CONTRACTION OF GLYCERINATED MUSCLE FIBERS AS A FUNCTION OF THE ATP CONCENTRATION

ROGER COOKE AND WILLIAM BIALEK, *Department of Biochemistry and Biophysics and Cardiovascular Research Institute, University of California, San Francisco, California 94143 U.S.A.*

ABSTRACT We have measured the force-velocity curves of glycerinated rabbit psoas fibers over a range of ATP concentration from 2.5 μM to 5 mM. As the ATP concentration is increased, the isometric tension increases to a maximum around 50 μM, then decreases to a plateau at 70% of the maximum by 1 mM ATP. At low ATP concentrations the maximum velocity of contraction is low and increases with increasing ATP, reaching a plateau at ~2 lengths per second by 1 mM ATP. Our studies suggest that the binding of ATP dissociates the myosin head from actin in the contracting muscle, a reaction similar to that seen in solution. We have constructed models of the actin-myosin-nucleotide interactions based on a kinetic scheme derived from solution studies. The fit of these models to the data shows that the rates of some reactions in the fiber must be considerably different from the rates of the analogous reactions in solution. The data is best fit by models in which head attachment occurs rapidly at the beginning of a power stroke, head detachment occurs rapidly at the end of the power stroke, and the force produced by a myosin head in a power stroke is independent of velocity.

INTRODUCTION

The force of contraction in a muscle cell is generated by the cyclic interaction of two proteins, actin and myosin. The free energy that drives this reaction is derived from the hydrolysis of ATP. Myosin has two heads that extend radially from the thick filament and that interact with the actin (or thin) filament. It is now thought that in a contracting muscle the myosin heads attach to the actin, execute a power stroke, and then detach.

The interactions of actin, myosin (or myosin heads), and ATP have been studied extensively in solution and a number of intermediate states and the transition rates between them have been identified (for review see Lymn 1974, 1975). When ATP binds to the myosin nucleotide site of an actomyosin complex, the myosin is rapidly released from the actin filament (Lymn and Taylor, 1971; Sleep and Taylor, 1976). Myosin splits the ATP and the myosin-products complex rebinds to actin, followed by product release (Finlayson et al., 1969). It is assumed that a similar cycle of events occurs in the muscle fiber; however, the

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Note added in proof: Ferenczi et al. (1979) have recently reported force-velocity curves for skinned frog muscle in 5 and 0.3 mM ATP. Their results are qualitatively similar to those reported here. They find that at 0.3 mM ATP V_m is 0.63 lengths/s, which is approximately a factor of two slower than we found for rabbit muscle. The rate constant for dissociation of frog actomyosin by ATP is also approximately two times slower than that for rabbit actomyosin (Ferenczi et al., 1978; White and Taylor, 1976; Sleep and Taylor, 1976). Thus the rate constant for the dissociation of actomyosin by ATP appears related to V_m measured at low ATP as predicted by the model presented above.

kinetic methods that have been used to establish this cycle in solution are difficult or impossible to apply to the organized filament array of the fiber.

The physiological properties of muscle fibers have been studied through measurements of force, contraction velocity, heat output, and ATPase activity (A. V. Hill, 1938, 1964; Curtin et al., 1974; Edman and Hwang, 1977). More recently, the transient responses of fibers to changes in length or tension have given additional data (Huxley and Simmons, 1971; Civan and Podolsky, 1966). A number of theories have been constructed that attempt to explain the characteristics of muscle fibers in terms of the kinetics of the myosin cross-bridges. A. F. Huxley (1957) was the first to propose such a theory and subsequent theories have been proposed by Podolsky and Nolan (1972), Huxley and Simmons (1972), Julian et al. (1974), and Borejdo (1978).[1] These theories have characterized the cross-bridge-actin interaction in terms of rates for attachment, power stroke execution, and detachment. They have not explicitly incorporated the interactions with nucleotides that are now well understood in the solution studies. A formalism to incorporate energetics and nucleotide interactions has been presented by Hill (1974), Hill et al. (1975), and Eisenberg and Hill (1978). However, applications of this approach are difficult because the cycle of the actomyosin-ATP interaction is complex, and rates measured in solution may not apply in the intact filament array of the muscle fiber. Studies of the mechanical responses and the energetics of intact fibers have not generated sufficient data to determine such a scheme. Data allowing one to link biochemical kinetics with force production are required before complex models of muscle contraction can be understood.

By varying the ATP concentration, we vary the rate of one reaction in the cycle of interactions that generates force, i.e., the rate of the reaction in which ATP first enters the cycle. Thus, by recording force-velocity relations at varying ATP concentrations we see how the properties of the muscle depend upon the transition rate between two biochemical states, and hence make one link between the biochemistry and physiology of the system. This link has allowed us to draw conclusions about the properties of the nucleotide interactions in the fiber, and these conclusions have led in turn to a more detailed picture of the general nature of force production.

METHODS

Thin strips of rabbit psoas fibers (1–2 mm Diam) were dissected and tied to wooden sticks. They were incubated at 0°C for 12 to 24 h in 50% glycerol/50% 0.1 M KCl, 10 mM K_2HPO_4, 5 mM $MgCl_2$, 2 mM EGTA, pH 7. The solution was changed and the fibers were then stored at −20°C. Fibers were used for up to 3 mo. Storage did not appear to affect their characteristics.

Fibers were dissected in the above solution on a cold stage (temperature, <0°C). Single fibers or bundles of up to three fibers (2–4 mm long) were mounted on a tensiometer using the same techniques described by Crooks and Cooke (1977) for actomyosin threads. The solution bathing the fibers contained 0.15 M KCl, 5 mM $MgCl_2$, 2 mM EGTA, 1.9 mM $CaCl_2$, 20 mM *N*-tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid, pH 7, pCa ~5 (buffer 1). In all experiments at 10 mM ATP the Mg concentration was increased to 10 mM and the ionic strength was adjusted to that of buffer 1 by decreasing the KCl concentration and by assuming that all the ATP was complexed to Mg. Contraction was initiated by addition of ATP. Fibers developed maximal tension within a few seconds, and could maintain a stable tension for more than 5 min. The tensiometer has been described by Crooks and Cooke

[1]Borejdo, J. Personal communication.

