THE EFFECT OF CROSS-BRIDGE CLUSTERING AND HEAD-HEAD COMPETITION ON THE MECHANICAL RESPONSE OF SKELETAL MUSCLE UNDER EQUILIBRIUM CONDITIONS

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ABSTRACT The effect of cross-bridge clustering and head-head competition on the mechanical response of skeletal muscle under equilibrium conditions is considered. For this purpose, the recent multiple site equilibrium cross-bridge model of Schoenberg (Schoenberg, M., 1985, Biophys. J., 48:467–475) is extended in accordance with the formalism of T. L. Hill (1974, Prog. Biophys. Mol. Biol.,28:267–340) to consider the case where groups of independent cross-bridge heads compete with each other for binding to multiple actin sites. Cooperative behavior between heads is not allowed. Computations indicate that for the double-headed cross-bridge with two independent equivalent heads, the time course of force decay after a stretch is similar to that for the single-headed cross-bridge; that is, the rate constant for force decay is approximately equal to the cross-bridge head detachment rate constant. The results also show that the force decay after a stretch becomes slower than the detachment rate constant of a single head when cross-bridge heads bind adjacently in clusters so that competition between heads for binding to the available actin sites increases. However, if one assumes that the detachment rate constant of an unstrained head in a fiber is comparable to that of an S1 molecule in solution, this effect is not large enough to explain why some of the rate constants for force decay after a stretch in rigor, or in the presence of ATP analogues such as adenyl-5'-yl imidodiphosphate, appear to be significantly slower than the detachment rate constant of S1 from actin in solution.

INTRODUCTION

According to the sliding filament theory, muscle shortening during contraction occurs by the relative sliding of the thick and thin filaments that comprise the basic contractile unit, the sarcomere (1, 2, 3). Myosin cross-bridges, composed of the subfragment-1 head (S1) and subfragment-2 (S2) portions of the myosin molecule, protrude from the thick filaments and, during contraction, attach and detach cyclically to actin sites along the thin filaments. Each myosin cross-bridge is composed of two S1 heads although the significance of this is not yet known. As the cross-bridge goes through its various configurations during the hydrolysis of ATP, nucleotide bound to a site on the myosin head also goes through a number of biochemical states.

Clearly, the behavior of the cross-bridge during active force generation is quite complex. In recent years, attempts have been made to study the behavior of cross-bridges by looking, not at contraction, but at the simpler case of equilibrium behavior (see, for example, references 4–6). Quite recently, Schoenberg (7) proposed a model to explain the dynamics of myosin cross-bridge binding to actin sites under equilibrium conditions. That model

allowed a cross-bridge to attach to more than a single actin site, but ignored the possibility that neighboring cross-bridge heads might compete for the various sites. A major conclusion of that model was that when an equilibrium population of cross-bridges is rapidly stretched so that a force is generated, the decay of that force should occur with rate constants very close to the cross-bridge detachment rate constants. If one assumes that the detachment rate constant of an unstrained cross-bridge head in a muscle is similar to that of an S1 molecule in solution (2) and if one assumes that distortion (strain) of the cross-bridge head only increases the detachment rate constant (6), then the rate constants for force decay in muscle fibers after stretch should be comparable to, or faster than, the detachment rate constant of S1 from actin in solution. The fact is that in some instances the rate constants for force decay in a fiber (6, 8) are, if anything, slower than the detachment rate constants in solution (9, 10) under comparable conditions.

Since the force decay in the model of Schoenberg is due to cross-bridge heads detaching from actin sites on which they are strained and then quickly attaching to actin sites in positions of lesser strain, one possible explanation for the

above discrepancy is that, in the actual fiber, cross-bridge heads that detach from sites on which they are strained may not readily be able to find suitable sites in position of lesser strain because adjacent or nearby heads are already attached to those sites. In other words, when several cross-bridge heads compete for binding to the same actin sites, the rate of tension decay after a strain (stretch) may be retarded since the competing cross-bridges may not be able to as readily redistribute back to the equilibrium, zero force, distribution.

Here, the multiple-site model developed by Schoenberg (7) is extended to include the effects of multiple cross-bridge heads competing for the same actin sites. In section 1, the competition between two cross-bridge heads having the same origin is considered. This is equivalent to considering the case of a double-headed cross-bridge with two independent heads. To rule out the possibility that the behavior of two competing cross-bridges having the same origin is due specifically to the assumption of identical cross-bridge origin, in the second section, we consider two competing cross-bridge heads separated by a distance equal to the distance between adjacent actin sites. In the last two sections, we consider three and finally four or more competing cross-bridge heads. Although the rapidly increasing complexity of the equations necessitates making additional simplifying assumptions as the number of cross-bridges considered increases, the trend of the results is clear. Whereas an increase in the number of competing cross-bridge heads does slow the rate of force decay after stretch, the effect is not nearly so large as to offer an explanation for the apparent discrepancy between force relaxation in fibers and S1 detachment rate constants in solution. Possible alternative explanations are discussed.

METHODS AND RESULTS

1. Two Competing Heads with Identical Origins: the Double-headed Cross-bridge with Independent Heads.

In this section we consider the behavior of a model identical to that of Schoenberg (7) except that the single-headed cross-bridge of that model is here replaced by a double-headed cross-bridge. We assume that the two heads of the cross-bridge are equivalent and do not interact with each other except in that they cannot both be attached to the same actin at the same time. This assumption in effect allows the double-headed cross-bridge to be treated as two equivalent single-headed cross-bridges protruding from the same location on the thick filament.

