On the Theory of Muscle Contraction: Filament Extensibility and the Development of Isometric Force and Stiffness

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ABSTRACT The newly discovered extensibility of actin and myosin filaments challenges the foundation of the theory of muscle mechanics. We have reformulated A. F. Huxley's sliding filament theory to explicitly take into account filament extensibility. During isometric force development, growing cross-bridge tractions transfer loads locally between filaments, causing them to extend and, therefore, to slide locally relative to one another. Even slight filament extensibility implies that 1) relative displacement between the two must be nonuniform along the region of filament overlap, 2) cross-bridge strain must vary systematically along the overlap region, and importantly, 3) the local shortening velocities, even at constant overall sarcomere length, reduce force below the level that would have developed if the filaments had been inextensible. The analysis shows that an extensible filament system with only two states (attached and detached) displays three important characteristics: 1) muscle stiffness leads force during force development; 2) cross-bridge stiffness is significantly higher than previously assessed by inextensible filament models; and 3) stiffness is prominently dissociated from the number of attached cross-bridges during force development. The analysis also implies that the local behavior of one myosin head must depend on the state of neighboring attachment sites. This coupling occurs exclusively through local sliding velocities, which can be significant, even during isometric force development. The resulting mechanical cooperativity is grounded in fiber mechanics and follows inevitably from filament extensibility.

INTRODUCTION

Based on early evidence from x-ray diffraction studies, it has long been held that both the thick and thin filaments of striated muscle are effectively inextensible. In this case, the relative displacement between thick and thin filaments should be uniform along the entire region of filament overlap. The implications of this premise are profound. Specifically: 1) stiffness of the muscle has been taken as a direct reflection of the number of cross-bridges attached (Julian and Sollins, 1975); 2) the quick release transients F_1 and F_2 (instantaneous force change and early transient force recovery, which follow a quick length change) have been taken as direct evidence of transitions between multiple binding states (Huxley and Simmons, 1971); and 3) the delay of force development relative to stiffness development has been taken to reflect transitions in the cross-bridge states from one that develops stiffness but no force to one that develops both stiffness and force. Most findings were readily assimilated into this framework of ideas, and it was almost universally presumed that our understanding of muscle biophysics could rest comfortably upon it. But in the past year this idea has been upset by findings made in two independent laboratories (H. E. Huxley et al., 1994; Wakabayashi et al., 1994). These findings have firmly established that the actin filament, and probably the myosin filament as well, exhibit extensibility comparable to or larger than that of the cross-bridge itself. Goldman and A. F. Huxley (1994) have pointed out that this new evidence invites reexamination of the theory of muscle contraction at the most basic level.

H. E. Huxley et al. (1994) and Wakabayashi et al. (1994) independently reexamined actin monomer spacing and myosin head spacing using x-ray diffraction techniques. They found that the 2.7-nm actin monomer spacing increased by some 0.2–0.3% during the development of full isometric force. Additionally, slow stretching of muscle was accompanied not only by increased force, but also by increased spacing over the isometric value. Net elongation of the actin filaments amounts to about 3–4 nm per half-sarcomere. This is direct quantitative evidence for the extensibility of the actin filaments.

The above authors also found evidence that the myosin filaments are also extensible, with a strain magnitude similar to that of actin when scaled to 100% tension. The net elongation of the thick filaments appears to be on the order of 2.1 nm per half-sarcomere at full activation. Taken together, these results show that some 70% of the compliance of the sarcomere resides in the extensibility of the thick and thin filaments. This is in direct contradiction to the conventional view of muscle, wherein the actin and myosin filaments are both assumed to be inextensible, and the overall stiffness of the sarcomere is presumed to be associated only with the attached cross-bridges themselves, whether they produce force or not.

This evidence concerning filament extensibility hardly lessens the likely importance of multiple binding states. It does, however, underscore the fact that Huxley's (1957) model, as well as all subsequent modifications of that model, would have

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mistakenly attributed to the behavior of the cross-bridge itself any appreciable effects of spatially nonuniform filament strain and, therefore, spatially nonuniform relative displacement and velocity along the overlap region. The magnitude and nature of such effects are unknown (Goldman and Huxley, 1994). Results offered below, however, indicate that the effects of fiber extensibility on muscle mechanics may be rather important. Quantification of this effect in the specific context of isometric force and stiffness development is the major objective of this report.

We show below that the major effects of filament extensibility on filament-filament interaction evolve from temporally and spatially distributed strains, which create local relative filament displacements and velocities, even when overall sarcomere length is held fixed. This follows necessarily from the nature of the load transfer between the filaments, hereafter called tractions and denoted τ (force per unit length transferred from one filament to the other). The tension (local force in a filament), and hence the strain, at any spatial position of a filament is the cumulative resultant of all the tractions conferred by cross-bridges from the filament's free end, where the tension and strain are zero, to the given location. Thus, the external load exerted on a sarcomere must be transferred from one filament to the other by local contact tractions generated by cross-bridges.

With this generalized concept, we reformulated A. F. Huxley's sliding filament theory, using the simplest possible model of local cross-bridge binding kinetics, which consists of the original two-state model of A. F. Huxley (1957), wherein cross-bridges are either unattached (and not force producing) or attached (contributing both to force and to stiffness) and, in addition, explicitly including filament extensibility. We focused on isometric force development because the effect of filament extensibility in this circumstance is significant and, as we will demonstrate, challenges the interpretation of experimental evidence that rests on current theories of muscle mechanics. We quantitatively assessed the nonuniform relative filament displacement, cross-bridge strain, and importantly, the local shortening velocities that—even at constant overall sarcomere length—depress force below that which would have developed if the filaments had been inextensible. We show that filament extensibility may be a major effect contributing to the difference in the timing of force versus the timing of stiffness development, and the evolving relationship between stiffness and number of cross-bridges attached.

METHODS

Formulation of the coordinate systems, strain fields, and field equations

For the analysis of load transfer in an extensible filament system we utilized the theory of filament-filament kinetics (Mijailovich et al., 1993, 1994). The spatial orientation of the actin and myosin filaments is such that the free end of one is apposed to the region of the other where tension and strain are maximal, and vice versa. Thus, over the overlap distance L, the tension in an actin filament changes from 0 at the free end to total force transferred $F(t)$ at the end of overlap closest to the Z band at any instant of time t. Similarly, in a myosin filament the tension changes from $F(t)$ to 0 (Fig. 1). Along L, the net force $F(t)$ is transferred entirely from actin to myosin filament via cross-bridges (A. F. Huxley, 1957; H. E. Huxley, 1969).

