 shows the concentration dependence of the reciprocal of molecular weight at less than 3 hours, at 24 hours, and at 3 days. The molecular weight of the 3-hour sample was determined by the Archibald technique; the weight average value of M_0 was 65,000, in good agreement with the value of 66,000 obtained by Kay (1960) in this solvent by light scattering. It can be seen from Figure 6 that the molecular weight increases with increasing concentration owing to aggregation. At 24 hours, heterogeneity was indicated by the nonlinear $dlnc/dx^2$ plots from sedimentation equilibrium data. A weight average molecular weight of 80,600 was obtained, in good agreement with the value of 80,000 obtained by Steiner *et al.* (1952) in this solvent by light scattering. After 3 days, a solution from which sodium ascorbate was omitted showed marked heterogeneity, and the extent of aggregation appeared to be pressure dependent. As can be seen in Figure 6, this is indicated by the fact that increasing the rotor speed from 3189 rpm to 5784 rpm, an increase of over 3-fold in centrifugal field, resulted in an increase of the weight average M_0 from 400,000 to 547,000 and also reversed the concentration dependence of the apparent molecular weights.

It is clear that inactivation of actin is slowly occurring with time in KI solutions, even in the presence of ATP and reducing agent, and that the inactivated actin is forming aggregates. It is apparent that little reliability can be attributed to molecular weights measured in this medium and that an ATP solution is the medium of choice.

Evaluation of Hydrodynamic Parameters.—While the molecular weight measurements demonstrate clearly that EDTA-inactivated actin forms a dimer, both the large increase in sedimentation constant and modest decrease in intrinsic viscosity (rather than an increase) are unexpected and indicate some further change in the molecule beyond simple association to give a dimer. In an attempt to evaluate this change, calculations can be made of the effective volume and axial ratio of an equivalent ellipsoid, using the Scheraga-Mandelkern (1953) function, β. Table I shows the results of such calculations for native and EDTA-inactivated actin. The striking figure is 0.64 cm³/g for the specific effective volume, V_e, of the inactivated protein. This is slightly less than the anhydrous partial specific volume, $\bar{V}$, and accounts for the low viscosity and high sedimentation constant compared with the native molecule, which has a specific effective volume four times larger. While the exact values of the derived dimensions of the equivalent ellipsoid are very sensitive to errors in experimental determinations, it is certainly safe to conclude that inactivation of actin by EDTA causes a marked

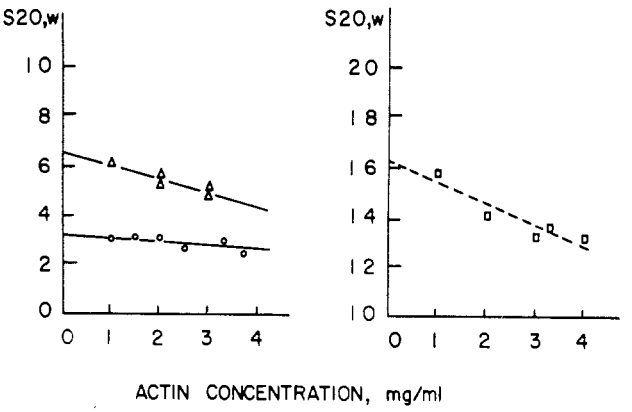

FIG. 4.—Concentration dependence of the sedimentation rate of G-actin. All solutions are 2 mM Tris, *p*H 8.0, at 25°. O–O, native G-actin in 0.5 mM ATP; Δ–Δ, inactivated G-actin in 0.5 mM EDTA; □–□, inactivated G-actin in 0.5 mM EDTA and 0.1 M KCl.

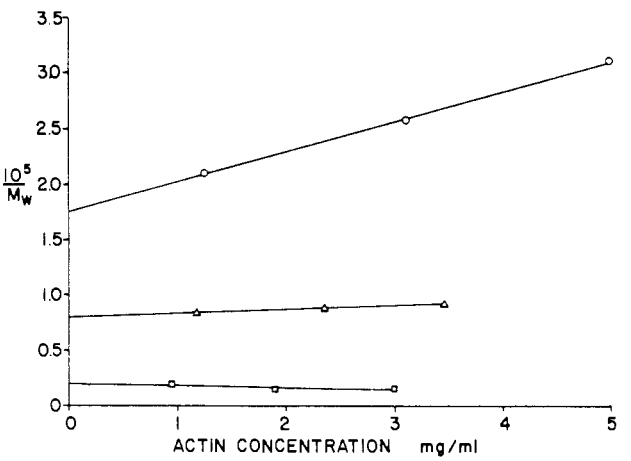

FIG. 5.—Concentration dependence of the reciprocal of the molecular weight of G-actin, determined by sedimentation equilibrium or Archibald procedures. All solutions are 2 mM Tris, *p*H 8.0, at 4°. O–O, native G-actin in 0.5 mM ATP (equilibrium); Δ–Δ, inactivated G-actin in 0.5 mM EDTA (equilibrium); □–□, inactivated G-actin in 0.5 mM EDTA and 0.1 M KCl (Archibald).