We assume that all the actin sites are equivalent. Since the pitch of the actin helix causes azimuthal twisting of the actin sites, sites separated by a distance s should not be equivalent. However, since it is not known how great an effect this has on cross-bridge binding, at present, we make the simplifying assumption that all actin sites are equivalent. We identify the actin sites available for attachment of

a particular cross-bridge with the integer index i, $i = 0$ referring to the actin closest to the unstrained modeled cross-bridge head as shown in Fig. 1 a. The index i increases from left to right so that the distance from the cross-bridge to the actin site i is equal to $x + is$, where x, as usual, denotes the distance from the cross-bridge to the nearest actin site $(-s/2 < x < s/2)$. The variable $n_{iu}(x, t)$ denotes the fraction of cross-bridges at a given x, at time t, with head A attached to actin site i and head B unattached. Similarly $n_{i(i+1)}$ denotes the fraction of cross-bridges in which head A is attached to the actin site i, and head B is attached to the site $i + 1$. It is assumed that when two heads are attached they can only be attached to adjacent actin sites. The fraction of cross-bridges with both heads unattached is denoted by n_{uu}.

We let $f = f(x)$ and $f' = f'(x)$ denote, respectively, the rates of attachment and detachment of a hypothetical, isolated single head to and from an actin site. If we assume that the attachment rate constant of a single head shows a Gaussian distribution with respect to x, namely,

$$f = f_o \exp(-x^2/2\sigma^2) \qquad (1)$$

the detachment rate constant f' is specified by the relationship

$$f'(x) = f(x) \exp[(A_o + 1/2Kx^2 - A_u)/kT], \qquad (2)$$

where f_o has the units of inverse time (s^{-1}), σ is in the length units, A_u is the basic free energy of an unattached single head, K is the stiffness of a cross-bridge head, $A_o + 1/2Kx^2$ is the basic free energy of an attached single head, and kT at 25°C is 4.1×10^{-14} dyn-cm (see reference 2 for details).
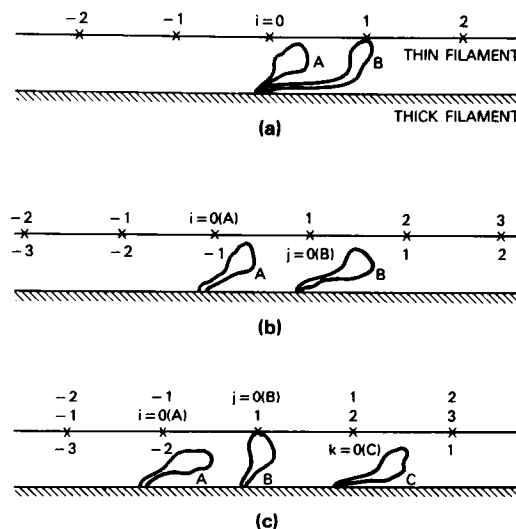

FIGURE 1 Schematic diagrams illustrating the modeled cross-bridge configurations and corresponding notation for the case of (a) two heads of identical origin competing for the same actin sites, (b) two heads separated by a distance s ($s = 5.5$ nm), competing for the same actin sites, (c) three heads, each separated by a distance s, competing for the same actin sites.

The kinetic equations governing the various double-headed cross-bridge states can be written in terms of these attachment and detachment rates since, as stated previously, we treat the double-headed cross-bridge with the heads totally independent as equivalent to two single-headed cross-bridges protruding from the same x-location. For example, it can be shown that

$$Dn_{iu}/Dt = f(x + is) n_{uu} + f'(x + (i + 1)s)n_{i(i+1)}$$
$$+ f'(x + (i - 1)s)n_{i(i-1)} - n_{iu} (f'(x + is)$$
$$+ f(x + (i + 1)s) + f(x + (i - 1)s), \quad (3)$$

where t is time and D/Dt is the total time derivative. The first term on the right-hand side represents the increase in the state n_{iu} due to attachment of head A of the doubly detached species, n_{uu}; the second and third terms represent the increase in n_{iu} due to detachment of head B of the two doubly attached species, $n_{i(i+1)}$ and $n_{i(i-1)}$; the last three terms represent the decrease of n_{iu} due to detachment of head A of this species or attachment of head B of this species to actins $i+1$ or $i-1$ to form a doubly attached species. Since, in fact, the heads A and B are considered indistinguishable, we define $n_i = n_{iu} + n_{ui}$ and $N_i = n_{i(i+1)} + n_{(i+1)i}$ where the variables n_i and N_i denote, respectively, the fractions of singly and doubly attached cross-bridges at a given x. The kinetic equations then become

$$DN_i/Dt = f(x + is) \cdot n_{i+1} + f(x + (i + 1)s) \cdot n_i$$
$$- N_i \cdot [f'(x + is) + f'(x + (i + 1)s)] \quad (4)$$

$$Dn_i/Dt = 2f(x + is) \cdot n_{uu} + f'(x + (i + 1)s) \cdot N_i$$
$$+ f'(x + (i - 1)s) \cdot N_{i-1}$$

$$- n_i \cdot [f'(x + is) + f(x + (i + 1)s) + f(x + (i - 1)s)]. \quad (5)$$

Since the sum of the probabilities for all states of the cross-bridge must add to one, an additional equation is

$$\sum_{i=-\alpha}^{\alpha} n_i + \sum_{i=-\alpha}^{\alpha-1} N_i + n_{uu} = 1, \quad (6)$$

where the symbol α denotes the maximum value of index i and $\sum_{i=-\alpha}^{\alpha}$ denotes summation over index i from $-\alpha$ to α. The maximum number of actin sites to which each cross-bridge can bind is therefore equal to $2\alpha + 1$. α is chosen large enough (typically 1 or 2) to insure that n_i and N_i are nearly zero for $|i| > \alpha$. The equations are easily solved by Gear's method (11) and we used a prepared subroutine, DGEAR, available on the National Institutes of Health computer (Model DEC-10; Digital Equipment Corp., Maynard, MA). Once n_i and N_i are determined, the average force per cross-bridge, $P(t)$, can be computed from the equation:

$$P = K\left[\int_{-s/2}^{s/2} \sum_i n_i(x)[x + is]dx \right.$$
$$\left. + \int_{-s/2}^{s/2} \sum_i [N_i(x)[(x + is) + (x + (i + 1)s)]]dx\right]/s, \quad (7)$$

where Σ denotes the summation over all actin sites considered.

