THE INFLUENCE OF DOUBLY ATTACHED CROSSBRIDGES ON THE MECHANICAL BEHAVIOR OF SKELETAL MUSCLE FIBERS UNDER EQUILIBRIUM CONDITIONS

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ABSTRACT A simple model of a double-headed crossbridge is introduced to explain the retardation of force decay after an imposed stretch in skeletal muscle fibers under equilibrium conditions. The critical assumption in the model is that once one of the heads of a crossbridge is attached to one of the available actin sites, the attachment of the second head will be restricted to a level of strain determined by the attachment of the first head. The crossbridge structure, namely the connection of both heads of a crossbridge to the same tail region, is assumed to impose this constraint on the spatial configurations of crossbridge heads. The unique feature of the model is the prediction that, in the presence of a ligand (PPi, ADP, AMP-PNP) and absence of Ca^{2+}, the halftime of force decay is many times larger than the inverse rate of detachment of a crossbridge head measured in solution. This prediction is in agreement with measured values of half-times of force decay in fibers under similar conditions (Schoenberg, M., and E. Eisenberg. 1985. *Biophys. J.* 48:863–871). It is predicted that a crossbridge head is more likely to re-attach to its previously strained position than remain unattached while the other head is attached, leading to the slow decay of force. Our computations also show that the apparent cooperativity in crossbridge binding observed in experiments (Brenner, B., L. C. Yu, L. E. Greene, E. Eisenberg, and M. Schoenberg. 1986. *Biophys. J.* 50:1101–1108) can be partially accounted by the double-headed crossbridge attachment. Our model predictions fit the aforementioned data best when the crossbridge stiffness does not change significantly with the dissociation of one of the two attached heads. This observation suggests that crossbridge stiffness is determined either by the extensibility (flexibility) of the double helical tail region or its junction to the thick filament backbone.

INTRODUCTION

The purpose of the present study is to investigate analytically the influence of doubly attached crossbridges on the mechanical response of skeletal muscle fibers under equilibrium conditions. By double attachment we mean the attachment of the two heads of a crossbridge to different actin sites possibly on different thin filaments. Specifically we seek to explain, in terms of double-headed attachment, the data on (*a*) the retardation of force decay after stretch in the presence of a ligand such as AMP-PNP and in the absence of Ca^{2+}; and (*b*) the apparent cooperativity in crossbridge binding observed in measurements of fiber stiffness under varying ionic strength conditions (1).

Recent experimental data indicate that the rate of force decay after stretch is considerably slower than the corresponding subfragment-1 (S-1) detachment rate measured in solution. For example, in rigor solution the detachment rate is on the order of 0.01 s^{-1} (Marston [2]). If the rate of force decay in rigor was comparable to S-1 dissociation rate, 50% of the force would decay in ~100 s. Recent experiments, however, indicate that the force generated by stretch can persist for hours (Kuhn [3], Schoenberg and Eisenberg [4]). Similarly, in the presence of AMP-PNP the apparent S-1 detachment rate at 5°C and ionic strength I = 125 mM is ~15 s^{-1} (Conrad and Goody [5]). Under similar conditions the halftime of force decay was ~3.5 s. Previous studies by White (6), Kuhn (3), and Clark and Treager (7) had indicated that fiber force could persist exceedingly long times under similar situations. In order to explain this behavior Kuhn (3) suggested that crossbridges bound in clusters become more stable than individual crossbridges bound separately, leading to slowing of force decay after stretch.

Recently Schoenberg (8) proposed a new model to explain crossbridge dynamics under equilibrium conditions. Following Hill (9) and Wood and Mann (10), Schoenberg allowed a modeled single-headed crossbridge to attach to one of the multiple actin sites available for attachment. This model predicts that the thick and thin filaments slide past one another. This produces a rise in tension due to straining of attached crossbridges. After stretch, detached crossbridges attach to actin sites which

would produce less strain and therefore the force induced by the stretch decays to zero. The model also predicts that the rate constants of force decay must be comparable to the detachment rate of S-1 from actin in solution and therefore does not explain the retardation of force decay.

