A Model of the Release of Myosin Heads from Actin in Rapidly Contracting Muscle Fibers

Roger Cooke,* Howard White,‡ and Edward Pate§

*Department of Biochemistry and Biophysics, & Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California 94143; ‡Department of Biochemistry, Eastern Virginia Medical School, Norfolk, Virginia 23501; and §Department of Pure and Applied Mathematics, Washington State University, Pullman, Washington 99164

ABSTRACT We describe a model that relates the maximum shortening velocity of a muscle fiber, V_m, to the kinetics of the dissociation of a myosin head from actin. At V_m, the positive work exerted by cross-bridges attached in the powerstroke must be balanced by cross-bridges that have been carried by movement of the filaments into a region where they exert a negative force. This balance allows one to relate V_m and the rate of cross-bridge detachment. Studies of actomyosin kinetics suggest that at high substrate, detachment should be limited by a slow protein isomerization ($\approx$50 s^{-1}) that precedes ADP release. This rate is too slow to be easily accomodated in existing models. However, a slow rate for cross-bridge dissociation, similar to that of the isomerization, is predicted if previous models are modified to include rapid detachment of cross-bridges that have been carried so far into the negative force region that their free energy exceeds that of the detached state. The model also explains another aspect of muscle contraction: at high shortening velocities, the observed rate of ATP hydrolysis is low, because a cross-bridge can interact with multiple actin binding sites before releasing the hydrolysis products and binding another ATP.

INTRODUCTION

A major goal in the field of muscle physiology has been to explain the mechanics of active fibers in terms of the kinetics and energetics of the actin–myosin–nucleotide interaction. This interaction has been studied extensively using isolated proteins in solution. A number of intermediate states in the biochemical cycle have been identified, and their free energies and the rates that connect them have been measured (reviewed in Cooke, 1986; Goldman, 1987; Brenner, 1990; Geeves, 1991). It is assumed that a similar cycle occurs in the intact, organized filament array. However, the steric constraints imposed by the filament array couple the decrease in chemical free energy within some of the states to the production of mechanical work. This coupling would be expected to alter significantly the energetic cascade and the kinetics of the actomyosin interaction within the fiber (Hill, 1974).

A number of investigations have found that transition rates between steps in the actomyosin-nucleotide interaction are similar in solution and in active muscle fibers. One approach to measuring kinetic rates in fibers has been the use of photolabile, "caged compounds" in isometric fibers (for review, see Homsher and Millar, 1990). These studies have suggested that the rates of ATP binding and the subsequent hydrolysis steps are both similar to the corresponding rates measured in solution (Goldman et al., 1984a, 1984b). Ligand concentrations can also be altered rapidly in isolated myofibrils due to the rapid diffusive equilibration across the 1 μm diameter, where again the kinetics and energetics of substrate binding and hydrolysis appear similar to those measured for acto-S1 in solution (Taylor, 1989; White, 1985; Houadjeto et al., 1992). A third approach, which is applicable to shortening fibers, has been to vary the concentration of substrate or competing ligands in skinned muscle fibers, and to measure the resulting perturbations in the steady-state mechanics. The dependence of the maximum velocity of shortening, V_m, upon substrate concentration requires that the rate of substrate binding in fibers be rapid and similar to that measured for acto-S1 (Cooke and Bialek, 1979; Ferenczi et al., 1984; Pate and Cooke, 1989). In addition, the values of K_i for the inhibition of velocity by ADP, AMPPNP, and PP_i are all similar to their affinity constants for acto-S1 in solution (Cooke and Pate, 1985; Pate and Cooke, 1985; Johnson, 1986). A variant to the above approach has been to perturb substrate binding rates in muscle fibers by using substrate analogs, or by using various myosin isoforms, and then to correlate the apparent rates of substrate binding measured in the fibers with the corresponding ones measured in solution (White et al., 1993; Pate et al., 1993a, 1993b). These studies have yet again concluded that the rates for the dissociation of the cross-bridge in the fiber resemble those measured for acto-S1 in solution. Although some rate constants must vary with the spatial distance between actin and a cross-bridge, and the rate of ADP release and of ATP binding have been observed to depend on stress (Goldman et al., 1984a, Dantzig et al., 1991), it appears that many of the rates found in solution are applicable to fibers. A current goal is to relate these rates to fiber mechanics in order to better understand cross-bridge function in the filament array of the fiber.

The most widely accepted cycle for a working cross-bridge derives from the original analysis by A. F. Huxley (1957). A free energy diagram for this model is given in Fig. 1 where the path traversed by a cross-bridge is illustrated by the open arrows. Binding sites enter from the right (A). A detached myosin cross-bridge attaches to an actin binding

Received for publication 08 April 1993 and in final form 13 December 1993.

Address reprint requests to Dr. Roger Cooke, Department of Biochemistry and Biophysics, B-0524, University of California, San Francisco, CA 94143-0448.

0006-3495/94/03/778/11 $2.00

site (B), and exerts a positive force through some powerstroke distance, h. The movement of the parallel filaments then carries the cross-bridge into a spatial configuration where it exerts a negative, resistive force (the dragstroke), prior to detaching (C) with some rate constant, g. The cross-bridge can then rebind to a nearby actin to commence a new working cycle. At V_m, the force produced by a muscle is zero. Stiffness measurements, however, indicate that at V_m an appreciable fraction of the cross-bridges are still attached to actin (Julian and Sollins, 1975; Ford et al., 1985). Thus, as originally postulated by Huxley (1957), V_m is the velocity at which the sum of the positive work produced by the attached, powerstroke cross-bridges is balanced by the sum of the negative work produced by dragstroke cross-bridges which have not yet detached. In the formulation by Huxley the detachment rate in the dragstroke was taken as a single constant. Later comparison of fiber mechanics and solution biochemistry has suggested that detachment in the dragstroke region involves two steps, ADP release from the actomyosin complex, with subsequent ATP binding detaching the cross-bridge (reviewed in Cooke, 1986; Goldman, 1987; Brenner, 1990; Geeves, 1991).

At high substrate concentration the rate of ATP binding is rapid, so preceding steps, e.g., ADP release, must limit cross-bridge detachment. The release of ADP from acto-S1 has been shown to be a two-step process involving an isomerization between two conformations of the A·M·ADP complex (A, actin; M, myosin), followed by dissociation of ADP (Taylor, 1991). In fast skeletal muscle, the rate of ADP dissociation is very rapid (>1000 s^{-1}, 10°C) (Zhang et al., 1992). The rate of the isomerization step is slow (30–90 s^{-1}, 10°C) (Taylor, 1991; Zang et al., 1992). Such a slow rate is difficult to reconcile with the experimentally observed rapid shortening. At a maximum shortening velocity of 1.6 muscle lengths/s (a relative filament velocity of 2000 nm/s) for rabbit psoas fibers at 10°C (Cooke et al., 1988), an attached myosin head will traverse 10 nm in 5 ms (10 nm is the distance of the order of magnitude of the dragstroke region). In the context of the above cross-bridge cycle, a dilemma arises, because during this time, only about 20% of the myosin heads will have undergone the isomerization step necessary to complete the hydrolysis cycle. This dilemma could be resolved in several ways. The distance traversed in the negative-force region could be very long (longer than the physical size of a myosin molecule), the rate of the isomerization could become more rapid for highly strained cross-bridges, or an entirely new model for cross-bridge function may be possible in which a slow step could be accomodated. In this paper we discuss this third possibility.

