The Interaction of Actin with Myosin and Heavy Meromyosin in Solution at Low Ionic Strength*

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SUMMARY

The interaction of actin with myosin and heavy meromyosin was studied in the presence of Mg++ ion and adenosine triphosphate at low ionic strength. When the heavy meromyosin ATPase was 17-fold activated by actin, the acto-heavy meromyosin complex responsible for this activation did not give rise to a measurable increase in the viscosity of the sample above that predicted for a noninteracting mixture of actin and myosin. In the case of myosin, simultaneous measurements of turbidity, viscosity, and ATPase activity in the presence of actin showed, first, that considerable activation of the myosin ATPase could occur in the absence of any measurable precipitation of the actomyosin, and second, that in contrast to the behavior of the acto-heavy meromyosin system, ATPase activation was always accompanied by a corresponding viscosity increase indicating the formation of a relatively stable actomyosin complex in the “cleared” state. Comparison of the ATPase activity of acto-heavy meromyosin and actomyosin, under conditions where both systems were in solution (0.1 M KCl), indicated a distinct difference in the response of the actomyosin and acto-heavy meromyosin ATPases to falling ATP concentration.

Methods

Myosin—Myosin A was prepared from rabbit skeletal muscle by a modification of the method of Szent-Györgyi (9). The myosin was reprecipitated four times, including three times by the method of Azuma and Watanabe (10), to avoid contamination with actin. It was stored at 2° in 0.5 M KCl and was always used within 5 days after the death of the rabbit.

1 The abbreviation used is: HMM, heavy meromyosin.
Heavy Meromyosin—Heavy meromyosin was prepared by the method of Lowey and Cohen (11) with a 250:1 weight ratio of myosin to trypsin (twice crystallized, Worthington). Digestion was allowed to proceed for 4 min at 25°C and was stopped with soybean trypsin inhibitor (Worthington) added at a 2:1 weight ratio to the trypsin. Sedimentation of the purified HMM in in the Spinco model E analytical ultracentrifuge showed a single symmetrical peak. The protein was stored at 2°C in 10 mM phosphate buffer, pH 7, and was used within 3 days of preparation.

Actin—Actin was extracted from acetone-dried muscle powder (9) at 0°C to avoid contamination with tropomyosin (12), and the actin was purified by the procedure of Uhreht et al. (13). The resulting F-actin pellets were stored no longer than 5 days at 2°C. Before use they were depolymerized into 0.1 mM ATP solution by brief sonication. The free nucleotide was then removed by addition of 0.05 volume of freshly washed Dowex 1-X8 (Cl−), followed by filtration, and the actin was repolymerized by making the solution 0.1 M in KCl and 1 mM in MgCl2. The final F-actin solution was always used within 3 days after preparation.

Preparation of Experimental Samples—Most of the experiments in this study involved comparisons of viscosity, turbidity, and ATPase activity in identical samples, and also, for each of these parameters, comparison of actomyosin or acto-HMM with actin and myosin or HMM separately. Special care was therefore necessary to ensure that the samples to be compared were truly identical, and the procedures used to accomplish this will be described in some detail. For each set of measurements, a large “stock” solution was prepared, which contained all components except the proteins, and equal aliquots of this stock solution were carefully measured out, one for each experimental sample. The actin and the myosin or HMM were added to the sample with syringes with Teflon needles, because it was found that sufficiently reproducible volumetric measurements of the viscous protein solutions could not be made with pipettes; and when one of the proteins was to be omitted from a given sample, exactly the same volume of the appropriate solvent was added in its place, again with a syringe. In all cases, the actin was added first, and a few minutes later the reaction was initiated by the addition of the myosin or HMM. To avoid any temperature differences among the samples, the ATPase vessel on the pH-stat was jacketed with circulating water at 25°C from the same thermostat bath which was used for the viscosity and turbidity measurements.

ATPase—The ATPase rate was measured by a method similar to that of Green and Mommaerts (14) with an automatic pH-stat apparatus (Radiometer model TTT1 titrator, SBR2 titrigraph, and SBU1 syringe burette assembly). A combination electrode (A. H. Thomas Company, No 4858-L15) was employed, and the titrating agent was 20 mM KOH delivered from a 0.5-m1 syringe. Since the samples used for ATPase measurements were identical with those used in viscosity and turbidity measurements, they contained 10 mM imidazole buffer at pH 7, but the rate of hydrogen ion production was sufficient in all experiments so that the presence of the buffer did not significantly affect the pH-stat measurements. The reaction mixture was placed on the pH-stat apparatus, and after the measured pH had stabilized satisfactorily (as indicated by an expanded scale pH recording), the ATPase reaction was started by the addition of myosin or HMM, except where otherwise noted. If actin was to be present in the sample, it was added only a few minutes before the myosin and HMM. The reaction was allowed to continue to completion to determine the total amount of KOH consumed during hydrolysis of the known total amount of ATP initially present, and from this value the equivalence between ATP hydrolyzed and KOH added was determined. In this way we could also determine the amount of ATP which had been hydrolyzed at any time during the reaction, and, in some of the figures to be presented here, the ATPase rate or viscosity is shown as a function of the ATP concentration remaining in the reaction mixture rather than as a function of time.

