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SEDIMENTATION STUDIES ON POLYMERISED ACTIN SOLUTIONS

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SUMMARY

1. Polymerised actin solutions contain two components (F_1- and F_2-actin) giving solution boundaries and a gel component (gel-actin). The proportion of the latter increases on aging, and on exposing the solution to ultrasonic vibration, in a manner similar to that known to occur at nearly isoelectric pH values.

2. The extrapolated sedimentation coefficients of F_1- and F_2-actin are 40 S and 98 S, respectively. The value for F_1-actin is independent of centrifugal field.

3. Assuming F_1-actin to consist of elongated linear molecules, their diameter is calculated as 62 ± 5Å, for an assumed axial ratio of between 50 and 200. It is shown to be likely that F_2-actin is a side-to-side dimer of F_1-actin.

4. Gel-actin undergoes reversible depolymerisation, and combines with myosin to give an ATP-sensitive complex, similar to the "gel-component" of actomyosin systems.

5. A small amount of non-polymerised material was always present in polymerised solutions. Its sedimentation–concentration dependence was anomalous, the sedimentation coefficient increasing with concentration.

INTRODUCTION

The sedimentation of polymerised (F-) actin solutions has not been described in detail despite all the work performed upon this protein. PORTZEHL, SCHRAMM AND WEBER[1] and JOHNSON AND LANDOLT[2] gave values for the sedimentation coefficient of F-actin at particular concentrations. MOMMAERTS[3,4], having described what has since become the most widely accepted method for the purification of actin by cyclic polymerisation, gave sedimentation diagrams for F-actin solutions over a range of pH conditions. The sedimentation coefficient was measured at four concentrations, and varied between 33 S and 41 S: an extrapolated value for at zero concentration was not, however given as the peaks observed were invariably skewed, were not fully reproducible in character and an apparent dependence of s_{20} upon applied centrifugal field was noted. These latter effects were attributed to gel structure in the solution. The presence of "a component of a heterogeneous nature and considerable turbidity" was also noted by MOMMAERTS[4], who further suggested that low pH's caused a random type of aggregation.

In the present work, two polymerised components (F_1- and F_2-actin) have been observed which give solution boundaries (see later) in polymerised actin solutions,

together with a distinct, gel-forming component, gel-actin. It is shown to be possible to obtain an extrapolated sedimentation coefficient for the former components, independent (at least for F_1-actin) of significant gel effects. From this value, diameters of the F_1-actin molecule, and of its probable dimer (F_2-actin) are calculated. The gel-actin component is shown to be distinct from F_1- and F_2-actin, to undergo reversible depolymerisation and to combine with myosin to give an actomyosin gel component.

EXPERIMENTAL

Materials

All water used was thrice-distilled[5] to remove all trace metal ions. Reagents were of analytical grade. ATP was supplied (from B.D.H.) as the disodium salt, and neutralised before use.

Preparations of actin

Muscle powder was usually prepared by the method of STRAUB[6] and SZENT-GYÖRGYI[7] from rabbit-skeletal muscle. Several preparations were also performed by the method of TSAO AND BAILEY[8], but these gave rather poor yields of actin, and except where explicitly stated, were not used in the work reported.

The dried muscle powder was extracted in the cold with a solution of ATP and ascorbic acid (both 40 mg/l) in water, adjusted to pH 8. A ratio of 25 ml solution to 1 g muscle powder was employed, the mixture was gently stirred for 30 min, and then filtered through paper. The filtrate was clarified by centrifuging where necessary, and then polymerised by the addition of KCl and $MgSO_4$ to final concentrations of 0.1 M and 0.001 M respectively. Purification of actin was then performed by the method of MOMMAERTS[3], using two polymerisation cycles. Depolymerisation of the F-actin pellets at each stage was effected in a rocking dialyser[9]. Gel-actin (*q.v.*) was removed from the polymer solution by centrifuging at 20000 $\times$ *g* for 20 min. Myosin was prepared as previously described[5].

Sedimentation studies

Ultracentrifuge runs were performed on a Spinco Model E analytical ultracentrifuge equipped with diagonal schlieren optics and facilities for continuous temperature measurement and control. In the more recent runs, a phase plate has been substituted for the schlieren diaphragm. Details of temperature measurement and of the evaluation of sedimentation coefficients were as previously described[5].

Area measurement

The areas of schlieren peaks on the sedimentation diagrams were estimated by enlarging and tracing the diagrams, and counting squares. Areas were then corrected for radial dilution.

