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Persisting in vitro motility of actin filaments at nanomolar ATP concentrations after ATP pretreatment

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Abstract

Muscle contraction is driven by the relative sliding of actin- and myosin-containing filaments. This process can be reconstituted in in vitro motility assays, where fluorescent actin filaments travel over a lawn of myosin heads. The velocity of the traveling actin filaments has been shown to depend strongly on the free adenosine triphosphate (ATP) concentration, but below 2–4 μM ATP, filament movement is consistently abolished. Here we report that after a brief exposure of actomyosin to 1 mM ATP, actin filament motility persists down to nanomolar ATP concentrations.

Keywords: Actin filament; Heavy meromyosin; Motility assay, in vitro; ATP; Energy production, unexplained

1. Introduction

One of the unsolved problems of muscle contraction is 'unexplained' energy [1]. The work + heat produced during unloaded rapid muscle shortening exceeds the energy that can be accounted for by simultaneous ATP splitting. Indeed, Ohno and Kodama [2] reported that unloaded shortening of myofibrils occurs with no significant ATP turnover. The low ATPase values accompanying shortening imply that the energy of ATP, at least in part, may be stored prior to contraction, and released gradually as the sarcomere shortens.

An ideal method for addressing the issue of unexplained energy production is the in vitro motility assay [3,4]. Actin-filament translation over the field of myosin molecules is thought to be analogous to the unloaded shortening of muscle [5]. Thus, actin filaments might be expected to move in vitro at negligible actomyosin ATPase activities. Interestingly, however, the ATP-dependence of filament velocity and actomyosin ATPase activity in the in vitro assay seems to show the opposite: the K_m of ATPase activity is 4 μM ATP [4,6] (6 μM for heavy meromyosin, HMM [6]), while that of filament velocity is 60 μM [7] (88.3 μM for HMM [5]). Thus, there is still significant ATP turnover below 2–4 μM ATP, while actin filament motility is already abolished.

One possible reason for this discrepancy is that experiments in the motility assay generally begin in the rigor state, where ATP is absent; then ATP is added to initiate motion. This situation is unnatural to the intact sarcomere, where ATP concentrations are consistently millimolar [8]. It has been shown that ATP causes conformational changes in both G- [9] and F-actin [10], and also in myosin [11]. Thus, by preexposing the contractile proteins in the motility assay to 1 mM ATP initially, we could presumably create a situation analogous to that in the intact sarcomere. We find, in such a case, that motility can persist even if ATP is subsequently lowered to nanomolar levels. Preliminary results of this work have been presented in the form of abstracts [12,13].

2. Materials and methods

2.1. Preparation of proteins

Skeletal myosin was prepared from rabbit. 100 g of back muscle was processed according to the method of

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Margossian and Lowey [14], with the following modifications. After cooling on ice, the muscle was minced in a pre-cooled meat grinder. The muscle mince was then extracted with buffer containing 0.3 M KCl, 0.09 M KH_2PO_4, 0.06 M K_2HPO_4 (pH 6.8), 0.2 mM ATP, 0.2 mM dithiothreitol (DTT), and 0.23 mM phenylmethylsulfonyl fluoride (PMSF). After filtration through cheesecloth, myosin was precipitated by diluting the filtrate ten times with ice-cold distilled water. The precipitated myosin was collected by centrifugation (20 000 × *g*, 4° C, 30 min), and the pellet was dissolved in 2 M KCl and 0.5 M potassium phosphate (pH 6.8). The precipitation-dissolution cycle was repeated twice. The pellet of the final step was dissolved in 0.5 M KCl and 0.05 M potassium phosphate (pH 6.8), to yield a myosin concentration of 30 mg/ml. Myosin was then stored in 50% glycerol at −20° C. Prior to further use, the stored myosin was further purified by a precipitation-dissolution cycle: 1 ml aliquot of myosin was precipitated with ice-cold 0.1 mM $NaHCO_3$, 0.1 mM EGTA and 1 mM DTT. The precipitated myosin was collected with low-speed centrifugation (5000 × *g*, 4° C, 30 min), and the pellet was dissolved in equivalent volume of 20 mM imidazole-HCl (pH 7.0), 1 M KCl, 4 mM $MgCl_2$, and 10 mM DTT.

