Reactivities of Actin as a

Contractile Protein

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ABSTRACT   The molecular basis for the mechanism of contraction in striated muscle, with primary emphasis on the interaction between the thick and thin filaments and the role of the thin (actin) filaments, is the theme presented. Recent information relating to actin-myosin interaction points up the fact that definitive statements cannot be made regarding the molecular interaction(s) that lead to contraction. Nevertheless, the properties of actin indicate that (a) actin in the monomeric state has properties differing markedly from actin in the polymer (filament) state; (b) these property differences may be significant in the contractile process, for they include changes in the reactivity of the bound nucleotide and actin-myosin complex formation; (c) the bound nucleotide seems to be required in the contraction process. For these, and other, reasons discussed, the tentative hypothesis is advanced that the contraction reaction involves local changes in the actin filament providing local monomer or monomer-like actin units in the reaction with myosin.

The current literature contains several fundamentally different hypotheses on the mechanism of muscle contraction, but as the contributors to this volume have made abundantly clear, the sliding filament model of A. F. Huxley and R. Niedergerke (1) and H. E. Huxley and J. Hanson (2) is by far the best-documented postulated mechanism. This mechanism calls for an interaction between the actin and myosin filaments, resulting in a series of small displacements of one filament relative to the other. This action produces muscle contraction and, therefore, movement.

The production of movement in a biological system does not require the presence of a double set of filaments in a finely ordered array as demonstrated in striated muscle. Thus, the slime mold *Physarum polycephalum* has a characteristic flowing motion of the protoplasm. From this system, both an actomyosin-like protein (3) and an actin-like protein (4) have been extracted. Thus, an actin-myosin interaction may be responsible for movement in this organism in the absence of interdigitating filaments. This provides hope for the test-tube physiologist that in vitro studies of these protein molecules

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without ordered filaments may still provide an understanding of the molecular basis of movement.

Of the two principal contractile proteins, myosin has been studied more intensively than actin until recent times. In our laboratory we have in recent years concentrated on the study of actin, which has been found in all types of muscle studied to date. It has been our hope that these studies would lead to some understanding at the molecular level of two requirements of the sliding filament model. These are the cyclic make-and-break of the attachments of actin to myosin and the possible structural changes related to this reaction. From our laboratory, I shall be describing mainly the work of my colleagues E. E. Clark, R. J. Grant, L. B. Cohen, and W. D. Cohen.

THE ACTIN MOLECULE

The classic work of Straub and his coworkers (5), who developed the technique of water extraction of acetone muscle powder, resulted in their demonstration of the fact that the actin as extracted in this way is a globular (G) protein. This molecule, which has a bound nucleotide in the form of ATP, will, under certain conditions, polymerize to form a fibrous (F) aggregate which contains bound ADP so that P_i is released in the polymerization process. The reaction may be depicted thus:

$$n \text{ G-ATP} \xrightarrow{\text{0.1 M KCl}} \text{F-ADP} + n \text{ P}_i$$

in which n for a complete polymer (F-ADP) may have a value of several hundred. The bound nucleotide–protein ratio has been determined by a number of investigators using several techniques (cf. references 6–8), and there is general agreement that 1 mole of nucleotide is bound to 57–60,000 g of protein. As will be seen later, there is some doubt that this figure can be assumed to represent the molecular weight of actin. Under the conditions shown, the release of inorganic phosphate coincides with the polymerization (9), and the ADP of the polymer formed seems firmly bound, as indicated by lack of exchange with added ATP (10). Exchange occurs readily with actin in the G-ATP form (8).

A third form of actin, G-ADP, has been isolated and studied (11, 12). In many ways, the G-ADP molecule is more fragile than the G-ATP or F-ADP forms, but it has certain properties which make it more amenable for study; namely, it polymerizes without an accompanying dephosphorylation, and the polymerization can be controlled simply by controlling the temperature. The bound ADP exchanges very rapidly with added ATP.

ACTIN AND CONTAMINATING PROTEINS

In order to obtain unequivocal information regarding actin-myosin inter-actions, one would prefer to start with pure components, but in the case of

actin a pure preparation has been difficult to obtain. It was recognized early (5) that the water extract of muscle powder contains other proteins besides actin. One of the most persistent of these contaminating proteins is tropomyosin (13).

The molecular weight of tropomyosin as recently determined (14, 15) is about 70,000. It is a highly charged molecule, and polymerizes readily under ionic conditions essentially opposite to those prevailing for actin. Tropomyosin also complexes with actin (16–18), and this property leads to its persistence in actin preparations. The recommended 0°C extraction (18) does not entirely eliminate the tropomyosin, and may leave as much as 5%, which complicates the characterization of the actin monomer.