We are interested in comparing the response of this double-headed model with the behavior of the simpler single-headed cross-bridge model (7). One way to do this is to look at the simplest case of the response to a step stretch when the detachment rate constant f' is independent of x. This occurs, from Eqs. 1 and 2, when $\sigma^2 = kT/K$. In reference 7 it was shown that for single-headed cross-bridge binding to equivalent multiple actin sites, when $df'/dx=0$, the rate of force decay after a step stretch is equal simply to f'. The aim in these computations is to see how differently the two-headed model behaves. The initial conditions required for solution of this problem are obtained by starting with the equilibrium distribution and shifting it to the right by an amount equal to the step size. The force, $P(t)$, which is zero before the step stretch, is maximal just after the application of the step stretch. The force then decays to zero, as the cross-bridges detach and reattach, redistributing back to their equilibrium distribution. The results of our computations are summarized in Table I. For the case $df'/dx = 0$, the solution is uniquely determined once the free energy of binding $(A_u - A_o)$ and the cross-bridge stiffness K are set. Table I shows the influence of these parameters on the peak force, $P(0)$, the fractions n and N of the cross-bridges attached with one or two heads respectively, and also the ratio of the fitted rate constant for force decay, β, to the uniform detachment rate constant f'. Parameters n and N were calculated according to

$$n = \left[\int_{-s/2}^{s/2} \left(\sum_i n_i(x)\right) dx\right]/s$$

$$N = \left[\int_{-s/2}^{s/2} \left(\sum_i N_i(x)\right) dx\right]/s.$$

The present computations show, as expected, that the sum, $N+n$, increases with increasing strength of binding $(A_u -$

TABLE I
INFLUENCE OF FREE ENERGY OF BINDING $(A_u - A_o)$
AND CROSS-BRIDGE STIFFNESS K ON THE
FORCE DECAY FOLLOWING STRETCH FOR THE
DOUBLE-HEADED CROSS-BRIDGE MODEL
PRESENTED IN SECTION 1

$(A_u - A_o)/kT$	K	$P(0)$	β/f'	N	n
	dyn/cm	$10^{-7} dyn$			
5	0.25	1.40	0.80	0.985	0.015
5	2.5	10.12	1.35	0.45	0.54
0	0.25	0.78	0.91	0.26	0.58
0	2.5	3.71	1.02	0.02	0.50

The numbers in the table were calculated for a step size of $\Delta = s/2$. $P(0)$ is the force immediately after stretch. n and N are, respectively, the fractions of cross-bridges with either one or two heads attached. f' is the uniform detachment rate constant. The rate constant β was determined from the best least-squares fit of the force decay, $P(t)$, to the equation, $P(t) = P(0)\exp(-\beta t)$.

A_0), saturating, of necessity, at the value $n+N = 1$. The relative values of n and N are influenced strongly by the cross-bridge stiffness K. As indicated in Table I, the fraction of doubly attached cross-bridges, N, decreases with increasing cross-bridge stiffness K. The reason for this is that after the first head attaches, more often than not to the most favorable actin site, the ability of the second head to attach to the adjacent, usually less favorable actin site, decreases with increasing cross-bridge stiffness. This happens because for large values of cross-bridge stiffness, the additional energy available from binding the second head is more than offset by the increased strain energy expended in the second head reaching an available actin site. This results in an increase in singly bound species.

Fig. 2 shows the equilibrium distribution of cross-bridge states for the case of $K = 2.5$ dyn/cm. The figure indicates that cross-bridges at $x = 0$ tend to be singly attached, whereas cross-bridges at $x = \pm s/2$ are mostly doubly attached. As argued above, when cross-bridge stiffness K is decreased, more cross-bridges, both at $x = 0$ and $x = \pm s/2$, become doubly attached.

As stated earlier, for each of the four cases shown in Table I, the force decay predicted by the simpler model studied by Schoenberg (7), is a single exponential with a rate constant f'. As Table I shows, this is approximately
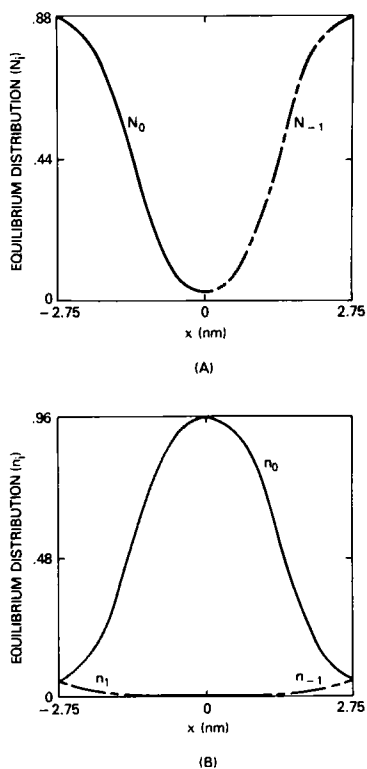
but not exactly true for the double-headed model considered here. Fig. 3 shows the time course of force decay for the two cases, $K = 0.25$ dyn/cm and $K = 2.5$ dyn/cm when $(A_u - A_0)/kT = 5$. We see that the force decay is slower than that of the simple model for $K = 0.25$ dyn/cm and faster for the larger value of K. With $K = 2.5$ dyn/cm, as Fig. 2 shows, approximately half of the cross-bridge population is singly attached. The reason for the greater than f' rate of decay in this case is that the imposed step stretch brings the unattached heads into favorable positions for attachment at negative values of x and the subsequent rapid attachment with negative strain quickly reduces the overall force exerted by the cross-bridges.

