Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 21–25, January 1982
Biochemistry

Inhibition of actomyosin ATPase by vanadate

(contractility)

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Communicated by Hewson Swift, August 10, 1981

ABSTRACT **Actin–myosin subfragment-1 (SF-1) or actin–heavy meromyosin is dissociated by the binding of ADP and vanadate (V_i) under conditions such that ADP alone does not dissociate the complex. The association constant of the stable complex M·ADP·V_i†, in which M indicates myosin [Goodno, C. C. (1979) *Proc. Natl. Acad. Sci. USA* 76, 2620–2624] with actin is smaller than the average association constant of the intermediate states of the actin–SF-1 ATPase cycle. Actin–SF-1 ATPase activity is 90% inhibited by ADP plus vanadate. The reaction of actin with M·ADP·V_i† produces a slow release of ADP and vanadate and quantitative recovery of ATPase activity. The rate of dissociation of ligands was almost linear in actin concentration; consequently, the rate constant of dissociation could only be roughly estimated as 0.5–1 sec^{-1}. The rate of dissociation of ADP and vanadate is thus increased by a factor of 10^5 compared to M·ADP·V_i†. The rate of release of ligands by regulated actin (actin–tropomyosin–troponin) was reduced to 1/10th to 1/20th by removal of calcium ion. Therefore the M·ADP·V_i† complex has the properties of a more stable analogue of the myosin–ADP–phosphate complex that is generated in the normal ATPase cycle. The activation of ligand release (ratio of rate of dissociation of ADP and vanadate from actomyosin relative to myosin) is much larger than the activation of myosin ATPase by actin, whereas the actual rates of the reactions are much slower.**

A recent study demonstrated the inhibition of myosin ATPase activity by vanadate (V_i) (1). The formation of an extremely stable myosin–ADP–vanadate complex ($t_{1/2}$ of 1–2 days) provides an explanation of the mechanism of inhibition. Further studies to be published elsewhere have shown that the normal myosin–ADP complex binds vanadate weakly and undergoes a slow isomerization to form a stable complex that is a competitive inhibitor

$$M\cdot ADP + V_i \underset{}{\overset{K = 10^3\ M^{-1}}{\rightleftharpoons}} M\cdot ADP\cdot V_i \xrightarrow{0.05\ sec^{-1}} M\cdot ADP\cdot V_i\dagger,$$

in which M indicates myosin. Considering the similarity in structure of vanadate and phosphate and that vanadate is bound stoichiometrically at or near the active site, the M·ADP·V_i† complex may be a stable analogue of the myosin–ADP–phosphate intermediate, which is believed to be a key intermediate in the myosin and actomyosin ATPase mechanism (2).

In the present study we have examined the reactions of vanadate with actomyosin to test the hypothesis that M·ADP·V_i† is indeed an analogue of the myosin–products intermediate. This state, which is generally referred to as the M·ADP·P_i**, state is characterized by the following properties. The complex is much more weakly bound to actin than is myosin alone or M·ADP (3, 4). The rates of dissociation of ADP and P_i are greatly increased by forming a complex with actin, and this increase in rate accounts for the activation of myosin ATPase activity by actin. The control of the ATPase activity of regulated actomyosin (AM) by calcium ion involves a change either in the association constant of M·ADP·P_i** to the actin–tropomyosin–troponin complex or in the rate of release of products from the AM·ADP·P_i** complex (5–7). The evidence to be presented here shows that the AM·ADP·V_i† complex has the same properties.

MATERIALS AND METHODS

Myosin was prepared from rabbit back and leg muscles by the method described by Perry (8); actin was made by the procedure of Spudich and Watt (9); heavy meromyosin (HMM) and subfragment-1 (SF-1) were obtained by chymotryptic digestion of myosin and purified by the method of Weeds and Taylor (10); native tropomyosin (the one-to-one complex of troponin and tropomyosin) was made by the procedure of Hitchcock (11).