Heavy Meromyosin (HMM) was prepared fresh from stored myosin according to the steps described by Kron et al. [15]. Often, the HMM sample was actin-affinity purified prior to the experiments to remove irreversible rigor heads. The purification was carried out according to Kron et al. [15].

Skeletal actin was prepared from rabbit back muscle by the method of Pardee and Spudich [16]. F-actin was fluorescently labelled with molar excess of tetramethylrhodamine-isothiocyanate-phalloidin (TRITC-Ph, Sigma Chemical Company, St. Louis, MO). Purity of the protein preparations was checked using electrophoresis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [17], using a 10% gel. Sigma SDS-6H kit was used as molecular-weight standard.

2.2. *In vitro motility assay*

The in vitro motility assay was performed essentially according to Kron et al. [15]. All experiments were performed at 30° C. Actin filaments were visualized in a Zeiss Axiovert epifluorescence microscope using a microchannel plate-intensified CCD camera (Electro-Optical Services, Charlottesville, VA), and the images were recorded using a computer-controlled Hi8mm video cassette recorder (SONY CCD-V5000). The time code was generated by an SMPTE time code generator (ESE, El Segundo, CA). Images from the tape were digitized by a QuickCapture frame-grabber board (Data Translation, Marlboro, MA). The images were processed with an Apple Macintosh IIfx computer using the public domain NIH Image v.1.47 program (written by Wayne Rasband at the US National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868). Filament velocity was measured by calculating the distance between coordinates of filament locations in successive digitized video images. Only filaments clearly moving on a path were evaluated.

The flow-through microchamber was fabricated from a coverslip sandwich similar to the one described by Warshaw et al. [18], but modified as follows: first, a 22 × 40 mm coverslip was coated with 0.5% nitrocellulose (in iso-amyl acetate). To achieve a microscopically smooth surface, we used the method of spin-coating. The method – widely used in the semiconductor industry for photoresist coating of wafers [19] – yields a surface smoother than the underlying substrate [20]. 100 μl nitrocellulose solution was pipetted onto the center of the coverslip spinning at 1500 rpm. The spinning was continued for 30 s, until the coverslip was dry. A second modification was that two strips of silicone rubber (127 μm thick, Dow Corning, Midland, MI) were placed on this coverslip parallel to each other so as to form a channel inbetween them, and a 18 × 18 mm coverslip was placed on the silicone rubber 'spacers.' Internal volume of the flow-through microchamber was approx. 10 μl.

Buffer conditions in the in vitro motility assay were 25 mM imidazole-HCl (pH 7.4), 25 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT. The solution was complemented with 0.5 mg/ml bovine serum albumin (BSA) to prevent nonspecific binding of actin to sites other than the myosin head; in some experiments, however, BSA was omitted from the buffer solution. To reduce photobleaching and the breakage of actin filaments, dissolved oxygen was scavenged by the addition of glucose oxidase (0.1 mg/ml), catalase (0.018 mg/ml), and glucose (3 mg/ml) [21], and the solutions were degassed. HMM was infused in the microchamber at a concentration of at least 50 μg/ml to achieve saturation of the nitrocellulose surface. 30 μg/ml HMM has been shown to be sufficient for saturation [22]. ATP concentration in the in vitro motility assay was adjusted by dilution from a 300 mM ATP stock solution. The ATP stock solution was prepared by the following steps. The pH of the ATP solution was adjusted to 6.8, and its exact ATP concentration was measured spectrophotometrically. Aliquots of the solution were then rapidly frozen in liquid nitrogen, and stored at −20° C for subsequent use.

ATP-reduction in the microchamber was achieved by sequential washes with rigor solution (100 μl each step). Washing of the microchamber was carried out by micropipetting solution to one end of the flow cell while drawing solution from the other end with filter paper. During the washout procedure, new actin filaments were not added; the original filaments were followed throughout. Alternatively, ATP concentration was reduced enzymatically, using apyrase (Grade V, Sigma Chemical Com-