For example, viscometric measurements on monomeric actin preparations by a number of workers give widely varying values of the intrinsic viscosity, ranging from 10 to 30 ml/g. These viscosity values indicate a considerable
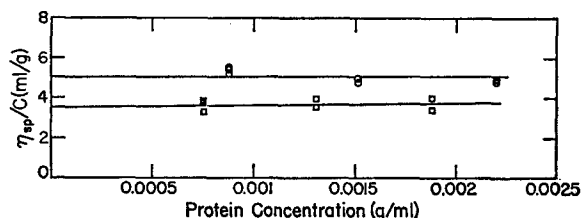

FIG. 1. Reduced viscosities of G-ADP actin in the absence and presence of salt. ⊙, 10 mM barbital, pH 9.1; 2 mM MgCl₂; 0°C. ⊡, 0.2 M KCl; 10 mM barbital, pH 9.1; 1.714 mM MgCl₂; 0°C. *Figure reprinted by permission from Archives of Biochemistry and Biophysics, 1966, 117:289.*

asymmetry of the actin monomer, and stand in paradoxical contrast to electron microscope evidence (19), which indicates the actin monomer to be a relatively spherical molecule with an axial ratio no larger than 3.

New and exhaustive purification methods, first begun in our laboratory by R. J. Grant (20) and extended by L. B. Cohen (21), reducing the tropomyosin contamination to trace amounts and utilizing refined viscometry, have resulted in unequivocal values of the intrinsic viscosity of globular actin. Figs. 1 and 2 show clearly that the intrinsic viscosity of G-ADP actin is 3.7 ml/g and that of G-ATP is 3.5 ml/g, both in the presence of salt. These values are identical within experimental limits, and establish firmly that monomeric actin in aqueous solution belongs to the class of spherical proteins. These data indicate also that the nature of the bound nucleotide does not make for a significant change in the conformation of the protein moiety, a conclusion which is confirmed by optical rotatory dispersion measurements. Optical rotatory dispersion spectra for G-ADP and G-ATP have been extended down to 220 mμ, and it is found that the curves for the two proteins are superimposable.[1]

[1] T. Hayashi and L. B. Cohen. Unpublished observations.

The results are in agreement with those of Nagy and Jencks (22) on a narrower wavelength range.

Intrinsic viscosity values of the order of 3.5 ml/g, if accepted for monomeric actin, lead to other difficulties. Sedimentation studies on monomeric actin have been done by a number of workers (e.g. reference 23), and an s_{20} value of 3.25–3.5 is well established. However, acceptance of this value and an intrinsic viscosity of 3.5 for G-actin indicates that all values for the molecular weight of G-actin are too high.[2]

E. E. Clark[3] has examined this question by studying the sedimentation characteristics of G-actin at pH 9.0 in the presence of salt, and reports that the molecular weight is about 50,000.

The association of actin and tropomyosin may be part of the natural structure of the thin filaments. Thus, purified preparations of tropomyosin show a
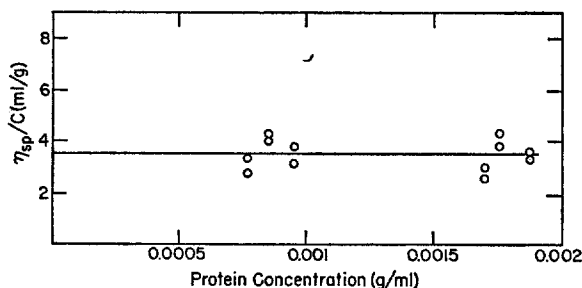

Figure 2. Reduced viscosity of G-ATP actin in 0.15 M KI, 2 mM MgCl₂, and 10 mM barbital, pH 9.0, at 0°C. From L. B. Cohen (personal communication).

characteristic periodicity of 380–400 A (25), and an axial periodicity of 400 A has been reported for the "I substance" of vertebrate striated muscle (26). Hanson (27) has reported that isolated, unpurified actin filaments show this same periodicity, but filaments formed of purified actin do not. Taken in sum, these observations indicate the presence of tropomyosin in the thin filaments.

