Actin Activation of Heavy Meromyosin Adenosine Triphosphatase

DEPENDENCE ON ADENOSINE TRIPHOSPHATE AND ACTIN CONCENTRATIONS*

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SUMMARY

Actin activation of the ATPase activity of heavy meromyosin, a tryptic digestion product of myosin, was studied over a wide range of actin and ATP concentrations with creatine kinase and creatine phosphate added to hold the ATP concentration constant. Double reciprocal plots of ATPase against ATP concentration at varied actin concentration and of ATPase against actin concentration at varied ATP concentration were linear, suggesting that the system obeys simple Michaelis kinetics. From extrapolation of these plots we found that not only does ATP increase the dissociation of actin from heavy meromyosin but, analogously, the Michaelis constant of the acto-heavy meromyosin ATPase is greater than that of the heavy meromyosin ATPase. However, whereas the dissociation of actin from the heavy meromyosin-ATP complex is markedly dependent on ionic strength, the Michaelis constant of the acto-heavy meromyosin ATPase shows no salt dependence whatever. These data have important implications for the kinetic model which we previously proposed for the actin-heavy meromyosin-ATP system.

It has long been known that actin activates the Mg-ATPase activity of myosin at low ionic strength, and there have been several studies on the ATP dependence of the actomyosin ATPase (2–4). However, complete kinetic studies involving variation of both the ATP and actin concentrations have been prevented by the fact that actomyosin is precipitated at low ionic strength, which makes systematic variation of the free actin concentration essentially impossible. Of course, this problem does not arise with heavy meromyosin which forms a soluble complex with actin at low ionic strength and Sekiya, Takeuchi, and Tonomura (5), did study the dependence of the HMM ATPase on actin and ATP concentration, although they did not attempt a detailed kinetic analysis. We have previously shown that double reciprocal plots of HMM ATPase against actin concentration are linear in the presence of excess ATP (6, 7), and we have now extended these studies to much lower ATP concentrations with the creatine kinase-creatine phosphate system (EC 2.7.3.2) added to hold the ATP concentration constant. We have found that, over a wide range of actin concentration, double reciprocal plots of ATPase against ATP concentration are linear, and the system can therefore be analyzed in terms of the steady state kinetics of a simple enzyme-substrate-modifier model as recently described by London (8).

METHODS

Protein Preparations—Actin and HMM were prepared as we described previously (6, 9). Creatine kinase was prepared from rabbit muscle by the method of Kuby, Noda, and Lardy (10) with the addition of a final treatment with Norit-A charcoal to remove contaminating nucleotide (11). All protein concentrations were determined as previously (9) by ultraviolet absorption at 280 nm. The extinction coefficient used for creatine kinase was 890 cm⁻¹ per g (12).

ATPase Activity—In all experiments the ATP concentration was maintained with the use of the creatine kinase-creatine phosphate system. In this coupled system, the rate of ATP hydrolysis is equal to the rate of breakdown of the creatine phosphate, and this reaction in turn consumes approximately 0.3 mole of H⁺ per mole of creatine phosphate degraded at pH 7; therefore, we could measure the rate of the reaction with the pH-stat with HCl as the titrant. As in our previous work (6, 9), we allowed the reaction to continue to completion to determine the total amount of HCl consumed during hydrolysis of a known total amount of creatine phosphate, and from this value we determined the exact equivalence between creatine phosphate breakdown and HCl added.

Since the measured ATPase rates were always constant throughout the reaction even when the creatine phosphate

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Actin Activation of Heavy Meromyosin ATPase Vol. 245, No. 9

FIG. 1. Dependence of the HMM ATPase on added ATP concentration at fixed actin concentrations. Conditions: 2.5 mM MgCl₂, 2.5 mM creatine phosphate, 1 mg of creatine kinase per ml, 0.036 mg of HMM per ml.

RESULTS

In Fig. 1, the measured ATPase rate of a mixture of actin and HMM is plotted against the added ATP concentration. The resulting curve appears to obey a simple Michaelis hyperbolic relation except that a small but significant ATPase activity is observed even when no ATP is added. This occurs because, at very low ATP concentrations and relatively high actin concentrations, the small amount of free nucleotide inevitably present in the actin solution makes a significant contribution. Not only is a small portion of the actin-bound ADP always released into solution, but it is also impossible to remove all traces of the free ATP which is added during preparation of the F-actin solution, and these small amounts of contaminant nucleotide are, of course, maintained as ATP by the creatine kinase-phosphocreatine system.

