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STEADY-STATE STUDIES OF THE ACTIN-ACTIVATED ADENOSINE TRIPHOSPHATASE ACTIVITY OF MYOSIN

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Summary

Reconstituted actomyosin (ATP phosphohydrolase, EC 3.6.1.3) (0.400 mg F-actin/mg myosin) in 10.0 μM ATP loses 96% of its specific ATPase activity when its reaction concentration is decreased from 42.0 μg/ml down to 0.700 μg/ml. The loss of specific activity at the very low enzyme concentrations is prevented by the addition of more F-actin to 17.6 μg/ml. It is concluded that at low actomyosin concentrations the complex dissociates into free myosin with a very low specific ATPase activity and free F-actin with no ATPase. The dissociation of the essential low molecular weight subunits of myosin from the heavy chains at very low actomyosin concentrations may be a contributing factor.

Actomyosin has its maximum specific activity at pH 7.8—8.2. The K_m for ATP is 9.4 μM, which is at least 20-fold greater than myosin's K_m for ATP. The actin-activated ATPase of myosin follows hyperbolic kinetics with varying F-actin concentrations. The K_m values for F-actin are 0.110 μM (4.95 μg/ml) at pH 7.4 and 0.241 μM (10.8 μg/ml) at pH 7.8. The actin-activated maximum turnover numbers for myosin are 9.3 s^{-1} at pH 7.4 and 11.6 s^{-1} at pH 7.8. The actomyosin ATPase is inhibited by KCl. This KCl inhibition is not competitive with respect to F-actin, and it is not a simple form of non-competitive inhibition.

Introduction

In the presence of Mg^{2+} myosin (ATP phosphohydrolase, EC 3.6.1.3) catalyzes a very low hydrolysis of ATP to yield ADP and P_i. Addition of F-actin to a solution of myosin results in the formation of actomyosin, which has a 50-fold greater ATPase activity. Bárány [1] has shown that the maximum shortening speed of muscles from a wide variety of animals is directly proportional to

the actin-activated ATPase activity of the myosins prepared from these muscles. This proportionality was also found for the various muscles of cats and sloths [2]. These findings helped establish that the muscle contraction speed is determined by the actin-activated ATPase of the muscle's myosin.

Most of our knowledge of the steady-state kinetics of the actin-activated ATPase of myosin comes from studies with heavy meromyosin and subfragment [3—5]. Levy and his associates [6,7] have reported studies of the effect of ATP variation on the ATPase and superprecipitation activity of natural actomyosin. However, little is known about the kinetic properties of the actin-activated ATPase of purified myosin. In fact Burke et al. [8] have recently observed, "It is usually contended that the aggregated state of myosin at low ionic strengths precludes a systematic kinetic study of the interaction particularly in terms of its enzymatic behavior". They presented data to show that the actin-activated ATPase of skeletal muscle myosin does yield normal Michaelis-Menten kinetics with respect to F-actin. It has also been assumed that the steady-state ATPase properties of acto-heavy meromyosin are identical to those of actomyosin.

A study of the actin-activated ATPase of myosin was undertaken to provide a better understanding of its catalytic properties. These studies show that actomyosin yields typical enzyme kinetic properties. While the actin-activated ATPase activities of myosin and heavy meromyosin are similar, they differ in their maximum turnover numbers, K_m values for F-actin, and mode of salt inhibition. A preliminary report of a portion of these results has been presented elsewhere [9].

Materials

The following materials were purchased: ATP from P-L Biochemicals, phosphocellulose from Bio-Rad and from Sigma Chemical Co. The [γ-^{32}P]ATP was synthesized and standardized as described previously [2].

Myosin was prepared from the back and hind leg muscles of male rabbits as described by Bárány and Oppenheimer [10]. The myosin was further purified by passage at 5° C through a column of Bio-Rad's phosphocellulose equilibrated in 0.040 M Tris · HCl and 0.40 M KCl at pH 7.8 as described by Harris and Suelter [11]. The protein's passage through the column in the above buffer was followed by its absorbance at 280 nm. The protein-containing fractions with an absorbance greater than 0.500 were combined and dialyzed overnight against 4 l of 0.01 M KCl at 5° C to precipitate the protein. The suspension was centrifuged at 36000 × g for 15 min and the supernatant was discarded. The precipitate was homogenized in a minimum amount of 3.0 M KCl and then diluted with water to yield 0.60 M KCl, pH 7.0. This solution was centrifuged at 92000 × g for 2 h to remove insoluble material. This chromatography procedure was originally employed to remove adenylic acid deaminase activity that contaminates the myosin. During this investigation it was found that Bio-Rad's phosphocellulose yields a myosin with a higher actin-activated V (1.3 μmol P_i formed/min per mg myosin) than does the phosphocellulose from Sigma (0.43 μmol P_i formed/min per mg myosin). Both phosphocelluloses remove the adenylic acid deaminase activity.

