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A RE-EXAMINATION OF THE MOLECULAR CHARACTERISTICS OF G-ACTIN

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SUMMARY

1. The molecular weight of ultracentrifugally homogeneous G-actin, prepared by the method of Tsao and Bailey, is 66,000 ± 2,000 by light scattering and 61,600 ± 6,000 by sedimentation viscosity.

2. The virtual identity of the extrapolated Hc/τ intercept found by light scattering for G-actin in either 0.5 M KI or H_2O–10^{-4} M ATP suggests that the same limit of dissociation is attained for the protein dissolved in either of these media.

3. The light scattering data do not support the earlier view, derived from osmotic pressure measurements, that both monomeric and dimeric G-actin exist in depolymerized actin systems.

4. The dispersion constant, $\lambda_0 = 223$ mμ, is unusually low for G-actin, reflecting an apparent lack of any extensive helical configuration in the molecule.

INTRODUCTION

The earliest attempts to characterize monomeric G-actin were limited by the lack of purity of the preparations used[1–3]. Most of these studies were carried out on preparations based on the original or modified procedures of Straub[4], and these were subsequently shown to contain as much as 40–60 % impurity[5,6]. In 1951 Mommaerts[5], using the method of successive polymerizations and ultracentrifugal separation of the polymerized actin, was able to obtain preparations of G-actin that showed a single broad peak in the ultracentrifuge, with an intrinsic sedimentation constant of 2.8 S when examined in the stabilizing medium of 10^{-4} M ATP, pH 8–8.2. The molecular weight as calculated from measurements of 90° scattering in this same medium was 57,000 (see ref. 1) as compared with a value of 80,000 obtained by STEINER, LAKI AND SPICER[3], also by light scattering, on unpurified preparations in 0.5 M KI, a medium known to favor the depolymerization of polymeric F-actin to G-actin. This discrepancy was no doubt due very largely to the difference in purity of the preparations, but what contribution the different media may have made to it remained an unanswered question.

In 1953 TSAO AND BAILEY[8] evolved their own variation of the STRAUB procedure to obtain electrophoretically homogeneous G-actin. In this work, the protein samples were not purified by fractional sedimentation in the ultracentrifuge, but, rather, the final extraction was made with 30 % acetone instead of pure water, a treatment which successfully suppresses the extraction of contaminants, notably tropomyosin. Various preparations were subjected to osmotic pressure measurements in 0.6 M KI (see ref. 9). The results were found to be somewhat erratic, indicating in some experiments the presence of a 74,000 unit, at other times one of 140,000, and in very dilute solution the transition of the latter to the former with progressive dilution. Viscosity determinations on a G-actin preparation, which by osmotic pressure measurements possessed a particle weight of 74,000, gave an intrinsic viscosity value of 0.21 dl/g, indicating, for a prolate ellipsoid of revolution, an axial ratio of about 12. The use of an assumed $\bar{v}$ value for actin of 0.74 in conjunction with this viscosity provided dimensions of 290 Å and 24 Å for the length and diameter of the monomer.

More recently TSAO's[9] work has come under severe criticism from GEIDUSCHEK AND HOLTZER[10], who point out that the osmotic pressure data must be discounted, because the extrapolation is not unique for the various samples examined, even though they were gathered under presumably identical conditions. The viscosity results are also questioned in view of their lack of parallellism with the osmotic pressure measurements. These reviewers argue that, since the osmotic pressure data on the identical sample in the same medium show the occurrence of distinct dissociation upon dilution of the protein, the viscosity curve should also show strong negative curvature in this region. The fact that it does not indicates that the two sets of measurements are inconsistent.

From the foregoing discussion it is apparent that although considerable progress has been made in elucidating the actin system, several "sore-spots" in the story must be re-explored. Among these is the question of the disparity in the fundamental particle weight of G-actin in the water–ATP system and in 0.5 M KI: to date none of the better preparations have been subjected to molecular weight determination by at least two different methods. Secondly, the evidence of TSAO for the existence of monomeric and dimeric G-actin in 0.6 M KI is far from conclusive and deserves re-investigating. For this purpose light scattering is the preferred method over osmotic pressure because of its accessibility to lower protein concentrations with a resultant smaller uncertainty associated in the extrapolated ordinate intercepts. The present investigation was carried out with a view to answering the above questions. For this purpose, a preparation of G-actin prepared by the method of TSAO AND BAILEY was studied, using sedimentation, viscosity, light scattering and pycnometric techniques.

Also, an estimate of the helical content of the protein was obtained through a study of its optical rotatory dispersion.

