THE INTERACTION OF ACTIN, MYOSIN AND ADENOSINE TRIPHOSPHATE

by

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It is now generally accepted that the system actin, myosin and adenosine triphosphate (ATP) contains the essential components of the contractile mechanism of muscle, but the nature of the interaction of the three, whether in solution, in gel form or *in situ*, is far from being elucidated. The present paper is concerned only with the physical changes in the state of aggregation of the isolated, purified protein components in solution during such interaction.

Since the interaction is especially pronounced when the actin partner is in the F-form, this will first be considered. The physical changes involved in the transformation of G-actin to F-actin by the addition of salt or by the lowering of pH have generally been regarded, on the basis of measurements of viscosity (STRAUB[1,2]), flow birefringence (STRAUB[1,2], BARBU AND JOLY[3]), sedimentation (PORTZEHL, SCHRAMM AND H. H. WEBER[4], H. H. WEBER[5]) and light scattering (JOHNSON AND LANDOLT[6], STEINER, LAKI AND SPICER[7]) as a process of linear polymerisation of globular actin molecules. The electron micrographs and X-ray photographs of F-actin (JAKUS AND HALL[8], ASTBURY, REED, PERRY AND SPARK[9], RÓZSA AND STAUDINGER[10], SNELLMAN AND ERDÖS[11], and RÓZSA, SZENT-GYÖRGYI AND WYCKOFF[12]), while providing visual evidence for this concept, involve an additional variable of drying and may therefore not be of direct relevance to the system under investigation.

The current view on the interaction of F-actin and myosin is that it involves complex formation, and that the action of ATP is to cause dissociation of the complex. The experimental evidence in support of this view is derived from measurements of viscosity (BANGA AND SZENT-GYÖRGYI[13], MOMMAERTS[14]), sedimentation (SNELLMAN AND ERDÖS[15], JOHNSON AND LANDOLT[16]) and light scattering (JOHNSON AND LANDOLT[6], MOMMAERTS[14]). Here too, electron optical observations in support of this thesis are available (PERRY, REED, ASTBURY AND SPARK[17]). There is, however, no agreement with regard to the mode of changes in configuration during such an interaction. Thus, light scattering alone has indicated an overall coiling (JORDON AND OSTER[18]) as well as an extension (BLUM[19]). Measurements of viscosity likewise suggest a greater complexity than has hitherto been considered (see for example LAKI AND CLARK[20]). While some difficulties are inherent from the complexity of the system, differences in the purity of the preparations and in the refinement of experimental techniques, the major difficulty may lie much deeper.

The thixotropy of F-actin and F-actomyosin has frequently been mentioned (see STRAUB[1,2], DUBUISSON[21], JAISLE[22]). All the physico-chemical studies so far carried out

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on the solution phase seem to be based on the assumption that by employing sufficiently dilute solutions (0.2–0.3% or lower) thixotropy can be annulled and that the seemingly constant physico-chemical properties of the whole system depend only on the characteristics of the individual protein molecules. The only recorded doubt seems to be that expressed by H. H. WEBER AND PORTZEHL[5] who suggested that the constancy of the viscosity in repeated viscometer runs could also be due to a balanced process between the destruction of structural viscosity during movement and a building up in the resting parts of the liquid.

It is possible to observe the gel structure of F-actin down to concentrations very much lower than 0.05%. When an apparent true solution, forced out of a very fine capillary under high pressure after the flow time is constant, is allowed to flow down the wall of a test tube, the stream is not homogeneous but can be seen against strong light to consist of microscopic lumps. The persistence of flow birefringence of a dilute solution of F-actin or F-actomyosin long after the cause of disturbance has ceased, likewise suggests some kind of structure in solution. It seems that the validity of the straightforward application of such techniques as viscosity, flow birefringence, light scattering, sedimentation etc. to such thixotropic systems exhibiting structure in solution has so far not been questioned.

In the following, some experimental evidence, derived from fluorescence-polarisation measurements, will be presented which appears difficult to interpret on conventional lines. It is better explained by changes in structure in solution rather than by the presence of static linear polymers in F-actin or of complex "copolymers" in F-actomyosin.