F-actin was prepared by the ATP and KI solution extraction of saline-insoluble rabbit muscle acetone powder [12] and by the Ca^{2+} and ATP solution extraction of the standard, myosin-free, rabbit muscle acetone powder [13]. The F-actin prepared by both extraction procedures was purified by solubilizing the protein pellet in Ca^{2+}, ATP, and thioglycerol solution to yield G-actin, clarification of the solution by ultracentrifugation, polymerization of the G-actin to F-actin, and collecting the F-actin pellet by ultracentrifugation [12]. The F-actin was homogenized in 0.10 M KCl containing 1.0 mM $MgCl_2$ and stored at 0° C. The F-actin prepared by both procedures yielded identical K_m and ΔV values for the actin-activated myosin ATPase.

The molecular weight for the monomer of F-actin is 45000 [13], and the molecular weight of myosin is 470000 [14] for the calculation of turnover numbers.

Methods

ATPase determination with [γ-^{32}P]ATP. When the concentration of ATP was less than 0.10 mM, the ATPase was determined using [γ-^{32}P]ATP as the substrate and following the formation of $^{32}P_i$. The reaction mixture consisted of 0.0200 M Tris · HCl, 1.00 mM $MgSO_4$, 6—30 mM NaCl plus KCl, myosin, F-actin, bovine serum albumin, and [γ-^{32}P]ATP at pH 7.4. The bovine serum albumin was added to protect the actomyosin from non-specific denaturation during high dilution. The total protein concentration was 0.0500 mg/ml. The reactions were initiated by addition of either the ATP solution or the actomyosin solution, which had been prepared by mixing myosin with F-actin in 0.30 M KCl. The reactions were incubated at 25° C. At varying time intervals 2.00-ml samples were assayed for $^{32}P_i$ by the procedure of Schliselfeld and Bárány [15].

Colorimetric ATPase determination. When the reaction mixture contained 1.00 mM ATP, the P_i formed was measured by a colorimetric procedure. Myosin in F-actin were mixed together in 0.30 M KCl to yield actomyosin. A 7.80 ml buffer mixture was prepared with the actomyosin and incubated at 25° C for 5 min or more. The reaction was initiated by the addition of 0.20 ml 40 mM ATP. The reaction contained 0.0200 M Tris · HCl, 1.00 mM $MgSO_4$, 6—115 mM NaCl and KCl, myosin, F-actin, and 1.00 mM ATP at designated pH values. The reaction mixtures were incubated at 25° C for a sufficient time period to form 0.4—3.5 μmol of P_i. The myosin concentration for actin-activated ATPase studies was 10—30 μg/ml, which is too low to detect settling out of the actomyosin. The reactions were stopped by the addition of 0.60 ml of 0.835 M H_2SO_4. Zero time samples were prepared by adding the 0.60 ml of 0.835 M H_2SO_4 before adding the ATP solution. Color was developed by adding to each acidified mixture 0.90 ml of 2.78% (w/v) ammonium molybdate in 2.22 M H_2SO_4 and 0.50 ml of the aminonaphthol sulfonic acid solution [2]. After 5 min at room temperature the solutions were read in the Klett-Summerson photoelectric colorimetric using filter No. 66. This assay can only be employed for reactions containing 0.6 mg or less protein, because high protein concentration precipitates the colored material that forms.

For the determination of the Mg^{2+}-ATPase of myosin without F-actin it was necessary to employ high myosin concentration. Then each reaction was stop-

ped by adding 1.00 ml of 5.0 M $HClO_4$ to the 8.00 ml reaction mixture. Each suspension was centrifuged in a clinical centrifuge, the supernatant was transferred to a clean test tube, and the color was developed by the addition of 0.50 ml 5.0% (w/v) ammonium molybdate and 0.50 ml of aminonaphthol sulphonic acid solution [16]. The solutions were read after 5 min in the Klett-Summerson photoelectric colorimeter using filter No. 66. No significant difference was found for the extinction coefficients in H_2SO_4 and $HClO_4$.