One possible role of the tropomyosin may be to stabilize the structure of the thin filaments. Grant (28) in our laboratory has found that, by adjustment of the ionic milieu, the polymerization of G-ADP (without dephosphorylation) is a temperature-reversible process, and is a true thermodynamic equilibrium. Fig. 3 depicts this temperature reversibility, and shows that at 0°C there is complete depolymerization, while at 29°C there is complete polymerization. This cold-induced depolymerization can be inhibited; that is, the polymer structure can be stabilized by certain agents. Table I shows the effect of the

[2] Adelstein et al. (24) have reported a molecular weight of 47,000 for G-ATP actin. However, the ultracentrifugal measurements were done in the absence of salt, and the authors admit that charge effects under these conditions would give low values.
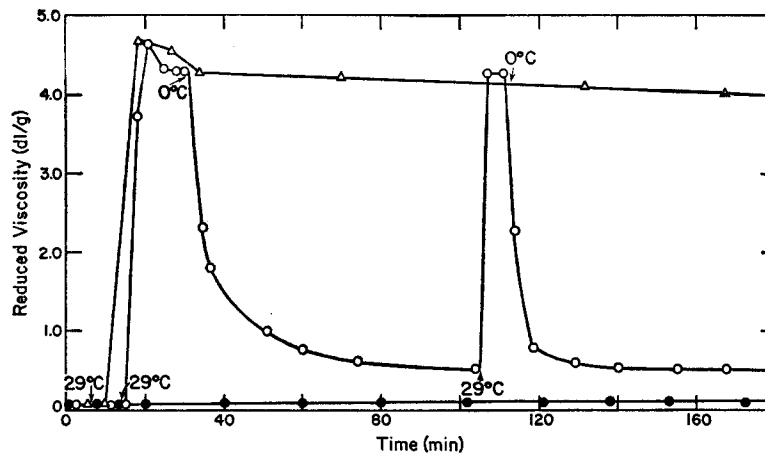
[3] E. E. Clark. Personal communication.

FIGURE 3. Temperature-induced polymerization-depolymerization of G-ADP actin. 0.53 mg/ml actin in 2 mM MgCl₂. ●, viscometer at 0°C full time; △, viscometer left at 29°C; ○, viscometer transferred to temperatures indicated by arrows. *Figure reprinted by permission from thesis of Grant (28).*

orthophosphate ion, which "locks" the polymer structure. This effect is quite specific, and is not shown by pyrophosphate or sulfate. Significantly, tropomyosin is also an effective agent for the inhibition of cold depolymerization, as in metin (29), a fibrous protein from muscle, which is probably largely tropomyosin. The most effective stabilization of the actin polymer results from the application of a combination of metin with orthophosphate.

TABLE I

EFFECT OF ORTHORPHOSPHATE ION
ON TEMPERATURE REVERSAL
OF ACTIN POLYMERIZATION

To each of seven viscometers at 0°C, each containing 1 ml of KH₂PO₄ at the molarity necessary to give the final concentrations listed below, was added a 5 ml sample of G-ADP in 2 mM MgCl₂. Constant viscosity values at different temperatures were determined. Time of depolymerization at 0°C, 70 min. Final actin concentration in all samples, 0.48 mg/ml (21).

Concentration of KH₂PO₄	Reduced viscosity			Reversal
	0°C	29°C	0°C	
M	$ml/g \times 10^{-2}$	$ml/g \times 10^{-2}$	$ml/g \times 10^{-2}$	%
0	0.16	4.24	0.41	94
16	0.16	5.30	1.48	74
33	0.18	4.10	2.04	54
66	0.22	4.26	2.57	42
100	0.20	5.25	3.44	36
133	0.26	4.57	3.41	26
200	0.30	4.92	4.95	0

Other proteins which affect the structure of the actin filament have been described. "Native tropomyosin" (30), which may consist of Bailey tropomyosin and another protein, troponine, not only confers EGTA [ethylene-dioxybis(ethyleneamino)tetraacetic acid] sensitivity upon reconstituted actomyosin, but also enhances the viscosity of actin solutions. α-Actinin (31) enhances network formation and the viscosity of polymerized actin; when removed, the actin in reaction with myosin is incapable of superprecipitation.

One other persistent protein contaminant in actin preparations may be mentioned. This is myokinase, which may be found in small amounts even in highly purified actin preparations.[4] Such preparations can be shown to polymerize extremely slowly at 0 °C, and this slow polymerization is enhanced by the addition of ADP and inhibited by the addition of AMP. Exhaustive purification will eliminate this slow 0 °C polymerization completely, under conditions in which equally purified G-ATP polymerizes readily.

THE ACTIN-MYOSIN INTERACTION

With this background, we may address ourselves to two questions: Does the polymerization-depolymerization cycle of actin have a physiological role in the contraction process? Does the bound nucleotide have a physiological role in the contraction process?

