EQUILIBRIUM MUSCLE CROSS-BRIDGE BEHAVIOR

Theoretical Considerations

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ABSTRACT We have developed a model for the equilibrium attachment and detachment of myosin cross-bridges to actin that takes into account the possibility that a given cross-bridge can bind to one of a number of actin monomers, as seems likely, rather than to a site on only a single actin monomer, as is often assumed. The behavior of this multiple site model in response to constant velocity, as well as instantaneous stretches, was studied and the influence of system parameters on the force response explored. It was found that in the multiple site model the detachment rate constant has considerably greater influence on the mechanical response than the attachment rate constant. It is shown that one can obtain information about the detachment rate constants either by examining the relationship between the apparent stiffness and duration of stretch for constant velocity stretches or by examining the force-decay rate constants following an instantaneous stretch. The main effect of the attachment rate constant is to scale the mechanical response by influencing the number of attached cross-bridges. The significance of the modeling for the interpretation of experimental results is discussed.

INTRODUCTION

The biochemical and structural changes occurring during muscle contraction appear to be quite complex, involving multiple steps, including binding of nucleotide to the cross-bridge active site, changes in state of the cross-bridge and nucleotide, and attachment and detachment of the cross-bridge to and from actin. However, under a variety of conditions, e.g., moderate to rapid stretch of an active muscle (Flitney and Hirst, 1978; Griffiths et al., 1980), rapid stretch of a relaxed muscle (Brenner et al., 1982; Schoenberg et al., 1984), and stretch of a muscle in the presence of nonhydrolysable analogues of ATP (Kuhn, 1978; Schoenberg et al., 1984), muscle behaves in a manner that suggests that it can be described partially or totally in terms of the equilibrium attachment and detachment of cross-bridges from actin without change of state.

The purpose of the present manuscript is to present a very simple model for equilibrium attachment and detachment of cross-bridges and to describe its behavior in response to the types of maneuvers employed in experimental studies. The goal is to try to gain insight into how equilibrium cross-bridge systems behave and how molecular cross-bridge parameters may be deduced from experimental measures. We look at the force response to constant velocity stretches, a procedure often used for studying muscle fibers, as well as the response to an instantaneous stretch. We find that it is possible to extract information about certain of the cross-bridge parameters either from a plot of apparent muscle stiffness vs. duration of stretch for constant-velocity stretches, or from the force response to an instantaneous stretch. The relative advantages of the two techniques are discussed.

METHODS

The Single Site Model

According to the theoretical formalism of T. L. Hill (1974), each cross-bridge state may be characterized by its basic free energy as a function of x, where x is the relative position of the cross-bridge to an actin site. In this section we set out the equations and nomenclature appropriate for a model in which a single myosin head (cross-bridge) can interact with but one actin site. In the next section we derive the equations appropriate to the case where the cross-bridge can interact with one of several actin sites, each separated by 5.5 nm. We consider only two cross-bridge states, attached, AMN, and unattached (detached), MN, where N indicates that in general the cross-bridge may have bound nucleotide. The free energy of MN, is taken as A_1, independent of x as required by the Hill formalism. If the attached cross-bridge has a linear spring constant K, then the total basic free energy of the attached state, AMN, is $A_0 + 1/2\, Kx^2$, where A_0 is its minimum free energy and $x = 0$ is the point of minimum strain of the attached cross-bridge. For simplicity we assume that the form of the cross-bridge attachment rate constant, $f\,(x)$, is a Gaussian centered about $x = 0$, with width, σ, i.e., $f = f_0\, e^{-1/2(x/\sigma)^2}$. In a few instances, non-Gaussian and nonsymmetrical attachment functions were studied with results similar to those to be reported herein. If each cross-bridge can interact with but one actin site, the general equation describing this interaction is, from Huxley, 1957, or Hill, 1974

$$\frac{\partial n}{\partial t} = f(x) \cdot [1 - n(x, t)] - f'(x) \cdot n(x, t) - V \cdot \frac{\partial n}{\partial x}, \quad (1)$$

where n is the probability density of attached cross-bridges (the fraction of attached bridges between x and $x + dx$), f and f' are the reversible rate constants of attachment and detachment, and V is the velocity of motion between thick and thin filaments (which is the same as the relative velocity between actin and myosin sites, defined positive for stretch).

Steady state solutions to Eq. 1 have previously been obtained (Huxley, 1957), simply by setting $\partial n/\partial t = 0$ in which case $\partial n/\partial x = \mathrm{d}n/\mathrm{d}x$. To find the non-steady state solution, Eq. 1 may conveniently be solved using the method of characteristics. That is, if we introduce the variable β, which describes the characteristic curve $\mathrm{d}t/\mathrm{d}\beta = 1$, $\mathrm{d}x/\mathrm{d}\beta = V$, where $t(0) = 0$ and $x(0) = x_0$, then for V constant, $t = \beta$, $x = x_0 + V\beta$ and

$$\frac{\mathrm{d}n}{\mathrm{d}\beta} = f(x_0 + V\beta)\cdot[1 - n(x_0, \beta)] - f'(x_0 + V\beta)\cdot n(x_0, \beta). \quad (2)$$

More details can be found in any treatise on differential equations (see also Zahalak, 1981) but, practically speaking, if one integrates along the characteristic line $t = \beta$, $x = x_0 + V\beta$, then Eq. 2 enables one to start with known values of $n(x_0, t_0)$ at time, t_0, and get the solution for $n(x, t)$ for all future time.