MATERIALS AND METHODS

Preparation of G-actin

The protein was prepared from rabbit skeletal muscle by the method of TSAO AND BAILEY[8]. Material prepared in this way was shown by the above workers to be electrophoretically homogeneous at pH 2 and 10. However, since electrophoresis patterns do not show up the presence of stepwise aggregates of the same molecule, ultracentrifugation was chosen as the criterion of purity in this study. An example of the ultracentrifugal pattern obtained for G-actin in water–10^{-4} M ATP at pH 8 is given in Fig. 1.

A single broad asymmetric peak, similar to that obtained by MOMMAERTS[5] for material prepared by his method, was observed. Although this is not regarded as a rigorous indication of purity, it must be stressed that there is a special difficulty in the case of G-actin: it is stable only at low ionic strength and mildly alkaline pH in the presence of ATP. MOMMAERTS has shown that under these conditions charge effects and the sedimentation of ATP itself complicate the interpretation of the boundary[7]. Furthermore, since ordinary preparations exhibit more than one peak under similar conditions, it may be inferred that the purification procedure has been at least partially successful. For most of the studies reported herein, solutions of G-actin in water–10^{-4} M ATP were dialyzed against repeated changes of KI solution (0.5 M KI, 0.05 M K_2HPO_4, 0.025 M KH_2PO_4, pH 7, ionic strength = 0.6). Representative photographs of a sedimentation–velocity run on such a preparation is shown in Fig. 2, from which it is seen that a single boundary was maintained throughout the course of the run.

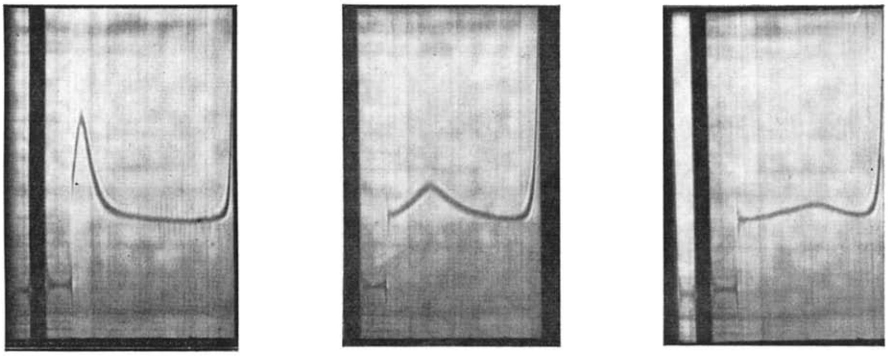

Fig. 1. Ultracentrifugal sedimentation patterns of 0.56 % G-actin in H_2O–ATP (10^{-4} M) at pH 8. Centrifugation at 59,780 rev./min in Kel-F cell; bar angle, 50°; temperature 23.5°; time after reaching full speed: a, 16 min; b, 80 min; c, 144 min.

Protein concentrations were determined by the micro-Kjeldahl method, taking 16.1 % for the total nitrogen[9]. Concentrations in the ultracentrifuge runs were usually checked by area measurements of tracings of enlarged patterns (10 ×) made with a photographic enlarger. The concentration of sedimenting material was calculated in accordance with the equation given by SCHACHMAN[11].

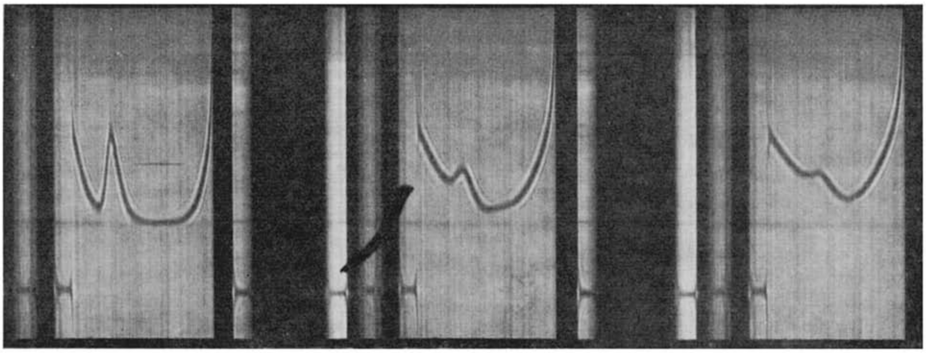

Fig. 2. Representative photographs obtained in a sedimentation-velocity run for 0.34 % G-actin in 0.5 *M* KI, I = 0.1, phosphate buffer, pH 7. Centrifugation at 59,780 rev./min in synthetic boundary cell; bar angle, 60°; temperature, 24.5°; time after reaching full speed: a, 0 time; b, 16 min; c, 32 min. Much of the curvature of the pattern on the left is due to the redistribution of the KI buffer solution.

