Presence of rigor or rigor-like myosin heads AM cannot, however, be detected by X-ray diffraction studies on contracting muscle. To give information about the state of myosin heads after the end of powerstroke, we studied changes in the state of myosin heads when single Ca-activated skinned muscle fibers were transferred into high-Ca rigor solution (pCa 4.4), by applying quick releases (0.5-1% of Lo, complete in 1-2ms followed by restretch) at various times after the transfer of the fiber into high-Ca rigor solution. Unexpectedly, the fibers exhibited distinct tension recovery Pr following quick release. The value of Pr relative to the maximum Ca-activated isometric tension Po (Pr/Po) was about 0.4 at 10s after the transfer of the fiber into rigor solution, and decreased with time to completely disappear in 10-20min. In the presence of 5-10mM EDTA, chelating Mg, the amplitude of Pr was markedly reduced, and disappeared in 5-10min. After disappearance of Pr, the fiber only showed tension drop coincident with quick release. These results suggest that AM-ADP myosin heads are responsible for the tension recovery following quick release, if, on the other hand, the ionic strength of rigor solution was reduced from 170 to 50mM by totally removing KCl, the amplitude of Pr was increased appreciably, and did not disappear for 20-30min. Concerning the cyclic actin-myosin interaction, it seems possible that, after the end of powerstroke, myosin heads take the form of AM-ADP having a long average lifetime.

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Non-Linear Cross-Bridge Elasticity, ATP-Independent Detachment and ATP-Velocity Relationships for Skeletal Muscle Actomyosin
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The idea that contraction of skeletal muscle and heart results from ATP-driven actomyosin cross-bridge cycles is generally accepted. However, operational details remain controversial. For instance, in conflict with most accepted views, evidence was recently presented [1] for appreciably non-linear elasticity with low stiffness for post-power-stroke cross-bridges. Moreover, a non-hyperbolic relationship was observed [2] between MgATP concentration and sliding velocity for actin filaments propelling in vitro by myosin subfragment 1 or full length myosin. Here we present convincing evidence for a hyperbolic [MgATP]-velocity relationship \( r^2=0.998 \); Michaelis-Menten constants, \( V_{max}=15.28 \pm 0.28 \) µm/s (mean ±2 SEM) and \( K_M=0.389 \pm 0.023 \) mM when actin filaments are propelled by heavy meromyosin from rabbit fast skeletal muscle myosin (28-29°C; >3 independent experiments). Because the hyperbolic [MgATP]-velocity relationship is not readily consistent with inter-head cooperativity the results were interpreted using a cross-bridge model with independent myosin heads. The inter-state transition rates were strain-dependent and the model had one detached state and five attached actomyosin (AM) states with either MgATP (AMATP) or MgADP and/or inorganic phosphate \( (\text{Pi})\) or no nucleotide at the active site. The AMADPPi state was a strongly bound pre-power-stroke state whereas the remaining states without Pi were post-power-stroke states required to account for strain-dependent MgADP release on the one hand and MgATP-dependence of velocity and competitive inhibition of MgATP binding by MgADP (AM, AMADP, AMATP) on the other. The MgATP induced detachment was supplemented by MgATP independent, but strain-dependent, detachment from the rigor (AM) state. This model predicts a hyperbolic [MgATP]-velocity relationship if the cross-bridge elasticity is non-linear but a non-hyperbolic [MgATP]-velocity relationship (cf. [2]) if cross-bridge elasticity is linear.

References:

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Nonlinear Elasticity of a Crossbridge in Sarcomere Lattice
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Crossbridge elasticity is an essential determinant of strain dependent transition rates in the actomyosin cycle. Recent estimates of myosin stiffness range from 1.5 to 3.2 pN/nm and are much larger than most previous estimates used in sliding filament models. These higher stiffnesses limit thermally-induced motions on unattached myosin heads, affect transition rates associated with power stroke and narrow the parabolic parts of energy landscapes. This in turn raises the energy barriers between actomyosin states reducing the probability of strain dependent transitions between them. Estimates of crossbridge stiffness derived from a study of its parts (S2, the lever arm, the “neck region”) could be helpful in informing this issue. We used the known atomic structures of crossbridge components in molecular dynamic simulations (CHARMM) to estimate the elasticity of the individual components. We then used nonlinear finite element analysis to estimate the crossbridge stiffness under a range of tensile and compressive forces in the context of the 3-D sarcomere lattice. Using estimated axial and lateral stiffnesses for S2 (of 60 pN/nm, and 0.01 pN/nm respectively), and a bending stiffness S1 of 3 pN/mm, we computed force displacement relationships for crossbridges under tension and in compression. As expected, crossbridge stiffness under tension was slightly below 3 pN/nm at any force. In contrast, stiffness under compression falls about 3-fold at 1 pN, and more than an order of magnitude at forces exceeding 3-4 pN. Consequently, the energy landscape is asymmetric and skewed toward negative crossbridge strains. Our data agree well with recent measurements of nonlinear cross-bridge compliance (Kaya et al., Science 329:686-688) and quantitatively define departures from these measurements in terms of azimuthal departures of the S1-S2 plane from the axial axis of myosin filament and increased inter-filament lattice spacings.

