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ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle

(transient kinetics/ATP hydrolysis/crossbridge)

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ABSTRACT **The rate constant for dissociation of ADP from actomyosin subfragment 1 (S1) has been measured in this laboratory and elsewhere for a variety of vertebrate muscle types. We have made the following observations: (*i*) In solution, the dissociation of ADP from actomyosin-S1 limits the rate of dissociation of actomyosin-S1–ADP by ATP and, presumably, also limits the rate of crossbridge detachment in contracting muscle. (*ii*) For muscle types in which the rate of ADP dissociation from actomyosin-S1 is slow enough to measure using stopped-flow methods, the rate constants are nearly the same as the theoretical value for the minimum allowable rate constant for dissociation of an attached crossbridge. Therefore, ADP dissociation is sufficiently slow to be the molecular step that limits the maximum shortening velocity of these muscles. (*iii*) Variation with muscle type of the rate constant for ADP dissociation may be a general phylogenetic mechanism for regulating shortening velocity.**

Muscle contraction is thought to occur as the result of a cyclic association and dissociation of crossbridges formed between myosin molecules in the thick filaments and F-actin molecules in the thin filament, involving concomitant hydrolysis of ATP. This cycle can lead to relative sliding of the actin and myosin filaments and result in the production of work. A rationale for studying the kinetic mechanism of ATP hydrolysis by actomyosin-S1 in solution is that it may directly relate to the observed physiological properties of muscle. A condensed version of the kinetic mechanism, consistent with recent observations (1, 2), is shown in Eq. 1. The second-order reactions of nucleotide and phosphate binding have been shown to be two-step reactions (3–5), but they are condensed here to single steps:

$$\begin{array}{ccccccc} \mathrm{AM} & \overset{\mathrm{ATP}}{\rightleftharpoons} & \mathrm{AM\text{–}ATP} & \rightleftharpoons & \mathrm{AM\text{–}ADP\text{-}P_i} & \overset{\mathrm{P_i}}{\rightleftharpoons} & \mathrm{AM\text{–}ADP} \overset{\mathrm{ADP}}{\rightleftharpoons} \mathrm{AM} \\ & & \downarrow\uparrow & & \downarrow\uparrow & & \\ & & \mathrm{M\text{–}ATP} & \rightleftharpoons & \mathrm{M\text{–}ADP\text{-}P_i} & & \end{array} \qquad [1]$$

where M represents myosin subfragment-1 (S1) and AM represents actomyosin S1.

Different types of vertebrate muscles have shortening velocities that vary by almost two orders of magnitude. Barany showed that the steady-state rate of hydrolysis of MgATP by actomyosin is correlated with muscle shortening velocity (6). However, it has not been demonstrated that the rate of ATP hydrolysis directly limits shortening velocity. Indeed, the observed correlation might be anticipated, as rapidly contracting muscles would be expected to produce more power and therefore hydrolyze ATP more rapidly than slowly contracting muscles. Nevertheless, it is reasonable to expect that one or more of the rate constants of the ATP hydrolysis mechanism limits the muscle contraction rate. For a molecular step to limit the shortening velocity of muscle, it must have the following attributes:

(*i*) The rate constant for such a step must be consistent with both the maximum working length of an individual crossbridge and the rate of axial displacement of the thick and thin filaments observed in contracting muscle. The minimum allowable rate constant, k_{min}, for the conversion of an attached crossbridge state in muscle (corresponding to the AM states in the top line of Eq. 1) to other attached or detached states (corresponding to the M states in the bottom line of Eq. 1) can be estimated from the maximum rate of contraction (the unloaded shortening velocity) and the distance over which a crossbridge can remain attached, using Eq. 2,

$$k_{min} = V_0 \cdot S_L \cdot d^{-1}, \qquad [2]$$

where V_0 is the unloaded shortening velocity of the muscle (muscle lengths per second), S_L is half the sarcomere length (11,000 Å in the vertebrate striated muscle), and d is the maximum allowed axial crossbridge translation, ≈100 A.*

(*ii*) The rate limiting molecular step should have similar dependence on changes in experimental conditions (such as temperature) as are observed for the shortening velocity of muscle.