Concentration measurements

Protein concentrations have been measured either by the micro-Kjeldahl method, assuming a nitrogen content of 15.2 % (see ref. 4), or by a modified biuret technique[10].

RESULTS

After polymerisation, solutions of actin showed two main peaks in the ultracentrifuge (Fig. 1). Slow-moving material (<6 S), presumed to be non-polymerised, accounted for 10% or less of the total protein content. All these components gave linear log x *versus* t plots (x being the distance of the boundary from the centre of rotation at time t), from which sedimentation coefficients were evaluated in the normal manner. Such boundaries are termed solution boundaries, by contrast with gel boundaries, whose properties are mentioned below.

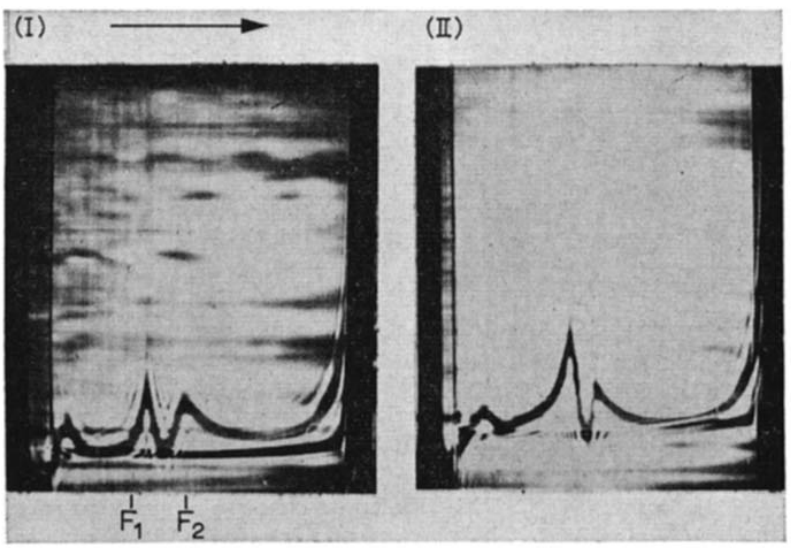

Fig. 1. Sedimentation diagrams at 29500 rev./min of polymerised actin in 0.1 M KCl, 0.001 M. $MgSO_4$

	Protein concentration (g/100 ml)	*Time since polymerisation began (h)*	*Time at 29500 rev./min (min)*
I	0.33	63	60
II	0.66	15	50

The dependence upon total protein concentration (at the boundary) of the sedimentation rate of the two major components is shown in Fig. 2a, from which values of 40 S and 98 S are yielded for the extrapolated sedimentation coefficients of these two components, designated as F_1-actin and F_2-actin, respectively. Over a two-fold range of centrifugal field, the sedimentation coefficient does not appear to vary with the field, at least in the case of F_1-actin. With F_2-actin, the scatter of points about the regression line is greater, and there is a tendency for runs performed at the higher fields to give higher s_{20} values. A sample of polymerised actin prepared from TSAO muscle powder, which contained almost all F_2-actin, showed a very highly skewed peak, and a marked dependence of s_{20} upon field at a particular concentration (Fig. 2a).

The s *versus* concentration dependence of the slow-moving material was anomalous, the sedimentation coefficient increasing with concentration (Fig. 2b). An extrapolated value of $s_{20} = 3.0$ S was yielded.

It was found that during storage of polymerised solutions the F_1-actin peak decreased in size, whilst the F_2-actin peak increased proportionately (Fig. 3). The total area (F_1 and F_2) showed only a small decrease. It may be concluded that F_1-actin is a precursor of F_2-actin. Whilst no distinct additional peaks other than F_1 and F_2