The following field equations governing the actin-myosin filament interaction are obtained from the filament stress-strain relations and equilibrium equations, and the cross-bridge tractions obtained from local solution of Huxley's (1957) rate equation.

Let the material displacements of the filaments at position X (laboratory frame fixed to the constrained end of the myosin filament) at time t be u_m and u_a. The small strains ϵ in the filaments are given by the gradients of the displacements:

$$\left.\begin{aligned}\epsilon_m(X, t) &= \partial u_m(X, t)/\partial X\\ \epsilon_a(X, t) &= \partial u_a(X, t)/\partial X\end{aligned}\right\}. \tag{1}$$

Let the stiffness (force per unit strain) of the myosin and actin filaments be denoted K_m and K_a, respectively. The tension in each filament is then given by the constitutive equations:

$$\left.\begin{aligned}T_m(X, t) &= K_m\epsilon_m(X, t)\\ T_a(X, t) &= K_a\epsilon_a(X, t)\end{aligned}\right\}. \tag{2}$$

The important point here is that, because of the presence of attached cross-bridges, the tensions, and hence the strains, in the filaments are not uniform in X, and may further be functions of time t. In particular, the change in filament tension from global position X to $X + dX$ is equal to the force conferred by the presence of cross-bridge traction developed at X, namely, $\tau(X,t)dX$, where τ is the traction, dimensionally force per unit length. The equilibrium equations for myosin and actin filaments, respectively, are therefore given by

$$\left.\begin{aligned}\frac{\partial T_m(X, t)}{\partial X} &= -\tau(X, t)\\ \frac{\partial T_a(X, t)}{\partial X} &= +\tau(X, t)\end{aligned}\right\}. \tag{3}$$

In addition, global force equilibrium implies that local tractions associated with attached cross-bridges at any spatial location (X) are the sum of the tensions of the actin ($T_a(X,t)$) and myosin ($T_m(X,t)$) filaments, which is spatially constant, i.e. $F(t) = T_a(X,t) + T_m(X,t)$.

The traction developed by the cross-bridges is assumed to be governed by the same springlike character associated with attached cross-bridges in the scheme of A. F. Huxley (1957). This is proportional to the first moment of the distribution of cross-bridges attached over the local coordinate x, which measures the position of the myosin head from its equilibrium position. Thus,

$$\tau(X, t) = c\int_{-\infty}^{+\infty} xn(X, x, t)\,dx, \tag{4}$$

where $c = k_{XB}/l_a l_m$, k_{XB} is cross-bridge stiffness; l_a and l_m are distances, respectively, between successive actin sites and successive myosin heads; and $n(X,x,t)$ is the fraction of cross-bridges (at global coordinate X) at local position x that are attached. The constant of proportionality arises as follows. The constant relating force per unit area to the first moment is $mk_{XB}(s/2)/l_a$ (Huxley, 1957), where m is the cross-bridge density (number per unit volume) and $s/2$ is the half-sarcomere length. Note that this force per unit area is the cumulative effect of the total number of cross-bridges n_{XB} per half-sarcomere. Converting from the force generated by all attached myosin heads on a single filament to that by a single myosin head thus involves multiplication by A_{myo}/n_{XB}, where A_{myo} is the cross-sectional area of a single filament. This force per l_m, the distance between heads, is the traction (force per unit length). The net result is $[mk_{XB}\,(s/2)/l_a][A_{myo}][n_{XB}]^{-1}[l_m]^{-1} = k_{XB}/l_a l_m = c$, because $m(s/2)A_{myo}$ is simply n_{XB}.

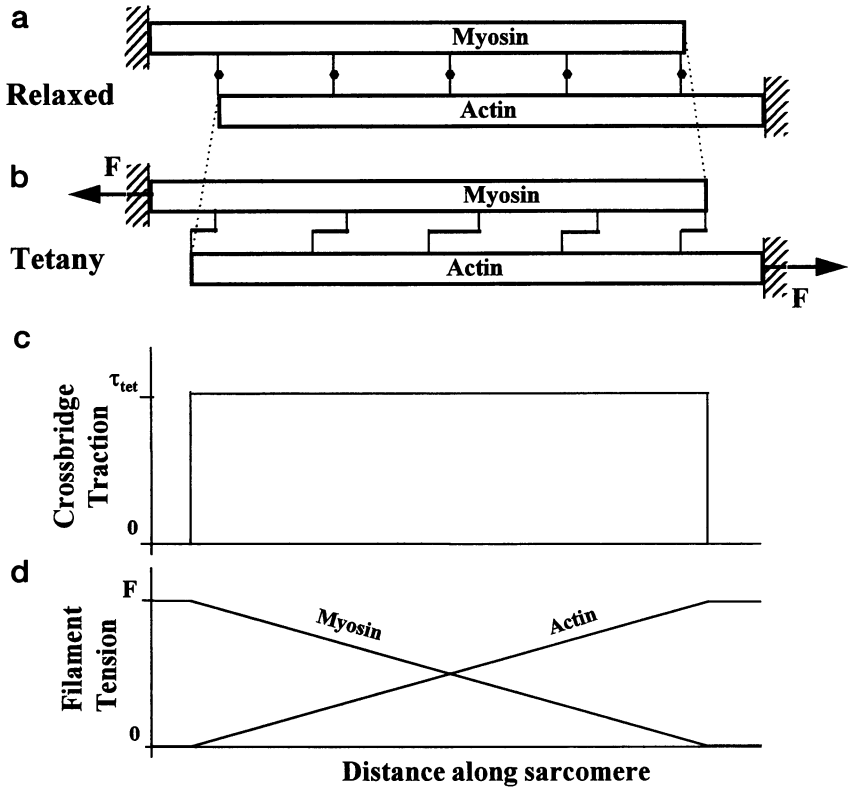

FIGURE 1 Schematic representation of the effect of cross-bridge traction on filament tension, strain, and displacement, for extensible filaments at fixed sarcomere length. One half sarcomere is shown. (*a*) Relaxed; no cross-bridge traction is generated, and therefore filament tensions, strains, and relative displacements are zero. The diagrammed case (*b*, *c*, *d*) represents full tetany. (*b*) Cross-bridge traction load is transferred between myosin and actin filaments, resulting in induced filament tension, strain, and relative displacement (exaggerated here for clarity). Total force developed (F) is borne by the myosin and actin filaments, respectively, at the isometric boundaries. The horizontal lines between the filaments represent the relative displacement of fiducial markers on the filaments. (*c*) Cross-bridge traction (force per unit length) as a function of distance along the sarcomere. In tetany, the cross-bridge traction is spatially uniform at τ_{tet}. (*d*) Tension (local force) in the myosin and actin filaments resulting from load transfer of cross-bridge tractions, also as a function of distance. Filament tensions are always zero at the free ends and equal to total force developed (F) at the opposite ends. Because the traction is uniform, it follows that filament tensions are linear, and (for Hookean elastic filaments) filament strains are also linear. The nonuniform displacements in this case are quadratic. Note that although the largest displacements are at the free filament ends, the largest relative displacements are in the middle. In the non-steady-state case, the distributions of tractions, tensions, strains, and displacements are complex, evolving functions of both space and time.