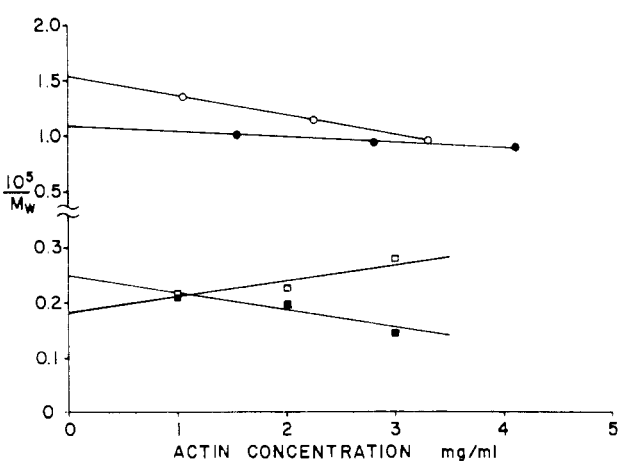

FIG. 6.—Concentration dependence of the reciprocal of the molecular weight of G-actin in 0.6 M KI solution, determined by sedimentation equilibrium or Archibald procedures. O–O, less than 3 hours at 9.5° (Archibald); ●–●, 24 hours at 4° (equilibrium); □–□, 3 days at 4° (equilibrium at 5784 rpm); ■–■, 3 days at 4° (equilibrium at 3189 rpm).

TABLE I

PHYSICAL CONSTANTS AND DERIVED PARAMETERS OF G-ACTIN

The derived parameters are for an assumed prolate ellipsoid having a specific effective volume, V_e, and an axial ratio, $1/p$, that will demonstrate the same hydrodynamic behavior as the real molecule. We are assuming that the ratio of specific effective volume to anhydrous partial specific volume, $V_e/\bar{V}$, gives a measure of the apparent swelling.

	Native	EDTA-Inactivated
M	57,200	125,000
$s^\circ_{20,w}$, Svedberg units	3.25	6.6
$[\eta]$, dl/g	0.10	0.07$_6$
$\bar{V}$, cm^3/g	0.716[a]	0.716[b]
$\beta \times 10^{-6}$	2.17	2.38
$1/p$	3.1	9.0
V_e, cm^3/g	2.67	0.64
$V_e/\bar{V}$	3.73	0.90

[a] Kay (1960). [b] Assumed value.

TABLE II

EFFECT OF EDTA ON OPTICAL ROTATORY DISPERSION OF G-ACTIN

Measurements were made at pH 8.0 and 20° with protein concentrations 0.3–0.4 mg/ml. When added, EDTA was present as 10 moles per mole of actin.

	$[\alpha]_D$		λ_c	
Preparation	Control	EDTA	Control	EDTA
1	−51°	−67°	243 mμ	231 mμ
2	−50°	−61°	268 mμ	245 mμ
3	−52°	−63°	275 mμ	253 mμ
4	−44°	−55°	285 mμ	251 mμ

decrease in the apparent swelling, $V_e/\bar{V}$. Further evidence of a change in internal organization is provided by the changes in specific rotation and λ_c illustrated in Table II.

DISCUSSION

The present study indicates that past measurements on actin may have to be reinterpreted as a consequence of the increased purity of actin preparations which is now possible. The properties most markedly altered are those most influenced by small amounts of tropomyosin. At low ionic strength, where tropomyosin forms long polymers, the viscosity and molecular weight measured physically are considerably lower for the purified actin; the molecular weight is essentially unchanged from that measured by nucleotide binding (Mommaerts, 1952b). In 0.6 M KI, in which tropomyosin no longer forms polymers, the viscosity and molecular weight measured physically should be the same for pure and impure preparations; this cannot be ascertained because the gradual inactivation of G-actin in KI solutions means that any results obtained in these solutions are unreliable.

The errors in experimental determinations allow the β for native G-actin to fall within the range 2.12–2.17. This allows great ambiguity as to the shape of this protein. It could be represented by a prolate ellipsoid as in Table I, by oblate ellipsoids with a wide range of axial ratios and effective volumes, or by a more flexible structure bearing little resemblance to any of these "equivalent ellipsoids."

To determine the most likely among these possible structures, we have turned to the electron microscopic evidence on the structure of F-actin for guidance. Hanson and Lowy (1962) have shown that F-actin consists of 56 A diameter units, arranged in a helical array. This picture is consistent with what is known from x-ray studies of F-actin in dry films (Astbury *et al.*, 1947; Cohen and Hanson, 1956), in dry muscle (Selby and Bear, 1956), and in wet muscle (Huxley, 1960). If it can be assumed that G-actin in dilute solution is comparable to wet F-actin, then it should also be comparable to dry F-actin observed in the electron microscope, since the x-ray studies show no difference between the wet and dry states.