If as suggested above, a slow step limits the rate of cross-bridge dissociation, then some attached cross-bridges will be carried far into the region of negative force as the actin and myosin filaments slide relative to each other. For sufficiently large cross-bridge strain, the mechanical potential energy of these cross-bridges will eventually exceed that of even the detached state. Here we consider a modification of previous models of cross-bridge action, which allows these highly strained cross-bridges to detach, equilibrating with the detached state. Thus in this model, the detachment of the myosin head is only "weakly coupled" to ATP binding in that many cross-bridges that interact with actin are detached mechanically without releasing ADP and binding a new ATP. This model is to be contrasted with the "strongly coupled" models in which detachment of each cross-bridge from a working stroke requires obligatory hydrolysis of a single ATP. The concept of weakly coupled models, in which cross-bridges may undergo repeated attachment-detachment cycles prior to ATP hydrolysis, has been introduced to explain measurements of the apparent distance traversed by an attached myosin head per ATP split during translation of actin filaments (reviewed in Burton, 1992; Yanagida, 1990). Unfortunately, rigorous, analytical descriptions of how weakly coupled models may function at the cross-bridge level remain lacking.

Here we consider implications of strongly coupled models in terms of actomyosin solution biochemistry and show that they naturally lead to a working, weakly coupled model. The weakly coupled model resolves the dilemma discussed above, allowing a slow isomerization step to be involved in the process of cross-bridge release, while also allowing us to explain an additional facet of muscle physiology. At high velocities, the model indicates that a majority of myosin heads can interact with actin several times before binding ATP. Hence, the apparent distance traversed by an attached myosin head *per ATP split* depends on the velocity of contraction as also observed experimentally (Higuchi and Goldman, 1991.). Additionally, this allows for the possibility that the predicted rate of ATP hydrolysis decreases in rapidly shortening cross-bridge systems, as has been observed experimentally for both muscle and myofibrils (Rall et al., 1976; Homsher et al., 1981; Ohno and Kodama, 1991; Kushmerick and Davies, 1969).

RESULTS

The interplay of the various factors present in a chemomechanical description of functioning muscle is obviously complex. To facilitate analysis, we first consider two simple cross-bridge models. Individually, they are formulated to emphasize the basic concepts involved in strongly coupled and weakly coupled cross-bridge systems. Their computational simplicity, however, allows, us to develop analytical expressions for relationships of interest. Thus we gain the considerable advantage of being able to quantitatively demonstrate the interplay of solution biochemistry and muscle physiology which is at the heart of our arguments. Subsequently, we consider a more complex, multistate chemomechanical model which bridges the gap between the weakly coupled and strongly coupled regimes.

Strongly coupled models

The model considered here is derived from that first proposed by A. F. Huxley in 1957. Following Huxley, we define a

probability, $n(x)$, that a myosin cross-bridge is attached to the nearest actin site which is located at spatial location x. If attached, a cross-bridge then exerts force equal to κx as a linearly elastic element. The elastic force constant, κ, is taken to be the same for both positive and negative forces. The cross-bridge cycle is detailed in Fig. 1 (open arrows). After traversing the powerstroke ($x > 0$) cross-bridges are then pulled into negative regions of x where they exert negative force (the dragstroke) before dissociating from actin with some rate constant, g. The rate, g, will be identified with some transition rate determined for the release of acto-S1 by ATP in solution. We make several modifications to the original Huxley model. The first, which is made for mathematical simplicity, is the assumption that all cross-bridges attach to actin at the point $x = h$, where the free energies of the attached and detached states are equal. After attachment, all cross-bridges execute the powerstroke from $x = h$ to $x = 0$. Then $n(x) = 1$ for $0 \leq x \leq h$ and the positive work per cross-bridge produced in the powerstroke, W_+, equals $\kappa h^2/2$. This is simply the energy contained in a spring with force constant κ pulled out by a distance h.

The mean negative work produced by a cross-bridge in the dragstroke is calculated from the following integral

$$W_- = \int_0^{-\infty} n(x) \cdot \kappa x \cdot dx. \qquad (1)$$

As x decreases in the region $x < 0$, the probability of remaining attached decays as $\exp(-gt)$, where t is the time the attached cross-bridge spends in the negative force region. We adopt the sign conventions that W_- is a positive quantity (to be equated to W_+), and that shortening velocity, V, is also a positive quantity (cross-bridge movement from higher to lower values of x). The probability of attachment can then be expressed as

$$n(x) = n(0)\exp(gx/V), \qquad x \leq 0. \qquad (2)$$

Substituting from Eq. 2, the integral in Eq. 1 is easily evaluated to give $W_- = \kappa V^2/g^2$. At the maximum velocity of shortening, $V = V_m$, the net work is zero, and $W_+ = W_-$. Equating the two expressions we obtain an expression which relates g and h to the maximum contraction velocity, V_m

$$gh/V_m = \sqrt{2}. \qquad (3)$$

Thus the amount of work exerted by a dragstroke cross-bridge can be readily calculated if one knows the velocity and the rate of cross-bridge dissociation from actin (Pate et al., 1993b). At low concentrations of substrate, shortening velocity is proportional to substrate concentration indicating that in this range, the rate of cross-bridge release from actin is limited by the rate of substrate binding (Cooke and Bialek, 1979; Ferenczi et al., 1984). Using a series of NUCLEOSIDE triphosphates, we have previously shown that there is an excellent correlation between the experimentally observed V_m for skinned fibers and the rate, k_t, of acto-S1 dissociation by the analog as measured in solution (White et al., 1993; Pate et al., 1993a). This correlation suggested that the energetics and kinetics of substrate binding are similar in solution and in slowly contracting fibers. The balance between positive and negative work at V_m (zero force) allowed us to calculate the length of the powerstroke, h, in terms of the fiber value of V_m and the solution value for k_t. Our calculations suggested that h is approximately 7 nm, a distance that can be traversed by a swinging cross-bridge the size of a myosin head.

The maximum velocity of shortening demonstrates saturation behavior with respect to ATP concentration (Cooke and Bialek, 1979; Ferenczi et al., 1984). Thus at high ATP, cross-bridge dissociation in the dragstroke region and sliding velocity must be limited by a step occurring prior to substrate binding. A reasonable assumption is that at high ATP, the rate of cross-bridge dissociation would be limited by the biochemical step that precedes ATP binding, the release of ADP (Siemankowski et al., 1985). As noted previously, however, studies of the dissociation of acto-S1 in solution have suggested that the release of ADP is itself a multistep process. For fast skeletal muscle, there is a slow isomerization step, followed by rapid dissociation of ADP, with respective rate constants of $\approx 50\ s^{-1}$ and $>1000\ s^{-1}$ (10°C). If one assumes that the powerstroke length determined at low substrate levels is applicable at high substrate levels, Eq. 3 can be used to determine the rate of the transition which governs muscle velocity at the high substrate levels, with g taken as the net dissociation rate of ADP from the actomyosin complex. With the maximum filament sliding velocity of 2000 nm/s^{-1}, and a powerstroke length equal to 7 nm, Eq. 3 gives a value for g of 400 s^{-1}. The obvious difficulty is that this

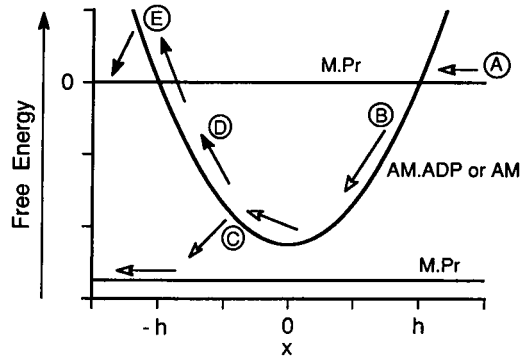

FIGURE 1 Strongly coupled model (open arrows). Binding sites enter from the right (A). The detached cross-bridges (M.Pr) initially attach (B) and enter a force-producing AM.ADP state at $x = h$. Attached cross-bridges are assumed to be linear elastic elements resulting in parabolic free energy profiles. Attached cross-bridges traverse the powerstroke from $x = h$ to $x = 0$ producing useful work W_+. For $x < 0$, continued attachment retards motion (dragstroke). Cross-bridges detach (C) with rate constant, g. This transition represents a composite of ADP release and subsequent ATP binding and is an obligatory step in the cycle. Weakly coupled model (additional closed arrows). Steps (A), (B), and (C) of the cycle are identical with those in the strongly coupled model. However, we consider the additional possibility (D) that cross-bridges, which do not detach for $-h < x < 0$ instead (E), find it energetically favorable to equilibrate with the detached M.Pr state *without* ATP hydrolysis.

rate is considerably faster than the isomerization step described above, and would suggest that this isomerization could not be a part of the pathway of cross-bridge dissociation at high velocities. On the other hand, the rate is also considerably less than the experimentally observed rate of dissociation of ADP from an acto-S1 complex. Thus this strongly coupled model provides no correspondence between solution kinetics and fiber mechanics at high substrate concentration.

