Force Generation, Work, and Coupling in Molecular Motors

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ABSTRACT A mechanism is proposed for molecular motors in which force is generated by a protein conformational change driven by binding energy (in muscle, that of myosin with actin as well as with ATP, ADP, or P_i). Work, the product of the force generated by one myosin or kinesin molecule (F) and the distance over which it acts (d), is a function of a ratio of dissociation constants before and after the contractile step: $F \cdot d < RT \ln(K_{Ae}/K_{Ac})$. From published data the ratio is $>2 \times 10^4$, which can be explained by conversion of a surface complex to an enclosed, or partly enclosed, complex. Although the complex performing the work stroke is in an unstrained conformation, the complex after the work stroke is much more stable, owing to binding forces; the latter, however, is destabilized by the load, which thereby opposes the contractile conformational change, countering the force-generating reaction. The connection between free energy release and work is implicit in the mechanism, inasmuch as coupling, like force generation, depends on conformational changes driven by binding energy (internal rather than external work being involved in coupling). The principles apply whether ATP or an ion gradient drives the system. At high load, in muscle, the mechanism allows for a summation of the forces generated by several myosin molecules.

INTRODUCTION

In muscle, the tension developed in the myofibril undoubtedly has its origin in intermolecular forces, but how the two are linked remains unclear. As Woledge et al. (1985) have remarked, the theory describing muscle contraction is divided: on one side, the reaction of ATP, involving molecular interactions; on the other side, shortening of the myofibril, involving events on the scale of the cell and tissue. The challenge is to connect the two. Ultimately, the explanation of a macroscopic force comes down to forces between atoms and molecules, which can be expressed in terms of binding energy and binding constants. Several aspects of the problem will be explored here: the origin of the force produced by a single molecular motor, the balance between force and the resisting load, and the connection between work and the release of free energy (from ATP or an ion gradient). The best understood molecular motor is muscle, and muscle will be the focus of attention in the following discussion.

The force of muscle contraction has been accounted for in various ways. In some models the binding of ATP produces a strained (i.e., a high-energy) conformation of the myosin molecule, on the analogy of a stretched spring (Taylor, 1993), and shortening is what happens when the strain is released. The hypothesis may be criticized on two grounds: first, a high-energy intermediate would act as a kinetic barrier in any reaction sequence, and second, the free energy of ATP hydrolysis cannot be transferred in any one step in a reaction, according to the principles governing energy transduction in biological systems (Hill and Eisenberg, 1981). The free energy is a function of the relative concentrations of reactants and products, and the driving force in muscle contraction or active transport is not the standard free energy of the reaction but the ongoing conversion of ATP to ADP and P_i, dependent on the continuous removal of the products by the ATP-synthesizing machinery of the cell. Any chemical reaction, whatever the energy level of the reactive bond, could drive the process (see Atkinson, 1977; de Meis, 1993). The standard free energy, a function of the equilibrium constant, is of importance only because in the steady state the product should be present at a concentration about as high as the reactant, to be handled conveniently by cellular enzymes and carriers; under these circumstances there will be a continuous net reaction in the forward direction only if the equilibrium greatly favors the products.

Force generation has also been explained by what appears to be an entirely different mechanism, a "thermal ratchet" (Braxton and Yount, 1989; Vale and Oosawa, 1990; Cordova et al., 1992; Magnasco, 1993; Maddox, 1994; Astumian and Bier, 1994). Thermal energy is seen as providing the impetus to move one component of the system (myosin) over a series of steps marked along the other component, the ratchet (the actin filament). Continuous movement in one direction, rather than random movement, depends on an asymmetric ratchet structure and on an input of free energy, which is supplied by ATP. The mechanism is not such a departure from ordinary biochemical thinking as it first appears to be. Only a perpetual motion machine could extract energy from Brownian motion to do work. But thermal energy does play an essential role, in any mechanism, being required to get the system across some energy barrier, in a process of vectorial displacement; this is true whether one component of the system is to be raised above a physical barrier erected in a second component (as with a ratchet), or whether the conformation of a protein molecule

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is to be shifted. That is, thermal energy is absorbed in forming the transition state but does not drive the reaction. Net displacement, in one direction, has to be explained by another component of the system that makes free energy available. Thus, a thermal ratchet can be thought of as an alternative view of a process describable in a more familiar and more helpful way, involving conformational changes. The great weakness in thermal ratchet theories is that ratchet movement remains isolated from ATP hydrolysis, no connection being made to the real energy source for force generation and displacement of the load—ATP and not thermal energy.

The general point may be made that a successful theory will have to explain, simultaneously, i) the connection between macroscopic and microscopic force and ii) the connection between work and the release of free energy. Also to be accounted for are the effects of the opposing load on the net force and on the ATP reaction, and in the case of muscle the shifting of loads greater than the force generated by one myosin molecule. What seems to be lacking are concepts relating force, as well as load, to elementary processes, expressible in physical-chemical equations, and giving rise to testable predictions. Such concepts will underlie any model of the system that is proposed.