Note that we explicitly assume that there is no effect of extensibility on the local coordinate x itself. This is justifiable on the grounds that, locally, the distribution of cross-bridge attachment sites varies only slightly with strain in the actin filaments, and that the primary effect of extensibility is in the distribution of n over the global coordinate X, and the associated local relative velocity between the filaments. Specifically, the small strains of the filaments (order 0.2%) imply shifts in the $n(x)$ distribution of order $0.002h$, where h is the limit of the positive attachment probability function (see below) and represents the order of magnitude of convective shifts inducing substantial changes in force. This should be compared with the relative velocity-induced cumulative convective shifts of the $n(x)$ distributions, which can be order h.

The local distribution of attached cross-bridges is assumed to follow the general first-order kinetic scheme of A. F. Huxley (1957). This relates the convective or material derivative of the fraction attached to the attachment probability $f(x)(1 - n(X,x,t))$ and the detachment probability $g(x)n(X,x,t)$. Thus,

$$\frac{\partial n(X, x, t)}{\partial t} - V(X, t)\frac{\partial n(X, x, t)}{\partial x} = f(x) - [f(x) + g(x)]n(X, x, t), \tag{5}$$

where $V(X,t)$ is the local relative (shortening) velocity between the filaments at global position X. This is explicitly given in terms of the derivatives of the displacements by

$$V(X, t) = \frac{\partial u_m(X, t)}{\partial t} - \frac{\partial u_a(X, t)}{\partial t}. \tag{6}$$

Note that V is positive for local shortening, and V is negative for local lengthening. Our choice for the attachment and detachment probability functions are the same as in the original work of A. F. Huxley (1957):

$$f(x) = \begin{cases} 0, & x < 0 \\ f_1 x, & 0 \le x \le h \\ 0, & h < x \end{cases} \tag{7}$$

$$g(x) = \begin{cases} g_2, & x < 0 \\ g_1 x, & 0 \le x \end{cases}$$

These field equations are applicable only in the limit of small strains, where the material derivatives and the spatial (or laboratory frame) derivatives are approximately equal. Moreover, the small displacement expected

in isometric contraction further simplified the problem by allowing us to neglect the difference between Lagrangian (material) coordinates and Eulerian coordinates.

The distribution of axial filament strains during isometric force development

The isometric preparation is one in which the sarcomere is held at fixed overall length. With inextensible filaments, this global isometric condition implies local isometric conditions. With extensible filaments, however, there will be systematic local length changes, and nonzero local relative velocities of actin versus myosin, even when overall sarcomere length is held fixed. These in turn will have an immediate effect both on the stiffness and the force developed, as these are direct functions of filament strain and local relative velocities.

We begin with a given set of distributions of attached cross-bridges $n(X,x,t)$. This might be zero for studying force and stiffness development, or it might be a constant for $0 < x < h$, beginning with full tetany before a quick shortening experiment. These distributions in x determine an initial set of tractions in X from Eq. 4. For this X distribution of tractions, we solve for the stresses and strains as follows. The first three field equations above can be combined in the following form for the displacements:

$$\left.\begin{aligned} K_m \frac{\partial^2 u_m(X,t)}{\partial X^2} &= -\tau(X,t) \\ K_a \frac{\partial^2 u_a(X,t)}{\partial X^2} &= +\tau(X,t) \end{aligned}\right\}. \quad (8)$$

The interface condition, Eq. 4, includes the distribution of attached cross-bridges, which are calculated from the first-order hyperbolic partial differential Eq. 5, and it depends on the local relative velocity between filaments. In turn, the deformation of filaments and, therefore, relative velocity, depends on traction distribution along the filaments. This makes the problem simultaneously dependent on the tractions defined at a local level and relative velocity defined at global level. The solution of the problem is obtained numerically by the method of characteristics for local tractions and by finite-element method for the actin and myosin filament deformations by using an iterative scheme described in the Appendix.

We solved the field equations for isometric force development beginning with the filaments in a resting state, with strain everywhere zero at $t = 0$. Furthermore, $n(X,x,0)$ was also taken to be 0. At $t = 0^+$, Ca^{2+} is assumed to have flooded the sarcomere, with its associated relief of the troponin/tropomyosin inhibitory complex, and allowing cross-bridges to begin attaching. We computed the force at sequential points in time, from 0 to at least 500 ms, at intervals of 1 μs, reporting values every 1 ms. We examined the time rate of evolution of both the stiffness and the force.

Force at any time was computed from the first moment of the distribution of attached states, and the stiffness of the sarcomere was computed from the ratio $\Delta F/\Delta L$, where ΔL represents a small step change in length. ΔF was obtained from the finite-element solution to the simultaneous pair of equations given by the change in tractions (from Eq. 4) due to an instantaneous shift in the local distribution $n(x,t)$, and the equilibrium equations (Eqs. 8) expressing how the local displacements are related to the tractions. This was done in a special routine after convergence at time t had been achieved (i.e., outside of the iteration loop). Because the simulated step length change and return to baseline occur in zero time, the local fraction distribution of cross-bridges attached $n(x,t)$ remained unchanged. Note that these stiffness calculations were not obtained at each time step ($\sim$1 μs), but after each 1000 time steps (i.e., at 1-ms intervals). From these results we displayed the evolution of force and muscle stiffness as a function of time. The net force developed $F(t)$ acts on the actin filament at the Z line and on the constrained end of the myosin filament and has a steady state, in the limit of large times, of F_∞ (at full tetany). The macroscopic sarcomere stiffness K, which is also time dependent, is defined by $K(t) = \lim_{\delta L \to 0} \delta F/(\delta L/L)$ (i.e., the increment in force per unit fractional change in length), having a steady state, in the limit of large times, of K_∞.