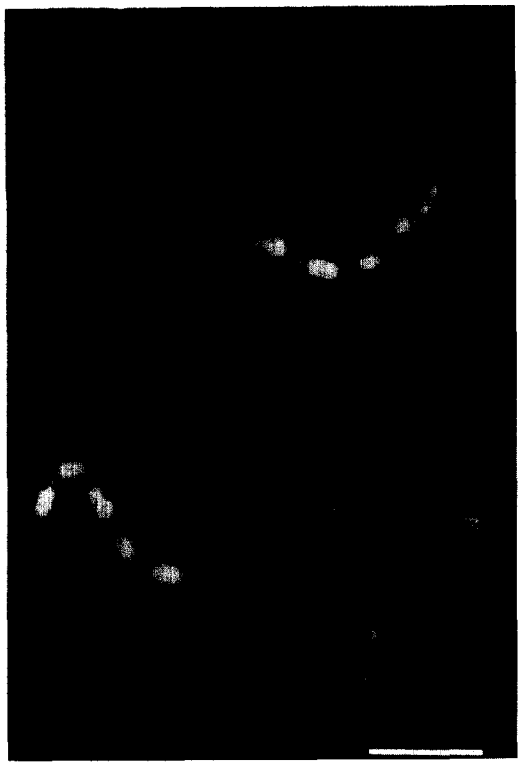


Fig. 1. Stroboscopic image of actin filaments after three washes with rigor solution subsequent to pretreatment with 1 mM ATP. Successive video frames of fluorescent actin filaments were superimposed using image processing software (NIH Image v.1.47). The elapsed time between individual frames is 3 s. Scale bar, 5 μm.

pany, St. Louis, MO, with an ADPase impurity 50% that of the ATPase activity). The apyrase experiments were carried out in the following way. First, ATP-digestion kinetics were established by monitoring the ATP concentration (by the luciferin luciferase method, see below) as a function of time. The apyrase level was adjusted so that the ATP concentration would fall from 1 mM to the nanomolar range in not more than 10 min. Choosing slower kinetics impaired the extended visibility of actin filaments in the motility assay, and faster kinetics yielded inaccurate velocity measurements. After establishing the kinetics of ATP digestion, actin-filament velocities were measured as a function of time from the addition of apyrase. Using the established apyrase enzyme kinetics and filament velocities, the appropriate ATP-velocity curves were plotted.

2.3. ATP measurement

Residual ATP in the microchamber was measured using the luciferin-luciferase assay [23]. After each rigor-solution wash, 10-μl samples were collected by adding rigor solution at one end of the microchamber and micropipetting from the other. Alternatively, the total content of the microchamber was collected. The collected samples were either diluted with assay buffer [0.5 mM $MgSO_4$ in 10 mM Tris-HCl (pH 7.75)] or injected directly into a luminometer (Packard, Laguna Hills, CA) containing a mixture of luciferin and luciferase. The luminometer was calibrated by fresh dilution series of ATP (1 nM–1 mM) before and after the washout experiments.

Scraping of the microchamber surface (for the purpose of collecting surface constituents) was accomplished by inserting a gel-loading micropipet tip (100 μm outer diameter, Brinkmann Instruments, Westbury, NY) into the microchamber. The pipet tip was moved laterally several times over the microchamber surface before drawing up the solution.

2.4. Chemicals

All chemicals were reagent grade. ATP used was 'special quality' ATP from Boehringer Mannheim (Indianapolis, IN, stock No. 519 987). Luciferin and luciferase were the product of Coral Biomedical (San Diego, CA).