From the solution of Eq. 2 for $n(x, t)$ for a given relative filament velocity, V, one can calculate the fraction of attached cross-bridges at any instant

$$\bar{n}(t) = \int_{-s/2}^{s/2} n(x, t)\,\mathrm{d}x \bigg/ \int_{-s/2}^{s/2} \mathrm{d}x \quad (3)$$

and also the average force per total number of cross-bridges

$$\bar{P}(t) = \int_{-s/2}^{s/2} n(x, t)\,Kx\,\mathrm{d}x \bigg/ \int_{-s/2}^{s/2} \mathrm{d}x, \quad (4)$$

where s is the repeat distance between actin sites.

A Multiple Site Model

Most previous computations of cross-bridge models have been concerned with modeling active force generation. Since those computations typically involve many states, it has been common to make the simplifying assumption that a cross-bridge can interact with but a single actin site (free energy diagram as in Fig. 1 *A*). However, here we are able to consider the more realistic model in which a cross-bridge can interact with one of several actin sites, each separated by a distance, $s \sim 5.5$ nm (free energy diagrams schematically shown in Figs. 1 *B* and *C*). Because the pitch of the actin helix causes azimuthal twisting of the actin sites about the actin filament, in general, sites separated by a distance s will not be totally equivalent (Fig. 1 *C*). However, since it is not at present known how great an effect the azimuthal orientation of the actin site has on cross-bridge binding, a reasonable first approximation would be to assume that all actin sites are equivalent (Fig 1 *B*). A detailed free energy diagram corresponding to equivalent actin sites is shown in Fig. 2. Here the free energy curves for binding to a central actin site and five of its nearest neighbors is shown. The assumption of equivalent actin sites offers considerable mathematical simplification since one can perform all integrations and averaging only over the 5.5 nm actin repeat rather than the 38.5 nm actin helix repeat.

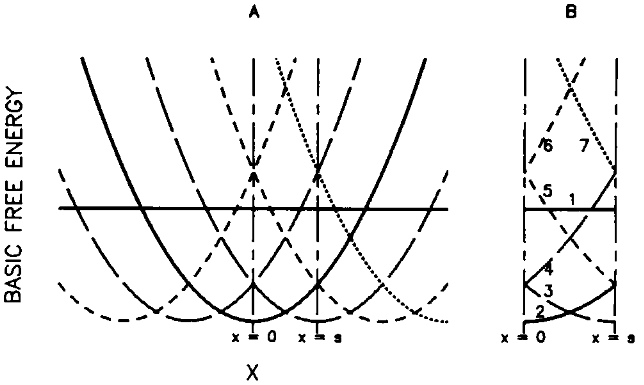

FIGURE 2 Complete free energy diagram for the multiple equivalent actin site model showing six equivalent actin sites. *Ordinate*, basic free energy; *abscissa*, relative position between the actin site and cross-bridge origin. $x = 0$, position of minimum strain of cross-bridges attached to the central actin; $x = \pm s$, position of minimum strain of cross-bridges attached to nearest neighboring actins, s being the actin repeat distance. Because actin sites are assumed equivalent, the diagram has a basic repeat each distance, s, and the repeating unit is shown in *B*. For notational convenience, each alternative energy level (line segment) between $x = 0$ and $x = s$ is connoted as a state, *1* being the off state with the other states as labeled. All cross-bridges properties need be averaged only over the interval 0 to s.

For the multiple site model, at a given value of x, an attached cross-bridge can have multiple values for its basic free energy, corresponding to attachment to different actins (see Fig. 2). It is notationally convenient to connote each alternative energy level as a different state. Seven such states are labeled in Fig. 2 *B*, 1 being the detached state. In theory there are an infinite number of such states but the ones of highest energy will not be significantly populated. (This is the same as saying that a cross-bridge will attach only to actins relatively nearby.) The allowable transitions between states are $1 \leftrightarrow 2$, $1 \leftrightarrow 3$, $\ldots$ $1 \leftrightarrow 7$. Therefore, for the seven states, we have six equations equivalent to Eq. 1, i.e.,

$$\frac{\partial n_i}{\partial t} = \alpha_{1i}\left(1 - \sum_{j=2}^{7} n_j\right) - \alpha_{i1} n_i - V\frac{\partial n_i}{\partial x} \qquad i = 2, \ldots 7 \quad (5)$$

and a seventh equation

$$\sum_{i=1}^{7} n_i = 1, \quad (6)$$

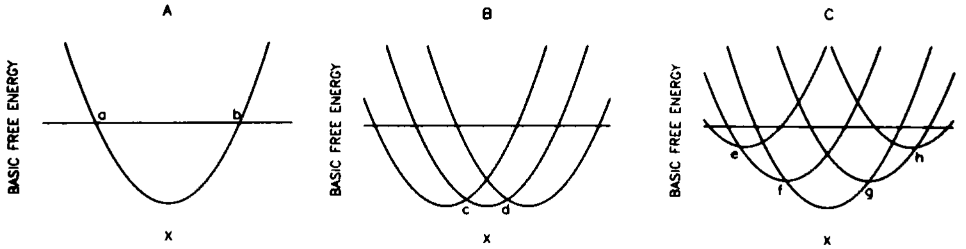

FIGURE 1 Schematic free energy diagrams for the single site (*A*), multiple equivalent site (*B*), and nonequivalent actin site models (*C*). The horizontal lines show the basic free energy of the detached cross-bridge; the parabolas show the basic free energy curves for a cross-bridge attached to a single isolated site in *A* or to one of several neighboring sites in *B* and *C*. The lower case letters connote crossover points in the free energy diagrams where significant transitions occur. (See Discussion.)

where n_i is the fraction of bridges in state i, α_{1i} is the (attachment) rate for the step (1 → i) and α_{i1} is the (detachment) rate for the step (i → 1).

