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The myosin step size: Measurement of the unit displacement per ATP hydrolyzed in an *in vitro* assay

(actin/crossbridge/muscle contraction)

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ABSTRACT **Chemomechanical coupling in muscle contraction may be due to "swinging crossbridges," such that a change in the angle at which the myosin head binds to the actin filament is tightly coupled to release of products of ATP hydrolysis. This model would limit the step size, the unit displacement of actin produced by a single ATP hydrolysis, to less than twice the chord length of the myosin head. Recent measurements have found the step size to be significantly larger than this geometric limit, bringing into question any direct correspondence between the crossbridge and ATP-hydrolysis cycles. We have measured the rate of ATP hydrolysis due to actin sliding movement in an *in vitro* motility assay consisting of purified actin and purified myosin. We have calculated an apparent myosin step size well within the geometric limit set by the size of the myosin head. These data are consistent with tight coupling between myosin crossbridge movement and ATP hydrolysis.**

In 1942, Albert Szent-Gyorgyi and colleagues (1) showed that threads formed from myosin, the principal ATPase of muscle, and actin, its activator protein, could shorten in the presence of ATP. That an amorphous protein gel displays the contractile property of muscle is striking, considering the highly ordered structure of the muscle sarcomere. In striated muscle, actin and myosin are arranged in a remarkable array of interdigitating thin and thick filaments and connecting crossbridges. The observation that the length of the sarcomere reflects the extent of overlap of these filaments inspired the sliding-filament theory (2, 3). H. Huxley's observations (4) demonstrated physical connections between thin and thick filaments, suggesting that filament sliding is mediated by crossbridges that project from the thick filament. These were imagined as independent force generators that cyclically bind, produce force, and release in their interaction with the thin filament. "Swinging" myosin crossbridges (5) have been the basis for most contemporary theories. By analysis of tension transients in muscle after small length changes, A. Huxley and Simmons (6) estimated the range of motion of crossbridges bound to the thin filament to be ≈10 nm.

Myosin and actin are principal components of the thick and thin filaments respectively. Myosin (7) is a 500-kDa heterooligomeric protein, divided functionally into two catalytically active head domains and a filament-forming rod domain. Significantly, that part of the crossbridge which binds to the thin filament is the myosin head. Cleavage of the head from the rod by mild proteolysis yields subfragment 1 (S1), a soluble 130-kDa actin-activated ATPase composed of the amino-terminal 95 kDa of the myosin heavy chain and two myosin light chains. The solution kinetics of ATP hydrolysis by S1 in the presence of actin has been studied extensively (8–10). In the absence of ATP, S1 binds with high affinity to the actin filament. The rate of release of S1 from this rigor complex is dependent upon the binding of ATP. In solution, S1·ATP undergoes rapid interconversion with S1·ADP·P_i. When S1·ADP·P_i binds to actin, release of P_i and then of ADP is stimulated, reforming the stable rigor complex.

One crossbridge model proposes that the myosin head operates via a mechanochemical cycle with tight coupling between kinetic steps in ATP hydrolysis and conformations of the myosin head. This model predicts a one-to-one relationship between the release of ATP hydrolysis products and a force-producing conformational change in myosin while bound to actin. When actin filaments interact with S1 fixed to a surface, they undergo ATP-dependent sliding movement (11). This yields an obvious constraint that displacement between binding and release of S1 from a moving filament is limited to no greater than twice the ≈20-nm chord length (12) of the myosin head. With respect to these geometric limits, tight coupling between binding and product release would limit the step size, the average displacement associated with an ATP hydrolysis, to an upper limit of ≈40 nm.

Surprisingly, measurements of the step size by Yanagida and colleagues (13–15) have yielded distances of ≥60 nm. Such values are inconsistent with tight coupling and demand multiple cycles of binding and dissociation between myosin heads and actin filaments with each ATP hydrolysis (16). Thus, we set out to examine whether an independent estimate of the step size would confirm the reported value of >40 nm. We have measured movement and ATP hydrolysis simultaneously to determine in a biochemically defined system, the myosin-coated surface assay (11, 17), the average displacement of actin filaments associated with the hydrolysis of one ATP by myosin.