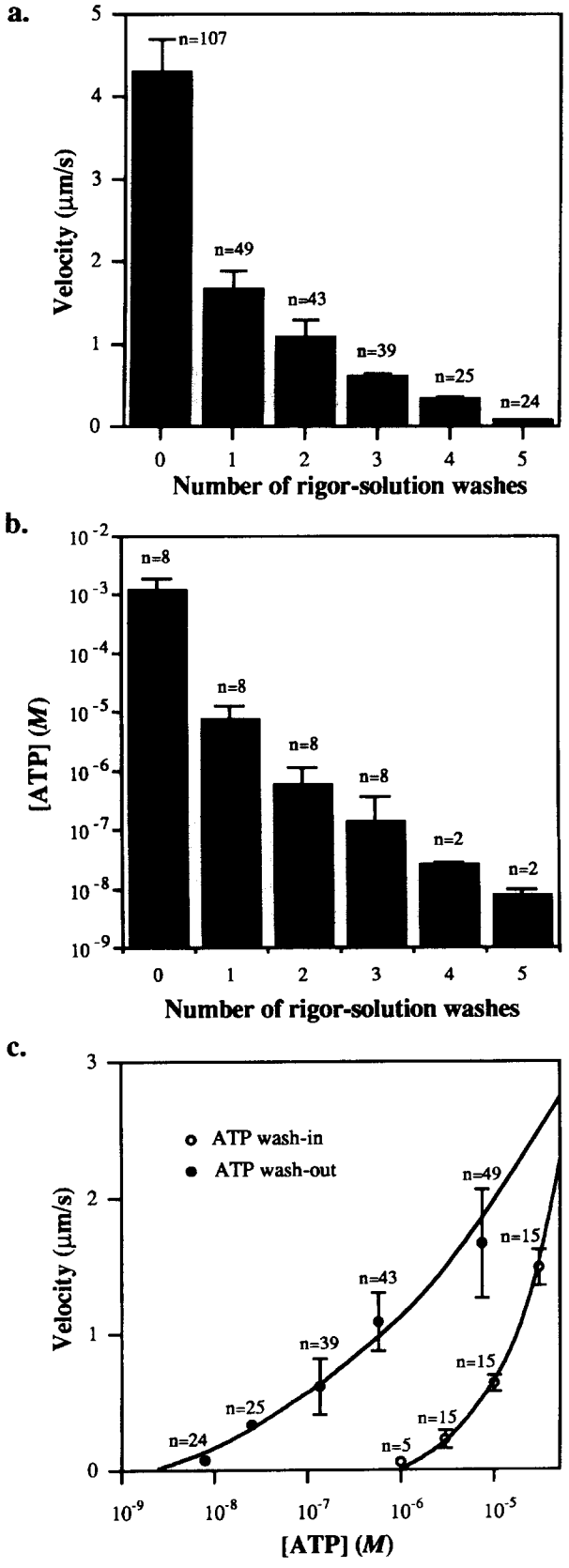
3. Results

Upon the addition of 1 mM ATP, actin filaments began to move with an average velocity of 4.3 μm/s. ATP concentration was then lowered by washing of the 10 μl microchamber with 100 μl rigor solution (devoid of ATP). Interestingly, actin filament movement did not stop, but continued with an average velocity of 1.7 μm/s. Upon further washes with rigor solution, filament motility persisted at progressively lower rates (Figs. 1, 2A). In fact, motility persisted after up to five washes with rigor solution (0.07 μm/s). The characteristics of actin movement following washes were mostly similar to those after simple ATP activation: movement occurred uniformly across the coverslip and the extent of filament breakage was similar to that in the standard assay. However, the percentage of non-moving filaments increased. Using actin-affinity-purified HMM reduced the fraction of non-moving filaments; however, the average velocity of the moving fraction did not change significantly. The use of a silicone-coated surface instead of nitrocellulose did not modify the result: filament movement persisted after multiple rigor-solution washes. Thus, contrary to the common expectation, infusion of vast amounts of rigor solution into the flow-through microchamber after ATP pretreatment did not halt the movement of actin filaments.

We measured the amount of ATP remaining in the flow-through microchamber after each rigor-solution wash. Fig. 2B shows that ATP concentration was progressively reduced with each wash. After five washes the average remaining ATP concentration in the microchamber was 8 nM.

As a control experiment, we attempted to induce actin-filament movement using ATP levels equal to the ones measured after the rigor-solution washes, but without the

ATP pretreatment. The result was identical to that of Harada et al. [7]: the filaments did not start to move until ATP concentration was raised above 1 μM (Fig. 2C).



Motility, therefore, cannot be induced with ATP concentration lower than 1 μM, but can be sustained at ATP concentrations more than hundred times lower following a brief exposure to 1 mM ATP.

In order to circumvent any uncertainties associated with the washout procedure, we implemented a different method to reduce ATP concentration: enzymatic digestion with apyrase. After determining the kinetics of ATP digestion by apyrase using the luciferin-luciferase assay, apyrase was added to the in vitro assay in the presence of 1 mM ATP. Actin filaments continued to move, though at a much reduced speed, at ATP concentrations down to 400 nM (Fig. 3). This concentration is 3-times lower than the threshold observed when ATP concentration was gradually increased, though not as low as found in the washout experiments. One possible reason for the discrepancy is that, unlike the washout protocol, apyrase digestion generates ADP, which inhibits motility [5]. Thus, filaments may have stopped prematurely because of a partial ADP build-up in the microchamber. Qualitatively, however, the apyrase experiments support the finding that following ATP pretreatment actin filaments move at lower-than-established ATP concentration.

