Myosin lever disposition during length oscillations when power stroke tilting is reduced

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Myosin motors are intracellular protein macromolecules, which function as actin-dependent transducers of ATP hydrolysis free energy into mechanical work. At least 17 classes of myosin exist, fulfilling many important motile roles in cell physiology, including cell division, cytokinesis, exo-, endo-, and pinocytosis, acuity adjustment of sensory receptors, and the contraction of all forms of muscle tissue (34). Their mechanism is thought to involve the active lever arm model of the power stroke. During the initial part of the release phase of the thin filament, the lever arm tilts, approaching its orientation at IM3,max and IM3 rises. If the tilting is great enough, the lever goes beyond IM3,max and the intensity starts to decrease, reaching a new minimum at the end of the release and increasing again when the preparation is restretched toward its resting length. Distortion at frequencies <1 kHz, IM3 signals become increasingly distorted, developing a secondary intensity minimum at maximum shortening (forming an intensity “double peak”; Ref. 2). This behavior can be accounted for within the framework of a tilting lever power stroke hypothesis. During the initial part of the release phase of the thin filament, the lever arm tilts, approaching its orientation at IM3,max and IM3 rises. If the tilting is great enough, the lever goes beyond IM3,max and the intensity starts to decrease, reaching a new minimum at the end of the release and increasing again when the preparation is restretched toward its resting length. Distortion at frequencies <1 kHz, IM3 signals become increasingly distorted, developing a secondary intensity minimum at maximum shortening (forming an intensity “double peak”; Ref. 2). This behavior can be accounted for within the framework of a tilting lever power stroke hypothesis. During the initial part of the release phase of the thin filament, the lever arm tilts, approaching its orientation at IM3,max and IM3 rises. If the tilting is great enough, the lever goes beyond IM3,max and the intensity starts to decrease, reaching a new minimum at the end of the release and increasing again when the preparation is restretched toward its resting length. Distortion at frequencies <1 kHz, IM3 signals become increasingly distorted, developing a secondary intensity minimum at maximum shortening (forming an intensity “double peak”; Ref. 2). This behavior can be accounted for within the framework of a tilting lever power stroke hypothesis. During the initial part of the release phase of the thin filament, the lever arm tilts, approaching its orientation at IM3,max and IM3 rises. If the tilting is great enough, the lever goes beyond IM3,max and the intensity starts to decrease, reaching a new minimum at the end of the release and increasing again when the preparation is restretched toward its resting length. Distortion at frequencies <1 kHz, IM3 signals become increasingly distorted, developing a secondary intensity minimum at maximum shortening (forming an intensity “double peak”; Ref. 2). This behavior can be accounted for within the framework of a tilting lever power stroke hypothesis. During the initial part of the release phase of the thin filament, the lever arm tilts, approaching its orientation at IM3,max and IM3 rises. If the tilting is great enough, the lever goes beyond IM3,max and the intensity starts to decrease, reaching a new minimum at the end of the release and increasing again when the preparation is restretched toward its resting length. Distortion at frequencies <1 kHz, IM3 signals become increasingly distorted, developing a secondary intensity minimum at maximum 0363-6143/05 $8.00 Copyright © 2005 the American Physiological Society
for the power stroke to progress appreciably, thus active tilting is reduced and the remaining elastic tilting may be insufficient to reach the ISM3,max orientation, resulting in an undistorted, quasi-sinusoidal ISM3 signal.

The appearance of a secondary intensity minimum allows the mean position of the lever arm to be determined relative to its ISM3,max position. To achieve this, the ISM3 response was simulated by a model, in which we varied the mean tilt of the lever arm domain, until a good fit to the corresponding, experimentally observed ISM3 distortion was obtained (12).

In principle, it is possible to account for the absence of distortion at high frequencies in other ways. It could be argued that ISM3 distortion results from a separate structural event accompanying the power stroke, but unrelated to lever arm disposition. This arrangement could also reduce ISM3 distortion at frequencies >1 kHz through power stroke reduction. In addition, it has been proposed that the lever arm may be shifted away from ISM3,max during oscillations at high frequencies (6), in which case an increased oscillation amplitude would be required for the lever arm to reach ISM3,max and to produce ISM3 distortion. These possibilities can be examined by measurement of experimental ISM3 changes at larger length oscillation amplitudes in the high frequency range; if the absence of distortion were simply due to reduction of power stroke tilting (and hence reduced total lever displacement), distortion should be restorable by an increase in the elastic component of lever arm movement. On the other hand, if the absence of distortion resulted from a shift in mean lever arm tilt away from its ISM3,max disposition during high-frequency oscillations, this should be apparent in the lever position required to simulate ISM3 signals for larger amplitude length oscillations. Finally, if distortion were solely dependent on the size of the power stroke event, then because of the reduced power stroke at the higher frequency, replication of the ISM3 distortion obtained at low frequencies would require an increase in oscillation amplitude in the high frequency domain sufficient to produce a power stroke movement of the same absolute size.