Before further kinetic studies could be carried out, it was necessary to determine the contaminant ATP concentration at each actin concentration so that we could know the true substrate concentration in the reaction mixtures. This was done by the following kinetic method, which is based on the assumption that the system does indeed obey Michaelis kinetics.

The Michaelis equation can be rearranged to give Equation 1, in which \( v \) = the ATPase rate; \( V'_{\text{max}} \) = the ATPase rate at infinite ATP concentration with the given actin concentration; \( K' \) = the apparent Michaelis constant; and the total ATP concentration is given by the sum of \( S' \), the added ATP concentration, and \( S'' \), the contaminant ATP concentration.

\[
S' = K'v/(V'_{\text{max}} - v) - S''
\]  

(1)

First, the contaminant ATP concentration was very roughly estimated by extrapolating plots like those in Fig. 1 to their intercepts on the abscissa. These values were used to calculate the approximate total ATP concentrations so that Lineweaver-Burk double reciprocal plots of ATPase rate against ATP concentration could be constructed for determination of \( V'_{\text{max}} \). Since these plots were most accurate at high ATP concentration, a precise value of \( V'_{\text{max}} \) could be obtained by extrapolation to the ordinate. With this value of \( V'_{\text{max}} \), the data could then be plotted as \( v/(V'_{\text{max}} - v) \) according to Equation 1 and extrapolated to the value of \(-S''\) given by the intercept on the ordinate. As illustrated in Fig. 2, these plots were indeed linear, confirming the applicability of Equation 1 and suggesting that the system does indeed obey Michaelis kinetics. The contaminant ATP concentration at each concentration of actin, obtained from the ordinate intercept of the corresponding plot in Fig. 2, plus the added ATP concentration gives the actual free ATP concentration present in the solution. In practice, since the HMM concentration was about 0.1 \( \mu \text{M} \), which is 0.2 concentration had dropped far below its Michaelis constant, it is clear that the rate of the over-all coupled reaction was not limited by the rate of the creatine kinase reaction; i.e. the creatine kinase concentration was sufficient to maintain essentially all of the free nucleotide as ATP. Furthermore, since creatine kinase has a relatively high turnover rate per mole of enzyme and a relatively weak affinity for ATP (13), the complexes of ADP and ATP with creatine kinase represented a negligible fraction of the total nucleotide even at the lowest ATP concentration used.
Fig. 3. Reciprocal plots of acto-HMM ATPase data under conditions of Fig. 1. a, dependence of ATPase rate on ATP concentration at various fixed actin concentrations. b, dependence of ATPase rate on actin concentration at various fixed ATP concentrations. (Points in b taken from curves in a.)

μM in ATP-binding sites (1, 14), we could only know the free ATP concentration with an accuracy of about ±0.2 μM. Therefore, for the double reciprocal plots which follow, data were not taken at ATP concentrations below about 1.5 μM.

Double reciprocal plots based on the data shown in Fig. 2, as well as data obtained at two intermediate actin concentrations, are shown in Fig. 3a. As can be seen, at all four actin concentrations the plots were linear over a range of ATP concentration from 1.5 μM to 50 μM. Furthermore, as shown in Fig. 4, Hill plots of the data are also linear with a slope equal to 1. It would therefore appear that, over a wide range of ATP and actin concentrations, the acto-HMM ATPase system obeys simple Michaelis-Menten kinetics.

In addition to the plots of 1/v against 1/ATP at varied actin concentration shown in Fig. 3a, a complete kinetic analysis requires plots of 1/v against 1/actin at various fixed ATP concentrations. Since the contaminant ATP concentration was different at different actin concentrations, it was difficult to vary the actin concentration at constant ATP concentration. Therefore, data were taken from the plots of 1/v against 1/ATP shown in Fig. 3a to construct the plots of 1/v against 1/actin which are shown in Fig. 3b. As can be seen, these plots were also linear over the range of ATP concentration tested. It was not possible to determine whether the sets of lines in Fig. 3, a or b, were parallel or whether they intersected somewhere in the lower left quadrant; however, it is clear that in neither Fig. 3a nor Fig. 3b do the plots intersect on the ordinate or in the upper left quadrant, a finding which is important for our later discussion of the velocity equations applicable to this system.