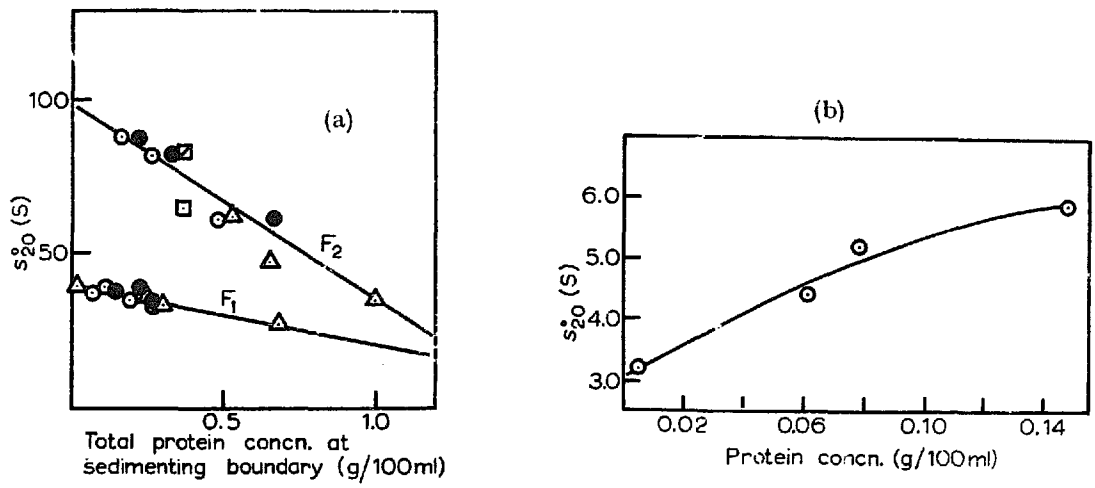

Fig. 2. Sedimentation coefficients of the components of polymerised actin, at various concentrations, in 0.1 M KCl, 0.001 M. $MgSO_4$. (a) Fast-moving components (F_1 and F_2). Field (approx.) in (rev./sec)/: ●, $5.59 \cdot 10^5$; ○, $3.02 \cdot 10^5$; △, $2.43 \cdot 10^5$ for STRAUB-actin. Square with line $7.90 \cdot 10^5$; ⊡, $3.02 \cdot 10^5$ for TSAO-actin. (b) The slow-moving component. Field (approx.): $5.59 \cdot 10^5$ (rev./sec)^2 for STRAUB-actin.

have been seen in actin solutions after polymerisation, it has been noticed that if a solution is centrifuged during the initial period of several hours following the addition of salt, then a quantity of poorly defined material sedimenting at a rate intermediate between that of unpolymerised and F_1-actin can often be observed (Fig. 1). This suggests that formation of F_1-actin occurs slowly through a range of intermediate polymers.

The F_2-actin peak was always extended on the solution side, indicating the presence of some heavier material. After standing, polymerised actin solutions at room temperature became markedly turbid and opalescent. Similar solutions were found to result if the actin was prepared from aged muscle powder (stored 6–18 months

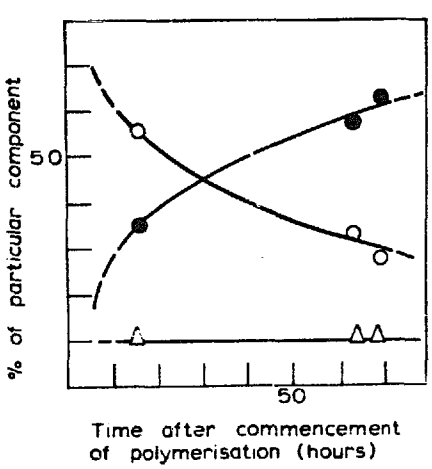

Fig. 3. The percentage of the components of polymerised actin solutions at various times after commencement of polymerisation. △—△, slow component; ○—○, F_1-actin; ●--●, F_2-actin.

at —20°), or if F-actin pellets obtained during purification were dispersed with the aid of ultrasonic vibration (40 kc/sec). Investigation in the ultracentrifuge showed that in all these cases the heavy material responsible for the turbidity formed a distinct gel interface, which was rapidly compressed to the bottom of the cell without the formation of a complete schlieren solution peak (Fig. 4a). We have designated this gel-forming material "gel-actin", and the associated boundaries as gel boundaries.