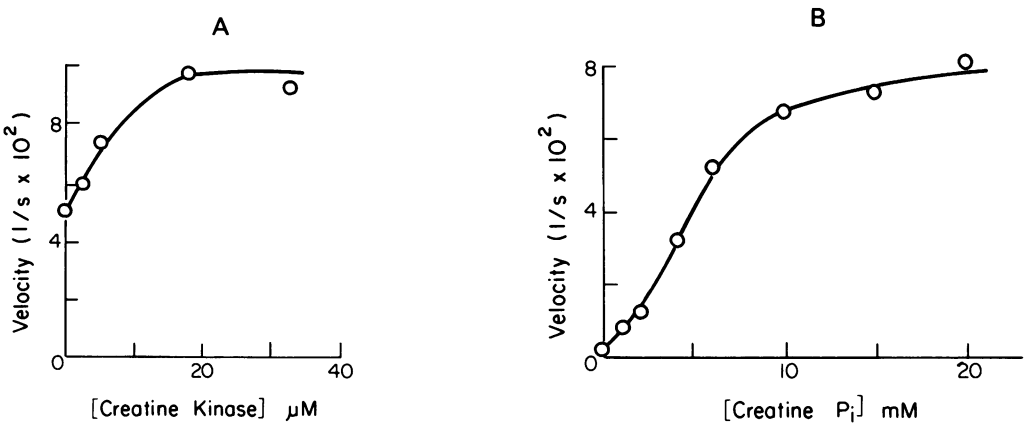


FIGURE 1 (*A*) The maximum velocity of contraction in lengths/second (l/s) is shown as a function of the concentration of creatine kinase added to the medium (buffer 1 plus 20 μM ATP and 20 mM creatine phosphate). (*B*) The velocity of contraction is shown as a function of the creatine phosphate. The medium contained buffer 1 plus 20 μM ATP and 30 μM creatine kinase. The temperature was 10 ± 0.5°C.

(1977). It was operated in an isometric mode until the tension stabilized after addition of ATP; the tension was then dropped rapidly (5 ms) and the length of the fiber recorded as a function of time. We found that the fibers gave reproducible velocity traces for approximately five contractions. The fiber then began to display a large initial drop in length after the tension step (>12 nm/half sarcomere) and the velocity decreased as the contraction proceeded. Thus, only the first 2–5 contractions per fiber were used, and the force-velocity curves shown are averages from several fibers. The diameters of the fibers were measured with a microscope at several positions along the fiber, and the values averaged. All force measurements were made at 10°C. Creatine kinase was bought from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

RESULTS

The diffusion of ATP into a muscle fiber is slow compared with its rate of hydrolysis; thus the level of ATP must be maintained by a feeder system. We choose to use the creatine kinase-creatine phosphate system because it operates in the living fiber. The velocity of contraction was much more sensitive to the level of ATP than was the isometric tension; thus the feeder system was characterized by studying the velocity of contraction as a function of the concentrations of creatine kinase and creatine phosphate. The results are shown in Figs. 1 A and B. At a low level of ATP (20 μM), the velocity of contraction was very low in the absence of a feeder system. As creatine phosphate was raised (in an excess of creatine kinase), the contraction velocity increased and then reached a plateau. A similar effect was seen for variation of creatine kinase. The increase in the contraction velocity that occurs upon the addition of creatine phosphate in the absence of creatine kinase indicates that some creatine kinase had remained bound to the fiber in spite of the glycerination procedure. Subsequent studies were performed with levels of creatine kinase and creatine phosphate that were more than sufficient to saturate the effect on the contraction velocity.

Fig. 2 shows the isometric tension as a function of ATP concentration. In the absence of added ATP the fiber exerted a low tension of about 0.01 N/mm^2 probably due to residual ATP in the fiber. As ATP was added, the tension rose to a maximum at ~30–50 μM ATP,

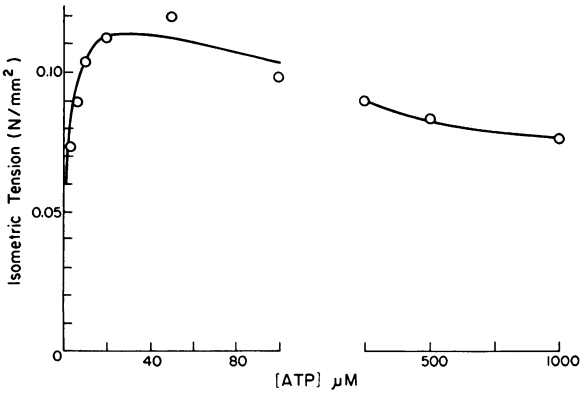


FIGURE 2

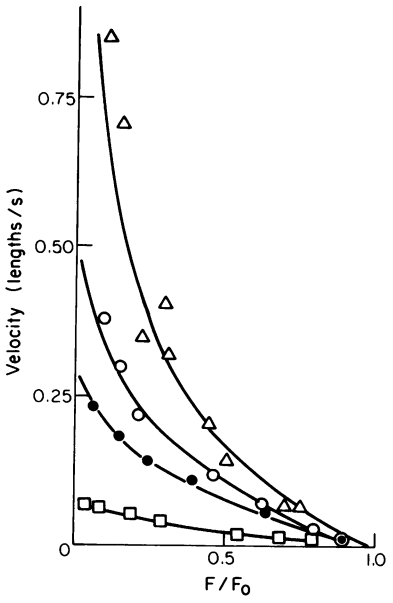


FIGURE 3

FIGURE 2 The isometric tension is plotted as a function of the ATP concentration. The medium contained buffer 1 plus 20 mM creatine phosphate and 30 μM creatine kinase. The temperature was controlled at 10 ± 0.5°C. The solid line represents a fit to the data as described in the text. The isometric tension at 5 mM ATP (not shown) was 85% of that at 1 mM ATP, and at 10 mM ATP it was 81% of that at 1 mM ATP. This drop was included in the analysis of the data.

FIGURE 3 Force-velocity curves for different ATP concentrations: 10 μM (□), 50 μM (•), 100 μM (○), and 500 μM (△). The conditions were the same as in Fig. 2. The solid lines represent a fit to the data as described in the text.

then decreased as ATP was raised to 1 mM. In contrast to the isometric tension, which is not a strong function of ATP concentration in the range 50 μM to 1 mM, the force-velocity relation was highly dependent on ATP concentration up to 1 mM ATP, (Figs. 3 and 5). To extrapolate the data to a maximum contraction velocity at $F = 0$, the force velocity curves were fit by a linearized version of the Hill equation. Several fits are shown in Fig. 4. The maximum contraction velocity, obtained from plots such as those shown in Fig. 4, is shown as a function of the ATP concentration in Fig. 5. The presence of a feeder system is required to obtain a maximum contraction velocity of 2 lengths/s. In the absence of the feeder system the maximum contraction velocity was ~0.7 lengths/s in the presence of either 4 or 10 mM ATP (Fig. 6). The fact that both ATP concentrations give identical force-velocity curves indicates that the build up of ADP and not the availability of ATP causes the decreased velocities compared with those obtained with a feeder system.