More recently Tozeren and Schoenberg (11) studied the effects of crossbridge clustering and head–head competition on force decay, as possible causes for the existence of a wide range of time constants observed in the time course of force decay after step stretch in a fiber under equilibrium conditions. Cooperative behavior between the crossbridge heads was not allowed. In their double-headed crossbridge model, once a crossbridge head is attached the other head was allowed to attach at one of two distinct levels of strain. With these assumptions the rate of force decay after stretch was found to be approximately equal to the S-1 detachment rate measured in solution. The reason for this is that in the model a crossbridge head is allowed to swing, that is, to detach and subsequently attach to a less straining position while the other head remains attached. Their computational results also indicated that, when crossbridge heads bind adjacently in clusters, so that competition for available actin sites increases, the force decay becomes slower. This effect, however, is not large enough to explain the spectrum of the time constants observed in experiments.

Another experimental fact that cannot be explained by the models described in references 8 and 11 is the apparent cooperativity in crossbridge binding observed by Brenner et al. (1). These authors showed that the stiffness of skinned rabbit fibers decreases with increasing ionic strength due to the weakening of the affinity of crossbridges for actin. In the absence of calcium, normalized stiffness decreased from 75% to 25% over an ionic strength range where ionic strength in solution weakens the binding constant of S-1 to actin by less than a factor of three. The authors have noted that a 10-fold change in binding constant would be required to produce the threefold decrease in stiffness in a noncooperative system.

In the present study we show that the complex mechanical behavior of skeletal muscle fibers under equilibrium conditions, briefly outlined above, can at least be partially accounted for by doubly attached crossbridges. The critical assumption of the present study which is different from previous models is that an unattached crossbridge head can attach only at a level of strain determined by the previous attachment of the other head. This assumption is reasonable because both heads are connected to the same coiled helical tail region (S-2). This structural arrangement introduces a spatial constraint on the degree of movement of crossbridge heads relative to each other. The impetus for this model came from the recent study of Marston (2) that investigates the kinetics of formation and dissociation of myosin from actin. Marston measured the dissociation rate of heavy meromyosin from actin in rigor and found it to be at least 20 times slower than that for S-1. Marston attributed this result to the low probability of both heads dissociating at the same time.

METHODS

In this study, a crossbridge is modeled as a macromolecule (M) which contains two equivalent sites for binding to actin (A) and two additional sites for binding to a nucleotide (N). Fig. 1 *a* shows the equilibrium crossbridge states assumed to characterize crossbridge kinetics in rigor. The present kinetic scheme is consistent with the observation that the kinetics of attachment of S-1 to actin in solution can be modeled satisfactorily by assuming that the association reaction involves a single step (2). Fig. 1 *b* shows the crossbridge states in the absence of Ca^{2+} and in the presence of a nucleotide at a concentration higher than required for saturation. In order to study the mechanical response of fibers in the presence of nucleotides at lower concentrations, all possible permutations of A and N, including the ones not shown in Fig. 1 *b*, must be taken into account.

Recent biochemical and structural studies indicate that both heads of the double-headed myosin moiety crosslink thin filaments in skeletal muscle myofibrils (12, 13). Whether or not the two heads are identical, the capability of a crossbridge to interact with two different actin sites, possibly along different thin filaments, must be taken into consideration. Fig. 2 *a* shows a schematic diagram illustrating the interaction of a double-headed crossbridge with actin sites on different thick filaments. We identify sets of actin sites available for attachment of a particular crossbridge with an integer i; the actin sites within the same set may belong to different thin filaments (Fig. 2 *a*). The distance between the crossbridge and any actin site in the same set is assumed to be the same. The index $i = 0$ refers to an actin site closest to the unstrained crossbridge, and i increases from left to right as shown in Fig. 2. The parameter x denotes, as usual, the distance from the crossbridge to the nearest set of actin sites and s denotes the repeat distance of actin sites ($-s/2 < x < s/2$). The distance from the crossbridge to an ith actin site is then given by $(x + is)$ where i can be a negative integer (11). It is assumed that both heads of a crossbridge can be in an attached configuration at the same time only if they are attached to different actin sites within the same set. The quantity $(x + is)$ is then considered to be the measure of strain of an attached crossbridge.