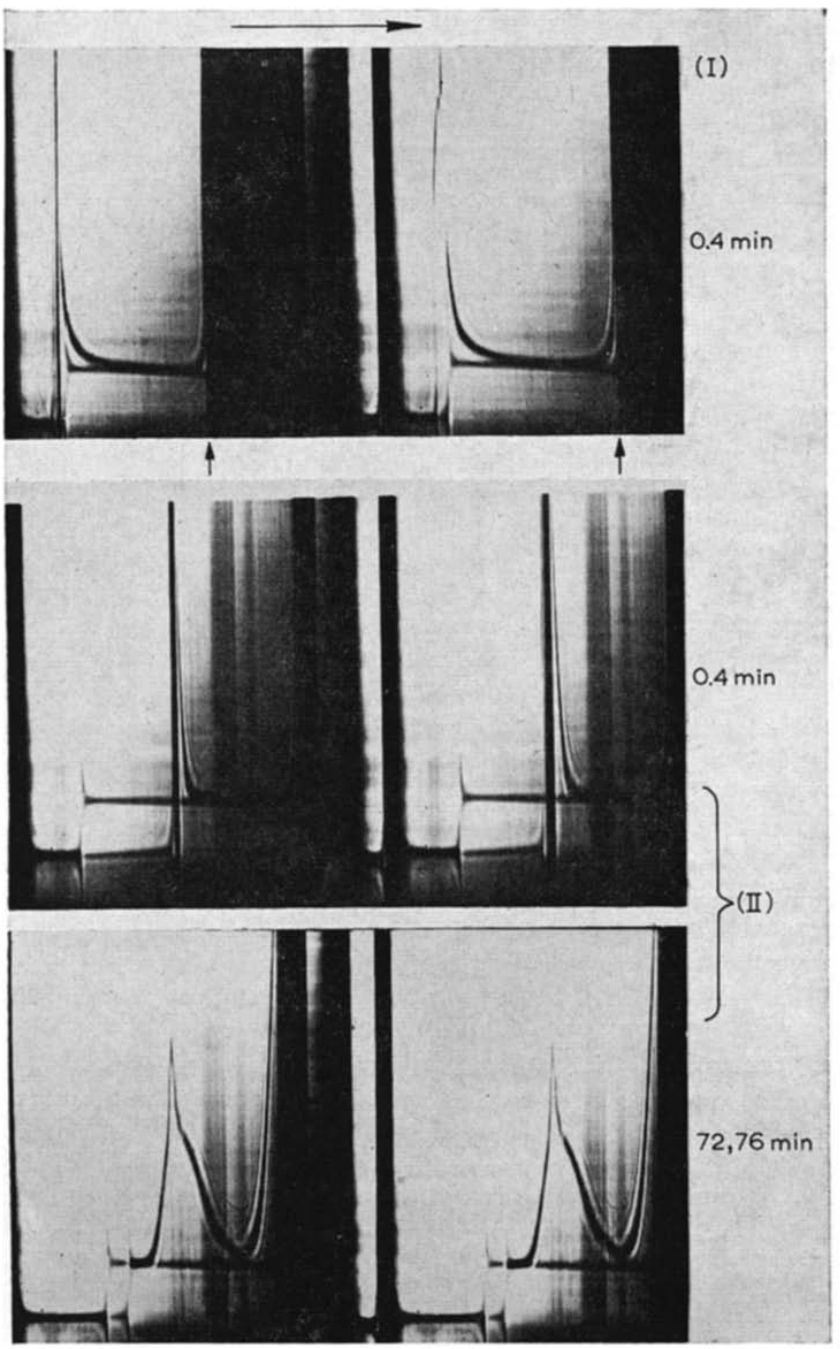

Fig. 4. Sedimentation diagrams of gel-actin. (I) Polymerised gel-actin, in 0.1 M KCl, 0.001 M $MgSO_4$; showing a heavy gel boundary (arrowed). (II) Depolymerised gel-actin, in 10^{-4} M ATP (pH 8); showing no gel boundary (first two pictures) but only slow-moving components (last two pictures). Times after reaching 42040 rev./min are given in minutes. Protein concentration 0.75 g/100 ml.

For such boundaries a plot of log x *versus* t deviates far from linearity (Fig. 5), a property which is possessed by gel systems (*e.g.* agar, gelatin) in general[11]. Like the F-actin which gives solutions peaks, it can be reversibly depolymerised by dialysis against dilute ATP solution: and the resulting depolymerised solution, though still opalescent, shows no fast-moving material (either gel or in solution) in the ultracentrifuge (Fig. 4b).

On adding myosin to gel-actin (at $I = 0.6$) a very turbid solution results. By following the packing of the gel in the ultracentrifuge, and by analysis of the protein content of the supernatant and gel component in parallel experiments, it has been