On the first question, there is considerable evidence against the idea that polymerization-depolymerization is involved in the contraction process. The most convincing evidence comes from the remarkable X-ray studies of Elliott, Lowy, and Millman (32) and Huxley, Brown, and Holmes (33) on living muscle in the resting and contracted states. All the data agree that there is no change in axial spacing in either the actin or myosin spacings in going from the relaxed to the contracted state, and quite thoroughly rule out any possibility of a wholesale polymerization or depolymerization of the actin-containing filaments during the contraction of muscle. However, Huxley et al. (33) point out that these measurements would not detect local changes, so that such changes are still a possibility.

There is another line of evidence against the idea of the polymerization-depolymerization of actin in muscle. The acetone powder from which G-ATP actin is extracted is found not to contain ATP (8), but only ADP and some AMP. Exposure of the powder to the action of water results in depolymerization of the F-ADP actin to G-ADP. Unbound ADP, also extracted from the powder, is converted to ATP by the action of the contaminating myokinase, and this ATP then displaces the bound ADP to form G-ATP. Thus, the globular actin may be considered to be an extraction artifact, and probably does not exist as such in muscle.

[4] L. B. Cohen. Personal communication.

There is, however, some experimental evidence that local structural changes may occur in the actin filament. It has been mentioned earlier that the bound ADP of F-actin does not exchange with added nucleotide. However, when the actin is combined with myosin and reacted with ATP in the reaction called superprecipitation, it can be shown that the bound ADP exchanges with the added ATP up to about 50% (34–36). Furthermore, Moos et al. (36) have shown that the bound nucleotide of F-ADP does actually exchange slowly, eventually to 20% of the total bound nucleotide, but that both the rate and extent of exchange are considerably enhanced by the presence of myosin. If nucleotide exchange is taken as an indication of local structural change in the actin filament, these data may be interpreted to mean that myosin brings about a "loosening" locally of the actin filament to make the bound nucleotide available to exchange with added ATP.

This influence of myosin on the structure of F-actin is shown in another way. Haga et al. (37) have demonstrated that, in the extraction of actomyosin from muscle mince using the standard Weber-Edsall solution, myosin appears in the medium first, with the subsequent appearance of actin in the form of actomyosin. Studying this further, they have found that the actin in the muscle mince is relatively insoluble and nonextractable, but that when myosin is present the actin becomes more soluble and is extracted out complexed with the myosin.

These changes in property toward increased extractability and greater availability of the bound nucleotide to exchange characterize actin when the F-actin is depolymerized to the G form. The effect of myosin on the structure of the F-actin filament may be, therefore, a localized rupture or "loosening" of the bonds of the polymer structure, so that, at these loci, the actin units take on the characteristics of the monomeric G form. Such local changes would not be detected by X-ray methods. That the effect is due to myosin and not to the added ATP (which is inevitably present) is indicated in the design of the experiments (36).

Further support for this interpretation comes from some recent studies in our laboratory. These studies take note of the fact that both actin and myosin can exist in several states: myosin as dispersed molecules in solution, or as molecular aggregates, and actin as monomers (G) or as polymers (F), which we may consider as dispersed (G) or aggregated (F). These may be caused to interact in the following combinations:

	Actin	*Myosin*
1.	Aggregate	Dispersed
2.	Aggregate	Aggregate
3.	Dispersed	Aggregate
4.	Dispersed	Dispersed

Of these four ways of combining actin and myosin, combinations 1 and 2 have been studied in detail in the electron microscope by H. E. Huxley (38). He shows clearly that, when these proteins are combined as in method 1, the result is the formation of "composite filaments" in the now well-known herringbone-patterned filament in which there seems to be a mole-to-mole association of the actin and myosin. Combination 2 results in a side-to-side
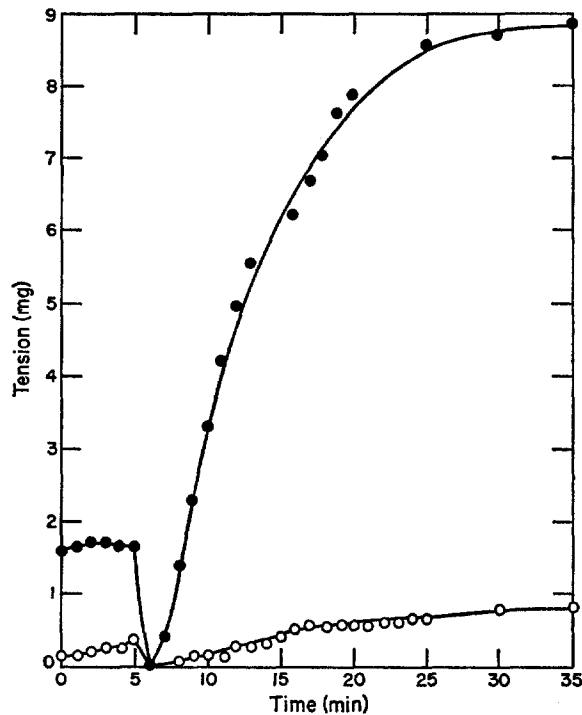