The myosin–ADP–vanadate complex was formed by incubation of the protein with 0.5 mM ADP and 1 or 2 mM vanadate for 15 min (1). Longer incubation times had no further effect on the state of the protein or the ability to quantitatively regenerate the ATPase activity. Free ADP and vanadate were removed when necessary by passage of the protein solution in pH 8.5 or pH 7.0 buffer over a 1-cm Dowex-1 × 8 column. Modification of HMM led to 95–99% inhibition of Mg^{2+}-ATPase activity and incorporation of 0.95–1.0 mol of vanadate per active site. Modification of SF-1 was generally less complete, but 90% inhibition of Mg^{2+}-ATPase and incorporation of 0.85–0.90 mol of vanadate was obtained routinely with freshly prepared SF-1.

In all experiments vanadate was added by dilution from a concentrated stock solution at pH 10 to avoid the possible formation of complex ions.

Free vanadate was determined colorimetrically by reaction with 4-(2-pyridylazo)resorcinol (PAR) as described (1). The rate of color development was first order in vanadate and PAR concentrations over the ranges used in the experiments. Because protein-bound vanadate does not react with PAR, the rate of vanadate dissociation at pH 7 was determined by adding PAR to the reaction mixture and measuring the rate of color development. This procedure was limited to first-order rates of less than 0.03 sec^{-1} because of the relatively slow rate of color development and the inhibition of ATPase activity by concentrations of PAR in excess of 0.2 mM. The procedure could not be used at pH 8.5 because of the much slower rate of color development. Aliquots were assayed by dilution to pH 6 and the absorbance was measured at 540 nm after a 5-min incubation (1).

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Abbreviations: V_i, vanadate; HMM, heavy meromyosin; acto, actin in complex; M, myosin in complex; AM, actomyosin in complex; SF-1, myosin subfragment-1; Mes, 4-morpholineethanesulfonic acid; PAR, 4-(2-pyridylazo)resorcinol; IAEDANS, 5-(iodoacetamidoethyl)-aminonaphthalene-1-sulfonic acid.

In the case of slow reactions at high ionic strength (90 mM NaCl) the rates of dissociation of acto·SF-1 and acto·HMM (acto, actin in complex) were measured by the change in turbidity at 320 nm or 400 nm if free vanadate was present. A correction for vanadate absorbance of less than 25% of the turbidity change was necessary in the latter case. Measurements were made in a Cary 14 equipped with a slide wire that gave a full-scale deflection for a change of 0.1 OD unit.

ATPase activity was measured either by the time course of phosphate formation assayed colorimetrically by the method of Taussky and Shorr (12) or in a Radiometer pH stat at pH 7.0 [2 mM Tris/2 mM 4-morpholineethanesulfonic acid (Mes) buffer] or pH 8.5 (2 mM Tris buffer). Actomyosin ATPase activity was also measured from the time interval for complete hydrolysis of a known quantity of MgATP determined by the regain in turbidity (2).

Faster reactions were measured in a stop-flow apparatus as described by Johnson and Taylor (2). ATP binding was determined by the enhancement of the tryptophan fluorescence of myosin; association and dissociation of actomyosin complexes were measured by light scattering at 90° and a wavelength of 340 nm or by the change in fluorescence of actin labeled with 5-(iodoacetamidoethyl)aminonaphthalene-1-sulfonic acid (IAEDANS). The labeled actin gave a maximal change in fluorescence emission of 25% for complete occupancy of actin sites by SF-1 or HMM. The fluorescence method was used to verify that light scattering changes in the presence of vanadate were actually caused by a change in association of the proteins. Rate measurements were in agreement for the two methods, and the results reported here are light scattering measurements.

RESULTS

Experiments were performed in a high ionic strength buffer (90 mM NaCl, pH 8.5), which was used in the initial study of myosin (1) and a low ionic strength buffer (20 mM Tris/20 mM Mes, pH 7.0) to increase the interaction of actin with M·ADP·V_i† because binding of myosin or myosin intermediates is known to be strongly dependant on ionic strength (3). The rate of inhibition of myosin ATPase by vanadate was faster at the lower pH. The difference may be attributed to the change in the state of ionization, because the pK_a of $H_2VO_4^-$ is approximately 8.2 (13).