The numerical values used in the simulations reported in the Results are as follows. The attachment and detachment kinetic rate constants of A. F. Huxley's scheme are $(f_1, g_1, g_2) = (43.3, 10, 209)\ s^{-1}$. Filament moduli are derived from H. E. Huxley's recent work $(K_m, K_a) = (1.76 \times 10^5, 1.28 \times 10^5)$ pN (Huxley et al., 1994; Kojima et al., 1994). The half-sarcomere length is 1.125 μm, with 0.7 μm actin-myosin overlap (0.125 μm and 0.3 μm free end lengths of myosin and actin, respectively, in the half-sarcomere). The myosin distortion displacement scale (h) in Huxley's notation was taken to be 3 nm, for both extensible and inextensible filament case. The reason why h is taken to be smaller than originally used by Huxley (1957) is that the h used here is consistent with force dropping to zero in the compliant filament system for an overall length change of 4 nm during sharp force transitions (Ford et al., 1977; this was denoted T_1 (force) transition by Huxley and Simmons, 1971, but note that T in our work refers to the spatially dependent tension along a filament and not to the overall net force). Accordingly, traction at full tetany is given by 0.726 pN/nm per myosin filament, which corresponds to muscle tension of 285 kN/m^2 (Ford et al., 1977). For a repeat distance of 43 nm, nine myosin molecules per repeat (Squire et al., 1990; Bagshaw, 1993), and one cross-bridge per molecule (we do not distinguish this on a per-head basis), this fraction gives a single cross-bridge force of about 3.4 pN. Furthermore, if at tetany 65% of cross-bridges are attached, we find approximately 5 pN per cross-bridge, similar to cross-bridge forces measured in vitro (Finer et al., 1994; Ishijima et al., 1994).

For the quantitative comparison of the predictions of the classical rigid filament model and the extensible filament model, we have used Huxley's (1957) attachment and detachment rate functions (Eq. 7). The only difference is that in the extensible model we have used the average estimates of the filament extensibility of H. E. Huxley et al. (1994) and Wakabayashi et al. (1994).

RESULTS

The distribution of axial filament tractions, tension, and relative velocity during isometric force development

The temporal evolution of the spatial distributions of tractions and tensions for both the rigid filament model and the extensible filament model are contrasted in Fig. 2. We display these distributions at 10, 20, 40, 80, and 160 ms after the onset of force development, and the asymptotic distributions for large times (labeled t = inf.). The cross-bridge tractions of the extensible filament system develop more slowly than those of the rigid filament system during early development, until about 50 ms; thereafter they develop faster than in the rigid case, until the tractions in both systems become equal at large times. The tractions of the extensible system are slightly nonuniform, having the largest value at the overlap end proximal to the Z band, and a minimum somewhere about the middle of the overlap region. Unlike the rigid filament system, in which the tractions are uniform, the filament extensibility contributes to the nonuniform traction distribution in two ways: 1) by the difference in stiffness of the actin and myosin filaments, giving smaller τ values at the free end of less stiff (actin) filament, and 2) by spatially distributed local relative velocities (i.e. displacements), giving a minimum τ in the region where the relative velocities are the largest (see right upper panel (τ) and right lower panel (V) of Fig. 2). The

