Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 2451–2455, March 1993
Biophysics

Determination of the myosin step size from mechanical and kinetic data

(muscle/motility/cross-bridge)

EDWARD PATE*, HOWARD WHITE†, AND ROGER COOKE‡

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

Communicated by Hugh E. Huxley, November 20, 1992 (received for review June 11, 1992)

ABSTRACT **During muscle contraction, work is generated when a myosin cross-bridge attaches to an actin filament and exerts a force on it through some power-stroke distance, *h*. At the end of this power stroke, attached myosin heads are carried into regions where they exert a negative force on the actin filament (the drag stroke) and where they are released rapidly from actin by ATP binding. Although the length of the power stroke remains controversial, average distance traversed in the drag-stroke region can be determined when one knows both rate of cross-bridge dissociation and filament-sliding velocity. At maximum contraction velocity, the average force exerted in the drag stroke must balance that exerted in the power stroke. We discuss here a simple model of cross-bridge interaction that allows one to calculate the force exerted in the drag stroke and to relate this to the power-stroke distance *h* traversed by cross-bridges in the positive-force region. Both the rate at which myosin can be dissociated from actin and the velocity at which an actin filament can be translated have been measured for a series of myosin isozymes and for different substrates, producing a wide range of values for each. Nonetheless, we show here that the rate of myosin dissociation from actin correlates well with the velocity of filament sliding, providing support for the simple model presented and suggesting that the power stroke is ≈10 nm in length.**

The prevailing model for cross-bridge function incorporates the concept of the cross-bridge power stroke, the distance *h* through which a single myosin molecule can exert a positive force on an actin filament during one interaction. Original estimates of this distance, based upon observations of the decrease in tension after rapid changes in muscle-fiber length, indicated a power stroke of ≈10 nm (1), a distance compatible with structural data (2). More recently, Uyeda *et al.* (3) have determined a value for *h* of 5–20 nm from measurements of the velocity and ATPase activity of actin filaments that are translated by a small number of immobilized myosin molecules. Observations on myofibrils have likewise suggested that the power stroke is ≈20 nm in length (4). Some recent work, however, has reached the conclusion that the cross-bridge working distance may be substantially larger than suggested above. Interpretations of the length transients following photolysis of caged nucleotides in rigor muscle fibers have suggested that the sum of the drag stroke and the power stroke depends on velocity and is >40 nm at high velocities (5). Simultaneous measurements of the velocity of filaments and ATPase activities in *in vitro* motility assays have suggested that the myosin step size is >100 nm and potentially >200 nm (6, 7). Values of *h* <20 nm could be traversed by a myosin head exerting force via a change in angle while attached to actin. Distances significantly greater than this are incompatible with such a model due to the steric constraints provided by the physical size of the myosin head (length = 17–20 nm; ref. 8), and they have led to models in which multiple attachment–detachment cycles occur per ATP hydrolyzed.

In 1957 A. F. Huxley (9) proposed a model of cross-bridge interaction that explained much of the steady-state mechanical data available at the time. In this model, an attached cross-bridge traverses a power stroke, during which it exerts a positive force on the actin filament. The myosin head is subsequently pulled into a drag-stroke region, now exerting a negative force that opposes muscle shortening. Thus, one of the many conceptual innovations of this model was a chemomechanical explanation for the existence of a maximum velocity of shortening. At the maximum velocity of shortening, tension is equal to zero, and thus the net positive force exerted by myosin heads attached in the power stroke equals the net negative force exerted by heads that have been carried into the drag-stroke region. Subsequent interpretation of mechanical data has suggested that a myosin head attached to actin can, indeed, exhibit both positive and negative forces (10–12). Although the distance traversed in the positive-force region has been difficult to define, the distance traversed in the negative-force region can be calculated when one knows both rate of head dissociation and velocity of filament translation. This is most clearly defined at low substrate concentration, where the rate of head dissociation is proportional to substrate concentration (13, 14). We discuss a model that allows one to relate the distance traversed in the region of negative force to the distance *h* traversed in the region of positive force. The rate of myosin head dissociation can be altered by varying experimental conditions, myosin isoform, and the nucleotide used as substrate. An extensive body of experimental data exists, relating substrate concentration, the steady-state filament, the sliding velocity, myosin isoform, and the kinetics of the actomyosin interaction, with parameters varying by over an order of magnitude. We show here that despite the variations in isozymes, substrates, sliding velocities, and kinetic rates, the data are consistent with a model for actomyosin chemomechanics in which the rate of cross-bridge detachment governs filament velocity. Furthermore, the data are consistent with a value for *h* of ≈10 nm.