DISCUSSION

General Procedure

The force-velocity curves we have measured provide constraints that help define models of the interaction of actin, myosin, and nucleotides in a contracting fiber. Biochemical experiments

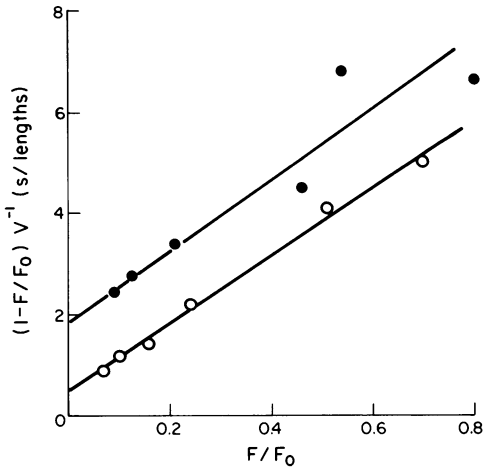


FIGURE 4

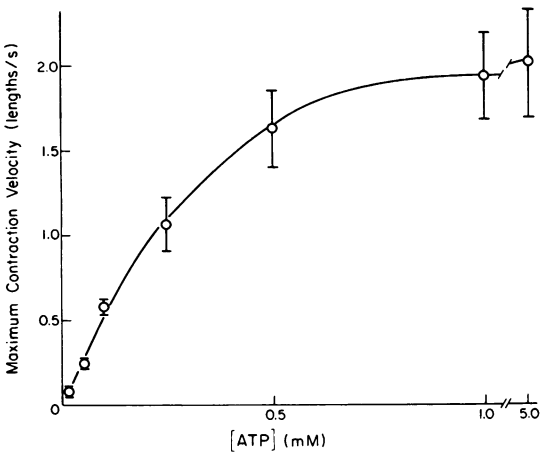


FIGURE 5

FIGURE 4 A fit of the Hill force-velocity relation to the data is shown for two ATP concentrations; (○) 1 mM and (●) 100 μM. The Hill equation (Hill, 1938) was plotted in the form: $(1 - F/F_0)v^{-1} = F/F_0b + a/F_0b$ where F, F_0, and v are the force, isometric force, and velocity of contraction, respectively, and a and b are two constants that define the hyperbolic force-velocity relationship. Extrapolation to $F = 0$ gives the maximum velocity $V_m = P_0b/a$. As ATP is lowered the force velocity curves still fit the hyperbolic relationship with a constant value of b and an increased value of a.

FIGURE 5 The maximum velocity of contraction is plotted as a function of the ATP concentration. The maximum contraction velocity was determined by fitting a linear form of the Hill equation as shown in Fig. 4. The error bars represent standard errors of the mean from at least 5 force-velocity curves.

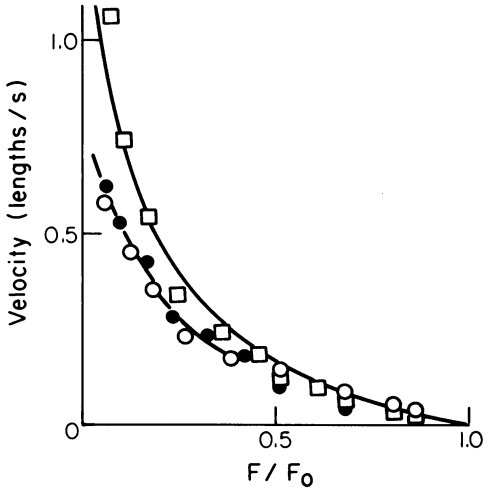


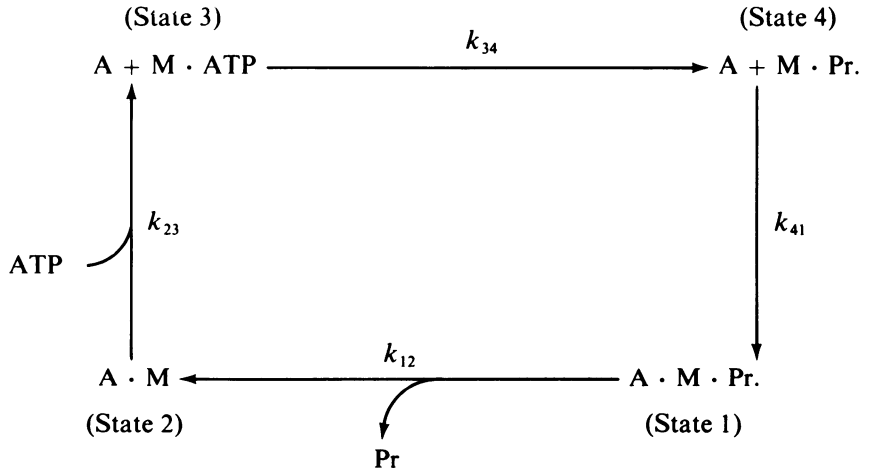
FIGURE 6 Force-velocity curves at high ATP concentrations in the absence and presence of an ATP regenerating system. The medium contained buffer 1 plus 4 mM ATP (○); buffer 1 plus 10 mM ATP with the ionic strength adjusted as described in Methods (●); or buffer 1 plus 5 mM ATP with 30 μM creatine kinase and 20 mM creatine phosphate (□). The upper curve, obtained in the presence of the ATP regenerating system, has been fit to the data as described in the text, while the lower curve was drawn by eye.

have suggested a kinetic scheme for this interaction in solution (Sleep and Taylor, 1976; White and Taylor, 1976; Chock et al., 1976; Lymn, 1974, 1975), and we use this kinetic scheme as a starting point for models of the analogous interaction in the fiber. Thus, we assume that force is produced by a cyclic interaction involving the dissociation of myosin and actin by ATP, the hydrolysis of the ATP by myosin while dissociated from actin, the rebinding of a myosin-products complex to the actin, followed by a power stroke. Recent evidence shows that the two myosin heads produce force independently in actomyosin threads (Cooke and Franks, 1978), and there is growing evidence that the two heads of each myosin molecule have identical primary chain sequences (Holt and Lowey, 1977; Schachat et al., 1977). Thus, the most reasonable model involves the independent action of each head in the force-producing interaction. We assume that one ATP is split during each cycle.

We first fit the isometric tension as a function of ATP concentration (Fig. 2), assuming that the cycle of events described above occurs in a fiber but that rates that involve an actomyosin complex may differ from those measured in solution. We show that this model can explain the isometric data. We then use energetic relations to derive some conclusions from the isotonic data taken at low ATP concentrations. The conclusions from both the isotonic and isometric data are then used to define a simple model that can explain all the data.

The Isometric Model

The complete kinetic scheme, constructed from the solution data, for the interaction of actin, myosin, and nucleotides, is too complex to be determined by the fiber data. Thus we simplify the kinetic scheme to four states, recognizing that some of these states are composites of several individual states. We ignore the back rate constants, so that the rates can be thought of as the flux from one state to the next. The kinetic scheme with which we will fit the isometric data is shown below:



where A represents actin, M represents myosin, and Pr represents either ADP or ADP + P_i. During an isometric contraction force is generated by myosin heads interacting with actins that are distributed throughout some force-producing region, and we assume that the above cycle can describe the interaction of all of these heads. Our present goal is to ask whether this

simple scheme will explain the curve of isometric tension vs. [ATP], and if so, to determine the rate constants of the model by the fit to the data.