A mechanism relating macroscopic force to interactions between atoms and molecules is necessarily constrained by physical and chemical principles, and its predictions, therefore, are not arbitrary. Thus, when a molecular motor exerts force on the surroundings, there must, as a minimum, be an equivalent shift in intermolecular forces. In the mechanism proposed here, force is related to well-understood concepts, those of binding energy and conformational changes. The idea that ligand-induced conformational changes underlie a variety of biological functions, including muscle contraction, was clearly expressed by Eisenberg and Hill (1985); the idea is now developed by explicitly relating the magnitude of changes in binding energy during conformational changes to the tightness of coupling and to the development of tension. In the case of muscle, the binding energy is that between myosin and what may be called, in a broad sense, its substrates—actin, which is to be translocated, and ATP, which provides the free energy for movement (the analogy is with an ATP-driven membrane pump that translocates substrate ions). Contraction does not depend on a sudden release of energy in the ATP reaction, and the contractile complex—the state of myosin which through a change in conformation draws actin up to a new position—is not in a strained conformation. Instead, the conformational change responsible for shortening is driven by substrate binding forces (the binding forces becoming stronger in the altered conformation). An equation is derived relating the tension produced by one myosin head to equilibria for complex formation between components of the system before and after contraction. The calculated increment in binding energy, based on experimental measurements of the force produced by myosin or kinesin molecules, is found to be well within the range of forces that can be exerted in such a complex. In principle, the mechanism may be tested by determining the binding energy (i.e., association constants) for components of the system at different stages of the reaction.

The link between ATP hydrolysis and force generation is implicit in this treatment, inasmuch as the mechanism by which ATP hydrolysis is coupled to a vectorial process also depends on conformational changes driven by substrate binding energy (Krupka, 1993a,b). Consequently, the equations relating binding energy to force and to the tightness of coupling are of similar form.

The proposed mechanism, with the equations for force, accords with the reversible nature of actual processes, accounting for both the generation of force and the effect of the load on the force-generating reaction. A model formulated so that only the forward direction is taken into account, with no term for the load appearing in the equations, would be of limited applicability and could not be related to free energy changes. Models in the literature do not appear to have included these terms and therefore would not be able to deal with a system under load.

With loads greater than the force developed by a single myosin molecule, a possible coordinated action is indicated. It is self-evident that if the load against which a fiber pulls is many times greater than the force that can be exerted by a single myosin head, then one myosin molecule, acting alone, could not shorten the fiber. If many myosin heads are intermittently poised to exert force, with no two being in this state at the same time, then again the contractile force at any moment will be insufficient to overcome the resistance of the load. Only during a period when several heads are poised for action could the resistance be overcome. At this time, as shortening occurs, the heads responsible would have to undergo whatever transformation underlies force generation. Thus, even if the myosin heads were independent, under load they could be constrained by the actin filaments to which they attach to move in the force-generating step as a group rather than singly. These myosin molecules could be at adjacent positions in a filament, or at distant positions, or even in different filaments. (A consequence of joint action is that the force should increase in a linear fashion with the number of myosin molecules, as found by Kishino and Yanagida (1988).) Equations will be derived for the net force (the difference between the total force generated and the load) under these conditions. When appended to existing models, the mechanisms should allow an extension to more realistic, and more complex cases, in which account is taken of varying load and of a possible interaction among myosin molecules in all sections of the fiber. In the literature, these problems do not appear to have been dealt with.

COUPLING FREE ENERGY TO A VECTORIAL PROCESS

The principle on which coupling is based is this: two processes will be coupled, and the free energy of one will drive

the other, if the reactions in each are constrained to flow through a single sequence. For example, ATP will drive a vectorial process—by which is meant the directed movement of a cellular component—provided the reaction is made to follow a path that involves both ATP hydrolysis and movement of some substrate; and an ion gradient will drive a vectorial process provided the reaction is made to follow a path that involves movement of both the driving ion and the driven substrate, as in secondary active transport. It becomes apparent, from such sequences, that deviation from the prescribed path allows ATP to be hydrolyzed, or the ion gradient to collapse, without substrate movement; this is referred to as slippage—the uncoupled reaction. The transport model in Fig. 1 illustrates the point. The sequence around the periphery of the diagram is the coupled reaction, the lower cycle an uncoupled hydrolysis of ATP. The latter is avoided if the initial complex with the translocated substrate S is immobile, unlike the free carrier and the ternary complex with ATP, which are mobile (f_2, $f_{-2} \ll f_1$, f_{-1}, f_3, f_{-3}); and the importance of the two paths depends on how sharply the mobility of the binary complex is curtailed.