The hysteresis in the ATP-velocity curve (Fig. 2C) implies that ATP remains stored after the initial exposure to 1 mM ATP, and this can later be used to fuel actin movement. ATP could remain in large quantities near the microchamber surface, escaping the rigor-solution washes. This could occur via either of two mechanisms. First, a high concentration of ATP could remain in unstirred solution layers near the microchamber surface, which would be a particularly realistic problem if a microscopically non-flat surface were used. In our case, however, the nitrocellulose surface was prepared by spin-coating that produces a flat surface, smoother than the underlying glass [20]. Further, upon collecting the total content of the microchamber (presumably including the unstirred solution layers), the measured ATP concentrations were as low as with the wash-through method. Thus, a high-concentration ATP source in the form of unstirred solution layers near the microchamber surface is unlikely.

The second possible mechanism for storage of ATP is in the form of ATP bound to proteins on the microchamber surface. To check for this possibility, a different sample-collection technique was applied. 10-μl samples were collected after scraping the chamber surface. Scraping

Fig. 2. (a) Actin-filament velocity after different degrees of washing with rigor solution. (b) Residual ATP concentration in the flow-through microchamber after different degrees of washing with rigor solution. (c) ATP-dependence of filament velocity in the case of ATP wash-in, and ATP wash-out. For the ATP wash-in data, velocities are plotted against the ATP concentrations of the wash-in solution; these concentrations were not significantly different from the concentrations in the microchamber following wash-in. Error bars refer to standard deviations for n filaments.

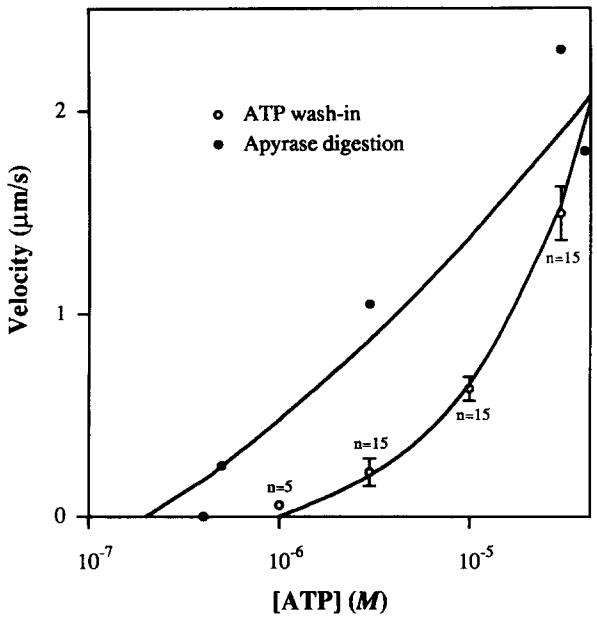


Fig. 3. Effect of ATP depletion by apyrase on the velocity of actin filaments. The ATP concentrations were calculated from the ATP-digestion kinetics measured immediately before the motility assay. Error bars refer to standard deviations for n filaments. For the apyrase experiments, pooled single observations of two experiments are shown.

perturbs the microchamber surface, releasing surface constituents. Using this method, significantly higher quantities of ATP were collected (Fig. 4), indicating that ATP did bind to proteins on the microchamber surface.

Of the proteins on the microchamber surface to which ATP could bind, the possibilities are: the two contractile proteins, or BSA. Actin is excluded, because we could obtain similar movement at nanomolar ATP when the HMM-coated surface was preexposed to the high ATP prior to the addition of actin. A more likely possibility is that the ATP pretreatment leaves behind the lawn of myosin heads saturated with ATP (HMM · ATP). When the free ATP concentration is subsequently lowered by washing, the remaining high concentration of HMM · ATP could fuel motility. To check this possibility, we calculated the rate of decay of HMM · ATP, and compared it to the measured decay in filament velocity (both following the third rigor-solution wash). If the initially high concentration of HMM · ATP drives movement, the decay of HMM-bound nucleotide should result in a parallel decay of filament velocity. HMM-bound ATP is quickly hydrolyzed to ADP and P_i, which remain associated with the myosin head, and can power motility [24]. The rate of HMM-nucleotide decay is thus determined by the dissociation of the hydrolysis products. In the absence of actin, ADP and P_i dissociate from the myosin head with a rate constant of 0.05 s^{-1} [24]. Thus, within 20 s, 63% of the myosin heads lose their tightly bound nucleotide. The decay proceeds until an equilibrium is reached with the free ATP remaining in the microchamber (e.g., after the third wash, 100 nM). In the case of our densely HMM-coated microchamber, assuming a 100% initial saturation with ATP, this equilibrium is reached in about 85 s. At this time, only 1.4% of the myosin heads contain associated nucleotide. Thus, by 85 s, filament velocity is expected to drop by 98.6%. Further, in a recent work, Sowerby et al. [25] measured the rate of ATP turnover by myosin filaments under in vitro motility assay conditions. Their measurements yielded an even higher rate constant (0.064 s^{-1} for skeletal myosin filaments). Considering the observations of Sowerby et al. [25], actin-filament movement