Since ATP is capable of dissociating the actomyosin complex, it is clear that there exists a species of that complex whose population tends to zero as the ATP concentration becomes large. The decrease in the isometric tension that occurs as the ATP concentration is increased above 50 μM can be explained if this dissociable species, which we write as A · M., is force producing. The tension does not fall to zero as the ATP concentration is increased, however, so that the data demand the existence of a second force-producing species of the actomyosin complex that cannot be dissociated by ATP. While this undissociable species may be constructed by having the myosin head assume an undissociable conformation after a rapid release of products, it seems more natural to allow the products to remain bound in the state A · M · Pr, thus protecting the head from dissociation by ATP as the data require. Thus, both the states in which actin and myosin are complexed in the above scheme must generate force.

The isometric tension will be proportional to the number of myosin heads capable of participating in the cyclic interaction with actin and to the expected force produced in the cycle. The expected force produced in the cycle is just $F = \Sigma f_i p_i$, where f_i is the force produced by the state i and p_i is the probability of being in the state $i (\Sigma p_i = 1)$. We shall assume that only the two attached states produce force, and that they produce equal force (the assumption of equal force will be discussed later). Hence:

$$F(0, T) = F_a(p_{\text{A·M·Pr}} + p_{\text{A·M}}) \text{ (probability of cyclic interaction)}, \tag{1}$$

where $F(0, T)$ is the tension at zero velocity and [ATP] = T. One can write down four differential equations that describe the rate of change of the population of each of the four states in terms of the rate constants, the populations of the states, and the concentration of ATP. At steady state the rate of change of each state is set equal to zero, and the equations solved to give $p_{\text{A·M·Pr}}$ and $p_{\text{A·M}}$. All factors affecting the probability of interaction that do not depend on [ATP] may be absorbed into the constant F_a. At very low [ATP], the tension is low because few myosin heads are participating in a contractile cycle. This is a complex phenomenon that represents a balance between the rate at which ATP transfers heads into force-generating states and the rate of relaxation of these heads back to states that are not generating a net force. A description of this process goes beyond our present scope and we describe the low tensions at low [ATP] phenomenologically by an apparent Michaelis Menten constant K_m. The isometric tension is given by:

$$F(0, T) = F_a \frac{k_{12} + k_{23}T}{k_{12} + k_{23}T + k_{12}k_{23}T\tau} \cdot \frac{T}{K_m + T}, \tag{2}$$

where $\tau = k_{34}^{-1} + k_{41}^{-1}$ represents the average time spent dissociated.

Eq. 2 was fit to the isometric tension as a function of the ATP concentration by a nonlinear least-squares method by using standard programs on a Hewlett-Packard 9825 desk-top calculator (Hewlett-Packard Co., Palo Alto, Calif.). The equation provides a good fit to the data, as shown in Fig. 2. K_m is determined by the increase in tension at low ATP concentrations, and is found to be 2.3 ± 0.5 μM. The first factor in Eq. 2 can be reduced to two variables by dividing both denominator and numerator by any one of the variables (e.g., k_{12}) and the portion of the curve at higher ATP concentrations in Fig. 2 contains sufficient

information to determine these two variables. The data thus fix the ratios of the rate constants k_{12}, k_{23}, and τ^{-1}:

$$k_{23}/k_{12} = 4.2 \pm 1.1 \times 10^3\ M^{-1}; \quad k_{12}\tau = 0.94 \pm 0.12. \tag{3}$$

The fit to the data provides narrow constraints on the parameters, as shown above, but does not contain sufficient information to uniquely determine all the rates in the model.

The absolute values of the above parameters can be determined if one knows the rate at which heads complete an entire cycle. This rate is the ATPase rate for the whole muscle divided by the fraction of heads, f_0, that are actively participating in a tension-generating cycle. The factor f_0 must be introduced because we do not know the fraction of heads that will have an actin site with which an ATP splitting interaction can occur during an isometric contraction, e.g., in the model presented in Fig. 8, f_0 is $(X_2 - X_1)/L$. The cycle time is easily evaluated to give the isometric ATPase at high ATP, R_{max}:

$$R_{max} = f_0[k_{12}^{-1} + \tau]^{-1}. \tag{4}$$

The value of the isometric ATPase has been measured by several workers (Takashi and Putnam, 1979; Steiger, 1977; Arata et al., 1977) and we take an average value to be ~0.3 s^{-1} (per head at 10°C). The value of f_0 cannot be determined exactly but can be constrained to lie within a certain range. It must be <1, and a lower limit can be estimated from the fact that, as the value decreases, fewer heads are attached and thus each must be generating more tension. Taking 0.2 N/mm^2 as the isometric tension and assuming that each head exerts appreciable force over 12 nm (Barden and Mason, 1978), and that it cannot perform more work than that available from one ATP (~50 kJ/mol, Curtin et al., 1974), one calculates that more than 17% of the heads must produce force simultaneously. Of those heads that can participate in the cycle, $(1 + k_{12}\tau)^{-1} = 0.52$ are attached at high [ATP], and thus, f_0 is approximately >0.3. Although this calculation is not an exact one, it provides a rationale for a lower limit on the number of attached heads and establishes a rough estimate of the actual limit. We note that this argument can be reversed to yield an upper bound for f_0. Since we know that the muscle is capable of operating at ~50% efficiency, the work per head must be at least half of the ATP energy. Thus, f_0 is $\lesssim 0.7$.

By using a range ($0.7 > f_0 > 0.3$), the kinetic parameters of the model are found to be:

$$\begin{aligned} 0.8 &< k_{12} < 1.8\ s^{-1} \\ 3.6 \times 10^3 &< k_{23} < 7.6 \times 10^3\ M^{-1}s^{-1} \\ 0.8 &< \tau^{-1} < 1.9\ \ s^{-1}. \end{aligned} \tag{5}$$

The value of k_{12} is lower than that which has been measured indirectly in solution (100 s^{-1} [Lymn, 1974]). The slow release of products insures that at high [ATP], the head will remain attached for sufficient time and thus the "tension cost" will be low. The value of k_{23} is about two orders of magnitude lower than that measured in solution (White and Taylor, 1976). The value of $k_{12}\tau$ determines the fraction of attached heads for those heads that are interacting with actin and producing force. This fraction depends on the assumption that A·M and A·M·Pr produce the same force. This assumption is certainly arbitrary and the forces generated could be different. If these forces differed by more than a factor of three a

satisfactory fit to the fraction of attached heads at high ATP could not be obtained. Variations of less magnitude will change primarily the factor $k_{12}\tau$ and estimates of f_0 but will not affect the overall conclusions drawn from this model.

