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Unfolding transitions in myosin give rise to the double-hyperbolic force–velocity relation in muscle

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Abstract

This work presents an extension to a recent model of muscle contraction that was based on entropic elasticity (Nielsen 2002 *J. Theor. Biol.* **219** 99–119). By using entropic elasticity as the origin of muscle force, various possibilities emerge that can account for the presence of the double-hyperbolic force–velocity relation in muscle that was observed by Edman (1988 *J. Physiol.* **404** 301–21). In the present work, it will be argued that a slight change (elongation) of the contour length of the entropic springs involved in their high-force regions is sufficient to produce such a double-hyperbolic profile. A sudden elongation would correspond to an unfolding event of a small region of the myosin molecule, which causes a sudden reduction of the tension that may be produced by the individual molecule. To obtain the double-hyperbolic profile, it is assumed that a gradual transition occurs in the entropic spring array from being mainly composed of non-unfolded myosin springs that have a short (i.e. normal) contour length to consisting of a mixture of myosin springs with short and long (unfolded) contour lengths.

1. Introduction

As the velocity of muscle contraction increases, there is a decrease in the maximal tension that a muscle may produce, an observation that is well captured by Hill's hyperbolic force–velocity relationship (Hill 1938), and which has been found to be valid for a wide variety of muscles and was until recently assumed to be valid also for all contraction velocities ($v \in [0, v_{max}]$). However, some careful experiments performed on single fibres isolated from the anterior tibialis muscle of *Rana temporaria* do seem to indicate the presence of a second hyperbola (Edman 1988) which is only effective at low contraction velocities ($v \leqslant 0.11 v_{max}$) corresponding to the high-force range of the muscle ($F \geqslant 0.78 F_{max}$). Essentially, the presence of this second hyperbola means that the muscle tension developed at low contraction velocity is slightly less than would be predicted by the Hill equation.

A significant breakthrough in understanding muscle function was achieved with the sliding filament model of muscle (Huxley and Niedergerke 1954, Huxley and Hanson 1954, Huxley 1957, 1973). Now it is well known that muscle contraction depends on a complicated interplay between myosin molecules and actin filaments which, very briefly, may be summarized as the following ATP-dependent three-step cycle: (1) myosin binds to actin, (2) myosin changes its conformation (e.g. by shortening or bending), (3) myosin unbinds (for a much more detailed description, see Lymn and Taylor 1971, Holmes 1996). During step 2, if one were to hold on to one end of the actin filament and to the unattached end of the myosin molecule, one would observe the generation of a small force (in the piconewton range) which depends on the separation between the ends. In a muscle, this small force contributes to the sum of all the forces generated by thousands of similar actin/myosin filaments that are arranged in parallel within the muscle's sarcomeres. If the sum of all these forces exceeds the load that is imposed on the muscle, then the muscle will start shortening due to the sliding motion of all these filaments. There are various ways in which such a system may be modelled, but essentially it is considered equivalent to a set of N springs that are arranged in parallel, and with lengths, x, distributed within some finite interval, $x \in [a, b]$. One modelling approach is to assume that the myosin/actin complex has a linear force–extension relationship (Huxley and Simmons 1971), which requires the use of complex spring length distributions which depend non-linearly on contraction velocity in order to obtain a hyperbolic force–velocity relation. However, this view has been challenged recently by a model which proposes that muscle force is dependent on the entropic elasticity of the myosin molecule (Nielsen 2002), and is thus highly non-linear, whereas the myosin–actin bonding probability is assumed linear in velocity, on the basis of the observation of certain geometrical regularities in muscle.

2. Modelling muscle

The total force, F, produced by a system of N springs arranged in parallel and with lengths evenly distributed in the interval $x \in [a, b]$, is

$$F = \frac{N(v)}{b(v) - a(v)} \int_{a(v)}^{b(v)} s(x)\,\mathrm{d}x \tag{1}$$

where $s(x)$ is the force–extension relation of the springs, v is the fibre sliding velocity, the lower bound of spring lengths as a function of velocity is given by $a(v)$, and $b(v)$ is the upper bound of spring lengths as a function of velocity (for further details about this derivation, consult Nielsen (2002)). Note that $N(v)$, $a(v)$, and $b(v)$ have been defined as functions of sliding velocity. This is due to the observation that the bond-formation probability necessarily depends on v because this is the factor which determines how much time will be available for bond formation. Essentially, the number of springs participating in force production, N, will be proportional to the time available for myosin–actin interaction according to the following equation:

$$N(v) = p_{bond} N_{tot} = \left(1 - \kappa \frac{|v|}{v_{max}}\right) N_{tot} \tag{2}$$

where $\kappa \in [0, 1]$ is an arbitrary parameter (which to some extent determines the predicted ATPase rate (Nielsen 2002)). A similar argument can be made for the spring length interval, where it seems appropriate to assume that a short delay will exist from the moment of initial actin–myosin bond formation to the moment at which the actin–myosin complex actually begins generating force. In that short delay, the attached cross-bridge will have displaced by a short distance which is dependent on v. According to the findings of Lymn and Taylor (1971)

and Holmes (1996), there seem to be two extremes of the position of the myosin head during contraction: the bond-formation, 90° or 'up' position, here represented by $x_{up} \sim 5.3$ nm; and the bond-breaking, 45° or 'down' position, here represented by $x_{down} \sim 0$ nm. To these observations one might add the finding that at a certain extension the actomyosin bond will inevitably break (Brown and Loeb 1999), at what could be termed the 'taut' position, here represented by $x_{taut} \sim 10.6$ nm. Finally, let x_{eff} be the effective initial length of the myosin spring at the moment at which it begins to generate force, following the previously mentioned short delay after bond formation. This effective length will depend on sliding velocity and on the initial bond-formation length, x_{up}, as follows:

$$x_{eff} = x_{up}\left(1 - \frac{v}{v_{max}}\right). \tag{3}$$

During muscle contraction, cross-bridges are formed at x_{up}, start generating force at x_{eff}, and are broken at x_{down}. During stretching, however, cross-bridges will form at x_{up} and start generating force at x_{eff} as before, but will break at x_{taut}. This can be used to determine the spring length interval ($x \in [a, b]$) precisely:

$$x \in [a(v), b(v)] = \begin{cases} [x_{down}, x_{eff}] & \text{during contraction} \\ [x_{eff}, x_{taut}] & \text{during stretching.} \end{cases} \tag{4}$$

So far this muscle model can produce a non-linear force–velocity relation even if using linear springs (Nielsen 2002). In general, however, the best fit to Hill's hyperbolic force–velocity relation is obtained with this model if one assumes that the actin–myosin spring has a force–extension relationship which depends on the protein's entropic elasticity. The following force–extension interpolation formula is based on an analysis of the worm-like chain (WLC) model and is often used to model entropic elasticity (Marko and Siggia 1995):

$$s(x) = \frac{k_B T}{A}\left(\frac{x}{L} - \frac{1}{4} + \frac{1}{4(1 - \frac{x}{L})^2}\right) \tag{5}$$

where k_B is Boltzmann's constant, T is the temperature, A is the 'persistence length', and L is the contour length of the force-producing region of the polymer chain. In Nielsen (2002) it was shown that by integrating equation (5) over the myosin spring length interval $x \in [a, b]$ according to equation (1), an expression is obtained which can account for various properties of muscle force production, including the single-hyperbolic Hill relationship (Hill 1938), the near-linear force–extension profile (Ford *et al* 1977, Lombardi and Piazzesi 1990), the ATP consumption rate (Shirakawa *et al* 2000, He *et al* 2000), the energy liberation rate (Hill 1964a), and the muscle efficiency (Hill 1964b). The full expression for the sarcomeric force is given here (adapted from Nielsen (2002)):

$$F(a, b, L) = D\left(\frac{b^2 - a^2}{2L} - \frac{1}{4}(b - a) + \frac{L}{4}\left(\frac{1}{1 - \frac{b}{L}} - \frac{1}{1 - \frac{a}{L}}\right)\right) \tag{6}$$

where D is defined as

$$D = \frac{N}{b - a}\frac{k_B T}{A}. \tag{7}$$

In the present work, this muscle model is extended to also encompass a molecular account of the events leading up to the development of a double-hyperbolic force–velocity relation as reported by Edman (1988).