The reason for the slower than f' rate of force decay when $K = 0.25$ dyn/cm is presumably because the heads interfere (compete) with each other while trying to attach to the actins where they would be attached with the lowest free energy. If this is occurring, one would expect the rate constants for force decay to get even slower as more and more heads compete for the same actin sites. In sections 3 and 4 we explore this possibility. In section 2 we explore whether the assumption that both heads have the same origin is critical for the results we have obtained here.

2. Two Separated Heads Competing for the Same Actin Sites

In the previous section we considered the competition between two cross-bridge heads having the same origin. With heads of the same origin, it is impossible for both heads of the modeled double-headed cross-bridge to be simultaneously attached with zero strain. To be certain that this inability of the heads to attach simultaneously with zero strain is not critical in determining the behavior of two competing heads, in this section we consider a case in which two heads can both attach simultaneously (to different actins) with zero strain. This is the case where the two cross-bridge heads are separated by a distance equal to the separation between two actins. We let n_{ij} identify the

(A)

(B)

FIGURE 2 Equilibrium cross-bridge distributions for the multiple actin site model with double-headed cross-bridges (section 1). $A_u - A_o = 5\,kT$, $K = 2.5$ dyn/cm. The fraction N_i corresponds to the configuration in which one head is attached to actin site i, the other to actin site $i+1$ (Fig. 2 A). The fraction n_i corresponds to the configuration in which one of the heads is attached to actin site i, the other one unattached (Fig. 2 B).
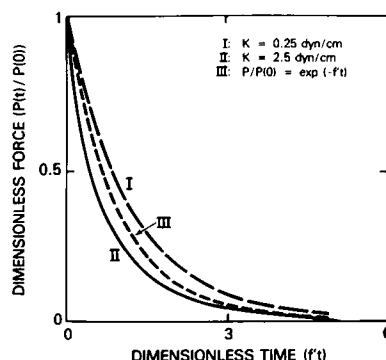

FIGURE 3 Force decay after a stretch step of size $\Delta = s/2$ for the multiple site model with double-headed cross-bridges (section 1). $A_u - A_o = 5\,kT$; $K = 0.25$ dyn/cm for curve I, $K = 2.5$ dyn/cm for curve II. Curve III shows the force decay for single-headed cross-bridges as in the simpler model of Schoenberg (7).

fraction of cross-bridges between x and $x+ dx$ for which the two heads, A and B, are attached to actin sites i and j, respectively, as shown in Fig. 1 b. The choice of the indices i and j identifying the actin sites for A and B are such that $i = j + 1$ for the same actin site. For example, in this notation, n_{00} denotes the fraction in which both of the competing heads are each attached to the closest actin site. The fraction of cross-bridges in the state in which both heads are unattached is denoted by n_{uu}.

As before, it is assumed that both cross-bridges heads cannot be attached to the same actin site at the same instant, which leads to the constraint: $n_{ij} = 0$ if $i = j + 1$. In this section there is no restriction that the heads must bind to adjacent actins. Note that $n_{ij} \neq n_{ji}$ in the present notation. The rate equations governing the fractions n_{iu}, n_{uj}, and n_{ij} can be written as

$$Dn_{iu}/Dt = f(x + is)n_{uu} + \sum_{\substack{j=-\alpha \\ j\neq(i-1)}}^{\alpha} f'(x + js)n_{ij}$$

$$- n_{iu}[f'(x + is) + \sum_{\substack{j=-\alpha \\ j\neq(i-1)}}^{\alpha} f(x + js)] \quad (8)$$

$$Dn_{uj}/Dt = f(x + js)n_{uu} + \sum_{\substack{i=-\alpha \\ i\neq(j-1)}}^{\alpha} f'(x + is)n_{ij}$$

$$- n_{uj}[f'(x + js) + \sum_{\substack{i=-\alpha \\ i\neq(j-1)}}^{\alpha} f(x + is)] \quad (9)$$

$$Dn_{ij}/Dt = f(x + is)n_{uj} + f(x + js)n_{iu}$$

$$- n_{ij}[f'(x + is) + f'(x + js)] \quad (10)$$

where again $2\alpha + 1$ is the number of actin sites to which each head can attach. As before, the sum of the probabilities of all the states must add up to 1, so that

$$\sum_{j=-\alpha}^{\alpha} (n_{ju} + n_{uj}) + \left(\sum_{i=-\alpha}^{\alpha} \sum_{j=-\alpha}^{\alpha} n_{ij}\right) + n_{uu} = 1. \quad (11)$$

The average force per pair of heads, $P(t)$, is

$$P = K \int_{-s/2}^{s/2} \left[\sum_{i=-\alpha}^{\alpha} (n_{iu} + n_{ui}) \cdot (x + is)\right.$$

$$\left. + \sum_{i=-\alpha}^{\alpha} \sum_{j=-\alpha}^{\alpha} n_{ij} \cdot ((x + is) + (x + js))\right]dx/s. \quad (12)$$

We again carry out the computations for the simplest case of uniform detachment rate constant, that is, the case $df'/dx = 0$. In the computations we consider only the case where the free energy of binding is large (i.e., $A_u - A_o = 5$ kT) so that the fraction of attached cross-bridge heads is always nearly 1. Any slowing in the rate of force decay due to inability of heads to find available actin sites should be more pronounced in this case in comparison with the case where fewer cross-bridges are attached.