Our model also predicts the dependence of the isometric ATPase on the ATP concentration. Those myosin heads that are participating in a contractile cycle will have an isometric ATPase that is given by:

$$R(T) = R_{max}\frac{T}{K_{app} + T}; \text{ where } K_{app} = \frac{k_{12}/k_{23}}{1 + k_{12}\tau}. \quad (6)$$

Above approximately 20 μM ATP the probability of participating in a contractile cycle is independent of the ATP concentration and R_{max} is thus a constant. The value of K_{app}, and, hence, the dependence of the isometric ATPase rate on ATP concentration is predicted by the fit of the model to the data of Fig. 2, and has a value of 125 ± 30 μM. This is much higher than the K_m for acto-myosin ATPase in solution, ~6 μM (Moos, 1972); or myofibril ATPase, ~15 μM (Goodno, 1978). This prediction was recently confirmed by Takashi and Putnam (1979) in a direct measurement of the ATPase of an isometrically contracting fiber. They observe that half saturation is reached at ~150 μM, an order of magnitude above estimates from solution studies and in excellent agreement with our prediction.

Isotonic Contractions

The first theory of myosin cross-bridge kinetics put forward to explain the characteristics of muscle contraction was proposed by A. F. Huxley in 1957. In this model the myosin is dissociated rapidly at the end of a power stroke with a rate g_2 in a region in which the head is exerting a negative tension (i.e., a tension that opposes contraction). The most straightforward modification of this theory that will allow it to describe data at various ATP concentrations is to make g_2 dependent on the ATP concentration. As [ATP] decreases, corresponding to lower values of g_2, the maximum contraction velocity in this model decreases in a manner similar to that observed. However, the shapes of the curves are distinctly different from the experimental ones, as shown in Fig. 7. At low ATP concentrations the theoretical curve is concave downward with a negative second derivative, while the observed curve is concave upward with a positive second derivative. Furthermore, it can be shown that modifications of the force-distance relation in the region of negative force cannot solve this problem. We thus conclude that dissociation of the head by ATP is not significant in the region in which negative work is done.

We have found that the experimental data can be fit by a model in which the myosin head is dissociated by ATP in a region that preceeds the region in which negative work is performed. We may assume (quite generally) that if the head is not dissociated while traversing this region (region III in Fig. 8) that it is mechanically dissociated by the movement of the filaments, requiring an average energy $\overline{W}_-$. Using this model, we can derive an equation that describes the variation of the maximum contraction velocity with ATP concentration. The general force-velocity relation may be derived by writing:

$F \cdot V$ = mechanical power =

(work per interaction) (cycle time)$^{-1}$ (number of heads). (7)

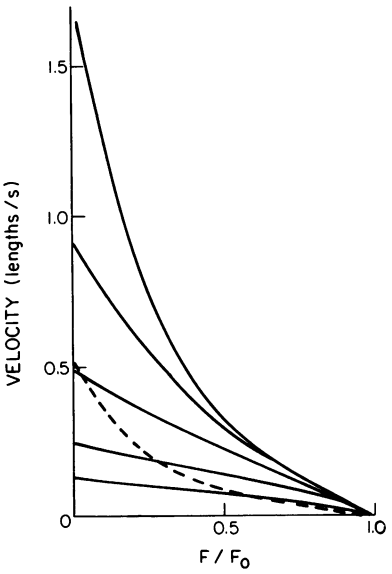


FIGURE 7

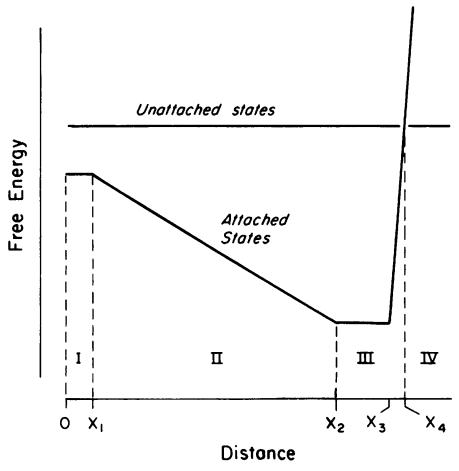


FIGURE 8

FIGURE 7 Force-velocity curves derived from the model proposed by A. F. Huxley (1957). The uppermost solid curve is given by the theoretical expression derived for his model (Eq. 11, A. F. Huxley, 1957) for f_1 85 s^{-1}, g_1 12 s^{-1}, and g_2 400 s^{-1}. The other solid curves are obtained by setting g_2 200, 100, and 25 s^{-1} (in decreasing values of V_m). The dashed line gives the observed data for 100 μM ATP.

FIGURE 8 Schematic model of the free energies for a simple model that explains both the isometric and isotonic contractions as a function of the ATP concentration. The horizontal line represents the free energy of unattached states while the lower curve represents the free energy of attached states, i.e., either A·M·Pr or A·M. The free energies are plotted as a function of x where x represents the distance of an actin site from the position at which it could first interact with a myosin head. Actin sites enter from the left. In region I there are fast rates for both attachment, f_1, and detachment, f'_1. ATP splitting is not involved in these transitions, and no force is produced. Region II is the "power stroke" region in which positive work is produced and is characterized by slow rates of attachment, f_2, and detachment, g_2, determined from the fit to the isometric data: $f_2 = \tau^{-1}$ and $g_2 = [k_{12}^{-1} + (k_{23}T)^{-1}]^{-1}$. In region III ADP is released rapidly, with rate k_D, and ATP binds rapidly, with a second order rate constant k_T, releasing the head from the actin. The rate of attachment is assumed negligible. Near the end of region III the free energy rises abruptly resulting in a negative force, as the head reaches the limit of its excursion. In region IV the energy of attached states exceeds that of unattached states, and the rate of detachment f'_4 is very rapid. The distances are defined as follows: $x_1 = 1$ nm, $x_2 - x_1 = 10$ nm, $x_3 - x_2 = 2$ nm, $x_4 - x_3 = 0.5$ nm. The rates of f and f' refer, respectively, to attachment and detachment processes that do not involve nucleotides, while the rates g and g' refer, respectively, to detachment and attachment processes that do involve nucleotides.

The work per interaction can be split into two components.[2] First, $\overline{W}$, the expected work done by a myosin head in traversing the power stroke, and second, $-\overline{W}_- f_u$, the work lost to the fiber each time a head has to be torn loose multiplied by the probability, f_u, that it will have to be torn loose. Hence:

[2]In the following we first separately average quantities such as the cycle time, work performed in a power stroke, etc., and we then manipulate these averages. This method, which takes the products of averages instead of the averages of products, will be a valid approximation when the distributions of these quantities are sharp. As will be seen below, these distributions will be sharp for the range of velocities we treat.

