

APPENDIX: Is the lever arm of myosin a molecular elastic element?

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The classic experiments of Huxley and Simmons (40) defined an elastic element in muscle that has been attributed to the myosin molecule. They measured the tension drop when a stimulated muscle held at a fixed length is rapidly shortened through a small distance and found that a component of the system behaves like a linear spring. Such an elastic element is fundamental to force generation because it allows strain to develop within the motor prior to movement of the cargo; relief of this strain then drives the relative displacement of the motor and the track along which it moves. While diagrammatic representations often show this elastic spring as being part of the myosin rod beyond the light-chain binding domain of the molecule, we consider here that the elastic element is the light-chain binding domain itself and may account quantitatively for the cross-bridge stiffness observed in muscle experiments.

The head domain of myosin, commonly called subfragment 1 or S1, is the only part of the myosin molecule required for movement *in vitro* (33) and for production of force similar to that seen in intact muscle (9, 42). An unusual structural feature of S1 is the ≈ 8 -nm-long light-chain binding domain that is at

the C terminus of the S1 moiety (2, 3). It has been suggested that this region of the myosin head could serve as a lever arm to amplify smaller conformational changes elsewhere in the motor domain (5, 13–15, 19–21, 43, and this paper). Indeed, fluorescence polarization experiments have shown that the light-chain binding region changes orientation by a minimum of 3° relative to the filament axis in muscle in response to quick length changes and during the transitions between states of the cross-bridge cycle associated with active force production (15). While this angle change would appear to be too small to account for a unitary displacement of several nanometers (6), it is a minimum value for technical reasons, and two other complementary studies strongly support the lever arm hypothesis. First, electron microscopy of decorated actin filaments showed that a rotation of the light-chain binding domain through $\approx 23^\circ$ accounts well for the two different conformations that S1 adopts depending on whether ADP is bound at the active site; the difference could account for as much as 3.5 nm of movement of the far C terminus of S1 (13, 14). Second, this paper used molecular genetic approaches to shorten, and importantly, to elongate the lever arm and demonstrate a linear relationship between the lever arm length and the velocity with which the myosin moves *in vitro*.

We argue here that the lever arm could also be the elastic element referred to above, since the elasticity of the light-chain binding domain is expected to be comparable to that measured in the rapid shortening experiments. Furthermore, the nature of the light chains and their interaction with the ≈ 8 -nm-long α -helical stretch of the heavy chain at the C terminus of S1 may determine the spring constant of the light-chain binding domain and therefore affect the force that the molecular motor can produce.

Consider a very simple model of the lever arm as a clamped beam of length L and flexural rigidity (the resistance to bending forces) equal to EI . If a transverse force F is applied at the free end, then this end will move through a distance x such that:

$$F = (3EI/L^3)x$$

(44). In other words, the beam has a stiffness

$$\kappa = 3EI/L^3 = 3kTL_p/L^3,$$

where $L_p = EI/kT$ is the persistence length (45), k is the Boltzmann constant, and T is temperature. The light-chain binding domain has a length of ≈ 8 nm. It seems reasonable to consider that the lever arm, which has two light chains wrapped around the long α -helix, has a rigidity similar to that of a coiled coil, which has two α -helices wrapped around each other. The persistence length of a coiled coil is ≈ 100 nm (J.H., unpublished measurements derived from the coiled-coil myosin rod domain). For comparison, the L_p of DNA, which has a dimension similar to these two protein structures, is ≈ 50 nm (46). Substituting $L = 8$ nm, $L_p = 100$ nm, and $kT = 4$ pN·nm, we obtain