Our findings show that distortion can be restored by increasing the oscillation amplitude so as to increase the elastic movement of the lever arm. Analysis of the results with the model indicate that 1) distortion is due to both elastic and active movement of the lever arm and 2) the mean lever arm disposition during high-frequency oscillations is similar to that determined for lower frequencies.

**METHODS**

**Preparation.** To reduce the heat overload of the stretcher motor caused by the high-amplitude length oscillation needed to test our hypotheses, we performed our experiments on tetanized fibers bundles from the dorsal interossei muscles (Rana temporaria), which are 30% shorter than tibialis. This allowed us to impose a larger percentage of the lever arm movement. On the other hand, if the absence of distortion resulted from a shift in mean lever arm tilt away from its ISM3,max disposition during high-frequency oscillations, this should be apparent in the lever position required to simulate ISM3 signals for larger amplitude length oscillations. Finally, if distortion were solely dependent on the size of the power stroke event, then because of the reduced power stroke at the higher frequency, replication of the ISM3 distortion obtained at low frequencies would require an increase in oscillation amplitude in the high frequency domain sufficient to produce a power stroke movement of the same absolute size.

For ISM3 model simulations, experimental sarcomere length and force records were used to generate ISM3 signals. We assumed that the applied length changes caused corresponding changes in both filament compliance and in lever tilt. Filament compliance at isometric tetanic force tension (P0) was taken to be 50% of total sarcomere compliance, which is 4 nm per half sarcomere (HS) per P0 (1, 24) and changes in length of this filament compliance were assumed to be proportional to the instantaneous force (P). Taking filament and cross-bridge compliance to act in series and to account for all the sarcomere compliance, then the average change in length of cross-bridge compliance is given by subtraction of the filament compliance length changes from the total sarcomere length change. We further assumed that all cross-bridge compliance is associated with passive tilting of the lever arm and therefore we varied lever tilt by an amount sufficient to displace the lever tip by an axial distance (zcg) corresponding to the instantaneous change in length of the cross-bridge compliance. A single S1 was represented by the spatial coordinates of the α-carbon atoms of both the heavy and light chains of nucleotide-free chicken skeletal myosin (31). We orientated S1 with respect to the actin filament axis for the best fit of this S1 X-ray crystallographic structure to cryoelectron microscopy images of decorated F-actin. The Fourier transform (F) of S1 structure was obtained by treating the jth α-carbon scatterer as a δ function shifted in real space by its separation from the center of gravity (zcg) of the motor domain [zcg(P) − zcg], measured along the actin filament axis (z-coordinate), then forming the sum over m α-carbons in S1.

X-ray data were collected at beamline SAXS (Elettra, Trieste, Italy). Radiation (λ = 0.15 nm, beam dimensions 0.5 mm × 3 mm) passed through the chamber horizontally through Kapton windows (12.5 μm thickness) placed within 0.2 mm of the bundle to minimize the X-ray path length in water. ISM3 was measured using a delay line, one-dimensional X-ray detector, positioned with its axis along the meridian of the X-ray pattern at a distance of 2.46 m from the preparation. A fast beam shutter enabled us to expose the fiber to radiation only during periods of data collection to minimize beam damage.

Tetanic contractions (500 to 600 ms duration) were evoked by trains of brief electric field pulses (0.2 ms, 12–16 Hz) at 180-s intervals. Length oscillation trains (350-ms total duration) were imposed on the preparation at the tetanus plateau. The experiment was terminated when the tetanic tension dropped by >15% compared with the initial control tetanus.

**Data acquisition.** Mechanical data were collected on a Pentium-based personal computer using a user-designed timing and a data-acquisition system. X-ray data were collected on a separate, dedicated machine, whose sampling was triggered by the data-acquisition system. X-ray and mechanical data readings were performed at intervals of 1/200th the oscillation period to ensure X-ray and mechanical sampling at exactly the same points in an oscillation period during an oscillation sequence. This permitted data to be summed over the whole sequence of individual oscillations, so as to obtain an averaged ISM3 signal over a single oscillation period. This, in turn, was then summed with the corresponding ISM3 signals from 10–40 tetani for a given preparation to obtain sufficient X-ray counts for the required time resolution (17.8 μs).

**Data processing and simulation.** Meridional X-ray patterns were analyzed with a Levenberg-Marquardt fitting algorithm (30) using a mathematical description of the meridional spectrum as a polynomial background intensity on which Gaussian reflections were superimposed, as described previously elsewhere (11). Possible bundle movement in the X-ray beam was checked by examination of the total spectrum intensity (which depends on the quantity of mass in the X-ray beam) and corrected for, if necessary, by scaling spectra to maintain total intensity constant. In agreement with previous data obtained in similar conditions (6), the application of length oscillations did not alter the width of the M3 reflection either along or across the meridian.