We shall present below a mathematical formulation of double-headed crossbridge attachment that is valid only for the case shown in Fig. 2 *a*, namely, a crossbridge interacting with two thin filaments. Our computations, however, indicate that a model of a double-headed crossbridge interacting with actin sites along the same thin filament would show

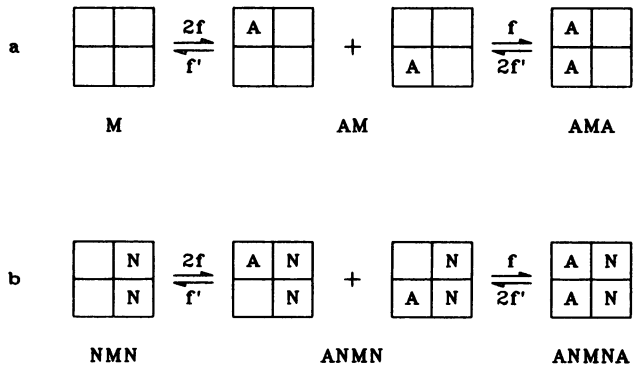

FIGURE 1 Modeled double-headed crossbridge cycle in rigor (*a*), and the corresponding cycle in the absence of Ca^{2+} but presence of a ligand (ADP, PPi, ANP-PNP) with concentration above saturation (*b*). M is a double-headed crossbridge with one actin (A) and one ligand (N) site per head. The crossbridge cycle shown in *b* is an approximation for high ligand concentration, because a ligand is assumed to be bound to each head at all times.

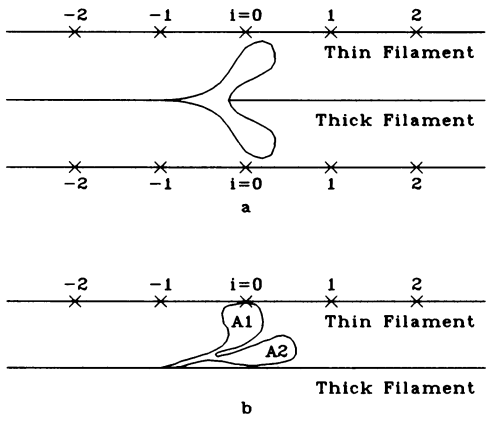

FIGURE 2 The schematic diagrams of the modeled crossbridge interacting with actin sites along different thin filaments (*a*) and along the same thin filament (*b*).

similar qualitative behavior if it is assumed that once the crossbridge head A1, shown in Fig. 2 *b*, is attached to the actin site *i*, then head A2 can only be attached to the site $i + 1$. The possibility of A1 attaching to site *i* and A2 attaching to site $i - 1$ must be excluded. A crossbridge head, is therefore not allowed to swing, that is, to detach and subsequently attach to a less straining position while the other head remains attached.

Present structural evidence is not sufficient to specify the origins of crossbridge stiffness (14). For this reason we consider two distinct models to describe the elastic properties of a double-headed crossbridge. In model 1 (stiffness invariant) the stiffness of a doubly attached crossbridge is assumed equal to the stiffness of a crossbridge with one head attached and the other head unattached. This model would be reasonable if the attachment of one head to an actin site stretches the double helical S-2 (or its connection to the thick filament backbone) to such an extent that the attachment of the second head does not cause a significant change in crossbridge stiffness.

In model 2 (stiffness doubling) the stiffness of a doubly attached crossbridge is assumed to be equal to twice the crossbridge stiffness with one head attached. This model would be reasonable if crossbridge stiffness were determined by the interaction at the actin-myosin binding site.