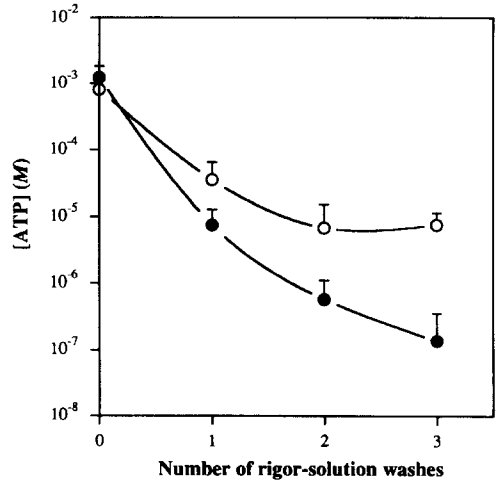


Fig. 4. Comparison of the amounts of ATP collected with the washing (●) and scraping (○) methods. Scraping the microchamber surface yielded a release of 'surplus' ATP. Error bars refer to standard deviations for three experiments.

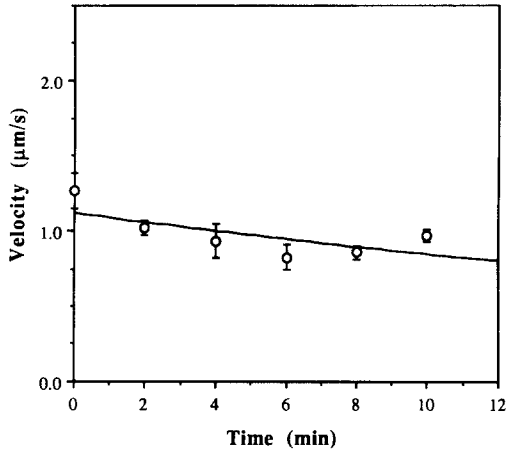


Fig. 5. Change in average filament velocity at 100 nM ATP concentration (after three rigor-solution washes), as a function of time. Error bars refer to standard deviations for five filaments. Curve was drawn by single exponential fit. Continued movement of actin filaments could be visually monitored for up to 15 minutes. At this time, however, the filaments were so faint and small that precise determination of their velocity was precluded.

should stop within a few tens of seconds following the third rigor-solution wash. By contrast, filament velocity hardly changed over a 10 min period; it decreased by less than 25% (Fig. 5). Thus, the HMM-bound nucleotide does not appear to be sufficient to support persistent motility.

The third and final possibility is that a reservoir of stored ATP resides in BSA. Indeed, BSA binds ATP with high affinity (K_d, 30–110 μM) [26]. To test whether BSA-bound ATP serves as an ATP source for motility, we performed experiments where BSA was entirely omitted. We obtained results identical to the original observations; i.e., motility persisted after the rigor-solution washes. Two experiments were performed. Actin-filament velocity after the third rigor-solution wash – corresponding to a free ATP level of 135 nM – was found to be as high as 1.22 μm/s ($\pm$0.16, $n = 14$). Thus, any ATP bound to BSA during pre-exposure appears to be stable, and unavailable to fuel motility.

4. Discussion

In this work, we have shown that the in vitro motility of actin filaments over a lawn of HMM can persist at ATP concentrations down to the nanomolar range, provided that the acto-HMM is pretreated with ATP in the millimolar or 'physiological' concentration range. In contrast, if the ATP concentration is increased from zero, filament movement begins only as the ATP concentration is raised above 1 μM, similarly to the previous results of Harada et al. [7]. Thus, one can observe different ATP-velocity functions depending on whether ATP concentration is being raised to, or decreased from, the 'physiological'.