EXPERIMENTAL

Preparation of actin. F-actin was prepared from rabbit skeletal muscle by the method described in a previous paper[23]. The preparation was electrophoretically homogeneous at pH 2 and 10. Judging from the "specific viscosity" and the actomyosin-forming ability, the activity was very much higher than the actin prepared according to FEUER, MOLNÁR, PETTKÓ AND STRAUB[24].

Preparation of myosin. Myosin was prepared by the method of SZENT-GYÖRGYI[25]. After the first and second precipitations and redissolving in 0.6 *M* KCl, the solution was filtered through paper pulp and precipitated for the third time. Only freshly prepared myosin was used for the experiments.

Methods. Measurements of viscosity were employed as a qualitative check on the activity of actin before and after the experiments and were carried out in the TSUDA[26] horizontal viscometer described in a previous paper[27]. The coupling of actin with 1-dimethyl-aminonaphthalene-5-sulphonyl chloride by the method of G. WEBER[28] is described in the accompanying paper[29]. When the coupling and the subsequent dialysis were carefully carried out at 0° with ATP as stabilizer, the properties of actin such as solubility, "polymerisability" and ability to form actomyosin underwent little change. The polarisation of the fluorescent radiation of the conjugate was measured by G. WEBER's technique[28,30,31]. In this paper the same notations will be used as those in the earlier papers.

Preliminary attempts to couple myosin with the fluorescent dye at low temperatures have not been successful. Even with the protection of ATP[32], myosin was invariably denatured.

RESULTS

"Polymerisation" of actin

G. WEBER[33] has studied the polarisation of fluorescent dyes in thixotropic gels at rest or on shaking or when the suspending medium set to a gel. In all cases the polarisation remained unchanged. It is therefore possible to study the rotational relaxation of fluorescent dyes even when the macroscopic medium resists rotation and diffusion.

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He has also shown[31] that conjugates of native bovine serum albumin and ovalbumin showed polarisations which were independent of the changes of translational diffusion with concentration.

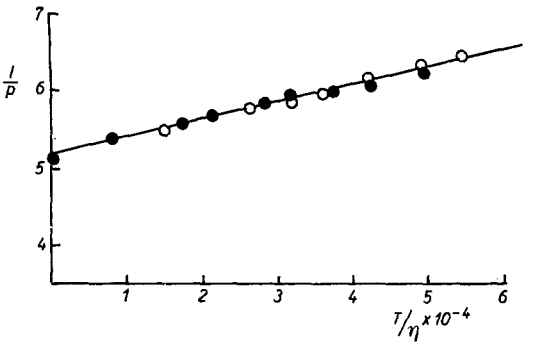

Fig. 1. Fluorescence-polarisation of F-actin in 0.1 M KCl + 10^{-4} M ATP at pH 7.
open circles – polarisation on heating up.
closed circles – polarisation on cooling down.

The polarisation of the fluorescent radiation of the actin-dye conjugate in 0.1 M KCl in the presence of 10^{-4} M ATP at neutral pH is given in Fig. 1. At this ionic strength, the degree of "polymerisation" of F-actin appears to be near a maximum (compare FEUER *et al.*[24], STEINER *et al.*[7], BARBU AND JOLY[3]). The thixotropy and flow birefringence of the labelled actin remained unchanged after the protein was heated to 45° C in the course of the experiments. The slope and intercept of the curve $1/p$–T/η, where p is the polarisation, T the absolute temperature and η the microscopic viscosity of the solvent, combine to give a rotational relaxation time $\varrho h = 31.6 \cdot 10^{-8}$ sec at 25° C. Calculated in the manner outlined in the previous paper[29] for an anhydrous particle, this value corresponds to a particle weight of 147,000, and for 39% hydration, 134,000.