FIGURE 4. Contraction of myosin fiber preincubated in G-actin. Same fiber, before and after incubation. O, incubated in actin solvent only (1.0 mM MgCl₂, 5.0 mM Tris, and 5.0 mM KCl, pH 7.4) at 0°C and tested for contraction. ●, removed from contraction medium, washed twice in actin solvent, reincubated in G-ADP actin, 2 mg/ml, at 0°C, and retested for contraction. *Figure reprinted by permission from thesis of Cohen (40).*

association of the F-actin filaments with the myosin aggregates. If ATP is added to the herringbone type of filament, it is transformed into the second type of arrangement, the filament-to-filament association.

The properties of the actin-myosin associations brought together in these combinations have been studied in other ways in our laboratory. Some years ago (39) it was shown that combination 1 could be formed into a fiber which contracted with ATP to produce movement, and that this contraction could be repeated if the fiber were "relaxed" by exposure to ionic medium partially going back to the conditions of combination 1 before returning to the low ionic

condition for the second exposure to ATP. Using the same techniques of fiber formation, W. D. Cohen (40) has studied combinations 2 and 3. This was accomplished by forming macrofibers of myosin (aggregate) and incubating them in solutions of either F-actin (combination 2) or G-actin (combination 3). In the latter case, G-ADP actin was used, since G-ATP actin is difficult to maintain in the monomer form in the presence of myosin in the ionic media used in these experiments. It may be remarked parenthetically that combination 2 is, at least superficially, closest to the situation found in striated muscle.
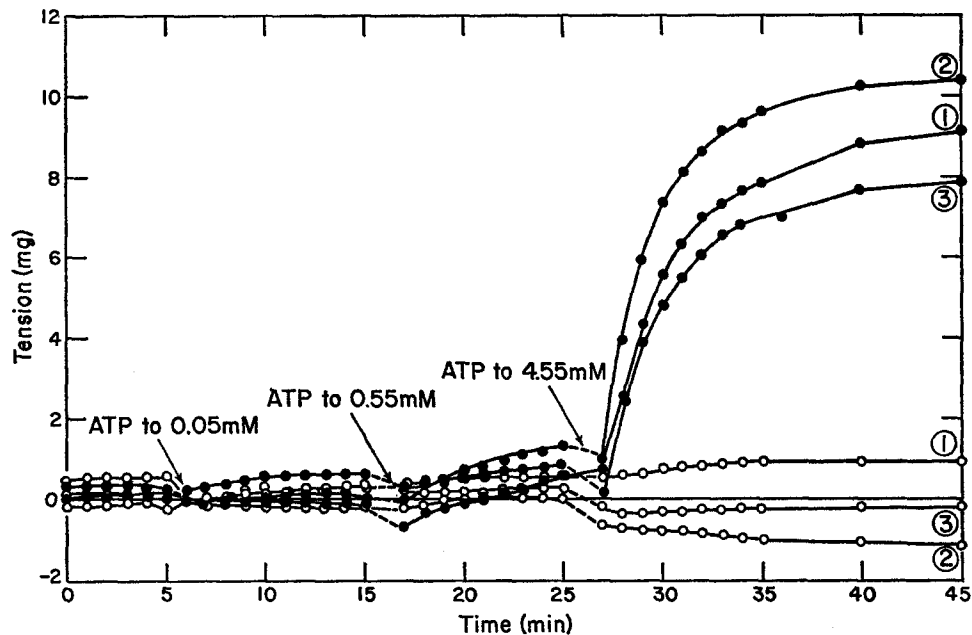

FIGURE 5. Comparison of behavior of G-actin–incubated (●) and F-actin–incubated (○) myosin fibers with graded additions of ATP. *Figure reprinted by permission from thesis of Cohen (40).*

The results of these experiments are clear, and surprising. Fig. 4 shows clearly that a myosin fiber exposed to G-ADP actin is capable of contraction upon exposure to ATP. Fig. 5 compares the behavior of G-ADP–incubated and F-ADP–incubated myosin fibers when treated with increasing concentrations of ATP. The G-myosin fiber contracts strongly when the critical level of ATP is reached, but what is surprising is that the F-myosin fiber does not contract. That this result is not due to the inactivation of the myosin by the F-actin is shown in Fig. 6. Here the F-actin–incubated fiber, after failure to contract with ATP, was washed and incubated with G-ADP actin, whereupon it contracted vigorously. From these results, it is quite clear that myosin aggregates exposed to preformed F-actin filaments are not capable of ATP-in-

duced contraction, whereas the same myosin fibers exposed to G-ADP actin will contract.