The results of the computations are shown in Fig. 4 and in Table II. In Table II, the peak average force per pair of heads, $P(0)$, the rate constant, β, of force decay after the

$(A_u - A_o)/kT$	K	P(0)	β/f'	N	n
	dyn/cm	10^{-7} dyn			
5	0.25	1.35	0.930	0.99	0.01
5	2.5	13.4	0.80	0.935	0.064

Step size $\Delta = s/2$. $P(0)$ is the force immediately after stretch. n is the fraction of groups of two heads having only one head attached and N is the fraction with both heads attached. f' is the uniform detachment rate-constant. The rate-constant β was determined from the best least-squares fit of the force decay, $P(t)$, to the equation, $P(t) = P(0)exp(-\beta t)$.

step stretch, and the fractions n and N are shown for two different values of cross-bridge stiffness K. For the model in this section, n and N correspond, respectively, to the fractions of groups having either one or two of the heads bound, a group consisting of two competing heads that are separated by a distance s

$$n = \sum_{i=-\alpha}^{\alpha} (n_{iu} + n_{ui}); \quad N = \sum_{i=-\alpha}^{\alpha} \sum_{j=-\alpha}^{\alpha} n_{ij}. \quad (13)$$

Fig. 4 and Table II show that the behavior of this model is very similar to that derived in section 1. Here too, the fraction N, the fraction with both heads attached, decreases with increasing cross-bridge stiffness. The effect is much smaller than seen for the model of section 1 because here, where both heads are able to attach simultaneously with zero strain, there is a greater tendency for double-headed binding at all values of stiffness.

Also as in the previous section, the rate constant for force decay, β, is approximately equal to f', with the rate of force decay slightly slower when the cross-bridge stiffness is larger. The reason for the retardation in force decay when K is larger is because, as the equilibrium distributions in Figs. 5 and 6 show, when cross-bridge stiffness is
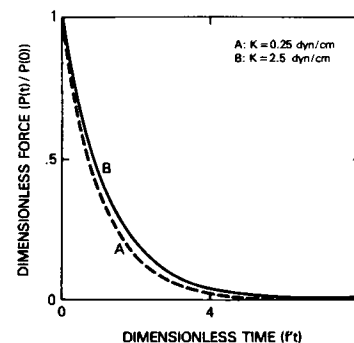

FIGURE 4 Force decay after a stretch step of size $\Delta = s/2$ for the multiple site model with two competing cross-bridge heads separated by a distance s (section 2). $A_u - A_o = 5$ kT. Dashed curve, $K = 0.25$ dyn/cm. Solid curve, $K = 2.5$ dyn/cm. The two curves shown in the fig. correspond to the two cases in Table II.
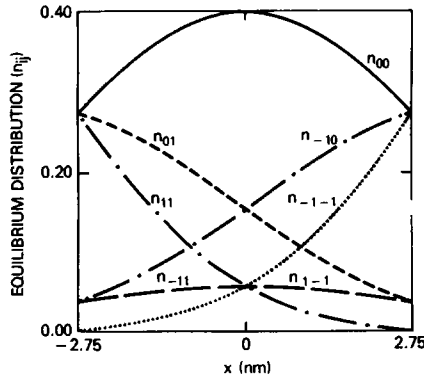
FIGURE 5 Equilibrium cross-bridge distributions for the multiple site model with two competing cross-bridge heads separated by a distance s (section 2) with $A_u - A_o = 5\,kT$, $K = 0.25$ dyn/cm.

larger, cross-bridge heads spend more of their time attached to the nearest actin sites. With larger K therefore, each head attaches to fewer actin sites, and this increases the probability that a head that detaches will likely reattach back to the actin site from which it came, without there being any drop in force. This occurs because the site the head would like to attach to is more often occupied by the competing head. Therefore, each head, on average, sustains force longer because of the presence of the competing head, and this retards the decay of force after stretch. To see how much this effect increases with an increase in the number of interacting cross-bridge heads, in the next section we consider the case where three heads separated by a distance s compete. Our method of analysis will have to be somewhat different because the number of possible attachment configurations dramatically increases in this case.

3. Three Cross-bridge Heads Competing for the Same Actin Sites

In this section we consider the behavior of three single-headed cross-bridges, each separated by a distance s, competing for binding to a series of actin sites. The symbol n_{ijk} denotes the fraction in which heads A, B, and C are
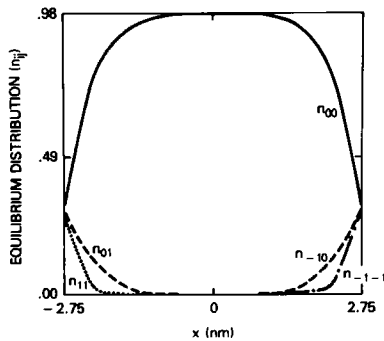

FIGUREL 6 Equilibrium cross-bridge distributions for the multiple site model with two competing cross-bridge heads separated by a distance s (section 2) with $A_u - A_o = 5\,kT$, $K = 2.5$ dyn/cm.

attached to actin sites i, j and k respectively (Fig. 1 c). As in the previous section $i = j + 1 = k + 2$ for the same actin site. If each head can attach to $2\alpha + 1$ actin sites, a single head will have a maximum of $2\alpha + 2$ configurations and the number of possible states for a group of three heads will be equal to $(2\alpha + 2)^3$ minus the number of impossible configurations due to the constraint $i \neq j+1 \neq k+2$. If each cross-bridge has only three actin sites available for attachment, the number of possible states is equal to 44. Because of the large number of states, we need to simplify further, and we do so by considering solely the case of strong binding so that the only significant states are those for which all three heads are attached to actin sites. The force decay after an external perturbation can then be thought of as occurring by transitions between states of this type, with each transition consisting of the detachment and subsequent reattachment of one of the heads. Our computations for two competing cross-bridge heads indicate that for the range of K values considered here, the number of actin sites available for each cross-bridge can be restricted to three without loss of accuracy. In this case, there will be 14 states in which all the heads are attached, and 17 intermediate states in which one of the three heads is detached. The intermediate states can be eliminated from the equations by assuming that they reach equilibrium faster than the others (see reference 3, for details).