In effect, the ordinate intercepts of the plots in Fig. 3a give a measure of the dependence of the ATPase rate on actin concentration at infinite ATP and, analogously, the ordinate intercepts of the plots in Fig. 3b give a measure of the dependence of the ATPase rate on ATP concentration at infinite actin. These intercepts are plotted in double reciprocal form in Fig. 5, a and b. In both of these figures, the intercept on the ordinate gives the ATPase activity when both the ATP and actin concentrations approach infinity, i.e. the true V_max of the acto-HMM, which...
turns out to be about 4 μmoles per mg-min, in agreement with the value for $V_{\text{max}}$ that we previously obtained from 1/$v$ against 1/actin plots at high concentrations of ATP (6).

The intercept on the abscissa of Fig. 5b gives the Michaelis constant of the acto-HMM ATPase and, analogously, the intercept on the abscissa of Fig. 5a gives the dissociation constant of actin from HMM at infinite ATP concentration, i.e. of actin from the HMM-ATP complex. Assuming a molecular weight of 47,000 for actin, the latter dissociation constant is found from Fig. 5a to be about 13 μM. This is a smaller dissociation constant than we found in our previous studies (6), but this was expected because the ionic strength was lower in the present work. Nevertheless, the dissociating effect of ATP on acto-HMM is still evident if this value is compared with the dissociation constant of about 2 μM obtained by Young in the absence of ATP (15), although it should be noted that Young’s dissociation constant was determined as a lower temperature and a higher ionic strength than we used here.

The Michaelis constant of the acto-HMM ATPase was found from the abscissa intercept of Fig. 5b to be about 6 μM, which is larger than the reported values of 0.5 to 1 μM for the Michaelis constant of the HMM ATPase (1, 14). It would therefore appear that, just as ATP increases the dissociation constant of

![Fig. 5](image-url)  
**Fig. 5.** a, reciprocal plots of ATPase rate at infinite ATP concentration ($V_{\text{max}}$) against actin concentration. Values of $V_{\text{max}}$ were obtained from ordinate intercepts in Fig. 3a. b, reciprocal plots of ATPase rate at infinite actin concentration ($V_{\text{max}}$) against ATP concentration. Values of $V_{\text{max}}$ were obtained from ordinate intercepts in Fig. 3b.

![Fig. 6](image-url)  
**Fig. 6.** Reciprocal plots of acto-HMM ATPase data at higher ionic strength. Conditions: same as in Fig. 3 with the addition of 20 mM KCl. a, dependence of ATPase rate on ATP concentration at various fixed actin concentrations. b, dependence of ATPase rate on actin concentration at various fixed ATP concentrations. (Points in b taken from curve 5 in a.)
actin from HMM, so too does actin increase the Michaelis constant of the HMM ATPase.

We next investigated the relative effects of ionic strength on the dissociation of actin from HMM-ATP and on the Michaelis constant of the acto-HMM ATPase. Fig. 6, a and b, shows plots of 1/v against 1/ATP at varied actin and 1/v against 1/actin at varied ATP, respectively, at an ionic strength about twice that used in Fig. 3, and here again both sets of plots are linear. The plots in Figs. 3b and 6b have markedly different slopes (note difference in abscissa scale), as is expected from our previous finding that the binding of actin to HMM-ATP shows a marked salt dependence (6); however, the plots of 1/v against 1/ATP in Figs. 3a and 6a have essentially identical slopes, which suggests that the Michaelis constant of the acto-HMM ATPase shows little or no salt dependence. These conclusions are confirmed in Fig. 7, a and b, which shows reciprocal plots of the ordinate intercepts of Fig. 6, a and b, respectively, as well as similar data at three other ionic strengths. In two of the other plots, the ionic strength was altered by varying the KC1 concentration, while in the third the creatine phosphate concentration was changed. The ordinate intercepts of Fig. 7, a and b, show that ionic strength has essentially no effect on Vmax, while the abscissa intercepts of Fig. 7a show the marked effect of ionic strength on the dissociation constant of actin from the HMM-ATP complex—both of these effects being in agreement with our previous findings from double reciprocal plots of ATPase against actin at high ATP concentration (6). However, the abscissa intercepts of Fig. 7b show, as we suggested above, that ionic strength has no significant effect on the Michaelis constant of the acto HMM ATPase. Thus, there is a clear difference in the effects of ionic strength on the dissociation constant of actin from HMM-ATP and on the Michaelis constant of the acto-HMM ATPase.