Specific activity calculations. The hydrolysis of ATP is a first-order reactior (Fig. 1). When the percent hydrolysis is equal to or less than 25% of the zero

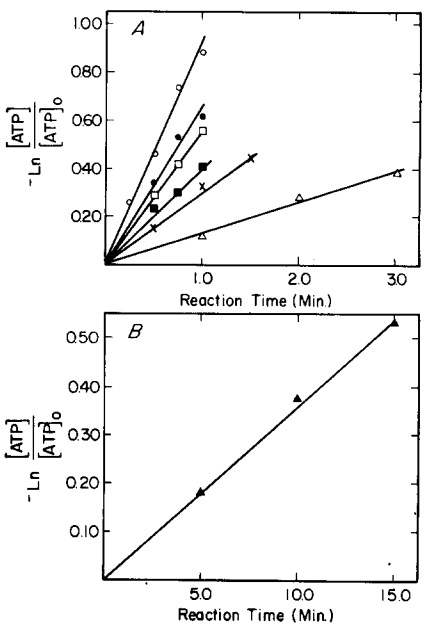

Fig. 1. Time course of ATP hydrolysis by actomyosin. (A) The substrate solution and actomyosin mixture in 0.30 M KCl are pre-incubated at 25° C for at least 5 min. The reactions are started by adding 0.10 ml of the thoroughly mixed actomyosin mixture to 1.90 ml of substrate solution. After mixing throughly the reaction mixtures are incubated at 25° C. At varying time intervals 0.40 ml of 5.0 M $HClO_4$ containing 0.500 mM P_i is added to each reaction mixture to stop the reaction. Zero time reactions are prepared by adding the 0.40 ml of 5.0 M $HClO_4$/500 mM P_i solution before adding the 0.10 ml of enzyme mixture. All reaction mixtures contain 0.020 M Tris · HCl, 1.00 mM $MgSO_4$, 0.0150 M KCl, 10.0 μM [γ-^{32}P]ATP, bovine serum albumin, myosin, and F-actin at pH 7.4. The actomyosin consists of 0.400 mg F-actin/mg myosin. The total protein concentration is 0.0500 mg/ml. The zero time samples and each reaction mixture after acidification are assayed for $^{32}P_i$ by the procedure of Schliselfeld and Bárány [15]. The fraction of ATP remaining, $[ATP]/[ATP]_0$, is equal to 1.00 minus the fraction of $^{32}P_i$ formed. The concentration of actomyosin used are as follows: ○, 42.0 μg/ml; ●, 33.6 μg/ml; □, 28.0 μg/ml; ■, 21.0 μg/ml; X, 16.8 μg/ml; and △, 14.0 μg/ml. The correlation coefficients of the first-order plots are equal to 0.993—1.00. (B) After a 5 min incubation at 25° C of the substrate solution and of the actomyosin mixture the reaction is started by adding the ATP solution ot the enzyme mixture. The reactions contain 0.020 M Tris · HCl, 1.00 mM $MgSO_4$, 7.5 mM KCl, 28.5 μg/ml myosin, 58.8 μg/ml F-actin, and 1.00 mM ATP at pH 7.4. The reactions are carried out at 25° C. At varying time intervals 8.00-ml reaction mixtures are stopped by the addition of 0.60 ml of 0.835 M H_2SO_4. Zero time sample is prepared by adding the acid before adding the ATP solution. Each acidified reaction mixture is then mixed with H_2SO_4/ammonium molybdate solution and aminonaphthol sulfonic acid solution to develop color for the P_i colorimetric assay described under Methods. No settling of the actomyosin occurs at this enzyme concentration. The ratio of $[ATP]/[ATP]_0$ is equal to 1.00 minus the fraction of P_i formed. The correlation coefficient of the first-order plot is 0.999.

time ATP, the ATPase velocity is equal to the total P_i formed divided by the reaction time. When more than 25% of the zero time ATP is hydrolyzed, the ATPase velocity is calculated by multiplying the slope of the first-order plot as seen in Fig. 1 by the ratio of the ATP concentration at zero time divided by myosin concentration. Specific activities are the μmol P_i formed per min per mg myosin.

Other analytical procedures. Protein concentration was determined by precipitation of the protein in 5% trichloroacetic acid and assaying the precipitate for biuret protein as described previously [15]. Radioactivity was determined either by counting in a thin window radiation counter [15] or by scintillation counting [16].

Results

Effect of actomyosin concentration on its ATPase

First-order plots for actomyosin ATPase ($-\ln[ATP]/[ATP]_0$ versus time) are shown in Fig. 1. Fig. 1A shows that actomyosin (0.400 mg F-actin/mg myosin and 14.0—42.0 μg/ml actomyosin protein) in 0.0100 mM [γ-^{32}P]ATP yields a linear first-order plot. Fig. 1B shows actomyosin (58.8 μg/ml F-actin and 28.5 μg/ml myosin) in 1.00 mM ATP also yields a linear first-order plot. All of these plots have correlation coefficients of 0.98 or greater, which establishes their excellent linearity.