It may be argued that the F-actin filaments, because of steric hindrance, do not combine with the myosin in sufficient amounts to bring about a contraction, whereas the G-actin, without such hindrance, can penetrate and combine with the myosin in amounts adequate to account for the contraction. This is denied by the fact that, when the fibers in question are washed and analyzed for the presence of nucleotide indicative of actin, both types of fibers show the presence of considerable amounts of actin, much more than needed to exhibit
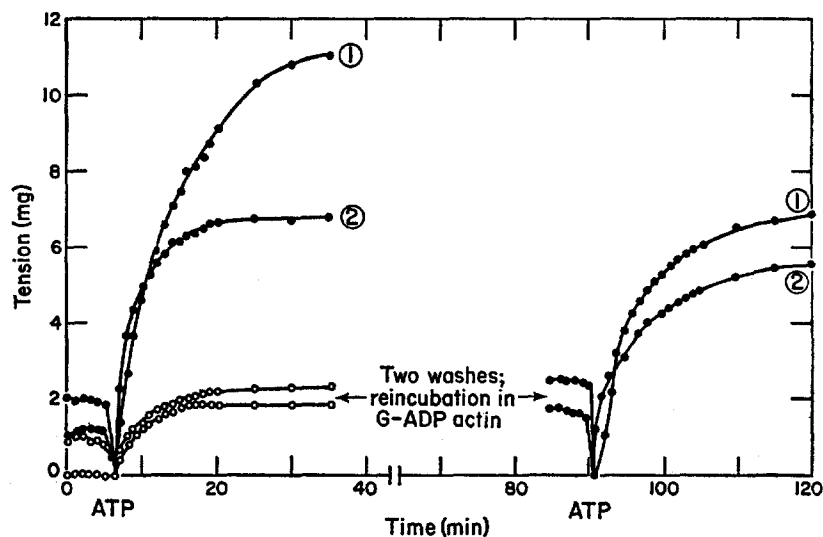

FIGURE 6.   Contraction of G-actin–incubated myosin fiber (●) and of F-actin–incubated myosin fiber only after subsequent incubation of the latter in G-actin (○). *Figure reprinted by permission from thesis of Cohen (40).*

a significant contraction when the two proteins are combined as in method 1 (41).

While more experiments are obviously needed, for the moment these results may be interpreted to mean that, when myosin and actin are combined under conditions 1 or 3, the actin may be in a relatively unstructured form so that, in the interaction with myosin and ATP, movement is produced. When they are combined as in method 2, the actin structure is in a condition not amenable to whatever interactions with myosin and ATP are necessary to bring about movement.

Combination 4, while seemingly simple, is technically difficult to achieve, and in initial experiments the interaction of G-actin with heavy meromyosin rather than myosin has been investigated (42). When these proteins are mixed in low salt (0.1 M) and studied in the ultracentrifuge, a surprising new property

difference between G- and F-actin is observed. It is well known that F-actin complexes with myosin (cf. reference 38), but in this case, if the actin is kept rigidly in the G form, it does not complex with the myosin. In the ultracentrifuge, two distinct sedimenting peaks can be observed, which can be identified as G-actin and heavy meromyosin. This failure of the G-actin to complex adds a third property difference to the two physical forms of actin. It may be stated that when the structured F-actin is transformed to the unstructured G form, there is a loss in the properties of insolubility, resistance to nucleotide exchange, and complex-forming ability.

If we relate this to the make-and-break cycle of the actin and myosin during contraction, it is possible that the structured F-actin filament undergoes local changes to an unstructured state, and that at these loci any or all of the property changes above may be involved in reactions at these loci to complete the cycle. In other words, the answer to the first question posed in this section is that, while wholesale polymerization-depolymerization of actin can be ruled out, there may be local (partial?) polymerization-depolymerization involved in the contraction process.