F-actin cannot be formed from stacked oblate ellipsoids of axial ratio less than ½, since such structures would have diameters less than 50 A. If G-actin were a prolate ellipsoid with axial ratio of 2 and with apparent swelling, $V_e/\bar{V}$, of 4, then each molecule could compose two of the 56 A repeating units in F-actin. However, this structure would give half as much actin as is present in muscle (Hanson and Lowy, 1962); furthermore, the x-ray pattern of a protein fiber with uniform swelling of this extent would be expected to show marked differences from its dry state, which are not found (Huxley, 1960). Thus, assuming that no major conformational change occurs from G-actin to F-actin, models of rigid oblate or prolate ellipsoids can be eliminated.

A third possibility remains, that native G-actin may be flexible and penetrable. Under these conditions a smaller molecule can be equivalent to a larger rigid and impenetrable ellipsoid. The large effective volume that is found in the "equivalent ellipsoid" would not measure either bound water or actual size of the molecule, but it would reflect the increased resistance to solvent flow of a penetrable domain within the molecule.

Utilizing this concept, we can reconcile the hydrodynamic data with the electron-microscopic data by postulating the existence of two realms within the native G-actin molecule: One realm is relatively rigid and has a diameter of 56 A. The other realm is flexible and penetrable; it is responsible for the large effective volume of the "equivalent ellipsoid." Upon denaturation in a poor solvent, such as water at neutral pH, this flexible realm will coil up compactly, becoming rigid and impenetrable. Such a process could produce the loss of apparent swelling which occurs upon inactivation of native G-actin. It has been pointed out (Kauzmann, 1959; Scheraga, 1961) that only upon denaturation in good solvents such as urea or guanidine does increased swelling take place.

It should be noted that other native proteins besides actin have very large effective volumes. For example, fibrinogen has an apparent swelling, $V_e/\bar{V}$, of about 5 (Edsall, 1954), which Yang (1961) has suggested could be explained on the basis of two different "equivalent ellipsoids" for viscosity and for sedimentation.

The optical rotatory dispersion measurements support the view that the inactivation of actin is a denaturation (Kauzmann, 1959). The drop in specific rotation to more negative values, and the displacement of λ_c to shorter wave lengths, are typical of a process of unfolding (Schellman, 1958) to a less helical configuration (Yang and Doty, 1957). Nagy and Jencks (1962) have reached a similar conclusion from elegant optical rotatory studies on purified actin preparations and correlated studies on susceptibility to proteolysis. This occurrence of a conformational change casts doubt on the assumption that the value of $\bar{V}$ remains at 0.716 in the EDTA-inactivated actin, giving a further possible source of error in calculations of the derived parameters in Table I.

The change of configuration and aggregation of actin

upon inactivation are analogous to the processes occurring in myosin upon covering of certain of its —SH groups (Kominz, 1961), except that in myosin the alterations are reversible. It appears as though the Ca-ATP prosthetic group is holding two parts of the native G-actin molecule in a metastable configuration, as suggested by Strohman and Samorodin (1962). It is not clear whether the role of the Ca-ATP prosthetic group in giving direction to the polymerization of native actin is simply to hold the molecular structure intact in this way, or whether it is to participate directly in bond formation, as inavailability to exchange of ADP in F-actin suggests (Martonosi *et al.*, 1960).

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The Molecular Weight of Rabbit Myosin A by Light Scattering

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A light scattering determination of the molecular weight of myosin was undertaken with particular attention to the measurement of auxiliary parameters, especially specific refractive index increment (dn/dc). A dn/dc, on a dry weight basis, of 0.192 ml/g was obtained. This was in good agreement with values found for other proteins but at variance with some values which have been used for myosin. A molecular weight of 5.2×10^5 g, including a 4% contribution due to bound salt, was obtained.

The recent proposal by Kielley and Harrington (1960) of a three-stranded model for the myosin molecule has stimulated new interest in the varying figures reported for the molecular weight of myosin.

Some recent studies using the Archibald analysis of sedimentation data (von Hippel *et al.*, 1958; Mommaerts and Aldrich, 1958) and light scattering (Brahms, 1959) have yielded molecular weights near 420,000. Two other light scattering determinations (Holtzer and Lowey, 1959; Holtzer *et al.*, 1962) gave values near 500,000 g/mole though widely differing numbers for the specific refractive index increment were employed. Molecular weights calculated from sedimentation (von Hippel *et al.*, 1958; Holtzer and Lowey, 1959; Parrish and Mommaerts, 1954; Johnson and Rowe, 1960) and diffusion (Parrish and Mommaerts, 1954; Johnson and Rowe, 1960) measurements cluster around 550,000 g. Kielley and Harrington's extensive studies using the Archibald method gave 619,000 g/mole.

The range of these figures, combined with a molecular weight of 206,000 g for the myosin subunit in guanidine·HCl (Kielley and Harrington, 1960) (the only value so far reported) is sufficient to include two-stranded as well as three-stranded models for native myosin.

We have undertaken yet another light scattering

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