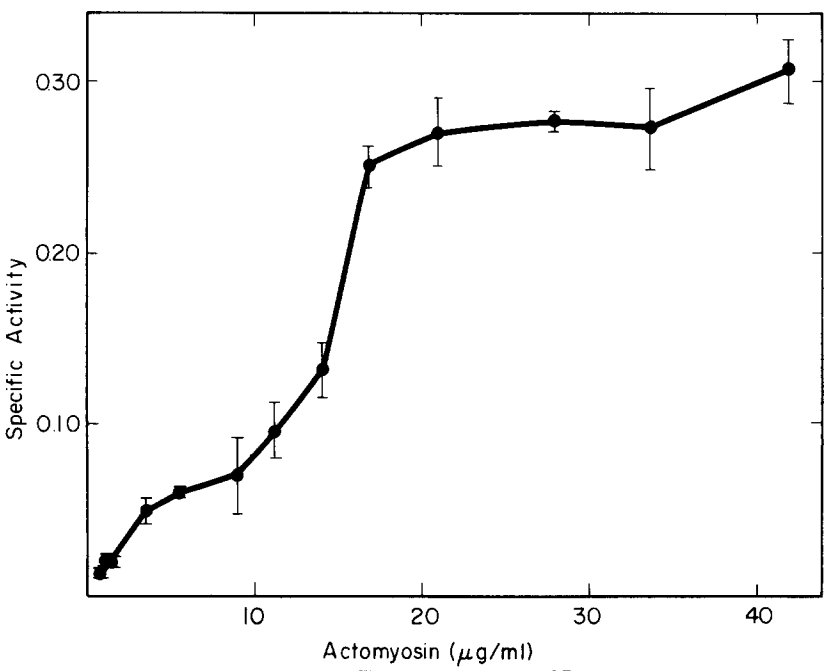

Fig. 2. Effect of actomyosin concentration on the ATPase specific activity. The activities for these results are determined in 10.0 μM [γ-^{32}P]ATP as described in the legend to Fig. 1A. The slopes of the resulting first-order plots are multiplied by the ratio $[ATP]_0$/myosin concentration to give the initial specific activities. The vertical bars of each point represent one standard error of the specific activity for each plot.

In an earlier study of the actin-activated ATPase activities of muscle myosins prepared from cats and sloths [2] a large difference in specific activities was found for actomyosin at high and very low concentrations. The effect of 0.700—42.0 μg/ml reconstituted rabbit skeletal muscle actomyosin on its specific activity is seen in Fig. 2. A sigmoid shaped curve is obtained. The specific activity decreases slowly from 0.307 μmol P_i/min per mg myosin at 42.0 μg/ml to 0.249 μmol P_i/min per mg at 16.8 μg/ml. Next the specific activity drops rapidly to 0.071 μmol P_i/min per mg at 8.95 μg/ml and finally to 0.012 μmol P_i/min per mg at 0.700 μg/ml. Therefore, reconstituted actomyosin, consisting of 0.400 mg F-actin/mg myosin, loses 96% of its specific activity in going from 42.0 down to 0.700 μg/ml.

These reactions were carried out at 10.0 μM ATP. It will be shown that this concentration corresponds to the K_m of actomyosin for ATP. Therefore, 10.0 μM ATP is sufficient to easily detect actomyosin ATPase activity up to 50% of the maximum velocity. At this concentration of ATP sufficient hydrolysis occurs at the very low enzyme concentrations within 60 min. If 1.00 mM ATP or 0.100 mM ATP levels had been used then the minimum necessary reaction times in the 0.700—12.8 μg/ml range would have to be, respectively, 33 h and 3 h 20 min. Such long reaction times are not satisfactory for a very highly diluted enzyme solution.