Since one of the properties concerned here is the exchangeability of the bound nucleotide, this brings us to the second question, whether this bound nucleotide is involved in the contraction process. Here again, recent investigations have adduced evidence against bound nucleotide involvement. The fact that G-ADP actin will polymerize without dephosphorylation (11, 12) is indicative of the nonparticipation of the bound nucleotide in polymerization. Subsequently, nucleotide-free actin has been prepared by Kasai et al. (43) and by Bárány et al. (44). The latter workers report that this actin will polymerize, complex with myosin, enhance the ATPase of the myosin, superprecipitate with myosin, and, upon exposure to ATP, bind the nucleotide 100%. This last finding indicates that nucleotide-free F-actin is rather "unstructured." Finally, while ATP binds readily to this form of actin, deoxy-ATP was found not to bind, in spite of the fact that the nucleotide-free actomyosin exhibits deoxy-ATPase activity and superprecipitates with deoxy-ATP. All of this evidence, therefore, points to the nonnecessity of the bound nucleotide for the biological activity of the actin-myosin complex.

Evidence to the contrary, however, is obtained from the following experiment.[1] Nucleotide-free actin is prepared according to Bárány et al. (44) and, as F-actin, is complexed with myosin in high salt (as in combination 1). This complex is then formed into a synthetic fiber (9) and, when tested for contraction with ATP, it contracts as shown by the development of considerable tension. However, a similar fiber, if tested with deoxy-ATP, is found *not* to contract. If, after failure to contract with deoxy-ATP, ATP is added to the reaction vessel, a vigorous contraction takes place (Fig. 7). A synthetic fiber formed of F-ADP actin complexed with myosin will contract with deoxy-

ATP, and subsequent addition of ATP may or may not cause a small additional contraction (data not shown).

These facts tell us that the bound nucleotide is obligatory for contraction, since deoxy-ATP, which does not bind to the actin, is incapable of producing contraction in the *absence* of bound nucleotide but does bring about a contraction in the *presence* of bound nucleotide. The contraction of nucleotide-free actomyosin with ATP is probably due to the fact that the ATP becomes bound
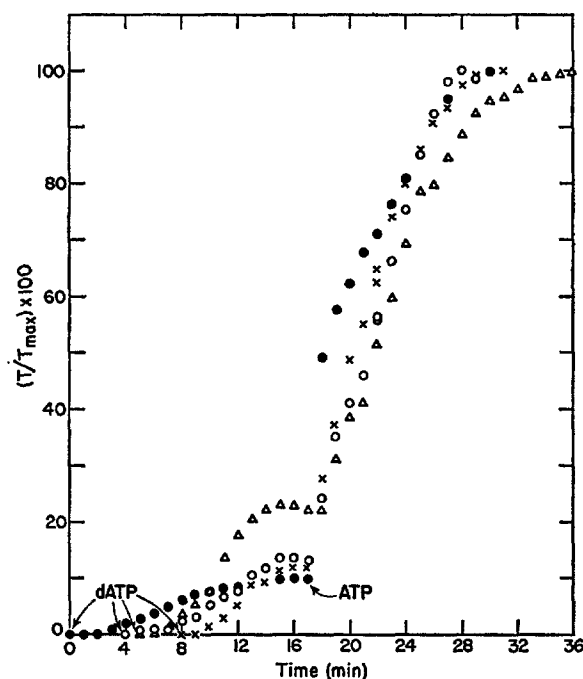

FIGURE 7.   Effect of deoxy-ATP (dATP) and ATP on nucleotide-free actomyosin fibers. ×, ○, △, and ● represent four different fibers. From T. Hayashi and L. B. Cohen (unpublished observations).

to the actin initially. The small amount of contraction of the deoxy-ATP-treated fibers observed in Fig. 7 correlates with the small amount of bound nucleotide left in the "nucleotide-free" actin as prepared by us. This amount is usually 10–12%, although in one case it was 20%, in agreement with the greater degree of contraction of this fiber. Further experiments on these observations are planned, but it seems quite clear that the bound nucleotide of the actin is in some way involved in the contraction process.

The above data are in agreement with the studies of Tonomura et al. (45). These workers found that when nucleotide-free actin and myosin are combined, the deoxy-ATPase of the myosin is not enhanced, but if F-ADP actin is combined with myosin, the deoxy-ATPase of the myosin is enhanced more than 20-fold. Their results on the enzymatic activity of the actomyosin par-

allel our findings on contraction, and point up the requirement for bound nucleotide in the actin enhancement of myosin deoxy-ATPase activity.