3. Results

From Edman's experiments (Edman 1988) it seems evident that in some preparations, a discontinuity is present at $\sim 0.78 F_{max}$ of the force–velocity relation. Essentially what Edman found was that the actual force produced by a muscle at low contraction velocities is lower than what the Hill equation predicts. Given the model presented earlier, there are a limited number of ways in which it may be possible to obtain a second hyperbola in the high-force region of the force–velocity relation which is more or less discontinuous with the main hyperbola, while maintaining the parameters of the model within biologically realistic bounds. A common observation often made in protein pulling experiments is that, at a certain critical pulling force, the tension suddenly drops to zero because a region of the protein unfolds (see Smith and Radford 2000 for a review). In a similar vein, one could argue that at the critical force found by Edman, an unfolding/refolding transition of some part of the myosin molecule could occur. In muscle, however, the total tension of the muscle does not drop to zero because thousands of filaments are acting in unison, and it is only a few of them at a time that reach the critical unfolding transition. Thus, according to the model presented here, myosin will exist in either of two states during contraction, distinguishable by the contour length of the molecule: the short folded state (F-state) at low force levels which has a force-producing contour length of L_F, or the longer unfolded state (U-state) at higher force levels which has a contour length of L_U. The difference in contour length between the F-state and the U-state need not be large to obtain the double-hyperbolic relation, but for a given difference it is necessary to adjust the way in which the transition from F- to U-state of the whole array of myosin springs occurs. Let $\phi_F(v)$ be the fraction of myosin springs that are in the F-state, defined as

$$\phi_F(v) = \begin{cases} 1 + c\left(\left(\dfrac{v_N}{v_{Edman}}\right) - 1\right) & \text{for } 0 \leqslant v_N < v_{Edman} \\ 1 & \text{for } v_{Edman} < v_N \leqslant 1 \end{cases} \tag{8}$$

where $v_N = v/v_{max}$ is the relative velocity, v_{Edman} (~ 0.11) corresponds to the critical velocity below which Edman's second hyperbola appears (velocity normalized to v_{max}), and $c \in [0, 1]$ corresponds to the fraction of myosin filaments to have completed the F–U-state transition at $v = 0$ (thus the parameter c defines the slope of the F–U-state transition; see the lower panel in figure 2). Let $\phi_U(v) = 1 - \phi_F(v)$ be the fraction of myosin springs in the U-state. The total force generated by such a system becomes

$$F_{tot} = \phi_F F(a, b, L_F) + \phi_U F(a, b, L_U) \tag{9}$$

where F is given by equation (6). This function has been plotted in figure 1, where it can be seen that it closely follows Edman's empirical formula for finding the force–velocity relation. Figure 2 shows a close-up of the critical region ($0 \leqslant v_N \leqslant v_{Edman}$) for a variety of parameters. It should be mentioned that at very small v, Edman's own curve fit (Edman 1988) is not entirely accurate, because at $v = 0$ it predicts an isometric tension equal to Hill's, even though Edman's experimental results clearly show that the experimental force–velocity curve intersects the force axis at an isometric force that is only a fraction (0.78) of that of Hill. This accounts for the small discrepancy at $v \sim 0$, observed between Edman's curve fit and the present WLC-based muscle model, the latter of which actually shows such an intersection.

4. Discussion

To obtain the results presented here, it was necessary to assume that the myosin molecule undergoes an unfolding transition near $v = 0.11 v_{max}$, rather than only at $v = 0$

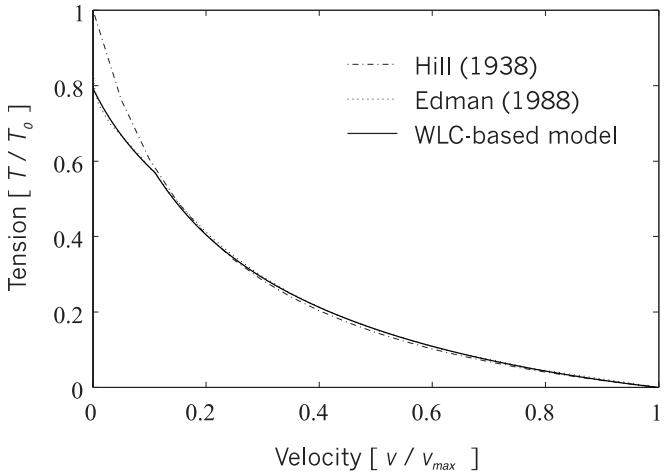

Figure 1. Comparison of Hill's, Edman's, and the force–velocity relation that results from using equation (9). The plots of Hill's and Edman's force–velocity relations are based on these authors' own curve-fitting equations as provided in Hill (1938) and in Edman (1988). In the present simulation the following parameters were used: $L_F = 5.95$ nm, $L_U = 2.23L_F$, and $c = 0.25$ (from equation (8)). The force predicted by the WLC model at $v_N = 0$ for $L = 5.95$ nm was used as the normalization force, F_0. Edman's observations were made on single fibres of the anterior tibialis muscle of *Rana temporaria*, at 1–11 °C, and with a sarcomere ranging from 1.85 to 2.6 μm.