It is clear from Fig. 2 that

$$\begin{aligned}\alpha_{12}(x) &= f(x)\\ \alpha_{13}(x) &= f(x - s)\\ \alpha_{14}(x) &= f(x + s)\\ \alpha_{15}(x) &= f(x - 2s)\\ \alpha_{16}(x) &= f(x + 2s)\\ \alpha_{17}(x) &= f(x - 3s)\end{aligned}$$

and α_{21}, α_{31}, etc., can be found from the corresponding $f'(x)$, i.e., $\alpha_{31} = f'(x - s)$.

Two final equations are the assumption that

$$f(x) = f_o e^{-1/2(x/\sigma)^2} \tag{7}$$

(see last section), and, from thermodynamic considerations (Hill, 1974)

$$f'(x) = f(x) \cdot \exp[(A_o - A_1 + 1/2\, Kx^2)/kT] \tag{8}$$

where kT at 25°C is 4.1×10^{-14} dyn-cm.

The equivalence of actin sites provides the boundary conditions

$$\begin{aligned}n_2(0, t) &= n_3(s, t)\\ n_3(0, t) &= n_5(s, t)\\ n_4(0, t) &= n_2(s, t)\\ n_5(0, t) &= n_7(s, t)\\ n_6(0, t) &= n_4(s, t),\end{aligned}$$

while the high free energy of states 6 and 7, allows the assumption $n_7(0, t) = 0$ and $n_6(s, t) = 0$. For some of the problems considered here, the high energy of binding $(A_1 - A_o)$ made the above assumption invalid. In this instance, two more actins for possible attachment were considered with the equivalent assumptions, $n_9(0, t) = 0$, $n_8(s, t) = 0$.

Using the method of characteristics, for a given relative velocity of the actin and myosin filaments, the above equations may be easily converted to a set of ordinary differential equations and boundary conditions that can be solved by Gear's method. This was done using a subroutine, DGEAR, provided by the National Institutes of Health (NIH) DEC-10 computing facility. From the values for $n_i(x, t)$, the fraction of attached cross-bridges, $\bar{n}(t)$, can be calculated as

$$\bar{n}(t) = \sum_{i=2}^{7} \int_0^s n_i \mathrm{d}x \Big/ \int_0^s \mathrm{d}x \tag{9}$$

and, corresponding to Eq. 4, the average force per total number of cross-bridges is

$$\bar{P}(t) = \int_0^s K\rho(x)\mathrm{d}x \Big/ \int_0^s \mathrm{d}x, \tag{10}$$

where $\rho(x) = n_2 \times x + n_3 \times (x - s) + n_4 \times (x + s) + n_5 \times (x - 2s) + n_6 \times (x + 2s) + n_7 \times (x - 3s)$.

RESULTS

Fig. 2 *A* shows the free energy diagram for the multiple equivalent actin site model. The diagram repeats every distance, s; the repeat unit is shown in Fig. 2 *B*. At a given value of x, the cross-bridge may be detached or attached to any number of actins and hence at a variety of energy levels. For convenience we have connoted each energy level as a different state. The cross-bridges will tend to populate the states with the lowest free energy; that is, at equilibrium, states 2 and 3 will be most heavily populated with the other states progressively less so. Initially the equilibrium force is zero, but upon stretch the entire cross-bridge distribution is shifted to the right, leading to an increase in force. One can see that the force response will be determined, on one hand by the velocity of stretch, how quickly the distribution is shifted away from equilibrium, and, on the other hand, by how fast the cross-bridges detach and go back toward their equilibrium distribution. We also see from Fig. 2 that the location $x = s/2$ (and the location $x = s$ to a lesser extent) has a special significance in this process because as the distribution is shifted towards the right upon stretch, cross-bridges that were in their most stable state for $x < s/2$ are no longer so for $x > s/2$; they will tend to detach and reattach to an actin where they have a more stable attachment. (With regard to Fig. 2, cross-bridges in state 2 pulled past $x = s/2$ will tend to detach and reattach in state 3.) It is clear that the detachment rate constant, $f'(x)$, and, in particular, the detachment rate constant, $f'(s/2)$, will play an important role in determining the force response. For this reason we choose a special symbol k_b, for $f'(s/2)$. Since, as argued above and as will be seen further on, the detachment rate constant is the primary determinant of the mechanical behavior, it will be convenient to separately discuss three cases: Case I, detachment rate constant, f', independent of x ($\mathrm{d}f'/\mathrm{d}x = 0$); Case II, f' decreasing with increasing x ($\mathrm{d}f'/\mathrm{d}x < 0$); and Case III, f' increasing with x ($\mathrm{d}f'/\mathrm{d}x > 0$).

Force Response to Constant-Velocity Stretch

Despite the seemingly large number of variables in the preceding equations, nondimensional analysis reveals that the nondimensional force, P/Ks, is actually a function of only four dimensionless parameters, V/sk_b, $(A_1 - A_o)/kT$, s^2/σ^2, and Ks^2/kT. The case where f' is independent of x is the case where $s^2/\sigma^2 = Ks^2/kT$. This case is by far the simplest and it is, in fact, possible to derive analytic expressions that closely describe the force response to constant velocity stretch. However, it is necessary to first derive the response to an instantaneous (step) stretch.

