Kinetics of the Interaction between Actin, ADP, and Cardiac Myosin-S1*

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The rate and equilibrium constants for the formation and dissociation of the bovine ventricular (BV) actomyosin-S1-ADP have been measured by stopped flow light scattering. A comparison of the rate constants obtained here with those for rabbit skeletal (RS) actomyosin-S1 indicates that there are large differences in several of the rate and equilibrium constants. 1) The rate constant of ADP dissociation from BV actomyosin-S1 is 65 ± 10 s⁻¹ at 15 °C compared to a lower limit of 500 s⁻¹ previously observed for RS actomyosin-S1. 2) The association constant for ADP binding to actomyosin-S1 is increased from 6 x 10⁸ M⁻¹ from RS to 1.5 x 10¹⁰ M⁻¹ for BV at 15 °C.

The following rate and equilibrium constants differ by less than a factor of 2 between RS and BV actomyosin-S1: 1) the second order rate constant for the dissociation of actomyosin-S1 by MgATP; 2) the second order rate constant of myosin-S1 and myosin-S1-ADP binding to actin; and 3) the association constant of myosin-S1 to actin.

The rate constant for ADP dissociation from BV actomyosin-S1 is at least 10-fold greater than the V₉₅ for the steady state ATPase and therefore cannot be the rate-limiting step of ATP hydrolysis. However, at physiological temperature, 38 °C, and ATP concentration, >3 mM, ADP dissociation is sufficiently slow to limit the rate of myosin-S1 dissociation from actin by ATP and is likely to be the rate-limiting step of cross-bridge dissociation in muscle. Moreover, the rate constant of ADP dissociation is sufficiently slow to be the molecular step which limits the unloaded shortening velocity in cardiac muscle.

The kinetic mechanism for the hydrolysis of ATP catalyzed by RS myosin-S1 and actomyosin-S1 has been extensively studied (1–5). Most of the rate and/or equilibrium constants

\[
\begin{align*}
K_{AT} & = K_{AH} \\
K_{-AT} & = K_{-AH} \\
K_{ADP} & = K_{-ADP} \\
K_{AD} & = K_{-AD} \\
AM & = AM-T \\
AM-D & = AM-D-P \\
K_{T} & = K_{T-A} \\
K_{P} & = K_{P-A} \\
M & = M-T \\
M-D & = M-D-P
\end{align*}
\]

The rate and equilibrium constants in the absence of actin (corresponding to the bottom line in Equation 1) have been previously measured for BV myosin-S1 (6, 7). In general, they are equal in magnitude to within a factor of 2 of those measured for RS myosin-S1. On the other hand, the steady state rate of hydrolysis at saturating concentrations of actin and ATP, V₉₅, is ~5-fold higher for RS actomyosin-S1 (6).

A primary rationale for studying the mechanism of the ATPase of actomyosin-S1 in solution is that the rate and equilibrium constants obtained may be related to the contractile properties of muscle. Several steady state and transient kinetic studies indicate that this is so. For example, there is a strong correlation between the steady state rate of hydrolysis of MgATP by actomyosin and the unloaded shortening velocity for a variety of skeletal muscles (8). Furthermore, the steady state rate of hydrolysis of CaATP by myosin isolated from a variety of mammalian hearts has been correlated with the rate of shortening of cardiac muscle in vivo (9). Marston and Taylor (10) found that, in the chicken, the V₉₅ for the actomyosin-S1 MgATPase increased in the following order: posterior latissimus dorsi > anterior latissimus dorsi > heart > gizzard. This same order is observed for the velocity of shortening of these chicken muscle types.

In this paper, we extend the kinetic studies of the mechanism of BV actomyosin-S1 MgATP hydrolysis by measurement of the rate and equilibrium constants of MgADP binding to actomyosin-S1 and analysis of the interaction between the ADP and actin-binding sites of BV myosin-S1. Implications for the molecular mechanism of muscle contraction are discussed.

MATERIALS AND METHODS

Proteins—Myosin was purified from the left ventricle of bovine hearts according to a modification of the procedure of Siemankowski 5045.
and Dreizen (11). Myosin was extracted from Triton-washed myofibrils for 5 min by a solution containing 0.25 M KCl, 7.5 mM potassium phosphate, 2.5 mM ATP, 0.25 mM MgCl₂, 1 mM EDTA, 1.0 mM dithiothreitol, pH 6.8 at 4 °C. After centrifugation to remove cellular debris, the crude myosin was precipitated by dialysis for 12 h versus 15 volumes of 0.1 M dithiothreitol. The precipitated myosin was dissolved in 0.5 M KCl, 0.1 mM potassium phosphate, 20 mM MgCl₂, 0.5 mM ATP, pH 7.0, and the light scattering centrifuged at 140,000 × g to remove actomyosin. The myosin was salted out by the resulting supernatant at 38 to 42% saturated ammonium sulfate. BV myosin-SI was prepared by a modification of the method of Flamin and Cusanovich (7). Myosin, 15 mg/ml, in 0.6 M NaCl, 10 mM potassium phosphate, pH 6.5, 1.5 mM EDTA, pH 7.0, was digested for 18 h by 0.05 mg/ml bovine pancreatic chymotrypsin (Sigma; type II). The digest was terminated by the addition of 0.1 M mg/ml lima bean trypsin inhibitor and dialyzed versus 50 mM KCl, 10 mM K-phosphate, 0.02% (w/v) NaN₃, 1 mM EDTA, pH 6.5. The resulting suspension was centrifuged for 1 h at 140,000 × g to pellet light meromyosin, myosin rod, and undigested myosin. The supernatant was fractionated with ammonium sulfate. The myosin-SI fraction, which precipitated between 50 and 55% saturated ammonium sulfate, was found to be entirely free from contamination by HMM as indicated by the absence of the 140-kDa peptide upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