Model 1 (Stiffness Invariant)

Let M denote the fraction of unattached crossbridges shown in Fig. 1. Let U_i denote the fraction of crossbridges in which only one head is attached to actin site i. Let V_i denote the fraction of crossbridges in which both heads are attached with equal strain. The kinetic equations governing these fractions can be written as

$$DU_i/Dt = 2f(x + is)M + 2f^1V_i - (f_0 + f^1)U_i \quad (1)$$

$$DV_i/Dt = f_0U_i - 2f^1V_i, \quad (2)$$

where D/Dt is the total time derivative, $f(x)$ and f^1 denote, respectively, the rates of attachment and detachment of a single crossbridge head, and $f_0 = f(x)$ at $x = 0$. The transition from U_i to V_i occurs with the rate constant f_0 because of the assumption that the attachment of the second head does not induce additional strain to the crossbridge. The rate parameter f^1 is assumed to be independent of x as in Tozeren and Schoenberg (11), because we would like to separate the effect on the time course of force decay after stretch, of double-headed attachment of crossbridges from that of a strain dependent rate of detachment. The rate parameters f_0 and f^1 can be estimated from the results of biochemical studies conducted in solution. With f^1 constant, the detailed balance requires that (9):

$$f(x) = f_0\,[\exp(-Kx^2/2\,kT)], \quad (3)$$

where K(dyn/cm) is the crossbridge stiffness and $kT = 3.8\ 10^{-14}$ dyn-cm at 6°C. The sum of probabilities appearing in Eq. 1 must add up to one:

$$M + \sum_{-j}^{j}(U_i + V_i) = 1, \quad (4)$$

where symbol j denotes the maximum value of index i and Σ denotes summation. The average force per crossbridge is then computed from the following equation:

$$P = \left[\sum_{-j}^{j}\int_{-s/2}^{s/2} K(x + is)(U_i + V_i)\,dx\right]\Big/s. \quad (5)$$

Eqs. 1–5 can be used to compute modeled force decay after a step stretch. The normalized fiber stiffness E(dyn/cm) can be expressed in this case as:

$$E = \left[\sum_{-j}^{j}\int_{-s/2}^{s/2} (U_i[x] + V_i[x])\,dx\right]\Big/s. \quad (6)$$

Note that $E = 1$ for a fiber in rigor solution, because all crossbridges are doubly attached in this case.

Model 2 (Stiffness Doubling)

In this model we also assume that both crossbridge heads can be attached only at the same level of strain, say $(x + is)$, but further assume that the stiffness of a doubly attached crossbridge is equal to twice the stiffness of a crossbridge attached with one head. The definitions of the parameters f_0, f^1, $f(x)$ and K are the same as in model 1 (stiffness invariant). The rate equations in this case become:

$$DU_i/Dt = 2f(x + is)M + 2f^1V_i - [f(x + is) + f^1]U_i \quad (7)$$

$$DV_i/Dt = f(x + is)U_i - 2f^1V_i. \quad (8)$$

The equations for P and E become, respectively,

$$P = \left(\sum_{-j}^{j}\int_{-s/2}^{s/2} K(x + is)[U_i(x) + 2V_i(x)]\,dx\right)\Big/s. \quad (9)$$

$$E = \left(\sum_{-j}^{j}\int_{-s/2}^{s/2} (U_i[x] + 2V_i[x])\,dx\right)\Big/2s. \quad (10)$$

Note that doubly attached crossbridge states are multiplied by two in Eqs. 9 and 10 because of the assumed doubling of the crossbridge stiffness when both heads are attached.

The equations presented above can be used to compute the time decay of fiber force after stretch, and also stiffness as a function of the strength of binding (f_0/f^1). Computational results and comparison with experimental data will be presented in the next section.

DISCUSSION

The parameters appearing in the kinetic equations presented in the previous section are s, K, f_0, and f^1. As in Tozeren and Schoenberg (11), the repeat distance between available actin sites is chosen as $s = 5.5$ nm, and stiffness coefficient K is considered to be on the order of 1 dyn/cm; in fact we choose $K = 1$ dyn/cm. The values of these parameters are kept constant in the computations

described below. In these computations we have assumed that a crossbridge head can attach to one of the actin sites available at locations $x - 2s$, $x - s$, x, $x + s$, and $x + 2s$. Increasing or decreasing the number of distinct actin sites by two changed the computed halftimes of force decay by <5%.