The problem has been to understand how the reaction path is controlled, and at what cost. The mechanisms have become clearer through an analysis of exchange transport, secondary active transport, and primary active transport (Krupka, 1989a, 1993a,b). From Fig. 1, the required control depends on abrupt shifts in the properties of the carrier as the reaction proceeds—in its mobility, and in its specificity in binding and catalysis. These shifting properties depend on conformational changes in the carrier protein, and the conformational changes are driven by substrate binding

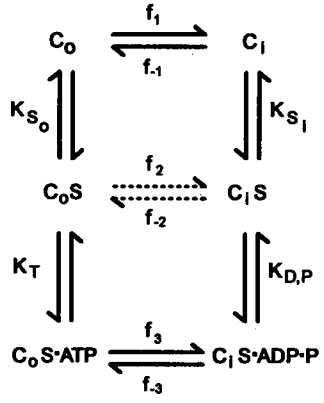

FIGURE 1 A carrier model for ATP-driven transport of substrate S. The substrates add in fixed order, S followed by ATP, and transport depends on rotation of the transport site (the site for S) between inward-facing and outward-facing forms, C_o and C_i, respectively. The ATP site, by contrast, is stationary. The transport site is exposed in the free carrier, whereas the ATP site becomes exposed only when S is bound. The outward-facing carrier-substrate complex, C_oS, specifically binds ATP, whereas the inward-facing carrier-substrate complex, C_iS, specifically binds ADP and P_i. In the ternary complex, ATP is hydrolyzed as the binding site for S rotates across the membrane. For coupling, the binary complex (C_oS and C_iS) must be immobile ($f_2 \ll f_1$).

forces. The cost of control, then, is paid in binding energy; hence the tightness of control may be expected to be related to the expense of binding energy, and this is found. A relationship exists between the ratio of coupled to uncoupled rates and the ratio of substrate dissociation constants before and after a conformational change:

$$K_{(\text{initial state})}/K_{(\text{final state})} > \text{Rate}_{(\text{coupled})}/\text{Rate}_{(\text{uncoupled})}. \quad (1)$$

Eq. 1 is general: it applies to primary and secondary active transport, as well as to exchange-only transport (antiport); it applies when the role of the substrate is to shift the equilibrium between two conformations by binding preferentially to one, and when the role is to increase the rate of a conformational change by binding strongly in the transition state; it applies to transport models with substrate sites alternately exposed on opposite sides of the membrane (as in the ordinary carrier model) and to models with sites simultaneously exposed on both sides, where the translocated substrate jumps from one site to the other; in active transport it applies to the driven substrate, such as a sugar molecule or calcium ion, and to the driving substrate, Na^+ or H^+ in secondary active transport, or ATP in primary active transport; and it applies whether the substrates add in random or fixed order, and whether the order on the two sides of the membrane is the same or different.

The principle of coupling in muscle, or any molecular motor, should not be different from that in active transport: ATP will drive the relative movement of myosin and actin filaments if the hydrolysis reaction and movement are intercalated in a single controlled sequence. That force has to be generated in one step in the reaction does not affect the problem of coupling; the requirement is still for a controlled reaction. The analysis suggests that the common denominator in coupling and force, and therefore the link between them, could be binding energy and induced conformational changes.

That coupling—of the ATP reaction to substrate movement—can be mediated, at long range, by conformational changes affecting distant regions of a pump protein, rather than through direct interaction between ATP and the substrate, has been demonstrated in studies of the calcium pump, where Ca^{2+} is bound in the membrane domain, and ATP in a domain outside the membrane (MacLennan and Toyofuku, 1992). Similarly, the ATP site in myosin and the site interacting with actin are separate (Rayment and Holden, 1994; Spudich, 1994).

THE GENERATION OF FORCE

Force and substrate binding energy

Internal work, unlike external work, is always involved in coupled transport, inasmuch as coupling depends on a ligand-induced switching of the conformation of the carrier, as pointed out above. The basis of these abrupt changes is indicated in Fig. 2, where a more stable conformation, C′, can be converted, at the expense of substrate binding en-

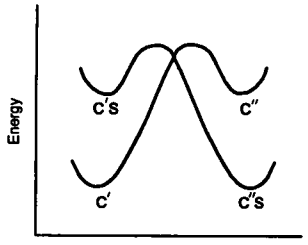

FIGURE 2 Energy profile illustrating the basis of the abrupt conformational change required for tight coupling. In this diagram and in those that follow the vertical dimension is energy, the horizontal dimension a reaction coordinate. Here, the free carrier is more stable in conformation C′ than C″; the substrate, by binding more strongly to C″ than to C′, shifts the conformation from C′S to C″S. As the resistance to the conformational change is that of the carrier protein itself, the work performed, at the expense of substrate binding energy, is internal.

ergy, to an inherently less stable conformation, C″. There is experimental evidence for such a two-state conformational equilibrium as the basis of ligand-induced switching. In studies of the acetylcholine receptor it is found that the ion channels, without acetylcholine bound, open spontaneously for brief periods, when they have the same intrinsic conductance and ion selectivity as the channels gated by acetylcholine (Jackson, 1994). The open conformation, therefore, must be formed, although at very low concentration, even in the absence of bound ligand. In Fig. 2, the greater substrate binding energy in conformation C″ exactly compensates for its lower stability, and the result is an abrupt switch from one state to another when the substrate is bound. The resistance to the transformation is that of the carrier protein itself, and the work is therefore internal.