**Discussion**

The foregoing data are considered in relation to the kinetic model which we have previously applied to this system (6, 7). This model is shown below, together with definitions of certain constants which we will find useful:

\[
\begin{align*}
&\text{AM} \\
&\text{M} \\
&\text{MS} \\
&\text{AMS} \\
&\text{M + products} \\
&\text{AM + products}
\end{align*}
\]

In this model, A represents actin, M represents HMM, and S represents the substrate, ATP. Depending on the relative values of the rate constants in the above kinetic model, many different rate equations based on the model can be written. Only a few of these equations, however, are consistent with linear 1/v against 1/S plots, and since we find linear 1/v against 1/S plots for our system, these are the only equations with which we need be concerned. In a recent paper, London (8) has listed the 11 velocity equations based on the above type of kinetic model which give linear plots of 1/v against 1/S at various concentrations of modifier (actin). Of these equations, some can be ruled out because they require that the plots of 1/v against 1/S at different actin concentrations intersect at a single point on the ordinate or in the upper left quadrant, which clearly does not occur with our system. Others can be ruled out either because they require actin to inhibit rather than activate the HMM ATPase, or because they require that K1 or K2 be infinite, which would contradict the obvious fact that HMM can bind to actin in the absence of nucleotide and can hydrolyze nucleotide in the absence of actin. Finally, several equations can be
ruled out because they are not consistent with our finding that plots of \(1/v\) against \(1/\text{actin}\) at varied ATP concentrations are linear. It therefore turns out that, in all likelihood, Equation 2 below is the only velocity equation which is consistent both with our data and with the above kinetic model.

\[
\frac{1}{v} = \frac{K_3'K_2 + K_1(A + (K_4 + A))S}{(k_3K_2 + k_4A)S}
\]  
Equation 2

Our experiments were done at actin concentrations comparable to \(K_s\), the dissociation constant of actin from the HMM-ATP complex (abscissa intercept of Fig. 5a or 7a). On the other hand, \(k_4\), which is the same as \(V_{max}\) in our plots, is some 200-fold larger than \(k_3\), the ATPase activity of HMM alone (6). Therefore, \(k_4K_4 < k_3A\), and Equation 2 is consistent with our finding of linear plots of \(1/v\) against \(1/\text{actin}\). It also predicts that all of the plots of \(1/v\) against \(1/S\) at different actin concentrations will intersect each other at a single point in the lower left quadrant of the graph, as will all of the plots of \(1/v\) against \(1/\text{actin}\). Our data are not accurate enough at low ATP and low actin concentration to permit these extrapolations; however, in preliminary experiments with ITP rather than ATP as the substrate, it does indeed appear that the plots of \(1/v\) against \(1/\text{ITP}\) intersect each other at a single point, as do the plots of \(1/v\) against \(1/\text{actin}\) in the presence of ITP when the plots are corrected for the appreciable ITPase activity of HMM alone.2

If we make the one further assumption that the rates of the reactions involving the binding of ATP or actin to HMM alone are not negligible in our steady state system, then Equation 3 below, which London (8) has described as an analogue of detailed balance, follows directly from Equation 2 and should hold true for our system.3

\[
\frac{K_3}{K_2} = \frac{K_4'}{K_1'}
\]  
Equation 3

The first point to be noted about this equation is that if the binding of ATP to HMM weakens the binding of actin, i.e. if \(K_3 > K_2\), then it is required that the Michaelis constant of the acto-HMM ATPase (\(K_4'\)) be greater than the Michaelis constant of the HMM ATPase (\(K_1'\)). In fact, as we noted earlier, the data from Fig. 5. a and b, indicate that this is indeed the case, at least qualitatively, for our system.

2 M. Wicha and C. Moos, unpublished data.
3 W. London, personal communication.

Equation 3 also makes another prediction. Since we found, as shown in Fig. 7b, that the Michaelis constant for the acto-HMM ATPase shows no dependence on ionic strength, and since the Michaelis constant of the HMM ATPase also shows no salt dependence (14), it follows that the left-hand side of Equation 3 must also be independent of ionic strength. Therefore, since we showed in Fig. 7a that the dissociation constant of actin from HMM-ATP shows a marked salt dependence, it follows from Equation 3 that the dissociation constant of actin from HMM in the absence of ATP must show the same marked salt dependence. It is not known at the present time whether the binding of actin to HMM does indeed show a marked dependence on ionic strength. If it does not, however, then it follows either that one or more reactions in the above kinetic model occur at negligible rates, or that this simple kinetic model is not sufficient to describe our system and a more complex model is required. Application of this test of our kinetic analysis will have to await an accurate independent measure of the binding between actin and HMM in the absence of nucleotide at varied ionic strength.

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