THE MOLECULAR DIMENSIONS AND THE MONOMER-DIMER TRANSFORMATION OF ACTIN

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The determination of the molecular size and configuration of actin is of great importance to our understanding of the quantitative aspects of such phenomena as the "polymerisation" of globular protein molecules, the formation of the actomyosin complex and thixotropy. Owing to the sensitivity of the degree of aggregation to neutral salts, and the difficulties involved in the purification of this protein, the application of physico-chemical methods to the study of molecular dimensions has not proved to be simple and straightforward. The globular nature of the protein has generally been accepted on the basis of low relative viscosity of G-actin solutions (STRAUB[1], see also H. H. WEBER[2]); and the molecular size of 68,000, 70,000 or 76,000 (FEUER, MOLNÁR, PETTKÓ AND STRAUB[3]; A. SZENT-GYÖRGYI[4,5,6]) was based on a highly inaccurate tryptophan content (PERRY[7]; see also[12]). SNELLMAN, ERDÖS AND TENOW[8] and SNELLMAN AND GELOTTE[9] have reported the sedimentation constants of actin depolymerised with 0.5 *M* KI, calgon (hexametaphosphate), 0.1 *M* KSCN, glycine buffer at pH 9, as well as that of the inactive component in active actin, the values varying from 2.7 to 4.0. The results do not seem to be definite, but these investigators, using the sedimentation and diffusion constants in hexametaphosphate solution ($S = 3.2$ and $D = 2.5 \cdot 10^{-7}$ respectively) arrived at a molecular weight of 150,000. JOHNSON AND LANDOLT[10], applying light-scattering technique to impure samples of actin, gave an order of magnitude of 120,000, while STEINER, LAKI AND SPICER[11], applying the same technique to actin in 0.5 *M* KI, arrived at a value of 80,000.

The present investigation was carried out with a view to defining the molecular dimensions of actin. For this purpose, an active, electrophoretically homogeneous preparation of actin (TSAO AND BAILEY[12]) was studied, using osmotic pressure, viscosity and fluorescence-polarisation techniques (G. WEBER[13,14,15]). It was found that molecules of actin exist in a monomeric form of particle weight 70,000 and a dimeric form of 140,000. The two monomers appear to be linked through the nucleotide prosthetic group and a divalent metal.

EXPERIMENTAL

Preparation of actin. The protein was prepared from rabbit skeletal muscle by the method described in a previous paper[12]. The preparation was electrophoretically homogeneous at pH 2 and 10.

Methods. Protein concentration, *C*, of solutions of actin were determined by a micro-Kjeldahl method (CHIBNALL, REES AND WILLIAMS[16]) and expressed as g protein/100 ml solution. Samples of

References p. 235.

actin, dialysed free of salt, freeze-dried and then dried at 120°, gave a value of 16.1 % for the total N. Hydration was determined by the method of ADAIR AND ROBINSON[17] which gave a value of 0.385 g water/g protein. Osmotic pressure measurements were carried out at 0° in the toluene osmometers of ADAIR[18]. In all the experiments, collodion membranes were used (ADAIR[19]). Viscosity measurements were made in the TSUDA[20] horizontal viscometer. The capillary had the following characteristics: radius 0.022 cm, length 28.60 cm, flow time for 1.128 ml of water at a pressure head of 21.80 cm tetrachloroethylene 100.0 sec.

Evaluation of molecular weight and shape. For the calculation of particle weight from osmotic pressure data, the ratio of pressure P (in mm Hg) and concentration C was plotted against C and the limiting value at zero concentration was employed (ADAIR AND ROBINSON[21]). The axial ratio was assessed by the use of SIMHA's equation[22] from the value of intrinsic viscosity $[\eta]$ which is lim $(\eta sp/C)$, where ηsp is $\eta r - 1$, ηr being the relative viscosity η/η°. In correcting for hydration (ONCLEY[23]) a probable value for the partial specific volume of actin, 0.74, was assumed.