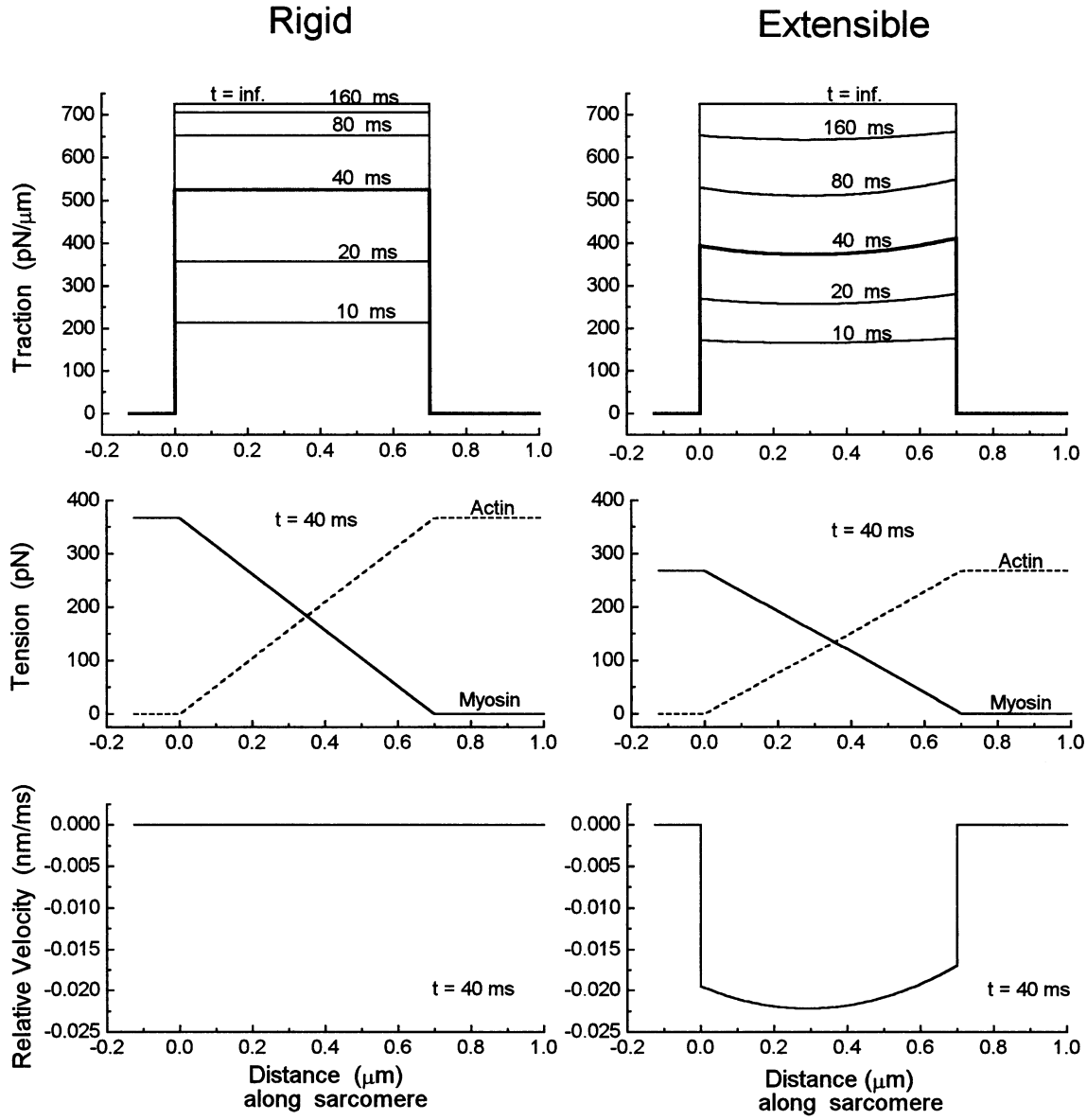

FIGURE 2 The axial distribution of tractions, tensions in actin and myosin filaments, and their relative sliding velocities as a function of axial position X, at selected times after the onset of muscle activation. The left panels show the evolving distributions for rigid filament systems; the right panels show the distributions for extensible filament systems. The notation t = inf. corresponds to the limiting distributions for $t = \infty$. At t = 500 ms, all tractions and forces for the extensible and rigid filament cases are, respectively, within 1% and 0.1% of their limiting values.

tension (Fig. 2, *middle panel*) is given by the integral of traction along the filament overlap. The tension measured from the free filament end increases linearly in the inextensible filament case and nearly linearly with extensible filaments.

The development in time of muscle stiffness and force

The time lag between the initial rise in stiffness and that of force

Fig. 3 shows the time development of force F and sarcomere stiffness k, both normalized to their values at large times (F_∞ and k_∞), for both the inextensible filament and the extensible filament cases. This figure parallels that of Fig. 2, but as a function of time rather than space. Also shown in the lower panel of Fig. 3 are experimental data points, redrawn from Bagni (Bagni et al., 1988), showing the observed evolution of normalized force and normalized stiffness. These data demonstrate the widely seen phenomenon that stiffness leads force. The behavior of the rigid filament model cannot account for this observation. Regardless of the values assigned to the model constants, the inextensible filament model has the wrong sign with respect to the temporal relationship of force and stiffness. By marked contrast, the inclusion of extensibility of the actin and myosin filaments

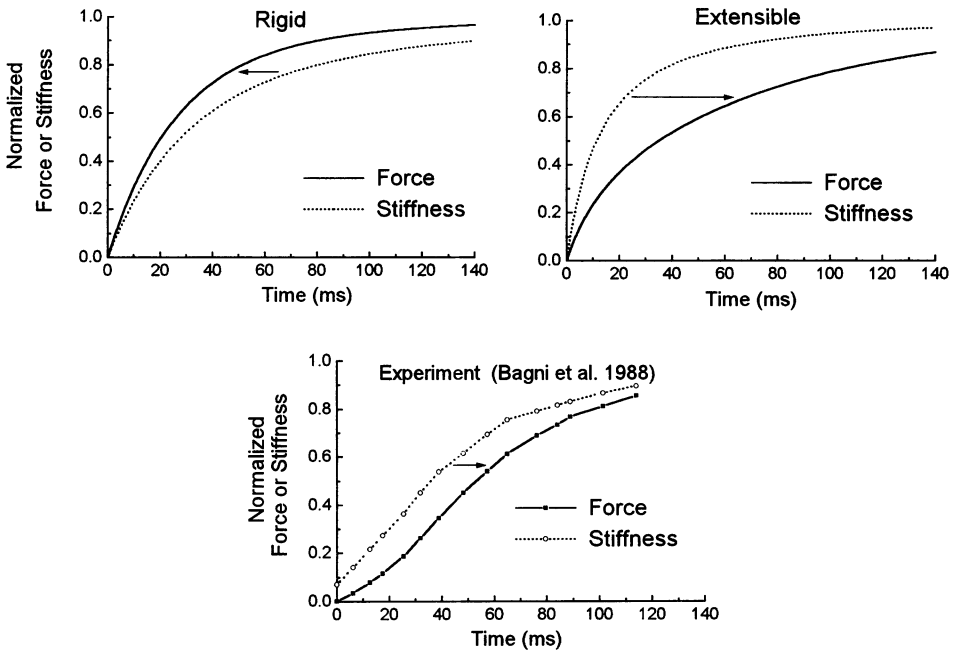

FIGURE 3 (*Top panels*) The time evolution of normalized force (indicated by the *dashed lines*) and normalized stiffness (*solid lines*) in the rigid and extensible filament models. Note that stiffness lags force in the rigid filament model and leads force in the extensible filament model. (*Bottom panel*) For comparison, the experimental points of Bagni et al. (1988) are shown. Note that stiffness leads force in the experimental preparation, which is consistent with the extensible filament model and inconsistent with the rigid filament model.

has not only reversed the sign, but has also brought the predicted values quantitatively close to the measured values of lag.

The relationship between stiffness and the number of attached cross-bridges

Fig. 4 shows the behavior of stiffness versus the number of cross-bridges attached as a function of time, for both the rigid filament model and the case including filament extensibility. As expected, the rigid filament model (*left*) shows a strict proportionality between stiffness and numbers of attached cross-bridges. By contrast, when filament extensibility is taken into account, there is a marked departure from this behavior. There are two effects to be noted in the right panel, showing the relationship of numbers attached and muscle stiffness. The first is that the overall stiffness is generally lower, typically by more than 60%. This is, of course, not surprising, insofar as the extensibility of the filaments has a serial elastance consequence that will lower the overall muscle stiffness. This is similar to the arguments of Bagni et al. (1990). The second is that with extensible filaments in a simple two-state binding model, we have the striking result that stiffness is prominently dissociated from the numbers of attached cross-bridges.