Viscosity—Viscosity measurements were performed with Ostwald viscometers having an outflow time for water of about 70 sec and a calculated velocity gradient of approximately 2300 sec−1. In all cases the reaction was initiated by the addition of the protein components to the complete reaction mixture in a test tube. (As in the ATPase samples, the myosin or HMM was always added after the actin.) After 30 sec of rapid mixing with a magnetic stirrer, 6 ml of the sample were pipetted into the viscometer. For actomyosin and acto-HMM samples, measurements were made as often as possible thereafter throughout the course of the reaction; whereas for control samples containing actin or myosin alone, five readings were taken and the results averaged.

Turbidity—Turbidity (9) was measured as absorbance at 675 μm in a Bausch and Lomb Spectronic 20 colorimeter, with a test tube (18 × 150 mm) as a cuvette. The sample, prepared in this test tube in the same manner as described above for the viscosity measurements, was constantly stirred in the water bath except for the intervals of a few seconds every minute or so when turbidity readings were being taken.

Theoretical Values for Complete Dissociation—In many cases the measured values for various properties of actomyosin and acto-HMM are reported under “Results” as multiples of the theoretical value expected in the absence of interaction between the actin and the myosin or HMM. For turbidity this theoretical value is taken to be equal to the sum of the turbidities of the actin and myosin measured individually; for ATPase it is taken to be equal to the ATPase of the myosin or HMM measured in the absence of added actin; and for viscosity the theoretical value of log ηrel is taken to be equal to the sum of log ηrel for the actin and the myosin or HMM measured individually (9).

In all cases, of course, the conditions for measurement of these parameters in the actin and the myosin or HMM individually were identical with the conditions used in the corresponding actomyosin or acto-HMM samples. In the experiments reported here, the theoretical values of ATPase rate varied between 0.009 and 0.012 amol per mg of myosin per min for actomyosin and between 0.018 and 0.025 amol per mg of HMM per min for acto-HMM. The theoretical value of viscosity (log ηrel) varied between 0.223 and 0.263 for actomyosin and between 0.152 and 0.170 for acto-HMM; and the theoretical value of actomyosin turbidity (A290) varied between 0.08 and 0.12.

Protein Concentration—Protein concentrations were routinely determined by ultraviolet absorption at 280 μm. The extinction coefficients used were 545 cm2 per g for myosin A (15), 647 cm2 per g for HMM (16), and 1149 cm2 per g for F-actin. This extinction coefficient for actin was determined by Dr. J. E. Estes in this laboratory with the use of the micro-Kjeldahl technique and an assumed nitrogen content of 16%.

Reagents—All reagents were of analytical grade, and deminer-
alized water was used for all solutions. The ATP was purchased from Sigma and was used without further purification. Stock solutions were prepared and neutralized to pH 7.0 with NaOH, and concentrations were determined spectrophotometrically.

**RESULTS**

**ATPase and Viscosity of Acto-HMM**—The first point we investigated was the relationship between the activation of the HMM ATPase by actin and the viscosity of the acto-HMM sample. Table I shows the initial ATPase and viscosity of acto-HMM at two different KCl concentrations, as well as the viscosity after all the ATP had been hydrolyzed. Both ATPase activity and viscosity are expressed as multiples of the theoretical values to be expected in the absence of any interaction between the actin and HMM. As can be seen, acto-HMM shows no detectable increase in its viscosity over this theoretical value when the ATPase is activated either 4-fold or 17-fold by actin, although a large viscosity rise did occur after the ATP was completely hydrolyzed. Therefore, under these conditions the acto-HMM complex responsible for the 17-fold ATPase activation does not give rise to a measurable increase in the viscosity of the sample above that predicted for a noninteracting mixture of actin and HMM.