(*iii*) For a molecular step to limit shortening velocity, it must follow an actomyosin intermediate (attached crossbridge) that is not in rapid equilibrium with a myosin intermediate (detached crossbridge). It follows from criterion one that if the crossbridge of an actomyosin intermediate preceding any step of the mechanism dissociates from actin with a rate constant that is $>>k_{min}$, then that step cannot limit the rate of movement between the actin and myosin filaments. Thus, even the slowest step of the ATP hydrolysis mechanism will not limit the shortening velocity if the intermediate preceding the step is in rapid equilibrium with a detached crossbridge state.

(*iv*) If the molecular rate constants measured in solution with actomyosin-S1 apply to muscle, they must be independent of the three-dimensional geometry imposed by the myofibrillar filament lattice of muscle.

(*v*) The molecular step must be on the predominant kinetic pathway of ATP hydrolysis by actomyosin.

We have previously shown (8, 9) that the only kinetically significant pathway for the dissociation of myosin-S1 from actomyosin-S1–ADP involves dissociation of ADP followed by binding of ATP and subsequent rapid dissociation of myo-

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Abbreviation: S1, subfragment 1.

*Estimates of the maximum axial displacement of an attached crossbridge of ≈100 Å have been made by Huxley and Simmons from the tension changes produced by rapid length changes (7).

sin-S1–ATP from actin as shown in Eq. 3.†

$$\text{A-S1–ADP} \underset{k_{AD}[ADP]}{\overset{k_{-AD}}{\rightleftharpoons}} \text{A-S1} \overset{K_{AT}[ATP]}{\rightleftharpoons} \text{A-S1–ATP} \overset{k_{-TA}}{\rightleftharpoons} \text{A + S1–ATP} \quad [3]$$

Although ADP dissociation from actomyosin-S1 is not sufficiently slow to limit the steady-state rate of ATP hydrolysis (8, 9), it is slow enough to be important during rapid muscle shortening. The extrapolated value for the rate constant of ADP dissociation, k_{-AD}, from bovine ventricle actomyosin-S1 is 550–800 sec^{-1} at 38°C (9). This value is approximately twice the minimum rate constant for crossbridge detachment (330 sec^{-1}) calculated from an *in vivo* estimate of the unloaded shortening velocity (10). The similarity between k_{-AD} and k_{min} suggested that ADP dissociation is sufficiently slow to limit the maximum shortening velocity of bovine ventricular muscle. In this paper, we report the rates of ADP dissociation from actomyosin-S1 from vertebrate muscles for which there are more precise *in vitro* measurements of the unloaded shortening velocity, using skinned muscle fiber preparations. We demonstrate here that the rate constant measured for ADP dissociation from actomyosin-S1 satisfies at least four of the five requirements for a molecular step that limits the unloaded shortening velocity in muscle. On the other hand, V_{max}, the maximum steady-state rate of actomyosin ATP hydrolysis, does not satisfy these requirements and is not likely to be the molecular step that limits the shortening velocity of muscle.

METHODS

Proteins. Myosin was prepared from rabbit psoas and soleus muscles using standard methods (11). Myosin was prepared from both ventricular walls and the interventricular septum of rat and rabbit hearts as described for bovine left ventricle (9), except that frozen hearts obtained from Pel-Freez were used. Myosin from rat and rabbit hearts salted out between 40% and 50% (vol/vol) of saturated ammonium sulfate, compared to 38% and 45% previously observed from bovine heart myosin.