A number of previous studies on cardiac myosin have shown that it is less stable than RS myosin (6, 7, 12). The preparative procedure described here yields BV myosin-SI, which exhibits 25 to 28% enhancement in steady state tryptophan fluorescence upon the addition of 20 mM MgATP. MgATP hydrolysis rate at standard conditions, 0.2 mg/ml actin, 10 mM KCl, 2 mM MgCl₂, 2 mM MgATP, pH 7, 25 °C, was observed to be 1.5 ± 0.3 s⁻¹ using a pH-stat. Active site titration, in which ATP hydrolysis was linked to NAD reduction by pyruvate kinase and lactate dehydrogenase reactions, required 0.75 ± 0.06 mol of ADP/mol of myosin-SI to obtain the maximum ATP hydrolysis rate, 0.012 ± 0.002 s⁻¹. Special handling was required to retain maximum enzymatic activity and prevent aggregation of BV myosin-SI. Immediately upon completion of purification, BV myosin-SI was dialyzed versus 0.1 M KCl, 5 mM MOPS, pH 7.0, 0.2 mM MgCl₂, 2 mM MgATP, pH 7, 25 °C, to be lyophilized in the presence of 0.5 M sucrose and stored at −20 °C over desiccant. Lyophilized myosin-SI was dialyzed for 18 h and used for experiments within 24 h. BV myosin-SI is unstable at temperatures greater than 15 °C in the absence of both nucleotide and actin. For instance, BV myosin-SI aggregates so extensively upon incubation for more than 30 min at 25 °C in 10−100 mM KCl, 5 mM MOPS, pH 7.0, 2 mM MgCl₂, 0.1 mM DTT that a 1 mg/ml solution becomes visibly turbid. Therefore, measurements requiring BV myosin-SI in the absence of both nucleotide and actin could not be done above 15 °C. Concentrated stock solutions of BV myosin-SI (20 mg/ml or greater) were stored in ice, 0.1 M KCl, 5 mM MOPS, pH 7.0, 0.2 mM DTT, 0.1 mM EDTA and diluted into reaction buffer of the required temperature immediately prior to use for kinetic experiments or were diluted into buffer on ice and the temperature was equilibrated in the drive syringes of the stopped-flow. In the latter case, temperature equilibration occurred within 10 min.

Actin was prepared from bovine ventricles using a modification of the method of Spudich & Watt (13). Crude G-actin was extracted from heart acetone powder and filtered successively through 3.0- and 0.2-μm Millipore filters prior to polymerization at 37 °C. Polymerization at temperatures less then 37 °C significantly reduced yield. RS myosin-SI and HMM were prepared using published procedures (14). HMM was prepared by digesting myosin with type VII TLCK-treated bovine pancreatic chymotrypsin (Sigma) and chromatographed on DEAE-cellulose.

Reagents—All solutions were prepared using glass-distilled water. Ammonium sulfate was absolute grade (Research Plus Laboratories). Vanadate-free ATP, ADP (dicyclohexylammonium salt, grade VI), Ala, hexokinase (type F-300), lima bean trypsin inhibitor, and reagent solutions were purchased from Sigma Chemical Co. Other reagents were of analytical grade.

Concentration Determinations—The concentrations of rabbit skeletal muscle myosin, myosin-SI, and HMM were determined from the absorption at 280 nm (corrected for Rayleigh light scattering) using extinction coefficients of 0.12, 0.13, 0.53, 0.57, 0.77, and 0.75 (w/v, 1 cm) for the respective proteins. Concentrations of BV myosin and myosin-SI were determined from absorption at 280 nm employing extinction coefficients of 0.55 and 0.64 (15). The concentration of BV actin was determined using the microbiuret procedure (16) standardized with bovine serum albumin. The molecular weights (in grams/mol) used for calculation of molar concentration of protein species were myosin-SI, 115,000; HMM, 320,000; and actin, 43,000. The following extinction coefficients at 295 nm were used to calculate concentrations of nucleotides: ATP and ADP, 15.4 mM⁻¹ cm⁻¹; A₂₆₀, 30.8 mM⁻¹ cm⁻¹.

Steady State Light Scattering Measurements—The intensity of light (530 nm) scattered at 90° from the incident beam was measured using the Spex Fluorolog spectrophotometer in the photon-counting mode. The temperature of the observation cell was regulated to within 0.5 °C by a refrigerated water bath.

Stopped Flow Measurements—Transient fluorescence and light scattering measurements were made with a stopped flow fluorimeter, equipped with a 20 by 1 nm observation cell, which was illuminated lengthwise though quartz end windows. The cell was recessed into a spherical mirror so as to improve the efficiency of light collection (17). Temperature of the drive syringes, mixer and observation cell was regulated to ±0.5 °C by a refrigerated water bath. Excitation wavelengths, 296 nm for fluorescence measurements or 340 nm for light scattering, were obtained by using interference filters (Oriel Corp.) to select the appropriate wavelength of a mercury arc (Osram 100W/2) or xenon (Osram 75W/2) lamp spectrum. For fluorescence measurements, emitted light was filtered through a Corning broad band glass filter (325–380 nm) in order to reduce scattered light. The photomultiplier tube output was smoothed using an active filter circuit having a reciprocal time constant at least 20 times the rate constant of the reaction being measured. Kinetic data were digitized and a Data Formac 1200 computer was used to plot graphs. Four or more data sets were taken at each experimental condition, summed together and fit to single or double exponential equations by the method of Foss (18). The difference between data and fit was examined to determine if the distribution of the residuals was random and the data were accurately described by a single exponential equation or if a double exponential equation was required to properly describe the data.

Steady State Kinetic Experiments—Steady state MgATPase rates were measured using a pH-stat (17). Standard reaction conditions were 10 mM KCl, 2 mM MgCl₂, 0.1 mM DTT, 2 mM MgATP, pH 7.0, unless specifically noted otherwise. Reactions were initiated by addition of myosin-SI. Steady state rates were obtained from initial rate measurements, which were linear up to hydrolysis of ~50% of the MgATP. V₅₀ and Kₘ₅ were estimated from the hydrolysis rates observed at actin concentrations from 2 to 100 μM using the method of Woolf (19). At I = 0.02, V₅₀ of the steady state actin-activated ATPase is readily measured, but at I = 0.11, ionic conditions approximate those found in muscle, V₅₀ cannot be measured due to the magnitude of the Kₘ₅ for actin, −200 μM.