For ISM3 model simulations, experimental sarcomere length and force records were used to generate ISM3 signals. We assumed that the applied length changes caused corresponding changes in both filament compliance and in lever tilt. Filament compliance at isometric tetanic force tension (P0) was taken to be 50% of total sarcomere compliance, which is 4 nm per half sarcomere (HS) per P0 (1, 24) and changes in length of this filament compliance were assumed to be proportional to the instantaneous force (P). Taking filament and cross-bridge compliance to act in series and to account for all the sarcomere compliance, then the average change in length of cross-bridge compliance is given by subtraction of the filament compliance length changes from the total sarcomere length change. We further assumed that all cross-bridge compliance is associated with passive tilting of the lever arm and therefore we varied lever tilt by an amount sufficient to displace the lever tip by an axial distance (zcg) corresponding to the instantaneous change in length of the cross-bridge compliance. A single S1 was represented by the spatial coordinates of the α-carbon atoms of both the heavy and light chains of nucleotide-free chicken skeletal myosin (31). We orientated S1 with respect to the actin filament axis for the best fit of this S1 X-ray crystallographic structure to cryoelectron microscopy images of decorated F-actin. The Fourier transform (F) of S1 structure was obtained by treating the jth α-carbon scatterer as a δ function shifted in real space by its separation from the center of gravity (zcg) of the motor domain [zcg(P) − zcg], measured along the actin filament axis (z-coordinate), then forming the sum over m α-carbons in S1 to give
where \( Z \) is the meridional reciprocal space coordinate. The lever arm tilting effect is accommodated by changing \( z_i \) (\( P \)) appropriately for the instantaneous tension for each \( \alpha \)-carbon belonging to the lever arm (\( z_{\alpha,s} \) for the motor domain were constants). Transforms were summed over 50 \( S_1 \) on either side of the M-line, each separated by an axial spacing of 14.55 nm, using a mirrored \( S_1 \) structure on opposite sides \( \mathcal{F}[S_1(z)], \mathcal{F}[S_1(-z)] \) and a 169.5-nm M-line gap (\( a \)), to obtain a transform of the thick filament

\[
\mathcal{F}[\text{Thick filament}] = \sum_{p=1}^{n} \mathcal{F}[S_1(z)] e^{2\pi j p(1+i/p_0)Z} + \mathcal{F}[S_1(-z)] e^{-2\pi j p(1+i/p_0)Z} \tag{2}
\]

where \( n \) is the number of 14.55 nm segments (\( l \)) in half of a thick filament. The displacement of the actin-bound motor domain, is accommodated in Eq. 2 by changing appropriately \( a \). The squared modulus of this transform, integrated over its whole profile, was taken as \( \Delta z_{\text{avg}} \).

Estimates of \( \Delta z \) (the lever tip displacement required to reach \( \text{IM}_{3,\text{max}} \)) were obtained from least-squares fitting of the displacement-intensity curves (Fig. 4) to the IM_{3} signals obtained during oscillations, using force and sarcomere length records to calculate lever arm tilting as outlined above. Values quoted in the text are means \( \pm \) SD.

RESULTS

Mechanical properties of dorsal interossei bundles. Because our previous studies of the effects of oscillations in IM_{3} had been conducted on tibialis anterior muscle, we first performed a comparative study of the mechanical properties of dorsal interossei and tibialis anterior. For improved resolution, the study was performed on single fibers with the use of a striation follower device to monitor the sarcomere length changes (14). We measured the compliance of both preparations as a function of oscillation frequency falls) of the two preparations. For dorsal interossei fibers, we obtained compliances of 8.98 (100 Hz), 7.04 (200 Hz), 5.99 (400 Hz), 5.25 (1 kHz), and 3.98 (3.4 kHz), all expressed in nanometers per HS/P_{0}. The corresponding measurements for tibialis anterior were 7.91 (100 Hz), 6.9 (200 Hz), 5.79 (400 Hz), 4.62 (1 kHz), and 3.91 (3.4 kHz), in nanometers per HS/P_{0}. The similarity of isometric tension development, absolute compliance measurements and of compliance dependency on oscillation frequency shows that the instantaneous stiffness and the power stroke kinetics of these two preparations are very similar.

Force and IM_{3} signals from dorsal interossei bundles during 2.8-kHz length oscillations. Figure 1 shows the force response to sinusoidal length oscillations at 2.8 kHz applied at tetanus plateau in a fiber bundle from interossei muscle. The two cycles shown in the figure represent the average of the 970 cycles applied during a single contraction. It can be seen that at this relatively high frequency of oscillation, force, and sarcomere length signals are in phase. Because both the synchronized power stroke and fiber inertia introduce a phase shift between force and length signals, this means that the force response is dominated by muscle elasticity.

Small-amplitude 2.8-kHz oscillations (peak to peak, 3–5 nm per HS, producing force oscillations less than or equal to isometric tension) similar to those applied previously to tibialis anterior muscle produced almost sinusoidal changes in IM_{3} with little or no distortion (Fig. 2A), in phase opposition to bundle length changes, with IM_{3,max} roughly coincident with maximum shortening. However, larger oscillation amplitudes (peak to peak, 5–7 nm per HS, producing force oscillations of 1.3–1.6 times isometric tension) gave rise to a clear secondary minimum in IM_{3} at maximum shortening (Fig. 2A), producing a double peak in intensity at this point similar to that seen previously at lower frequencies (2) or higher temperatures (12).