When one applies an instantaneous step stretch of δ nm to a muscle, each cross-bridge is strained an additional amount δ, and the force rises by the amount $n_b K\delta$, where n_b is the number of attached cross-bridges. Subsequently, those attached cross-bridges, that are no longer attached to the actin that provides the most stable attachment, will, on average, detach and reattach to actins where they are less strained. As this happens, the force induced by the step stretch will decay towards zero as the cross-bridge distribution tends toward the initial equilibrium distribution. Assuming that the strength of binding is strong, i.e., A_1 −

$A_0 >> 0$, which implies that most of the cross-bridges are attached and $f >> k_b$, then if a displacement of $\delta = Bu_{-1}(t)$, where $u_{-1}(t)$ is the Heaviside step function, is applied to the end of the fiber, the force response should be

$$P_1 = n_b K B u_{-1}(t) \exp(-k_b t). \tag{11}$$

Assuming this response is linear in B, superposition holds, and the solution for the constant velocity stretch $\lambda = Vt$ is simply

$$P(t) = n_b K V k_b^{-1} [1 - \exp(-k_b t)]. \tag{12}$$

This relationship is plotted in Fig. 3 for a number of stretch velocities. Although in deriving this analytic expression we made use of the argument $f >> k_b$, the equation is, in fact, more generally valid and accurately describes the force response in Case I in instances where $k_b > f$ (for example, open circles, Fig. 3.). Although Eqs. 11 and 12 are not exact solutions of the full equations, they do exceedingly accurately describe most cases of interest[1] and for all intents and purposes may be taken as appropriately describing the behavior of the muscle model in Case I.

For Case II, $df'/dx < 0$, and Case III, $df'/dx > 0$, the behavior of the muscle model must be derived from numerical integration of the full equations, Eqs. 5–10. The results are shown in Figs. 4 and 5. From Eq. 12 and Fig. 3 it is seen that in the simplest case, Case I, the force response to a constant velocity stretch scales with velocity and the curves all have similar shape. The rate constant for cross-bridge detachment may be obtained from the rate constant with which the force response reaches a plateau. In Case II, $df'/dx < 0$, the curves nearly scale with velocity, particularly at short times. However, there is no plateau to the force response. In Case III, $df'/dx > 0$, there is a plateau in the force response, but the curves do not scale with velocity, they do not all have the same shape, and the force response is not even necessarily monotonic, i.e., dP/dt may be < 0. The curves of Figs. 3–5 are of interest, in and of themselves, in that they describe the range of force responses one can see when one stretches a population of attached equilibrium cross-bridges.

Also of interest is the inverse problem. That is, given the force response to stretch, how may one extract the cross-bridge parameters. Ideally one would like to learn the cross-bridge stiffness, the range of attachment and detachment rate constants, and whether the detachment rate constants go up or down with x. It is not quite clear, for example, how, except in Case I, one might deduce from the force responses shown in Figs. 3–5 even k_b, the dominant detachment rate constant. In the sections that follow, we show a way of characterising the force response to constant velocity stretch that provides information about the muscle stiffness ($n_b K$), k_b, and also, some information about the range of detachment rate constants. We then examine the response to an instantaneous stretch, which provides further information about the range of detachment rate constants.

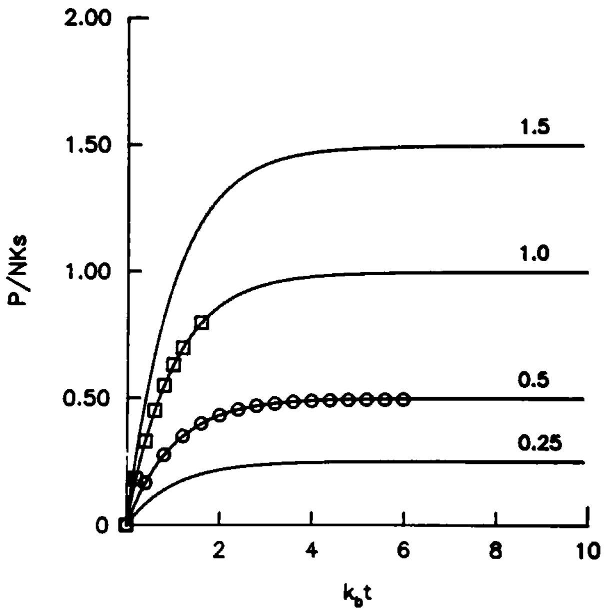

FIGURE 3 Case I. Force, P, vs. time, t, constant velocity stretches, $df'/dx = 0$. The number next to each curve gives the velocity of stretch in units of V/sk_b. In this and the other figures, N is the total number of cross-bridges per half-sarcomere. Curves calculated from Eq. 12. Squares (□), calculated from full equations for $(A_1 - A_0)/kT = 10$, $s^2/\sigma^2 = 1.84$, and $Ks^2/kT = 1.84$. Circles (○), 11.89 times the solution of the full equations for $(A_1 - A_0)/kT = -3$, $s^2/\sigma^2 = 1.84$, $Ks^2/kT = 1.84$, where $N/11.89$ is the number of cross-bridges attached for this case. Note agreement between derived and computed curves.