In an interesting contrast to our findings, the ATP dependence of tension generation by skinned single muscle fibers under isometric and low-Ca^{2+} conditions shows the opposite: when increasing ATP concentration from zero (rigor state), tension begins to rise at an ATP level as low as a few tens of nanomolar, and peaks at around 2 μM [27,28]. Upon further increase in ATP, the muscle relaxes. When ATP is decreased from the millimolar concentration range (relaxed state), however, tension responses to stretch increase at as high an ATP level as 200 μM [29]. The difference between the observations in the in vitro actomyosin motility and the whole muscle fiber could be caused by several factors (e.g., presence of regulatory proteins and titin in the muscle fiber, unloaded conditions and low ionic strength in the in vitro assay versus isometric conditions and high ionic strength in the muscle fiber). Nevertheless, the findings of Reuben et al. [27] and Goldman et al. [28] indicate that the interaction of actin and myosin can indeed lead to contraction at submicromolar ATP concentrations.

As far as the mechanism of the persisting in vitro actin motility is concerned, it might be caused by an ATP storage and release during the ATP pretreatment and subsequent washouts; alternatively, the phenomenon could be the result of a physiologically interesting mechanism, much like the one underlying the problem of 'unexplained energy'. We examined the problem of ATP storage-release through a series of experiments, giving consideration to all candidates we could conceive of. However, our results imply that ATP stored during preexposure is largely unavailable to drive subsequent motility. The amount available must therefore be determined by the solution-ATP levels, which were extremely low. We explored the possibility that this low ATP level itself might be sufficient to drive motility. We did this by estimating the myosin step size – the amount of filament translation driven by each myosin head, per ATP split. This was calculated assuming Michaelis-Menten kinetics and using the approach of both Toyoshima et al. [30] and Harada et al. [31]. At 8 nM ATP and 0.07 μm/s average filament velocity, the approach of Toyoshima et al. [30] yielded a step size of 93 nm, while that of Harada et al. [31] gave 440 nm. (These calculations do not take into account the numerous nucleotide-free myosin heads expected at these low ATP levels, which will restrain motility. In the absence of such restraint, the step size would be even larger.) Since steps of this size are excessively large according to current views [32], it is clear that the 8 nM ATP concentration is insufficient to drive motility. In order to fuel the motion observed at nanomolar ATP concentrations, therefore, some form of potential energy must be conferred to the system by the ATP pretreatment.

The nature of the potential energy is not yet clear. The energy could be stored in actomyosin in the form of elastic or conformational energy [9–11,33]. Another possibility is that the ATP preexposure replenishes energy-rich phosphoryl groups on the basic amino acids of myosin [34]. Alternatively, the preexposure could induce changes in the free energy of actomyosin's hydration shell [35]. A recent work by Elliott and Worthington [36] offers another mechanism for a possible energy storage: the myosin molecule is charged by ATP in a slow process, and the discharge of myosin – hence the impulsive, force-generating step – takes place as a rapid process which can occur at a later time. An intriguing possibility within the framework of the cross-bridge mechanism is that the ATP pretreatment could 'cock' the myosin heads, leaving them poised to stroke.

Whatever the mechanism, the results imply energy storage: when ATP is diminished from an initially high level, motility persists at far lower ATP concentrations than when ATP is elevated from an initially low level. Functionally, this implies that in actomyosin motile systems, energy may be mobilized in advance of the contractile event. In the intact sarcomere, this advance mobilization could in principle account for the unexplained gap [1,2] between the energy delivered and the energy accounted for by concurrent splitting of ATP. The gap could be filled by the stored energy.