Power stroke contribution to lever movement suppression at high frequencies. For low-amplitude oscillations at frequencies approaching the frequency domain of the power stroke, we suggest that active tilting is reduced and the remaining elastic tilting may be insufficient to reach IM_{3,max}. As a result, there is no distortion of the IM_{3} signal. The changes in total lever arm excursion and the power stroke contribution, calculated in the range of 200 Hz to 3 kHz by assuming a lever arm compliance of 2 nm per HS/P_{0} and shown in Fig. 3, demonstrate that the active movement is greatly attenuated at high frequencies.

For example, we found that 69% of lever tip movement (\( z_{\text{tip}} \)) during 200 Hz oscillations was active tilting, whereas at 2.8 kHz this component fell to only 24%. However, because at 2.8 kHz, \( z_{\text{tip}} \) was also reduced to only 45% of its value at 200 Hz, consequently absolute lever displacement due to the power stroke was reduced by 85% when oscillation frequency was increased from 200 Hz to 2.8 kHz.

\( \text{IM}_{3} \) signal amplitude during oscillations. In a previous study, we have simulated IM_{3} changes by representing \( S_1 \) as a column
observed intensity signals could be better simulated using the molecular structure of S1. In addition, we studied the effect of two additional parameters that could influence simulated intensity: 1) disordering of the period of S1 binding to the thin filament due to the mismatch of actin and myosin helices; 2) effects of a detached S1 moiety in each actin-bound myosin dimer. See the APPENDIX for a detailed mathematical treatment of these effects.

We assume here that S1 lever displacement contributes all cross-bridge compliance and that the tip of the lever arm remains at an ordered axial period of 14.55 nm. The motor domain is assumed to bind to the nearest actin monomer (axial period ~5.5 nm), leading to a disordered axial period for this domain in the range 14.55 ± 2.75 nm, whereas lever arm tilt is adjusted to link the position of its tip to its pivot in the actin-bound motor domain. We calculated IM3 as a function of the axial \( \Delta z \) for both ordered and disordered axial periods of the motor domain (Fig. 4) with the use of the S1 structure and actin-bound motor domain orientation proposed by Rayment et al. (31). The comparison shown in Fig. 4 indicates that the shape of this function was similar in both cases, in agreement with an earlier study (22) and was also similar to that obtained from our simulations of S1 structure by overlapping spheres (2, 12). IM3,max was reduced to 72% by disordering whereas the filament displacement required to reduce IM3,max intensity by 20% was slightly reduced from 2.93 to 2.81 nm.

Inclusion of an intensity contribution from a detached S1 moiety for each actin-bound myosin dimer was simulated assuming that the tips of actin-bound and free S1 lever arms coincided at all times and that free S1 orientation was independent of changes in tilt of the actin-bound S1 lever. We first examined the effect of the orientation of free S1s on the change in lever arm tip position along the fiber axis needed to reach IM3,max (\( \Delta z \)). We found that IM3,max occurs at roughly maximum overlap of the mass projections of the actin-bound and free S1 motor domains, so \( \Delta z \) is rather sensitive to axial displacement of the free head motor domain with respect to its actin-bound partner. Because the measured \( \Delta z \) needed to reach

of overlapping spheres (2). This simulation reproduced well the shape of experimentally observed IM3 signals, but not their absolute amplitude, in fact simulated intensity changes were substantially smaller (mean 52 ± 17%) than those actually observed. We therefore examined whether the amplitude of

![Image](image1.jpg)

**Fig. 2.** Tension (continuous line) and M3 reflection intensity (IM3) signals from bundles of activated dorsal interossei muscle fibers during sinusoidal length oscillations of different amplitudes at 2.8 kHz. Peak-to-peak force oscillations were 0.72 P0 (A), 1.17 P0 (B), and 1.42 P0 (C). Corresponding IM3 changes were 0.128, 0.17, and 0.30 maximum IM3 (IM3,max). Force and IM3 signals were averaged over one oscillation period, but plotted over two periods to clarify waveform shape. Length oscillation amplitudes were (peak to peak) 3.32 nm per HS (A), 5.36 nm per HS (B), and 6.62 nm per HS (C). In B and C, an increasingly evident distortion of the IM3 signal is present in the form of a new intensity minimum close to the point of maximum shortening. The peak of this intensity minimum lagged slightly behind the force minimum. Vertical calibration bar corresponds to 2.5 P0 and 0.2 IM3,max.

![Image](image2.jpg)

**Fig. 3.** Calculated maximum peak-to-peak excursion of the lever arm tip (•) during oscillations in the frequency range from 200 Hz to 3 kHz, expressed relative to the value of 5.64 nm per HS calculated at 200 Hz. Displacement was evaluated for a peak-to-peak force oscillation amplitude equal to isometric tension at each frequency, assuming 50% of total sarcomere compliance resides in the myofilaments and it is simply in series with cross-bridge compliance. ⊙, Calculated relative contribution to the total lever tip excursion by the active tilting at each frequency.