RESULTS

Steady State Light Scattering of Bovine Ventricle Actomyosin-SI—The increase in light scattering upon the addition of BV myosin-SI to actin is shown in Fig. 1. The increase is proportional to the amount of myosin-SI added up to a maximum scattering intensity which occurs at a stoichiometry of approximately 1 mol of myosin-SI/mol of actin subunit. These results are similar to those observed previously for RS actomyosin-SI (14). At very low actin concentration, 50 nm, the break from linear increase to plateau level is less sharp than at high actin concentrations, as would be expected from mass action considerations. The amount of light scattered at the saturating level of myosin-SI is proportional to the actin subunit concentration over the range from 5 × 10⁻⁵ to 5 × 10⁻⁴ M. The observed data are fit well by an association constant on the order of 10⁶ M⁻¹ at an ionic strength of 0.11 (solid curves). Attempts to estimate the association constant at ionic strength of 0.02 were unsuccessful as the reaction was essentially stoichiometric even at 50 nm actin (data not shown). However, a lower limit of the association constant was estimated to be ~10⁶ M⁻¹. Kₐ for the association of BV myosin-SI to F-actin is comparable to literature values for actomyosin-SI from RS and chicken anterior latissimus dorsi, posterior latissimus cardiac, and smooth muscles pro-
vided that the dependence of the association constant upon ionic strength is taken into consideration.

**Kinetics of Myosin-S1 Binding to Actin**—The kinetics of binding of BV myosin-S1 to F-actin was measured from the increase in light scattering observed upon mixing actin and myosin-S1 in a stopped flow fluorimeter. The observed increase was fit adequately using a single exponential equation as shown in Fig. 2. \( k_{\text{obs}} \) increases linearly with the concentration of myosin-S1 up to at least 100 s\(^{-1} \) (Fig. 3). The data are therefore interpreted in terms of a simple one-step binding mechanism in which the gradient of the dependence of \( k_{\text{obs}} \) upon myosin-S1 concentration is the bimolecular rate constant, \( k_A \), in Equation 2.

\[
A + S1 \overset{k_A}{\underset{k_{-A}}{\rightleftharpoons}} A-S1
\]  

At standard low ionic strength conditions (I = 0.02), the second order rate constant \( k_A \) is observed to be 2 \( \times 10^8 \) M\(^{-1}\) s\(^{-1} \). Increasing the ionic strength to 0.11 decreases \( k_A \) to 8 \( \times 10^7 \) M\(^{-1}\) s\(^{-1} \). These values observed for BV actomyosin-S1 are compared to values reported in the literature for actomyosin-S1 from a variety of muscle types in Fig. 4. The logarithm of \( k_A \) (and \( K_A \)) for actomyosin-S1 from various muscles is proportional to the square root of ionic strength, \( I^{1/2} \), from I = 0.02 to 0.5. The dependence of \( k_A \) and \( K_A \) upon ionic strength satisfies the Debye-Hückel relationship for the rate of reaction between two uniformly charged spheres. From the slope of the plots, the product of the charges on actin and myosin-S1 is calculated to be from -4 to -5. An exception is the \( K_A \) for smooth muscle actomyosin-S1, which has a much lower dependence upon ionic strength (20). The second order rate constant for myosin-S1 binding to F-actin extrapolated to zero ionic strength, \( 10^{-10} \) M\(^{-1}\) s\(^{-1} \), is at (or near) the limit of diffusion for molecules having the axial ratio and molecular weight of F-actin and myosin-S1 (see discussion in Ref. 14).

The observed ionic strength dependence of \( K_A \) at temperatures from 15 to 20 °C, and at pH 7 to 8 for the various actomyosin-S1 species is shown by the solid symbols in Fig. 4. \( K_A \) for BV is within the values of the previous determinations for RS actomyosin-S1. However, differences of up to 5-fold could be accommodated within the range of the data. There is good agreement between the values obtained by methods which depend upon measurement of the concentration of the actomyosin-S1 complex (light scattering and kinetic) whereas methods measuring the concentration of free myosin-S1 (centrifugation and fluorescence depolarization) tend to give somewhat lower values. Both the apparent second order rate constant \( k_A \) and association constant \( K_A \) observed for various actomyosin-S1 species display essentially the same ionic

**Fig. 1.** Equilibrium binding of bovine ventricular S1 to actin. Three-ml aliquots of actin, 50 (●) and 400 nM (●), in 0.1 M KCl, 5 mM MOPS, 2 mM MgCl\(_2\), pH 7, 15 °C were titrated with myosin-S1. The increase in light scattering intensity (\( I_{\text{LSI}} \)) was measured after successive additions of myosin-S1. Following the final addition of myosin-S1, MgATP was added to a final concentration of 20 μM. The amount of light scattered by actomyosin-S1 in the presence of ATP is the same as the sum of the light scattered by myosin-S1 and actin measured individually. The theoretical lines through the data are for a simple equilibrium mechanism with an association constant of 1 \( \times 10^8 \) M\(^{-1}\).

**Fig. 2.** Stopped flow kinetic measurements of myosin-S1 and myosin-S1-ADP binding to actin. The increase in the intensity of scattered light was measured upon mixing myosin-S1 (A and B) and myosin-S1-ADP (C and D) with F-actin. Smooth curves drawn through the data are computer fits to a single exponential equation. Conditions: 2 μM actin, 0.1 mg/ml hexokinase, 10 mM glucose, 5 mM MOPS, 10 mM (A and C) or 100 mM KCl (B and D) pH 7.0, 15 °C. The concentrations of myosin-S1, MgADP, \( k_{\text{obs}} \) and the time scale are: A, 5 μM S1, 0 ADP, 96 s\(^{-1} \), 38 ms; B, 12.5 μM S1, 0 ADP, 15 s\(^{-1} \), 230 ms; C, 5 μM S1, 25 μM ADP, 26 s\(^{-1} \), 160 ms; D, 12.5 μM S1, 32.5 μM ADP, 2 s\(^{-1} \), 2.7 s.
The increase in light-scattering due to the binding of BV myosin-S1 from actin, and myosin-S1-ADP concentrations were varied as indicated [ADP] = 0 (C, D); [ADP] = (myosin-S1) + 20 μM, ( ), [KCl] = 10 mM (left) or 100 mM (right). Solid lines through the data correspond to second order rate constants of 8 x 10^6 (100 mM KCl) and 2 x 10^8 (10 mM KCl) M^-1 s^-1 for myosin-S1 binding to actin and 4 x 10^6 (10 mM KCl) and 1 x 10^8 (100 mM KCl) M^-1 s^-1 for myosin-S1-ADP.