Sedimentation

Sedimentation velocity studies of G-actin were carried out in a Spinco model E ultracentrifuge equipped with temperature control. Photographic plates were measured with a Gaertner micro comparator and calculated sedimentation coefficients were corrected to a standard state of water at 20°.

Intrinsic viscosity

Ostwald-Fenske viscometers requiring 6.5 ml and having a water flow time of about 240 sec at 25° were used to measure the viscosity of G-actin solutions. The intrinsic viscosity was obtained by extrapolating to zero concentration, a plot of reduced specific viscosity, η_{sp}/c, against c in g/100 ml.

Light scattering

Light scattering measurements were performed at a wavelength of 436 mμ with the Brice-Phoenix light scattering photometer. Prior to the measurements the protein solutions were clarified by filtration through millipore filters as described by KAY AND EDSALL[13]. Turbidities on the pure solvents were determined in a similar fashion and were subtracted from the readings obtained with actin solutions. Scattering dissymmetries at 45° and 135° and depolarization measurements were carried out in the Brice instrument in the prescribed manner. The specific refractive index increment of G-actin, determined in a Phoenix differential refractometer[14], was 0.172 at 4358 Å and 25°, in good agreement with the previously published figure[7].

Partial specific volume

Partial specific volume determinations were made at 20 ± 0.01° with pycnometers of 10 ml capacity, which were calibrated with air-free distilled water. Partial specific volumes were calculated from the equation[15]:

$$1 - \bar{v}\rho = \frac{1 - W_2}{m} \cdot \frac{dm}{dW_2} \qquad (1)$$

where *m* is the mass of solution, W_2 is the weight fraction of the protein (0.01 times the concentration by weight) and ρ is the density of the solution.

*Optical rotary dispersion measurements**

Optical rotations were measured with a Rudolph automatic recording spectropolarimeter, Model 260. The light source was a Xenon compact arc lamp and the wavelength range investigated extended from 300 to 650 mμ. The concentration of the protein solution, contained in 1-dm tubes, was 0.3 % and the temperature of the measurement was 29°.

RESULTS AND DISCUSSION

Sedimentation

A plot of the calculated $S_{20,w}$ values against concentration for G-actin in the KI–phosphate buffer medium (pH 7, I = 0.6) is given in Fig. 3. Also included are 3 sedimentation values obtained for different concentrations of the protein in the H_2O–10^{-4} *M* ATP system. These latter values are susceptible to serious error due to charge effects, but do not appear to be out of line with the values obtained in the presence of salt. All of the data can be represented by the single full line of Fig. 3 derived by the method of least squares. The sedimentation constant extrapolated to zero concentration ($S_{20,w}$) is 3.02, as compared with the values of 2.8–3.1 obtained by MOMMAERTS for material prepared by his method.

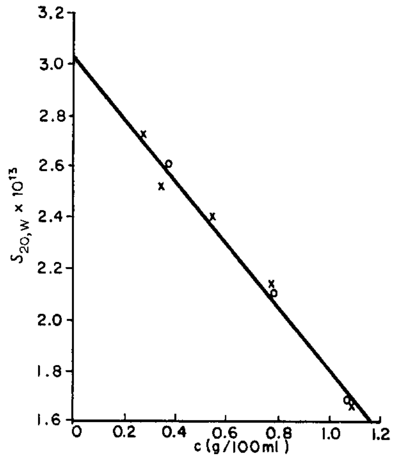

Fig. 3. Concentration dependence of sedimentation of G-actin prepared by the method of TSAO AND BAILEY[8]. The crosses represent a solvent medium of 0.5 *M* KI, 0.1 *M* K phosphate (pH 7, I = 0.6) and the circles a medium of H_2O–10^{-4} *M* ATP at pH 8.

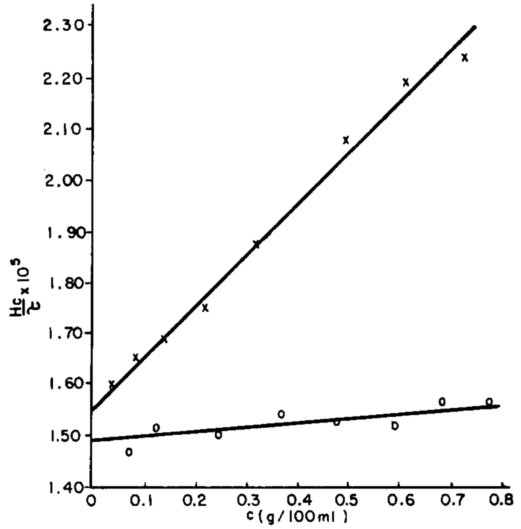

Fig. 4 *Hc/τ versus c* data for G-actin in H_2O–10^{-4} *M* ATP (×) and in phosphate buffered KI of pH 7 and ionic strength 0.6(○).