![Image](image3.jpg)

**Fig. 4.** Dependence of simulated IM3 (normalized to the value of IM3,max) on lever arm displacement for a single ordered (●) and disordered subfragment 1 (S1; ⬤). Inset, crystallographic S1 structure with the lever arm tip tilted by ~10 nm (left), ~6.3 nm (mean position during isometric contractions; middle) from its nucleotide-free orientation and at the nucleotide-free orientation (0.0 nm; right), respectively. Values for the ordered state, calculated from Eq. 5; for the disordered state, calculated from Eq. 7. IM3 is simulated for a lever tip displacement (\( \Delta z \)) of 10 nm in the direction of the power stroke; the lever orientation at 10 nm displacement corresponds to that in the nucleotide-free crystallographic structure of S1 (31). The apparently paradoxical, increased steepness of the disordered curve results from the proportionally greater reduction in the intensity contribution from the "smeared out" mass projection of the motor domain.
I_{M3,\text{max}} is small (2), this would constrain the axial location of the second S1 motor domain to be close to that of the isometric, actin-bound structure. To simulate this arrangement, we first selected the nucleotide-free S1 structure and orientation proposed by Rayment et al. (31) for the actin-bound S1, but we tilted the lever domain so that its tip was displaced by 6 nm toward the M-line [shown in Fig. 5A as a clockwise rotation of the lever (red) by $39.2^\circ$ in the y-z plane about its pivot in the motor domain], to account for the ~6 nm $\Delta z$ required to discharge all force recovery after a large step release [the “T2” intercept of Huxley and Simmons (15), assuming 50% filament compliance], taking the unloaded post power stroke state to correspond to the rigor structure.

This orientates the lever at close to $90^\circ$ to the fiber axis, which would be expected for the most efficient conversion of tilting into translational motion. The free S1 was orientated as for the actin-bound structure, but the lever was displaced a further 2 nm (i.e., tilted through a further $10^\circ$ clockwise in the same sense as in Fig. 5A) to represent a prepower stroke state.

Finally, the whole free S1 was rotated $15^\circ$ anticlockwise in the y-z plane about the tip of the lever, an orientation giving a $\Delta z$ of 1.0 nm. The disposition of the free S1 lever was close to that reported for the relaxed state in fluorescence depolarization studies (3, 5). The two-headed complex is shown in Fig. 5B.

The plot of I_{M3} as a function of tip displacement now became much steeper than that for a single head shown in Fig. 4, the amplitude of tip displacement required to reduce $I_{M3,\text{max}}$ by 20% being reduced to 2.4 nm. Absolute maximum intensity increased by 3.9-fold compared with that of the ordered single head. Disordering of the bound head, as before, reduced $I_{M3,\text{max}}$ by 3.3-fold compared with the ordered single head, but had little effect on the shape or the position of the relation between tip displacement and intensity. Finally, giving to the free head an angular standard deviation of $37^\circ$ (the proposed angular dispersion in the relaxed state, Ref. 5), reduced $I_{M3,\text{max}}$ to 1.26-fold that of the ordered single head without altering significantly the tip displacement required to reduce $I_{M3,\text{max}}$ by 20%, which remained 2.4 nm. However, the position of $I_{M3,\text{max}}$ now returns to being dominated by the lever orientation of the bound head; that is, similar to the single head situation. This is because the axial mass projection of the free head is increasingly smeared out with distance from the lever tip by rotational disorder; hence, the densest region of the mass projection is at its tip, which is coincident with the tip of the actin-bound lever.

The increased steepness of the $I_{M3}$ dependence on axial displacement for all double-headed myosin models is in better agreement with the absolute intensity changes we observed experimentally during sinusoidal oscillations, implying that detached S1 may make a significant contribution to I_{M3} signals, in accordance with previous observations (29). In all cases studied, calculations with the model showed, in agreement with previous results (25), that the interference between diffracting units on either side of the M-line had no significant effect (<1%) on total M3 intensity.

**Estimation of $\Delta z$ from $I_{M3}$ signals during length oscillations.** Estimation of $\Delta z$ was obtained by fitting the theoretical $I_{M3}$ changes, calculated for both single and double-headed myosin structures as outlined in METHODS, to the experimental data. Data were fitted with the use of a least-squares algorithm (30), varying $\Delta z$, and adjusting the amplitude of the simulated signal to minimize the residuals. To compare these values for $\Delta z$ with those at lower frequencies, we analyzed our earlier data obtained at 200 Hz from tibialis fibers in the same manner. For a single, ordered S1 (open symbols in Fig. 4) we obtained a mean $\Delta z$ at 2.8 kHz of 1.33 ± 0.58 nm. For tibialis data at 200 Hz, we obtained a $\Delta z$ of 1.17 ± 0.47 nm. The difference in these values was not statistically significant ($P = 0.621$). For double-headed myosin, with the actin-bound S1 disordered and the free S1 ordered, we obtained a $\Delta z$ of 1.24 ± 0.49 nm at 2.8 kHz and 1.11 ± 0.39 nm at 200 Hz ($P = 0.638$). In both cases, the absolute intensity changes were smaller in simulations than those measured experimentally, although the double-headed myosin gave a closer approximation of the experimental signal amplitude. However, we also found that the best fits for single or double-headed myosin to the experimental data consistently appeared to underestimate the amplitude of the distortion and of the secondary minimum (Fig. 6, A and B), suggesting that the true dependence of intensity on tip displacement might be a sharper function than accounted for by our present modeling.