Myosin-S1 was obtained by digestion of myosin with bovine pancreatic chymotrypsin at low ionic strength according to the digestion procedure of Weeds and Taylor (12), except that digestions were terminated by the addition of a 2-fold (wt/wt) excess of lima bean trypsin inhibitor. Myosin-S1 from rabbit psoas and soleus muscles was purified by chromatography on diethylaminoethyl cellulose (12). Only psoas myosin-S1 fractions containing >95% LC1 and soleus myosin-S1 fractions containing intact LC1a and LC1b light chains were used for kinetic experiments. Rat and rabbit cardiac myosin-S1 were separated from other digestion products by low ionic strength precipitation and salting-out with ammonium sulfate as described (9). Cardiac myosin-S1 was used immediately after purification or was lyophilized in the presence of 0.5 M sucrose and stored at −20°C over a dessicant to avoid irreversible aggregation (9). The subunit composition on $NaDodSO_4$/polyacrylamide gel electrophoresis of myosin and myosin-S1 preparations from cardiac tissues is shown in Fig. 1.

†The following nomenclature is used to identify rate and equilibrium constants. Positive subscripts identify rate and equilibrium constants of association; negative subscripts identify rate and equilibrium constants of dissociation. Single subscripts (A, actin; D, ADP; T, ATP; P, inorganic phosphate) refer to the affinity of the respective ligand to myosin-S1, which is denoted as M. For multiple subscripts, the final letter of the string identifies the ligand associating to (or dissociating from) myosin-S1; all other letters refer to ligands already bound.

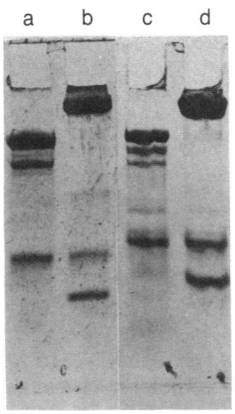

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of myosin and myosin-S1 preparations from rabbit and rat cardiac tissue. Samples (25 μg) of rabbit cardiac myosin-S1 (lane a), myosin (lane b), and rat cardiac myosin-S1 (lane c), and myosin (lane d) were run on 10% (wt/vol) acrylamide gels.

Actin from rabbit skeletal muscle was prepared from acetone powder using the method of Spudich and Watt (13), except that G-actin was filtered successively through 3.0- and 0.3-μm Millipore filters.

Reagents. All solutions were prepared using glass distilled water. Ammonium sulfate was absolute grade (Research Plus Laboratories, Bayonne, NJ). Vanadium-free ATP, ADP, (dicyclohexylammonium salt, grade VI), P^1,P^5-di(adenosine-5′)pentaphosphate (A_5A), chymotrypsin, and lima bean trypsin inhibitor were obtained from Sigma. Other reagents were of analytical grade.

Concentration Determinations. Concentrations of cardiac myosin and myosin-S1 were determined from absorption at 280 nm using extinction coefficients of 0.53 and 0.64, respectively (14). The concentration of rabbit skeletal muscle actin was determined using the micro-Biuret procedure (15) standardized with bovine serum albumin. The molecular weights (in g/mol) used for calculation of molar concentration of protein species were 115,000 (myosin-S1) and 43,000 (actin). The following extinction coefficients at 259 nm were used to calculate concentrations of nucleotides: ATP and ADP, 15.4 $mM^{-1}\cdot cm^{-1}$; A_5A, 30.8 $mM^{-1}\cdot cm^{-1}$.

Kinetics of the Dissociation of Actomyosin-S1 and Actomyosin-S1–ADP by MgATP. The ternary actomyosin-S1–ADP complex can be indirectly observed from the reduction by MgADP of the rate of dissociation of actomyosin-S1 by MgATP. Actomyosin-S1 [2 μM rabbit skeletal muscle actin, 1.75 μM myosin-S1, and 10 μM A_5A (to inhibit myokinase activity)], containing the desired amount of ADP, was mixed with ATP in a stopped-flow spectrofluorimeter. The change in the intensity of 340-nm light scattered at 90° from the incident beam was measured in a 20-mm path length cell (16). Temperature of the drive syringes, mixer, and observation cell was regulated to ±0.5°C by a refrigerated water bath. Kinetic data were recorded and stored on floppy disks using a Nicolet model 206 oscilloscope. Four or more data sets were taken at each experimental condition, summed together, and fit with a single exponential equation using an analog-fitting procedure. The difference between data and the theoretical curve was examined to determine whether the distribution of residuals was random and the data were accurately described by a single exponential equation. Standard experimental conditions were 0.1 M KCl/5 mM $MgCl_2$/5 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.0 (at the temperature indicated)/0.1 mM dithiothreitol.