At low ionic strength MgADP produced less than a 10% dissociation of acto·SF-1, and the addition of phosphate (1–3 mM) did not increase the degree of dissociation. The addition of 1 mM vanadate produced a dissociation of 60–70%. As shown in Fig. 1, vanadate alone does not dissociate the complex; the combination of ADP and vanadate is necessary. The order of addition did not affect the rate of dissociation, and because the rates of transitions of ADP with acto·SF-1 are fast compared to the observed rate of dissociation, the reaction rate is probably determined by an isomerization of an AM·ADP·V_i or M·ADP·V_i complex to a state in which actin is weakly bound.

Measurement of the degree of dissociation (Fig. 2) as a function of vanadate concentration showed that dissociation is nearly complete at 3–4 mM vanadate. Thus the association constant of M·ADP·V_i† with actin must be very small, approximately $1.5 \times 10^3\ M^{-1}$, to account for the degree of association obtained at 20 μM actin. The association constant is smaller than the constant for M·ADP·P_i** at the same ionic strength (4). Actin alone did not bind vanadate, and F-actin was not depolymerized by the concentrations of vanadate used in these experiments.

The rate of dissociation was measured by the decrease in light scattering in the stop-flow apparatus. The rate was independent of vanadate concentration in the range of 1 mM to 4 mM and had a value of 0.03 sec^{-1} (20°C, pH 7).

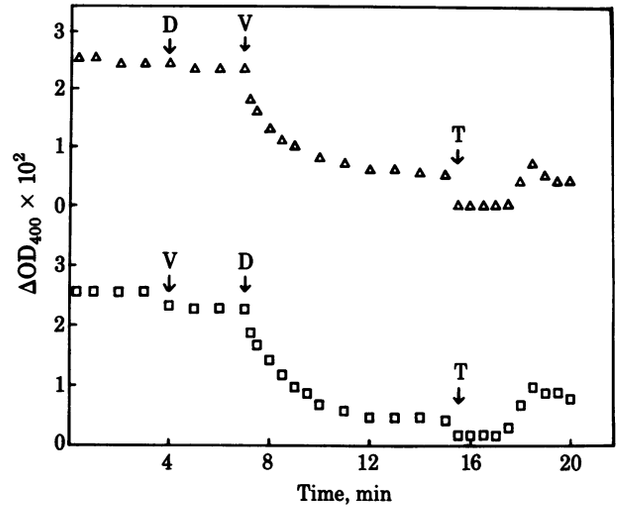

FIG. 1. Dissociation of acto·SF-1 by ADP plus vanadate (two separate experiments). Ordinate: turbidity at 400 nm, 1-cm path length, Cary 14. D, addition of MgADP to a concentration of 100 μM; V, addition of vanadate to 1 mM; T, addition of MgATP to 200 μM. Experimental conditions: 22°C, 10 mM Tris/10 mM Mes (pH 7), 1 mM $MgCl_2$, 5 μM SF-1, 10 μM actin.

Vanadate also inhibits acto-SF-1 ATPase activity, as would be expected from the formation of a M·ADP·V_i† complex in the course of the reaction. The degree of dissociation of acto·SF-1 by ADP plus vanadate and the inhibition of ATPase activity have a similar dependance on vanadate concentration (Fig. 2), although the two sets of measurements are not superimposable. The concentration of vanadate for half-maximal effect was 1.2 mM for inhibiton of ATPase activity and 0.7 mM for dissociation of acto·SF-1. The conditions were not identical because 1 mM ADP was present in the dissociation experiments and the distribution of intermediate states was different than in the presence of ATP.

A significant difference in the behavior of acto·SF-1 ATPase compared to SF-1 ATPase is the rate at which inhibition occurs when vanadate is added in the presence of ATP. A constant level