DISCUSSION

Development of stiffness and force

One of the most striking features of muscle behavior during the onset of an isometric contraction is that stiffness, as measured by the ratio of force to length changes during sinusoidal oscillation, develops before force (Bagni et al., 1988). By contrast, the predictions of Huxley's (1957) model led to two ideas: first, that the force must necessarily lead stiffness if there is only one force-producing attached state, and second, that stiffness is a direct measure of the number of cross-bridges attached. The first idea follows from the fact that stiffness and force are given by the zeroth and first moments of the $n(x,t)$ distribution; because the attachment rate function $f(x)$ increases with x, it follows that

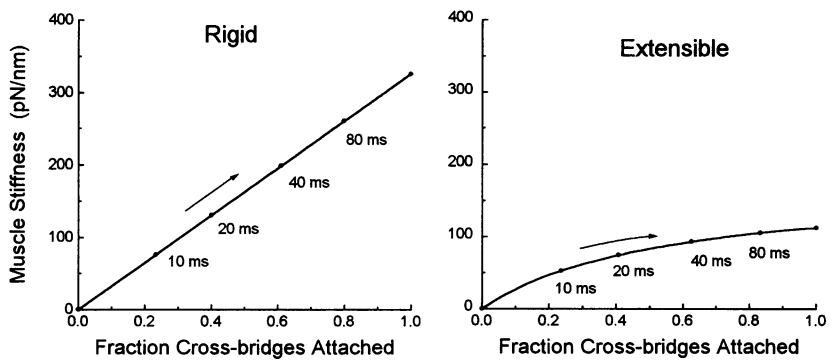

FIGURE 4 The relationship of developing overall muscle stiffness (K) to the total number of cross-bridges attached (n), in both the rigid filament model (*left*) and extensible filament model (*right*). Note that K is strictly proportional to n in the rigid case, whereas there is a marked dissociation of K from n in the extensible filament case.

during force development, $n(x,t)$ is also an increasing function of x, which implies force leading stiffness. The disagreement between predictions and experiments has led to the widely accepted notion that cross-bridges can be attached in more than one state, namely an early state that is non-force-producing and, later, through a conformational change, a second state that is force-producing (Huxley and Simmons, 1971; Ford et al., 1977, 1981). Although this mechanism provides an explanation for the sign of the difference in stiffness and force development, the force lag is quantitatively too short (Bagni et al., 1988). Other, longer-lasting non-force-producing states may exist, and there may be mechanisms of long-range cooperativity (Bagni et al., 1988) that could account for the observed lag. On the other hand, we propose an alternative mechanism (which does not preclude the existence of multiple binding states or cooperativity, but does not require such postulates) found simply in the consequences of extensible filaments, even with a single force-producing binding state. Indeed, Fig. 3 illustrates just such an effect and, more importantly, shows that the magnitude of the time delay is consistent with experimental observations. For example, the calculated lag in force development is on the order of 20 ms, similar to the lag observed in frog muscle at 4°C (Bagni et al., 1988). The critical point of this alternative mechanism is that, even though the overall length is held constant, extensible filaments can slide locally relative to one another, because of increasing contact tractions induced by an increasing number of attached cross-bridges. In other words, globally isometric preparations are not locally isometric if the filaments are extensible; the resulting local velocities decrease the force that would have developed if the filaments were rigid. This results in a lag in the developed force relative to stiffness.

The spatial distribution of cross-bridge tractions is uniform in an inextensible filament system, but is nonuniform if the filaments are extensible. For both systems, mechanical equilibrium requires that the load transfer be balanced by gradients in local filament tensions (Mijailovich et al., 1993, 1994; White and Thorson, 1973; Thorson and White, 1969). For linearly elastic filaments, this results in nonzero strains that are proportional to the local tensions. Experimentally, the magnitude of strains is small (on the order of 0.2–0.3%; Huxley et al., 1994; Wakabayashi et al., 1994), and although it is true that the nonuniformity of very small strains may not, by itself, contribute significantly to muscle mechanics (Ford et al., 1981), it does not follow that the strain *rates* must be small, especially during transient events. Furthermore, the effect of relative filament velocities on cross-bridge kinetics associated with the redistribution of nonuniform strains also need not be small.

During the three different protocols of force development, force recovery after a period of high-velocity shortening, and relaxation, there is a nearly one-to-one relationship of force and stiffness (Bagni et al., 1988). This is a striking result insofar as both stiffness and force follow very different time courses in these experiments. In particular, the rise in $n(x)$ during force development and its fall during relaxation might be expected to be sufficiently different that, with or without a serial elastance, the zeroth and first moments of $n(x)$ will be correspondingly different, and therefore force and stiffness would not show this one-to-one character. Filament extensibility and nonuniform shortening and lengthening will certainly change this relationship, but whether our analysis will also display a virtual lack of hysteresis is not currently known; the fundamental origin of these findings remains an open question.

Local shortening velocities in isometric preparations

Are the local shortening velocities associated with extensible filaments quantitatively important in force reduction? Consider the example shown in Fig. 2, where we display the local velocities at 40 ms after activation. These velocities are on the order of 0.02 nm/ms. For maximum shortening velocities on the order of a sarcomere length per second, this translates into only about 2% of the maximum velocity of shortening, which, by the force-velocity relationship of Hill (1938), would be expected to have a negligible effect on force. Nevertheless, we find a substantial decrease in the tractions, and hence in the developed force, persisting at this relatively intermediate time after activation. This seemingly paradoxical result (H. E. Huxley, personal communication) can be explained by noting that during force development, the distribution of attached cross-bridges $n(x,t)$ is far from the steady-state distribution that would obtain for the (local) shortening velocity given by $V(t)$. It follows that it is inappropriate to compare the decrease in force associated with Hill's force-velocity relationship with those that obtain during non-steady-state force development. The specific mechanism may be seen as follows. During force development, the distribution of attached cross-bridges $n(x,t)$ is biased toward larger values of x (distortions of the myosin head from its equilibrium position), because the attachment rate function $f(x)$ is an increasing function of x. Simultaneously, there is a convective shifting of the $n(x,t)$ distribution due to local shortening. Because force is proportional to the first moment of $n(x,t)$, this shift is of sufficient magnitude to exact a substantial decrement in force, even with the modest local velocities displayed in Fig. 2.

The relationship of sarcomere stiffness to number of attached cross-bridges

It is interesting to consider how the developing stiffness reflects the growing numbers of attached cross-bridges, even in the simple two-state model. In the rigid filament case, stiffness can only vary in direct proportion to the numbers of cross-bridges attached, and therefore, a plot of stiffness versus the total number attached would be strictly linear (Julian and Sollins, 1975). In contrast to this commonly accepted proportionality, our results show that with

extensible filaments this is not the case. Stiffness is prominently dissociated from the numbers of attached cross-bridges. This dissociation, an example of which is shown in Fig. 4, is caused by at least two factors: 1) Even in the simplified limit of lumped filaments (of constant stiffness) in series with lumped cross-bridges (of stiffness increasing strictly proportional to cross-bridge number), the overall sarcomere stiffness is nonlinearly related to cross-bridge number (the reciprocals of the lumped stiffnesses are additive). In this circumstance, overall stiffness is proportional to cross-bridge number only at very low numbers of attached cross-bridges. 2) The non-steady-state distribution of number of cross-bridges attached $n(X,x,t)$ is nonuniform with respect to the global coordinate X.