Steady-State Kinetic Experiments. Steady-state MgATPase rates were measured using a pH-stat (17). Standard reaction conditions were 10 mM KCl/2 mM $MgCl_2$/0.1 mM dithio-

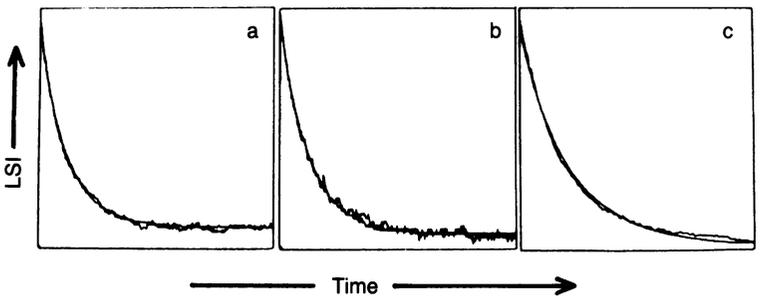

FIG. 2. Change in light scattering intensity (LSI) on mixing actomyosin-S1–ADP with ATP. Actomyosin-S1–ADP mixed with 1 mM ATP in a stopped-flow fluorimeter. The type of myosin-S1, final concentration of ADP, time scale, and value for the observed rate constant are as follows: (*a*) rabbit cardiac, 60 μM ADP, 102 msec, and 83 sec^{-1}; (*b*) rat cardiac, 100 μM ADP, 51 msec, 166 sec^{-1}; (*c*) rabbit soleus, 50 μM ADP, 83 msec, 64 sec^{-1}. Curves drawn through data are the best fit to a single exponential equation. Experimental conditions were 100 mM KCl/5 $MgCl_2$/5 mM 3-(*N*-morpholino)propanesulfonic acid/0.1 mM dithiothreitol, pH 7.0, 15°C.

threitol/2 mM MgATP, pH 7.0, unless noted otherwise. Reactions were initiated by addition of myosin-S1. Rates were obtained from initial velocity measurements, which were linear up to hydrolysis of ≈50% of the MgATP. V_{max} and K_{app} were estimated from the hydrolysis rates observed at actin concentrations from 2 to 100 μM from plots of k_{obs} versus k_{obs}/[actin] (18).

RESULTS

Kinetic Measurements of the Dissociation of ADP from Actomyosin-S1. The observed rate constants for the dissociation of actomyosin-S1 and actomyosin-S1–ADP by ATP were determined from the decrease in light scattering after mixing in a stopped-flow fluorimeter as described in *Methods*. In the absence of ADP, the kinetics of dissociation of actomyosin-S1 by ATP are the same within experimental error for actomyosin-S1 from rabbit psoas and soleus and from rat and rabbit cardiac muscles (data not shown). The observed rate constant for dissociation increases linearly with ATP concentration up to at least 400 sec^{-1} and the second-order rate constant is $2.0 \pm 0.5 \times 10^6$ $M^{-1} \cdot sec^{-1}$ at 15°C. The same rate constants have been reported for actomyosin-S1 from chicken anterior latissimus dorsi, posterior latissimus dorsi, and cardiac muscles (17), and for bovine cardiac (9) and rabbit skeletal muscles (19). Thus, in the absence of ADP, ATP concentration >500 μM would be expected to dissociate actomyosin-S1 at ≈1000 sec^{-1}, and the reaction would be complete within the mixing dead time of the stopped-flow instrument used in these experiments (3–4 msec). However, in the presence of ADP at concentrations >10 μM, the rate of dissociation of actomyosin-S1 from rabbit soleus and cardiac muscle and from rat cardiac muscle is easily observable at 500 μM ATP, as shown in Fig. 2. The dissociation rate in the presence of ADP was also measured at 1 and 2 mM ATP. In general, the rate observed at 2 mM ATP was <30% greater than at 500 μM ATP. Plots of $1/k_{obs}$ versus 1/ATP thus required only a small extrapolation to obtain limiting values for k_{-AD} of 70, 115, and 220 sec^{-1} at 15°C for rabbit soleus, and rabbit and rat cardiac actomyosin-S1, respectively. Only a lower limit of 400 sec^{-1} for k_{-AD} was obtained for psoas actomyosin-S1 at 15°C, 1 mM ADP, and 6.4 mM ATP.