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References

[1] Homsher, E. (1987) Annu. Rev. Physiol. 49, 673–690.
[2] Ohno, T. and Kodama, T. (1991) J. Physiol. (Lond) 441, 685–702.
[3] Yanagida, T., Nakase, M., Nishiyama, K. and Oosawa, F. (1984) Nature 307, 58–60.
[4] Kron, S.J. and Spudich, J.A. (1986) Proc. Natl. Acad. Sci. USA 83, 6272–6276.
[5] Homsher, E., Wang, F. and Sellers, J.R. (1992) Am. J. Physiol. 262, C714–C723.
[6] Moos, C. (1973) Cold Spring Harbor Symp. Quant. Biol. 37, 137–143.
[7] Harada, Y., Noguchi, A., Kishino, A. and Yanagida, T. (1987) Nature 326, 805–808.
[8] Gordon, A.M. (1989) in Textbook of Physiology (Patton, H.D., Fuchs, H.D., Hille, B., Scher, A.M. and Steiner, R., eds.), Vol. pp. 196–213, W.B. Saunders, Philadelphia.
[9] Hozumi, T. (1988) Biochem. Int. 17, 171–178.
[10] Ménétret, J.-F., Hoffmann, W., Schröder, R.R., Rapp, G. and Goody, R.S. (1991) J. Mol. Biol. 219, 139–144.
[11] Nag, S., Suzuki, H., Sosinski, J. and Seidel, J.C. (1987) Prog. Clin. Biol. Res. 245, 91–108.
[12] Kellermayer, M.S.Z., Hinds, T.R. and Pollack, G.H. (1993) Biophys. J. 64, A232.
[13] Kellermayer, M.S.Z. and Pollack, G.H. (1993) in 11th International Biophysics Congress, pp. 98, Budapest.
[14] Margossian, S.S. and Lowey, S. (1982) Methods Enzymol. 85, 55–72.
[15] Kron, S.J., Toyoshima, Y.Y., Uyeda, T.Q. and Spudich, J.A. (1991) Methods Enzymol. 196, 399–416.
[16] Pardee, J.D. and Spudich, J.A. (1982) Methods Enzymol. 85, 164–182.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Warshaw, D.M., Desrosiers, J.M., Work, S.S. and Trybus, K.M. (1990) J. Cell Biol. 111, 453–463.
[19] Hershcovitz, M. and Klein, I.E. (1993) Microelectron. Reliability 33, 869–80.
[20] Yonkoski, R.K. and Soane, D.S. (1991) in Materials Science of High Temperature Polymers for Microelectronics Symposium (Grubb, D.T., Mita, I. and Yoon, D.Y., eds.), pp. 259–264, Mater. Res. Soc., Pittsburgh.
[21] Kishino, A. and Yanagida, T. (1988) Nature 334, 74–76.
[22] Uyeda, T.Q., Kron, S.J. and Spudich, J.A. (1990) J. Mol. Biol. 214, 699–710.
[23] Wu, L., Hinds, T.R. and Vincenzi, F.F. (1992) Biochim. Biophys. Acta 1106, 56–62.
[24] Lymn, R.W. and Taylor, E.W. (1971) Biochemistry 10, 4617–4624.
[25] Sowerby, A.J., Seehra, C.K., Lee, M. and Bagshaw, C.R. (1993) J. Mol. Biol. 234, 114–123.
[26] Bauer, M., Baumann, J. and Trommer, W.E. (1992) FEBS Lett. 313, 288–290.
[27] Reuben, J.P., Brandt, P.W., Berman, M. and Grundfest, H. (1971) J. Gen. Physiol. 57, 385–407.
[28] Goldman, Y.E., Hibberd, M.G. and Trentham, D.R. (1984) J. Physiol. (London) 354, 577–604.
[29] Ushitani, H., Iwamoto, H. and Sugi, H. (1994) J. Muscle Res. Cell Motil. 15, 354–355.
[30] Toyoshima, Y.Y., Kron, S.J. and Spudich, J.A. (1990) Proc. Natl. Acad. Sci. USA 87, 7130–4.
[31] Harada, Y., Sakurada, K., Aoki, T., Thomas, D.D. and Yanagida, T. (1990) J. Mol. Biol. 216, 49–68.
[32] Huxley, A.F. and Simmons, R.M. (1971) Nature 233, 533–538.
[33] Burlacu, S., Janmey, P.A. and Borejdo, J. (1992) Am. J. Physiol. 262, C569–C577.
[34] Fazekas, S., Berentey, G., Fehér, G. and Székessy-Hermann, V. (1988) Acta Physiol. Hung. 71, 183–201.
[35] Oplatka, A. (1989) in International Conference on Muscle Energetics (Paul, R.J., Elzinga, G. and Yamada, K., eds.), pp. 45–49, A.R. Liss, Yufuin, Japan.
[36] Elliott, G.F. and Worthington, C.R. (1994) Biochim. Biophys. Acta 1200, 109–116.