METHODS

Protein Purification. Myosin was purified from rabbit back muscle (18) and stored in sealed tubes at −20°C in 50% (vol/vol) glycerol for <3 months. Chymotryptic heavy meromyosin (HMM) was prepared essentially as described (11) and used within 3 days. In our previous HMM preparation (11), the proteolytic nicking of heavy and/or light chains resulted in increased sliding speeds. Here, the incubation time was optimized to maximize intact 170-kDa heavy chain, resulting in a speed like that of native myosin bound to nitrocellulose (11). Rabbit skeletal muscle actin was purified (19) and labeled with tetramethylrhodamine (Me_4R) conjugated to phalloidin (Me_4RP; Molecular Probes) as described (17). Severin was purified from *Dictyostelium discoideum* (20). For minimum-length determination, short actin filaments were prepared by mixing Me_4RP-actin with severin to give a 1:100 molar ratio, then diluting in assay buffer to 2.2

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Abbreviations: Me_4RP, tetramethylrhodamine-conjugated phalloidin; HMM, heavy meromyosin; S1, myosin subfragment 1; MLF, minimum-length filament; BSA, bovine serum albumin.

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μg/ml final concentration of actin. For measurement of dependence of sliding velocity on filament length, labeled actin filaments were sheared by pipetting. Protein concentrations were determined by absorbance (21, 22) or by a dye-binding protein assay (23).

Motility Assay. Movement of Me_4RP-actin over HMM-coated nitrocellulose surfaces was examined by video epifluorescence microscopy (17) in a flow cell. The flow channel was constructed on a microscope slide that had been cleaned, blocked with bovine serum albumin (BSA) at 0.5 mg/ml in distilled H_2O, rinsed, and dried. A 22-mm square nitrocellulose-coated coverslip (11) was laid over parallel lines of grease (Apiezon M) separated by 15 mm and was supported by two fragments of no. 2 coverslip. The resulting channel, 0.25 × 15 × 22 mm, had an internal volume of 80 ± 5 μl.

Actin movement was observed at 30°C in assay buffer (25 mM imidazole hydrochloride, pH 7.4/25 mM KCl/4 mM $MgCl_2$/1 mM EGTA/1 mM dithiothreitol) with 1 mM ATP. In brief, the nitrocellulose film was coated with HMM and then blocked with BSA. In the absence of ATP, 2.2 μM Me_4RP-actin was allowed to bind to the HMM immobilized on the nitrocellulose film at 24°C. The flow cell was then equilibrated to 30°C. Upon addition of ATP, filament sliding movement was observed. Average filament sliding velocity, v, was measured from VHS videotapes of filament movement as described (24).

ATPase Assay. To determine the NH_4^+/EDTA-ATPase activity of HMM immobilized on the nitrocellulose film, for each HMM concentration one flow cell volume, 80 μl, of a dilution of HMM in assay buffer was applied for 1 min. The flow cell was washed with 240 μl of BSA (0.5 mg/ml) in 0.2 M NH_4Cl/5 mM EDTA/25 mM imidazole hydrochloride, pH 7.4, equilibrated to 30°C; 80 μl of 1 mM ATP in this buffer was infused and the slide was incubated. The solution was withdrawn, mixed with an equal volume of 0.6 M perchloric acid and diluted with 0.3 M perchloric acid, and the phosphate release in the flow cell was measured (25). The HMM content of the flow cell was then estimated by comparing the ATPase rate to that of a known concentration of HMM in solution.

The total ATPase activity associated with actin sliding movement in the flow cell, V, was measured as follows. First, 80 μl of HMM (15 μg/ml) in assay buffer was infused and allowed to bind for 1 min, and excess HMM was washed away with 160 μl of BSA (0.5 mg/ml) in assay buffer. Then, 240 μl of unfragmented Me_4RP-actin filaments (2.2 μg/ml) was infused, the filaments were allowed to bind for 1 min, and the excess actin was washed away with 160 μl of assay buffer. The flow cell was equilibrated to 30°C and observed under the fluorescence microscope. Then, 80 μl of 1 mM ATP in assay buffer was infused and movement was observed. The reaction was stopped by withdrawing 60 μl of solution from the flow cell and mixing it with 120 μl of ice-cold distilled water and then 180 μl of malachite green reagent (25). The mixture was incubated 5 min at 25°C and the absorbance at 650 nm was determined. The apparent Mg^{2+}-ATPase of the bound HMM in identical flow cells in the absence of actin was measured similarly and used for background correction.