**Fig. 4.** Dependence of the rate and equilibrium constants of myosin-S1 binding to actin upon ionic strength. Data obtained here for BV myosin-S1 are compared with literature values for other myosin types. Nominal conditions are pH 7–8, 2–5 mM MgCl₂, 15 ± 5°C plus buffer and potassium chloride to give the indicated ionic strength. Equilibrium association constants, Kₐ, actin + myosin-S1 = actomyosin-S1, are represented by open symbols. Apparent second order rate constants of binding, kₐ, actin + myosin-S1 → actomyosin-S1, are represented by filled symbols. Myosin-S1 type and experimental method are: BV, stopped flow and steady state light scattering, this paper (C, D); RS, stopped flow light scattering and fluorescence (21) ( ); RS, stopped flow light scattering (14) ( ); RS, stopped flow light scattering, Kₐ values determined according to detailed balance from values for Kₐ, KₐN, and Kₐ measured by filtration and sedimentation (24) ( ); RS, analytical ultracentrifugation (×) (37); RS, fluorescence depolarization (38) (); chicken posterior latissimus dorsi, stopped flow light scattering (10) (); chicken anterol latissimus dorsi, cardiac and gizzard, stopped flow light scattering (10) ( ). Linear regression lines drawn through the data for the dependence of kₐ and Kₐ upon I° are log kₐ = 7.6 ± 4.1 (I)° and log Kₐ = 9.8 ± 5.0 (I)°, respectively.

In order to more directly determine the rate of dissociation of BV myosin-S1 from actin, the increase in light-scattering intensity was measured upon mixing BV actomyosin-S1 (2 μM actin, 3 μM myosin-S1) in 0.1 M KCl, 5 mM MOPS, 2 mM MgCl₂, pH 7, 15°C with 1 μM RS HMM. The kinetics was first order, provided that all of the actin sites were saturated with myosin-S1 prior to addition of HMM. The rate constant observed, 0.005 to 0.009 s⁻¹, is slightly lower than previously observed for RS actomyosin-S1 using the same method (14), 0.025 s⁻¹, and also by a fluorescence method (21), 0.012 s⁻¹, and is similar to the value predicted from kₐ/Kₐ from Fig. 4. The displacement of myosin-S1 from actin by HMM is a valid measure of the rate of myosin-S1 dissociation only if myosin-S1 dissociation is the rate-limiting step of HMM binding. The good agreement observed between the equilibrium constant measured by the light-scattering titration, 1.0 x 10^8 M⁻¹, and that calculated from kₐ/kₐ, (-1.6 x 10^8 M⁻¹), suggests that displacement of myosin-S1 from actin by HMM is a valid method for estimating the rate of myosin-S1 dissociation from actin. At I = 0.02, BV myosin-S1 was not measurably displaced from BV actin by RS HMM (less than 10%) and a reliable estimate for kₐ could not be obtained. We have been unable to determine that this is due to very slow dissociation of BV myosin-S1 from BV actin at I = 0.02 or to a higher equilibrium affinity of BV myosin-S1 than RS HMM for BV actin. At I = 0.11, RS HMM displaces ~2 times as much RS as BV myosin-S1 from BV actin. These results are consistent with the data in Table I, which show that Kₐ for the binding of BV myosin-S1 to BV actin is approximately twice that for the binding of RS myosin-S1 to RS actin. Therefore, the relative affinity of myosin-S1 for actin does not seem to depend on the species of actin used.

**Table I**

| Parameter | Units | Bovine ventricle* | Rabbit fast skeletal muscle |
|-----------|-------|-------------------|---------------------------|
| K₀        | M⁻¹ s⁻¹ | 3 x 10⁶ (6)        | 1.5 x 10⁷ (3)             |
| Kₐ        | M⁻¹ s⁻¹ | 6 x 10⁶ (6, 7)     | 1 x 10⁷ (3)               |
| Kₐ        | M⁻¹ s⁻¹ | 2 x 10⁷           | 1.4 x 10⁷ (14)            |
| Kₐ        | s⁻¹   | 5 x 10⁻⁴          | 2 x 10⁻⁵ (14)             |
| Kₐ        | s⁻¹   | 4 x 10⁶           | 7 x 10⁶ (14)              |
| kₐ        | M⁻¹ s⁻¹ | 1 x 10⁻³ (6, 7)    | 1 x 10⁻⁴                 |
| kₐ        | s⁻¹   | 1 x 10⁻⁴          | 0.7 x 10⁻⁴               |
| kₐ        | s⁻¹   | 1.4 x 10⁻⁴        | 1 x 10⁻⁴                 |
| kₐ        | s⁻¹   | 55                | >500 (25)                 |
| kₐ        | s⁻¹   | 2.5 x 10⁶         | 6 x 10⁶ (23)              |
| kₐ/kₐ     | M⁻¹ s⁻¹ | 10               | 50                        |
| kₐ/kₐ     | 24     | 20                | 150                       |
| kₐ/kₐ     | 55     | 65                | >500 (25)                 |
| kₐ/kₐ     | 10     | 16                | 50                        |
| kₐ/kₐ     | 24     | 20                | 150                       |

*Based on data obtained at 100 mM KC1 (6, 7) and corrected assuming the same ionic strength dependence as observed for rabbit fast skeletal muscle.

Assumed to be the same as observed at 100 mM KC1.

Calculated from kₐ/kₐ.

Calculated from kₐ/kₐ.

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Calculated from kₐ/kₐ.

Calculated from kₐ/kₐ.
myosin-S1 as the association constant of ADP by BV myosin-S1, $K_{AD}$ is $3 \times 10^8$ M$^{-1}$ (6, 7). Increasing the concentration of MgADP to 75 μM in excess over myosin-S1 does not further decrease the rate of binding of myosin-S1 to actin (data not shown). At concentrations of myosin-S1 greater than $10^{-6}$ M, the change in light-scattering intensity observed upon mixing myosin-S1-ADP with F-actin is not fit adequately by a single exponential equation, as shown in Fig. 2D. Use of a double exponential equation considerably improves the fit. However, the magnitudes of the rates constants obtained by fitting with a double exponential equation differ by less than a factor of 3. As a result, a unique fit of two rates and amplitudes cannot be obtained. In general, the larger rate constant is 30 to 50% greater than the value obtained by fitting the data to a single exponential equation and the lower rate constant is approximately 50% of the best single exponential fit value. The $k_{obs}$ values plotted in Fig. 3 were obtained by fitting the data to the closest single exponential equation. The apparent second order rate constant for BV myosin-S1-ADP binding to actin using these values for the $k_{obs}$ is $1 \times 10^8$ M$^{-1}$ s$^{-1}$ at $I = 0.11$ and $4 \times 10^7$ M$^{-1}$ s$^{-1}$ at $I = 0.02$.