We therefore fitted the single, ordered S1 model to the experimental data by eye, adjusting the value of $\Delta z$ to obtain a good match between the depths of the secondary minimum and the sinusoidal part of the $I_{M3}$ signal (Fig. 6C), as we had done in previous studies (2, 12). This method reduced the mean $\Delta z$ (200 Hz, 0.98 ± 0.19 nm; 2.8 kHz, 0.76 ± 0.20 nm, $P = 0.73$). The absence of a significant difference between paired values of $\Delta z$ determined at 2.8 kHz and 200 Hz for the different structures and fits suggests that the absence of distortion in previous oscillation studies in the kilohertz frequency domain (6, 10) appears not to have been due to suppression of the power stroke per se, nor to a shift in the mean position of the lever arm away from $I_{M3,\text{max}}$ during high frequency oscillations (6), but to the application of an insufficiently large filament displacement.

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**Fig. 5.** A: actin-bound S1 structure at isometric tension. Only $\alpha$-carbon chains are shown. Lever arm domain (red) is tilted at an angle of $39.2^\circ$ clockwise in the y-z plane about its pivot from its orientation in the nucleotide-free structure. Motor domain is shown in blue, light chains in green. B: free S1 superimposed on the actin-bound structure. Lever arm (yellow) is tilted at $49.2^\circ$ clockwise from its orientation in the nucleotide free structure with respect to its motor domain (light green) and the whole free S1 structure then rotated $15^\circ$ anti-clockwise about the lever arm tip to obtain a $\Delta z$ of 1.0 nm. Light chains are shown in sky blue. The actin filament would lie horizontally in the y-z plane at the top of the figure and the M-line would be located to the far left, the Z-line to the far right.
DISCUSSION

The most widely accepted mechanism for the myosin power stroke is an angular displacement of part or all of myosin S1 moiety (17, 19). Considerable evidence from electron microscopy (9), X-ray diffraction (22), and optical probes (4, 5, 35) has recently emerged in favor of this mechanism from both muscle and nonmuscle myosins. The meridional M3 X-ray reflection in the skeletal muscle pattern, with a lattice spacing derived from the axial period of S1 projections from the thick filament, is sensitive to the synchronized power stroke event that results from a rapid length change. We were particularly interested in the response of this reflection to sinusoidal length changes; first, because they provide a constantly changing length signal over which the relation between IM3 and length changes can be examined; second, because they permit averaging over a single oscillation period, potentially allowing very high time resolution of IM3 changes; and third, because they allow exploration of the frequency dependence of the active tilting contribution to intensity signals. Because, during oscillations, bundle length increases during half of the sinusoid period and decreases during the other half, oscillations also allow evaluation of the responses to stretches and releases in the same record.

In a previous study, we (12) showed that the shape of the IM3 signal obtained during sinusoidal length oscillations was dependent on temperature and oscillation frequency. Elevation of temperature or reduction of oscillation frequency increased the double peak distortion of the IM3 signal. No IM3 signal distortion was observed during oscillations at high frequencies (>1 kHz) and low temperature (6, 10), conditions under which the lever arm active tilting during the cycle would be greatly reduced. The temperature dependence of the distortion, as a consequence of temperature-dependent variation in the force per active S1 (12) and its frequency dependence as evidence of a time-dependent contribution to the IM3 signal (2), were interpreted using a rotating lever arm model of the power stroke (21).

The present findings indicate that the IM3 distortion observed for low frequency length oscillations is also present at higher frequencies, provided that a sufficiently large amplitude is applied. At 2.8 kHz, where our simulations indicate that the power stroke displacement of the lever arm is only 15% of its amplitude at 200 Hz for the same peak-to-peak force change, IM3 distortion has the same form as that detected for low frequency oscillations, where, for example at 200 Hz, the power stroke contributes almost 70% of total lever displacement (Fig. 3). This strongly suggests that the increased elastic excursion of the lever arm in our present experiments is able to replicate the power stroke component of the total excursion at the lower frequency, i.e., that both elastic and active force responses arise from angular displacement of the lever domain of myosin. Our findings therefore support the interpretation of Irving et al. (21) that active and passive displacements of the lever arm occur in response to length changes and have cumulative effects on IM3. If IM3 distortion had arisen exclusively from other structural changes in S1 associated with the power stroke, then the distortion at 2.8 kHz could only duplicate that at 200 Hz by imposition of an oscillation amplitude sufficient to restore the absolute amplitude of the power stroke event. Because the power stroke contribution at 2.8 kHz was only 17% of its size at 200 Hz, we would expect to require a much larger oscillation amplitude to restore the IM3 signal distortion. In fact, this was achieved by an oscillation amplitude approximately equal to that applied at 200 Hz. Furthermore, by simulation of the distorted IM3 signal at 2.8 kHz, we obtained estimates of ΔZ, which accord well with values obtained at 200 Hz, indicating that high frequency oscillations appear not to cause displacement of the mean position of the lever arm.