Model

We consider the cross-bridge model that has been the predominate view of the field. It is a modified version of the model proposed by A. F. Huxley in 1957 (12). As we wish to provide an analytical framework in which to consider the available data, it remains important to carefully detail the nature of the cross-bridge cycle. We make the following assumptions:

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

(*i*) Cross-bridge kinetics are dependent upon a spatial variable, x, denoting the distance between a myosin cross-bridge and actin site (Fig. 1).

(*ii*) Cross-bridges attach at the beginning of the power stroke, entering a force-producing state at $x = h$. They then progress through the working stroke ($0 < x < h$) without detaching.

(*iii*) While attached, cross-bridges produce force as linearly elastic elements (i.e., force is proportional to strain x). The elastic modulus is the same for both positive and negative values of x.

(*iv*) When $x < 0$, and continued attachment would resist useful motion, ADP is released from the actomyosin cross-bridge with rate constant k_d, and ATP binds with an apparent second-order rate constant k_t, detaching the cross-bridge. The original formulation by Huxley considered only the physiological substrate concentration, and thus the transition rate for $x < 0$ was taken as a constant, g_2. Here we wish to investigate the implications of variation in substrate concentration and, thus, consider a two-step process.

(*v*) The kinetic values k_t and k_d are the values measured for ATP binding to and ADP dissociation from acto-S1 (actomyosin subfragment-1) in solution. Fig. 1 gives a schematic of the cross-bridge cycle described above.

At the maximum shortening velocity V_{max}, no net force will be produced by the cross-bridge system. This condition requires that the positive force produced by those attached cross-bridges with $x > 0$ exactly balance the resistive force of the attached, negatively strained ($x < 0$) cross-bridges. From the above model, analytical expressions can be developed for these forces (see *Appendix*). Upon equating these two expressions, it is then possible to determine V_{max} as a function of the two kinetic rate constants k_t and k_d and the concentration of ATP:

$$V_{max} = \frac{V_\infty[ATP]}{K_m + [ATP]} M(K_m, [ATP]). \quad [1]$$

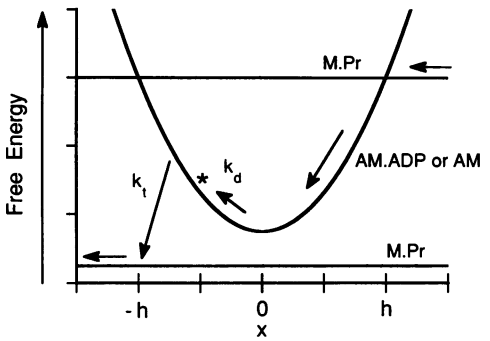

FIG. 1. Free energies of the states involved in the working cross-bridge cycle are shown as a function of the spatial variable x. Binding sites enter from the right (arrow). Detached cross-bridges [myosin–products (M.Pr)] initially attach and enter a force-producing [actomyosin–ADP (AM.ADP)] state at $x = h$. Attached cross-bridges are assumed to be linear elastic elements resulting in parabolic free-energy profiles. Attached cross-bridges traverse the power stroke from $x = h$ to $x = 0$, producing useful work equal to $\kappa h^2/2$, where κ is the cross-bridge elastic modulus. For $x < 0$, continued attachment retards motion. The ADP off-rate is taken as k_d, resulting in ADP release, illustrated as occurring at some point (*). ATP subsequently binds to the actomyosin complex (second-order rate constant k_t), and the cross-bridge dissociates to begin a new cycle. The cross-bridge populations as a function of x can be determined by solving the appropriate mass-action differential equations. The velocity at which positive and negative forces balance, V_{max}, can then be determined, yielding in Eq. 1. Additional details are provided in the *Appendix*. The free-energy difference between subsequent M.Pr states represents the free energy of hydrolysis of ATP.

Here $V_\infty = k_d h/\sqrt{2}$ is introduced to represent the maximum shortening velocity at infinite [MgATP], $K_m = k_d/k_t$, and the multiplicative function

$$M(K_m, [ATP]) = \{1 - K_m[ATP]/(K_m + [ATP])^2\}^{-1/2}$$

can be shown to be in the range [1.0, 1.15] for all values of K_m and [ATP].