$$\kappa \approx 2 \text{ pN/nm}.$$

On the other hand, the rapid shortening experiments indicate a muscle stiffness equal to 0.27 pN/nm when normalized to the total number of myosin heads per half sarcomere [a shortening of 6 nm per half sarcomere drops the force from 1.6 pN per head to zero (47)]. Since only about half the compliance in muscle resides in the myosin heads and the other half resides in the actin filaments (e.g., see ref. 48), this value for the stiffness needs to be doubled to ≈ 0.5 pN/nm per myosin head. If only a quarter of the myosin heads were attached during isometric contraction (duty ratio of ≈ 0.25 ; refs. 6 and 16), then the stiffness per attached head would be ≈ 2 pN/nm, equal to that derived above! Clearly, this equality could be fortuitous

given the large uncertainties in both the experimental and theoretical stiffnesses. The assumptions made, however, are not unreasonable, and the calculations do show that it is quite plausible that the elasticity of myosin resides within the light-chain binding domain, which corresponds to the lever arm. Indeed, one expects the light-chain binding domain to contribute some compliance to the myosin molecule.

There are three interesting predictions that follow from the hypothesis that the lever arm is the elastic element.

(i) The motor force should be inversely proportional to the square of the length of the lever arm. To see this, let the force-generating conformational change be a rotation, through an angle $\Delta\theta$, of the insertion point of the lever into the motor domain. Thus, in the absence of a restoring force, the tip of the lever arm (the C terminus of S1) would move through a distance

$$\Delta x = L\Delta\theta,$$

On the other hand if there were a restoring force (F_{\max}) that prevented the C terminus of the lever arm from moving, then

$$F_{\max} = (3kTL_p/L^3)\Delta x = (3kTL_p/L^3)L\Delta\theta = 3kTL_p\Delta\theta/L^2.$$

Since the angular change $\Delta\theta$ is independent of the length of the lever arm, it follows that the maximum force is proportional to L^{-2} . On the other hand, if the lever arm acted as a rigid rod and the elasticity were due to a pivotal spring (49) located at the point of insertion into the motor domain, then the maximum force would depend on L^{-1} .

(ii) The maximum work should be inversely proportional to the lever length (L^{-1}). To see this, note that if the restoring force (F_o) is less than the maximum force, then the tip will move through a distance

$$\Delta x - F_o/\kappa \text{ (the working stroke),}$$

and the amount of work done will equal

$$W = F_o(\Delta x - F_o/\kappa) = F_o\Delta x - F_o^2/\kappa.$$

The maximum work occurs when $F_o = F_{\max}/2$, and

$$W_{\max} = F_{\max}\Delta x/4 = (3/4)kTL_p\Delta\theta^2/L.$$

That is, the maximum work is inversely proportional to the lever length. This leads to a paradox at the shortest lever arm lengths where the work might get so large as to exceed the theoretical maximum force. Presumably a motor with a very short lever arm will fail at high forces (the rotation through $\Delta\theta$ would not take place).

(iii) The maximum force will depend on the stiffness of the lever arm. For example, if the link between the ELC and the catalytic domain of S1 and/or the link between the ELC and RLC domains were flexible, we would expect a smaller stiffness and thus a smaller force. Thus, the properties of the light chains may affect the flexural rigidity of the lever arm, thereby regulating the force produced by a particular myosin isoform.

The establishment of laser trap technologies to measure directly the force and work produced by a single myosin molecule (6, 50) and systems that allow genetic engineering of the molecular motor myosin to produce myosins with different lever arm lengths (this paper) should allow critical testing of whether force production is inversely proportional to the lever arm length squared, as predicted by the elastic lever arm model.

The same approaches should allow testing of the concept that the nature of the light chains modulates the spring constant of the elastic lever arm and therefore the amount of force that can be produced by different isoforms of myosin,

which have different light chains. Indeed, even skeletal myosin binds two alternate forms of RLC, for reasons that have been unclear. Moreover, myosin light chains are altered by post-translational modifications, such as phosphorylation in the case of smooth muscle myosin and *Dictyostelium* myosin (for a review, see ref. 51) and binding of Ca^{2+} in the case of scallop myosin (52). One goal then is to use molecular genetics and laser trap technology to gain detailed molecular information about the physiological relevance of altered myosin types.

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