Apparent Chord Stiffness vs. Log Duration of Stretch

If a muscle is stretched exceptionally rapidly (duration of stretch approaching zero), so that almost no cross-bridges have time to detach during the stretch, then the ratio of the size of the force response, ΔP_1, to the size of the stretch, d, is a direct measure of the muscle stiffness. If one stretches the muscle the same amount, d, over a more extended time, t_d, and the force at time t_d is found to be half of ΔP_1, then one might argue that in time t_d, one-half of the cross-bridges had detached and redistributed toward their equilibrium distribution. From this reasoning it is seen that a plot of $\Delta P/d$ vs. t_d should give a measure both of cross-bridge stiffness and cross-bridge detachment rate con-

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[1] The one exception where Eq. 11 differed perceptibly from the fully computed solution of the equations for Case I is the case $(A_1 - A_0)/kT << Ks^2/kT$, $\delta/s << 1$. This is the case where the free energy curve for the attached state just intersects that of the unattached state so that, in mathematical terms, the free energy of the attached state at $x = 0$ lies below that of the detached state, but at $x = s/2$, it lies above. In this instance, following a stretch, the force decay is somewhat more rapid than k_b as new (initially detached) cross-bridges attach in the region of negative x. However, since for this condition f is the same order of magnitude as k_b in the regions where cross-bridges attach, the deviation from Eq. 11 is not large.

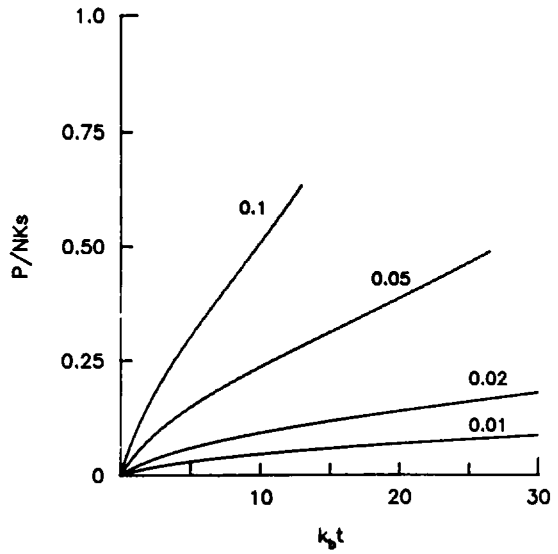

FIGURE 4 Case II. Force vs. time for $df'/dx < 0$, constant velocity stretches. Velocity given next to each curve as V/sk_b. $(A_1 - A_0)/kT = 10$; $s^2/\sigma^2 = 4.6$; $Ks^2/kT = 1.84$.

stants. Since the relationship between P and d is, in general, not linear, the ratio, $\Delta P/d$, which has the units of stiffness, can be called a chord stiffness. However, since $\Delta P/d$ is not a true stiffness, as it is a function of t_d, we refer to $\Delta P/d$ as the apparent chord stiffness, S_c.

In Case I, an expression for S_c may be derived from Eq. 12 simply by setting $d = Vt_d$. This yields

$$S_c^d = (n_b K/k_b t_d)\,[1 - \exp(-k_b t_d)], \tag{13}$$

where the superscript d in S_c^d implies that the chord stiffness (and t_d) are measured over the stretch distance d.

Fig. 6 A shows the calculated S_c vs. $(-\log t_d)$ relationship for Case I. Figs. 6 B and C show computed S_c vs. $(-\log t_d)$ relationships for Case II and Case III. Curves for two different chord distances, $d/s = 0.2$ and $d/s = 1.0$ are displayed. It is clear that the muscle stiffness, $n_b K$, can be obtained simply from the height of the S_c-log t_d relationship. If the horizontal position of the S_c-log t_d response were insensitive to the attachment rate constant, f, then the S_c-log t_d response could be used to also extract information about the detachment rate constants. We now proceed to show that this is indeed the case.

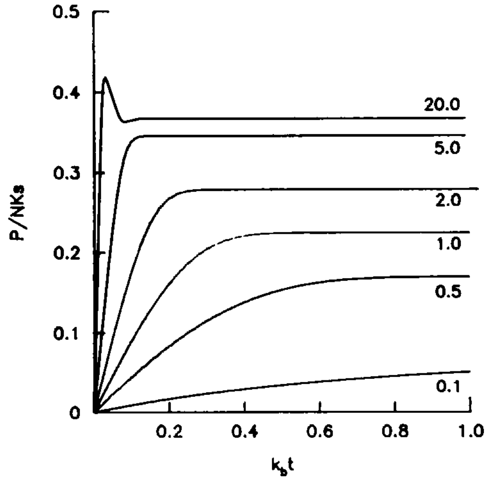

FIGURE 5 Case III. Force vs. time for $df'/dx > 0$, constant velocity stretches, velocity given as V/sk_b. $(A_1 - A_0)/kT = 10$; $s^2/\sigma^2 = 10^{-4}$; $Ks^2/kT = 18.4$.

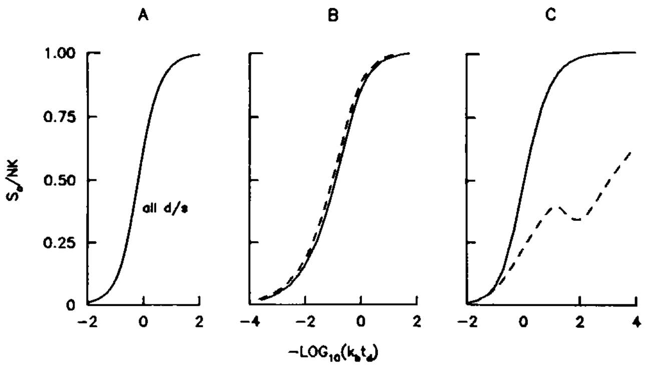

FIGURE 6 Relationship between apparent chord stiffness, S_c, and duration of stretch, t_d, for constant velocity stretches. *Ordinate*, S_c/NK; *abscissa*, $-\log_{10} k_b t_d = \log_{10} (\{k_b t_d\}^{-1})$. Displayed this way, velocity of stretch increases to the right. (A) $df'/dx = 0$. (B) $df'/dx < 0$. (C) $df'dx > 0$. Parameters as in Figs. 3–5; solid curves, $d/s = 0.2$; dashed curves, $d/s = 1.0$.