Fluorescence-polarisation measurements. Actin was coupled with 1-dimethylaminonaphthalene-5-sulphonyl chloride at 0° in the presence of 10 % acetone and 10^{-4} M sodium ATP. The product was allowed to stand in the ice chest overnight and the excess dye dialysed away against repeated changes of $M/10$ KCl and 10^{-4} M ATP. After coupling, the properties of actin, such as solubility, "polymerisability", ability to form actomyosin etc., undergo little change. Before each experiment, the conjugate was dialysed against the required solvent for two days. The polarisation of the fluorescent radiation of the conjugate was measured (G. WEBER[13,14,15]). The reciprocal of the polarisation $1/p$ was plotted against the temperature-viscosity ratio T/η, and the rotational relaxation time ϱh evaluated from the slope S and intercept $1/p^\circ$ [13,14,15].

RESULTS

Osmotic pressure studies of depolymerised actin. The depolymerising action of KI (> 0.5 M), first observed by STRAUB[1], has now been well established (see for example, GUBA[24], SNELLMAN *et al.*[8,9], STEINER *et al.*[11]). The depolymerisation is generally accompanied by inactivation, but is entirely reversible if ATP is present (A. G. SZENT-GYÖRGYI[25]). For osmotic pressure measurements, actin was dialysed against repeated changes of KI solution (0.6 M KI, 0.05 M KH_2PO_4, 0.006 M $Na_2S_2O_3$, titrated with NaOH to pH 6.5) before putting into the collodion membranes. Equilibration was usually reached overnight, but readings were taken over quite extended periods to ensure that the solutions were truly equilibrated and that there were no further changes in the state of aggregation of actin. The results are given in Fig. 1. The different curves refer to different preparations. The reason for the difference in the osmotic behaviour from sample to sample is not yet entirely clear, although all preparations were electrophoretically homogeneous, and appeared to be identical in other properties. Curves (*a*), (*b*) and (*c*) tend to the same value of $(P/C) = 2.32$ which represents a particle weight 74,000; while in (*d*), in the concentration range 1–2 % the values of P/C would indicate a particle weight of 140,000. Considered in conjunction with other evidence to be presented later in the paper, there seem to be a monomeric as well as a dimeric state, and in

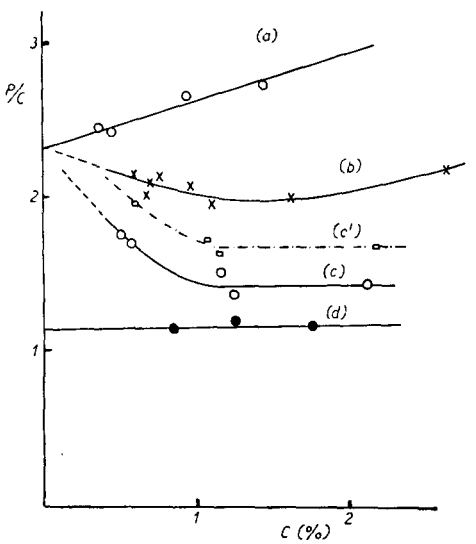

Fig. 1. Osmotic pressure of depolymerised actin at 0° C.
——— in 0.6 M KI, 0.05 M KH_2PO_4, 0.006 M $Na_2S_2O_3$, pH adjusted to 6.5; (a), (b), (c) and (d) for 4 different samples; (b) in presence of 10^{-4} M ATP.
–·–·– in 0.104 M boric acid, 0.1 N NaOH, 0.1 M KCl, pH 10.8.

References p. 235.

the cases of (*b*) and (*c*) the dissociation took place on dilution. In the series of experiments represented by (*b*), 10^{-4} *M* ATP was present in the system. Since ATP may be the prosthetic group of G-actin (STRAUB AND FEUER[26], LAKI, BOWEN AND SPICER[27]; see also [28]), it would seem that the transformation is in some way related to ATP, an inference borne out by the fluorescence-polarisation experiments.

An osmotic pressure curve similar to Fig. 1 (*c*) was also obtained in a borate buffer, pH 10.8, and is included in Fig. 1 as (*c'*).