The resulting rate equations for the remaining states are as follows:

$$\frac{Dn_{111}}{Dt} = f'\{\beta_1(n_{011} + n_{-111}) + \gamma_1 n_{1-11} - (2 - \beta_1 - \gamma_1)n_{111}\}$$

$$\frac{Dn_{011}}{Dt} = f'\{\beta_0(n_{111} + n_{-111}) + \gamma_1 n_{01-1}$$
$$+ \alpha_1 n_{001} - (3 - \alpha_1 - \gamma_1 - \beta_0)n_{011}\}$$

$$\frac{Dn_{1-11}}{Dt} = f'\{\alpha_1 n_{1-10} + \gamma_1 n_{-1-11}$$
$$+ \gamma_{-1}n_{111} - (3 - \alpha_1 - \gamma_1 - \gamma_{-1})n_{1-11}\}$$

$$\frac{Dn_{1-10}}{Dt} = f'\{\alpha_0 n_{1-11} + \gamma_1 n_{-1-10} - (2 - \alpha_0 - \gamma_1)n_{1-10}\}$$

$$\frac{Dn_{001}}{Dt} = f'\{\alpha_0(n_{011} + n_{-101}) + \alpha_1 n_{000} - (3 - 2\alpha_0 - \alpha_1)n_{001}\}$$

$$\frac{Dn_{-101}}{Dt} = f'\{\phi_{-1}n_{001} + \beta_0(n_{-1-11} + n_{-111}) + \alpha_1 n_{-100}$$
$$- (3 - \phi_{-1} - \beta_0 - \alpha_1)n_{-101}\}$$

$$\frac{Dn_{000}}{Dt} = f'\{\alpha_0 n_{001} + \phi_0 n_{-100} - (2 - \alpha_0 - \phi_0)n_{000}\}$$

$$\frac{Dn_{-100}}{Dt} = f'\{\phi_{-1}n_{000} + \alpha_0 n_{-101}$$
$$+ \phi_0 n_{-1-10} - (3 - \phi_{-1} - \alpha_0 - \phi_0)n_{-100}\}$$

$$\frac{Dn_{-11-1}}{Dt} = f'\{\gamma_1 n_{-1-1-1} + \gamma_{-1}n_{-111} + \phi_{-1}n_{01-1}$$
$$- (3 - \gamma_1 - \gamma_{-1} - \phi_{-1})n_{-11-1}\}$$

$$\frac{Dn_{-1-1-1}}{Dt} = f'\{\beta_{-1}(n_{-1-11} + n_{-1-10})$$

$$+ \gamma_{-1}n_{-11-1} - (2 - \gamma_{-1} - \beta_{-1})n_{-1-1-1}\}$$

$$\frac{Dn_{-1-11}}{Dt} = f'\{\beta_{-1}(n_{-101} + n_{-111})$$

$$+ \beta_1(n_{-1-10} + n_{-1-1-1}) + \gamma_{-1}n_{1-11}$$

$$- (3 - \gamma_{-1} - \beta_{-1} - \beta_1)n_{-1-11}\}$$

$$\frac{Dn_{-1-10}}{Dt} = f'\{\gamma_{-1}n_{1-10} + \phi_{-1}n_{-100} + \beta_0(n_{-1-11} + n_{-1-1-1})$$

$$- (3 - \gamma_{-1} - \phi_{-1} - \beta_0)n_{-1-10}\}$$

$$\frac{Dn_{-111}}{Dt} = f'\{\beta_{-1}(n_{111} + n_{011})$$

$$+ \beta_1(n_{-101} + n_{-1-11}) + \gamma_1 n_{-11-1}$$

$$- (3 - \beta_1 - \beta_{-1} - \gamma_1)n_{-111}\}. \tag{14}$$

The remaining fraction, n_{01-1}, is determined by the condition that the sum of probabilities of all cross-bridge states must be equal to 1. The constants α_0, β_0, α_{-1}, ... etc. appearing in Eq. 14 are defined as follows:

$$\alpha_i = f(x + is)/[f(x) + f(x + s)]$$
$$\beta_i = f(x + is)/[f(x) + f(x + s) + f(x - s)]$$
$$\gamma_i = f(x + is)/[f(x + s) + f(x - s)]$$
$$\phi_i = f(x + is)/[f(x) + f(x - s)], \tag{15}$$

where $i = +1, 0, -1$. Eqs. 14 and 15 indicate that the rates of change of the various fractions are governed by a multitude of rate constants, all smaller than or equal to the uniform detachment rate constant f'.

Using Eqs. 14, we have determined the time course of force decay after a step stretch. The initial condition was taken as $n_{111} = 1$ with all other $n_{ijk} = 0$. This distribution is not the same as that that would be reached by displacing the equilibrium distribution by an amount equal to the magnitude of the step. However, since only the case of strong binding is considered, all the heads will be attached and, at equilibrium, they will tend to spend most of their time on the nearest actin sites. Therefore, the assumption that $n_{000} = 1$ before stretch and $n_{111} = 1$ after stretch appears to be a reasonable approximation. Fig. 7 shows the dimensionless force $P(t)/P(0)$, as a function of time, for $K = 0.25$ dyn/cm and also an exponential decay curve with a rate constant of f'. The rate constant of force decay in Fig. 7 is $\beta = 0.73 f'$. The rate of force decay was nearly identical for K' values in the range $0.2 < K < 0.6$ dyn/cm. Larger values of K were not studied in order to be consistent with the assumption that nearly all the heads in the modeled fiber are attached at equilibrium. Clearly the results here are again similar to those of the previous two sections and not very different from those for the simpler model of reference 7.