If the protein were to offer no resistance at all, as when the initial and final conformations are equally stable in the absence of the substrate, the whole increase in binding energy between one state and the other would be available for external work. In Fig. 3, a contractile protein M (myosin), which forms a complex with substrate A (actin), exists in two equally stable conformations, extended and contracted, M_e and M_c, respectively. If the binding forces are stronger in M_c than in M_e, it should be possible, as illustrated in Fig. 4 *a*, to harness the conformational change A_eM_e to A_cM_c, the available free energy being a function of the equilibrium constant $K_2 = [A_cM_c]/[A_eM_e]$.

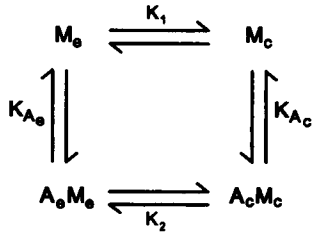

FIGURE 3 Conformational equilibrium of two forms of protein M, which forms a complex with A (M and A can represent myosin and actin, respectively). The conformations are extended, M_e, and contracted, M_c; K_{Ae} and K_{Ac} are dissociation constants.

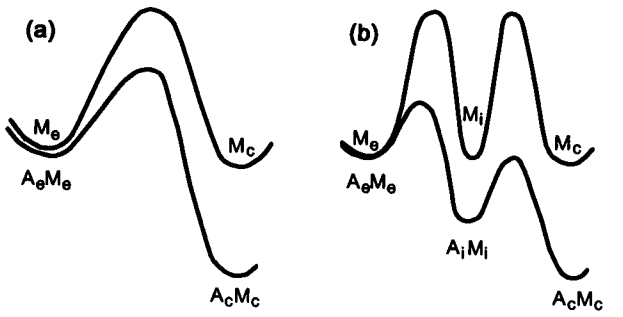

FIGURE 4 Energy profile for a conformational change in which the work generated by the binding forces can be external. Substrate A forms a complex with protein M, and in doing so stabilizes a new conformation (as in Fig. 3, A and M can represent actin and myosin, respectively). (*a*) Protein M exists in two conformations, M_e and M_c, which are equally stable in the free form but not when complexed with A; A binds more tightly to M_c, and consequently A_cM_c is more stable than A_eM_e. In shifting the conformation, the substrate binding forces are not opposed by internal resistance of the protein and are therefore available to drive an external process. (*b*) Similar to *a*, except for an intermediate conformation, M_i and A_iM_i.

A disadvantage should be noted, however, in having M_e and M_c equally stable (with $K_1 = [M_c]/[M_e] = 1$). First, only part of the protein, M_e, would be available to generate force, and second, the contracted complex could form directly from the contracted state of unattached myosin, $M_c \rightarrow A_cM_c$, bypassing the route through which tension is generated, $M_e \rightarrow A_eM_e \rightarrow A_cM_c$. K_1, therefore, may be less than unity, when only part of the increased binding energy in A_cM_c relative to A_eM_e is available for external work.

A work function may be found as follows. With $K_1 < 1$,

$$K_2 = [A_c \cdot M_c]/[A_e \cdot M_e] = K_1 \cdot K_{Ae}/K_{Ac} < K_{Ae}/K_{Ac}. \quad (2)$$

The standard free energy of the conformational change is

$$-\Delta G^\circ = RT \ln K_2 < RT \ln(K_{Ae}/K_{Ac}). \quad (3)$$

If the force developed is F and the distance over which it acts is d, the work that can be performed is $F \cdot d$. In any real system the conversion of energy into work is incomplete; hence

$$F \cdot d < RT \ln K_2 < RT \ln(K_{Ae}/K_{Ac}) \quad (4)$$

$$K_{Ae}/K_{Ac} > K_2 > e^{F \cdot d/RT}. \quad (5)$$

Work is done by the system when the conformational change $A_e \cdot M_e$ to $A_c \cdot M_c$ in Fig. 3 is opposed by an external resistance, the load. And the load, by drawing against tension in the fiber, counteracts the conformational change, $A_e \cdot M_e$ to $A_c \cdot M_c$, reducing the stability of the contracted relative to the extended conformation, as illustrated in Fig. 5. If the load borne by one myosin molecule is L (which, properly, includes both internal friction and external load), the net force is $F - L$ (because the two forces are in opposition). The opposing force shifts the equilibrium, K_2, between the extended and contracted conformations (Fig. 5). From Eq. 5, K_2 under load, $(K_2)_L$,

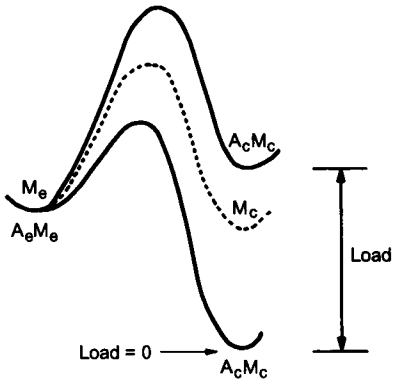

FIGURE 5 Energy profile illustrating the effect of an applied load on the relative stability of the extended and contracted conformations of the actin-myosin complex, A_eM_e and A_cM_c. The conformational change can be harnessed to do external work, drawing against the external load and displacing the actin filament relative to the myosin filament; reciprocally, the load counteracts the conformational change. The load has no effect on the conformational equilibrium of free myosin, M (*middle curve, dotted*). As in Fig. 4 *a*, the complex is inherently more stable in the contracted form, the difference in energy levels being available to work against the load (*lower curve*). The load, drawing against the conformational change, can make the contracted form less stable than the extended form (*upper curve*).