Fluorescence-polarisation studies of F-actin depolymerised in various media. Labelled actin, preserved as a thixotropic F-actin gel, was dialysed against 10^{-4} *M* ATP at pH 8 until it was converted (reversibly) into globular actin. In the absence of ATP, actin was inactivated. For the complete depolymerisation of actin for the studies of particle dimensions, 0.6 *M* KI was used. In addition, the conventional solvents which are

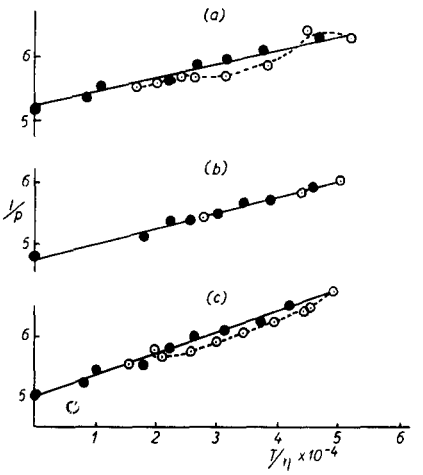

Fig. 2. Fluorescence-polarisation of depolymerised actin (sample 1)
(a) in water at pH 7,
(b) in $5 \cdot 10^{-4}$ *M* $MgSO_4$, pH 7,
(c) in 10^{-4} *M* Na ATP, pH 8.

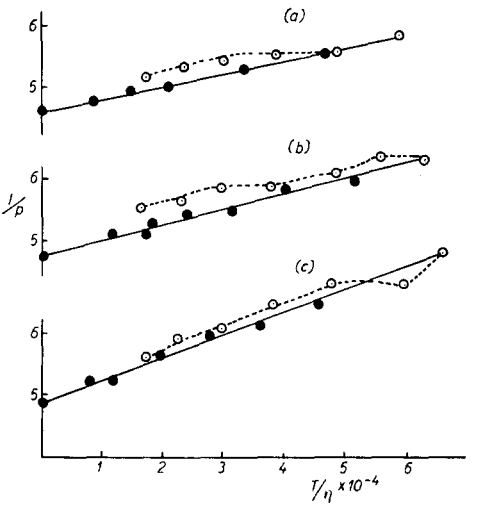

Fig. 3. Fluorescence-polarisation of depolymerised actin (sample 2)
(a) in 0.1 *M* glycine, 0.1 *M* KCl, titrated with HCl to pH 2.2,
(b) in 0.12 *M* boric acid, 0.1 *N* NaOH, pH 10,
(c) in 0.1 *M* boric acid, 0.1 *N* NaOH, pH 11.

generally used for the fission of H-bonds (dilute acid, alkali and concentrated urea solution) were also employed. The effect of sodium versenate and polyphosphates such as pyrophosphate, hexametaphosphate, ADP and ATP were examined with a view to elucidation of the role of the prosthetic group and of divalent metals in the fundamental units of actin. In all these experiments, a 0.2–0.3% solution was used.

The results are given in Figs. 2, 3, 4 and 5, where the open circles denote the polarisation when actin was gradually warmed up to 45° and the closed circles that on cooling down. The heating curves sometimes showed a kink to a large or small extent which was furthermore different from one sample to another under identical conditions, whilst the cooling curves were usually straight lines and entirely reproducible; if now the samples were heated up again, the points followed the cooling curves. It would seem that some loose aggregates in solution are disaggregated when the temperature rises;

References p. 235.

and for the evaluation of the rotational relaxation times of the depolymerised units, only the slopes and intercepts of the cooling curves were considered. These were obtained from the experimental points by the method of least squares. The intercepts were also obtained experimentally by measuring the polarisation in 60% sucrose (G. WEBER[15]), and the agreement is in general very good. The results are given in Table I. The increase in the $1/p$ value at higher temperatures and in the intercept in concentrated urea solution may be interpreted as an increase in the rotational freedom of the coupled molecule due to decrease in the binding power of the protein (G. WEBER[14]).

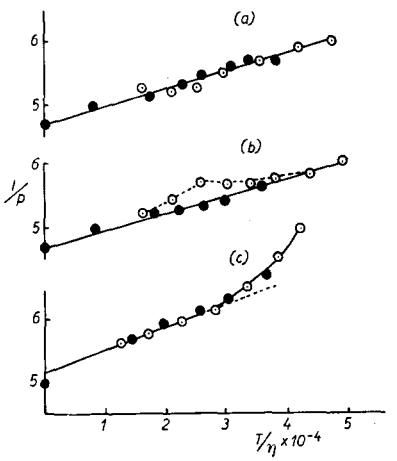

Fig. 4. Fluorescence-polarisation of depolymerised actin (sample 3)
(a) in 0.6 *M* KI, 10^{-4} *M* ATP,
(b) in 10^{-4} *M* $Na_4P_2O_7$, pH 8.
(c) in 6 *M* urea.