We reemphasize that given the cross-bridge cycle described above, no mathematical approximations are involved in the derivation of Eq. 1. We now make our only analytical approximation, an approximation that is actually a reflection of experimental reality. To within the accuracy with which the experiments considered in this study can be done (≈20%), the function $M(K_m,[ATP])$ can be approximated as equal to 1.00, and the model analysis is consistent with experimental observations that maximum shortening velocity obeys classical, Michaelian saturation behavior with respect to substrate concentration. We also note that due to the symmetry considerations involving positively and negatively strained cross-bridges, V_{max} is independent of both the cross-bridge elastic modulus and the number of cross-bridges that attach at the beginning of the power stroke. It depends only upon the kinetic parameters determining the rate of cross-bridge release and the length of the power stroke. The fact that our analysis is independent of the number of attached cross-bridges represents a significant difference from some other analyses of step length that require knowledge of either the fraction attached or the ATPase rate per working head.

Eq. 1 suggests two sets of experimental conditions in which the power-stroke length h can be determined from the biochemical and mechanical parameters. At low ATP concentrations, V_{max} will be determined by the rate of substrate binding to the actomyosin complex. Using the definitions of V_∞ and K_m above, and solving for h, one obtains

$$h = \frac{\sqrt{2}\, V_\infty}{K_m\, k_t}. \quad [2]$$

At sufficiently high substrate concentration, the cross-bridge step(s) involving substrate binding can no longer be rate limiting. It has been suggested that at high substrate concentration, the release of the diphosphate form is the rate-limiting step (15). Then from Eq. 1, at high substrate, $V_{max} = V_\infty = k_d h/\sqrt{2}$, yielding

$$h = \frac{\sqrt{2}\, V_\infty}{k_d}. \quad [3]$$

Because $K_m = k_d/k_t$, Eqs. 2 and 3 are equivalent expressions. However, they now allow independent determinations of h based upon different kinetic and mechanical measurements.

Determination of *h*

Both muscle mechanics and actomyosin biochemistry vary with substrate, as well as temperature and ionic strength. The most convincing arguments can be drawn from comparisons involving widely varying mechanics and biochemistry but measured under identical experimental conditions. Such studies are those of Cooke and Pate (16), White *et al.* (17), and Pate *et al.* (18) comparing rabbit psoas muscle mechanics and solution biochemistry from a series of substrates (Table 1). The remarkable observation is that despite the fact that k_t, K_m, and V_∞ vary by an order of magnitude, the calculated step sizes h are similar. This observation is strong evidence that the binding of substrate limits the rate of cross-bridge dissociation, which in turn limits sliding velocity. The length of the

Table 1. Power stroke determined by variation of substrate

Substrate	V_∞, nm/s	K_m, μM	k_t, $M^{-1}\cdot s^{-1}$	h, nm
ATP	2040	150	2.7×10^6	7.1
CTP	1360	1900	1.4×10^5	7.1
Aza-ATP	470	375	3.4×10^5	5.2

Calculated step length h from Eq. 2 for a single muscle type (rabbit psoas) with experiments conducted under similar conditions (μ = 200–210 mM, T = 10°C) using substrates with widely varying kinetics and mechanics. V_∞ is based upon a sarcomere length of 2.4 μm.

power stroke determined by these data is sufficiently short to be traversed by a myosin cross-bridge that changes its angle by <45°.

As noted, Table 1 constitutes the only data set with the solution biochemistry and fiber mechanics investigated under identical conditions. However, additional data sets are available in which sliding velocity was measured as a function of substrate concentration to determine values for K_m and V_∞. These can be combined with measurements of k_t obtained under similar, but not identical, conditions. Both kinetics and mechanics vary with myosin isoform, experimental temperature, and ionic strength. Table 2 gives additional data, appropriately adjusted for differences in temperature. As would be expected, a comparison of data between different experimental laboratories and conditions produces results with greater variability. Nonetheless, the data in Table 2 again yield a "short" power stroke, compatible with the size of a myosin molecule. Although the *in vitro* assays of filament velocity from different laboratories have previously been interpreted to yield widely disparate values of h (3, 7), the values they yield in the present analysis are in line with those determined by using skinned fiber data in Table 1. In particular, although Harada *et al.* (7) find a much faster V_∞ than other investigators, they also have a proportionally greater value for K_m, producing a similar value for h by our analysis (lower section of Table 2).