Weakly coupled model

The inability of the above models to explain fiber velocity in terms of acto-S1 kinetics, leads one to consider the fate of the cross-bridges that remain attached to actin over a considerable distance in the negative-force region at the higher shortening velocities. The solid line in Fig. 2 plots the distribution of attached cross-bridges as a function of cross-bridge strain, x, for V_m given by Eq. 3. As is evident, in the original Huxley-type analysis, some cross-bridges become sufficiently strained (i.e., $x < -h$) that the free energy of the attached, negative-strain state actually *exceeds* the free energy of the detached state. Indeed, it is straightforward to show that for the model considered above, almost 60% of the total negative work is produced by these highly strained cross-bridges. This suggests the prospect of an alternative dissociation pathway into the detached state, driven by mass action and free energy differences, as opposed to the chemomechanically less efficient strong-coupling pathway requiring triphosphate hydrolysis. This path is demonstrated in Fig. 1. The initial portion of the cross-bridge cycle is identical with that in the strongly coupled model (open arrows). Binding sites enter from the right (A) and attached cross-bridges traverse the working powerstroke (B). In the dragstroke region, the strongly coupled detachment process (C) may still occur. However, for cross-bridges that do not detach via path (C), an alternative pathway (D, closed arrows) allows cross-bridges to be additionally strained by other attached, working, powerstroke cross-bridges. Cross-bridges strained into the region $x < -h$ have free energies that rapidly rise above that of the detached state. Energetically, these cross-bridges are unstable with respect to the detached state, and it would appear reasonable to believe that such cross-bridges would make the transition back to a detached state (E), essentially being pulled off of the actin ("mechanically detached") as their free energy rises and the attached state becomes less stable. There is also a good teleological reason for believing that such a detachment would occur: as the free energy of the attached state rises above that of the detached state, additional negative work, which further impedes the progress of one filament past another is unnecessary, and the splitting of an ATP molecule to produce detachment is also unnecessary. Thus, allowing rapid detachment of these highly strained cross-bridges will allow the muscle to contract at a more rapid velocity, and it will also decrease the use of ATP at high velocities. In more current terminology, this is an example of the widely postulated equilibration between a detached state and a strong-binding, force-producing state (Brenner, 1991). We now consider the implications of this modified cross-bridge scheme.

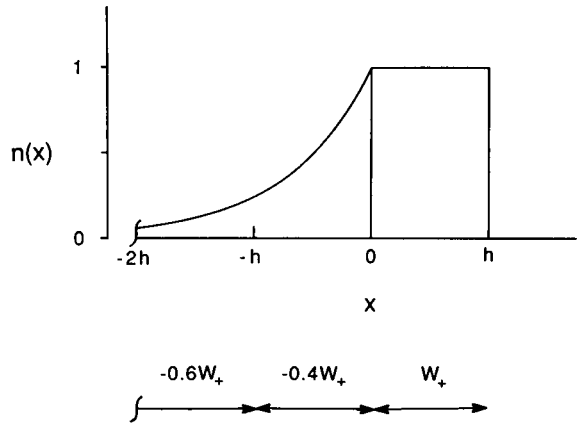

FIGURE 2 The fraction of attached cross-bridges, $n(x)$, for the strongly coupled model. Cross-bridges initially attach at $x = h$, with a constant distribution through the powerstroke, $h > x > 0$. In the dragstroke, $x < 0$, cross-bridges detach with first-order rate constant, g, and the attached population is a distributed as a decaying exponential, $\exp[gx/V]$. The figure shows this decay for $V = V_m$. Note that many of the cross-bridges are stretched beyond $x = -h$ before detaching The amounts of work done in each region are given below the figure. For this model, 60% of the negative work is performed by cross-bridges that are strained beyond $x = -h$. As seen in Fig. 1, the free energy of these highly strained cross-bridges actually exceeds that of the detached state. This provides motivation for the alternative, weakly coupled model.

Analysis is fundamentally as before, with $W_+ = W_-$ at V_m. However, W_- now consists of two distinct portions: W_{1-}, that in the strongly coupled region, $-h \le x \le 0$, and W_{2-}, that in the highly strained region, $x < -h$, where detachment occurs due to equilibration with the detached state. The mean negative work per cross-bridge in the region $-h \le x \le 0$ is easily calculated by integrating the expression in Eq. 3 over the new region of interest, obtaining

$$W_{1-} = \kappa(V^2/g^2)(1 - [gh/V + 1]\exp[-gh/V]). \quad (4)$$

As shown above, W_{1-} can be calculated in terms of the parameters of the model and related to W_+. However, the calculation of W_{2-}, the mean negative work generated in the region $-\infty < x \le -h$ is more difficult, and it depends upon the nature of the rapid equilibration between attached and detached states. The following calculation provides an estimate of the magnitude of the amount of negative work produced during the mechanical dissociation of a myosin head. We assume that for $x \le -h$, attached cross-bridges can be mechanically detached with a rate, k_{off}, equilibrating rapidly with the detached state. We further assume that the equilibration occurs more quickly than the release of ADP, and subsequent binding of ATP. Hence, we have only one process occurring in this region. This greatly simplifies the mathematics without significantly changing the final result. This restriction will be eliminated in the more detailed, multistate model considered subsequently. For

$x \leq -h$, again let $n(x)$ be the probability of being in the attached fraction. The probability of being attached as a function of x is now determined by solving the differential equation

$$-\frac{V\,dn(x)}{dx} = -n(x)\cdot k_{off} + (1 - n(x))\cdot k_{off}\cdot \exp\left(-\frac{\kappa[x^2 - h^2]}{kT}\right), \quad (5)$$

where k is the Boltzmann constant and T is absolute temperature. The exponential factor in the last term results from the fact that the forward (detachment) and reverse (attachment) rates are related by the free energy difference between the attached and detached states (Hill, 1974). To fix parameters, we take $k_{off} = 10^4\ s^{-1}$ in the range suggested for the rapid equilibration at the beginning of the working powerstroke (Schoenberg, 1988). Setting V = 2000 nm/s, one can solve Eq. 5 using standard numerical methods (Pate and Cooke, 1989), and then integrate to determine the negative work, obtaining $W_{2-} \approx 2$ kT. Obviously, simplifying assumptions have been used in obtaining this result, and their implications will be considered subsequently. Our major point with this calculation is to show that the additional energetic cost of mechanically dissociating a cross-bridge in this fashion is small, with the negative work amounting to only a few kT. In the discussion below we take $W_{2-} = 2$ kT. We note in advance that this value is close to that obtained from a more complex, multistate model to be subsequently considered.