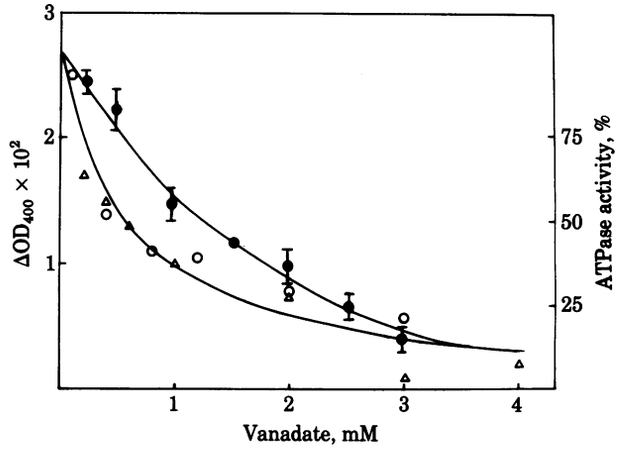

FIG. 2. Dissociation of acto·SF-1 and inhibition of acto·SF-1 ATPase as a function of vanadate concentration. Percent ATPase activity (right-hand scale) is relative to acto·SF-1. ○ and △, turbidity of acto·SF-1 (two separate experiments), 1 mM MgADP, 5 μM SF-1, 10 μM actin, pH 7, 22°C; ●, ATPase activity, same conditions except no ADP added, and 5 mM MgATP present. Error bars indicate range of duplicate assays.

of inhibition was reached in 10 sec for acto·SF-1 and 1 mM vanadate, whereas the inhibition process took 20 min for SF-1 alone.

Interaction of M·ADP·V_i† complex with actin

The first set of experiments showed that M·ADP·V_i† is weakly bound by actin. Consequently actomyosin is dissociated by ADP plus vanadate and ATPase is inhibited because of the low affinity of M·ADP·V_i† for actin. However, inhibition was not complete even at high vanadate concentrations; consequently, a slow ATPase cycle must still occur. This could be explained by the displacement of ADP and vanadate from the M·ADP·V_i† complex by actin. To investigate this reaction the free vanadate present in the M·ADP·V_i† solution was reduced to less than 0.5 μM by treatment with Dowex-1 just prior to use. In the high ionic strength buffer the rate of release of vanadate from M·ADP·V_i† was extremely slow (first-order rate constant approximately 5×10^{-6} sec^{-1}), but the addition of actin markedly increased the rate of release (Fig. 3). The time course fitted a single exponential process for actin concentrations of 25 μM or larger and the pseudo-first-order rate constant increased linearly with actin concentration over the range investigated (up to 75 μM in actin) to a value of approximately 10^{-3} sec^{-1}. Although this represents a 200-fold increase in the rate of dissociation of vanadate the rate had not begun to reach a maximum value.

The dissociation of vanadate was accompanied by an increase in turbidity to the level of the acto·SF-1 complex. This slow association occurred at the same rate as vanadate release, but the apparent second-order rate constant of 10 M^{-1} sec^{-1} is much too small to be attributed to a simple association reaction of the two proteins (2).

The ATPase activity was also regenerated at the same rate as the release of vanadate. Fig. 4 shows a representative experiment using HMM and an actin concentration of 17 μM. Vanadate dissociation and regain of ATPase activity both occurred with a pseudo-first-order rate constant of 10^{-4} sec^{-1} and complete recovery of ATPase activity was obtained. In other experiments (not shown) the release of [^{3}H]ADP from the M·ADP·V_i† complex occurred at the same rate as vanadate release.

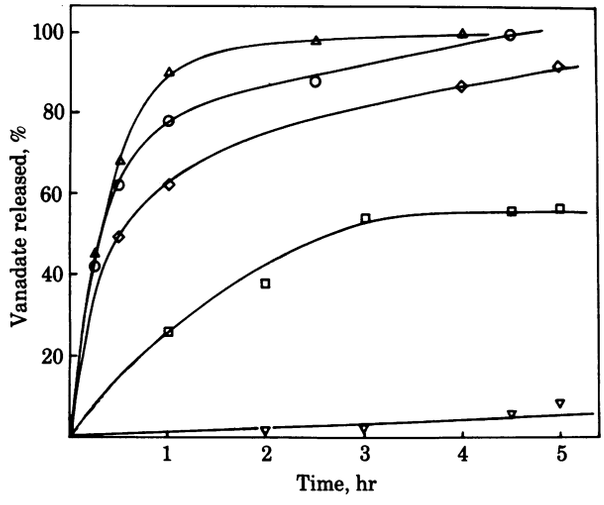

FIG. 3. Time course of vanadate release for a range of actin concentrations. Free vanadate was determined colorimetrically. Conditions: 25°C, 90 mM NaCl, 20 mM Tris (pH 8.5), 5 mM $MgCl_2$, 10 μM M·ADP·V_i†. ▽, No actin; □, 10 μM actin; ◇, 25 μM actin; ○, 50 μM actin; △, 75 μM actin. At the highest actin concentration the correction to absorbance of the dye from actin turbidity was 25%.