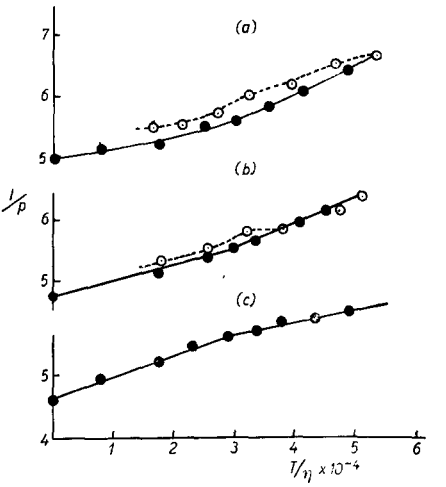

Fig. 5. Fluorescence-polarisation of depolymerised actin (sample 4), monomer-dimer transformation with temperature.
(a) in 10^{-3} *M* sodium versenate, pH 8.
(b) in 10^{-4} *M* sodium ADP, pH 8.
(c) in 10^{-4} *M* $(NaPO_3)_6$, pH 8.

TABLE I

FLUORESCENCE-POLARISATION OF DEPOLYMERISED ACTIN (1)*

Condition	*Solvent*	$\left(\frac{I}{P_0}\right)_{extr.}$	$\left(\frac{I}{P_0}\right)_{obs.}$	*Slope* · 10^5	$\varrho h \cdot 10^8$ *sec.* *(25° C)*
pH 7	Water	5.18	5.18	2.30	30.5
pH 2.2	0.1 *M* glycine, 0.1 *M* KCl, titrated with HCl	4.60	4.63	2.02	31.0
pH 10	0.12 *M* boric acid, 0.1 *N* NaOH	4.81	4.81	2.23	29.3
Mg^{+2}	$5 \cdot 10^{-4}$ *M* $MgSO_4$	4.73	4.77	2.55	25.3
Pyroph.	10^{-4} *M* $Na_4P_2O_7$	4.70	4.69	2.65	24.1
KI	0.6 *M* KI + 10^{-4} *M* ATP	4.69	4.66	2.83	22.6
pH 11	0.1 *M* boric acid, 0.1 *N* NaOH	4.88	4.88	3.50	20.2
Urea	6 *M* urea	5.11	5.00	3.57	19.3
ATP	10^{-4} *M* sodium ATP, pH 8	5.00	5.08	3.50	19.3

* For the meaning of the symbols, see text.

In versenate, ADP and hexametaphosphate, the polarisation showed a distinct change of slope with temperature. In the case of hexametaphosphate the slope dim-

References p. 235.

inishes as the temperature increases, indicating association, while in the other two cases, the reverse is true. The relaxation times of the associated and dissociated products are given in Table II.

TABLE II

FLUORESCENCE-POLARISATION OF DEPOLYMERISED ACTIN (2)*

Monomer-dimer transformation

Condition	*Solvent*	$\left(\frac{1}{P_o}\right)_{extr.}$	$\left(\frac{1}{P_o}\right)_{obs.}$	*Slope* $\cdot 10^5$	$\varrho h \cdot 10^8$ *sec.* (25° C)
Versenate < 21° C	10^{-3} *M* versenate, pH 8	4.93	4.96	2.12	31.5
Versenate > 21° C	10^{-3} *M* versenate, pH 8			4.84	13.8
ADP, < 21° C	10^{-4} *M* sodium ADP, pH 8	4.82	4.79	2.45	26.7
ADP, > 21° C	10^{-4} *M* sodium ADP, pH 8			3.94	16.6
Calgon, < 21° C	10^{-4} *M* $(NaPO_3)_6$, pH 8	4.67	4.67	3.35	18.9
Calgon, > 21° C	10^{-4} *M* $(NaPO_3)_6$, pH 8			2.10	30.2

* For the meaning of the symbols, see text. Absolute values of ϱh been have calculated using $1.4 \cdot 10^{-8}$ sec for the lifetime of the excited state.