Now equating the magnitude of negative work calculated above, with the positive work calculated previously, at maximum shortening velocity V_m,

$$W_{1-} + W_{2-} = W_{+}.$$

Let E (> 0) be the magnitude of the difference between the free energy of the detached state and minimum free energy of the attached state in units of kT (i.e., the free energy at the minimum of the parabola in Fig. 1). Then $E = \kappa h^2/2$. Using the above expressions and rearranging, one obtains a new expression that relates V_m, g, and h

$$2E\left(\frac{V_m^2}{g^2}\right)\left[1 - \exp\left(-\frac{gh}{V_m}\right)\left(\frac{gh}{V_m} + 1\right)\right] + 2\,\text{kT} = E. \quad (6)$$

This equation has two variables, $W_+ = E = \kappa h^2/2$, and gh/V_m. W_+ can be estimated from the efficiency of actively shortening muscle. Actively shortening muscle exhibits high efficiency, 50%–60%. Taking the free energy available from ATP hydrolysis as 23 kT, $W_+ = 15$ kT defines an efficiency within the above limits. Thus with knowledge of W_+ (or equivalently E), Eq. 6 becomes transcendental in the single variable, gh/V_m. Physically, this variable represents the ratio of g, the first-order rate constant for cross-bridge dissociation, to V_m/h, the time for passage through the region $x = 0$ to $x = -h$. For given g and h, the magnitude of this variable determines how rapidly a fiber can shorten. Standard numerical procedures yield a root for Eq. 6 of

$$gh/V_m = 0.2. \quad (7)$$

The crucial observation is that this value is dramatically different from the value we obtained in the absence of the rapid equilibration. In that case (see Eq. 2) we obtained, $gh/V_m = \sqrt{2}$, more than a factor of 7 greater. Thus, for fixed values of g and h, Eq. 7 indicates that the weakly coupled model predicts a considerably (sevenfold) higher shortening velocity. Alternatively, for given values of h and V_m, the detachment rate, g, can be considerably slower in the region $x = 0$ to $x = -h$ in the weakly coupled model. Although the strongly and weakly coupled models are applicable at different substrate regimes, we assume that they share the same powerstroke length. Using the values for $V_m = 2000$ nm/s and the previously taken value for h of 7 nm from the strongly coupled model, Eq. 6 gives a value for g of 57 s^{-1}. This value is in the range of the rate constant for the slow isomerization, which has been experimentally observed to precede the much more rapid ADP release step in rabbit fast muscle acto-S1 (Taylor, 1991; Zhang et al., 1992). The slower step should be rate limiting at high substrate concentration. Thus unlike the previous strongly coupled model, the isomerization rate can be part of the weakly coupled cross-bridge cycle. In fact, it fits very nicely into the rate predicted by the model.

As noted previously, the weakly coupled model has the property that not every cross-bridge that attaches to actin requires an ATP in order to detach. The fraction of cross-bridges that do not hydrolyze ATP is given by the population of cross-bridges that traverse the dragstroke and remain attached at $x = -h$. From Eq. 2, this is equal to $\exp[-gh/V_m]$. For $gh/V_m = 0.2$, we find that about 80% of the attached cross-bridges do not detach via the ATP-hydrolysis step, but instead must be mechanically detached when $x < -h$. Fig. 3 shows the fraction of attached cross-bridges as a function of x for the weakly coupled model (solid line). As is evident in the figure, as a cross-bridge passes through the dragstroke,

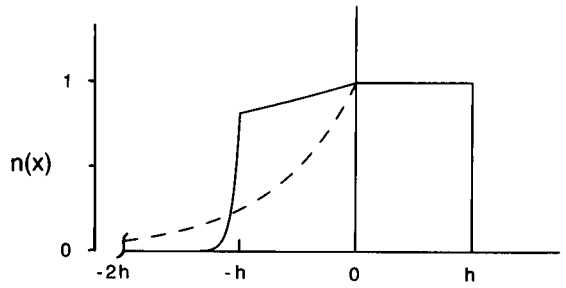

FIGURE 3 Solid line is the population of cross-bridges attached to actin as a function of strain, x, for the weakly coupled cross-bridge model. For comparison, the dashed line is the attached cross-bridge fraction for the strongly coupled model as in Fig. 2. In the weakly coupled model, the off-rate, g, is significantly less and thus significantly fewer cross-bridges detach via ATP hydrolysis in the region $-h < x < 0$. For $x < -h$, the free energy of the attached state (quadratic in x) rises rapidly above that of the detached state. Equilibration on the basis of free energy results in rapid depletion of the attached state. The populations are shown for shortening at V_m.

little detachment occurs for $-h < x < 0$. Significant, rapid detachment occurs only after the free energy of the attached state exceeds that of the detached state ($x < -h$). In order to more dramatically demonstrate the differences between the predictions of the weakly and strongly coupled models, the attached fraction in the dragstroke region predicted by the strongly coupled model for $V = V_m$ from Fig. 2 is also shown in Fig. 3 (dashed line).

Another significant aspect of the weakly coupled model becomes evident by considering in greater detail the fraction, f, of cross-bridges that detach via the ATP hydrolysis pathway as a function of normalized shortening velocity, $v = V/V_m$. From Eq. 2 and 7, the value of f is given by

$$f = 1 - \exp[-0.2/v]. \quad (8)$$

Fig. 4 (left vertical axis) plots f, or equivalently the number of ATP hydrolyzed per cross-bridge per working interaction, as a function of V. As would be expected, as shortening velocity increases, the hydrolysis rate per attached cross-bridge decreases due to the shorter time period an individual cross-bridge spends in the spatial region $-h \leq x \leq 0$. At V_m, only one cross-bridge in five binds an ATP during each interaction, with four cross-bridges in five recycling into another attachment cycle without ATP hydrolysis. These 80% of the total cross-bridges have been termed "passenger" cross-bridges (Irving, 1991). Passenger cross-bridges occur for lower velocities as well. Fig. 4, also plots the average total distance traversed by a cross-bridge while attached to actin per ATP hydrolyzed (right vertical axis). As is evident, due to increasing numbers of attachment-detachment cycles without hydrolysis as velocity increases, the model predicts that the distance traveled per ATP increases in a roughly linear fashion with increasing shortening velocity. The curve is similar to that obtained by Higuchi and Goldman (1991), who measured the distance shortened by a muscle following release of ATP by photolysis in skinned muscle fibers. The

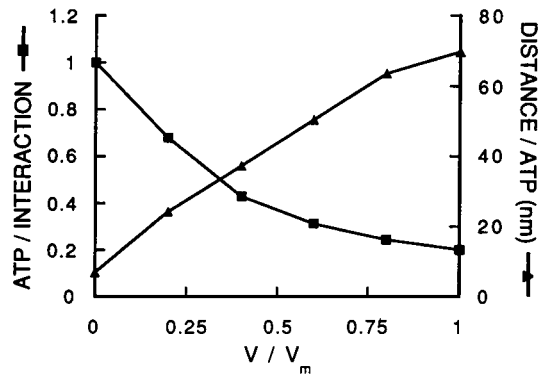

FIGURE 4 ATP hydrolyzed per cross-bridge working interaction (squares, left vertical axis) and distance traveled per ATP hydrolyzed (triangles, right vertical axis) plotted as functions of velocity for the weakly coupled model. Velocities are given relative to V_m. The hydrolysis rate substantially decreases as velocity increases. There is a corresponding, approximatley linear increase in the distance traveled per ATP hydrolysis. Parameters: $h = 7$ nm, $V_m = 2000$ nm/s, $g = 60$ s^{-1}.

limiting value of approximately 70 nm we obtain at V_m is furthermore compatible with their estimate of >60 nm.