**ATPase Activity during Clearing**—In the hope of correlating the studies on acto-HMM with similar studies on actomyosin in a dissolved state, we turned to an investigation of the interaction of actin and myosin during clearing. That the myosin ATPase can indeed be activated by actin during clearing is exhibited in Fig. 1, which shows the changes in ATPase activity and turbidity of two actomyosin samples. Both turbidity and ATPase activity are expressed as multiples of the theoretical values to be expected in the absence of interaction between the two proteins. In the sample shown by the dotted curves in Fig. 1, although the ATPase activity was initially 4 times that of the myosin and rose during the course of the reaction, the turbidity remained equal to the theoretical value until the ATPase rate reached approximately 8 times that of the myosin. At this point the turbidity rose abruptly to more than 7 times its theoretical value as the actomyosin superprecipitated. The solid curves in Fig. 1 show that when superprecipitation was delayed 30 min to a higher ionic strength, the ATPase rate was nearly 3 times that of the myosin alone, while the turbidity was again essentially equal to the theoretical value for no interaction. Moreover, throughout the 30 min of clearing, the ATPase rate and turbidity remained constant, an indication that the actin can activate the myosin ATPase for long periods without incipient superprecipitation occurring.

The fact that the turbidity of the actomyosin during clearing is essentially equal to that of actin and myosin alone, both of which are soluble under these conditions, suggests that the interaction of actin and myosin does not lead to any precipitation. To confirm this, an actomyosin sample was prepared which cleared for 6 min, during which time the myosin ATPase was activated five to ten times by the actin. Despite this enzymatic interaction of the actin and myosin, when an identical sample was centrifuged at approximately 5000 × g for 5 min, starting 30 sec after the addition of the myosin, no sediment appeared. On the other hand, at 7 min, 1 min after the actomyosin superprecipitated, all of the protein was sedimentable by 15 sec of centrifugation. This further confirms that the interaction between actin and myosin during clearing does indeed take place in the dissolved state.

It must be noted, however, that in order to show the interaction between actin and myosin in solution in this manner, two requirements of experimental technique must be satisfied. First, the myosin rather than the ATP must be the last component added to the sample, and second, very fresh myosin must be used. With regard to the first requirement, the solid curves in Fig. 2, a and b, show that when the actomyosin gel was preformed and the reaction was then initiated by the addition of ATP, both the initial turbidity and the ATPase were higher than was observed when the reaction was initiated by the addition of myosin. Moreover, in the former case there was a gradual rise in turbidity throughout the reaction, in contrast to the latter case in which the turbidity remained essentially constant until superprecipita-

![Fig. 1](http://www.jbc.org/DownloadedFrom)
Effect of adding ATP to the sample after myosin or of using aged myosin. a, ATPase; b, turbidity. Conditions were as follows: 2 mM ATP, 1 mM MgCl₂, 10 mM imidazole buffer (pH 7.0), 0.11 M KCl, 0.5 mg per ml of actin, 2 mg per ml of myosin. O and Δ, fresh myosin added to sample last, after ATP; ○ and △, 10-day-old myosin added to sample last. See “Methods” for theoretical values.

The observation may be related to the finding of Tonomura and Yoshimura (3) that addition of excess ATP to superprecipitated actomyosin did not return it to the cleared state. With regard to the requirement for fresh myosin, the dotted curve in Fig. 2b shows that with 10-day-old myosin, gradual superprecipitation occurred even when the myosin was added to the sample last. This may be related to the finding that the oxidation of myosin sulfhydryl groups, which is known to occur with aging, causes inhibition of the clearing response (3, 4, 6), or it may be related to the finding that myosin gradually aggregates with time (17, 18). At any rate, as long as fresh myosin is used and the myosin is added to the sample last, essentially no change in turbidity occurs during clearing.

Viscosity of Actomyosin during Clearing—To determine whether the interaction of actin and myosin in solution, like that of actin and HMM, occurs without a viscosity increase, the viscosity of an actomyosin sample was compared with that of the actin and myosin measured individually. Fig. 3 shows the time course of the changes in viscosity and turbidity of a typical actomyosin sample, and it can be seen that whereas the sample remains completely cleared with no evidence of precipitation, the viscosity is initially 1.17 times the theoretical value and rises throughout the period of clearing. While an actin-myosin interaction sufficient to lead to a viscosity increase might be expected to give rise to changes in light scattering as well, it should be pointed out that the turbidity measurements used here are only sensitive enough to detect precipitation of the actomyosin and not the smaller light scattering changes which might accompany the formation of the actomyosin complex in solution.