$$F \cdot V = [\overline{W} - \overline{W}_{-} f_u] \text{ (cycle time)}^{-1} \text{ (number of heads)}, \quad (8)$$

where $\overline{W}$, $\overline{W}_{-}$ and f_u are all functions of velocity and ATP concentration. Now we may rearrange to give:

$$F(V, T) = [\overline{W} - \overline{W}_{-} f_u] (\overline{X})^{-1} (\overline{X}/V) \text{ (cycle time)}^{-1} \text{ (number of heads)}, \quad (9)$$

where $\overline{X}$ is the expected distance traveled by a myosin head while attached to actin. But $\overline{X}/V$ is just the time spent attached; so $(\overline{X}/V)$ (cycle time)$^{-1}$ is the fraction of time the myosin head spends attached to actin, or simply the probability of being attached. Thus we have:

$$F(V, T) = [\overline{W} - f_u \overline{W}_{-}] \overline{X}^{-1} n(V, T), \quad (10)$$

where $n(V, T)$ is the number of attached heads at velocity V. Because neither $n(V, T)$ nor $\overline{X}^{-1}$ can approach zero as F approaches zero, the maximum contraction velocity, V_m, is determined by a balance between heads exerting positive and negative forces. Thus the term in square brackets in Eq. 10 must equal zero at V_m. At saturating ATP concentrations the maximum contraction velocity is determined by some process, such as a finite dissociation rate for ADP, that is independent of ATP. However, at low concentrations of ATP the step that determines the magnitude of f_u is the binding of ATP. Since we know from our analysis of the isometric data that head dissociation is slow in much of the power stroke, we assume that rapid head dissociation due to ATP binding occurs over a distance ΔX near the end of the power stroke with a second order rate constant k_T (for the binding of ATP); we assume that dissociation occurs very rapidly following ATP binding. If the fiber is contracting at velocity V, ATP has a time $\Delta t = \Delta X/V$ in which to bind, which means that the fraction of heads that are not dissociated by ATP is $f_u = \exp(-k_T[\text{ATP}]\Delta t) = \exp(-k_T[\text{ATP}]\Delta X/V)$. At V_m the term in brackets in Eq. 10 is zero, and rearranging we have:

$$V_m = [\text{ATP}]\{(k_T \Delta X)/\ln(\overline{W}_{-}/\overline{W})\}. \quad (11)$$

This expression gives a linear dependence of V_m on ATP, as shown by the data in Fig. 5 at [ATP] < 200 μM, if the expression in brackets is a constant. This expression will be a constant if and only if the ratio of the positive and negative work is a constant.

The above approach allows us to draw some important conclusions without relying heavily on the details of some model. Assuming only that head dissociation by the rapid binding of ATP occurs in a region ΔX with rate k_T we can calculate the probability that a head traverses this region undissociated. Eq. 11 then allows us to relate $k_T \Delta X$, V_m, and [ATP] to the unknown quantities $\overline{W}$. and $\overline{W}_{-}$, and shows that their ratio evaluated at V_m is a constant up to a velocity of approximately 1 length/s. Because $\overline{W}$ and $\overline{W}_{-}$ are determined by different factors the most obvious conclusion is that they are each constant. Although the above does not exclude the possibility that $\overline{W}$ and $\overline{W}_{-}$ change together in such a way as to keep their ratio a constant, the assumption that they do so leads to unreasonable models. As velocity increases, any change in $\overline{W}_{-}$ will be an increase, since heads torn off at higher velocities would be expected to possess more kinetic energy after detachment. Thus, $\overline{W}$ would have to also increase. This would require that the force generated increase, since $\overline{X}$ will not be a strong function of velocity at V_m where a large fraction of heads traverse the entire power stroke due to a slow rate of detachment found from the isometric data. Since it is unlikely that the ability of a head to generate force increases with velocity, the most reasonable assumption is that $\overline{W}$

and $\overline{W}_-$ are each independent of velocity up to ~1 length/s. If both $\overline{W}$ and $\overline{X}$ are independent of V_m, then $\overline{F}$, the average force generated by a head in a power stroke, is also independent of V_m. Furthermore, in the above model, the events that produce the force are not affected by the fact that the muscle is contracting at V_m; hence this conclusion can be generalized to any velocity, and we conclude that $\overline{F}$ is independent of the velocity of contraction.

A Simple Model to Fit Both Isometric and Isotonic Data

In the above we have analyzed the isometric data in terms of a four-state kinetic cycle and we have used some simple energetic relations to draw conclusions about force production in isotonic contractions. Together these two approaches provide several constraints that more detailed models must satisfy, and in this section we discuss these constraints and derive one simple model, outlined in Fig. 8, which incorporates them.

During an isometric contraction the low rate of ATPase demands that some transitions in the cycle be slow. The data of Fig. 2 are best explained by assuming a shift from attached force-producing states to unattached states at ATP increases. Thus, there must be sizeable fractions of both attached and unattached states at high ATP, implying that both the overall rates of detachment and attachment are slow. On the other hand, the isotonic data show that the work performed by each bridge is independent of velocity, which requires a fast rate of attachment. Thus the isotonic and isometric data must be reflecting rates of attachment and detachment of the head in different regions of head orientation, i.e., different values of X in Fig. 8. In fact, the apparent contradiction can be resolved by having a rapid rate of attachment preceding the power stroke (region I), and a slow rate in the power stroke (region II). The isometric data will be determined by the events occurring in region II, thus the rates in this region (f_2 and g_2) are determined from our four-state model of isometric contractions. In the above model, heads attach rapidly near the beginning of the power stroke in isotonic contractions, and, because of the slow detachment rates in region II, most heads will traverse its full length, thus doing a constant amount of work. Obviously, as v approaches zero $\overline{W}$ must also go to zero, and this process begins as v becomes less than $g_2(X_2 - X_1)$. We have argued that at the end of the power stroke there is a small region in which rapid dissociation of the myosin head by ATP can take place. Rapid release of products must also be occurring in this region, since product release is slow in region II but must precede ATP binding. Thus there must be fast rates for these processes in a small region (region III, assumed, for reasons discussed later, to be 2 nm long) that follows the power stroke. At the end of region III the head comes to the limit of its possible excursion and the free energy increases rapidly over a short distance. When the free energy of the attached head exceeds that of an unattached head we postulate a very rapid rate of detachment that does not involve ATP.

Fig. 8 shows an energy level diagram of a model that incorporates the constraints discussed above. To introduce the nucleotide interactions one must have more than two states. We assume that there is only one unattached state equivalent to M·Pr, i.e., the hydrolysis step, k_{34}, is fast compared with other rates. We assume that there are two attached states, A·M·Pr and A·M, which have the same free energy, shown as a function of X (the position of the actin relative to the myosin head) in Fig. 8.