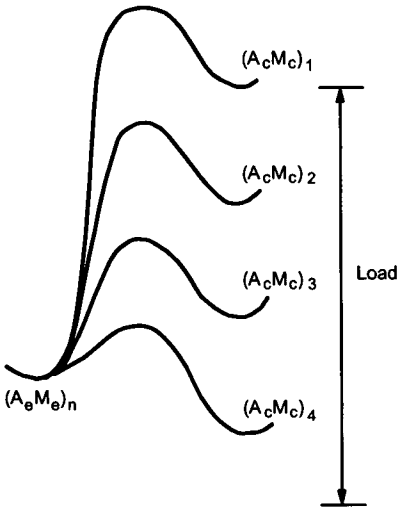

FIGURE 6 Energy profile illustrating the effect of the load on the relative stability of the extended and contracted conformations of myosin, in the hypothetical case in which several myosin heads can attach to the actin filament as the contractile complex. A load far larger than the force developed by a single myosin head raises the energy level of the contracted conformation of a single complex $(A_cM_c)_1$, making it far less stable than the extended conformation A_eM_e and therefore blocking the contraction step (extended to contracted conformation). Each additional complex between myosin and actin reduces the combined energy level of the contracted conformation of the attached heads. When, with n myosin heads engaged, the contracted conformation of the whole becomes more stable than the extended conformation, and contraction, assumed to depend on a concerted conformational change, becomes possible: $(A_eM_e)_n$ to $(A_cM_c)_n$.

is given by

$$(K_{Ae}/K_{Ac})_L > (K_2)_L > e^{(F-L)\cdot d/RT}. \quad (6)$$

Note that if $L \gg F$ then $(K_2)_L \ll 1$; that is, the equilibrium favors the initial state, before contraction, and the contractile event is effectively blocked.

Because the constants in a cyclic reaction are interrelated ($K_2 = K_1 \cdot K_{Ae}/K_{Ac}$), and because K_1 is unaffected by the load, the decrease in K_2 under load alters the relative affinities, K_{Ac} and K_{Ae}, weakening binding in the contracted relative to the extended state.

Force generation at high load

With a load several times the force developed by one myosin head the contractile conformational change would be blocked (Eq. 6). What are the implications of the mechanism under these conditions, when, as suggested in the Introduction, shortening could depend on the action of several attached cross-bridges, whose combined tension exceeds the load?

By hypothesis, the net tension, the load, and the number of contractile complexes will be interrelated (see Fig. 6). Let the load, L, be m times the force F developed by one contractile complex: $L = m \cdot F$; and assume that the tension developed by a number, n, of contractile complexes is additive: the total contractile force is $n \cdot F$. With the two forces in opposition, the net force is $n \cdot F - m \cdot F = F(n - m)$.

The intrinsic constant for the driving conformational change in a single complex under zero load is K_2, and the corresponding free energy change is $-\Delta G°$. Let the effective constant for the combination of n complexes, under the load $m \cdot F$, be $(\bar{K}_2)_{n,L}$, corresponding to an overall free energy change $(-\Delta \bar{G}°)_{n,L}$. Their ratio, from Eq. 5, is

$$(\bar{K}_2)_{n,L}/K_2 = e^{F(n-m)d/RT}/e^{F\cdot d/RT} = e^{F(n-m-1)d/RT}. \quad (7)$$

When $n \ll m$, the ratio is much less than unity; the contractile force under load is then much smaller than that from a single unloaded complex, and the conformational change involved in the work stroke is blocked. But when $n = m + 1$ the ratio is unity and the net force is the same as for a single unloaded complex; the contraction step, involving all the contractile complexes, should then ensue.

$(\bar{K}_2)_{n,L}$ and $(-\Delta \bar{G}°)_{n,L}$, as defined above, are related to the aggregate force driving the conformational change in a group of n contractile complexes. The equilibrium constant of an individual complex in the group, $(K_2)_{n,L}$, may be found as follows. Assuming that the free energy $(-\Delta \bar{G}°)_{n,L}$ is equally partitioned among the n complexes, the free energy of each conformational change would be $(-\Delta \bar{G}°)_{n,L}/n$. Then, from the relationship between the standard free energy and the equilibrium constant, $-\Delta G° = RT \ln K$, it follows that

$$\ln(K_2)_{n,L} = (1/n)\ln(\bar{K}_2)_{n,L} \quad (8)$$

$$(K_{Ae}/K_{Ac})_{n,L} > (K_2)_{n,L} = \{(\bar{K}_2)_{n,L}\}^{1/n}. \quad (9)$$

As an illustration, suppose that the load is 40 times the force developed by one myosin head, and that 41 complexes

combine to overcome this load; from Eq. 7, with $n = 41$ and $m = 40$, the net force is the same as that of a single head at zero load: $(\bar{K}_2)_{n,L} = K_2$. Under these conditions, with the fiber contracting, what is the value of K_2 for an individual complex ($(K_2)_{n,L}$)? From Eq. 9, if the intrinsic value of K_2 is taken to be 10^6, $(K_2)_{n,L} = 10^{6/41} = 1.4$. That is, whereas at zero load the equilibrium ratio of the contracted to extended conformations for an individual complex is 10^6, the ratio is only 1.4 in isotonic contraction. In isometric contraction, where $m > n$, the ratio of constants falls below unity (Eq. 7), which favors the extended conformation.