The computations of this and previous sections indicate that the retardation of force decay does increase with the
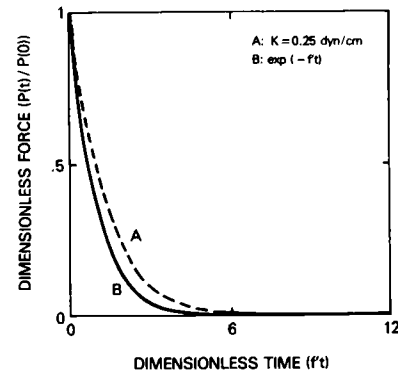

FIGURE 7   Force decay after a stretch step of size $\Delta = s/2$ for the multiple site model with three cross-bridge heads competing for the same actin sites (section 3). Curve A (dashed), $A_u - A_o = 5\ kT$, $K = 0.25$ dyn/cm. Curve B (solid), exponential decay with a rate constant f'. The best exponential fit to curve A gives a rate constant $\beta = 0.73 f'$.

number of cross-bridge heads competing for the same actin sites. However, this effect appears to be small in the case of clusters of two or three cross-bridge heads. In the next section, a simple statistical model is introduced to derive an upper bound for the influence of cross-bridge clustering when the number of clustered bridges is larger.

4. Many Competing Cross-bridge Heads

In this section, we develop a simple statistical model to study how the number of heads in a cluster influences the rate of force decay. We assume that each cluster is composed of cross-bridge heads separated by a distance s. We assume further that heads attach only to the closest actin site and remain attached, after a mechanical perturbation, in a strained configuration until the actin site that is now closest becomes available for attachment. With these assumptions, all heads will be bound to actin sites $i = +1$ immediately after a step stretch of size s. This results in a force, which, in time, will decay to zero as heads detach and reattach to actin sites where they are less strained. The force decay corresponding to this statistical model will be slower initially than the equilibrium models considered in sections 3 and 4, because (a), force reduction by detachment from $i = 1$ sites before an $i = 0$ site becomes available is not allowed; and (b), binding to $i = -1$ sites is not allowed. Because of these restrictions, the model considered in this section is not consistent with the thermodynamic formalism of Hill (2), but it is nonetheless useful in that it provides an upper bound for the slowing of force decay after a stretch due to clustering. For a cluster containing four heads, the equations governing transitions between states can be shown to be

$$\frac{dn_{1111}}{dt} = -f'n_{1111}; \quad \frac{dn_{0111}}{dt} = f'(n_{1111} - n_{0111})$$

$$\frac{dn_{0011}}{dt} = f'(n_{0111} - n_{0011}); \quad \frac{dn_{0001}}{dt} = f'(n_{0011} - n_{0001}), \tag{16}$$

where $n_{0000} = 1 - (n_{1111} + n_{0111} + n_{0011} + n_{0001})$.

If we consider only the case of heads attached at $x = 0$, the force $P(t)$ can be written as

$$P(t) = Ks[4n_{1111} + 3n_{0111} + 2n_{0011} + n_{0001}]. \qquad (17)$$

It is straightforward to derive analytical expressions for $P(t)$ corresponding to any number of heads in a cluster. For example, it can be shown that

$P(t) = P(0) \exp(-f't) \quad \text{for } \lambda = 1$

$P(t) = P(0) \exp(-f't) (1 + (f't)/2) \quad \text{for } \lambda = 2$

$P(t) = P(0) \exp(-f't) (1 + 2(f't)/3 + (f't)^2/6) \quad \text{for } \lambda = 3$

$P(t) = P(0) \exp(-f't) (1 + 3(f't)/4 + (f't)^2/4$

$$+ (f't)^3/24) \quad \text{for } \lambda = 4, \quad (18)$$

where λ is the number of cross-bridge heads in a cluster and $P(0)$ is the initial force at time $t = 0$. In Fig. 8, the decay of force is shown for $\lambda = 1, 2, 3, 4$. As shown in the figure, the rate constant for force decay becomes slower with increasing λ values. The half-time for force decay is approximately $0.7f'^{-1}$, $1.2f'^{-1}$, $1.6f'^{-1}$, $2.1f'-1$, $2.6f^{-1}$, and $3.1f'^{-1}$ for $\lambda = 1, \ldots 6$, respectively. Since the assumptions made cause an underestimate of the rate of force decay, these results indicate that while the rate of force decay after a step stretch decreases with increasing numbers of heads in a cluster, the effect is not a dramatic one and the rate constant for force decay is only of the order of $f'/4.4$ for a cluster of 6 heads.

DISCUSSION

Vertebrate muscle fibers are made up of thick myosin containing filaments arranged in a hexagonal array with thin actin containing filaments in the trigonal positions. Each thin filament, composed of a helical array of repeating actin monomers 5.5 nm apart, is surrounded by 3 thick filaments, each with 3 double-headed cross-bridges protruding every 14.3 nm, again in helical array, with the myosin helical repeat being quite different from the actin repeat. Because of this complexity, it has been common,
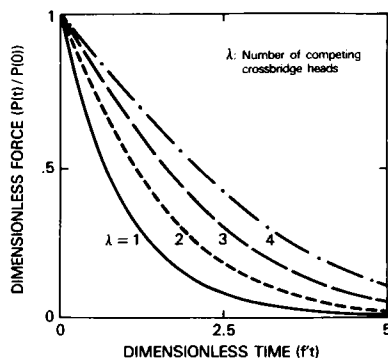

FIGURE 8 Force decay for the simple model discussed in Section 4. Parameter λ denotes the number of cross-bridges in the cluster competing for the same actin sites.

particularly in models with large numbers of states, to make the simplifying assumption that one myosin head can interact with but one actin site. However, it seems quite likely that a given cross-bridge head can bind to one of several possible actin monomers (12). This feature was incorporated into the model of Schoenberg (7) that was used to explain the interaction of cross-bridge heads with actin under equilibrium conditions. One conclusion of that model was that the force decay after a step stretch should occur with rate constants that reflect the cross-bridge detachment rate constants.