There are two possible reasons for the loss of specific activity at very low

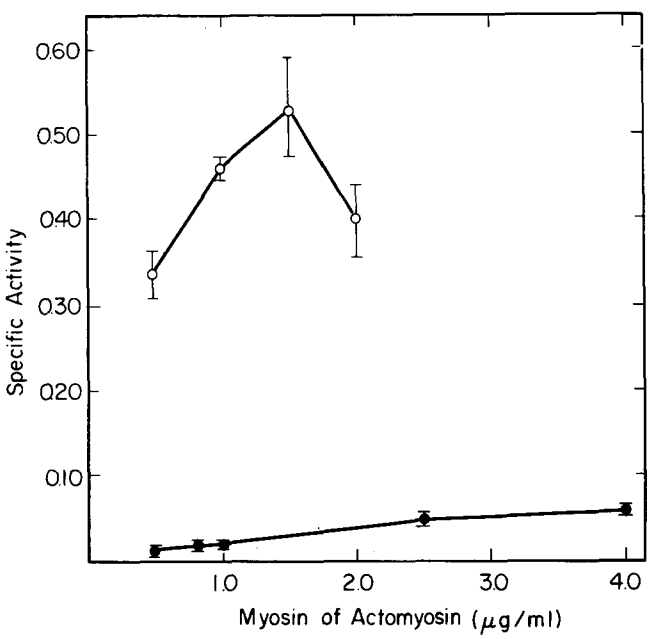

Fig. 3. Prevention by F-actin of specific activity losses at very low actomyosin concentrations. These reactions are performed as described in the legend to Fig. 1A in 10.0 μM [γ-^{32}P]ATP. The specific activity values are calculated as described in the legend to Fig. 2. The abscissa gives the concentration of only the myosin portion of the actomyosin preparations. (●) The lower curve gives the specific activity values of actomyosin consisting of 0.400 mg F-actin/mg myosin. The concentrations of F-actin in this curve varies from a low value of 0.200 up to 1.60 μg/ml. (○) The upper curve gives the specific activity values of actomyosin reactions all of which contain 17.6 μg/ml F-actin (0.392 μM).

actomyosin concentrations (Fig. 2). A non-dissociating enzyme should yield a constant specific activity at varying enzyme concentrations. This suggests that at high dilution actomyosin dissociates into free myosin, with a specific activity of 0.02 μmol P_i/min per mg, and free F-actin, with no ATPase activity. If this is correct then increasing the F-actin concentration should restore the lost actomyosin specific activity. Fig. 3 compares the specific activities of two reconstituted actomyosin preparations consisting of 0.400 mg F-actin/mg myosin and of a constant 17.6 μg/ml F-actin in the concentration range of 0.500—2.00 μg/ml myosin in actomyosin. It is seen that the high F-actin-actomyosin preparation has a 20-fold greater specific activity than the low F-actin-actomyosin. A student's *t*-test of the high F-actin-actomyosin data at a 5% two tail significance level led to the conclusion that there is no significant deviation of the points from their mean value ± S.E., 0.432 ± 0.042 μmol P_i/min per mg [17]. Multiplication of this value by 2 to correct for submaximum ATP concentration gives a V of 0.86 ± 0.08 μmol P_i/min per mg myosin.

The actomyosin concentration range that covers the low specific activities in Fig. 3 corresponds to myosin concentrations of $1.1 \cdot 10^{-9}$—$8.8 \cdot 10^{-9}$ M. At such low concentrations the low molecular weight subunits essential for ATPase activity may dissociate from the heavy chains. Such a dissociation of the essential light subunits would cause a loss of specific activity. The binding of myosin to F-actin might partially prevent this dissociation.

K_m of actomyosin for ATP

Fig. 4 contains plots for actomyosin 1/(ATPase) vs. 1/(ATP concentration) at F-actin concentrations of 0.0525 μM (2.36 μg/ml) and 1.05 μM (49.5 μg/ml). Both plots yield straight lines whose correlation coefficients are greater than 0.98. The reciprocal of the K_m values are 121 ± 29 μM^{-1} at 0.0525 μM F-actin and 91.3 ± 15.5 μm^{-1} at 1.05 μM F-actin. A *t*-test at a 5% two tail significance

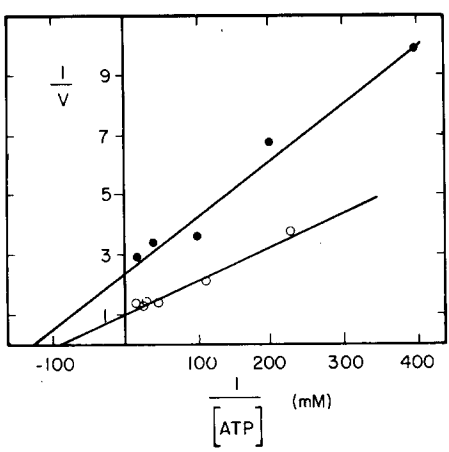

Fig. 4. Double reciprocal plots of actomyosin specific activity versus ATP concentration. The reactions were carried out as described under Methods using [γ-^{32}P]ATP. Each reaction contains 0.020 M Tris · HCl, 1.00 mM $MgSO_4$, NaCl or KCl, bovine serum albumin, F-actin, myosin, and [γ-^{32}P]ATP at pH 7.4 and at 25°C. (•) 0.0525 μM F-actin (2.36 μg/ml), 5.88 μg/ml myosin, 50.0 μg/ml bovine serum albumin and 12.5 mM NaCl; correlation coefficient = 0.987. (○) 1.05 μM F-actin (49.5 μg/ml), 0.600 μg/ml myosin, and 21 mM KCl; correlation coefficient = 0.986.