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* These were kindly performed by M. M. MARSH of the Lilly Research Laboratories, Indianapolis, Ind. (U.S.A.).

Light scattering

The values of Hc/τ as obtained for G-actin in the solvent media explored are plotted as a function of c in Fig. 4, where the best straight lines have been drawn by the method of least squares.

Turbidity measurements made at 45° and 135° to the incident beam indicated that no dissymmetry corrections were necessary and that the protein molecules were smaller in size than 0.1 × the wavelength of the light employed, which is approximately 3250 Å (4358/1.34). Depolarization was also negligible; hence no depolarization correction was required. The intercept of Hc/τ at zero concentration is the reciprocal of the molecular weight, M, according to the equation:

$$\frac{Hc}{\tau} = \frac{1}{\mathrm{M}} + 2Bc \qquad (2)$$

It is to be noted that in the water–ATP medium at pH 8 the second virial coefficient, B, has a high positive value, reflecting the repulsive forces between the charged protein molecules. On the other hand, as is usual in protein solutions at high ionic strengths, there is little concentration dependence of Hc/τ in the phosphate-buffered KI solvent (pH 7, ionic strength 0.6). The observed ordinate intercept values of 1.55 ($\pm$ 0.04)$\cdot 10^{-5}$ and 1.49 ($\pm$ 0.05)$\cdot 10^{-5}$ for G-actin in the water–ATP medium and in the KI buffer solution respectively are within experimental error; for this reason an average figure of 1.52 ($\pm$ 0.04)$\cdot 10^{-5}$ was chosen, corresponding to a molecular weight of 66,000 ± 2000. This is considerably higher than the value of 57,000 obtained by MOMMAERTS for material prepared by his technique[7]. However, the latter figure includes a depolarization correction which, in view of GEIDUSCHEK's work[16], is probably better omitted. If this is done, use of the intercept alone gives a value of M for G-actin of about 62,000, in good agreement with the value obtained in this study. Furthermore the virtual identity of the extrapolated ordinate intercept found for the same preparation in the 2 media employed suggests that the same limit of dissociation is attained for G-actin dissolved in either 0.5 M KI or H_2O–10^{-4} M ATP. The unusually high molecular weight of 80,000 found by STEINER *et al.*[3] for G-actin in 0.5 M KI must therefore be attributed to the impure preparations used rather than to the possibility of varying degrees of depolymerization in the different media employed.

One additional point emerges from this light scattering study: TSAO has inferred, on the basis of some of his osmotic pressure experiments where negative curvature was observed in the reduced osmotic pressure plot at higher protein dilutions, that higher protein concentrations favor a dimer and that dilution causes a disaggregation to the monomer[9]. The principle criticisms of these data have already been alluded to in the essay of GEIDUSCHEK AND HOLTZER[10]. In addition, the present study is at variance with the above inference, because the light scattering curves in both media do not exhibit any upsweep in the values of Hc/τ upon dilution. Since these measurements were extended to a protein concentration some 10 times lower than the osmotic pressure work, their greater reliability is unquestioned.

Viscosity

Results of viscosity measurements on a G-actin preparation in 0.5 M KI–0.1 M phosphate at pH 7 are shown in Fig. 5.

As in most proteins at high ionic strength, there is little concentration dependence of η_{sp}/c. The intrinsic viscosity obtained, 0.14 dl/g, is at variance with the value of 0.21 reported by TSAO for G-actin in 0.6 M KI + 10^{-4} M ATP. However, the latter figure has been questioned in view of the inconsistencies observed between TSAO's osmotic pressure and viscosity measurements[10]. Moreover, since in this study good reproducibility was obtained in the viscosity determinations made at each dilution, the figure of 0.14 dl/g will be considered the more reliable and will be used in all calculations involving $[\eta]$.

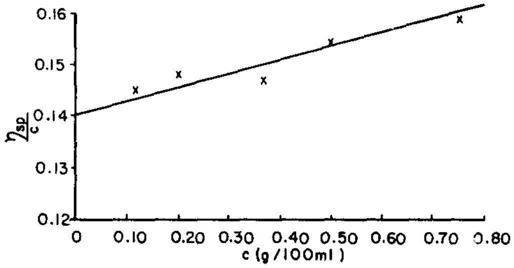

Fig. 5. Reduced specific viscosity of G-actin in phosphate-buffered KI (pH 7, ionic strength 0.6) as a function of protein concentration.