We first consider the dependence of normalized fiber stiffness E as a function of the strength of binding (f_0/f^1) as shown in Fig. 3. Note that E depends on the ratio (f_0/f^1) but is not sensitive to the actual values of these rate constants f_0 and f^1 because E is determined by the equilibrium crossbridge distribution. The curves I and II shown in Fig. 3 correspond to model 1 (stiffness invariant) and model 2 (stiffness doubling), respectively. Our computations show that if a single-headed crossbridge model with exactly the same parameter values were used, the resultant $E = E(f_0/f^1)$ curve would be very similar to the curve II. We would like to compare these theoretically obtained curves with the experiments (Fig. 3) of Brenner et al. (1) in which E was measured as a function of ionic strength (I) in the presence of 4 mM MgPPi at 6°C. The binding strength (f_0/f^1) used in our computations was estimated by multiplying the value of binding constant $K3$ obtained in solution by the effective actin concentration $[A]$. The definition and the representative values of $K3$ are given in Conrad and Goody (5) and Biosca et al. (13). Recent experimental studies indicate that $[A]$ is in the millimolar range (1, 14, 16). Biosca et al. (15) estimated that $K3$ = 600 M^{-1} for I = 170 mM and $K3$ = 300 M^{-1} for I = 240 mM. We use the following relation by Conrad and Goody (5) to estimate $K3$ for other valus of I:

$$\log 10(K3) = b + m\,(I)^{1/2}, \qquad (11)$$

where $b = 4.34$ and $m = -0.12$. The discrete points shown in Fig. 3 are the values estimated with this procedure from the experimental data of Brenner et al. (1) for $[A]$ = 0.6 mM. These experimental points indicate that normalized stiffness E increases from 25 to 75% with a slightly < threefold increase in binding strength (f_0/f^1). A single-headed crossbridge model would require 10-fold increase in (f_0/f^1) in order to achieve the same change in stiffness. Fig. 3 also shows that (f_0/f^1) must increase sixfold for model 1 (stiffness invariant) and 10-fold for model 2 in order to increase E from 25% to 75%. Hence part of the observed apparent cooperativity in crossbridge binding might be due to the double-headed attachment as described by model 1. According to this model the stiffness of a doubly attached crossbridge does not decrease significantly with the detachment of one of the heads, making the system appear to be cooperative. Other possible causes of cooperative binding of crossbridge heads are discussed in Brenner et al. (1), Green and Eisenberg (16), and Hill et al. (17). The effective actin concentration $[A]$ = 0.6 mM used in the present study is very close to the value given by Cooke (14) ($[A]$ = 0.5 mM) but less than the value predicted by Brenner et al. (1) ($[A]$ = 1.5 mM). After the procedure described in reference 1 we have estimated $[A]$ as the ratio of $K3$ to (f_0/f^1) corresponding to 50% crossbridge head attachment both in solution and in the intact fiber.

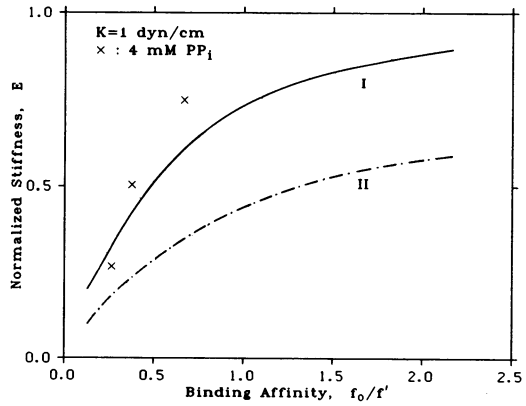

FIGURE 3 The normalized stiffness (E) as a function of strength of binding (f_0/f^1). E = 1 corresponds to the fiber stiffness in rigor at 6°C and at I = 40 mM. The curves I and II are the predictions of model 1 and model 2, respectively. The predictions of models discussed in Schoenberg (8) and Tozeren and Schoenberg are very similar to curve II. The discrete points shown in the figure are taken from Brenner et al. (1) (see text).