The biphasic kinetics observed for the binding of BV myosin-S1-ADP to actin suggests that there may be more than one significant conformation of either BV myosin-S1-ADP or actomyosin-S1-ADP. There is considerable empirical evidence from the literature that RS myosin$^+$ may exist in more than one conformation in solution depending on experimental conditions, temperature, ionic strength, pH, and bound nucleotides. Trybus and Taylor (22) have observed biphasic kinetics for ADP binding to both RS myosin-S1 and actomyosin-S1 and for myosin-S1-ADP binding to actin. Shriver and Sykes (23) have observed two phosphate peaks in the $^3$P NMR spectrum of ADP bound to RS myosin-S1 at 0 °C (but not at 20 °C). They have interpreted this result as an indication that two conformations of the myosin-S1-ADP complex can exist. In this paper, we have chosen not to study the complex equilibria between multiple conformers of the interaction between BV myosin-S1, ADP, and actin but rather to determine apparent rate constants of association and dissociation between each of the predominant stoichiometric forms in Equation 1. Although this approach is an obvious oversimplification, the apparent rate and equilibrium constants describe the reaction between the dominant conformers. The validity of this approximation is confirmed by the internal consistency of the data and the general absence of biphasic kinetics, except for myosin-S1-ADP binding to actin.

Kinetics of the Dissociation of BV Actomyosin-S1 and Actomyosin-S1-ADP by MgATP—The decrease in light-scattering intensity observed upon mixing actomyosin (with and without ADP) with MgATP is shown in Fig. 5. A single exponential equation adequately fits the data over a wide range of nucleotide concentrations. The dependence of the rate of dissociation of BV actomyosin-S1 upon the concentration of MgATP is shown in Fig. 6. Taylor and Weeds (6) reported that $k_{obs}$ increased proportionally to increasing concentration of MgATP and reached a maximum of approximately 2000 s$^{-1}$. We confirm that the rate of dissociation increases linearly with increasing concentration of MgATP to at least 300 s$^{-1}$. The apparent second order rate constant is observed to be $2 \times 10^8$ and $6 \times 10^7$ M$^{-1}$ s$^{-1}$ at ionic strengths of 0.11 and 0.02, respectively.

There is no measurable change in intrinsic fluorescence intensity upon the addition of up to 1 mM MgADP to BV actomyosin-S1 and, therefore, actomyosin-S1-ADP cannot be directly observed using this method. However, 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. Concentrations of MgADP greater than 5 μM appreciably decrease the rate of dissociation of BV actomyosin-S1 as shown in Fig. 6. The apparent second order rate constant for dissociation is decreased 50-fold from $2 \times 10^6$ M$^{-1}$ s$^{-1}$ in the absence of MgADP to $4 \times 10^5$ M$^{-1}$ s$^{-1}$ by the presence of 250 μM MgADP. Moreover, the rate constant for dissociation reaches a plateau at $k_{obs}$ of $\sim 65$ s$^{-1}$ in the presence of MgADP greater than 5 μM. Marston and Taylor (10) have observed a similar plateau is the rate of dissociation of actomyosin-S1 by ATP in the presence of ADP for actomyosin-S1 from chicken anterior latissimus dorsi and gizzard muscles. These results can be quantitatively described by a model in which ADP acts as a competitive inhibitor of ATP-binding at the active site of actomyosin-S1, as shown in Equation 3.

$$AM-ADP \xrightarrow{k_{-AD}} AM + ATP \xrightarrow{k_{AT}[ATP]} AM-ATP$$

The apparent second order rate constant for dissociation of BV actomyosin-S1 upon the concentration of MgATP is shown in Fig. 6. The apparent second order rate constant for dissociation is decreased 50-fold from $2 \times 10^6$ M$^{-1}$ s$^{-1}$ in the absence of MgADP to $4 \times 10^5$ M$^{-1}$ s$^{-1}$ by the presence of 250 μM MgADP. Moreover, the rate constant for dissociation reaches a plateau at $k_{obs}$ of $\sim 65$ s$^{-1}$ in the presence of MgADP greater than 5 μM. Marston and Taylor (10) have observed a similar plateau in the rate of dissociation of actomyosin-S1 by ATP in the presence of ADP for actomyosin-S1 from chicken anterior latissimus dorsi and gizzard muscles. These results can be quantitatively described by a model in which ADP acts as a competitive inhibitor of ATP-binding at the active site of actomyosin-S1, as shown in Equation 3.

$AM-ADP \xrightarrow{k_{-AD}} AM + ATP \xrightarrow{k_{AT}[ATP]} AM-ATP$
The association constant of ADP for BV actomyosin-S1, calculated by solving Equation 4 for $K_{AD}$, is $1.5 \pm 0.2 \times 10^8 \text{ M}^{-1}$. $k_{AD}$ is $1.0 \times 10^8 \text{ s}^{-1}$. The second order rate constant for ADP binding to BV actomyosin-S1, $k_{AD}$, may be calculated from $K_{AD} \cdot k_{AD}$ to be $1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. The rate and equilibrium constants of ADP binding to BV actomyosin-S1 are unchanged by decreasing the ionic strength from 0.11 to 0.02. Under similar conditions, ADP binds to RS actomyosin-S1 with an association constant of $6 \times 10^8 \text{ M}^{-1}$, which is approximately 30-fold smaller than that measured here for BV actomyosin-S1 (24, 25). The rate constant for ADP dissociation from RS actomyosin-S1 (14), $>500 \text{ s}^{-1}$, is at least eight times larger than measured here for BV actomyosin-S1. The difference observed between the association constants of ADP for BV and RS actomyosin-S1 is attributable in large part to the larger rate constant for dissociation of ADP from RS actomyosin-S1.