In the previous paper[29] on depolymerised actin, it has been shown that for monomeric actin the particle weight is 70,000 and the rotational relaxation time $\sim 15 \cdot 10^{-8}$ sec; and the corresponding values for the dimeric form, 140,000 and $30.5 \cdot 10^{-8}$ sec respectively. *It is apparent therefore that the kinetic units in F-actin are the actin dimers.* In 0.5 M KCl (the myosin solvent) in the presence of 10^{-4} M ATP, the behaviour of F-actin was found to be similar to that in 0.1 M KCl (+ 10^{-4} M ATP) (Fig. 2); the ϱ values, *i.e.*, 32.1 and $31.6 \cdot 10^{-8}$ sec respectively, agree with each other within the experimental error.

From these results, it would appear that, in F-actin solutions, there are no polymeric fibrils or aggregates such as the "ovoids" of particle weight $\sim 1.5 \cdot 10^6$ observed by ROZSA *et al.*[12] in the electron microscope. There are, however, two possible alternative explanations for the experimental observations: (1) The units forming the linear polymer of F-actin possess some degree of free rotation along the fibre axis. (2) The polarisation is due to small, labelled actin molecules which have not taken part in polymerisation and which remain free among the network of F-actin fibrils. For alternative (1) one would expect a temperature dependence of free rotation and correspondingly an upward curvature of the

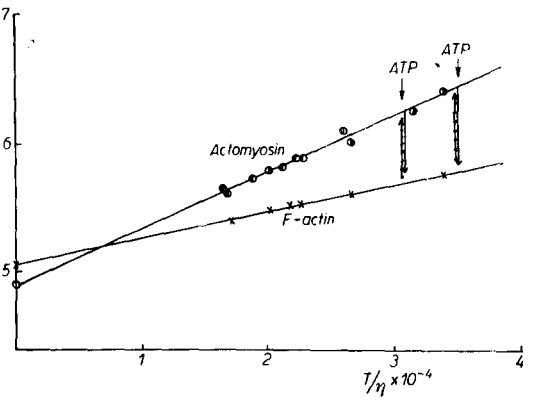

Fig. 2. Fluorescence-polarisation of F-actin and F-actomyosin in 0.5 M KCl + 0.006 M phosphate, pH 7. (F-actin in presence of 10^{-4} M ATP)
◐ actin: myosin = 1:2 (weight ratio)
◑ actin: myosin = 1:4 (weight ratio)
Effect of ATP shown for 26° (see also Fig. 3) and 22° C.

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$1/p$–T/η curve. However, the experimental points covering a temperature range from 1° to 45° follow a strictly linear course. Alternative (2) is not likely on two grounds: (a) Ultracentrifugal experiments on labelled F-actin carried out in collaboration with Dr P. JOHNSON[34] have shown that when the fast moving gel components were spun down leaving only the "unpolymerised" actin of $S = 3$–4, the fluorescence is associated almost entirely with the gel phase. (b) The polarisation of the fluorescent radiation of depolymerised actin, whether active or inactive, does not change on the addition of myosin. With F-actin, there was an instantaneous, ATP-sensitive change, concomitant with other changes in physico-chemical characteristics, which will be described in detail in the next section. Therefore, the observed polarisation in F-actin must be due predominately to those actin particles exhibiting F-actin characteristics but not to the interstitial "unpolymerised" actin molecules.

If the kinetic units of F-actin are those of the dimer, it is necessary to find alternative interpretations for the very high values of the particle weight of actin derived from other physico-chemical techniques. The suggestion is made here that these higher values are based on observations which are derived from structures in solution and which do not represent the true microscopic state of the individual particles.