Temperature Dependence of ADP Dissociation from Actomyosin-S1, k_{-AD} and the Maximum Steady-State Rate for ATP Hydrolysis, V_{max}. The temperature dependence of k_{-AD} for rat and rabbit cardiac actomyosin-S1 is shown in Fig. 3 (solid symbols). Linear Arrhenius plots are observed from 0°C to 25°C, yielding activation energies of 64 and 76 kJ/mol for rat and rabbit cardiac actomyosin-S1, respectively. These activation energies are within experimental error of the value previously measured for bovine cardiac actomyosin-S1 (9), even though the rate constants are 2 to 4 times greater. Values for k_{-AD} for rabbit psoas and soleus actomyosin-S1 obtained at 15°C are also shown in Fig. 3.

The temperature dependence of the V_{max} for steady-state ATP hydrolysis by actomyosin-S1 from rabbit and rat hearts is shown in Fig. 3 (open symbols). Data for actomyosin-S1 from rabbit psoas and soleus muscles were only obtained at 15°C. Linear Arrhenius plots are observed from 0°C to 25°C, yielding activation energies of 122 and 105 kJ/mol for the steady-state V_{max} of rat and rabbit cardiac actomyosin-S1, respectively. These activation energies are within experimental error of the values previously reported for bovine cardiac and rabbit skeletal actomyosin-S1 (9, 20). The results indicate that, although there are large tissue-specific variations in the absolute rates of k_{-AD} and V_{max}, the activation energies appear similar for actomyosin-S1 from many muscle types. We also found that the activation energies of k_{-AD} and V_{max} are 65–75 and 105–125 kJ/mol, respectively, for actomyosin-S1 from bovine, porcine, and canine cardiac muscles (unpublished data).

The steady-state rate of ATP hydrolysis catalyzed by actomyosin extracted from a variety of muscles was shown by Barany (6) to correlate with the maximum shortening velocity. However, Barany pointed out that the activation energy of the ATP hydrolysis rate is approximately twice the apparent activation energy of the shortening velocity. Workers in several laboratories have recently extended Barany's observation by demonstrating that the activation energy of the rate of ATP hydrolysis in contracting muscle fibers and myo-