The load, in lowering K_2, lowers the K_{Ae}/K_{Ac} ratio (Fig. 3, Eq. 2), raising the affinity of myosin for actin in the extended relative to the contracted conformation. This may make it easier to form the contractile complex $A_e \cdot M_e$; the greater the load, the greater the effect, suggesting a regulatory mechanism in which more cross-bridges are recruited as heavier loads require larger numbers for contraction.

With no load (and ignoring controls on the activity of the contractile apparatus imposed by other components of muscle) the system would fall into an energy well, that involving the strongly interacting and therefore very stable contracted conformation, $A_c \cdot M_c$ (Fig. 4 *a*). This state is to be avoided because slow dissociation of the complex in the next step in the reaction cycle could be slow, limiting the turnover rate. Under load, where the stability of A_cM_c and the affinity of myosin for actin decline, dissociation can be fast.

Because the translocation step $[A_eM_e] \rightarrow [A_cM_c]$ occurs when the combined tension of several myosin heads comes to exceed the load, the net contractile force at varying load (the excess of tension over resistance) may be fairly constant; furthermore, the hydrolysis of ATP may be automatically adjusted to meet the requirement of the load that is applied.

The contractile complex

It is often assumed that myosin cross-bridges attach to the actin filament in strained configurations and perform work as the strain is released, with movement toward configurations of minimum energy. As noted in the Introduction, however, a high-energy intermediate, which is by definition unstable, can be a bottleneck in a reaction sequence. The reason is that the concentration of an intermediate tends to be inversely related to its energy level: high energy implies low concentration and therefore a low rate of reaction. On the other hand a very stable intermediate, which reacts slowly, can also be a bottleneck. Both, therefore, are to be avoided in the interest of kinetic efficiency (see Jencks, 1980). The present mechanism has the advantage of not involving a high-energy contractile complex: before contraction, the attached and unattached states of myosin, A_eM_e and M_e, are assumed to have very similar, unstrained conformations. Contraction occurs as the conformation changes from extended to contracted, A_eM_e to A_cM_c, and in the transition external work can be done because the contracted complex, stabilized by strong binding forces, is much more stable than the extended complex. But owing to its inherent stability, the contracted complex A_cM_c could become a bottleneck in the reaction, were it not destabilized by the load, as explained above. As the system is undergoing contraction the load tends to make the contracted and extended complexes comparably stable, which is what is wanted for high kinetic efficiency. Figs. 4 *a* and 5 illustrate these points.

The intermediate involved in the generation of force, which may be called the contractile complex, is a complex of myosin with actin that may also contain ATP or a product of the ATP reaction, depending on the reaction sequence. The binding forces that drive this step can be between myosin (M) and actin (A) and between myosin and ATP or its products (X). The contractile conformational change may be written

$$A_e \cdot M_e \cdot X \rightleftharpoons A_c \cdot M_c \cdot X. \quad (10)$$

The conformations of both myosin and actin could be altered in the contractile step (M_e to M_c and A_e to A_c); indeed, actin has been proposed as the generator of tension (Schutt and Lindberg, 1992). The conformational changes displacing the actin relative to myosin filament should not be confused, however, with the driving force behind the conformational change, which is binding energy.

Having only two conformations of the complex—extended and contracted—may be too simple. In line with a mechanism proposed by Huxley and Simmons (1971), there could be intermediate states, A_i and M_i:

$$A_e \cdot M_e \cdot X \rightleftharpoons A_i \cdot M_i \cdot X \rightleftharpoons A_c \cdot M_c \cdot X. \quad (11)$$

The energy profile for the conformational changes involved would be a series of wells, as in Fig. 4 *b*.

ACCOUNTING FOR THE MEASURED FORCE

The force generated by a single myosin head acting on an actin filament was found to be about 3.4 pN at 21°C and the length of the working stroke about 11 nm (Finer et al., 1994). From Eq. 5 (with $R = 8.3143 \times 10^7$ erg K^{-1} mol^{-1}, $T \approx 300$, and Avogadro's number $N_o = 6.0225 \times 10^{23}$), $K_{Ae}/K_{Ac} > 1 \times 10^4$. With kinesin the force at room temperature was 5 pN, the length of the working stroke 8.2 nm (Svoboda et al., 1993), from which $K_{Ae}/K_{Ac} > 2 \times 10^4$. The ratio of dissociation constants should be calculable in another way, from the efficiency with which the free energy of ATP hydrolysis is used in muscle contraction, about 50% of 50 kJ/mol: with $R = 8.31433$ joule K^{-1} mol^{-1}, $K_{Ae}/K_{Ac} > 2 \times 10^4$.