In the case of viscosity, flow birefringence and light scattering, this may not be difficult to visualize. The most serious objection, however, may come from sedimentation in the ultracentrifuge. Studies from different laboratories[4, 16, 35] using the classical preparation of STRAUB[2, 24] showed more or less reproducible sedimentation velocity at pH 7 (50–65 S) for F-actin. However, it may be significant that SNELLMAN, ERDÖS AND TENOW[36] observed a variation of sedimentation "constant" with speed. That sedimentation with relatively definite velocity in the ultracentrifuge need not necessarily arise from the true physical characteristics of the individual solute particles in all soluble systems but may result from structures in solution or other causes can be illustrated by the behaviour of concentrated urea solution or 0.6 M KI. Experiments carried out in collaboration with Dr A. G. OGSTON[32] on 6.7 M urea itself (+ 0.075 M phosphate, 0.3 M KCl, pH 6.5) have shown that urea (molecular weight 60) sediments with $S = 1.2$ Svedberg units pertaining to particle weight of several tens of thousands. The possibility of the existence of structure in concentrated urea solution has previously been demonstrated with viscosity measurements (TSAO *et al.*[27]). Similarly, experiments carried out in collaboration with Dr P. JOHNSON[34] on 0.6 M KI (molecular weight 166) show demonstrated with viscosity measurements (TSAO *et al.*[27]). Similarly, experiments carried a very rapid sedimentation in the ultracentrifuge; but the exact sedimentation constant has not been evaluated.

Interaction of actin, myosin and ATP

The actomyosin-forming ability of labelled F-actin is comparable to that of unlabelled actin. If this interaction is due to complex formation, the attachment of particles of molecular weight 10^6 to the fluorescent units of particle weight 140,000 would increase the molecular volume and relaxation time so enormously that a horizontal $1/p$–T/η straight line would be expected from G. WEBER's theory[30]. It has not been possible to obtain labelled myosin without denaturation, but the polarisation of the labelled "framework of myosin" (*i.e.* myosin from which open chain subunits of average particle weight 16,000 have been removed by urea treatment), for which ultracentrifuge data[32] indicated a sedimentation constant $S = 8 - 7$ Svedberg units, does

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follow a horizontal line as predicted by WEBER's theory. In the case of F-actomyosin, however, the slope, contrary to expectation, is increased relative to that for F-actin (Fig. 2). The slope and intercept combine to give a relaxation time $\varrho h = 14.9 \cdot 10^{-8}$ sec, identical to that for the actin monomer, $15 \cdot 10^{-8}$ sec.

When ATP is added to the system, there is a pronounced rise in polarisation simultaneous with the visible drop in viscosity and flow birefringence. The polarisation gradually recovers as the ATP is progressively hydrolysed and the system responds once more to fresh addition of ATP. When ATP is present in great excess, the recovery is extremely slow, when in small amount, very fast. Fig. 3 is reminiscent of the changes induced by ATP on the viscosity and flow birefringence (DAINTY, KLEINZELLER, LAWRENCE, MIALL, NEEDHAM, NEEDHAM AND SHEN[37]) and light scattering (JOHNSON AND LANDOLT[6]) of actomyosin.

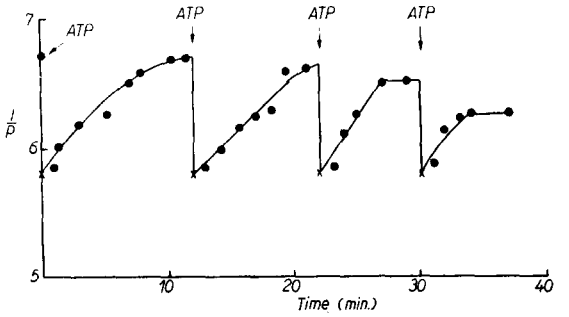

Fig. 3. Effect of ATP on the fluorescence-polarisation of F-actomyosin in 0.5 *M* KCl + 0.006 *M* phosphate, pH 7.0, 26.0° C.

If the exact magnitude of the polarisation, however, is analysed, and compared with that for F-actin, a quite extraordinary correlation stands out. For a maximal rise of polarisation (*i.e.* drop in $1/p$), the values seem to be identical with that of F-actin (Fig. 2), and it appears that *the kinetic unit of F-actin is that of a dimer which dissociates into monomers when interacting with myosin. Under the influence of ATP, the actin monomers are again reunited to form the dimer.*

The tentative conclusion seems to be, whatever the nature of the interaction between actin, myosin and ATP, and whatever the changes induced in myosin, that there appears to be no complex formation between actin and myosin in the conventional sense of the term and that this interaction involves, on the part of actin, a monomer-dimer transformation.