It has been assumed that the detachment rate constant of an unstrained cross-bridge head in a fiber should be similar to the rate constant for S1 dissociation from filamentous actin in solution (3) and qualitatively this appears to be true. When ATP is bound to the active site of myosin, the S1 detachment rate constant in solution is fast (13), as is the rate of force decay after a stretch (14). With ATP analogues such as AMP-PNP or PP_i bound to the active site, the S1 detachment rate and rate of force decay are both considerably slower (6, 9, 10, 15). When no ATP or analogue is bound to the active site (as in rigor), both the rate constants are slower still.

Quantitatively, however, there appears to be a problem. In the presence of AMP-PNP, the apparent S1 detachment rate constant in solution, at a temperature of 5°C and ionic strength of 0.125 M, is ~ 15 s^{-1} (9). Under a very similar condition, Schoenberg and Eisenberg (6) found that the rate of force decay in a fiber is not described by a single rate constant, but is multiexponential, described by a range of rate constants from 0.01 s^{-1} to 10 s^{-1}. The fastest rate constants appear comparable to the S1 detachment rate constant in solution but, clearly, there are also much slower components to the force decay. One way of explaining this is to assume that, in the fiber, the detachment rate constant of a strained cross-bridge head is slower than that of an unstrained head. This would also explain why there is a range of rate constants in the fiber since not all the attached cross-bridges would have the same strain. However, this does not seem to be the correct explanation since Schoenberg and Eisenberg (6) found that in the presence of AMP-PNP the detachment rate constants actually appear to increase with increasing strain.

It is known that the assumption in Schoenberg's model (7) that a single head can bind to a set of actin sites without any competition from neighboring heads is an oversimplification. Firstly, each myosin cross-bridge is double-headed, and secondly, in rigor at least, it has been found that the cross-bridges tend to bind along the actin filament in clusters (16). Since there are, in vertebrate skeletal muscle, only about 2 actin monomers for every myosin head, and since not all monomers may have appropriate orientation relative to the projecting heads suitable for attachment, it seems possible that in a region where large numbers of cross-bridges are clustered together, there may be few remaining unoccupied actin sites suitable for myosin

attachment. In this case, when a cross-bridge head detaches after stretch of a muscle fiber, it might not immediately be able to attach to the actin site at which it would mostly be attached once equilibrium is reestablished. This might slow down the decay of force after a stretch and could conceivably offer an explanation for why some of the rate constants for force decay in a fiber after stretch are somewhat slower than the S1 detachment rate constant in solution. This study was undertaken to explore this possibility.

As might be expected from the above arguments, we did find that as the number of cross-bridge heads binding in a cluster increases, the rate of force decay after a step stretch decreases. However this effect was not as large as anticipated. For the models defined in sections 1–3, which have two or three competing heads per cluster, the rate of force decay after a stretch is slower by less than a factor of 2 from what it would be if there were no head competition. The simplified model of section 4, shows that even when 6 out of a total of 7 actins have bound bridges, the time for the cross-bridges to redistribute back toward the equilibrium distribution and relax the force is increased less than a factor of 5 compared to the time it would take if there were just a single bridge. We conclude therefore, that cross-bridge clustering, without cooperativity, does not account for the slowness of some of the force decay rate constants relative to the S1 detachment rate constant. It should be mentioned that the above effect is not limited to the case of AMP-PNP. In rigor, for example, the S1 detachment rate constant is ≈ 0.01 s^{-1} (15) and yet the force generated by stretch can persist for hours.

If simple cross-bridge clustering does not account for these effects, what may? Pate and Brokaw (17) attempted to explain the slow decay of force in rigor by making the detachment rate constant extremely small for certain values of cross-bridge strain. However, there appears to be little evidence in skeletal muscle supporting this ad hoc assumption. With regard to other explanations, in section 1, we saw that when the two heads of a cross-bridge were assumed independent, the force exerted by a double-headed cross-bridge was quickly relaxed as the more strained head detached, swiveled around the less strained head, and reattached to an actin in a position of zero or negative strain. It is possible that such totally independent head action cannot occur. It might be that when two heads of a cross-bridge are bound, the rate constants for head detachment are somewhat slower than when one head is bound. Another possibility is that it might be necessary for both heads of a cross-bridge to detach before either head can rebind in a position of lesser strain (Anderson M. L., and M. Schoenberg, manuscript in preparation). A third possibility, one previously suggested by Kuhn (18), is that there is not cooperativity between the heads of a cross-bridge, but rather between cross-bridges in a cluster. Kuhn suggested that if cross-bridges bound clustered, the overall energy of binding of the cluster might be greater than if the

individual cross-bridges bound separately, thereby increasing the stability of the cluster and leading to slowing of the rate of force decay after stretch. As a final possibility, the assumption that an unstrained cross-bridge detaches with the same rate constant as S1 in solution could be wrong. More experimentation is needed to sort out these possibilities.

In summary, when the two heads of a cross-bridge are assumed independent, the behavior of the double-headed cross-bridge is very similar to that of the single-headed bridge, at least under equilibrium conditions. When independent cross-bridge heads bind clustered together, competition between the heads leads to some slowing in the force decay after stretch, but this effect is not large enough to explain why some of the rate constants for the decay are nearly two orders of magnitude slower than the S1 detachment rate constant measured in solution.

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