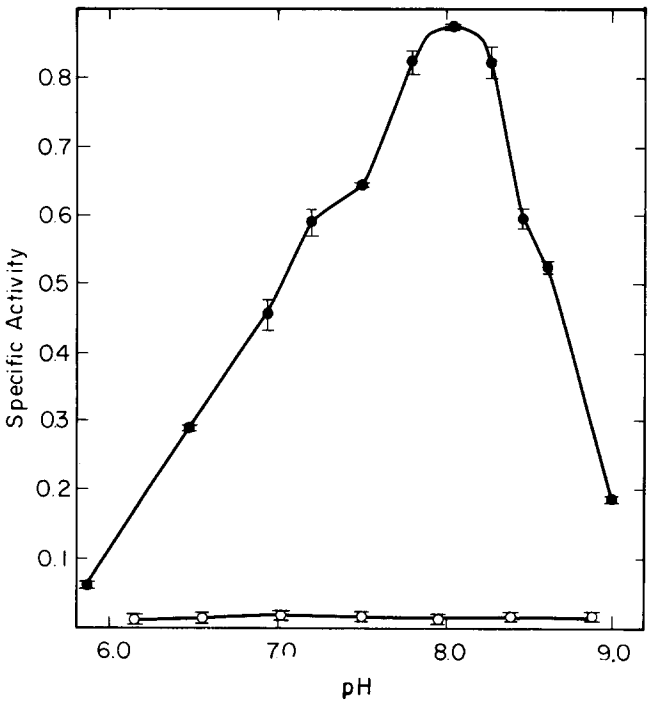

Fig. 5. Effect of pH variation on the specific activities of actomyosin and myosin. (o) Actomyosin specific activities are determined by the H_2SO_4-colorimetric assay procedure described under Methods. Each 8.00 ml reaction sample contains 0.020 M Tris, 1.00 mM $MgSO_4$, 45 mM HCl + NaCl + KCl, 1.00 mM ATP, 10.0 μg/ml myosin, and 1.50 μM F-actin (67.6 μg/ml) at varying pH values. The reaction mixture pH plotted on the abscissa is measured with a Beckman research pH meter on separate 8.00-ml reaction mixtures that have been incubated at 25° C along with the mixtures assayed for P_i. Each point gives the average specific activity value obtained from duplicate assays, and the vertical bars represent the deviation of the actual specific activity values from their average value. (●) Myosin specific activities are determined by the $HClO_4$-colorimetric procedure described under Methods. Each 8.00 ml reaction mixture contains 0.020 M Tris, 1.00 mM $MgSO_4$, 35 mM HCl + NaCl + KCl, 1.00 mM ATP, and 248 μg/ml myosin at varying pH values. The reaction mixture pH is measured with a Beckman research pH meter on separate 8.00-ml reaction mixtures that have been incubated at 25° C along with the mixtures assayed for P_i. The vertical bars represent the duplicate sample deviations from the average specific activity value.

level shows these values are equal [18]. Their weighted average K_m is 9.4 μM. The V values for actomyosin ATPase are 0.41 μmol P_i/min per mg at 0.0525 μM F-actin and 0.91 μmol P_i/min per mg at 1.05 μM F-actin. These are much greater than the V values for myosin of 0.02 μmol P_i/min per mg.

Effect of pH and F-actin concentration on the actin-activated myosin ATPase

The effect of pH on the ATPase activities of actomyosin and myosin are shown in Fig. 5. The total monovalent salt concentration of each reaction (Tris · HCl, KCl, and NaCl) is 0.035 M for myosin and 0.045 M for actomyosin (1.50 μM F-actin). Actomyosin yields a bell-shaped curve that is somewhat skewed at pH values below 7.8 and with a maximum specific activity between pH 7.8 and 8.2 of 0.87 μmol P_i/min per mg myosin. Myosin has a specific activity of 0.0144—0.0154 μmol P_i/min per mg myosin between pH 6.99 and 8.88. As the pH drops from 6.99 to 6.14 the specific activity drops to 0.0112 μmol P_i/min per mg.