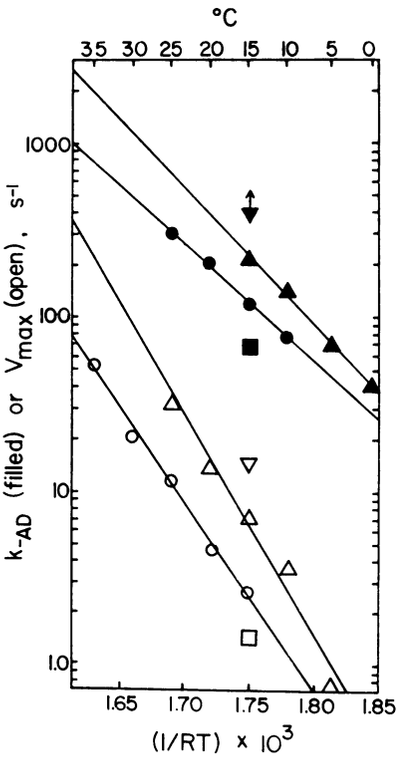

FIG. 3. Temperature dependence of the rate constant for the dissociation of ADP from actomyosin-S1, k_{-AD}, and of the maximum steady-state rate of actomyosin-S1 ATP hydrolysis, V_{max}. k_{-AD} (closed symbols) and V_{max} (open symbols) were measured as described in Fig. 2 and the text for rat cardiac (▲, △), rabbit cardiac (●, ○), rabbit psoas (▼, ▽), and rabbit soleus (■, □) actomyosin-S1 except the temperature was varied as indicated. Solid lines through the data were calculated by linear regression.

fibrils is similar to the activation energy of the maximum rate of ATP hydrolysis by actomyosin-S1 (21, 22). The temperature dependence of the velocity of unloaded shortening has been measured for a number of muscle types, including frog sartorius, tortoise iliofibularis, and rat extensor digitorum longus, to be 50–70 kJ/mol (22–25). Although corresponding measurements have not been made for k_{-AD} and unloaded shortening in the same muscle types, the temperature dependence of k_{-AD} and the unloaded shortening velocity are approximately the same. Moreover, the activation energy of V_{max} for steady-state ATP hydrolysis, ≈115 kJ/mol, is considerably larger than the apparent activation energy of the maximum shortening velocity. Thus, the activation energy of the unloaded shortening velocity is consistent with ADP dissociation being the molecular step that limits the rate of shortening in muscle but not the step that limits the steady-state rate of ATP hydrolysis.

The Correlation Between k_{-AD}, k_{min}, and the Velocity of Unloaded Muscle Shortening. To make a proper comparison between rate constants, measurements must be made under similar experimental conditions. Interpolation and extrapolation of the temperature dependence observed for the rate of k_{-AD} of rabbit and rat heart actomyosin-S1 (Fig. 3) was, therefore, required for the comparison to values in the literature for unloaded shortening velocity. Fig. 4 shows that the value calculated from the unloaded shortening velocity for k_{min} using Eq. 2 is equal, within experimental error, to the rate observed for ADP dissociation from actomyosin-S1–ADP for a variety of muscle types. A possible exception is rabbit psoas muscle for which only a lower limit for the rate of k_{-AD} can be measured. The close correlation between

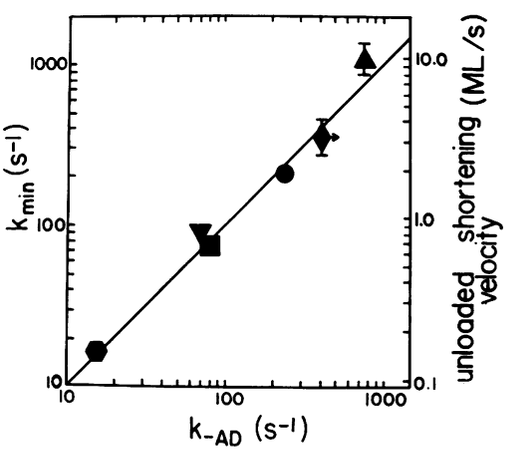

FIG. 4. Correlation between unloaded shortening velocity, theoretical value for minimum rate constant of a molecular step occurring between attached crossbridge states during muscle contraction, k_{min}, and rate constant for dissociation of ADP from actomyosin-S1. k_{min} is calculated from observed unloaded shortening velocity using Eq. 2. Solid line is for $k_{min} = k_{-AD}$. Muscle type, temperature, and references for the observed value for unloaded shortening velocity and k_{-AD} are as follows: chicken anterior latissimus dorsi (■), 20°C (17, 26); rabbit soleus (▼), 15°C (Fig. 3; ref. 27); rabbit heart (●), 22°C (Fig. 3; ref. 28); rat heart (▲), 26°C (Fig. 3; ref. 29); rabbit psoas (◆), 15°C (Fig. 3; ref. 27); chicken gizzard (⬢), 20°C (Robert Barsoti, personal communication). [Although smooth muscle cells do not have the easily recognizable sarcomere structure of striated muscle cells, several pieces of evidence suggest a sarcomere-like organization in smooth muscle having similar dimensions to sarcomeres in striated muscle cells. Myofibril-like structures with an axial repeat of ≈1.5 μm have been observed in chicken gizzard muscle cells (30). The mean distance between the dense bodies of toad stomach cells has been observed to decrease from 2.2 to 1.4 μm on contraction (31). Dense bodies may be analogous to the Z-line in striated muscle cells (Bond, M., Somlyo, A. V., Butler, T. M. & Somlyo, A. P., International Biophysics Congress, August 23–28, 1981, Mexico City, Mexico, p. 46, abstr.).