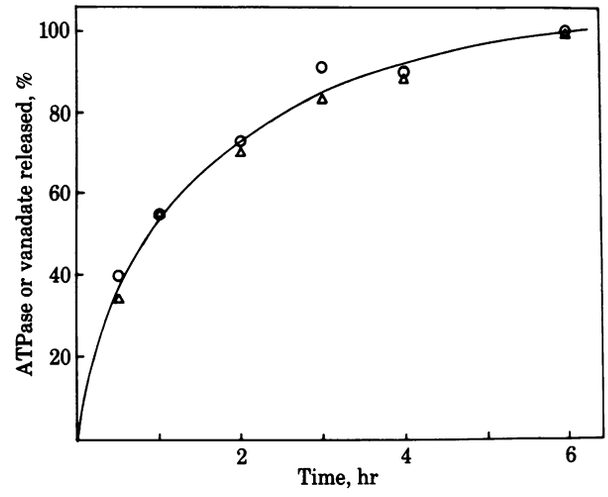

FIG. 4. Release of vanadate (△) and regeneration of ATPase activity (○). Vanadate–ADP complex of HMM, 10 μM sites, was mixed with 17 μM actin (final concentrations). Aliquots were taken at indicated times for determination of free vanadate colorimetrically, and ATPase activity was determined in a 20-min assay. Conditions: 25°C, 20 mM Tris (pH 8.5), 90 mM NaCl. The data points were fitted to a single exponential process, apparent pseudo-first-order rate constant $k = 1.4 \times 10^{-4}$ sec^{-1}.

This series of experiments shows that interaction with actin leads to release of vanadate and ADP and the formation of a normal acto·SF-1 or acto·HMM complex with complete restoration of ATPase activity. The process is still very slow at an ionic strength of 100 mM, but interaction of actin with myosin-nucleotide intermediates is strongly dependent on ionic strength. The properties of the system were examined in the absence of added KCl and the rate increased by 100-fold.

The apparent rate constant of dissociation of vanadate exceeded 0.045 sec^{-1} at 50 μM actin (20 mM Tris/20 mM Mes, pH 7) and appeared to be reaching a maximum. However, the rate of color development by the PAR dye is comparable to the measured rates of vanadate release, and this technique underestimates the true value. To determine the actual rates, stop-flow kinetic methods were employed. Association with actin was measured by increase in light scattering and by change in fluorescence emission of actin labeled with IAEDANS. The release of vanadate and ADP from the binding site was determined by taking advantage of the increase in tryptophan fluorescence emission which accompanies formation of the M·ADP·P_i** complex. The fluorescence emission of M·ADP·V_i† is essentially the same as for myosin alone (2% enhancement), whereas a 20% increase relative to myosin is obtained for the mixture of states present in a solution of myosin, actin, and ATP. Therefore, mixing of M·ADP·V_i† with actin plus ATP leads to enhancement of fluorescence as ADP and vanadate are released and ATP binds to the active site.

Typical stop-flow experiments are shown in Fig. 5. In Fig. 5A the association as measured by light scattering (the upper curve labeled LS in the figure) showed an initial small jump followed by binding at a pseudo-first-order rate of 0.06 sec^{-1} (10 μM actin). The small jump is explained by the presence of some unmodified SF-1, because this step is largely absent when ATP is added with the actin (lower LS curve). The fluorescence signal in the presence of ATP (F) had the same rate as the light scattering signal in the absence of ATP. Fig. 5*B* shows the light scattering and fluorescence signals on a longer time scale to illustrate reassociation when the ATP is completely hydrolyzed. The rate of increase in fluorescence emission is the same as the rate of association in the absence of ATP (0.09 sec^{-1} at 15 μM