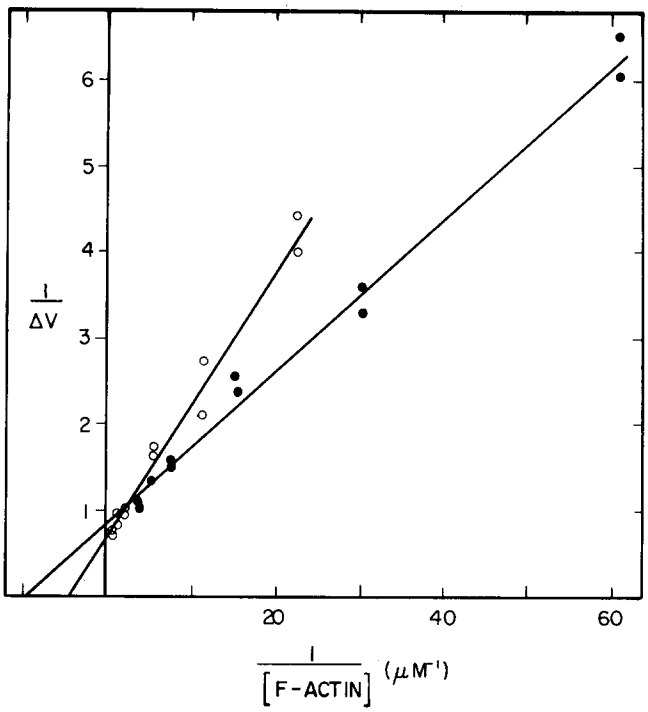

Fig. 6. Double reciprocal plots of actin-activated myosin ATPase as a function of F-actin concentration. The reactions are carried out at 25°C by the H_2SO_4-colorimetric procedure described under Methods. The actin-activated ATPase, Δv, is equal to the specific activity of myosin in the presence of F-actin minus the specific activity of myosin in the absence of F-actin. (●) pH 7.4 reactions contain 0.0200 M Tris · HCl, 1.00 mM $MgSO_4$, 14.1 mM NaCl, 1.0 mM ATP, 10.0 μg/ml myosin, and 0.00—0.262 μM (0.0—11.8 μg/ml) F-actin. (○) pH 7.8 reactions contain 0.0200 M Tris · HCl, 1.00 mM $MgSO_4$, 6.25 mM NaCl, 1.0 mM ATP, 14.8 μg/ml myosin, and 0.00—1.32 μM (0.0—59.4 μg/ml) F-actin.

Double reciprocal plots of actin-activated myosin ATPase versus F-actin concentration at pH 7.4 and pH 7.8 are shown in Fig. 6. The actin-activated ATPase is equal to the total specific activity with F-actin minus the specific activity without F-actin (Δv). At both pH values the experimental points of the double reciprocal plots yield straight lines with correlation coefficients greater than 0.98. Linear regression analysis yields at pH 7.4 a reciprocal of the K_m for F-actin of 9.07 ± 1.27 μM^{-1} (K_m = 0.110 μM or 4.95 μg/ml), and at pH 7.8 a reciprocal of the K_m for F-actin of 4.15 ± 0.54 μM^{-1} (K_m = 0.241 μM or 10.8 μg/ml). A *t*-test at the 5% two tail significance level shows that the K_m at pH 7.4 is not equal to the K_m at pH 7.8 [18]. The ΔV values are 1.19 μmol P_i/min per mg myosin at pH 7.4 and 1.48 μmol P_i/min per mg myosin at pH 7.8. The ΔV values are equal to turnover numbers of 9.3 s^{-1} at pH 7.4 and 11.6 s^{-1} at pH 7.8.

Salt inhibition of actomyosin ATPase

KCl is a competitive inhibitor of the actin-activated heavy meromyosin ATPase with respect to F-actin [3,4]. Fig. 7 shows plots of the reciprocal of the fraction of actomyosin ATPase remaining against increasing concentrations of KCl at 0.121 μM (5.45 μg/ml) and 1.21 μM (54.5 μg/ml) F-actin. Both plots

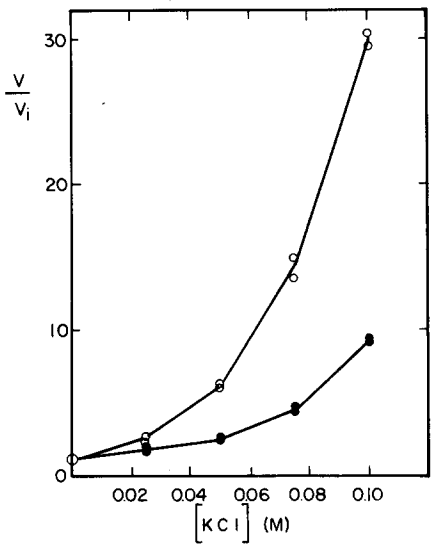

Fig. 7. KCl inhibition of actomyosin ATPase. The reaction mixtures are incubated at 25° C and assayed for P_i by the H_2SO_4-colorimetric procedure. All reaction mixtures contain 0.0200 M Tris · HCl, 1.00 mM $MgSO_4$, 21.8 μg/ml myosin, and 1.0 mM ATP at pH 7.4. The ordinate is the reciprocal of the fraction of ATPase activity remaining at the KCl concentration indicated on the abscissa. (●) Reactions contain 0.121 μM (5.45 μg/ml) F-actin and its specific activity is 0.362 μmol P_i/min per mg myosin. (○) Reactions contain 1.21 μM (54.4 μg/ml) F-actin and its specific activity is 0.874 μmol P_i/min per mg myosin.

yield positive slopes showing that KCl inhibits the ATPase activity. A competitive inhibitor with respect to F-actin should yield a line whose slope decreases with increasing F-actin concentration. A non-competitive inhibitor should give one line that is independent of F-actin concentration. The two plots in Fig. 7 show that the slope increases with increasing F-actin concentration. Therefore, KCl is not a competitive inhibitor with respect to F-actin, and it cannot be a simple non-competitive inhibitor.