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and essential light chain of the lever. The chicken S1 crystal structure lies near one end of the range of flexion observed. The Gaussian spread of angles of flexion suggests that flexibility is driven thermally, from which a torsion spring constant of ~23 pN nm/ rad2 is estimated on average for all S1 types, which is similar to myosin-5 measured in solution. This is equivalent to an apparent cantilever-type stiffness at the tip of the lever of 0.37 pN/nm. Because this stiffness is lower than recent estimates from myosin-2 heads attached to actin, we cantilever-type stiffness at the tip of the lever of 0.37 pN/nm. Because this stiffness is lower than recent estimates from myosin-2 heads attached to actin, we cantilever-type stiffness at the tip of the lever of 0.37 pN/nm. Because this stiffness is lower than recent estimates from myosin-2 heads attached to actin, we cantilever-type stiffness at the tip of the lever of 0.37 pN/nm. Because this stiffness is lower than recent estimates from myosin-2 heads attached to actin, we can...

Phosphorylation-Induced Structural Change in CMYBP-C affects its function. We used electron microscopy (EM) to study the effect of phosphorylation on CMYBP-C structure and on its modulation of tropomyosin position on thin filaments. Rotary shadowing and negative staining of CMYBP-C reveals that the molecule is flexible and has hinge points near its middle and close to one end. By comparison with the N-terminal fragment C0-C3, we deduce that the latter is near the M-domain. We investigated the structural effects of phosphorylation by EM of PKA-treated C0-C3 and of C0-C3 phosphomimetic molecules, in which the phosphorylatable serines in the M-domain have been mutated to alanine (C0-C3-AAAA, dephosphomimetic) or aspartic acid (C0-C3-DDDD, phosphomimetic). PKA-treated C0-C3 and the C0-C3-DDDD molecules had a smaller angle between the two arms of the M-domain hinge than wild type C0-C3 and C0-C3-AAAA. To investigate whether this structural change affects the displacement of tropomyosin on thin filaments that occurs when unphosphorylated CMYBP-C binds to thin filaments, the 3D structure of C0C3-DDDD-decorated thin filaments was compared with that of C0C3-AAAA and wild-type C0C3. C0C3-AAAA showed clear decoration and its binding induced a shift of tropomyosin from the blocked to the closed position, as found previously with wild-type C0C3. In contrast, C0C3-DDDD showed fewer decorated filaments, suggesting weaker binding and the 3D structure showed no movement of tropomyosin. These results suggest that phosphorylation reduces CMYBP-C’s displacement of tropomyosin and its activation of thin filaments. We have used rapid mixing negative stain electron microscopy (EM) to observe myosin heads (S1) bound to F-actin and thin filaments in the presence of ATP. Myosin V S1 was mixed with excess ATP, incubated for 2 sec to allow binding and hydrolysis, and then mixed with either F-actin or cardiac thin filaments. This mixture was applied to an EM grid and negatively stained with uranyl acetate ~20 ms later. Short times on the grid allow protein concentrations as high as 10 μM to be used, which is critical to observe weakly binding particles, such as S1-ADP-Pi with actin filaments. This approach has advantages compared to hand mixed solutions. It has improved time resolution (0.3 s vs 3 s between mixing and staining), the mixing is ~1000x faster compared to hand mixing, and protein concentration mixing is much more uniform. The entire automated procedure used an apparatus with computer controlled stepper motors previously built for time-resolved cryo-EM (White et al, J Struct Biol:144, 246-52, 2003), which was modified for negative staining. Slow and non-uniform mixing produced by hand mixing can suggest cooperative binding. After automated mixing, S1-ADP-Pi with cardiac thin filaments at low calcium concentrations, S1 bound randomly and only rarely were bare or fully decorated filaments observed. Without ATP, thin filaments were mostly either bare or fully decorated, suggestive of cooperativity. These results may explain the apparently contradictory observations that binding of S1 to thin filaments appeared to be cooperative in micrographs, whereas kinetic and structural measurements indicate that individual rigor heads activate 12-14 actin subunits.

Phosphorylation-Induced Structural Change in CMYBP-C affects its Thin Filament Binding and Modulation of Tropomyosin Position

Myosin binding protein C is a thick filament accessory protein of vertebrate striated muscle. The cardiac isoform (cMYBP-C), comprising eleven immunoglobulin-like and fibronectin-like domains (C0-C10) and a cMYBP-C-specific M-domain, plays a key role in increased contractility of the heart in response to β-adrenergic stimulation. Phosphorylation of the M-domain is an important post-translational modification of cMYBP-C that modulates its function. We used electron microscopy (EM) to study the effect of phosphorylation on cMYBP-C structure and on its modulation of tropomyosin position on thin filaments. Rotary shadowing and negative staining of cMYBP-C reveals that the molecule is flexible and has hinge points near its middle and close to one end. By comparison with the N-terminal fragment C0-C3, we deduce that the latter is near the M-domain. We investigated the structural effects of phosphorylation by EM of PKA-treated C0-C3 and of C0-C3 phosphomimetic molecules, in which the phosphorylatable serines in the M-domain have been mutated to alanine (C0-C3-AAAA, dephosphomimetic) or aspartic acid (C0-C3-DDDD, phosphomimetic). PKA-treated C0-C3 and the C0-C3-DDDD molecules had a smaller angle between the two arms of the M-domain hinge than wild type C0-C3 and C0-C3-AAAA. To investigate whether this structural change affects the displacement of tropomyosin on thin filaments that occurs when unphosphorylated cMYBP-C binds to thin filaments, the 3D structure of C0C3-DDDD-decorated thin filaments was compared with that of C0C3-AAAA and wild-type C0C3. C0C3-AAAA showed clear decoration and its binding induced a shift of tropomyosin from the blocked to the closed position, as found previously with wild-type C0C3. In contrast, C0C3-DDDD showed fewer decorated filaments, suggesting weaker binding and the 3D structure showed no movement of tropomyosin. These results suggest that phosphorylation reduces cMYBP-C’s displacement of tropomyosin and its activation of thin filaments.