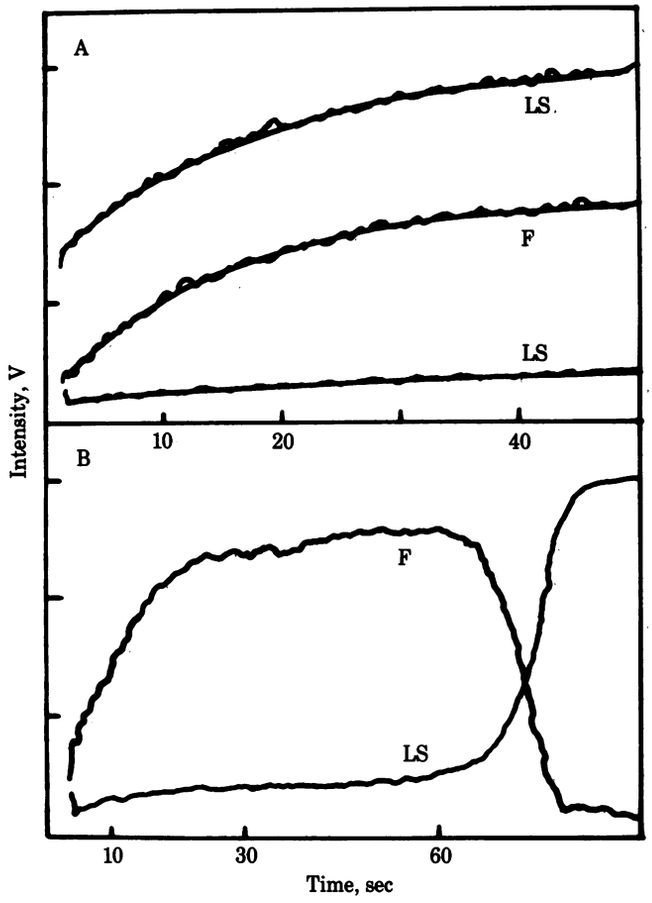

FIG. 5. Light scattering and fluorescence signals for the reaction of M·ADP·V_i† with actin. (*A*) Upper curve, light scattering signal (LS) for association of M·ADP·V_i† with 10 μM actin; the smooth curve drawn through the experimental trace is the computer fit to a first-order reaction, $k = 0.056$ sec^{-1}; lower curves, tryptophan fluorescence (F) and light scattering (LS) for the same reaction but with 0.5 mM MgATP present, $k = 0.064$ sec^{-1} for the fluorescence signal. (*B*) Reaction of M·ADP·V_i† with 15 μM actin plus 0.5 mM MgATP; a longer time interval is shown to illustrate reassociation when the ATP is hydrolyzed; rate as measured by the fluorescence signal, $k = 0.085$ sec^{-1}. Experimental conditions: 20°C, 20 mM Tris/20 mM Mes (pH 7), 1 mM $MgCl_2$, 2.5 μM SF-1. The voltage change (arbitrary units) is proportional to the change in light scattering intensity or the fluorescence intensity. The gain was adjusted to give similar amplitudes for the two signals.

actin). The slow increase in light scattering before the main reassociation step occurs is explained by the conversion of M·ADP·V_i† to M·ADP·P_i**, which is more tightly bound to actin. Association is accompanied by release of ADP and P_i, and consequently fluorescence emission decreases. The indirect method using fluorescence is preferable to light scattering for rate measurements at high actin concentrations because there is some slow aggregation of actomyosin solutions that contributes to the light scattering signal.

Double mixing experiments were undertaken to test for accumulation of a nondissociable intermediate. M·ADP·V_i† was mixed with actin and then with ATP after a variable time delay, which corresponded to the period in which dissociation of vanadate takes place. The second mixing with ATP produced very fast dissociation to the level obtained by including ATP with actin. Thus the complex AM·ADP·V_i† is not present in appreciable amounts during the period of formation of associated actomyosin states.

Regeneration of ATPase activity was also measured at low ionic strength for both SF-1 and HMM. Complete recovery of actin-activated ATPase was obtained at the same rate as vanadate release. The rate measurements for release of ADP and vanadate, regain of ATPase, and formation of actomyosin are summarized in Fig. 6. The various processes occurred at essentially the same rate. The largest measured value at 30 μM actin was 0.2 sec^{-1}. The plot of rate versus actin concentration exhibits little curvature; consequently, the rate constant of the first-order reaction of dissociation of vanadate could not be determined. By analogy with the reactions of M·ADP or M·ADP·P_i with actin (6), the association occurs in at least two steps: the very rapid formation of a weakly bound complex with actin, followed by release of ligand with the generation of the much more strongly bound actin–myosin complex. The first-order rate constant is approximately equal to the observed rate divided by the initial degree of association. At the highest actin concentration employed here (30 μM) there was an initial association of 10–20% of the myosin–ligand complex. Thus, the rate constant of the dissociation step is 5–10 times larger than the observed rate, or roughly 1 sec^{-1}.