Partial specific volume

Fig. 6 shows a plot of m, the mass of solution held by the pycnometer to w_2, the weight fraction of solute for G-actin in phosphate-buffered KI (pH 7, ionic strength 0.6).

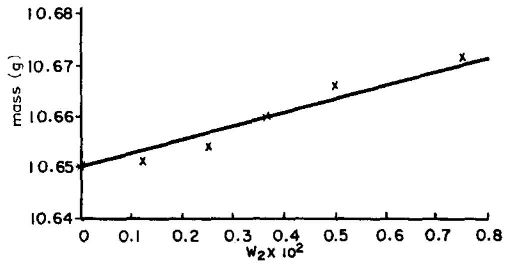

Fig. 6. Plot of m (mass of a given volume of solution of G-actin for which the solute weight fraction is w_2) against w_2.

The slope of the line, dm/dw_2, was drawn according to the method of least squares and when used in equation (1) gave a value of 0.716 ± 0.005 for the partial specific volume.

Molecular weight from sedimentation–viscosity

Another estimate of the molecular weight can be obtained from sedimentation and viscosity data by use of the SCHERAGA-MANDELKERN equation[17]:

$$\mathrm{M} = \left[\frac{Ns[\eta]^{1/3}\eta_0}{\beta(1-\bar{v}\rho)}\right]^{3/2} \tag{3}$$

In this equation N is Avogadro's number, s is the measured sedimentation coefficient extrapolated to infinite dilution of the protein, $[\eta]$ the weight intrinsic viscosity, η_0 the viscosity of the solvent, $\bar{v}$ the partial specific volume of the protein in the solvent and ρ the solvent density. The parameter β depends on the hydrodynamic characteristics of the solvated protein molecule and is derivable from the viscosity increment, γ, of the protein which is given by the following relation:

$$\gamma = \frac{[\eta] \cdot 100}{\bar{v}} \tag{4}$$

A $[\eta]$ of 0.14 dl/g and a $\bar{v}$ value of 0.716 g/ml correspond to a viscosity increment of 19.5 for G-actin. Using this value in conjunction with the table provided by SCHACHMAN[18] leads to a β value of $2.5 \cdot 10^6$, assuming a prolate ellipsoid of revolution model, Substituting this value for β in equation (3) along with $3.02 \cdot 10^{-13}$ sec for s, 0.14 dl/g for $[\eta]$, 0.00975 poise for η_0, 0.716 ml/g for $\bar{v}$, 1.065 g/ml for ρ and solving for M, leads to a value of 61,600 ± 6000, in good agreement with the 66,000 ± 2000 value deduced by light scattering.

Optical rotation

The optical rotatory dispersion for G-actin in 0.5 *M* KI-0.1 M phosphate buffer of pH 7, shown in Fig. 7, was plotted using the simple one-term DRUDE equation: $[\alpha]_\lambda = k/(\lambda^2 - \lambda_0^2)$ and modified plot suggested by YANG AND DOTY[19]. Although the specific rotation at 550 mμ is within the range of globular proteins, *i.e.* $[\alpha]_{550} = -60°$, the dispersion constant, $\lambda_0 = 223$ mμ, derived from the slope of the plot of $\lambda^2[\alpha]$ *versus* $[\alpha]$, is unusually low, reflecting an apparent lack of any extensive helical configuration in the molecule. The percent helix was estimated from the λ_0 value, in accordance with the method described by YANG AND DOTY, as 8 %. This low helix value is no doubt correlated with the high proline content in the molecule (29 residues/molecular weight of 65,000 (see ref. 20)). If these were distributed statistically along the polypeptide chain, only short helical segments could exist, since the presence of a prolyl residue in the chain interrupts the continuity of the helix.

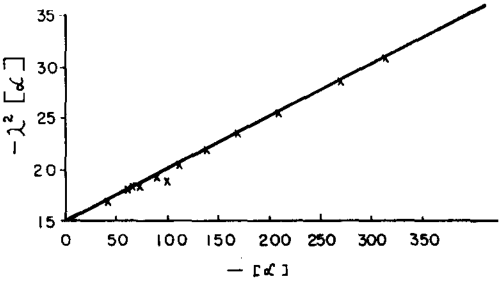

Fig. 7. A modified DRUDE plot of the rotatory dispersion of G-actin in 0.5 *M*, KI–0.1 *M* phosphate buffer of pH 7.

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