The lowest relaxation times are given by actin in versenate and in ADP at temperatures > 21°, *i.e.* $13.8 \cdot 10^{-8}$ sec, respectively (average $15.2 \cdot 10^{-8}$); while the average value for the highest relaxation times in versenate (< 21°), calgon (> 21°), water, glycine buffer at pH 2 and borate buffer at pH 10 (31.5, 30.2, 30.5, 31.0 and $29.3 \cdot 10^{-8}$ sec, respectively) is $30.5 \cdot 10^{-8}$ sec. The change in relaxation time may arise either from changes in size or shape or both. Anticipating the results of axial asymmetry from viscosity measurements to be discussed presently, and making allowance for the asymmetry factor in relaxation times (G. WEBER[13,15]), the molecular volumes V and molecular weights M are evaluated and given in Table III.

TABLE III

PARTICLE WEIGHT OF MONOMERIC AND DIMERIC ACTIN FROM FLUORESCENCE-POLARISATION MEASUREMENTS

Condition	$\varrho h \cdot 10^8$ *sec.*	*a/b* *	$\varrho o/\varrho h$ **	$\varrho o \cdot 10^8$ *sec.*	*V*	*d* ***	*M*	*Note*
Versenate > 21° C	13.8	~12	0.41	5.66	52,000	1.35	71,000	
						1.23	64,000	
ADP > 21° C	16.6	~12	0.41	6.80	62,700	1.35	85,000	
						1.23	77,000	
Average for dimer	30.5	~24	0.375	11.44	106,000	1.35	143,000	Calculated for end to end
						1.23	130,000	
		~6	0.44	13.4	124,000	1.35	167,000	Calculated for side by side
						1.23	152,000	

* a/b axial ratio.

** ϱo — the relaxation time of a sphere of volume equal to that of the actin particle; $\varrho o/\varrho h$ depending only on shape (G. WEBER[13,15]).

*** d, assumed density of protein (partial specific volume 0.74). The anhydrous density is given first, then that of the protein with 39% hydration.

References p. 235.

The results confirm the conclusion drawn from osmotic pressure measurements regarding the presence of a monomeric and a dimeric state for actin, and are highly suggestive that the units of the dimer may be held by a divalent metal (Mg^{+2} or Ca^{+2}) through the nucleotide prosthetic group. ATP, ADP, pyrophosphate and polyphosphates compete for the metal and cause dissociation, very high pH dissociates by virtue of the electrostatic repulsion between the two monomers, while versenate removes the divalent metal through coordination, leaving actin entirely in the monomeric form.

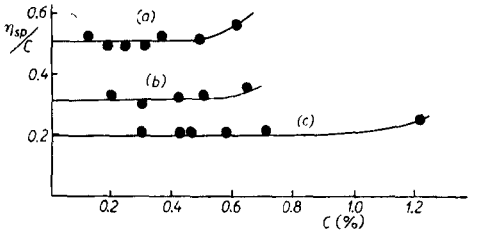

Fig. 6. Viscosity of actin at 20° C.
(a) Actin depolymerised in 0.1 *M* boric acid, 0.063 *N* NaOH, 0.1 *M* NaCl, pH 9.5, for 40 days at 0° C.
(b) Aqueous solution of G-actin prepared according to FEUER *et al.*[3].
(c) Actin depolymerised in 0.6 *M* KI, 0.05 *M* KH_2PO_4, 0.006 *M* $Na_2S_2O_3$, 10^{-4} *M* sodium ATP, pH adjusted to 6.5 with NaOH.

Viscosity studies of depolymerised actin. From the earlier viscosity data of STRAUB[1] for G-actin, which gave a specific viscosity 0.03 for a solution of concentration 3 mg/ml, it can be calculated that the intrinsic viscosity of actin is of the order of 0.03/0.3 = 0.1, indicating particles of considerable asymmetry. A remeasurement of an aqueous solution of actin, prepared according to FEUER *et al.*[3] gave a somewhat higher value, 0.3 (Fig. 6). Since both the electroviscous effect and the presence of impurities contribute to the total viscosity, the exact magnitude of the axial asymmetry of actin cannot be ascertained from these data.