In Fig. 4 changes in viscosity are shown together with simultaneous measurements of ATPase activity for two different actomyosin samples with different durations of clearing. Here again, turbidity measurements (not shown in the figure) detected no evidence of precipitation until the full superprecipitation occurred at the time marked by the arrow on each curve. It can be seen that in both cases, increases in the activation of the myosin ATPase were accompanied by corresponding increases in the viscosity of the solution. However, in the sample shown by the solid curves in Fig. 4, although both the ATPase and viscosity were initially above theoretical levels, no significant change in viscosity occurred during the period in which the ATPase was constant, an indication that the rise in viscosity is not a time-dependent process unrelated to ATPase activation.

To rule out the possibility that the increase in viscosity of the actomyosin sample was simply due to aggregation of the myosin as the ATP concentration decreased, the viscosity of a sample of myosin alone was measured (Fig. 5, solid curve), and essentially no change in viscosity occurred as the ATP was hydrolyzed. Furthermore, a control sample of actomyosin in 0.5 M KCl, in the presence of ATP (Fig. 5, dotted curve), showed essentially a theoretical value.
Fig. 4. Viscosity and ATPase of actomyosin. Conditions were as follows: 2 mM ATP, 1 mM MgCl₂, 10 mM imidazole buffer (pH 7.0), 0.5 mg per ml of actin, 2 mg per ml of myosin. ▲ and △, ATPase; ■ and ○, viscosity; solid symbols, 0.105 M KCl; open symbols, 0.13 M KCl. Arrows indicate the time of onset of superprecipitation. See “Methods” for theoretical values.

Theoretical value for viscosity despite repeated measurements (until the ATP concentration fell to zero); this demonstrates that under conditions of complete dissociation the measured value is indeed equal to the calculated theoretical value for no interaction and is not altered by repeated passage of the solution through the capillary tube of the viscometer.

In actomyosin at low ionic strength, an increase in ATPase activation occurs, not only as the ATP concentration falls in the course of the reaction, but also when the ionic strength of the sample is lowered. Table II shows an experiment in which the initial ATPase, turbidity, and viscosity of cleared actomyosin were compared at various KCl concentrations, all measurements being made within 3 min after the start of the reaction. It can be seen that increased activation of the myosin ATPase by actin was accompanied in all cases by an increase in the initial viscosity of the actomyosin sample. Fig. 6 summarizes this type of data for several different protein preparations. Initial viscosity and turbidity are plotted against initial ATPase, which was varied by changing the KCl concentration of the sample. It can be seen that, even with various preparations of the proteins, there is a roughly linear relationship between the activation of the myosin ATPase by actin and the viscosity of the sample, and that essentially no change in turbidity accompanies this activation. All of these data, then, demonstrate that, unlike the situation in the acto-HMM system, a measurable viscosity increase always accompanies activation of the myosin ATPase by actin.

The increased viscosity during clearing leads to the conclusion that the transition from clearing to superprecipitation is not an all-or-none transition from complete dissociation to association, but rather a precipitation of protein which occurs only after a certain critical amount of binding of actin to myosin has occurred. If this is in fact the case, since both ATP and KCl tend to decrease the binding of actin to myosin, it might be expected that at higher KCl concentration the onset of superprecipitation would occur at a lower ATP concentration. As shown in the last column of Table II, this is indeed the case and confirms for actomyosin the relationship between the KCl concentration and "threshold" ATP concentration which we observed previously for the syneresis of myofibrillar fragments (19). We can therefore conclude that actin and myosin can form a stable viscous complex while in solution at low ionic strength, and that superprecipitation occurs only after a critical level of interaction, sufficient to precipitate the actomyosin complex, is reached.

Fig. 5. Viscosity of myosin A at low ionic strength and actomyosin at high ionic strength. Conditions were as follows: 2 mM ATP, 1 mM MgCl₂, 10 mM imidazole buffer (pH 7.0), 2 mg per ml of myosin. □, left ordinate, myosin viscosity, 0.11 M KCl; ■, right ordinate, actomyosin viscosity, 0.5 M KCl, 0.5 mg per ml of actin. See “Methods” for theoretical value.

### Table II

<table>
<thead>
<tr>
<th>Concentration of KCl</th>
<th>Multiples of theoretical values</th>
<th>Clearing time</th>
<th>ATP concentration at superprecipitation</th>
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<td>0.99</td>
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<td>9.0</td>
<td>14.5</td>
<td>2.33</td>
</tr>
</tbody>
</table>

* After superprecipitation.