Length of Actin Filaments. The lengths of individual actin filaments were determined by two methods. Me_4RP-actin filaments of length ≥1 μm could be accurately measured from their fluorescence image by using a video micrometer (24). When long filaments were used, few if any filaments <1 μm in length were observed undergoing sliding movement. For experiments with severin-fragmented filaments, the lengths were obtained from measurements of electron micrographs of negatively stained specimens.

The total length of all actin in the flow cell, L, was measured from hardcopy images of digitized video frames captured at 17–20 locations within each flow cell representing ≈0.005% of the coverslip surface.

Electron Microscopy. For electron microscopy, nitrocellulose films were prepared by sandwiching an electron microscope grid between the film and coverslip. As judged by video microscopy, infusion of 80 μl of 40% (vol/vol) ethylene glycol in assay buffer followed by assay buffer without ATP was found to stop actin sliding movement without dissociation, fragmentation, or change in length of filaments. However, the possibility of dissociation of some short actin filaments from the surface could not be ruled out. Grids were negatively stained with aqueous uranyl acetate, carbon-coated, and visualized by transmission electron microscopy in a Philips EM201 at 80 kV.

RESULTS

Rationale. Filament sliding occurs in a hydrodynamic regime where inertial effects are negligible and Brownian motion is dominant. Actin filaments move forward only if continuously subjected to the impulse of a myosin head (13). The speed of moving actin filaments does not result from a balance of forces between the impulses of myosin heads and external viscous loads (17, 26) but reflects a kinetic limit set by the rate of release of myosin heads from actin filaments. Our goal was to use the relationship between sliding velocity and myosin ATPase kinetics to calculate the step size.

Consider the following premises about the nature of actin sliding movement under standard conditions. (*i*) The rate of ATP hydrolysis by myosin heads interacting with actively sliding actin filaments is the same as the rate of ATP hydrolysis by myosin in the presence of infinite actin concentration, V_m. Myosin heads with ATP hydrolysis products bound associate with actin and dissociate after rebinding ATP. (*ii*) For any density of heads, the length of an actin filament determines the number of heads interacting with that filament and thereby the number of hydrolysis events per unit time. (*iii*) For any myosin density, actin filaments of any length may bind to the myosin-coated surface by stable rigor linkages. Upon infusion of ATP, those filaments too short to be in continuous contact with active myosin heads will dissociate from the surface. (*iv*) Conversely, there is a minimum length of actin filament, l, sufficient to interact with a minimum number of heads such that the filament will stay associated with the surface and be continuously acted upon by myosin heads in the presence of ATP. Filaments greater than or equal to the minimum length will move at the maximum sliding velocity, v_0.

If the above properties hold, an approach to determining the step size, d, from the size of the minimum-length filament (MLF), l, is available. A continuously moving filament must be acted on at all times by one or more heads, yet each head must be able to release and rebind the filament as it moves. Two independent characteristic times describe the interaction of myosin heads with ATP and with actin: t_c is the time required to complete an entire ATP hydrolysis cycle, and t_s is the time within a cycle that the myosin head resides on the actin filament in a strongly bound state during which the putative power stroke occurs. The cycle time for hydrolysis of one ATP by a single myosin head, t_c, is the inverse of V_m, the maximum rate of actin-activated ATP hydrolysis. According to one model, the maximum filament velocity, v_0, is limited to the step size, d, divided by the strongly-bound-state (stroke) time, t_s. Thus, t_s is equal to d/v_0.

If t_c is greater than t_s, then, from consideration of premise *iii*, a single myosin head will not be able to support the continuous movement of an actin filament. As a first approximation, the minimum number of sequentially activated heads that would drive the continuous movement of a minimum length actin filament would be t_c/t_s, which is v_0/dV_m.