The force produced as a function of velocity and ATP concentration can be calculated from the model quite simply by methods outlined in the Appendix. Force-velocity curves were

TABLE I
MODEL PARAMETERS

Determined from force-velocity curves	Determined from isometric tension
$(f_1 + f_1')\, x_1 = 0.15\ s^{-1}$ lengths^{-1}	$k_{12} = 1\ s^{-1}$
$f_1/(f_1 + f_1') = 0.55$	$k_{23} = 7{,}000\ M^{-1}\ s^{-1}$
$k_D \Delta x = 1.5\ s^{-1}$ lengths	$\tau^{-1} = 2\ s^{-1}$
$k_T \Delta x = 3{,}000\ M^{-1}\ s^{-1}$ lengths	
$W_-/W_+ = 1.5$	

Values of the parameters of the model, outlined in Fig. 8, used to fit the force-velocity curves shown in Figs. 3 and 6. The three parameters in the second column were constrained to lie within the limits outlined in Eqs. 5. The values of these three parameters, which are chosen here to fit both isometric and isotonic data, do not provide as good a fit to the data of Fig. 2 as do the values given in Eq. 3. In the equations used to fit the data some parameters can be grouped, e.g., in several instances we fit the product of a rate constant and a length, and the expressions listed above were the ones used. The maximum positive and negative work in regions II and III, W_+ and W_-, are given as follows: $W_+ = F_+ (x_2 - x_1)$, $W_- = F_- (x_4 - x_3)$, where F_+ and F_- are, respectively, the positive force generated in region II and the negative force generated in regions III and IV.

simulated on a Hewlett-Packard 97 calculator, and fit to the data. The values of the parameters that provided the best fit of the data are shown in Table I, and the curves are shown in Figs. 3 and 6. The fit at saturating ATP concentrations required that $(f_1 + f_1')X_1$ be small so that the fraction of attached heads decreased with velocity and the negative force term was appreciable only at higher velocities. At low ATP concentrations the force-velocity curves were determined by the values of $k_T \Delta X$ and W_-/W_+. The value of k_T should be comparable to that measured in solution, since the region ΔX represents the region of minimum free energy of the actomyosin complex, and if we assume $\Delta X \sim 2$ nm we find $k_T \sim 2 \times 10^6 M^{-1} s^{-1}$, in excellent agreement both with the solution measurement of $2\text{–}4 \times 10^6 M^{-1} s^{-1}$ (Sleep and Taylor, 1976, White and Taylor, 1976) and with our earlier conclusion that ΔX is small compared with the power stroke (~10 nm). The energy required to break the actomyosin bond at the end of the power stroke is about 1.5 times greater than the work produced in the power stroke. Approximating W_- as the standard free energy of an actomyosin bond measured in solution $W_- \sim 35$ kJ/mol, (for review, see Margossian and Lowey, 1978), W_+ is found to be ~25 kJ/mol. The maximum efficiency of the model is given by $W_+/\Delta G_{ATP}$, where $\Delta G_{ATP} \sim 50$ kJ/mol is the free energy available from ATP hydrolysis. Thus, the maximum efficiency is ~50%, in reasonable agreement with physiological measurements (Hill, 1938). We note here that modifications of this model discussed in the next section will increase the maximum efficiency of the model.

The value of $k_D \Delta X$ is not well constrained in our model since it plays an appreciable role only at high velocities and high ATP concentrations. The value given has been constrained to be too high because in our model the negative work required to break the actomyosin bond does not depend on the presence of bound products. If this simplification is relaxed the value of $k_D \Delta X$ will be decreased. Values of f_4' less than $10^3 s^{-1}$ did not fit the data, and the best fits were obtained for $f_4' > 10^4 s^{-1}$. We have argued above that the amount of negative work performed by an undissociated head is independent of the velocity. This will be the case if $f_4' > V/(X_4 - X_3)$. Taking $V = 1$ length/s and $X_4 - X_3 = 0.5$ nm $= 4 \times 10^{-4}$ lengths, we find

that $V/(X_4 - X_3) = 2.5 \times 10^3\ s^{-1}$, and thus the best fit of the model to the data again requires that the negative work performed by an undissociated head is a constant. The bond dissociated in region IV has been distorted mechanically so that the equilibrium constant for binding is approximately unity, a condition in which very rapid dissociation rates are not unreasonable.

The model discussed above is obviously an oversimplified one, and its details cannot be considered significant. Rather, it is designed to show that the qualitative conclusions we drew from the analysis of the isometric and isotonic data can be incorporated into a single model within the framework originally proposed by Huxley (1957) and since used by many other workers. Because we have fit a whole family of force-velocity relations, we have been able to specify the rates for nucleotide interactions, and have arrived at different rate constants than those of previous workers. Although the complete model of Fig. 8 may appear complex, its qualitative features are quite clear. Heads attach rapidly at the beginning of the power stroke, move through it with almost no dissociation, and are dissociated rapidly by ATP at the end of the power stroke. Undissociated bonds are then broken by the motion of the filaments. Such a mechanism operates with high efficiency across a broad range of velocities, and the rate of ATP hydrolysis is tied in part to the rate at which work is performed, as first observed by Fenn (1923).

Modifications of the Model

In this section we consider several modifications of the simple model discussed above, beginning with the incorporation of thermodynamic restrictions that link the kinetic and energetic parameters. Hill and co-workers have discussed the application of these constraints to models such as the one above and in the following we shall follow their notation and methodology (Hill et al., 1976). For each rate f and g we introduce a reverse rate f' and g'. The rates are then connected to the free energies through:

$$f/f' = \exp(+\Delta)$$
$$g/g' = \exp(-\Delta + \Delta_T), \tag{12}$$

where Δ is the free energy of unattached states minus that of attached states and Δ_T is the free energy obtained from the hydrolysis of ATP (free energies are expressed in units of kT throughout). From the fit to the data we found that $f_1 \sim f_1'$, thus in region I Δ is approximately zero. At X_3 we approximate Δ as the free energy required to break an actomyosin bond, $\Delta \sim 14$. Thus in region II Δ varies linearly from ~ 0 at X_1 to 14 at X_2. The back rate f_2' can now be defined in terms of f_2 and Eq. 12 above:

$$f_2' = 2 \exp[-14(X - X_1)/(X_2 - X_1)],$$

where $f_2 = 2\ s^{-1}$. Thus f_2' will decay rapidly as X increases. Due to the low values of f_2 and f_2' the effect of f_2' will mainly be felt in the number of attached heads in region II during an isometric contraction, and it will change this number by $<5\%$. Because Δ_T is estimated to be ~ 20, (Curtin et al., 1974), which is larger than Δ throughout regions II and III, the back rate g_2' and its equivalent in region III will be negligible. Thus, introducing the above rates will not greatly effect the number of attached heads in regions II and III. However, the fit to the data now requires that we modify our view of what happens in region IV. Using the ratio $W_-/W_+ = 1.5$ and our estimate of Δ at X_3 we calculate that heads must be dissociated with

energies that exceed that of the unattached states by ~6. This can be accomplished by moving X_4 approximately 0.2 nm to the right in Fig. 8. Taking $f'_4 = 10^4 s^{-1}$ as before, we find that f_4 is 24 s^{-1} at X_4 and decreases rapidly as X increases. A simple calculation shows that the number of attached heads once again decays rapidly with X in region IV and the net negative work performed is still a constant. While these modifications produce some further insights into this model they have not changed the basic curves calculated from the more simple model proposed in the section above.

Certain additions to the model could be introduced to make it more realistic biochemically. Among these is the insertion of a finite rate for the hydrolysis step (or for the transition from a refractory to a nonrefractory state: Chock et al., 1976) that would give rise to two significant detached states. In addition, the states A·M and A·M·Pr should be given different free energies so that there will be a finite driving force for product release. As stated above, this would decrease our estimate of k_D. Both of these modifications would require considerably more data to determine the new parameters they would introduce. Thus, we feel that the simple model presented provides a satisfactory physical explanation of our data. It sacrifices a more detailed and realistic framework but allows simplicity of calculations leading to insight into its mechanisms.