No ATP.
Activation of HMM ATPase by Actin—Several studies on the ATPase activity of acto-HMM have recently been reported (7, 8, 20, 21), but it is still quite unclear how this ATPase activity compares with that of actomyosin under identical conditions. One of the difficulties in comparing the interaction of actin and myosin with that of actin and HMM at low ionic strength is that the former generally occurs in the precipitated state, while the latter occurs in solution. However, by making the comparison under conditions in which actin and myosin also interact in solution, this difficulty can be avoided. A striking difference between myosin and HMM in their interaction with actin is shown in Fig. 7, where the change in the actomyosin and acto-HMM ATPase with time, i.e., as the ATP concentration falls, is compared. The ATPase rates are plotted against the concentration of ATP remaining in the solution, rather than against time, so that the ATPase activities of the different samples can be compared at identical ATP and ADP concentrations. The point at which the actomyosin superprecipitated is marked with an arrow, and prior to this point the actomyosin was completely in solution. As can be seen, although under these conditions the ATPase activity of the actomyosin steadily increased as the ATP was hydrolyzed, the ATPase activity of the acto-HMM remained absolutely constant, which shows that acto-HMM does not respond to changes in the ATP and ADP concentrations as does actomyosin. Moreover, this difference in response to changing ATP and ADP concentrations occurred even when the ionic strength was adjusted so that the myosin and HMM were activated to the same extent at the start of the reaction (solid symbols in Fig. 7). It has been reported by Yagi, Nakata, and Sakashibara (20) that the ATPase of the acto-HMM does show a small increase as ATP is hydrolyzed, but these experiments were performed at a much lower KCl concentration than the experiments shown in Fig. 7. The distinct difference in the responses of actomyosin and acto-HMM to falling ATP concentration at 0.1 M KCl indicates that HMM differs from myosin, not simply in its solubility properties, but also in its interaction with actin.

**DISCUSSION**

At low ionic strength both myosin (22, 23) and actin are present in solution as long filaments, and all of the above data are consistent with the view that the formation of bonds between these actin and myosin filaments gives rise both to activation of the myosin ATPase and to the formation of a cross-linked network of actin and myosin filaments, which in turn gives rise to the increase in viscosity seen in the actomyosin solution. The formation of such a network would, of course, be a consequence of cross linking of separate actin filaments by the multiple binding sites of the myosin filaments and vice versa. At a relatively high KCl or ATP concentration, the filaments would be completely dissociated, but at a lower KCl or ATP concentration some bonds would form between the filaments, giving rise to both an increase in the ATPase and an increase in the viscosity due to network formation. Finally, at a still lower ATP or KCl concentration, the actomyosin network would be sufficiently cross-linked to undergo a phase transition to the gel state, which would lead immediately to superprecipitation under the influence of the remaining ATP. This suggests that the transition from clearing to superprecipitation may...
not always reflect an increase in actin-myosin interaction, but rather, under certain conditions, might result from an increase in network formation unrelated to an increase in the number of actin-myosin bonds. For example, the effect of the protein α-actinin on the transition from clearing to super-precipitation (24), which is much larger than its effect on the actomyosin ATPase (25), might be due to its ability to cross-link F-actin (26), rather than to any direct effect on the actin-myosin interaction.

If the viscosity increase of cleared actomyosin at low ionic strength is indeed due to network formation between actin and myosin filaments, then a similar viscosity increase would not be expected in the acto-HMM system, since HMM molecules occur as monomers and therefore would not be expected to form cross-links between actin filaments. The activation of the myosin and HMM ATPase may, therefore, be due to binding of actin to the same site on both proteins, with the difference in the viscosity change in the two systems being due simply to network formation in actomyosin and the absence of such network formation in acto-HMM in the presence of ATP. The viscosity increase in acto-HMM in the absence of ATP might then be explained by an increased interaction between the actin filaments themselves, when they are complexed with HMM in the absence of ATP, as Kitagawa and Gergely (27) have suggested in connection with their observation of turbidity changes in the acto-HMM system.

Although the difference in the viscosity of actomyosin and acto-HMM at low ionic strength in the presence of ATP may only reflect differences in the aggregation of myosin and HMM, we also found that, although the actomyosin ATPase markedly increased as the ATP concentration fell, there was no increase in the acto-HMM ATPase. Presumably, the increasing ATPase activation of actomyosin with decreasing ATP concentration results from the dissociative effect of ATP on the actomyosin complex. If so, then it follows that the difference in the response of actomyosin and acto-HMM to changing ATP concentration results from a difference in dissociability between the two systems. However, the possibility that the actomyosin and acto-HMM differ in other respects is certainly not ruled out, and further comparison of the actomyosin and acto-HMM under conditions where both are soluble will be necessary to establish the exact relationship between the two systems.

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The Interaction of Actin with Myosin and Heavy Meromyosin in Solution at Low Ionic Strength
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