Consider a MLF, of length l, to which t_c/t_s heads bind sequentially to exert continuous force, with their release from actin contingent upon the release of ATP hydrolysis prod-

ucts. Such a filament has a single myosin head bound in a force-producing state at any moment. The step size can therefore be expressed as the ratio of the velocity to the rate of ATP hydrolysis associated with the movement of that MLF. From premise *ii*, the total ATPase activity in the flow cell, V, should be directly proportional to the total length of actin in the flow cell, L. If individual filaments are treated as segments of an infinitely long filament, this proportionality extends to each filament independent of its length. (A correction for filament ends is presented in *Discussion*.) Thus the ATPase activity of a single MLF can be estimated as $(l/L)V$. If the velocity of the MLF, the measured average velocity of sliding movement in the flow cell, and v_0 are equal, the step size, d, can be directly computed from the total filament length, L, total ATPase, V, average velocity, v, and the length of the MLF, l:

$$d = n_{av}(vL)/(Vl),$$

where n_{av} is the average number of heads bound to a MLF at any instant. For t_c/t_s sequentially acting heads, $n_{av} = 1$. An estimate of n_{av} for stochastically acting heads is presented in *Discussion*.

Experimental Results. Nitrocellulose films coated with HMM support the sliding movement of Me_4RP-actin filaments in the presence of ATP as previously observed (11). With this system, the four parameters necessary for the step size calculation were obtained.

The density of active HMM molecules bound to the surface was estimated by measuring the NH_4^+/EDTA-ATPase of the HMM immobilized on the nitrocellulose film. When the concentration of HMM applied to the film was increased over the range from 0 to 50 μg/ml, the measured ATPase and, therefore, the surface density of active HMM increased linearly (Fig. 1). In contrast, actin sliding movement showed a complex dependence on HMM density. Below 5 μg of applied HMM per ml, actin filaments that bound to the surface in rigor links in the absence of ATP dissociated from the surface upon addition of ATP. Between 5 and 50 μg of HMM per ml, actin filaments did not dissociate upon ATP infusion but moved with constant and maximal speed.

Filaments polymerized from purified actin and labeled with Me_4RP typically range in length from 5 to 40 μm. These filaments can be further fragmented by shear or treatment with severin, a Ca^{2+}-dependent actin-filament-severing protein. Actin filaments of all lengths could bind to the HMM-coated surface in rigor. The addition of ATP resulted in sliding movement of >99% of those actin filaments that remained bound to the surface. These filaments moved at a characteristic speed independent of length (Fig. 2). However, filaments shorter than a minimum filament length did not remain associated with the surface. Minimum filament length for movement was found to depend on the surface density of HMM. As noted above, when <5 μg of HMM per ml was applied, no filaments of any length were observed to move. At 6.6 μg of applied HMM per ml, the minimum filament length was found to be 2.1 μm, while at 7.5 μg/ml, the minimum filament length was 1.1 μm.

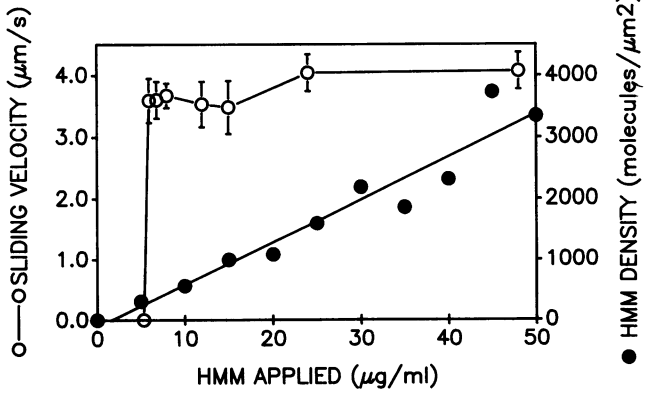

FIG. 1. Relationship among applied concentration of HMM, resulting surface density of HMM, and velocity of actin filament sliding movement. Dilutions of HMM were applied to nitrocellulose films as described in *Methods*. These films were either assayed for NH_4^+/EDTA-ATPase of the immobilized HMM or were used as substrates for actin sliding movement. The ATPase was normalized to that of HMM in solution. The average velocity and standard deviation of sliding movement of 60 filaments were determined at each concentration of applied HMM. Below 5 μg/ml, no actin sliding movement was observed.