Model appropriate for intermediate velocities and substrate concentrations

The previous strongly and weakly coupled models have been developed in order to explain the properties of muscle contracting at V_m for two distinctly different regimes–those of low and high substrate concentrations, respectively. Difficulties also exist at intermediate shortening velocities for both simple models. The strongly coupled model predicts a force-velocity curve that is concave downward with respect to increasing tension, unlike the experimentally observed concave upward trace. Additionally, both analyses assumed that all cross-bridges attach at $x = h$ and then pass through the powerstroke, i.e., $n(x) = 1$ for $0 \leq x \leq h$. Thus neither model predicts the correct fraction of attached cross-bridges as a function of shortening velocity. We now consider a model that can overcome these difficulties, and at the same time provides additional insight. Because this model bridges the strongly coupled and weakly coupled substrate regimes, incorporating the fundamental assumptions of each, we term it the *composite* model.

A model incorporating more realistic assumptions about the individual states is required. The following additional assumptions are made. In addition to a detached state (state 1), both the powerstroke and dragstroke in the model are assumed to be composed of three attached states: a preisomerization A·M*·D state (state 2), a postisomerization A·M·D state (state 3) and an A·M state (state 4), which is reached following ADP release. A finite attachment rate from state 1 to state 2 is assumed at the beginning of the powerstroke. Additionally, state transition rates in the powerstroke, $0 \leq x \leq h$ are now assumed to be nonzero in order to accomodate the isometric ATPase rate. The free energy difference between the pre- and postisomerization states, A·M*·D and A·M·D is taken to be 4kT, in the range suggested by solution data (Taylor, 1991). In the dragstroke, the isomerization rate is taken to be 60 s^{-1}, close to the value of 57 s^{-1} determined previously. Subsequent ADP dissociation, with an assumed rate constant of 2000 s^{-1}, then results in transition to the A·M state. The binding of MgATP, with a second-order rate constant of 3×10^6 M^{-1} s^{-1} as observed in solution (White et al., 1993), results in cross-bridge detachment via the tightly coupled pathway. Alternatively, A·M*·D cross-bridges (state 2) detach via the weakly coupled pathway at the end of the dragstroke, equilibrating with the detached fraction. In our previous estimation of W_{2-}, the weakly coupled equilibration was assumed to start at $x = -h$. For the present model, the best fit to the mechanical data was obtained by assuming the equilibration to start at an x value, which resulted in the free energy of the A·M*·D state being 2 kT above that of the detached state.

Fig. 5 shows the simulation results. The precise numerical parameters used are given in the legend. Simulations were done using numerical techniques previously em-

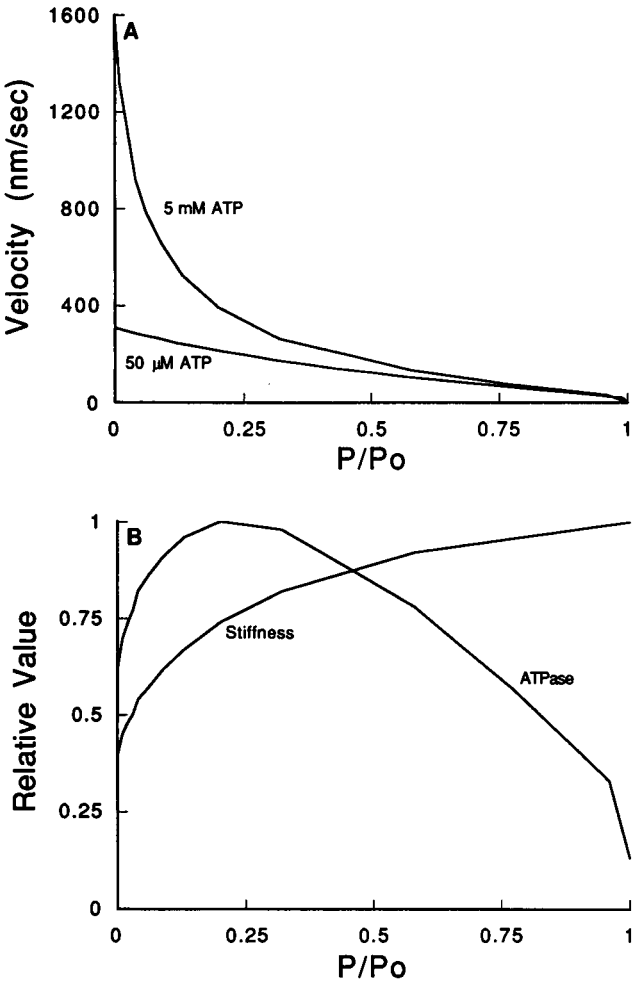

FIGURE 5 Simulation results from a composite model. (*a*) Velocity (nm/s) at simulated [ATP] of 5 mM (upper plot) and 50 μM (lower plot) as a function of normalized tension. (*b*) Simulated relative ATPase and stiffness for 5 mM ATP as a function of relative tension. Parameters for the states 1–4 as defined in text: Let $G_i(x)$ be the relative free energy of state i in units of kT, $\kappa = 0.3$ kT/nm^2 be the cross-bridge elastic force constant, and $R_{ji}(x)$ be the transition rate from state i to state j. $G_1(x) = 0$, $G_2(x) = -15 + \kappa x^2$, $G_3(x) = -19 + \kappa x^2$, $G_4(x) = -20 + \kappa x^2$, $\Delta G_{ATP} = -23$. For 0 nm $< x <$ 7 nm, $R_{12} = 1.5$ s^{-1}, $R_{23} = 6$ s^{-1}, $R_{34} = 3$ s^{-1}, $R_{41} = 3 \times 10^6$ M^{-1} s^{-1}; for $x \geq 7$ nm, rates are identical with $0 < x < 7$ nm, except $R_{12} = 200$ s^{-1}; for -7.5 nm $\leq x \leq 0$ nm, $R_{12} = 0$, $R_{23} = 60$ s^{-1}, $R_{34} = 2000$ s^{-1}, $R_{41} = 3 \times 10^6$ M^{-1} s^{-1}; for $x \leq -7.5$ nm, rates are identical with -7.5 nm $\leq x \leq 0$ nm, except $R_{23} = 10^5$ s^{-1}. In all cases the reverse transition rates are determined as $R_{ji}(x) = R_{ij}(x)\exp[G_j(x) - G_i(x)]$.

ployed for multistate cross-bridge systems (Pate and Cooke, 1989). Fig. 5a shows shortening velocity as a function of normalized tension for simulated substrate concentrations of 5 mM and 50 μM. Fig. 5b shows relative ATPase and mechanical stiffness as a function of normalized tension during releases assuming a physiological, 5 mM ATP concentration. For Fig. 5b, ATPase is taken as being proportional to the fraction of cross-bridges, which detach via the tightly coupled pathway; stiffness is taken to be proportional to the fraction of attached cross-bridges (states 2–4).

The composite model demonstrated saturation behavior for V_m as a function of ATP concentration as is observed experimentally (Cooke and Bialek, 1979, Ferenczi et al., 1984). The upper force-velocity plot gives a V_m of 1600 nm/s, and the substrate concentration giving half-maximal shortening velocity was 190 μM. Thus, both values are close to the experimentally observed values of 2000 nm/s (Cooke et al., 1988) and 150 μM (Pate and Cooke, 1985) observed, respectively, for rabbit fast skeletal muscle. We note that at 5 mM ATP the modeled force-velocity curves are concave upward but with a slightly higher curvature than generally observed. In the strongly coupled regime, plots remain concave upward for low release tensions. At relative shortening tensions very close to one, i.e., low velocities, the force-velocity curves switch from concave upward to concave downward. Such behavior has previously been noted for living muscle preparations at physiological ATP concentrations (Edman et al., 1976).