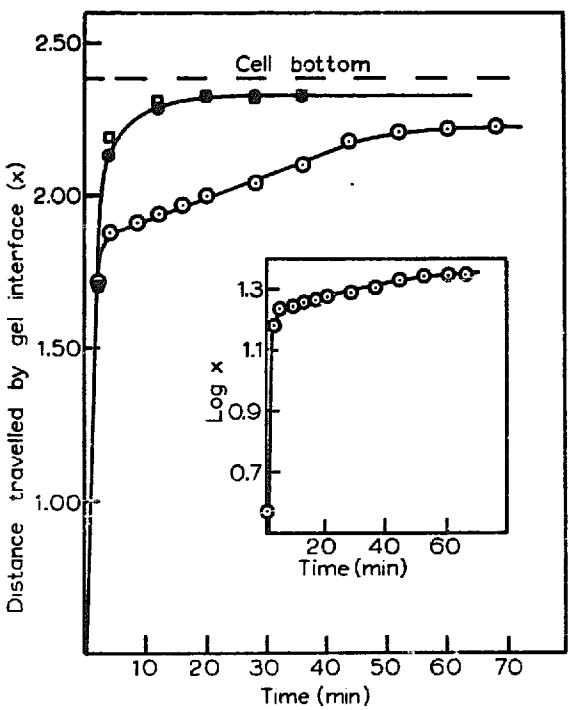

Fig. 5. The packing of the gel component in gel-actin and gel-actomyosin. ●—●, gel-actin; ○—○, gel-actin + myosin; □—□, gel-actin + myosin + ATP. All conditions as in Table I. Inset: log x *versus* t plot for the gel actomyosin sample, showing a marked deviation from linearity. Speed, 40040 rev./min.

TABLE I

THE AMOUNT OF PROTEIN PRESENT AS GEL-COMPONENT IN POLYMERISED ACTIN SOLUTIONS, TREATED WITH ULTRASONIC VIBRATION TO FORM GEL-ACTIN AND ALLOWED TO REACT WITH MYOSIN

All solutions in KCl–phosphate at $I = 0.6$ (pH 6.7). Solutions were centrifuged at 22000 × g for 15 min, a sample of the supernatant then removed and the protein content measured. Duplicate solutions were investigated in the analytical ultracentrifuge, and the packed volume of gel component estimated (Fig. 4).

Protein in 1 ml of original solution (mg)		*ATP (μmoles in 1 ml)*	*Protein recovered per millilitre original solution*		*Volume of gel pad (ml/l original solution)*
Polymerised actin	*Myosin*		*Supernatant*	*Gel pad*	
2.50	—	—	0.80	1.70	29
2.50	3.99	—	3.29	3.20	67
2.50	3.99	2.5	4.60	1.89	31

found that myosin combines with the gel-actin to give a gel-actomyosin, and that this combination is reversed by addition of ATP (Fig. 5 and Table I).

DISCUSSION

The F_1-actin peak corresponds in sedimentation rate to the component seen in F-actin sedimentation diagrams by MOMMAERTS[4]; but, after polymerisation is complete, it is much more symmetrical in shape, and its sedimentation rate does not depend significantly upon centrifugal field. These last two observations may well be related, if the skewed form of the peaks observed by MOMMAERTS (and of some of the F_2-actin peaks in the present work) is due to the presence of gel structure in the solution. There is no doubt that a component strongly involved in a gel structure would not give rise to a normal solution peak, and a non-linear log x *versus* t plot would be obtained. The effect, however, of a very weak gel-forming tendency in a component on its sedimentation pattern and rate is more difficult to predict. The solute molecules sedimenting near the boundary would be constrained in their movement by the solute molecules in the bulk of the solution (*cf.* BUTLER AND JAMES[12]). In a radially inhomogeneous field, the boundary would tend to be accelerated (drag effect) due to the higher sedimentation rate of the bulk solute molecules. The aggregation involved in partial gelation would give a higher effective molecular weight but this effect could be opposed by an increased effective frictional constant. Properties such as the structural rigidity of the gel would also be involved. Except in the case where these various forces were exactly balanced, a net acceleration or deceleration of the boundary (accompanied probably by skewing) would result. In experiments with agar and gelatin[11], gel boundaries have been found to sediment more rapidly than the same material in solution at lower concentration, but this may not be true invariably.

TABLE II

THE DIAMETERS OF F_1- AND F_2-ACTIN, CALCULATED FOR VARIOUS ASSUMED VALUES OF THE AXIAL RATIO OF F_1-ACTIN

In calculating the F_2 diameter it is assumed that F_2-actin is a side-to-side dimer of F_1-actin.

Axial ratio assumed for F_1-actin	*Diameter F_1-actin ($2a_1$)*	*Diameter F_2-actin ($2a_2$)*	*Mass ratio $(a_2/a_1)^2$*
50	58.0	87.4	2.27
100	62.2	94.2	2.29
150	64.6	97.8	2.29
200	66.0	100.4	2.30

These considerations suggest that there may be a certain inaccuracy in the value of s_{20} given for F_2-actin, since peaks due to this component showed both skewness and some variation of sedimentation coefficient with centrifugal field. By the same criteria, the value of s_{20} given for F_1-actin can be taken as a genuine, extrapolated sedimentation coefficient, upon which hydrodynamic calculations may be based.