Relatively high HMM concentrations were necessary in order to measure actin activation of ATPase in the flow cell. However, at concentrations >15 μg/ml, soon after the addition of ATP, actin filaments longer than several micrometers would rapidly break into smaller moving fragments (17). Thus 15 μg/ml was used as the standard concentration. Ethylene glycol quenching of motility and electron microscopy of negatively stained actin filaments on nitrocellulose films were used to determine the minimum length for surfaces coated with HMM at 15 μg/ml. Actin filaments were fragmented with severin and applied to an HMM-coated surface. The distribution of lengths of actin filaments bound to the surface was examined both before and after addition of ATP. The lengths of 547 actin filaments bound in the absence of ATP were distributed around 200 nm, and a large number of these filaments were <150 nm long (Fig. 3). However, with inflow of ATP, many of the short filaments dissociated from the surface and the distribution of lengths of those filaments which remained bound and underwent sliding movement had a broad peak near 500 nm (Fig. 3). A very small proportion of these filaments were <350 nm in length. Of 270 filaments measured, only 1 was between 150 and 175 nm in length and 6 were between 175 and 200 nm. From these experiments, it was estimated that the minimum length, l, of actin filaments for movement over a surface coated with HMM at 15 μg/ml was no shorter than 150 nm.

In experiments with unfragmented actin under standard conditions, ATP infusion did not elute actin filaments from the surface. For unfragmented actin, the total length of all actin filaments bound to the surface, L, was measured immediately prior to infusion of ATP. The calculated value of L was 86 m (SE = 15 m, determined from 17 images with total area 1.5×10^4 μm^2).

The average velocity of actin movement for long filaments, v, after infusion of ATP was 4.6 μm/s (SD = 0.4 μm/s, n = 92).

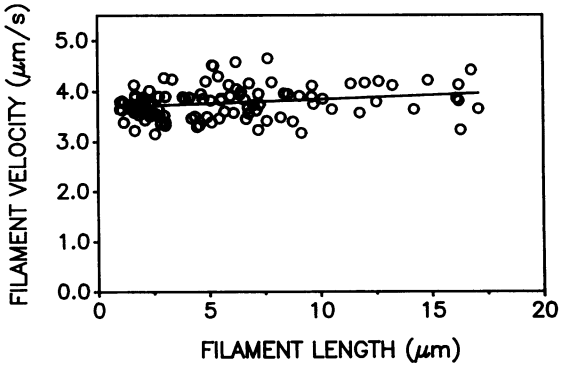

FIG. 2. Effect of filament length on sliding velocity. Actin filaments were sheared by pipetting. The length and sliding velocity of 104 Me_4RP-actin filaments were determined for movement over a surface coated with HMM at 12 μg/ml. The minimum filament length at this density was less than the minimum length, 1 μm, that could be measured accurately from the fluorescence image.

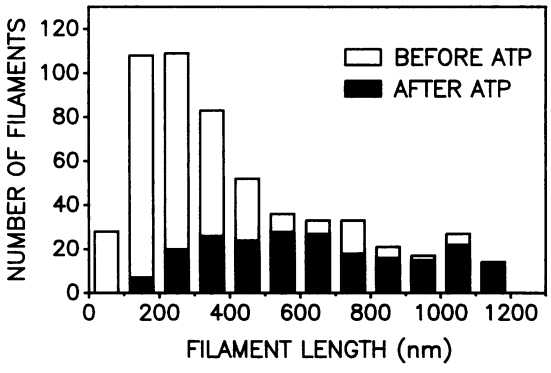

FIG. 3. Length distribution of actin filament fragments before and after initiation of movement. Actin filament fragments were prepared as described in *Methods*. Actin length was measured by electron microscopy of negatively stained nitrocellulose films prepared from flow cells quenched immediately before and 2 min after infusion of ATP to start sliding movement. Data are shown for 547 of 600 filaments measured before ATP addition and 202 of 270 filaments measured after ATP addition. The filaments not plotted were between 1.2 and 3 μm in length.

The rate of ATP hydrolysis associated with actin sliding movement, V, was found to be essentially constant for the first 8 min after ATP infusion (Fig. 4). Under standard conditions, the average rate of actin-activated phosphate release, after subtraction of the phosphate release with HMM bound to the coverslip in the absence of actin, was 3.4×10^{11} s^{-1} (SE = 0.4×10^{11} s^{-1}).

By using values of $v = 4.6 \pm 0.4$ μm/s, $L = 86 \pm 15$ m, $V = 3.4 \pm 0.4 \times 10^{11}$ s^{-1}, and a lower limit minimum length, $l =$ 150 nm, an upper limit of the average displacement of the minimum length actin filament per ATP hydrolyzed can be calculated, $vL/Vl = 8 \pm 2$ nm. If n_{av}, the average number of active myosin heads bound to the MLF, equals 1, then 8 nm represents a direct measure of the step size of the myosin head.