Next we compare the halftimes of force decay after step stretch predicted by the models with experimental data of Schoenberg and Eisenberg (4). The dimensionless halftime $f^1(t)½$ is defined as the actual halftime $(t)½$ multiplied by the rate of detachment f^1. Fig. 4 shows $f^1(t)½$ as a function of (f_0/f^1) computed by using K = 1 dyn/cm and s = 5.5 nm for a step size of $s/2$. Similar step sizes were used in the experiments. The curves I, II, and III in Fig. 4 correspond, respectively, to the predictions of model 1

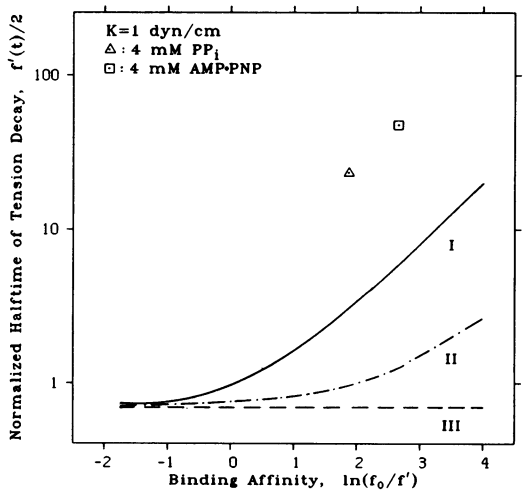

FIGURE 4 The dimensionless halftime of force decay $[f^1(t)½]$ corresponding to 50% force decay as a function of ln (f/f^1). The curves I, II, and III correspond, respectively, to model 1 (stiffness invariant), model 2 (stiffness-doubling), and Schoenberg's model (8). The predictions of models discussed in Tozeren and Schoenberg (11) are almost identical to curve III. The discrete points shown in the figure are taken from reference 4 (see text).

(stiffness invariant) and model 2 (stiffness doubling) of the present study, and to the model discussed in Schoenberg (8) (single-headed crossbridge model). The models described in Tozeren and Schoenberg (11) predict force decay curves very similar to curve III. The two discrete points shown in Fig. 3 are the experimental estimates of halftimes in the presence of 4 mM PPi (△) and 4 mM of AMP-PNP (□) at 6°C and at moderate ionic strength. Schoenberg and Eisenberg (4) found that $(t)\frac{1}{2} = 0.24$ s in the presence of 4 mM PPi, and $(t)\frac{1}{2} = 3.5$ s in the presence of 4 mM AMP-PNP. We multiplied these values with $f^1 = 100\ s^{-1}$ for PPi, and $f^1 = 15\ s^{-1}$ for AMP-PNP in order to obtain values for the dimensionless half time $f^1(t)\frac{1}{2}$. These f^1 values are comparable to the rates of dissociation of S-1 from actin observed in solution (2, 4). The values of (f_0/f^1) corresponding to these experiments can be obtained by mulitplying $K3$ with $[A] = 0.6$ mM as discussed above. Conrad and Goody (5) evaluated $K3$ as $2.8\ 10^4\ M^{-1}$ for AMP-PNP at 6°C, but did not measure the binding constant in the presence of PPi. Under similar conditions but at 24°C Biosca et al. (15) found that $K3 = 5 \cdot 10^4\ M^{-1}$ for AMP-PNP and $K3 = 3.4\ 10^4\ M^{-1}$ for PPi, respectively. We have estimated $K3$ for PPi which is valid at 6°C by assuming proportionality: $K3 = (2.8)(3.4/5.0)\ 10^4\ M^{-1}$. The discrete points in Fig. 4, obtained from experimental data with the procedure described above, represent upper bounds because Schoenberg and Eisenberg (4) point out that there is no evidence that the analog effect saturates as the ligand concentration is increased from 1 to 4 mM. We would also like to note that the computed force decay curves are nearly exponential (Fig. 5) and therefore do not follow closely the multiexponential curves measured by Schoenberg and Eisenberg (4) for ligand concentrations from 1 to 4 mM. Nonetheless Fig. 4 indicates that the rate of force decay predicted by the double-headed crossbridge model is many times slower than the rate of detachment of a crossbridge head, as observed experimentally.