The general solution to the kinetic model of Equation 3 is a sum of three exponentials (Equation 5). This solution was obtained using Laplace transforms.

$$I(t) = I_0 e^{-k_B t} + I_1 e^{-k_{AD} t} + I_2 e^{-k_{BS} t} + C$$

(5)

The dependence of the amplitudes, $I_0$, $I_1$, and $I_2$, and observed rate constants, $k_B$, $k_{AD}$, and $k_{BS}$, upon ADP and ATP concentration and the values of the rate constants in Equation 3 were evaluated by a Fortran program. Initial calculations were made using the following values for the rate constants of Equation 3 obtained semi-empirically (Equation 4 and Fig. 6): $k_{AD} = 65 \text{ s}^{-1}$; $K_{AD} = 1 \times 10^7 \text{ M}^{-1} s^{-1}$; $k_{TA} k_AT = k_AT = 2 \times 10^{-3} \text{ M}^{-1} s^{-1}$ at least 90% of the total amplitude $I_1 + I_2 + I_3$ is associated with a single dominant exponential term of Equation 5 if $k_{TA} k_AT / [ATP] / k_AT > k_{AD} + k_{AD} [ADP]$. If, however, $k_{TA} k_AT / [ATP] / k_AT = k_{AD} + k_{AD} [ADP]$, a second exponential term in the solution to Equation 5 becomes significant and slightly biphasic kinetics is predicted. Biphasic kinetics is observed under these conditions. However, the deviation of the data from a single exponential is insufficient to obtain unique fits for two exponential terms and the observed data were therefore fit to a single exponential equation. The theoretical lines calculated for the dependence of the dominant term of Equation 5 upon ATP and ADP concentration fit the data reasonably well as is shown in Fig. 6. Although no attempt was made to produce the best fit by covariance methods, a slight improvement in the fit over the semi-empirical approach could be obtained by increasing the value of $k_{AD}$ to 75 s$^{-1}$ and reducing the value of $K_{AD}$ to $8 \times 10^8 \text{ M}^{-1} s^{-1}$ as shown in Fig. 6. The good agreement between the theoretical curves and the data is strong evidence for the mechanism of Equation 3 and the validity of Equation 4. The theoretical values calculated for the amplitudes and rate constants of the dominant term of Equation 5 are not sensitive to the details of the kinetic mechanism of the dissociation of actomyosin-S1 by MgATP. The only requirement is that the individual rate constants be chosen such that the apparent second order rate constant of dissociation of actomyosin-S1 by ATP is $2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and that the maximum rate of dissociation, $k_{TA}$, is $>2000 \text{ s}^{-1}$.

**Temperature Dependence of $K_{AD}$, $k_{AD}$, and the Turnover Number, $V_{max}$.**—The maximum rate of dissociation of ADP from BV actomyosin-S1 at 15 °C is $65 \text{ s}^{-1}$. This indicates that ADP dissociation is not sufficiently slow to limit the steady state rate of ATP hydrolysis by actomyosin-S1, $V_{max}$ is 1.5 s$^{-1}$ at 15 °C. However, the rate of ADP dissociation from actomyosin-S1 is sufficiently slow to be a kinetically significant step in muscle contraction. It is therefore important to know the temperature dependence of the rate and equilibrium constants for ADP dissociation and for the steady state $V_{max}$ in order to evaluate the thermodynamic parameters of the reaction and to estimate the rate and equilibrium constants at physiological temperature, 38 °C. The rate and equilibrium

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2 A. Strand, unpublished results.
constants of ADP dissociation from BV actomyosin-S1 have been measured at temperatures from 5 to 30 °C (using the same methodology as experiments shown in Figs. 5 and 6). The temperature dependence of $k_{AD}$ and $V_{max}$ for the actin-activated ATP hydrolysis are compared in Fig. 7. Depending slightly on ionic strength, $k_{AD}$ increases from $\sim 10^{-1}$ at 5 °C to $\sim 800$ s$^{-1}$ at 30 °C. Linear regression analysis of the Arrhenius plots indicates activation energies of 20 and 16 kcal/mol at $I = 0.11$ and 0.02, respectively. Extrapolation of the observed data to 38 °C predicts rate constants of 800 and 550 s$^{-1}$ at $I = 0.11$ and 0.02, respectively. At $I = 0.11$, the association constant for ADP binding to actomyosin-S1, $K_{AD}$, is observed to be $1.5 \times 10^4$ and $3.1 \times 10^4$ M$^{-1}$ at 15 and 30 °C, respectively (data not shown). These values suggest an enthalpy of $\sim 18$ kcal/mol. Since both $k_{AD}$ and $K_{AD}$ are observed to have very similar temperature dependence, the rate constant for binding of ADP to actomyosin-S1, $k_{AD}$, would be expected to have only slight temperature dependence. From linear regression analysis of the Arrhenius plot, $V_{max}$ for the steady state rate of hydrolysis of MgATP is estimated to be $60$ s$^{-1}$ at 38 °C and $I = 0.02$, and has an activation energy of 28 kcal/mol.

Dissociation of BV Actomyosin-S1 by MgADP.—The dissociation of BV actomyosin-S1 by MgADP was measured from the decrease in light-scattering intensity observed upon mixing of actomyosin-S1 with MgADP. Commercial ADP contains up to several per cent ATP as an impurity. Therefore, the following precautions were taken to ensure that the observed dissociation of actomyosin-S1 was due to ADP rather than contaminating ATP. Lots of ADP were selected in which the ATP concentration was observed to be less than 1% as measured by isotachophoresis (26); the dicyclohexylammonium salt proved best. Stock ADP solutions, $\sim 200$ mM, were treated by incubation with $10 \mu$g/ml hexokinase and 10 mM glucose in order to convert any ATP to ADP. The decrease in light-scattering intensity observed upon mixing BV actomyosin-S1 with treated ADP followed first order kinetics. Mixing of untreated ADP with BV actomyosin-S1 produced a more rapid dissociation followed by a slow reassociation to the same final level of light-scattering intensity as observed with hexokinase-treated ADP. The dissociation and reassociation of actomyosin-S1, which would be expected during a single turnover of ATP by actomyosin-S1 (14) are thus eliminated by pretreatment of ADP with hexokinase. 25 to 70 $\mu$M ADP reduces the light-scattering intensity of $5 \times 10^{-4}$ M BV actomyosin-S1 by an amount, which is 40% the decrease caused by the same concentrations of ATP. If dissociation were due to contaminating ATP, the extent of dissociation would be expected to be larger at higher concentrations of ADP (and thus contaminating ATP). However, this was not observed. Moreover, the observed rate constants for dissociation of actomyosin-S1 by hexokinase-treated ADP, 0.01-0.02 s$^{-1}$, are of an order of magnitude lower than that calculated using Equation 3 for the dissociation of BV actomyosin-S1 by stoichiometric ATP. For example, the calculated rate of dissociation of $5 \times 10^{-4}$ M BV actomyosin-S1 by $5 \times 10^{-5}$ M ATP in the presence of 25 $\mu$M ADP is $\sim 0.3$ s$^{-1}$, which is 20 times faster than the observed rate of dissociation by 25 $\mu$M ADP, 0.015 s$^{-1}$. Thus, the kinetics and extent of the dissociation of actomyosin-S1 by ADP are inconsistent with dissociation by contaminating ATP and indicate that the dissociation is due to ADP.