It may be, as Tonomura et al. have suggested (45), that the role of the actin (with bound nucleotide) is to activate the dissociation of phosphorylated myosin in the enzyme activity of this protein. How this produces contraction is not known. The specific role of the bound nucleotide of the actin both in enhancement of enzymatic activity and in the contraction process is also not known, but the evidence presented points to its necessity in both processes.

In conclusion, we may say that while a wholesale polymerization-depolymerization of actin may not occur in the contraction process, there is still the possibility that local changes in structure of the actin filaments may be involved, an idea advanced originally by Asakura et al. (46). These local changes would bring about a reversion of the properties of the F-actin filament locally toward those of the G form. The reversions directly concerned may be increased solubility, increased exchangeability of the bound nucleotide, loss of ability to complex with myosin, or some other, as yet unknown, change of property. Whatever the case, such a change of property locally, as well as the bound nucleotide of the actin, both seem to be required to bring about contraction in the actin-myosin interaction.

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Discussion

Dr. Bárány: I am just dealing with that part of Dr. Hayashi's presentation which is related to the nucleotide-free F-actin prepared by us. We had given our procedure to Dr. Hayashi, prior to publication, and I had pointed out that 8–10 sonications are needed to remove the bound ADP completely. Therefore, the 10–20% bound ADP remaining in Dr. Hayashi's preparation reflects only the insufficient number of sonications. This residual nucleotide did not appear in our preparations.

The nucleotide-free F-actin prepared by us rebound ATP in both the absence and presence of myosin. Thus, an evaluation of the role of the F-actin–bound ADP in the superprecipitation of actomyosin became difficult, since in this system ATP (ADP) was rebound. However, we have found that deoxy-ATP and CTP are not

bound by nucleotide-free actomyosin, although these nucleotides induced super-precipitation of this actomyosin. Only deoxy-ATP was used by Dr. Hayashi as evidence for a role of the F-actin–bound ADP in contraction. I would suggest that CTP should also be tested in order to substantiate his findings with deoxy-ATP.

In recent experiments with Dr. Gary Bailin, we were able to prepare actins which showed normal nucleotide content but lost their ability to interact with myosin at both high and low ionic strength. It appears that one can prepare two kinds of actins: one which does not contain bound nucleotide but combines with myosin; another which maintains its bound nucleotide but does not react with myosin. This comparison clearly argues against the role of the bound nucleotide in the physiologically important properties of actin.

I agree with Dr. Hayashi that the bound nucleotide may be used as an index for changes in the structure of actin in various reactions of actomyosin. Indeed, I believe that the role of the bound nucleotide is to stabilize the structure of actin, as it is known, for example, that AMP stabilizes the conformation of some enzymes.

Finally, I would like to mention that the exchange of the F-actin–bound ADP during superprecipitation of actomyosin or myofibrils, shown by Dr. Andrew Szent-Györgyi, is extremely slow. The turnover of this exchange is only a small fraction of that of the cross-bridges, 50–100/sec, in a muscle shortening with maximal speed. Therefore, we have no good reason to attribute any role for the actin-bound nucleotide in the contractile process.

Dr. Hayashi: I would like to comment on that. In the first place, I was careful *not* to say that nucleotide exchange per se was involved in the contraction process. It is unlikely that nucleotide exchange occurs during contraction, for the very reasons Dr. Bárány has given, as well as other evidence. But the increase of exchangeability of the bound nucleotide seems to me to be an indication of structural change in the actin filament, and it was in this connection that nucleotide exchange was used in this presentation.

However, there is an unsettled question on the enhancement of the deoxy-ATPase of myosin by nucleotide-free actin. Bárány et al. (44) reported about a 4-fold enhancement, but Tonomura et al. (45) reported no enhancement with nucleotide-free actin, and a 26-fold enhancement with bound nucleotide present. This difference in result may be due to different methods of preparation of the actin, or different conditions under which the tests were made. Our results are in agreement with those of Tonomura et al.

Finally, it may be pointed out that the fiber system we have employed measures contraction directly on an actomyosin system, and not some reaction which is adjudged to be the equivalent of contraction. For this contraction, the data show that the bound nucleotide is necessary.

Dr. Perry: I would like to just raise one point, Dr. Hayashi. I was very struck with your experiments showing the depolymerization of F-ADP actin on cooling, and it struck me that this is possibly an explanation of the dissociation of actomyosin which occurs on cooling. I think this effect was first shown by Laki a long time ago.

Dr. Hayashi: That is an idea for some experiments, I think, because I am not suggesting that a wholesale depolymerization takes place, but enough loosening could take place with a lowering of temperature in order to bring about a dissociation, and therefore possibly a change in the state of the contraction.