Fig. 5b also shows that the finite attachment rate at the beginning of the powerstroke results in a decrease in stiffness with increasing shortening velocity, contrary to that predicted by the previously considered strongly and weakly coupled models. At V_m, the fraction of attached cross-bridges has decreased to approximately 40% of that obtained for simulated isometric conditions. This value is again in the range suggested from living frog fibers (Julian and Sollins, 1975; Ford et al., 1985). The decreasing fraction of attached cross-bridges, which occurs as shortening velocity increases, coupled with the ability of cross-bridges to cycle via the weakly coupled pathway several times prior to substrate hydrolysis has important implications. The ATPase initially rises with increasing shortening velocity. A maximum is reached at an intermediate velocity, and then ATPase decreases as the shortening velocity approaches V_m. The factor of 7 difference in ATPase we observe between the isometric value and the maximum value differs by less than a factor of 2 from the experimentally observed fourfold difference. Two additional comparisons between the weakly coupled and composite models are important. Despite the presence of the additional states, composite model simulations give a value for W_{2-} of 1.5 kT per attached cross-bridge and a value for the fraction of ATP hydrolyzed per working interaction (f, Eq.8) of 0.23 at V_m for 5 mM ATP. These are very close to those obtained in the weakly coupled analysis indicating that our original assumptions remain valid.

In summary, our goal in this section has been to demonstrate that simple modifications to the strongly and weakly coupled analyses produce a model that is consistent with physiological data taken at intermediate shortening velocities and at intermediate substrate concentrations, while maintaining compatibility in the high and low substrate regimes. The composite model examined most certainly does not represent a unique solution to the problem of actomyosin chemomechanics, because obvious simplifications have been made in

even this case, e.g., the omission of an initial, weakly attached state, which previous analyses have shown to provide more realistic curvature to the Hill force-velocity curve at high shortening velocities (Pate and Cooke, 1989). Nonetheless, our analysis does suggest that the composite model can provide a consistent, unifying framework in which to consider actomyosin chemomechanics as a function of substrate concentration.

DISCUSSION

The model proposed by Huxley in 1957 did not explicitly incorporate substrate binding. However, subsequent models have used actomyosin kinetics to define transitions between states. In many of these models, dissociation of the myosin head was *strongly coupled* to ATP binding and subsequent hydrolysis. These models have problems explaining two observations. 1) At high velocities of shortening, the ATPase activity of the fiber is very low in spite of an appreciable fraction of attached cross-bridges. 2) Recent studies of kinetics in solution suggest that an obligatory, slow, isomerization step occurs prior to ADP release. To address these problems we have explored here a simple modification of the Huxley model in which highly strained cross-bridges are allowed to be detached mechanically. We show that with this addition, ATP binding is *weakly coupled* to cross-bridge detachment, and that both of the above problems can be resolved. The major differences between strongly and weakly coupled models are discussed in more detail below.

The rate of dissociation of negatively strained cross-bridges

A salient difference between the strongly and weakly coupled models lies in the rate of dissociation of a cross-bridge carried into the region of negative force $-h \leq x \leq 0$. For the strongly coupled model at saturating [ATP], Eq. 3 shows that this rate must be high, $\approx 400\ s^{-1}$, in order to attain a plausible maximum shortening velocity of 1.6 lengths/s. For the weakly coupled model, the same velocity of shortening can be attained with a rate that is almost an order of magnitude slower. The rate predicted by the model is of the magnitude of the rate for an isomerization between actin·S1·ADP states that has been observed using purified proteins in solution (Taylor, 1991). Although rates measured in solution may not apply in the intact fiber, differences observed to date have been modest (Goldman et al., 1984a; Dantzig et al., 1991).

The differences between the strongly and weakly coupled models could be tested directly by measuring the rate of cross-bridge dissociation in the region of negative force. Such a measurement could be made following a large step decrease in fiber length (e.g., 12 nm/half sarcomere) when the population of attached and negatively strained cross-bridges is large. The weakly coupled model would predict that the rate of dissociation of these cross-bridge is slow, $\approx$50/s, while the strongly coupled models would predict a much faster rate.

The rate of ATP hydrolysis at high shortening velocities

The energetics of rapidly shortening muscle has been investigated by Homsher and coworkers in living frog muscle (Rall et al., 1976; Homsher et al., 1981) For shortening velocities near V_m, they found that the turnover rate for substrate was very low. Very low hydrolysis rates were also found by Ohno and Kodama (1991) for myofibrils shortening at V_m. Thus actomyosin systems appear to contract with high economy as shortening velocity approaches V_m. In contrast to the low rates of substrate turnover observed experimentally at V_m, the strongly coupled cross-bridge models predict high ATPase activities at V_m. If the binding of one ATP is assumed to be required for each cross-bridge dissociation in the Huxley model, the ATPase rate rises monotonically with shortening velocity, reaching a maximum at V_m (Huxley, 1957). If the association of cross-bridges with actin is modeled as a two-step process, the decrease in ATPase at high velocities can fit well with the existing data on fiber energetics (Huxley, 1973). In this case, however, the ATPase activity decreases due to the attachment of significantly fewer cross-bridges. The very low ATPase activities found in more recent work cannot be explained easily by this mechanism, while simultaneously maintaining the appreciable fraction of attached cross-bridges (>30% of the isometric value), which has been suggested by measurements of fiber stiffness (Julian and Sollins, 1975; Ford et al., 1985) during shortening. Subsequent, strongly coupled models have also failed to predict a decrease in ATPase activity at high velocities, cf. Fig. 10 of ref. (Eisenberg et al., 1980). Thus these models lead to very inefficient conversion of chemical energy to mechanical work at high shortening velocities.

In contrast to the strongly coupled models, a major prediction of the present model is that the number of ATP molecules hydrolyzed per cross-bridge interaction actually decreases as the velocity of filament motion increases. As the composite model demonstrated, the presence of passenger cross-bridges, coupled with a plausible decrease in the fraction of attached cross-bridges at V_m, provides a straightforward explanation for the decrease in the ATPase activity of rapidly contracting muscle fibers. The model implies that during a period of rapid shortening, only about one out of five cross-bridge interactions with actin would result in substrate hydrolysis. The other four would be mechanically dissociated prior to the isomerization step. This result, in fact, is in excellent agreement with experimental observations. Assuming that a working cross-bridge spends 5% of its time attached to actin while shortening at V_m (the duty cycle; Uyeda et al., 1991), and the powerstroke length is 7 nm, one can calculate from the observed hydrolysis rates of Homsher

and coworkers that a cross-bridge would hydrolyze one ATP for every 4.2 interactions with actin (Homsher et al., 1981).

The low ATPase rate per attached cross-bridge in rapidly contracting muscle, predicted by the weakly coupled model, assumes that a cross-bridge that detaches mechanically without hydrolyzing ATP is able to reattach and undergo a subsequent powerstroke. This assumption places certain restrictions on the nature of the force-generating interaction. In particular, it would suggest models in which the conformational change(s), which lead to force production, are driven by the formation of the actomyosin bond. The release of this bond, either by the binding of ATP or by mechanical energy obtained from filament motion, allows the detached myosin to assume a conformation appropriate to that of a detached state, and to subsequently bind at the beginning of another powerstroke. It should be noted that another class of models also fulfills this assumption. In this class of models the myosin cross-bridge works as a thermal ratchet (Vale and Oosawa, 1990; Cordova et al., 1992). Indeed, the model upon which the present analysis is built, the original model of Huxley (1957), was a member of this class. A cross-bridge is assumed to act as a spring. Thermal fluctuations in the aqueous environment allow the cross-bridge to randomly extend to strain values representing positive values of x. When this occurs, binding to actin locks the spring in place where it exerts a positive force on the actin filament. As filament sliding carries the attached cross-bridge into regions of negative force, it can be released from the actin when the free energy of the spring exceeds that of the actomyosin bond. This cross-bridge would return to its initial detached configuration, thereby becoming capable of participating in another powerstroke. Thus, a number of models of the actomyosin interaction would be compatible with the assumption that detached cross-bridges can recycle. From a biochemical perspective, upon detachment these cross-bridges would be largely in the biochemical state that precedes the slow isomerization discussed above.