For a more precise determination of the shape of actin, the viscosity behaviour of the purified actin dispersed in 0.6 *M* KI (+ 10^{-4} *M* ATP) was examined. For this purpose, the same solution was used in which osmotic pressure data (Fig. 1 (*b*)) indicated actin in the monomeric form. After osmotic equilibrium was reached, a portion of the solution was analysed for protein concentration while another was used for viscosity measurements. The results are again given in Fig. 6 (Curve (*c*)). Making allowance for the contribution of hydration (0.39 g/g protein) to the asymmetry factor and assuming a probable value 0.74 for the partial specific volume of actin, the value of intrinsic viscosity, 0.21, indicates for a prolate ellipsoid of revolution an axial ratio ~ 12. The approximate dimensions for monomeric actin are therefore ~ 290 A long and ~ 24 A wide.

The axial asymmetry of the dimeric actin is much less certain. An actin solution was dialysed against a borate buffer of pH 9.5 (0.1 *M* boric acid, 0.063 *N* NaOH, 0.1 *M* NaCl) for 40 days at 0°. Although this completely inactivated actin solution appeared fluid like freshly extracted G-actin, the intrinsic viscosity was in fact near 0.5 (Fig. 6*a*), indicating an approximate axial ratio of ~ 22.

Fluorescence-polarisation measurements have demonstrated that when the temperature was raised actin changes from its dimeric to monomeric state in the presence of versenate, and that the transition occurred at about 21°. This change was also reflected

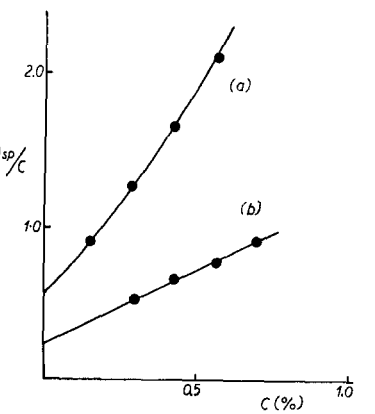

Fig. 7. Viscosity of actin in versenate: 10^{-3} *M* sodium versenate, 0.05 *M* Na_2HPO_4, pH 9.
(a) at 20.0° C,
(b) at 33.5° C.

References p. 235.

in the viscosity behaviour. Measurements carried out at 20° and 33.5° are given in Fig. 7. The values of intrinsic viscosity for the dimer (0.52) and for the monomer (0.24) agree well with those obtained in alkali and 0.6 *M* KI (+ ATP) respectively. Although the possibilities for the change of hydration of the protein and for the presence of fragments of higher degree of association have yet to be examined more rigorously, this agreement may not be altogether fortuitous. The results seem to suggest that the two monomers are linked end to end in the formation of the dimer.

DISCUSSION

The "polymerisation" of actin with neutral salts is a fast reaction at pH 6–8 whereas the reverse process is extremely slow and accompanied usually by inactivation. In the presence of ATP, the "polymerisation-depolymerisation" can be rendered reversible (A. G. SZENT-GYÖRGYI[25], MOMMAERTS[29]) but the rate of the "depolymerisation" process still remains comparatively slow. This difference in rates and the need of the presence of ATP and Mg^{++} in the system seem to indicate that specific interactions of some kind, other than purely electrostatic effects, are involved.

While the present investigation is not concerned with the mechanism of "polymerisation–depolymerisation", it has nevertheless been possible to demonstrate in the final depolymerised actin system a monomer-dimer transformation.

The demonstration of this change in the fundamental units of actin may help to resolve the differences between the values of molecular weight obtained by ultracentrifugal and analytical ultrafiltration methods. Depending upon the conditions of analysis, either one or both of these values or an intermediate value can be obtained. The component with $S = 3.7$ has been invoked to give the molecular weight of actin as both 150,000 and 70,000 (SNELLMAN *et al.*[8,9]; H. H. WEBER[28,30]). In the latter references, however, the diffusion constants have not been mentioned. It is of interest that SNELLMAN *et al.* observed a slower component with $S = 1.7$ when actin was dispersed in hexametaphosphate. If the possibility of impurities can be excluded, this component may be the monomer and the variation of sedimentation constants from preparation to preparation and from one solvent to another and the broad diffused peaks of depolymerised actin may have their origin in this transformation.