The demonstration of a monomer-dimer transformation in depolymerised actin has been described in the previous paper[29]. It was shown there that the two monomers are probably linked together at the nucleotide prosthetic group of actin through a divalent metallic ion, and that ATP or other polyphosphates compete for the ion, causing dissociation of the dimer. It may be argued that the observed changes in actomyosin may in fact be the monomer-dimer transformation of those actin particles which have not taken part in the "polymerisation" and interaction with myosin. This cannot be so for the following reasons: (1) The monomer-dimer transformation demonstrated in depolymerised actin was very slow and was induced under rather irreversible conditions, whereas the changes observed in actomyosin were *instantaneous* and remained reversible for many cycles. (2) The action of ATP on depolymerised actin was to dissociate the dimer into the monomers whereas the effect induced in actomyosin, as far as the actin partner is concerned, was to favour the recombination of the monomers into the dimer. (3) When myosin was added to labelled actin, which had been "depolymerised", whether reversibly or irreversibly, into the "globular" form, there was no change in the polarisation.

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Point (2) may sound self-contradictory. It is, however, not at all improbable in a complex system which contains actin, myosin, ATP and divalent metals, any two of which exhibit strong interaction with each other. In the presence of myosin (in actomyosin) or neutral electrolytes (in F-actin), for example, the balance of forces (whatever their nature) is so altered that ATP may very well influence the system in such ways as to favour the association of actin monomers; while under other conditions, competition for the divalent metals held by actin is the only possible pathway of interaction, and ATP favours the dissociation of the dimer.

DISCUSSION

Current investigations on the physico-chemical changes of the systems F-actin and F-actomyosin-ATP rely upon methods such as viscosity, flow birefringence, light scattering and sedimentation which are likely to be strongly influenced by the macroscopic state of the system as a whole. Since F-actin and F-actomyosin are thixotropic, the interpretation of the results obtained with the aid of the above-mentioned methods are open to some doubt unless due allowance is made for the contribution of structures in solution to the observed properties.

The technique of fluorescent-polarisation permits the study of the microscopic changes, relatively independent of the macroscopic state of the system. In the present study, the actin partner was labelled with a fluorescent dye and remained fully active, so that the physical changes of actin alone can be traced side by side with the aggregate changes of the system. Although the conclusions are as yet tentative, it seems that the interaction is accompanied by a monomer-dimer transformation in actin, involving, however, no complex formation between the actin and the myosin partners.

This later conclusion is also reached by BLUM[19,38] who applied light-scattering technique to the crude actomyosin extracts obtained by 5 or 24 hours' extraction of muscle mince. In BLUM's system, the interacting units shown possess particle weights of the order $20 \cdot 10^6$ (compare actin 70,000 and myosin 840,000). It would be interesting if BLUM's results could be repeated on simpler and purified actomyosin systems under conditions in which some experimental devices could be made to destroy existing structures in solution. It might be profitable to investigate better known thixotropic systems using modern physico-chemical methods, including electron optical observations, and to apply the findings to the interpretations of the structure of F-actin and F-actomyosin both in solution and after drying.

The preliminary results obtained in this investigation do not justify premature speculations as to the nature of the interaction in the G–F transformation of actin nor of the formation of actomyosin and its apparent reversal by ATP. It is not improbable that the interactions may in the last analysis rest to some extent upon long range forces which are strongly influenced by minute changes in the protein molecules or in the dielectric media in which the interaction takes place. The changes in the mechanical properties of actomyosin in solution, in extruded gel or *in situ* induced by such agents as ATP may in a large part be due to the changes in the relative packing, alignment and aggregation of the asymmetrical protein components, rather than to any association-dissociation in the conventional chemical sense.

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SUMMARY

1. Purified actin has been coupled with a fluorescent dye, 1-dimethylaminonaphthalene-5-sulphonyl chloride, which enables the molecular state of aggregation of F-actin and of F-actomyosin to be investigated by the fluorescence-polarisation technique of G. WEBER. The coupling does not modify the physico-chemical properties of actin with respect to "polymerisability" and ability to form actomyosin.