Factors affecting the degree of coupling

Solution of Eq. 6 requires the estimation of two parameters, W_+ and W_{2-}, both of which influence the value of gh/V_m. The smaller the magnitude of W_+, or the larger the magnitude of W_{2-}, the larger the value of gh/V_m. Additionally, the solution of Eq. 6 shows that gh/V_m is a steep function of the term $W_+ - W_{2-}$.

In the initially considered models, the value of W_+ is the maximum possible because all cross-bridges are assumed to attach at $x = h$ and produce positive work through the entire powerstroke region. If one instead assumes a finite rate for cross-bridge attachment, many cross-bridges now attach in the middle of the powerstroke and do less positive work, decreasing the value of W_+. For example, in the strongly coupled Huxley model (1957), Brokaw (1976) has shown that the steady-state shortening of vertebrate fast skeletal muscle at physiological substrate concentration is best fit with an attachment rate in the powerstroke region, $0 \leq x \leq h$, that is equal to $65\ x/h\ s^{-1}$. In this case, the value of W_+ at V_m is only two-thirds that obtained in the current model. This would lead to a value of gh/V_m, which is considerably larger than the value of 0.2 obtained here, now equal to 1.3, and requiring a greater than sixfold increase in g. A similar conclusion was reached in the analysis by Brokaw (1976), who found a dragstroke detachment rate of $300\ s^{-1}$ provided the best fit to vertebrate muscle data for the Huxley model.

As Eq. 6 indicates, estimates of W_{2--} are also model-dependent, and this is the factor, which is the least easy to define from experimental data. In calculating the work done between $x = -h$ and $x = -\infty$, we have assumed that there is rapid equilibration between attached and detached cross-bridge states. The magnitude of the rate constant associated with the equilibration with the detached cross-bridge state can affect W_{2-}. The smallest value possible for W_{2-}, 0.7 kT, is obtained for equilibration initially starting at $x = -h$, with $k_{off} > 10^6\ s^{-1}$. With W_+ equal to its maximum, this leads to a lower limit for the value of gh/V_m of 0.07. For $V_m = 2000$ nm/s and $h = 7$ nm, this value of gh/V_m is obtained if $g = 20\ s^{-1}$. Alternatively, W_{2-} can be affected by the value for x at which equilibration begins. In the composite model, data were best fit requiring the equilibration to begin at an x yielding a free energy slightly higher than that of the detached state.

If the value of W_{2-} is larger, or if the value of W_+ is smaller, the value of gh/V_m will be larger than that calculated above. Ultimately gh/V_m must be determined experimentally. Indeed, one estimate can already be obtained from the experimental data of Higuchi and Goldman (1991), who measured the distance traversed by a cross-bridge per ATP hydrolyzed in muscle fibers. Using their value of $\approx$60 nm at V_m, gh/V_m can be calculated to be 0.24, a value very compatible with the estimate given above, and providing additional support for our conclusions.

The length of *h* determined at low substrate

The strongly coupled model provides a relationship between g, h, and V_m (Eq. 3), which has been used to estimate the value of h (Pate et al., 1993b). Will the inclusion of rapid detachment of highly strained cross-bridges (weak-coupling) decrease the value of the dimensionless ratio gh/V_m, and thus decrease the previous estimate of h? We argue below that weak coupling plays little role at low substrate levels. At low substrate concentration, and low shortening velocity, the cross-bridges performing the major portion of the negative work will be rigor, A·M cross-bridges, and the rate limiting transition in the dragstroke, g, will now be substrate binding. Our present analysis allows us to estimate the amount of negative work done by these highly negatively strained, rigor cross-bridges prior to dissociation. As has been demonstrated, the value of gh/V_m depends strongly on the amount of negative work required for cross-bridge dissociation at $x < -h$. The amount of work required to dissociate a rigor cross-bridge is significantly greater than that required to dissociate an A·M·ADP cross-bridge. This was shown dramati-

cally by Warshaw and co-workers (1990). They found that a very small fraction (<1%) of rigor, A·M cross-bridges severely inhibits the translation of actin filaments using an in vitro assay. Thus the data indicate that the value of W_{2-} is much greater for rigor heads, and for the above data, increases the calculated value of gh/V_m from 0.2 to well above 1.0, in the range suggested by the strongly coupled analysis.

Relation to the length of the power stroke

A number of investigators have attempted to determine the value of h by measuring the velocity of filament translocation, the number of attached cross-bridges, and the ATPase activity of the system. The values have been widely divergent (reviewed in Burton, 1992; Yanagida, 1990). The model discussed here suggests that this analysis may be more complicated than previously appreciated, because a myosin cross-bridge may not bind and hydrolyze an ATP during each interaction with actin. Thus some of these previous studies may not have measured h, but instead measured some "interaction distance." This distance represents the sum of the distances traversed by an attached myosin during the several interactions that occur between successive binding of a substrate molecule. It must be remembered, however, that the presence of such multiple interactions requires an ensemble of cross-bridges to interact with each filament, because in order for movement to occur, at least one cross-bridge must be undergoing an ATP utilizing interaction with actin. Thus the weakly coupled model discussed here may not explain the long distances measured in some experimental systems where each actin filament interacts with only a few cross-bridges (Yanagida, 1990; Harada et al., 1990). However, our model does suggest that this approach to measuring h should work most effectively under conditions where the velocity is low and few cross-bridges are dissociated mechanically. Such data has been obtained in muscle shortening under high load by Curtin et al. (1974), and these data indicate a low value for h. Alternatively, h may be accurately determined from the velocity of filaments that are translated by low numbers of myosin heads, a regime where mechanical dissociation does not occur and where again the value of h is found to be small (Uyeda et al., 1991).

In summary, the model considered here was motivated by an effort to reconcile the mechanics of active fibers with the kinetics of the actomyosin interaction observed in solution. A simple modification to existing models was found to reconcile mechanics with biochemistry as a function of substrate concentration, and to also explain the high economy of rapidly shortening fibers. The modification involves the dissociation of a highly strained cross-bridge, a transition that is reasonable on both energetic and teleological grounds.

We thank C. Brokaw and J. Sleep for comments on previous versions of the manuscript.

This work was supported by NIH grants HL41776 (H.W.), AR39643 (E.P.), HL32145 (R.C.), a grant from the American Heart Association (H.W.), and a grant from the Muscular Dystrophy Association (R.C.). E.P. is an American Heart Association Established Investigator.

REFERENCES

Brenner, B. 1990. Muscle mechanics and biochemical kinetics. *In* Molecular Mechanisms in Muscular Contraction. J. M. Squire, editor. Macmillan Press, London. 77–149.

Brenner, B. 1991. Rapid association and dissociation of acto-myosin cross-bridges during force generation,- a newly observed facet of cross-bridge action in muscle. *Proc. Natl. Acad Sci. (USA)* 88:490–498.

Brokaw, C. J. 1976. Computer simulation of movement generating cross-bridges. *Biophys. J.* 16:1013–1027.

Burton, K. 1992. Myosin step size: estimates from motility assays and shortening muscle. *J. Muscle Res. Cell Motil.* 13:590–607.

Cooke, R., and W. Bialek. 1979. Contraction of glycerinated muscle fibers as a function of MgATP concentration. *Biophys. J.* 28:241–258.

Cooke, R., and E. Pate. 1985. The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys. J.* 48:789–798.

Cooke, R. 1986. The mechanism of muscle contraction. *CRC Crit. Rev. Biochem.* 21:53–118.

Cooke, R., K. Franks, G. Lucianni, and E. Pate. 1988. The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J. Physiol.* 395:77–97.