Eq. 3 provides an additional method for determining h. In Eq. 3, k_d is the kinetic rate that limits head dissociation at high substrate concentration. We have tested the assumption that k_d is identical to the rate of diphosphate dissociation from acto-S1 measured in solution. If this assumption is true, then h determined by this alternative method should be equivalent to that determined before. However, we find a much larger variability in the values obtained for h from V_∞ and k_d; this may be, in part, because the measurements are more difficult. Measurements of k_d values for fast skeletal actomyosin are in the range 600 s^{-1} to 2000 s^{-1} and, thus, are approaching the limits of rate constants reliably measured by stopped-flow methods. Reported values for V_∞ measured in fibers and from *in vitro* motility measurements show a much wider range of values than does V_∞/K_m. As a result, values of h obtained by using Eq. 3 varied over a wider range than observed in Tables 1 and 2. For example, rabbit soleus muscle with V_∞ = 840 nm/s (22) and k_d = 70 s^{-1} (15) at 15°C gave a high value for h of 17 nm, whereas a low value of 2.6 nm was obtained for rabbit psoas fibers activated in high concentrations of ATP at 10°C (V_∞ = 2040 nm/s, k_d = 1100 s^{-1}). The *in vitro* motility data of Harada *et al.* (7) at 22°C (V_∞ = 8300 nm/s, k_d = 2500 s^{-1}) give an intermediate value of 4.6 nm. The low values for h may suggest that velocity may be determined by slower steps of the mechanism that precede nucleoside diphosphate dissociation (23, 24). In this case, k_d would be a composite of at least two rates, the rate of ADP release and the rate of an isomerization that preceded it. Thus, the kinetics of the release of myosin heads is more complicated at high substrate, and additional work will be required to model it fully. Nevertheless, the length of the power stroke determined by this method is clearly not large.

Discussion

Our analysis, based upon the original cross-bridge model of Huxley (12), provides a simple framework in which to relate the rate of actomyosin dissociation to the maximum velocity at which myosin heads can translate an actin filament. From this it is possible to determine a power-stroke length, h. This determination can be made because at V_{max} there is no net force, and thus the positive force and the negative force exerted by attached, strained cross-bridges must be equal. With knowledge of the two rate constants involved in actomyosin dissociation, k_d and k_t, one can easily calculate the average negative force independently of other features of the model. From this, the requisite balancing force in the positive strain region determines h.

Despite the wide range of myosin types, substrates, experimental protocols, and sliding velocities in Tables 1 and 2, we obtain remarkably consistent values for the power-stroke length, providing support for our approach. The values cluster around 10 nm and are within the limits imposed by the experimentally observed size of the myosin head. At low substrate concentration there is a linear relationship between velocity and substrate. Our analysis is most valid in this regime because it is here that the rate-limiting step—substrate binding with subsequent, rapid cross-bridge dissociation—can be most convincingly identified. Indeed, it is for precisely these data that we get the tightest correlation, especially for those data taken under identical conditions (Table 1).

Table 2. Power stroke determined with different isoforms and assay conditions

Myosin type (ref.)	V_∞, nm/s	K_m, mM (°C)	k_t, $M^{-1}\cdot s^{-1}$ (°C)	h, nm
Muscle fibers				
Frog semitendinosus (13, 19)	2590	0.47 (0)	7.4×10^5 (0)	10.4
Rabbit semimembranosus (20)	708	0.02 (10)	4.1×10^6 (10)	12.1
Rabbit soleus (15, 20)	860	0.02 (10)	2.0×10^6 (15)	13.7
In vitro assays of filament velocity				
Rabbit fast (17, 21)	2800	0.05 (24)	5.7×10^6 (24)	13.9
Rabbit fast (7, 17)	8300	0.18 (22)	5.1×10^6 (24)	13.1

Calculated power-stroke length (Eq. 2) for various myosin isoforms based upon sliding velocity as a function of [ATP]. For each myosin isoform, the first two columns indicate V_∞, K_m, and the temperature at which the observations were made; the third column gives k_t and the temperature of experimental observation. A Q_{10} of 2 was assumed for k_t, and the k_t is the experimentally measured value, adjusted to correspond to the temperature of the mechanical observations. The adjusted value is used to calculate h. For example, our adjustment to the temperature of the mechanical observations (24°C and 22°C) results in different values for k_t in the last two rows (*in vitro* assays), even though the k_t values were both obtained at 24°C. Ionic strengths are varied between 50 and 200 mM. No adjustment was made for ionic-strength differences in calculating h. References for experimental observations are given in parentheses.