On the assumption that F_1-actin and F_2-actin are both elongated and rigid linear polymers, their effective diameters may be calculated from their sedimentation coefficients, on the basis of approximate values for the axial ratio, using the relation[13]

$$s_{20} = 0.222 \frac{(1 - \bar{v}\rho)}{\bar{v}\eta} a^2 \ln \frac{b}{a} \qquad (1)$$

where b and a are respectively the major and minor semi-axes of an equivalent ellipsoid of revolution, η and ρ the solvent viscosity and density, and $\bar{v}$ the partial specific volume of the protein. Because of rheological effects, the intrinsic viscosity of F-actin is too ill-defined for a reasonable estimate of the axial ratio to be yielded by that technique[14]. On the basis of general properties, a range of values for the axial ratio has therefore been assumed, and the respective diameters calculated from Eqn. 1 (Table II). If $50 < b/a < 200$, then the diameter can be given as 62 ± 5 Å.

In the case of F_2-actin, the value of the sedimentation coefficient suggests that it may be a side-to-side dimer of F_1-actin. On this hypothesis, the effective axial ratio would decrease according to

$$(b/a)_{F_2} = \frac{1}{\sqrt{2}} (b/a)_{F_1} \qquad (2)$$

ignoring any possible effective volume changes in dimerisation. Using $(b/a)_{F_2}$ from Eqn. 2, the diameter of F_2-actin has been calculated as before, for the range of assumed values of the axial ratio of F_1-actin given in Table II, yielding a value of 94 ± 7 Å. The mass ratio per unit length of F_2 to that for F_1 is 2.29, which is independent of assumed axial ratio, and is in reasonable accord with the dimerisation hypothesis, bearing in mind the experimental and theoretical uncertainties involved.

The diameter here calculated for F_1-actin may be compared with that recently derived by HANSON AND LOWY[18] in their electron microscope study of F-actin fibrils. They conclude that the single fibril, of maximum diameter 80 Å, is equivalent to a close-packed cylinder of diameter about 60 Å; in excellent agreement with our estimate for F_1-actin. HANSON AND LOWY further observed that many of their primary fibrils were aggregated side-to-side, in agreement with the explanation here advanced for the presence in solution of F_2-actin.

If, then, it is accepted that the elongated F_1-actin molecules can associate side-to-side to form a dimer (F_2-actin), then it is not unreasonable to suppose that by means of more extensive (and perhaps less ordered) cross-linking, a definite, stable gel structure could arise in solution, giving rise to gel-actin as suggested by MOMMAERTS[4]. In this latter state, the G-actin monomers must be either slightly denatured or else to some extent irreversibly associated, since the depolymerised solution on re-polymerisation gives rise to gel-actin directly, and cannot apparently form linear F-actin. It is unlikely, however, that major structural changes are involved, since two main distinctive properties of actin, that is, of reversibly polymerising, and of combining in the polymerised state with myosin to form an ATP-sensitive complex, are retained.

It is clear that since gel-actin does depolymerise, the procedure described by MOMMAERTS for removal of heavy material (centrifuging the depolymerised solution) must fail to remove this component; and in fact, MOMMAERTS' final purified preparations clearly contained a very considerable proportion of gel-actin. As described earlier (p. 369), gel-actin may be removed by differential centrifugation of the polymerised solutions; but, even in the cold, further amounts are slowly formed, and a total protein determination must always over-estimate the quantity of F_1- and F_2-actin present.

The combination of gel-actin with myosin provides an explanation for the gel component observed in actomyosin systems. The detailed relation of the components of actin solutions to those of natural and synthetic actomyosin solutions is discussed elsewhere[15,16].

The extrapolated sedimentation coefficient obtained for the unpolymerised material (3.0 S) is similar to that obtained for G-actin by MOMMAERTS[4] in ATP solution (2.8–3.1 S) and by KAY[17] in potassium iodide solution (3.02 S). The increase in s_{20} with increasing concentration suggests that the material undergoes reversible polymerisation in salt solution, possibly to dimers or trimers. It is uncertain whether it is inactive actin, contaminating protein, or active, polymerisable actin in a slowly established equilibrium with the F.-actin components.

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