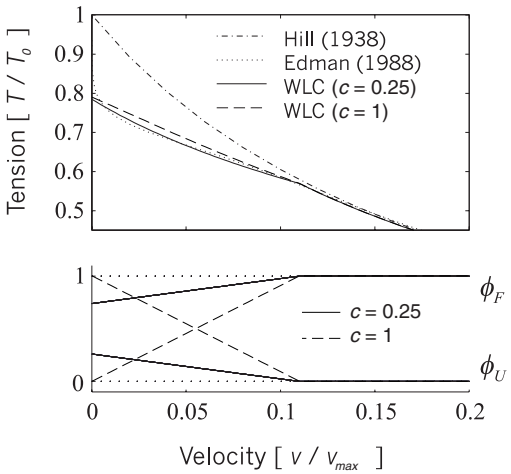

Figure 2. A close-up of the second hyperbola in the critical region $0 \leqslant v \leqslant v_{Edman}$. The upper panel shows a comparison of Hill's, Edman's, and the force–velocity relation that results from using equation (9) in the critical region for various model parameters. The lower panel shows the fraction of myosin molecules in the F-state, ϕ_F, and the fraction in the U-state, ϕ_U, for different values of the parameter c. For the full line in the upper panel, the following parameters were used: $L_F = 5.95$ nm, $L_U = 2.23L_F$, and $c = 0.25$, which yields a curve which closely follows Edman's fit. For the dashed curve in the upper panel, the following parameters were used: $L_F = 5.95$ nm, $L_U = 1.046L_F$, and $c = 1$. Notice that the force–velocity relationship in both cases is qualitatively similar, which allows for a wide range of selection of L_U.

(as was proposed in Nielsen (2002)). According to the present model, the maximal tension to which a single myosin molecule is normally subjected occurs right after bonding, at length x_{up}.

If the maximal tension exceeds a certain threshold, then the force-producing region of the myosin molecule will unfold slightly, bringing the molecule from the F- to the U-state, thereby increasing its contour length (from L_F to L_U), and hence reducing the force contribution of that particular molecule. As the tension on the whole muscle increases, the fraction of myosin molecules that transfer from the F- to the U-state will also increase, according to equation (8), thereby reducing the maximal force that the muscle can produce, and giving rise to the second hyperbola in the force–velocity relation that was described by Edman (1988). In this scenario, the Hill model (Hill 1938) corresponds to a muscle devoid of an F–U-state transition during contraction (i.e., where L is constant for all contraction velocities).

As was shown in figures 1 and 2, a close fit to Edman's results (Edman 1988) is attainable using equation (9) for a wide range of parameters, adding to the list of phenomena that this entropic model of muscle is capable of reproducing (Nielsen 2002), namely the near-linear force–extension profile derived from short mechanical transients (Ford *et al* 1977, Lombardi and Piazzesi 1990), the ATP consumption rate (He *et al* 1999, 2000, Chaen *et al* 1997, 1998, Shirakawa *et al* 2000), the extra energy liberation rate (Hill 1964a), and the muscle efficiency (Hill 1964b, He *et al* 1999, 2000). Although this in itself does not count as proof that the model is correct (only experiment will do that), it does lend credence to the approach taken, in that a large number of predictions can be made while keeping the number of free parameters at a minimum, and while remaining within the constraints imposed by experiment. Several outstanding questions still remain, particularly with respect to the events and forces produced by a muscle at velocities very close to zero, where a transition is assumed to occur from contraction to extension of the myosin filaments. Also, there remains the question about what significance should be given to the fact that myosin is two-headed, and one might also worry about the effects of filaments such as titin, which are serially connected to the thick filaments of the sarcomere. These are issues that will be taken up in subsequent works.

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