The studies from which Table 1 was developed also investigated GTP as a substrate for the actomyosin system (17, 18). The mechanical and biochemical data from GTP provide a much shorter value for h of 0.3 nm. These studies also showed, however, that GTP is an extremely poor substrate for the actomyosin system, with a dramatically perturbed cross-bridge cycle. In particular, a significant fraction of the attached cross-bridges are in a bound, triphosphate state. Thus, V_∞ is limited by factors other than substrate binding, as assumed in the present analysis, and these data have not been included in Table 1.

It is important to consider further the degree to which our conclusions depend upon specific aspects of the analysis. As noted, Eqs. 2 and 3 result from the fact that at V_{max}, total force is zero, requiring that the negative force exerted in the region $x < 0$ must equal the positive force exerted in the region $x > 0$. In the above model all working heads attach at $x = h$ and progress through the power stroke to $x = 0$. This assumption determines the positive force that is balanced at V_{max}. Other assumptions regarding the attachment of cross-bridges for $x > 0$ lead to similar relationships. However, the constant of proportionality can vary. For example, if for $x > 0$, the heads attach and detach with rate constants f and g that are proportional to x, as in the original formulation by A. F. Huxley (12), the cross-bridge distribution in the region $x > 0$ is no longer spatially uniform, and the multiplicative constant in Eq. 3 becomes 2 instead of $\sqrt{2}$. Thus reasonable modifications of the spatial distribution of the cross-bridges may alter the calculated value of h by less than a factor of 2 but will not alter the general conclusions drawn from our data. A similar conclusion is reached from simulations incorporating a more realistic inclusion of the initial weakly attached, pre-power-stroke state, as in Pate and Cooke (25). As was the case in the original Huxley cross-bridge model, we have made the additional assumption that the elastic modulus of an attached cross-bridge is the same in both positive and negative regions of force. Although this assumption would be true if the myosin head operated as a simple Hookian spring, it might not necessarily be true for the more complex, asymmetric macromolecules involved. The effect of unequal elastic moduli can be determined from the integral relationships for force in the *Appendix* (last equation). With elastic moduli κ_1 ($x < 0$) and κ_2 ($x > 0$), it is straightforward to show that our expression for h (Eq. 2) is multiplied by κ_1/κ_2, the ratio of the elastic moduli. Hence if a cross-bridge is more stiff in the negative region, our value for h is an underestimate; if it is less stiff, our value is an overestimate. In the above analysis we also assumed that the rates of ADP release and ATP-dependent dissociation for $x < 0$ are constants. In fibers, these ligand-dependent rate constants could depend on the value of x. If they were to become slower as x became increasingly more negative, the calculated value of h would become longer. However, it seems unlikely that the rate of head dissociation would become slower for more highly strained cross-bridges, and experimental observation of tension transients after release of caged ATP suggests modestly increased transient rates in highly strained myosin cross-bridges (26). If the rate constant for dissociation does increase as x becomes more negative, the value we have determined for h is an overestimate. Thus, although the exact value of h is model dependent, a substantial change in the value of h would require large changes in the cross-bridge parameters, changes not supported by current experimental data.

Table 3. Summary of cross-bridge distance measurements

Ref.	Value	Unit
This work	5–14	nm
Huxley and Simmons (1)	10	nm
Huxley and Kress (2)	4–12	nm
Taylor (4)	20	nm
Uyeda *et al.* (3)	5–20	nm/ATP
Higuchi and Goldman (5)	10–40	nm/ATP
Yanagida *et al.* (6)	120	nm/ATP
Harada *et al.* (7)	>200	nm/ATP

The distance traversed by an attached myosin head has been estimated by using a variety of experimental data from various laboratories (Table 3). In some of these experiments the distance measured represents h, whereas in others it represents the sum of the power stroke plus the drag stroke, with the exact relationship dependent on the model assumed. The value obtained by Higuchi and Goldman (5) is the sum of power and drag strokes, whereas the distance measured in the other studies is most probably related to the length of the power stroke. Three of these values are longer than that determined here (5–7). As noted in Table 3, however, these values are intrinsically different from the one measured here. They are all measurements of a distance traversed per attached myosin head per ATP hydrolyzed, whereas the value of h found here is determined by a comparison of kinetic and mechanical data.