This ratio may be compared with the ratio for coupling in transport, as given in Eq. 1. The coupling ratio for the anion exchanger of red cells, exchange relative to net flow, is about 4×10^4 (Fröhlich, 1984; Fröhlich and King, 1987), which makes the ratio of substrate dissociation constants

involved in the coupling step, $K_{\text{Initial state}}/K_{\text{Final state}}$, greater than 4×10^4. The similarity in the ratios for coupling and for force generation is understandable if similar processes are involved, as both are dependent on protein conformational changes driven by intermolecular binding forces.

The increment in binding energy required—a factor of 10^5 or more—can be explained by the formation of a chelate, or host-guest complex (Krupka, 1989b). The host, or enclosing, molecule interacts with the guest, or enclosed, molecule at several points, and the favorable entropy of multiple interactions makes the complex unusually stable. When two initially separate molecules, free to move in solution, come together in a complex, one of them, in effect, loses its freedom of translational and rotational motion. The binding energy is the difference between the unfavorable entropy, which is large, and the attractive force. But subsequent interactions within the complex, occurring without this loss in freedom of movement, can have their full effect. Any pre-organization of the binding region adds to the stability of the complex, because the price of aligning subsites has been paid for in its synthesis (Cram, 1988; Miyamoto and Kollman, 1993). Similar effects are seen in intermolecular reactions: reactions within molecules, even simple molecules, can be 10^5 times faster than those between molecules, and if the interacting groups are held in the right position, as much as 10^8 times faster (Page and Jencks, 1971). The catalytic efficiency of enzymes depends on such effects, as does the stabilization of the folded state of polypeptide chains (Creighton, 1984).

The general theory does not predict which reactants in the complex should undergo force-generating increments in binding energy: in muscle, actin could be involved, as well as the driving substrate (from the evidence, ADP and P_i rather than ATP, the latter acting in cross-bridge detachment). In either case, two types of complex would be formed, the first loose, the second tight. As seen above, increased binding energy with a small molecule such as ADP can be accounted for by its enclosure within a cavity in the protein, enlarging the surface of interaction. Some comparable process could occur with actin. The myosin head and actin, initially linked at one point, would presumably form additional linkages over a larger surface, following a conformational change. Binding at the first stage would be weak, the link flexible; binding at the second stage would be strong, the link rigid. The conformational change accompanying the formation of the rigid complex would, it is assumed, have the effect of shifting the angle between actin and myosin, displacing the thin relative to the thick filament, as in current models for force generation.

The experimental observations on muscle agree with the theory. ADP and P_i, before the force-generating step, are bound deep in an active site cleft; as the cleft closes the conformation of myosin is changed, producing a bend in the myosin head and, possibly, shifting the attached actin filament (Rayment and Holden, 1994). Actin, too, may be involved. The binding of actin to myosin occurs in two steps, the second involving a conformational change (Greeves et al., 1984; Taylor, 1991, 1993), and a myosin conformational change of the kind predicted, in which the surface of contact between actin and myosin is substantially enlarged, has been suggested (Spudich, 1994; Rayment and Holden, 1994). Weak and strong cross-bridge attachment states are detected (Brenner et al., 1982), the former undergoing rapid, the latter slower, attachment and detachment (Brenner et al., 1991); furthermore, force develops only after the cross-bridges have attached in the weakly bound state (Brenner et al., 1991). As force is being generated the structure of the myofibril changes, movement of the myosin heads being synchronous with the elementary force-generating process (Irving et al., 1992). Before the work stroke, the interaction between the myosin head and actin is nonstereospecific and involves a small number of electrostatic interactions, the myosin head being weakly bound and rotationally mobile; afterward, the interactions are much more extensive, with the myosin head rotationally immobile (Rayment et al., 1993; Berger and Thomas, 1993; Hirose et al., 1993; Berger and Thomas, 1994; Thomas et al., 1995; Ostap et al., 1995). It appears that force is generated during the transition, as dynamically disordered cross-bridges become rigid and stereospecific. These findings conform closely to the theory, which says that a force exerted externally must be derived from internal forces, through increased binding energy among components of the system; increased binding energy, in turn, depends on increased surfaces of interaction and, consequently, diminished freedom of movement. If the actin-to-myosin bond does play a role in generating force, then it is the weakly bound disordered state, the so-called pre-force phase, that has the potential to generate force, a potential exhausted in the strongly bound ordered state. The latter has been thought of as the force-generating phase but might better be called the post-force phase.

The theory has other implications, some regarding the kinetics of the system. Because the load necessarily opposes the contractile step (Eqs. 5 and 6), this step, even if rapid in the unloaded system, becomes rate-limiting under sufficient load. Hence measured rates, which are determined by the slowest steps in the reaction cycle, could vary, depending on the assay conditions. In a mobility assay, movement is necessarily opposed by a load and therefore force generation could be the rate-limiting step; on the other hand, in solution, in an ATPase assay, no load is applied, no work is done, and the force-generating step could be fast. The differing behavior of myosin mutants when measured in ATPase and mobility assays (Spudich, 1994) could possibly be explained in this way.