To further elucidate the origin of the relationship between sarcomere stiffness and number of attached cross-bridges, we compared our results with the following two simplified cases: 1) Lumped filaments (of constant stiffness) in series with lumped cross-bridges (of stiffness increasing strictly proportional to cross-bridge number), and 2) Filaments of constant stiffness connected along the overlap region by a uniform distribution of cross-bridges, which have a stiffness proportional to the number of attached cross-bridges.

The equivalent filament and cross-bridge stiffnesses for both cases were chosen to give identical results at tetany. The solution to case 1, as mentioned above, is simply given by the reciprocal additivity of stiffnesses in series. The solution to case 2 was given by Ford et al. (1981) and White and Thorson (1973), in terms of elementary transcendental functions. We found that the results for both cases 1 and 2 were within a few percent of the results we obtained using the full evolutionary Huxley equations. That 1 and 2 are similar is due to the imposed uniformity of the initial cross-bridge distribution as a function of the global coordinate X. That 2 (and hence 1) should also be similar to the results we present in this paper (see Fig. 4) is somewhat surprising, but it is a consequence of the fact that the global distribution of cross-bridge stiffness during force development is approximately uniform (similar to the tractions shown in Fig. 2). However, this extent of similarity is strongly dependent on the approximate uniformity of $n(X,x,t)$ with respect to X. Although this obtains during force development, the distribution of $n(X,x,t)$ during other non-steady-state protocols may not, and therefore we may expect marked departures of the full analysis from either of the simplified cases above during, for example, oscillatory loading or during recovery after quick changes in length.

There are both steady-state and non-steady-state implications of filament extensibility. First, our results (and, as argued above, the results assuming a model with a lumped extensible filament in series with parallel cross-bridges; Goldman and Huxley, 1994) implies that cross-bridge stiffness inferred from measurements of overall stiffness at high numbers of attached cross-bridges (e.g., tetany, rigor) must be some 2.5 times higher than assessed from data of transient responses to rapid mechanical perturbations (Ford et al. 1977; Lombardi and Piazzesi, 1990). Second, the extent of dissociation of stiffness from numbers attached in non-steady-state preparations depends on the distribution of attached cross-bridges, which in turn depends on the nature of the experimental protocol (e.g., force development from the relaxed state versus tension recovery after small but quick length changes). However, this protocol dependence has yet to be quantified. Taken together, these results support the view of Goldman and Huxley (1994) that sarcomere properties depend on filament extensibility, in some cases strongly, and that therefore the interpretations of experiments in light of current hypotheses may need reexamination.

Mechanical cooperativity among cross-bridges

The original theory of A. F. Huxley (1957), and the theories that have followed, are variations on the development or modulation of the numbers of cross-bridges attached. More recent theories include multiple binding states (e.g., Huxley, 1973, 1974; Lymn and Taylor, 1971; Eisenberg and Hill, 1985; Eisenberg et al., 1980), with kinetic schemes that govern the transition rates between these states. The most important feature of all these models, from the point of view of the potential effects of filament extensibility, is that they are all local, in the sense that the relevant length scale is that of the intrinsic movement of the myosin head around its local equilibrium position. This microscale view of muscle mechanics can be directly extended to the macroscale, if and only if 1) the cross-bridges are independent force generators and 2) the filaments are inextensible. On the other hand, the extensibility of both actin and myosin filaments implies that the local behavior of one myosin head must depend on the state of neighboring attachment sites. This is true because of the coupling through local velocities of shortening and lengthening that only, at filament ends, represent the macroscopic velocities of filament sliding. This is a real cross-bridge cooperativity that is grounded in filament mechanics, and follows inevitably from filament extensibility.

Such mechanical cooperativity arises from the following interactions. The local tractions between the filaments are conferred by attached cross-bridges. These in turn obey probabilistic laws of attachment and detachment which, following Huxley (1957), depend only on the local deformation of the cross-bridge from its equilibrium position through the rate functions $f(x)$ and $g(x)$; importantly, the time evolution of $n(x)$ also depends on the relative velocity of sliding locally. This velocity is simply the local difference in the rates of displacements of the filaments; the gradients of the displacements are the filament strains, and these change because of the load transfer associated with the attached cross-bridges. This scheme is thus fully coupled: no one component can be isolated, as in the case of rigid filaments. In particular, we conclude that for extensible filaments, except for isometric steady force maintenance, cross-bridges are not independent force generators.

SUMMARY

We have shown that the extensibility of actin and myosin filaments has implications for several of the key interpretations of experimental results that form the foundation of our understanding of muscle mechanics. Extensibility leads naturally to the dissociation of force and stiffness development, without the necessity of multiple binding states, to the dissociation of stiffness from numbers of cross-bridges attached, and to a simple mechanism by which cross-bridge cooperativity can be realized.

The temporal relationship of developing stiffness versus force is an important part of the interpretation of experimental evidence in terms of the kinetics of myosin attachment rates, the multistate hypothesis, and the observation that multistate binding is necessary to account for the experimental observations in rigid filament models of muscle mechanics. By contrast, extensible filaments have the property that globally isometric preparations need not be locally isometric, and that the relative velocity of actin and myosin filaments locally can sharply influence the developing tension through the velocity effects on cross-bridge tractions. Although the filament extensibility significantly affects the cross-bridge kinetics scheme and its experimentally assessed rate constants, it is unlikely that filament extensibility, coupled with simple first-order interaction kinetics, will suffice to account for all the experimental observations. Nevertheless, we have demonstrated the importance in the specific context of a non-steady state and concur with A. F. Huxley that filament extensibility invites further reexamination of the data relating to the kinetics and nature of the binding state(s) of myosin and actin.