Are these conclusions incompatible? Although a complete resolution of this question is beyond the scope of the present paper, we think that, at least, some of these observations are not incompatible with the present model. A simple extension of the current model may be capable of explaining the long values found for the distance traversed per attached head per ATP hydrolyzed in muscle fibers. In the model we have considered, one ATP is hydrolyzed by each cross-bridge that traverses the working power stroke. This assumption, however, can be eliminated allowing multiple interactions per ATP hydrolyzed. With reference to Fig. 1, consider the situation where ADP release, or some isomerization that precedes it, may be slow. Then some cross-bridges do not release ADP and detach via ATP binding. Instead, they will be carried up through the region of negative force until $x < -h$, and the free energy of the attached state is greater than that of the detached state. If these cross-bridges can dissociate "mechanically" in such a fashion that they are then able to reattach to a different actin at the beginning of the power stroke and begin a new cycle without ATP hydrolysis, they would, on average, produce little net force per cycle and, thus, would have little influence on fiber mechanics. The cross-bridges would be counted as attached to actin, but they would not contribute to ATPase activity. Rapid detachment and reattachment of strongly bound cross-bridges have been suggested by a number of workers on the basis of both experimental and theoretical grounds (14, 27–29). Some preliminary calculations suggest that this modification to the model is feasible, and that it could explain the long distances traversed by a myosin head per ATP split in muscle fibers where large numbers of myosin heads interact with each actin filament (5). However, this model would not explain the long distances traversed per ATP per attached head in experimental systems where very few (less than five) myosin heads interact with each actin filament. Such may be the case in the last two entries in Table 3 (6, 7). This modification to the model would not alter our conclusion that at low substrate, filament velocities are limited by the drag provided by heads that have not yet bound ATP in the region of negative force.

Considering a wide range of myosin isoforms and substrates, our analysis is consistent with the conclusion that the sliding velocity of actin generated by myosin is limited by the dissociation rate of the actomyosin complex as measured in solution. This correlation holds when the dissociation is limited by substrate binding. At high substrate concentration the rate-limiting step determining velocity is less certain. At V_{max}, the positive and negative forces of the cross-bridges

must balance. Equating these allows us to determine a value for the power-stroke length h. At low concentrations of substrate, the rate-limiting step is best defined, and it is under these conditions that the analysis provides the most consistent values for h. The exact value determined for h depends upon the specific details of the model; however, it is unlikely that realistic modifications can alter our basic conclusion—h is sufficiently short to be traversed by a single stroke of a rotating myosin head. In summary, we note that the h values in Tables 1 and 2 yield a power-stroke length that ranges from 5 to 14 nm. By considering the most reliable comparisons—where the data are taken under identical conditions, Table 1 provides a power-stroke length of 6.5 ± 0.8 nm (mean ± SEM).

Appendix

Our analysis follows the basic framework of the original cross-bridge models presented by A. F. Huxley (12). With reference to Fig. 1, let $n(x)$, $r(x)$, and $s(x)$ be the fractions of cross-bridges in [myosin–products (M.Pr), actomyosin–ADP (AM.ADP), and actomyosin (AM)] states as a function of x. Let fraction p attach in state AM.ADP at the beginning of the power stroke. Then for $0 < x < h$, $n_+(x) = 1 - p$, $r_+(x) = p$, and $s_+(x) = 0$. As shown in Fig. 1, we adopt the original sign conventions of Huxley, with binding sites entering from the right. For $x < 0$ and steady-state shortening velocity (V) > 0, the attached fractions of cross-bridges must satisfy differential equations of the form

$$-V dr_-(x)/dx = -k_d r_-(x)$$

and

$$-V ds_-(x)/dx = k_d r_-(x) - k_t T s_-(x),$$

with conditions at $x = 0$ given above. Here, subscripts + and − have been included to denote the solutions in the positive and negative regions of x. These equations are the two-attached-state analogs to the equation directly preceding equation 4 in ref. 12, stating that attached cross-bridge fractions as a function of x are altered by both kinetic transitions and filament translation. For more compact notation, we use T to represent [ATP]. Solving sequentially,

$$r_-(x) = p \exp(k_d x/V),$$

and then

$$s_-(x) = p\, k_d[\exp(k_t T x/V) - \exp(k_d x/V)]/(k_d - k_t T).$$

Attached cross-bridges are taken to produce force as linearly elastic elements with elastic modulus κ. At $V = V_{max}$, the positive and negative forces produced by attached cross-bridges must sum to zero. Integrating the attached cross-bridge populations times the force produced (κx) over the relevant spatial region, this requires

$$\int_{-\infty}^{0} [r_-(x) + s_-(x)]\kappa x\, dx + \int_0^h r_+(x)\kappa x\, dx = 0.$$