Relative Importance of f and f'. An important result that emerges from this study is that, in the equilibrium system, the cross-bridge detachment rate constant, $f'(x)$, is the major determinant of the mechanical response to stretch, with the attachment rate constant, f, serving mainly to scale the response by influencing the number of attached cross-bridges. This is easily seen for Case I, $df'/dx = 0$, as the analytic solutions, Eqs. 12 and 13, are independent of f. The sole influence of f is through the parameter, n_b; that is, as stated above, the influence of f is simply to scale the mechanical response by influencing the number of attached cross-bridges. For the more complicated cases, Cases II and III, a similar result concerning the relative importance of f and f' may be derived as follows.

As stated earlier, there are four nondimensional parameters that determine the behavior of our equilibrium cross-bridge model. Changes in f affect only the parameter $(A_1 - A_0)/kT$. One way of comparing the relative importance of f and f' is to change $(A_1 - A_0)/kT$ first by changing f and then by changing f' (in the latter case, the parameter, V/sk_b, also changes). For example, starting from the case $(A_1 - A_0)/kT = 10$, $s^2/\sigma^2 = 10^{-4}$ and $Ks^2/kT = 18.4$ (Case III), changing $(A_1 - A_0)/kT$ to -2 by decreasing f shifts the S_c-log t_d relationship no more than 0.5 decades to the left (towards longer durations). On the other hand, changing $(A_1 - A_0)/kT$ to -2 by increasing f' results in a shift to the left of ~5.7 decades! A similar situation exists in Case II. Starting from $(A_1 - A_0)/kT = 10$, $s^2/\sigma^2 = 36.8$, $Ks^2/kT = 18.4$, changing $(A_1 - A_0)/kT$ to -2 by decreasing f shifts the S_c-log t_d relationship 0.5 decades to the right. Changing $(A_1 - A_0)/kT$ to -2 by increasing f' shifts the S_c-log t_d relationship 4.7 decades to the left. Clearly then, for the type of cross-bridge model studied here, the mechanical response is much more sensitive to f' than to f. This facilitates learning about the detachment rate constants from the mechanical response but means, of course, that relatively little can be learned about the attachment rate constants.

Extracting Cross-Bridge Parameters from the S_c-log t_d Relationship. From the results of the previous sections, it is clear that the horizontal position of the S_c-log t_d relationship provides information about the rate constants for cross-bridge detachment. In Case I, for example, the value of the detachment rate constant may be extracted from the S_c-log t_d relationship since the approximate midpoint of the S_c-log t_d relationship (actually the point $S_c/n_bK = 1 - 1/e = 0.63$) occurs at the value of $t_d = 1/k_b$. In Cases II and III, the horizontal position of the S_c-log t_d relationship also gives the cross-bridge detachment rate constants. It is also seen that for a small amplitude stretch (e.g., $d/s = 0.2$), the S_c-log t_d curve in Case II lies just to the left of $t_d = 1/k_b$, showing the detachment of some of the cross-bridges with rate constant k_b and others at somewhat slower rates because of the decrease in detachment rate constant with increasing x. In Case III, the S_c-log t_d curve lies just to the right of $t_d = 1/k_b$, reflecting the increase in f' with the increasing strain seen during stretch. We see then, that the horizontal position of the S_c-log t_d relationship does provide information about the rate constants with which the cross-bridges detach during stretch. What we shall also see, however, is that it does not provide good information about the x values at which the cross-bridges detach.

It would seem from the arguments above that the width of the S_c-log t_d relationship should provide information about the range of the detachment rate constants and, in fact, it does. However, this information is complicated since the width of the S_c-log t_d relationship does not mirror the range of detachment rate constants over the distance of stretch. For example, when one stretches a muscle 5 nm at constant velocity, many of the cross-bridges that detach may detach well before they are strained 5 nm. This is especially true in Case II, $df'/dx < 0$, where the rate constants for detachment at $x = s/2$ are faster than those at $x = s/2 + 5$ nm; the majority of cross-bridges that detach with a 5-nm stretch in this instance will do so over a much narrower region near $x = s/2$. The width of the S_c-log t_d relationship will, in all cases, reflect the range of detachment rate constants for the region across which the cross-bridges actually detach. The fact that this region is narrow in Case II explains why the S_c-log t_d relationship for $d = s$ in Fig. 6 *B* is about the same as for $d = 0.2s$ and not nearly as broad as would be expected from the range of detachment rate constants over the interval from $x = s/2$ to $x = s/2 + s$. This problem is less acute in Case III, as can be seen by comparing Figs. 6 *B* and *C*. We conclude that, in general, whereas the position and width of the S_c-log t_d curve give reliable information about the range of rate constants with which the cross-bridges detach during a stretch, they do not provide, in a simple way, information about the strains (x values) of the detaching bridges.

Force Decay Following on Instantaneous Stretch

Another technique for studying cross-bridge parameters involves the instantaneous stretch of a muscle fiber and measurement of the subsequent rate of force decay. From the arguments that have preceded, it is clear that the rate of force decay will directly reflect the cross-bridge detachment rate constants. There will again be some uncertainty about the range of x values of the cross-bridges that detach, since, a priori, the initial distribution is unknown; but unlike the case with constant-velocity stretches, one can be certain that when one instantaneously applies a stretch of δ nm to a muscle, each cross-bridge is strained an additional amount δ.