CONCLUSIONS

In the experimental study of muscle, events such as contraction may be related to particular steps in a reaction sequence, and on this basis a kinetic model of the system may be formulated. At this stage the underlying mecha-

nisms of force generation or of coupling are not necessarily specified. Force may be taken to be a function of the movement of cross-bridges, or of attachment and detachment of cross-bridges, its value being a function of assigned rate and equilibrium constants in key steps in the reaction. Similarly, although coupling depends on abruptly shifting properties of components of the system as the reaction proceeds, the basis of the changes need not be explained. With values for rate and equilibrium constants assigned, the reaction sequence may mimic selected features of the experimental behavior, even though the underlying mechanisms remain obscure.

In further study, force production and shortening of the fiber may be traced to protein conformational changes, and if these can be shown to be driven by specific molecular interactions accounting for the force developed by the whole system, a detailed mechanism might be worked out. A mechanism, in this sense, relates a given phenomenon to molecular interactions, which in principle are independently measurable. The force-generating mechanism proposed here may enter as one step in a model for muscle contraction, whereas the coupling mechanism would enter at steps where the properties of myosin shift abruptly. The introduction of these mechanisms would enlarge the reaction scheme for any given model (Krupka, 1994a). Obviously, an expanded scheme can give rise to the behavior found for the original, although additional, implicit properties could be revealed, as in the case of the calcium pump (Krupka, 1994b). The importance of the expanded formulation would be to bring out the role played throughout by substrate binding energy, both in coupling and in force generation—in particular, in predicting relationships between experimental behavior (the tightness of coupling, the force generated) and changes in binding energy and conformation in different intermediates. The new tests that are suggested would involve studies of the physical properties of components at different stages of the reaction. With the basis of force and coupling more firmly established, modeling of an entire system could lead to deeper insight.

Vectorial coupling mechanisms always depend on a controlled reaction sequence, achieved through substrate-induced conformational changes, and the principles are identical in primary and secondary active transport, with free energy drawn from either ATP hydrolysis or an ion gradient (Krupka, 1993a,b). Likewise, coupling in a molecular motor will be based on the same principles whatever the source of free energy, and Eq. 1 will be applicable. Similarly, the mechanism for generating force, being connected to the coupled reaction through the involvement, in both, of substrate-induced conformational changes, should be independent of the energy source, whether ATP or an ion gradient. And in active transport, if force has to be exerted and work done in moving an ion against a membrane potential, the principles should not be different.

The central issue throughout this discussion has been binding energy. Binding energy drives the conformational change involved in force production and drives the conformational changes responsible for a coupled reaction sequence. The equation for coupling and the equation for force are of similar form (Eqs. 1 and 5), both setting a minimum increment in binding energy for a given tightness of coupling or a given performance of work. The lower limit, an expression of the necessarily incomplete conversion of free energy into work, suggests that "tightness" is a function analogous to work. Binding energy and free energy, it should be noted, are distinct entities, the first generating force in a single event, the second allowing a series of such events to continue.

That force has its origin in binding energy seems plausible for several reasons. These may now be summarized. First, on this hypothesis the measured force produced by myosin or kinesin can be accounted for by well-understood principles of host-guest chemistry. Second, the connection between free energy release and work is implicit in the mechanism, inasmuch as tightness (in coupling) and tension are closely related functions; likewise, the connection between load and free energy release is implicit in the mechanism. Third, the mechanism involves no high-energy contractile complex, to which there are objections. The very stable complex formed after the work stroke, which could be a bottleneck in the reaction, is destabilized by the load; thus, not only binding energy (Jencks, 1980) but an external load can be used to enhance kinetic efficiency. And fourth, a summation of forces at high load is implicit in the mechanism.

Finally, it may be noted that in biological systems interactions dependent on binding energy form a web of interconnected functions: 1) Enzymes catalyze chemical reactions by binding their substrates far more tightly in the transition state than in the ground state (Pauling, 1946). 2) In enzymic reactions and coupled vectorial processes, differences in binding energy can be used to make reactions reversible that are effectively irreversible in solution, and in general, to keep the relative populations of different states comparable, avoiding a bottleneck at overly stable or overly unstable intermediates (Jencks, 1980, 1989). 3) In a coupled vectorial process, the role of either substrate, driving or driven, may be to increase the rate of a protein conformational change, which it can do by becoming bound more tightly in the transition state than in the initial or final state (Klingenberg, 1985; Krupka, 1989a). Here, the protein reacts, the substrate is the catalyst; with enzymes, the substrate reacts, the protein is the catalyst. 4) A ligand can shift the equilibrium between two conformations of a protein by binding preferentially to one (Weber, 1972; Monod et al., 1965). The reaction sequence involved in vectorial coupling can be controlled in this way (Krupka, 1993a). 5) It may now be seen that the force developed by a molecular motor may have its origin in binding energy.

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