DISCUSSION

Our goal was to determine the displacement of an actin filament associated with the hydrolysis of one ATP by a myosin head. We have used a biochemically simple system, the HMM-coated surface assay (11, 17), and have simultaneously measured sliding movement and ATP hydrolysis. One limitation to this approach is that the sliding movement

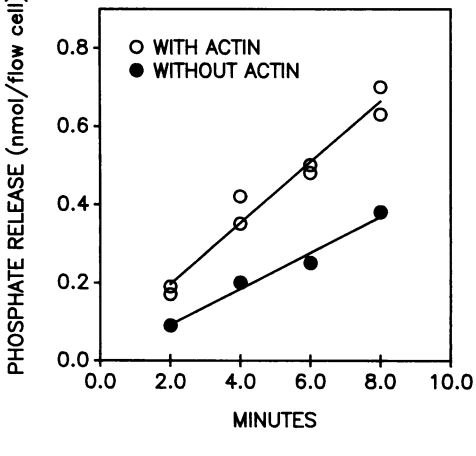

FIG. 4. ATPase activity coupled to actin sliding movement. The P_i release in flow cells prepared under standard conditions, with ATP infused at time 0, was measured at the indicated times. The data are from eight identical flow cells with actin (2.2 μg/ml) added and four identical flow cells without added actin. The slopes of the linear regressions are 0.078 nmol of P_i/min per flow cell ($r = 0.98$) with actin and 0.046 nmol of P_i/min per flow cell ($r = 0.99$) without actin.

of actin filaments in this assay is produced by many heads working in concert. We have been unable to measure the activity of a single head. Thus we have examined the movement of filaments at limiting numbers of myosin heads, the condition of the minimum length actin filament.

We have demonstrated that over a range of densities of HMM immobilized on nitrocellulose surfaces, there exists a corresponding length of actin filament, the MLF, which is the minimum for continuous sliding movement. However, the nonlinear dependence of minimum length on HMM density was not predicted (see *Rationale*). This discrepancy may reflect the effects of the bending and torsional flexibility of actin on effective transfer of force along the filament length, effects that may be diminished at high HMM densities. A second caveat is that under the conditions used here, the MLF was smaller than the resolution limit of the fluorescence microscope and thus its size and velocity were not directly measured.

From the minimum length for actin movement and a normalized activity of ATP hydrolysis per unit length of moving actin, we have calculated the displacement associated with a single ATP hydrolysis as an estimate for the step size of the myosin head. This calculation presumes that the movement of the MLF results from a continuous series of sequential impulses from myosin heads. However, cycling heads probably bind to sliding actin filaments in a stochastic manner. Formally, this precludes continuous interaction of myosin heads with sliding filaments of finite length. Nonetheless, a minimum filament length exists. This suggests that continuous interaction between the surface and filament is not strictly necessary. However, it is likely that the MLF is on average bound by more than a single actively stroking head ($n_{av} > 1$), thereby limiting the time during which the filament can dissociate from the surface. Estimating n_{av} will indicate to what degree 8 nm underestimates the step size.

Yanagida (15) has pointed out the relationship between the velocity of the MLF and n_{av}. If the average velocity of movement of long filaments, v, equals v_0, the speed of a MLF can be expressed as fv, where f is the fraction of time with at least one stroking head bound. For finite n_{av}, $f < 1$. The relationship between f and n_{av} is derived to be‡

$$f = 1 - [(v - dV_m)/v]^{n_{av}(v/dV_m)}.$$

From $d = n_{av}(vL/Vl) = n_{av} \times 8$ nm, it is clear that unless $n_{av} > 5$, the corrected step size will not exceed the geometric limit for tight coupling of 40 nm. Two arguments that the sliding movement is relatively insensitive to n_{av} suggest that an n_{av} of 5 or larger may not be required to support sliding movement of the MLF. First, since the standard error (SE) in the velocity measurement is 0.1, for SE = SD/v, the expected velocities when $n_{av} = 3$, where $f = 0.9$, and when $n_{av} = 5$, where $f = 0.996$, would each be indistinguishable from the average velocity, v. Second, when the sliding of short (<1 μm, but greater than minimum length) actin filament fragments was compared to that of longer (>2 μm) filament fragments, the average velocity of the shorter filaments (2.9 μm/s, $n = 20$) was 0.85 the sliding speed of the longer filaments (3.5 μm/s, $n = 15$), consistent with an $n_{av} <$ 3. Notably, all of these filaments were, by virtue of their length, in contact with a higher average number of heads than the MLF.