Fig. 7 shows the rate of tension decay following two instantaneous steps, one of size $\delta/s = 0.2$ and the other $\delta/s = 1$, for the same system parameters as in the example shown in Fig. 6 *B*. We see that, unlike the situation for Fig. 6 *B*, here the decay after the $d/s = 1.0$ step shows a wider range of cross-bridge detachment rate constants than seen after a $d/s = 0.2$ stretch. With instantaneous stretches, unlike the situation with constant-velocity stretches, the cross-bridges always see a wider range of detachment rate

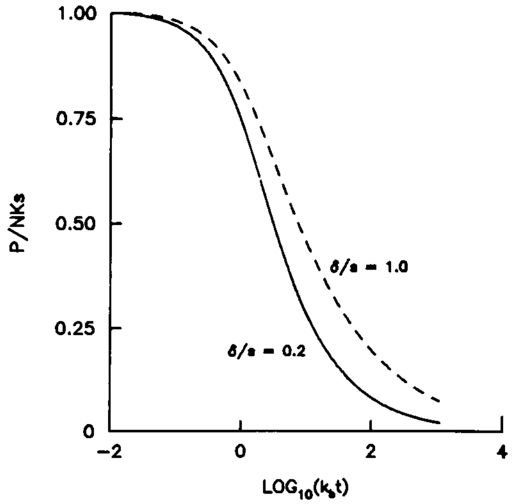

FIGURE 7 Force decay following an instantaneous step stretch. Solid curve, step size, $\delta/s = 0.2$; dashed curve, $\delta/s = 1.0$; *ordinate*, P/NKs; *abscissa*, k_bt. Parameter values as in Figs. 4 and 6*B*.

constants following a larger step, and the decay will reflect this (except, of course, in Case I).

DISCUSSION

Recently we reported that in the presence of ATP but absence of Ca^{2+}, large numbers of cross-bridges are attached in a relaxed muscle fiber at low ionic strength (Brenner et al., 1982; Schoenberg et al., 1984). Since the apparent stiffness in those fibers depended upon velocity of stretch, we suggested that the cross-bridges attached to actin in the relaxed fiber were not statically attached, but rather, were attached with a rapid equilibrium between attached and detached states. The model developed here was developed to provide insight into the behavior of such systems. The model chosen, one briefly discussed by T. L. Hill (1974), is more realistic than many previous cross-bridge models in that it allows a cross-bridge to interact with more than a single actin, as seems likely.

One of the major insights of our modeling is that the behavior of the equilibrium cross-bridge system can be deduced by considering the transitions that occur at regions near the crossover points of the free energy diagrams. Thus, looking at Fig. 1 *B*, in our model the response is dominated by the detachment rate constants at points *c* and *d*. We also see that for our model, in most instances, the equilibrium that exists is effectively one between attached states. A cross-bridge attached to one actin will detach and then rapidly reattach to a second actin. The unattached state effectively serves only as a transient intermediate.

One alternative model to the one we have developed is the simpler single site model (Fig. 1 *A*). While probably not as realistic as the one we have developed, it does largely behave similarly. The equilibrium in a single site model (Fig. 1 *A*) is clearly between the attached and unattached states. The stretch response in this instance, as seen from Fig. 1 *A*, is dominated by the detachment rate constant at point *b* and the attachment rate constant at point *a*. Although the attachment rate constant comes into the mechanical response for the single site model more explicitly than in the model we have been discussing, this does not always lead to big differences in behavior. This is because, from free energy considerations (Eq. 8), the detachment and attachment rate constants at point *a* must be equal. Thus, for slow velocities of stretch, the responses of the single site model are very similar to those of the multiple site model (Schoenberg, M., unpublished computations). One clear difference between the single site model and the model we have been discussing is that, with the single site model, at high velocities of stretch the number of attached cross-bridges tends to decrease. This is because, at high velocities of stretch, attached cross-bridges will detach, but unattached cross-bridges will not have time to attach during the time they are pulled across the interval between points *a* and *b*. This decrease in the number of attached cross-bridges at high velocities of stretch is quite different from the behavior of the multiple site model.[2]

It is also of interest to compare the behavior of our model with that of the next more sophisticated one. We have ignored the effect of azimuthal rotation of adjacent actin sites. When this is taken into account (Fig. 1 *C*), adjacent actin sites are no longer equivalent. Most, if not all, of the conclusions reached with regard to the equivalent site model should apply to that model as well. However, one additional interesting feature of that model is that whereas the transitions in the equivalent site model occur near $x = \pm s/2$ (points *c*, *d*), for a nonequivalent site model with free energy diagrams as drawn in Fig. 1 *C*, the transitions occur both near $x = 0$ (points *e*, *f*) and $x = s$ (points *g*, *h*). For a given x dependence of f', this should tend to give a much broader range of detachment rate constants since cross-bridges will detach, not just over a range of rate constants near a single x value, but over two ranges of rate constants near two different x values.

Advantages of the Techniques

We have shown how cross-bridge parameters may be extracted from the response to constant-velocity stretches or to instantaneous stretches. While neither provides much information regarding the cross-bridge attachment rate constants, both yield the detachment rate constants seen during the maneuver although not the precise x value (strain) of the detaching cross-bridges. This means that deduction of the detachment rate constant as a precise function of strain (x) is model dependent. In this regard, information provided from the response to a step stretch is easier to interpret since each cross-bridge is strained a known additional amount. On the other hand, using that technique requires being able to rapidly stretch a muscle on a time scale that does not allow significant cross-bridge detachment and also being able to hold it completely isometric following the stretch (Schoenberg and Eisenberg, 1985).