Interaction of M·ADP·V_i† with regulated actin

Regulated actin, the complex of actin with the proteins tropomyosin and troponin, activates myosin ATPase in the presence of calcium ions, but the activation is 95% inhibited by removal of calcium. In the absence of calcium the apparent second-order rate constant for the association of M·ADP or M·ADP·P_i** is reduced to 1/10th (6) and the equilibrium constant for association of M·ADP is 1/100th (7). Regulation of ATPase activity may arise from a reduction of the affinity of myosin intermediates for regulated actin or a reduction in the rate of release of the ligands ADP and phosphate from the regulated actin–myosin–ADP–phosphate complex. Either effect reduces the apparent second-order rate constant of binding. To test for an effect of the regulation system on the interaction of M·ADP·V_i† it was necessary to make the measurements in 30 mM KCl and 5 mM $MgCl_2$ to ensure that the regulatory proteins remain bound to actin. Under these conditions the apparent second-order rate constant for the binding of M·ADP·V_i† to pure

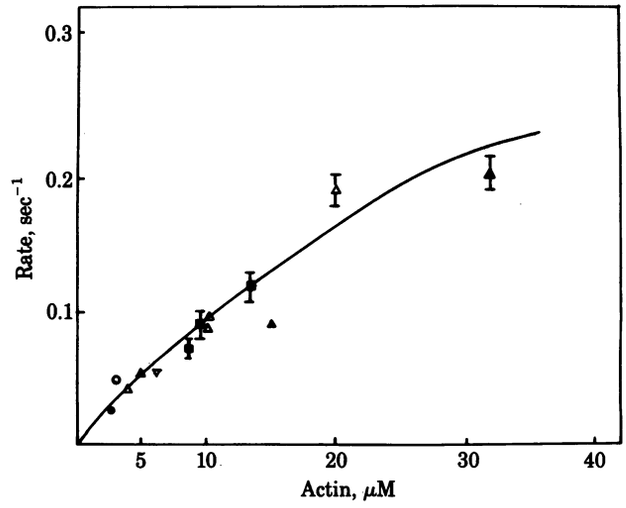

FIG. 6. Rate of reaction of M·ADP·V_i† with actin as a function of actin concentration. ● and ○, rate of regeneration of ATPase activity for SF-1 (●) and HMM (○); ▽, rate of release of vanadate (colorimetric). Rate of association with actin was determined by light scattering for SF-1 (▲) and HMM (△); ■, rate of increase in protein fluorescence (equals the rate of dissociation of ADP and vanadate). The ordinate is the pseudo-first-order rate constant for the various processes. Experimental conditions: 20°C, 20 mM Tris/20 mM Mes (pH 7), 1 mM $MgCl_2$. Error bars indicate range of duplicate or triplicate determinations.

actin is reduced to 1/10th compared to the results obtained in the absence of KCl. The three types of measurements that have been described for pure actin were repeated for the regulated actin in the presence or absence of calcium, namely the rate of formation of a complex between actin and M·ADP·V_i†, the rate of release of vanadate ion by actin, and the rate of regeneration of ATPase activity. The rates of the three processes were proportional to actin concentration in the range from 10–20 μM actin. The apparent second-order rate constants were approximately equal for regulated actin plus calcium and pure actin, but the rates of the three reactions were reduced to 1/10th to 1/20th by removal of calcium ion. Thus the regulatory proteins have a similar effect on the M·ADP·V_i† complex as on the myosin–ADP–phosphate intermediate of the normal ATPase cycle.