It can be noted that the plots in Fig. 7 are both curved in a manner that suggests KCl may be a homotrophic inhibitor. However, this possible explanation for the curvature should be taken with caution since there are other possible explanations that cannot be ruled out at present.

Discussion

During an earlier study of myosins prepared from cat and sloth muscles, large discrepancies were observed in their maximum actin-activated ATPases determined at high and vary low enzyme concentrations [2]. These studies had been carried out with natural actomyosins and reconstituted actomyosins, and it was assumed that the actomyosin did not dissociate under these reaction conditions. Because of the discrepancies of the observed ATPases, a study was started on the actin-activated ATPase of rabbit skeletal muscle myosin. These studies have verified the loss of reconstituted actomyosin specific activity at very low enzyme concentrations. The specific activity dropped from 0.307 μmol P_i/min per mg myosin at 42.0 μg/ml down to 0.012 μmol P_i/min per mg

at 0.700 μg/ml (Fig. 2). This is equal to a 96% loss of the actomyosin's specific activity. This loss of specific activity is prevented by the addition of more F-actin (Fig. 3).

There are two explanations for the above finding. The best explanation is that actomyosin at very low concentrations dissociates into free myosin with a very low ATPase, and into free F-actin with no ATPase. For example at 16.8 μg/ml of reconstituted actomyosin, 0.400 mg F-actin/mg myosin, the concentration of F-actin is 0.107 μM. This is equal to the K_m value of myosin for F-actin, which means that it should yield only 50% of the maximum obtainable specific activity for the given ATP concentration. Decreasing the concentration of reconstituted actomyosin would decrease the F-actin concentration and cause a further loss of specific activity. This type of dissociation is very different from the ATP-dependent dissociation of acto-heavy meromyosin observed by Fraser et al. [19], which yields heavy meromyosin in a refractory state that has a high ATPase activity.

A second explanation for the loss of specific activity is that at very low enzyme concentrations the essential low molecular weight light subunits of myosin will dissociate from the heavy chains to result in a loss of enzyme activity. The binding of F-actin to myosin would prevent this dissociation.

There are many investigations which require a knowledge of the maximum specific activities of diverse actomyosins. It should be realized that the concentration of F-actin and not the ratio of F-actin to myosin determines the specific activity. To ensure near maximum specific activities the F-actin concentration should be 2.0 μM (94 μg/ml) or higher.

The actin-activated ATPase of myosin has an optimal specific activity at pH 7.8—8.2. At pH 7.4 the ATPase follows hyperbolic kinetics with respect to ATP and F-actin. The K_m for ATP, 9.4 μM, is at least 20-fold greater than the K_m of myosin for ATP under identical conditions [15]. These results are in agreement with the K_m values for ATP obtained for acto-heavy meromyosin ATPase [3,4], natural actomyosin ATPase [6,7], and the $(NH_4)_2SO_4$-precipitable complex of actomyosin with [^{14}C]ATP [12]. However, there are three significant differences between the actin-activated ATPases of myosin and heavy meromyosin.

The first difference concerns the maximum turnover numbers, which are the following: 9.3—11.6 s^{-1} for actomyosin and 23—31.8 s^{-1} for acto-heavy meromyosin [3,4]. It has been suggested that aggregated myosin at low salt concentration has about 50% of its ATP binding sites blocked by steric hindrance [20]. The steric hindrance by the aggregated protein of a portion of the catalytic sites would cause a lower turnover number for actomyosin than is given by acto-heavy meromyosin.

The second difference concerns the K_m values for F-actin, which are 0.110—0.241 μM with myosin, and 13—25.5 μM with heavy meromyosin [3,4]. This difference may be due to the greater insolubility of actomyosin compared to acto-heavy meromyosin. The insolubility of actomyosin would enhance the affinity of myosin with F-actin.

The last difference is in the mode of monovalent salt inhibition of the ATPase activities. KCl has been shown to be a competitive inhibitor of acto-heavy meromyosin ATPase with respect to F-actin [3,4]. KCl is also an inhibitor of

the ATPase of actomyosin, but this inhibition is neither competitive with respect to F-actin nor a simple non-competitive reaction. Burke et al. [8] have reported that KCl inhibits actomyosin ATPase by decreasing both the ΔV and the K_m for F-actin.

The actomyosin ATPase in 0.1 M KCl is inhibited 89% or more. This lost ATPase cannot be restored by adding more F-actin. This suggests that salt is an inhibitor of muscle contraction, or the muscle must contain a factor that reverses the salt inhibition. The latter explanation is supported by the report of Danker [21] that mixing actomyosin with α-actinin prevents the salt inhibition.

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