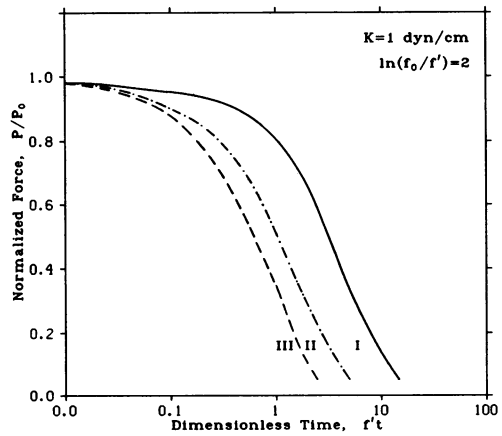

FIGURE 5 The time course of force decay after stretch. $P = P(t)$ denotes average crossbridge force at time t after the imposed stretch. The curves I, II, and III are defined as in Fig. 4.

Recently Pate and Brokaw (18) proposed a crossbridge model which assumes two distinct biochemical states for attached crossbridges to represent muscle behavior in rigor. These authors have explained the slow decay of force in rigor by making the detachment rates extremely small for certain values of crossbridge strain. More recently a model for the interaction of crossbridges with ligands that compete with ATP was introduced by Pate and Cooke (19). This model predicts that states with bound ligand are shifted axially so that they occur earlier in the power stroke than the rigor state. The authors conclude that ligands cause only small changes in the crossbridge configuration and do not detach from actin during their powerstrokes. We have not considered in detail the influence of this proposed axial shift in crossbridge configuration because our specific aim was to focus on the influence of double attachment. The cooperativity between crossbridges in a cluster has been suggested by Kuhn (4) as another possible cause of slow decay of force. Recently we have shown that crossbridge clustering has little effect on the time course of force decay in the absence of cooperativity between crossbridge heads (11), but this paper shows that cooperative-like behavior can be produced without clustering by double attachment.

The main conclusions of this study are: (*a*) The predictions of model 1 (stiffness invariant) are closest to the experimental data shown in Figs. 3 and 4. This model correctly predicts that the rate of force decay is many times slower than the rate of detachment f^1 measured in solution. The stiffness vs. strength of binding curve predicted by this model shows an apparent cooperativity in crossbridge binding as observed in experiments. (*b*) Model 2 (stiffness doubling) also predicts a retardation of force decay after stretch under equilibrium conditions. However, this effect is less pronounced in model 2 than in model 1 (Fig. 4). Model 2's prediction of the influence of binding strength on normalized stiffness is similar to a single-headed crossbridge model with identical parameter values. The single-headed crossbridge model discussed by Schoenberg (8) predicts that the rate of force decay is equal to the rate of detachment f^1 and therefore does not explain the retardation of force decay observed in experiments. No significant improvement is achieved in model predictions if the doubly-attached crossbridge model allows one of the heads to swing, namely to detach and subsequently attach to a less straining position while the other head is still attached, as assumed in Tozeren and Schoenberg (11). (*c*) Our numerical computations show that the prediction of retardation of force decay by models 1 and 2 of the present study is not dependent on the assumption of multiple actin sites but is the result of the double attachment hypothesis.

The results of the present study indicate the possibility that a double-headed crossbridge model may account for the complex behavior of fibers observed under equilibrium conditions (1, 4). For a more critical comparison with existing experimental data, crossbridge states correspond-

ing to all possible permutations of A, N, and M must be considered. This type of modeling effort may yield important information on the deformability characteristics of crossbridges, complementing the results of x-ray diffraction studies (20).

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