k_{-AD} and k_{min} is observed over a 40-fold range of shortening velocities. On the other hand, if the step that limits the steady-state V_{max} also limits the velocity of unloaded shortening, then muscle could have a maximum shortening velocity of only 1/10th what is actually observed. This apparent contradiction can be resolved if the step that limits V_{max} does not limit the unloaded shortening velocity of muscle, and it indicates that there is not a direct molecular basis for the correlation between the rate of actomyosin ATP hydrolysis and shortening velocity observed by Barany (6). Kinetic data from several laboratories (4, 19) also indicate that the rate-limiting step for ATP hydrolysis occurs on a myosin intermediate that rapidly binds to and dissociates from actin. Such intermediates can break and reform crossbridges many times before the rate-limiting step occurs. Therefore, they would not be expected to limit the relative sliding motion between the actin and myosin filaments. Conversely, myosin–ADP is not in rapid equilibrium with actin, because k_{-DA} is <0.1 sec^{-1} (9, 32). The only kinetically significant pathway available for myosin-S1–ADP dissociation from actin is ADP dissociation followed by ATP binding and dissociation of myosin-S1–ATP (9). Thus, ADP dissociation from actomyosin-S1 has attributes *i–iii* (enumerated in the introduction) that are required for a step of the mechanism to limit the maximum shortening velocity in muscle, whereas the maximum rate of ATP hydrolysis does not have these attributes.

DISCUSSION

Both in solution and during unloaded muscle shortening, actomyosin crossbridges would be expected to be under little, if any, tension. Therefore, the kinetic mechanism observed for actomyosin-S1 may be a reasonable approximation for the mechanism that occurs in muscle undergoing unloaded shortening. The dissociation constant for ADP binding to rabbit psoas and bovine cardiac myofibrils has been measured to be 200 and 7 μM, respectively (33). These values are comparable to those for rabbit fast skeletal and bovine cardiac muscle actomyosin-S1 in solution, 160 μM (8, 34) and 10 μM (9), respectively. Therefore, unless there are fortuitously compensating changes in the rates of both association and dissociation of ADP from myofibrils, the available evidence indicates that the rate constants for ADP dissociation from actomyosin-S1 and from crossbridges in muscle are likely to be similar. In addition, etheno-2-aza-ADP (a fluorescent ADP analogue) dissociates with a rate constant of 20 sec^{-1} from bovine cardiac actomyosin-S1 and 18 sec^{-1} from bovine cardiac myofibrils (S. J. Smith, personal communication). These results indicate that the rate constants measured for ADP dissociation from actomyosin-S1 are likely to be a reasonably good approximation for those occurring in muscle and satisfy criteria *iv* from the introduction of this paper. We have so far demonstrated that ADP dissociation satisfies the first four criteria listed in the introduction to be the molecular step that limits shortening velocity in vertebrate muscle. There is, however, no evidence demonstrating whether the intermediate formed by adding ADP to actomyosin-S1 (or contracting muscle) is on the predominant hydrolytic pathway. Sleep and Hutton (35) have shown that the rate of catalysis of the exchange of medium phosphate into ATP by rabbit skeletal actomyosin-S1 is independent of ADP concentration. This indicates that phosphate does not exchange into medium ATP by binding directly to the equilibrium actomyosin-S1–ADP intermediate, which is formed from ADP binding to actomyosin-S1. The exchange reaction must, therefore, occur by phosphate binding to another intermediate, actomyosin-S1–ADP^+, that is present during steady-state hydrolysis. Sleep and Hutton's results are consistent