Cordova, N. J., G. B. Ermentrout, and G. F. Oster. 1992. Dynamics of single-motor molecules: the thermal rachet model. *Proc. Natl. Acad. Sci. (USA)* 89:339–343.

Curtin, N. A., C. Gilbert, K. M. Kretzschmar, and D. R. Wilkie. 1974. The effect of the performance of work on total energy output and metabolism during muscular contraction. *J. Physiol.* 238:455–472.

Dantzig, J. A., M. G. Hibberd, D. R. Trentham, and Y. E. Goldman. 1991. Cross-bridge kinetics in the presence of MgADP investigated by photolysis of caged ATP in rabbit psoas muscle fibres. *J. Physiol.* 432: 639–680.

Edman, K. A. P., L. A. Mulieri, and B. Scubon-Muleri. 1976. Non-hyperbolic force-velocity relationship in single muscle fibres. *Acta Physiol. Scand.* 98:143–156.

Eisenberg, E., T. L. Hill, and Y. Chen. 1980. Cross-bridge model of muscle contraction, *Biophys. J.* 29:195–227.

Ferenczi, M. A., Y. E. Goldman, and R. Simmons. 1984. The dependence of force and shortening velocity upon substrate concentration in skinned fibers of *Rana temporaria*. *J. Physiol.* 350:519–543.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1985. Tension transients during steady shortening of frog muscle fibres. *J. Physiol.* 361:131–150.

Geeves, M. A. 1991. The dynamics of actin and myosin association and the crossbridge model of muscle contraction. *Biochem. J.* 274:1–14.

Goldman, Y. E., M. G. Hibberd, and D. R. Trentham. 1984a. Relaxation of rabbit psoas muscle fibres from rigor by photochemical generation of adenosine-5′-triphosphate. *J. Physiol.* 354:577–604.

Goldman, Y. E., M. G. Hibberd, and D. R. Trentham. 1984b. Initiation of active contraction by photogeneration of adenosine-5′-triphosphate in rabbit psoas muscle fibres. *J. Physiol.* 354:605–624.

Goldman, Y. E. 1987. Kinetics of the actomyosin ATPase in muscle fibers. *Ann. Rev. Physiol.* 49:637–654.

Harada, Y., K. Sakurada, T. Aoki, D. D. Thomas, and T. Yanagida. 1990. Mechanochemical coupling in actomyosin energy transduction studied in an in vitro movement assay. *J. Mol. Biol.* 216:49–68.

Higuchi, H., and Y. E. Goldman. 1991. Sliding distance between actin and myosin filaments per ATP molecule hydrolysed in skinned muscle fibres. *Nature* 352:352–354.

Hill, T. L. 1974. Theoretical formalism for the sliding filament model of contraction of striated muscle, Part I. *Prog. Biophys. Molec. Biol.* 28: 267–340.

Homsher, E., M. Irving, and A. Wallner. 1981. High-energy phosphate metabolism and energy liberation associated with rapid shortening in frog skeletal muscle. *J. Physiol.* 321:423–436.

Homsher, E., and N. C. Millar. 1990. Caged compounds and striated muscle contraction. *Ann. Rev. Physiol.* 52:875–896.

Houadejeto, M., F. Travers, and T. Barman. 1992. Ca^{2+}-activated myofibrillar ATPase: transient kinetics and the titration of active sites. *Biochemistry.* 31:1564–1569.

Huxley, A. F. 1957. Muscle structure and theories of contraction. *Prog. Biophys.* 7:255–318.

Huxley, A. F. 1973. A note suggesting that the cross-bridge attachment during muscle contraction may take place in two stages. *Proc. R. Soc. Lond. B.* 183:83–86.

Irving, M. 1991. Biomechanics goes quantum. *Nature* 352:284–286.

Johnson, R. E. 1986. Effect of ethylene glycol and Ca^{2+} on the binding of Mg^{2+}adenyl-5′-yl imidodiphosphate to rabbit skeletal myofibrils. *J. Biol. Chem.* 261:728–732.

Julian, F. J., and M. R. Sollins. 1975. Variation of muscle stiffness with force at increasing speeds of shortening. *J. Gen. Physiol.* 66:287–302.

Kushmerick, M. J., and R. E. Davies. 1969. The chemical energetics of muscle contraction. 1. Activation heat, heat of shortening and ATP utilization for activation-relaxation processes. *Proc. R. Soc. Lond. Ser. B* 174:293–313.

Ohno, T., and T. Kodama. 1991. Kinetics of adenosine triphosphate hydrolysis by shortening myofibrils from rabbit psoas muscle. 1. *Biophys. J.* 441:685.

Pate, E., and R. Cooke. 1985. The inhibition of muscle contrction by adenosine 5′(beta, gamma-imido) triphosphate and by pyrophosphate. *Biophys. J.* 47:773–780.

Pate, E., and R. Cooke. 1989. A model of cross-bridge action: the effects of ATP, ADP and Pi. *J. Muscle Res. Cell Motil.* 10:181–196.

Pate, E., K. Franks, H. White, and R. Cooke. 1993a. The use of differing nucleotides to investigate cross-bridge kinetics. *J. Biol. Chem.* 268:10046–10053.

Pate, E., H. White, and R. Cooke. 1993b. Determination of the myosin step size from mechanical and kinetic data. *Proc. Natl. Acad. Sci. (USA)* 90:2451–2455.

Rall, J. A., E. Homsher, A. Wallner, and W. H. F. M. Mommaerts. 1976. A temporal dissociation of energy liberation and high energy phosphate splitting during shortening in frog skeletal muscles. *J. Gen. Physiol.* 68: 13–27.

Schoenberg, M. 1988. Characterization of the myosin adenosine triphosphate (M·ATP) crossbridge in rabbit and frog skeletal muscle fibers. *Biophys. J.* 54:135–148.

Siemankowski, R. F., M. O. Wiseman, and H. D. White. 1985. ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc. Natl. Acad. Sci. (USA)* 82:658–662.

Taylor, E. W. 1989. Actomyosin ATPase mechanism and muscle contraction. *In* Muscle Energetics. R. J. Paul, G. Elzinga, and K. Yamada, editors. Alan R. Liss, Inc., N. Y. 9–14.

Taylor, E. W. 1991. Kinetic studies on the association and dissociation of myosin subfragment 1 and actin. *J. Biol. Chem.* 266:294–302.

Uyeda, T. Q. P., H. Warrick, S. J. Kron, and J. A. Spudich. 1991. Quantized velocities at low myosin densities in an in vitro motility assay. *Nature* 352:307–311.

Vale, R. D., and F. Oosawa. 1990. Protein motors and Maxwell's demons: does mechanochemical transduction involve a thermal rachet? *Adv. Biophys.* 26:97–134.

Warshaw, D. M., J. M. Desrosiers, S. S. Work, and K. M. Trybus. 1990. Smooth muscle myosin cross-bridge interactions modulate actin filament sliding velocity in vitro. *J. Cell Biol.* 111:453–463.

White, H. D. 1985. Kinetics of tryptophan fluorescence enhancement in myofibrils during ATP hydrolysis. *J. Biol. Chem.* 260:982–986.

White, H. D., B. Belknap, and W. Jiang. 1993. Kinetics of binding and hydrolysis of a series of nucleoside triphosphates by actomyosin-S1: relationship between solution rate constants and properties of muscle fibers. *J. Biol. Chem.* 268:10039–10045.

Yanagida, T. 1990. Loose coupling between chemical and mechanical reactions in actomyosin energy transductions. *Adv. Biophys.* 26: 75–95.

Zhang, X., W. Jiang, and H. D. White. 1992. Kinetic mechanism of the interaction between myosin subfragment-1 (S1), nucleoside diphosphates, and actin. *Biophys. J.* 61:440a.