An advantage of using an S_c-log t_d plot to extract the cross-bridge detachment rate constants relates to the fact that the S_c-log t_d relationship is relatively insensitive to the shape of the stretch waveform.[3] This makes it particularly valuable for the study of cross-bridge systems where the detachment rate constants are so fast that on a time scale

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[2] In the multiple site model, in Cases I and II, the number of attached cross-bridges remains more or less constant during stretch. In Case III, there is sometimes a decrease in the number of attached cross-bridges during the phase where $dP/dt < 0$, although, depending upon the parameter values, dP/dt can be negative without net detachment of cross-bridges.

[3] It may be shown mathematically for Case I, that, relative to the constant-velocity stretch $\lambda = At$, the S_c-log t_d is shifted only 0.18 units to the left for the stretch $\lambda = At^2$ and 0.3 units to the left for the stretch $\lambda = At^3$. By computation the shift was found to be the same magnitude in Case II, and only ~50% larger for Case III.

the precise shape of the rapid stretch waveform cannot be controlled. An experimental difficulty in using apparent stiffness concerns the influence of series compliance. The effect this has on the $S_c - \log t_d$ relationship can be understood by considering the case, $df'/dx = 0$. In this case the behavior of the cross-bridge system is identical to that of a spring and dashpot (viscous element) is series, with the former having a spring constant of $n_b K$ and the latter a viscous coefficient of $n_b K/k_b$. An additional compliance in series, therefore, will cause an apparent decrease in both stiffness and detachment rate constant. Experimentally it is often possible to minimize or eliminate stray compliance and, in that case, measurement of apparent stiffness provides useful information regarding stiffness and detachment rate constants.

Applying the Techniques

Whereas the stimulus for developing the model discussed here was provided by our experiments on the rapid equilibrium of relaxed cross-bridges with ATP bound, the results would seem to have much broader applicability. Even in active muscle where a much more complex model would be necessary to totally describe the system, phenomena similar to those modeled here have been reported (i.e, compare Fig. 2 of Flitney and Hirst, 1978, or Fig. 6 of Griffiths et al., 1980). Recently we have shown that our equilibrium cross-bridge model also seems to qualitatively explain the behavior of cross-bridges with pyrophosphate ($MgPP_i$) or adenyl-5'-yl-imidodiphosphate (MgAMP-PNP) bound to the active sight. One of the most salient features of those results (Schoenberg et al., 1983, 1984) was the large shift to the left (towards longer durations) of the S_c-log t_d relationship when MgATP was replaced with $MgPP_i$. The results presented here suggest that the rather large effect of replacing MgATP with $MgPP_i$ must be due to a rather substantial change in the detachment rate constants with nucleotide or analogue. It is interesting that this interpetation agrees with biochemical studies that suggest also that the main effect of nucleotide at the active site is upon the detachment step (Marston, 1982).

A second salient feature of our stiffness measurements with $MgPP_i$ or MgAMP-PNP was the large span of the S_c-log t_d relationship. From the results presented here, this would seem to imply a rather broad range of detachment rate constants in the fiber. Using reasonable values of cross-bridge stiffness and assuming $df'/dx > 0$ (Schoenberg and Eisenberg, 1985), the model developed here does not, in fact, produce such a wide S_c-log t_d curve as measured experimentally. However, as discussed earlier, the more realistic nonequivalent actin site model may correct this deficiency.

One particularly interesting question is whether our results on the behavior of relaxed cross-bridges with ATP bound (Brenner et al., 1982) are related to the experiments of Ford et al. (1977) on the apparent viscosity of resting intact frog fibers. It must be kept in mind that the viscosity of resting muscle is not likely to be due to the hydrodynamic viscous drag of two sets of myofilaments sliding past one another. All computations of the magnitude of such an effect, even when one considers myosin projections, suggest that it can account for <1% of the observed apparent viscosity (Schoenberg, M., unpublished calculations; Huxley, A. F., unpublished calculations, reported in Ford et al., 1977). On the other hand, as Figs. 3–5 show, the equilibrium model considered here gives viscouslike behavior and it is of interest to compute the possible magnitude of this behavior.

Ford et al. (1977) report that stretching a muscle ~30 muscle lengths per second produces an apparent viscous force of $\sim 10^{+5}$ dyn/cm^2. Assuming that the stiffness of a single cross-bridge is in the neighborhood of 1 dyn/cm (Huxley and Simmons, 1971; Eisenberg et al., 1980), that there are ~300 cross-bridge heads per half-sarcomere per myosin filament, and that there are 7×10^{10} myosin filaments/cm^2, then, even assuming that the detachment rate constant for the relaxed cross-bridge is possibly as high as 10^5 s^{-1} (Brenner, B., J. Chalovich, L. Greene, E. Eisenberg, and M. Schoenberg, manuscript submitted for publication), Eq. 12 would predict an apparent viscous force as high as seen in the Ford et al. (1977) experiments if as few as 15% of the cross-bridge heads were attached in the relaxed muscle. Since 10^5 s^{-1} is probably a high estimate of the detachment rate constants, it seems quite possible that the apparent viscosity of relaxed intact frog fibers may be due to the same rapid equilibrium phenomena we have described for relaxed skinned rabbit psoas fibers.

In summary, our rather simplified cross-bridge model seems capable of describing and providing considerable insight into a number of interesting cross-bridge phenomena. Future work will involve considering precisely how close the agreement between theory and experiment is, and also, perhaps, extending the model towards consideration of more complex factors, such as nonequivalent actin sites, or the possibility of multiple cross-bridge heads competing for actin sites.

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