The mechanism of ADP dissociation of actomyosin-S1 can be described by the minimal mechanism shown in Equation 6a. ADP binding to BV actomyosin-S1 is in rapid equilibrium compared to the rate of S1-ADP dissociation, that is $k_{AD} \gg k_{DAD}$.

Therefore, the dependence of the observed rate of dissociation upon [ADP] and [A] can be described by Equation 6b (27), which simplifies to Equation 6c for $[D] \gg K_D$. A value for $k_{AD}$ of 0.01 s$^{-1}$ is obtained from Fig. 8 by extrapolating $k_{obs}$ to 0 protein concentration. At higher concentrations of actomyosin-S1, the observed rate constant is equal to the sum of the forward and reverse rate constants.

**Coupled Equilibria between the Binding of MgADP and F-actin to Myosin-S1**—The rate and equilibrium constants determined here from the data of Figs. 1-8 may be used together with previously reported data for the binding of MgADP to BV actomyosin-S1 (6, 7) to provide a kinetic description of the interaction between BV myosin-S1, actin, and MgADP. The data are summarized in Table I and a minimal kinetic scheme is presented in Equation 7.

$$
S_1^D = S_1^A + D^A \\
S_1^A + D^A + A \rightarrow S_1^D + A \\
S_1^A \rightarrow S_1^D + A
$$

An interaction constant between actin and ADP binding to myosin-S1, $K_{int}$, is obtained by applying the principle of detailed balance to Equation 7, as shown in Equation 8.

$$
K_{int} = \frac{k_A k_{DA} k_{D} k_{A}}{k_{AD} k_{D} k_{AD}}
$$

The observed agreement to within a factor of 2 for the two expressions for $K_{int}$ in Table I indicates that the data are internally consistent at both $I = 0.02$ and $I = 0.11$. The free energy of interaction, $-RT \ln K_{int}$, is 1.5 ± 0.2 kcal/mol. This value for BV actomyosin-S1 is considerably lower than previously measured for RS actomyosin-S1, 2.0 to 2.9 kcal/mol by Greene and Eisenberg (24) using sedimentation methods, and calculated from data obtained in this laboratory by kinetic

**Fig. 8. Dissociation of actomyosin-S1 by MgADP.** Actomyosin-S1, [actin] = 1.25 × [myosin-S1], was mixed with 50 $\mu$M ADP. The decrease in light-scattering intensity was fit to a single exponential equation in order to determine $k_{obs}$. Solvent conditions were identical with Figure 5 at [KCl] = 100 mM. The rate constant of dissociation of myosin-S1-ADP, 0.01 s$^{-1}$, was estimated by extrapolation of $k_{obs}$ to [actomyosin-S1] = 0, as indicated by the linear regression line.
methods. Previously, Highsmith (28) and Beinfeld and Martonosi (29) had also shown that ADP reduces the affinity of myosin-S1 for actin.

**DISCUSSION**

We have completed the analysis of the rate and equilibrium constants of a minimal mechanism (Equation 7) describing the interaction of ADP and F-actin with BV myosin-S1.

The interaction between ADP and actin binding to BV myosin-S1 is qualitatively the same as for RS myosin-S1: ADP decreases the affinity of myosin-S1 for actin and actin decreases the affinity of myosin-S1 for ADP (24, 25, 28, 29). However, there are 10- to 20-fold differences between actomyosin-S1 from BV and RS muscles in the rate and equilibrium constants for the dissociation of the ternary actomyosin-S1-ADP complexes. At 15 °C, k-AD is observed to be ~65 s^{-1} for BV actomyosin-S1, but is too rapid to be measured for RS actomyosin-S1 and, therefore, only a lower limit of >500 s^{-1} was obtained (14). The rate constant of the dissociation of actin from BV actomyosin-S1-ADP, 0.91 s^{-1}, is at least 10 times slower than has been measured for RS proteins at comparable conditions (14, 21). Thus, microscopic equilibrium is preserved in large part by the compensating changes in k_{AD} and k_{DA}. There are much smaller changes (generally 2-fold, or less) in k_{AD}, k_{DA}, K_{A}, and K_{P} (Table I). Therefore, BV actomyosin-S1-ADP is at least 10-fold more stable, kinetically and energetically, than RS actomyosin-S1-ADP. The increased stability of the BV ternary complex is ~70% attributable to a smaller \( \Delta G^\circ \) between ADP and actin binding to BV myosin-S1. Actin destabilizes the binding of ADP to BV myosin-S1 (and vice versa) approximately 20-fold whereas the corresponding destabilization for RS is ~80-fold (24, 25).

Taylor and Weeds (6) observed that the rate of dissociation of actin from the BV ternary actomyosin-S1-ATP complex (\( k_{-TA} \) in Equation 1) is slower for BV than for RS actomyosin-S1. Although the absolute magnitude of \( k_{-TA} \) is ~10^{6} larger than \( k_{DA} \), the same structural difference which decreases the rate of dissociation of BV S1-ADP from actin relative to RS myosin-S1 ADP may also decrease the rate of BV S1-ATP dissociation from actin. Alternatively, the parallel changes in rate constants may mean that efficient contraction requires the ratio of different steady state intermediates to be maintained constant by muscles that contract at different rates, as pointed out by Taylor and Weeds (6).

The rate and equilibrium constants for the dissociation of the binary complexes, BV myosin-S1-ADP and actomyosin-S1, are similar to those measured for RS myosin-S1. The small observed differences may merely reflect differences in experimental conditions or experimental error. The rate of binding of BV myosin-S1, with or without liganded ADP (and presumably ATP, also), to actin is far more sensitive to ionic strength than any other step of the reaction sequence thus far observed. Others (30) have observed similar ionic strength dependence of binding of RS myosin-S1 to actin. The magnitude of the steady state \( V_{\text{max}}/K_{\text{app}} \) for both BV and RS (31) actomyosin-S1 species is likewise observed to have a large dependence upon ionic strength. Since \( V_{\text{max}} \) is relatively insensitive to changes in ionic strength (31), it is likely that the ionic strength dependence of the steady state ATPase can be attributed in large part to a step involving binding of myosin-S1 (with liganded nucleotide) to actin.