with reaction mechanisms in which the equilibrium actomyosin-S1–ADP intermediate occurs either after the actomyosin-S1–ADP^+ intermediate (Eq. **4**) or is not on the pathway. Although it remains important to show that the equilibrium actomyosin–ADP intermediate is on the predominant catalytic pathway of ATP hydrolysis, the weight of the evidence indicates that ADP dissociation from actomyosin-S1–ADP is a good candidate for the molecular step that limits shortening velocity in vertebrate muscle.

$$\begin{array}{ccccccc} AM + ATP \rightleftharpoons AM\text{–}ATP & \rightleftharpoons & AM\text{–}ADP\text{-}P & \rightleftharpoons & AM\text{–}ADP^+ & \rightleftharpoons & AM \\ \Updownarrow & & \Updownarrow & & \searrow\!\!\nearrow & & \\ M\text{–}ATP & \rightleftharpoons & M\text{–}ADP\text{-}P & & AM\text{–}ADP & & \end{array} \quad [4]$$

Several measurements of the mechanical properties of muscle fibers are consistent with ADP dissociation limiting the unloaded shortening velocity in vertebrate muscles. Dantzig *et al.* (36) have shown that ADP reduces the rate of tension decrease in skinned rabbit psoas fibers obtained after the photolysis of caged ATP. Ferenczi *et al.* (37) have found that the unloaded shortening velocity in skinned frog muscle fibers increases with MgATP concentration. Moreover, k_{min} calculated from the unloaded shortening velocity was similar to k_{obs} for dissociation of frog actomyosin-S1 at low MgATP concentration. At >500 μM MgATP, k_{min} reaches a plateau of $\approx$280 sec^{-1}. In contrast, k_{obs} continues to increase linearly with MgATP concentration to >360 sec^{-1}. They concluded that an additional step, which is not present during dissociation of actomyosin-S1 in solution (such as product release), limits the rate of crossbridge dissociation and the shortening velocity in muscle. The data presented here provide evidence that ADP dissociation is the additional step. Kawai (38) has measured a similar rate-limiting process in mechanically oscillated rabbit psoas and soleus fibers.

According to models of muscle contraction such as that of A. F. Huxley, the rate of crossbridge detachment limits the maximum velocity of shortening (39). In these models, crossbridge detachment is blocked by an unknown mechanism at the beginning of the power stroke but proceeds rapidly at the end of the power stroke. Such a mechanism improves efficiency by avoiding early dissociation of the crossbridge before the power stroke is completed. However, with the exception of actomyosin-S1 from smooth muscle, the rate constant of dissociation of myosin-S1–ATP from actin has been measured to be at least 1000 sec^{-1} and could be as fast as 6000 sec^{-1} ($K_{AT}\,k_{-TA} \times 3$ mM) at physiological concentrations of ATP. Rapid rates of dissociation of myosin–ATP from actin such as are observed in solution would only be expected at the end of the crossbridge power stroke in muscle. We have demonstrated that ADP bound to actomyosin-S1 reduces the rate of ATP binding and subsequent dissociation of myosin-S1–ATP from actin in solution and hence would be expected to reduce the rate of crossbridge dissociation by ATP in muscle by the same mechanism. This result provides experimental evidence for the model proposed by Hill and Eisenberg (40) in which ADP blocks the dissociation of the crossbridge by ATP at the beginning of the power stroke. The strong correlation observed in a variety of muscles between the rate of ADP dissociation from actomyosin-S1 and unloaded shortening velocity suggests that variation of the rate of ADP dissociation may provide a mechanism for kinetic coupling of the rate of the power stroke to the rate of the shortening velocity in different muscle types.

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