DISCUSSION

The stable M·ADP·V_i† complex appears to have properties similar to the M·ADP·P_i** intermediate of the actomyosin ATPase mechanism except that its half-life in the absence of actin is 1–2 days instead of 10 sec. The association constant with actin is the order of 1–5 $\times$ 10^3 M^{-1}, which is smaller than the value for the product intermediate state M·ADP·P_i**. The rate constant of dissociation of ADP and vanadate from an AM·ADP·V_i† complex was not accurately measured because of the low affinity for actin, but the first-order rate constant exceeds 0.2 sec^{-1} and could be the order of 1 sec^{-1}. Because the rate constant of dissociation of ADP and vanadate from myosin is approximately 10^{-5} sec^{-1}, actin binding "activates" the rate of ligand release by a factor of 10^4 to 10^5. There is some controversy concerning the rate-limiting step of actomyosin ATPase (4), but the maximal rate of turnover per actin residue at a saturating SF-1 concentration is less than 50 sec^{-1}. Thus for the same ionic strength used in the present experiments the rate of dissociation of ADP and P_i from actomyosin is the order of 50 sec^{-1}, whereas it is 0.05 sec^{-1} for myosin, which corresponds to an activation factor of 10^3. Consequently actin is at least 10 times more effective in accelerating the release of ADP and vanadate compared to ADP and P_i.

The apparent rate of association of actin and M·ADP·V_i† and of release of vanadate is 1/10th to 1/20th for regulated actin in the relaxed state (no calcium) compared to the active state. This ratio is similar to the ratio of rates of SF-1 binding in the presence and absence of calcium obtained by Trybus and Taylor (6).

The reactions are described by the following mechanism

$$\begin{array}{ccccc} V_i + AM\cdot ADP & \overset{K_1'}{\rightleftharpoons} & AM\cdot ADP\cdot V_i & \underset{k_{-2}'}{\overset{k_2'}{\rightleftharpoons}} & AM\cdot ADP\cdot V_i^{\dagger} \\ \Big\uparrow K_\alpha \quad + A & & \Big\uparrow K_\beta \quad + A & & \Big\uparrow K_\gamma \quad + A \\ V_i + M\cdot ADP & \overset{K_1}{\rightleftharpoons} & M\cdot ADP\cdot V_i & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & M\cdot ADP\cdot V_i^{\dagger} \end{array}$$

in which K_α, K_β, and K_γ are the association constants (M^{-1}) for the binding of actin (A) to the myosin intermediates. In the M·ADP·V_i state the bound vanadate is in rapid equilibrium with free vanadate, whereas M·ADP·V_i† refers to a state in which ADP and V_i are essentially trapped. At low ionic strength, $K_1 \approx 10^3$ M^{-1}, $k_2 = 0.05$ sec^{-1}, $k_{-2} \approx 10^{-5}$ sec^{-1} [data from Goodno (1) and unpublished], $K_\alpha \approx 10^6$ M^{-1} (calculated from the degree of dissociation at high ADP), $K_\gamma \approx 5 \times 10^3$ M^{-1} (calculated from the degree of dissociation at high ADP and vanadate), and $k'_{-2} \approx 1$ sec^{-1}. The apparent second-order rate constant for association of M·ADP·V_i† with actin is 10^4 M^{-1} sec^{-1} $= K_\gamma k'_{-2}$, which is consistent with this choice of constants. The main pathway for association passes through the AM·ADP·V_i† state but very weak actin binding prevents appreciable accumulation of this intermediate. The pathway of dissociation of AM·ADP by vanadate is less clear, because the maximal measured rate of 0.03 sec^{-1} is the same magnitude as k_2. The rate of spontaneous dissociation of actin from AM·ADP is approximately 0.5 sec^{-1} (14); consequently dissociation could occur from all three actomyosin states.

The extraordinary increase in rate of dissociation of ADP and vanadate induced by actin binding emphasizes a general property of the actomyosin system. The rates of dissociation of all nucleotide complexes are enhanced by actin binding. The 500- to 1000-fold activation of myosin ATPase by actin is simply one example of this general property. The ratio for ATP is 10^5 [calculated from measurements of ATP binding (2, 15) to myosin and ATP release from actomyosin (16)], and for ADP and adenosine 5'-[β, γ-imido]triphosphate the values are 500 and 10^4, respectively (14).

This work was supported by Program Project Grant HL 20592 from the National Institutes of Health and a grant from the Muscular Dystrophy Association.

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