The expressions for $r_-(x)$, $s_-(x)$, and $r_+(x)$ contain the variable V and, thus, so do the evaluated integrals. It is a straightforward but lengthy calculation to solve for V in terms of the other parameters and show it equivalent to Eq. 1. Because this V is determined at zero net force, it is our value for V_{max}. Note that each term in the integral relationship contains multiplicative factors of p and κ, making the result invariant with respect to the particular values chosen. For additional details, we note that discussion of the mathematical analysis of the Huxley model can be found in chapter 4 of ref. 30.

This work was supported by National Institutes of Health 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.

1. Huxley, A. F. & Simmons, R. M. (1971) *Nature (London)* **233,** 533–538.
2. Huxley, H. E. & Kress, M. (1985) *J. Muscle Res. Cell Motil.* **6,** 153–161.
3. Uyeda, T. Q. P., Warrick, H. M., Kron, S. J. & Spudich, J. A. (1991) *Science* **352,** 307–311.
4. Taylor, E. W. (1989) in *Muscle Energetics*, eds. Paul, R. J., Elzinga, G. & Yamada, K. (Liss, New York).
5. Higuchi, H. & Goldman, Y. E. (1991) *Nature (London)* **352,** 352–354.
6. Yanagida, T., Arata, T. & Oosawa, F. (1985) *Nature (London)* **316,** 366–369.
7. Harada, Y., Sakurada, K., Aoki, T., Thomas, D. D. & Yanagida, T. (1990) *J. Mol. Biol.* **216,** 49–68.
8. Walker, M. & Trinick, J. (1988) *J. Muscle Res. Cell Motil.* **9,** 359–366.
9. Huxley, A. F. (1957) *Prog. Biophys.* **7,** 255–318.
10. Goldman, Y. E., McCray, J. A. & Vallette, D. P. (1988) *J. Physiol. (London)* **398,** 72P.
11. Edman, K. A. P. (1979) *J. Physiol. (London)* **291,** 143–159.
12. Ford, L., Huxley, A. F. & Simmons, R. (1977) *J. Physiol. (London)* **269,** 441–515.
13. Ferenczi, M. A., Goldman, Y. E. & Simmons, R. M. (1984) *J. Physiol. (London)* **350,** 519–543.
14. Cooke, R. & Bialek, W. (1979) *Biophys. J.* **28,** 241–258.
15. Siemankowski, R. F., Wiseman, M. O. & White, H. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 658–662.
16. Cooke, R. & Pate, E. (1985) *Biophys. J.* **48,** 789–798.
17. White, H., Belknap, B. & Jiang, W. (1993) *J. Biol. Chem.*, in press.
18. Pate, E., White, H., Franks-Skiba, K. & Cooke, R. (1993) *J. Biol. Chem.*, in press.
19. Ferenczi, M. A., Homsher, E., Simmons, R. M. & Trentham, D. R. (1978) *Biochem. J.* **171,** 165–175.
20. Pate, E., Lin, M., Franks-Skiba, K. & Cooke, R. (1992) *Am. J. Physiol.* **262,** C1039–C1046.
21. Kron, S. J. & Spudich, J. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6272–6276.
22. Julian, F. J., Moss, R. L. & Waller, G. S. (1981) *J. Physiol. (London)* **311,** 201–218.
23. Taylor, E. W. (1991) *J. Biol. Chem.* **266,** 294–302.
24. Geeves, M. A. (1989) *Biochem. J.* **256,** 41–46.
25. Pate, E. & Cooke, R. (1989) *J. Muscle Res. Cell Motil.* **10,** 181–196.
26. Goldman, Y. E., Hibberd, M. G. & Trentham, D. R. (1984) *J. Physiol. (London)* **354,** 577–604.
27. Brenner, B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10490–10494.
28. Lombardi, V. & Piazzesi, G. (1990) *J. Physiol. (London)* **431,** 141–171.
29. Lombardi, V., Piazzesi, G. & Linari, M. (1992) *Nature (London)* **355,** 638–641.
30. McMahon, T. A. (1984) *Muscles, Reflexes, and Locomotion* (Princeton Univ. Press, Princeton, NJ).