Our simulations treated the lever arm domain as a rigid structure, although it has been calculated that the bending stiffness of the lever arm could be quite close to the total S1 axial stiffness (13). However, simulation with the bending lever arm during 6 nm lever displacement changed IM3 signals by only 4% and an even smaller value would be obtained if the free S1 in each myosin dimer were to contribute to IM3 signal.

In general, IM3,max for the single head case occurs when the lever is vertically aligned with the motor domain, with or without disorder. The addition of a second head shifts IM3,max to the position at which the axial mass projections of both motor domains coincide, irrespective of lever angle. However, when the second head is disordered, its axial mass projection is smeared out and the position of IM3,max is shifted back toward the point where the lever of the actin-bound S1 is vertically aligned with its motor domain, as in the single head structure.

The ratio of high and low angle peaks in the M3 substructure (caused by interference across the M-line) following a step release, has a complex dependence on release amplitude, de-
creasing to a minimum of 0.22–0.3 (29). Simulations of this ratio for our double-headed structure of Fig. 5B, showed it to be very sensitive to free head disorder. This occurs because a quick release will move actin filaments (along with actin-bound S1 motor domains) toward the center of the sarcomere. This reduces the separation of the arrays of actin-bound motor domains in each half sarcomere and changes the phase relation between scattered X-rays from each array. The free S1 associated with each cross-bridge are not displaced by the release and their contribution to M3 modulates the change in interference from the displacement of actin-bound motor arrays. For our free, disordered S1 simulation, we were unable to simulate the observed minimum in the M3 substructure ratio (29); instead, the ratio declined to zero for large releases (Fig. 7). In contrast, simulation with the ordered free head produced a ratio minimum of 0.27, close to that determined experimentally (29). This leads us to favor our ordered, detached S1 model as the more realistic approximation to the real structure, although limited angular disorder of up to 12° also gave acceptable values for the ratio minimum.

Simulation of IM3 signals using the double-headed myosin, with both ordered and disordered free S1s, gave intensity changes close to but always smaller than that observed experimentally. Because our estimates of $\Delta z$ were based on the shape of the IM3 response and not on its absolute amplitude, this does not affect our comparisons of the value of this parameter at 200 Hz and 2.8 kHz, but, coupled with the poor fitting of the secondary minimum in the IM3 signal, this implies that the true relation between filament displacement and IM3 is steeper than that used in our present modeling. Such a steeper relation might result from a longer lever (33) or structural changes in and/or tilting of the motor domain (32).

Figure 5 differs from a previous double-headed structure proposed by Juanhuix et al. (23). That structure was based on both S1s having rigor conformations, while we have assumed this conformation to be reached only at the end of the power stroke. In addition, the Juanhuix et al. (23) structure was deduced from interference effects between opposite halves of the sarcomere in several harmonics of M3, but it is possible that some of these reflections arise from structures other than S1, because not all of them respond to length steps with a change in intensity (20). For the free head be ordered when its partner is actin bound, there must be communication between the heads of the myosin dimer. In nonmuscle myosins and in kinesin, interaction between the heads of a myosin dimer is essential to maintain attachment with the actin filament. A similar interaction in muscle myosin might order and position the free head to ensure rapid actin binding on the dissociation of its partner. Such a rapid binding of the second myosin head has been proposed as an explanation of the mechanical “re-priming,” which occurs after a step length change (16). The purpose of a double-headed myosin type in muscle contraction has long been debated and definition of the free head’s structural state and orientation are important steps in determining its role. Our data allow us to propose a possible free head orientation and to provide useful constraints for future structural models of the working myosin dimer.

There are two factors relating to S1 kinetics, which could influence our estimate of $\Delta z$. First, after a step length change, the power stroke event is reprimed with a rate constant of $-150$ s$^{-1}$ for a release and 30 s$^{-1}$ for a stretch (26), a process thought to involve the rapid detachment of strained S1, followed by a rapid reattachment and restoration of the ability to execute a new power stroke. However, repriming is unlikely to be responsible for the distortion of IM3 signals at maximum shortening during oscillations because to do so it would require an abrupt onset of repriming at the point where IM3 starts to fall, followed by a symmetrical reversal of repriming after maximum shortening to account for the rise in IM3 during the restretch. In addition, if the distortion of IM3 signal were due to cross-bridge detachment and attachment, the amplitude of force oscillations would be modulated by the cross-bridge number, resulting in force distortion similarly to IM3 signal. However, as shown clearly in Figs. 1, 2, and 6, such distortion is not present on our force signals.