In general, the remaining rate constants for the kinetic mechanism of BV actomyosin-S1 measured by Taylor and Weeds (6), \( k_{DA} \) and \( k_{-DA} \), are similar to those observed for RS myosin-S1 as shown in Table I. On the other hand, \( V_{\text{max}} \), the maximum steady state rate of actin-activated ATP hydrolysis, is 4- to 5-fold slower for BV actomyosin-S1 over a wide range of experimental conditions (31).

The rate constant for the dissociation of BV myosin-S1-ADP from actin, \( k_{DA} \), is only 2-fold faster than the rate constant of myosin-S1 dissociation from actin, \( k_{-DA} \). The 15-fold decrease in the equilibrium constant of binding of BV myosin-S1-ADP to actin, \( K_{A} \), relative to myosin-S1, \( K_{A} \), is primarily due to an 8-fold reduction in the observed second order rate constant of BV myosin-S1-ADP binding to actin, \( k_{AD} \). These results are similar to those observed for RS actomyosin-S1 by Marston (21).

The apparent second order rate constant for myosin-S1-ADP binding to actin, \( k_{DA} \), is 1 \times 10^{6} M^{-1} s^{-1} at \( I = 0.1 \). This value is considerably lower than expected for a diffusion-limited reaction. Therefore, binding must either occur according to a two-step binding mechanism or else a conformational equilibrium must occur prior to binding (Equations 9a and 9b). Although the data presented here do not distinguish between the two mechanisms, the conformation equilibria observed in myosin-S1-ADP by Shriver and Sykes (25) may be taken to favor the preequilibrium mechanism of Equation 9a.

\[
M-X \leftrightarrow M'-X + \text{actin} \leftrightarrow \text{actin}-M'-X \tag{9a}
\]

\[
M-X + \text{actin} \leftrightarrow \text{actin}-M'-X \tag{9b}
\]

The rate constant for the dissociation of ADP from cardiac actomyosin-S1, \( k_{AD} \), is ~65 s^{-1} at 15 °C. This rate is nearly 50-fold greater than \( V_{\text{max}} \) for the steady state actin-activated ATP hydrolysis, 1.5 s^{-1}. The values for \( k_{-AD} \) and \( V_{\text{max}} \) at 38 °C and \( I = 0.02 \) obtained by extrapolation of Arrhenius plots are ~550 and ~60 s^{-1}, respectively. A similar result is obtained for RS actomyosin-S1 at 15 °C, where the rate constant of ADP dissociation, >500 s^{-1}, is at least 20 times the \( V_{\text{max}} \, 15 \text{ s}^{-1} \). Therefore, dissociation of ADP from actomyosin-S1 cannot be the rate-limiting step of ATP hydrolysis in either RS or BV actomyosin-S1.

Physiological concentrations of MgATP in muscle, ~3 mM, might be expected to dissociate BV actomyosin-S1 with a first order rate constant in the range of 2 \times 10^{4} to 6 \times 10^{4} s^{-1}. The lower limit was obtained by Taylor and Weeds (6) from the residual amplitude of light scattering observed upon mixing BV actomyosin-S1 with MgATP in a stopped flow fluorimeter with a deadtime of 1 ms. The upper limit is estimated by multiplying the apparent second order rate constant measured in Fig. 6, 2 \times 10^{6} M^{-1} s^{-1}, by the concentration of MgATP, 3 mM. However, during steady state hydrolysis, ADP must dissociate from actomyosin-S1-ADP before ATP binds. Therefore, the rate of dissociation of actomyosin-S1 by MgATP per se is not an appropriate measure of the rate of dissociation of myosin-S1 from actin by MgATP during the steady state. During steady state ATP hydrolysis, the relatively slow rate of ADP dissociation from actomyosin-S1 limits the rate of ATP binding and subsequent dissociation of myosin-S1-ATP. These results suggest that \( k_{AD} \) is the molecular step, which corresponds to the detachment rate, \( g \), in A. F. Huxley's model, when there is no force on the crossbridge (32).

Actomyosin-S1 seems a reasonable model for an unloaded muscle contraction in that little tension would be developed between the myosin heads and actin in either case. However, as pointed out by Hill (33), in muscle the rates and equilibrium constants of chemical steps (such as ADP dissociation) may depend upon the precise spatial orientation between each actin and myosin subunit. In solution, the orientation of the

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4 H. D. White, unpublished results.
5 R. F. Siemankowski and H. D. White, unpublished results.
myosin-S1-ADP with respect to actin would be expected to be the most thermodynamically stable state. However, myosin-ADP in a muscle may be constrained to less stable angles relative to the actin and myosin filaments have different helical repeats. BV myofibrils, which maintain the three-dimensional lattice of the actin and myosin filaments of muscle, bind ADP with an association constant of, $1 \times 10^6$ M$^{-1}$ at 23°C (34). This value is in good agreement with the association constants of ADP to BV myofibrils, which cancel in the equilibrium constant. Nevertheless, the rate constant of ADP dissociation from actomyosin-S1 in solution would appear to be a good first approximation of the rate of ADP dissociation from cross-bridges in muscle.

The unloaded shortening velocity of bovine ventricle has been estimated by Goldman et al. (35) to be $2.2 \times 10^5$ center of the cell. The shortening velocity at the cellular level would be expected to be at least 3.3 half-sarcomeres/s, as cardiac muscle cells are not oriented parallel to the center of the ventricular wall. Mechanical measurements of Huxley and Simmons (36) indicate that a cross-bridge has a working dimension of approximately 100 Å. Thus, the minimum rate constant allowed for a process occurring on an attached cross-bridge, $k_{min}$, can be estimated to be $360$ s$^{-1}$ in the bovine ventricle using Equation 10

$$k_{min} = V_0 \cdot S_c \cdot d^{-1} \sim V_0 - 110$$  

where $V_0$ = unloaded shortening velocity (half-sarcomere/s); $S_c$ = half-sarcomere length ($1.1 \times 10^4$ Å in bovine ventricle); and d = maximum axial displacement of the cross-bridge ($\sim 100$ Å).

The rate constant estimated for ADP dissociation from BV actomyosin-S1 at 38°C, 550–800 s$^{-1}$, is only twice the minimum allowable rate constant. Therefore, within the limits of these approximate calculations, the rate constant of ADP dissociation from actomyosin-S1 in solution (and hence from cross-bridges in muscle) is sufficiently slow to be the molecular step that limits the rate of cross-bridge detachment and the unloaded shortening velocity in bovine cardiac muscle. We have also observed that $k_{ADP}$ may limit the velocity of unloaded shortening of a wide variety of other muscle types.

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R F Siemankowski and H D White

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