APPENDIX: NUMERICAL SOLUTION OF THE SIMULTANEOUS HUXLEY AND MACROSCALE EQUILIBRIUM EQUATIONS

The overall scheme

There are two separate issues involved in the numerical implementation of the solution to the coupled set of Eqs. 4 to 8. First, we note that the time and space derivatives enter in different ways. The first-order time derivative in the Huxley equation represents the continuing evolution of the full spatial solution, whereas the spatial derivatives enter in the equilibrium condition for local force balance. These must be treated separately. Second, as noted in the text, we must distinguish between the macroscale variable of axial lengths along the fibers, generally denoted by X, and the microscale variable x describing the departure of the myosin head from its equilibrium position. Thus, x is the spatially independent variable in the Huxley equation, whereas X enters only as a parameter through the relative velocity term. Similarly, X is the spatially independent variable in the equilibrium and constitutive Eqs. 2–4, whereas x does not enter these equations at all.

We construct the independent coordinate system for X as a discretized Lagrangian coordinate. That is, we let X_p be the laboratory position of the (discretized) material point labeled p. Typically we take $p = 1, 2, \ldots, 2P$, where $2P$ is the total number of finite element nodes on actin and myosin filaments (P nodes on each filament, typically $P = 101$, corresponding to 100 finite elements per filament). Similarly, the microscopic axial coordinate is discretized again as a Lagrangian system, with points $x_{p,q}$ being the displacement, at the pth macroscale position on myosin, of the moving actin binding site labeled q from its position where a bound myosin head would be in equilibrium. Typically, $q = 1, 2, \ldots, Q$, where Q is the number of discrete points along x, typically 200. Note that the macroscale coordinate evolves in time, $X_p = X_p(t)$, and that the microscale coordinate both evolves in time and is a function of the macroscale position, $x_{p,q} = x_{p,q}(t)$. This construction is equivalent to the use of the method of characteristics to solve the Huxley equation.

Iterative scheme for the evolving solution

Let the fraction of actin sites bound to myosin at macroscale position X_p, microscale position $x_{p,q}$ at time t be $n_{p,q}(t)$. For the macroscale material point labeled p, let the traction be $\tau_p(t)$, the displacement be $U_p(t)$ (separately computed for both filaments), the strains and tensions be $\epsilon_p(t)$ and $T_p(t)$ (again computed separately for each filament), and the relative velocity between myosin and actin filaments at location X_p be $V_p(t)$. Note that the sum of the tension in the myosin and actin filaments at any station X_p necessarily equals the external applied force. In this appendix, $T_p(t)$ refers to the tension in the myosin filament. With these definitions, the convective version of the Huxley equation (Twizell, 1984), discretized in time, leads to the following expression for the increment in time:

$$\delta n_{p,q}(t) = \mathcal{F}_{p,q}(t)\delta t, \tag{A1}$$

where

$$\mathcal{F}_{p,q}(t) = f(x_{p,q}) - [f(x_{p,q}) + g(x_{p,q})]n_{p,q}(t). \tag{A2}$$

The tractions are given by the first moment of the number distributions, Eq. 4, and their increment in time is $\delta\tau_p(t)$. This was computed by trapezoidal integration of the x-weighted $n_{p,q}(t)$ over all q. The load transfer equation, Eq. 4, then implies increments in the filament tensions and hence strains, respectively, $\delta T_p(t)$ and $\delta\epsilon_p(t)$. This in turn yields increments in displacements $\delta U_p(t)$ (for both filaments) and relative velocities between the filaments $\delta V_p(t)$. The displacement increments were computed from the strains by a finite-element incremental method (Newton-Raphson iteration scheme; Bathe, 1982) over the macroscale variable X_p.

In principle, this suffices to obtain the velocity at time $t + \delta t$ by using the explicit estimate $V_p(t + \delta t) = V_p(t) + \delta V_p(t)$, thereby advancing to the next time step. However, the stability of first-order hyperbolic equations is known to be markedly improved by using implicit or semi-implicit methods (Press et al., 1986), wherein certain functions are evaluated either at time $t + \delta t$ (rather than at t) or as their average at t and $t + \delta t$. The improved stability, however, comes at a cost of using the desired result (namely at $t + \delta t$) in the calculations to determine the result at $t + \delta t$. This therefore requires an iterative scheme to be employed within each time step. The values of variables at iteration number i are denoted with the superscript (i). To approximate the integral of dx/dt and dn/dt we evaluated the microscale variables $x_{p,q}(t)$ and $n_{p,q}(t)$ semi-implicitly (Twizell, 1984), with the following iterative procedure:

$$x_{p,q}^{(i+1)}(t + \delta t) = x_{p,q}(t) + \frac{1}{2}(V_p^{(i)}(t + \delta t) + V_p(t))\delta t \tag{A3a}$$

$$n_{p,q}^{(i+1)}(t + \delta t) = n_{p,q}(t) + \frac{1}{2}(\mathcal{F}_{p,q}^{(i)}(t + \delta t) + \mathcal{F}_{p,q}(t))\delta t. \tag{A3b}$$

Note that there is no iteration number labeling variables V_p and $\mathcal{F}_{p,q}$ evaluated at t, because these are the values to which the previous iteration converged.

Similarly, we used a fully implicit Newton-Raphson scheme for the displacements (Bathe, 1982). The stresses, through their constitutive relationship with the strains, were evaluated at time $t + \delta t$, thus ensuring that the incremental displacements $\delta U_p(t)$ (for both filaments)

would at each successive time interval satisfy finite element equilibrium conditions

$$\nabla T_p(t) - \langle \tau_p(t) \rangle = 0,$$
$$T_1(t) - F(t) = 0, \qquad \text{(A4)}$$
$$T_P(t) = 0,$$

where ∇T_p and $\langle \tau_p(t) \rangle$ represent the discretized gradient of filament tension and integrated cross-bridge tractions over the element lengths characterized by node p. These were computed by length-weighted linear interpolation. This is the discretized, or finite element, version of Eq. 8. The boundary conditions are expressed by the fact that tension in myosin at the free end of actin ($p = 1$) must be equal to the applied force, and that at the free end of myosin ($p = P$), the tension equals zero. Also at the loaded ends, displacements can be prescribed instead of $F(t)$. This finite-element representation was used for each node p, and its solution was implemented using the Newton-Raphson scheme (Bathe, 1982).

Being an implicit scheme, the simultaneous satisfaction of the above equations A1–A4 requires iteration at each time step such that all equations are satisfied to a preset tolerance. This was done by considering the left-hand side of Eq. A4 as a $2P$-dimensional vector and $x_{p,q}(t)$ and $n_{p,q}(t)$ as $2P$ by Q matrices, and computing the respective vector and matrix L^2 norms. Iteration was continued until all three norms were changing by less than a relative tolerance typically set to 10^{-10}. Each time step typically required 5–10 iterations to achieve this criterion.

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