‡Derivation: From t_c and t_s as defined, $1 - f$, the probability of the filament being free of the surface at any instant, for a filament interacting with a single head is $1 - t_s/t_c$, or $(v - dV_m)/v$. For N independent myosin heads, $1 - f = [(v - dV_m)/v]^N$. It follows that when the average number of heads bound, n_{av}, is unity, $N = t_c/t_s$ or v/dV_m heads, and for n_{av} other than unity, $N = n_{av}(v/dV_m)$. Thus the general expression for the probability of a filament being free of the surface is $f = 1 - [(v - dV_m)/v]^{n_{av}(v/dV_m)}$.

A counterargument suggests that the approach used here overestimates the step size. In the development of the step-size formula, the ATP hydrolysis during sliding movement of the MLF was predicted from the measured ATPase during movement of long filaments. Special properties of the leading ends of sliding filaments were ignored.

The front tip of a sliding filament continuously encounters "cocked" ADP·P_i heads as it moves over the surface. The rate of phosphate release (and strokes from myosin heads) at the filament tip may be limited only by the product of filament velocity and HMM density rather than by the ATP turnover rate of myosin. In general, the kinetics at the tip of the filament may be analogous to a quench-flow nucleotide release experiment while that of the rest of the filament is analogous to a steady-state ATPase experiment. For long actin filaments, the non-steady-state activity at the filament tip is a small part of the total ATP hydrolysis. However, V/L, the normalized activity per unit length derived from long filaments, may significantly underestimate the ATPase activity per unit length of short filaments.

The product of the normalized activity, V/L, and the MLF length, l, predicts the activity of steady-state cycling heads along the length of the MLF. The product of the filament velocity, v, and the number of heads encountered per length of linear path, $(V/L)/V_m$, derived from the normalized activity of long filaments, predicts the activity due to product release at the filament tip, with the assumption that all "cocked" ADP·P_i heads encountered interact productively. The sum of the two sources of P_i release would approximate the total hydrolysis rate. A corrected minimum filament length, l', can be derived from $l'V/L = (lV/L) + (vV)/(LV_m)$. From the data presented above, l' equals 0.38 μm and predicts, from $d = vL/Vl$ and $n_{av} = 1$, a lower-limit step size of 3 nm.

Consider the situation where the myosin density is such that the spacing between accessible pairs of myosin heads is less than the step size. Under these conditions, it is possible that each head encountered by the actin filament tip imparts an impulse of a single unit step, which would allow the filament to be pulled along by the front end alone, as it is passed from one "cocked" ADP·P_i head to the next. This suggests that the spacing between heads is the upper limit for the step size that can be determined from a normalized activity and a minimum filament length. For the experiments reported here, in which HMM was applied at 15 μg/ml, the average nearest-neighbor distance of HMM molecules, $\approx$30 nm, is considerably greater than the calculated step size (for $n_{av} = 1$) of 8 nm. In contrast, Harada and Yanagida (14) used a myosin-coated surface with a nearest-neighbor distance of only 11 nm yet reported a step size of >50 nm.

In conclusion, we have used a novel approach to determine the unit displacement of the actin filament associated with a single ATP hydrolysis by myosin. The formula used to calculate the step size requires the measurement of only four parameters: total ATP hydrolysis rate, total length of moving actin, average filament velocity, and the minimum length of actin filaments capable of sliding movement. Applying this equation to the data presented above, we have calculated a step size of 8 ± 2 nm. This value must be considered an initial estimate since reasonable arguments can be made that the formula applied here yields alternately an overestimate or an underestimate. However, with respect to the question of comparison with previously published step-size measurements (6, 13–15), the experiments reported here do not confirm a step size >40 nm in length. Tight coupling of binding and release cycles to ATP hydrolysis is not ruled out, as the range of values consistent with the data presented here is well within the geometric constraint for conformational change imposed by the size of the myosin head. Further characterization of properties of movement of actin filaments over myosin-coated surfaces will be necessary to refine this approach and to conclusively determine the step size of myosin.

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