The idea that divalent ions such as Mg^{+2} or Ca^{+2} may form an integral part of protein molecules is gaining more and more ground in many fields of biochemistry, especially in the studies of enzyme-substrate interactions (see for example CAVALINI[31]). In the conventional preparations of actin, Ca and Mg exist to the extent of 0.22% and $\sim$ 0.05% respectively (FEUER *et al.*[3]). Straub and his co-workers have also demonstrated the indispensibility of Mg^{+2} in the native properties of actin. That these ions may be held by the nucleotide prosthetic groups is all the more likely in view of the work of NEUBERG and his associates[32,33] who demonstrated the remarkable properties of ATP and inorganic pyro-, meta- and polyphosphates, nucleic acids and nucleotides to form stable complexes with metal ions. The fluorescence-polarisation results can best be explained if one pictures the two monomers linked together by a divalent ion such as Ca^{+2} or Mg^{+2} through the nucleotide prosthetic groups. This can probably be expressed in more quantitative terms after analysis are made on the Ca, Mg and nucleotide contents of purified active actin.

In the present investigation, the chief attention was directed at obtaining infor-

References p. 235.

mation on the static structure of the actin molecule, and less concerned with the time factor of the monomer-dimer transformation, and with the extent of reversibility. Further complications may be expected to occur when actin "polymerises" or when it is involved in complex formation with myosin. Some aspects of these problems will be reported in a subsequent communication[34].

ACKNOWLEDGEMENTS

The author is especially indebted to Dr K. BAILEY, F.R.S. and to Dr G. WEBER for their unfailing help; and to Imperial Chemical Industries for a grant made available to Dr K. BAILEY.

SUMMARY

1. The particle size and shape of a homogeneous preparation of actin, after depolymerisation in various media, have been investigated by osmotic pressure, fluorescence-polarisation and viscosity methods.

2. It has been possible to demonstrate in the final depolymerised actin system a monomer-dimer transformation.

3. The actin monomer has a molecular weight of 74,000 (by osmotic pressure) or 70,000 (average value by fluorescence-polarisation), rotational relaxation time at 25° C, $15 \cdot 10^{-8}$ sec and axial ratio ~ 12. The dimer has a particle weight of 140,000 by osmotic pressure and rotational relaxation time $30.5 \cdot 10^{-8}$ sec.

4. The two monomers appear to be linked by a divalent metal (Mg^{+2} or Ca^{+2}) through the nucleotide prosthetic group.

RÉSUMÉ

1. La taille et la forme des particules d'une préparation homogène d'actine ont été déterminées, après dépolymérisation dans divers milieux, par pression osmotique, fluorescence-polarisation, et viscosimétrie.

2. Il est possible de démontrer dans le système final actine dépolymérisée une transformation monomère-dimère.

3. L'actine monomère a un poids moléculaire de 74,000 (déterminé par pression osmotique) ou de 70,000 (valeur moyenne déterminée par fluorescence-polarisation), un temps de relaxation rotationnelle à 25° de $15 \cdot 10^{-8}$ sec et un rapport axial ~ 12. Le dimère se présente en particules de poids 140,000 (déterminé par pression osmotique) et de temps de relaxation rotationnelle $30.5 \cdot 10^{-8}$ sec.

4. Il semble que les deux monomères sont liés par un métal divalent (Mg^{+2} ou Ca^{+2}) par l'intermédiaire du groupement prosthétique nucléotidique.

ZUSAMMENFASSUNG

1. Es wurde die Teilchengrösse und die Form eines homogenen Actinpräparates nach der Depolymerisation in verschiedenen Medien mit Hilfe des osmotischen Druckes, der Fluoreszenzpolarisation und viskosimetrischen Methoden untersucht.

2. Es war möglich in dem depolymerisierten Actin-Endsystem eine Umwandlung des Monomeren in das Dimere und umgekehrt zu zeigen.

3. Das Actinmonomere hat ein Molekulargewicht von 74,000 (durch osmotische Druckmessungen bestimmt) oder 70,000 (der Durchschnittswert, den die Fluoreszenzpolarisation ergibt), eine Rotationsrelaxationszeit von $15 \cdot 10^{-8}$ sec bei 25° und ein Achsenverhältnis von ~ 12. Das Dimere ligt ein Teilchengewicht von 140,000 (nach osmotischen Druckmessungen) und eine Rotationsrelaxationszeit von $30.5 \cdot 10^{-8}$ sec.

4. Die beiden Monomeren scheinen durch ein zweiwertiges Metall (Mg^{+2} oder Ca^{+2}) über eine prosthetische Nukleotidgruppe verbunden zu sein.

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Received November 13th, 1952