Relation to Other Work

We have worked with glycerinated rabbit psoas muscle fibers at 10°C, and one question concerns whether our results may be generalized to living fibers. At high ATP our fibers contract at up to 2 lengths/s, which is close to what one expects for the living fiber at this temperature. The glycerinated fibers exert ~0.1 N/mm^2, which is lower than found for most living fibers. This is probably because we worked at 10°C, since at 20°C the fibers will produce ~0.2 N/mm^2. We chose to work at the lower temperature because the tension of the fibers is more stable and the velocities are slow enough to measure. Data on the ATP dependence of isometric tension have been taken on cardiac fibers (Best et al., 1977), and on skeletal frog fibers (Magid, 1974). Although the drop in tension at [ATP] < 50 μM appears to depend on the type of preparation (Kawai and Brandt, 1976), in all cases a drop of ~30% in tension is observed as the ATP concentration is raised from 50 μM up to 1 mM. Arata et al. (1977) also find a maximum in the isometric tension of psoas fibers at 20–30 μM ATP. We know of no published data on the ATP dependence of the force-velocity relation in other muscle systems; however, the velocity of contraction of myofibrils increases as ATP concentration increases over a wide range of ATP concentrations (Arata et al., 1977). In addition, Kawai (1978) finds that the frequency response of muscle fibers, and therefore some of the rate constants relating to the velocity of contraction, are sensitive to ATP at high ATP concentrations (1–5 mM). The frequency of oscillation of flagella is also dependent on the ATP concentration in a manner similar to that observed here for fibers (Brokaw, 1975), and this process has been modeled in some detail by Brokaw and Rintala (1977). Thus it appears that the maximum velocity of both flagella and muscle is controlled up to high substrate concentration by a similar rate-limiting step, the binding of substrate.

CONCLUSION

When the ATP concentration inside a fiber is varied, it produces complex changes in isometric and isotonic contractions. Our main conclusion is that these changes can be adequately

explained by simple theories, consistent with the known biochemistry of the contractile interaction, in which the action of ATP is to dissociate an actomyosin bond. The isometric data can be explained by a simple four-state model. We find a rapid rate for the step in which ATP binding dissociates a myosin head from actin, but the overall rate of head dissociation is slow, indicating that some mechanism operates to protect the head from dissociation. A prediction that the ATPase of an isometrically contracting fiber saturates at a higher level of ATP than that measured for actomyosin in solution found experimental justification in the results of Takashi and Putnam (1979). As the ATP concentration is decreased below 1 mM the maximum contraction velocity, V_m, decreases dramatically at concentrations where the isometric tension is not greatly affected. The isotonic data at low ATP concentrations indicated that rapid dissociation of the actomyosin bond occurs in a small region that follows the power stroke, with a rate constant that is close to that measured for the analogous reaction in solution. An analysis on this basis concluded that the force generated by a head in a power stroke is independent of the velocity of contraction (up to ~1 length/s). The above conclusions were incorporated into a simple model of the actomyosin-nucleotide interaction, which explains both isometric and isotonic data. The essential features of this model are that in an isotonic contraction heads attach rapidly at the beginning of the power stroke, move through it with almost no dissociation, and are dissociated rapidly by ATP at the end of the power stroke. Bonds not dissociated by ATP must be broken by the motion of the filaments.

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APPENDIX

We here derive an expression for the force as a function of velocity, ATP concentration, and the parameters of the model outlined in Fig. 8. The method is completely analogous to that used by Huxley (1957). Starting in region I, the fraction of attached heads, $n(x)$, is related to the rate constants by:

$$V\frac{\partial n}{\partial x} = (1 - n)f_1 - nf_1' \tag{1}$$

solving by integration with the boundary condition, $n(0) = 0$, we find:

$$n(x) = \{1 - \exp[-(f_1 + f_1')x/V]\}[f_1/(f_1 + f_1')]. \tag{2}$$

Using similar procedures we find that the number of attached heads in region II relaxes exponentially from $n(x_1)$ to its equilibrium value $f_2/(f_2 + g_2)$. Integrating from x_1 to x_2 and dividing by the actin spacing, L, we find the total number of attached heads in region II:

$$N_{II} = \frac{f_2(x_2 - x_1)}{(f_2 + g_2)L}\left\{1 - \frac{V}{f_2(x_2 - x_1)}\left[\frac{f_2}{f_2 + g_2} - \frac{f_1}{f_1 + f_1'}\right.\right.$$
$$\left.\left.(1 - \exp(-(f_1 + f_1')x_1/V))\right][1 - \exp(-(f_2 + g_2)(x_2 - x_1)/V)]\right\}. \tag{3}$$

The positive force exerted will be equal to F_+N_{II} where F_+ is the force generated by each attached head in region II. The negative force will be given by $F_- \int_{x_4}^{\infty} n(x)\mathrm{d}x/L$. To simplify this expression we assume that few heads are dissociated over the small distance x_3 to x_4 and thus the force in this region is $F_-n(x_3)(x_4 - x_3)/L$. The value of $n(x_3)$ is obtained by noting that in region III $n(x)$ decays from $n(x_2)$ as a result of two processes: ADP release, and subsequent ATP binding with dissociation. Evaluating the integral:

$$\text{probability head off at } x = \int_{x_2}^{x} (\text{probability ADP released at } y)\,(\text{probability that ATP binds between } y \text{ and } x)\,\mathrm{d}y. \quad (4)$$

Then $n(x_2)$(1-probability head off at x_3) we find:

$$n(x_3) = \left\{\exp(-k_D\Delta X/V) - \frac{k_D}{k_TT - k_D}[\exp(-k_TT\Delta X/V) - \exp(-k_D\Delta X/V)]\right\} \left\{\frac{f_2}{f_2 + g_2}\left\{1 - \left[1 - \frac{(f_2 + g_2)f_1}{(f_1 + f_1')f_2}(1 - \exp(-(f_1 + f_1')X_1/(V))\right]\right\} \exp(-(f_2 + g_2)(x_2 - x_1)/V)\right\}. \quad (5)$$

The negative force exerted in region IV is given by:

$$F_- \int_{x_4}^{\infty} n(x)\,\mathrm{d}x/L \quad (6)$$

which can be evaluated to give:

$$F_- \frac{n(x_3)}{L}\frac{V}{f_4'}. \quad (7)$$

The average force generated by a myosin head is now calculated from:

$$F = F_+n_{II} - F_-n(x_3)(x_4 - x_3)/L - F_-n(x_3)V/Lf_4'. \quad (8)$$

We normalize our curves to the force produced at $V = 0$: $F = (f_2/(f_2 + g_2)\,[(x_2 - x_1)/L]\,F_+$.

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