2. The kinetic units in F-actin are those of the actin dimers of particle weight 140,000 and rotational relaxation time $\sim 32 \cdot 10^{-8}$ sec. There is no evidence for the existence in solution of polymerised fibrils such as are seen after drying nor of the primary large aggregates ("ovoids") which might act as their precursors.

3. There appears to be no complex formation between myosin and F-actin. In F-actomyosin, the actin partner exists in the monomeric form.

4. Alongside the macroscopic changes in viscosity, flow birefringence, light scattering and sedimentation, ATP appear to cause instantaneous re-dimerisation of the actin partner.

5. These observations and the published results of other investigators using different techniques are discussed in relation to the alternative views of polymerisation as against the formation of dynamic structures in solution.

RÉSUMÉ

1. Le couplage de l'actine purifiée avec un colorant fluorescent, le chlorure de 1-diméthylaminonaphtalène-5-sulfonyle, permet d'étudier l'état moléculaire d'aggrégation de la F-actine et de la F-actomyosine par la technique de fluorescence-polarisation de G. WEBER. Le couplage ne modifie ni la "polymérisabilité" de l'actine ni sa capacité à former de l'actomyosine.

2. Les unités cinétiques dans la F-actine sont celles de dimères d'actine, le poids des particules étant 140,000 et le temps de relaxation rotationnelle $32 \cdot 10^{-8}$ sec. L'existence en solution de fibrilles polymérisées telles qu'on en voit après dessication ou de gros aggrégats primaires ("ovoïdes") qui pourraient leur servir de précurseurs, n'a pu être démontrée.

3. Il semble qu'il n'y a pas formation de complexe entre la myosine et la F-actine. Dans la F-actomyosine, la moitié active existe sous forme monomère.

4. En même temps qu'il produit des modifications macroscopiques dans la viscosité, la biréfringence d'écoulement, la diffraction de la lumière et la sédimentation, l'ATP semble provoquer une redimérisation instantanée de la moitié active.

5. Ces observations, ainsi que les résultats publiés par d'autres auteurs utilisant des techniques différentes, sont discutées par rapport aux hypothèses sur la polymérisation et la formation de structures dynamiques en solution.

ZUSAMMENFASSUNG

1. Gereinigtes Actin wurde mit einem Fluoreszenzfarbstoff, dem Dimethylaminonaphtalin-5-sulfonsäurechlorid, gekuppelt, das die Untersuchung des molekularen Aggregatzustandes von F-Actin und von F-Actomyosin mit der Fluoreszenzpolarisationsmethode von G. WEBER ermöglicht. Die Kupplung verändert nicht die physikalisch-chemischen Eigenschaften des Actins bezüglich seiner "Polymerisationsfähigkeit" und der Fähigkeit Actomyosin zu bilden.

2. Die kinetischen Einheiten in F-Actin sind dieselben wie die des dimeren Actins mit dem Teilchengewicht 140,000 und der Rotationsrelaxationszeit $\sim 32 \cdot 10^{-8}$ sec. Es ist kein Beweis vorhanden für das Bestehen von polymerisierten Fibrillen in Lösung, wie sie nach dem Trocknen beobachtet werden, noch für ursprüngliche grosse Anhäufungen ("Ovoide"), die als ihre Vorläufer dienen könnten.

3. Es scheint keine Komplexbindung zwischen dem Myosin und dem F-Actin zu bestehen. Im F-Actomyosin besteht der Actinpartner in der monomeren Form.

4. Neben den makroskopischen Veränderungen der Viskosität, der Strömungsdoppelbrechung,

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der Lichtstreuung und der Sedimentation scheint die ATP eine augenblickliche Rückdimerisation des Actinpartners zu verursachen.

5. Diese Beobachtungen und die veröffentlichen Ergebnisse anderer Forscher, die verschiedene Methoden benützten, werden besprochen in Bezug auf die sich gegenseitig ausschliessenden Ansichten über die Polymerisation und entgegen der Bildung dynamischer Strukturen in Lösung.

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