The effects of axial disordering of the myofilament lattice as a possible cause of IM3 distortion can be rejected on similar grounds. Such disorder effects have not been observed in the IM3 response to length changes and would also require abrupt and symmetrical onset and reversal during an oscillation period to account for the observed IM3 distortion.

The second factor is the asymmetry of the synchronized power stroke recovery of force after a length step, which is faster for releases than for stretches. Oscillations therefore should increase mean tetanic tension because of the smaller force recovery during the stretch phase of the sinusoid, displacing the lever arm closer to its IM3,max orientation. At 2.8 kHz, mean force increase was 5% or less in all fibers studied (Fig. 1); at lower frequencies the mean force increase was insignificant. A 5% force increase reduces $\Delta z$ by only 0.1–0.13 nm (based on the expected filament compliance extension and also from the effect of temperature on IM3 and force; Ref. 12) and would be contrary to an earlier report proposing an increase in $\Delta z$ during high frequency oscillations (6). Correction of $\Delta z$ for this reduction would therefore only increase its value by 0.1–0.13 nm, which would still be insignificantly different from $\Delta z$ values obtained at lower frequencies.

In conclusion, our findings indicate that $\Delta z$ measured during high-frequency (2.8 kHz) oscillations accords well with our estimates of $\Delta z$ at <1 kHz, despite the suppression of the power stroke contribution to IM3 at this frequency and suggest...
a disposition of the working lever arm somewhat closer to \( I_{M3,\text{max}} \) than that previously reported (22). They also show that dynamic \( I_M \) signals during late oscillations provide a method of determining changes in lever arm position within the intact, working myofibrillar lattice of a living muscle cell, complementary to and independent of methods based on static intensity measurements, such as Fourier reconstruction. This method is a specific probe of lever arm disposition and is less sensitive to complications such as lattice disorder or interference between adjacent halves of the sarcomere. It may also permit lever arm position to be investigated in situ, both for myosin states, in which \( S1 \) structure has been determined crystallographically and also for possible states as yet not obtainable in crystalline form.

APPENDIX

Disordering due to mismatch of actin and myosin periodicities. For computations, Eq. 2 may be simplified by noting that the complex quantity \( \mathcal{F}[S1(z)] \) can be expressed as the product of amplitude and phase spectra

\[
\mathcal{F}[S1(z)] = |\mathcal{F}[S1(z)]| e^{i\phi}
\]

where \( \phi \) is the ratio of its imaginary and real parts. We now rearrange (2) to obtain the form

\[
\mathcal{F}[\text{Thick filament}] = |\mathcal{F}[S1(z)]| e^{i\pi a n z + \phi} \sum_{p=1}^{n} e^{i\pi n (p-1) Z}
\]

\[+ e^{-i \pi a n z + \phi} \sum_{p=1}^{n} e^{-i \pi n (p-1) Z} \]

Noting that \( \mathcal{F}[S1(z)] \) from opposite sides of the M-line are complex conjugates and therefore have identical moduli. The summed terms are also complex conjugates and are equal to

\[ e^{i \pi (n-1) Z} \frac{\sin(\pi n Z)}{\sin(\pi Z)} \] and \[ e^{-i \pi (n-1) Z} \frac{\sin(\pi n Z)}{\sin(\pi Z)} \]

respectively, so we may further simplify to obtain

\[
\mathcal{F}[\text{Thick filament}] = 2|\mathcal{F}[S1(z)]| \frac{\sin(\pi n Z)}{\sin(\pi Z)} \times \cos[\pi (a + (n-1)f)Z + \phi]
\]

as the filament transform (23), the squared modulus of which is proportional to intensity. Disorder is now introduced by modification of \( \mathcal{F}[S1(z)] \).

We assume that the probability distribution for actin binding of the motor domain is uniform over a range, \( c \), of \( \pm 2.75 \text{ nm} \) from its axial period and zero outside that range and that motor domain orientation is specific for the attached state and unaffected by its position in the range (Fig. 8). The averaging of a population of \( S1 \) occupying equally all possible locations within the actin binding range corresponds to a convolution of motor domain structure with the probability distribution function, which becomes a product of the transforms of these functions in reciprocal space. The transform of the rectangular probability distribution \( \omega(Z) \) is given by \( \sin(2 \pi n Z) / 2 \pi n Z \). Because \( S2 \) is rigid, the lever arm must tilt at the appropriate angle to link the disordered motor domain to the thick filament period. Translational disordering of the motor domain is therefore replaced by angular disordering of the lever (Fig. 8), which is introduced by convolution
where the $k$th $\alpha$-carbon is the lever fulcrum, taken as residue 707 and is used to compute new amplitude and frequency spectra in Eq. A1.

Intensity contribution from a detached S1 moiety in the myosin lever arm disposition in relaxed skeletal muscle fibers. Biochim Biophys Acta 2004; 164: 1356–1366.

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