In order to understand how myosin interacts with ATP and actin to produce motion during structural rearrangements corresponding to progression from one biochemical/structural state to the next in the ATPase crossbridge cycle, we examined the energetic differences in interfaces within structural models of myosin. So that these stages of the crossbridge cycle are logically and energetically realistically connected we generated, by interpolating in an energetically realistic way, structures between existing crystal structure states of individual myosin heads representative of different biochemical/structural states of the crossbridge cycle. We computationally alanine scanned[1] these structures to objectively examine myosin energetics and test hypotheses of force production and regulation. Combining these computed energies with displacement measurements as examined by distance displacement contact maps, we gained important insights into the complex interplay of energy, strain, elastic energy storage and release, and force generation within myosin and how structural elements interact to create the complex regulated mechanochemical behavior of the myosin motor. In particular, we focused on the recovery stroke connecting the Dictyostelium myosin structures 1MMD and 1VOM using intermediates generated by the conjugate peak refinement method[2] to find areas of strain creation, strain maintenance (elastic energy storage) and strain release, as well as elastic recovery. This contributes to a quantitative view of the regulatory mechanisms; interface formations, deformations and dissolutions; force producing motions, and the energy landscape on which these changes take place.

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Molecular Dynamics Simulations Of The Hydrolysis Transition State Intermediate In Myosin

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We have used molecular dynamics simulations to analyze the nucleotide site of myosin and its interaction with ATP and a catalytic water. Simulations used the Dictyostelium myosin ADP●VO4 x-ray crystal structure. This structure is widely hypothesized to be an analog of the hydrolysis transition state intermediate for an in-line water attack on the γ-phosphate position. The trigonal bi-pyramidal VO4 moiety was replaced by PO3 covalently bound to ADP and a water molecule oxygen. Surprisingly, the MD simulation indicated that the x-ray structure was not capable of controlling the position of the modeled attacking water as required for hydrolysis. Instead the water molecule rattled around a catalytic pocket formed by the γ-phosphate of ATP, elements of switch 1, switch 2, and the salt-bridge between R238 and E459. The salt-bridge has been postulated to serve to help stabilize the closed conformation of switch 2. The simulated double alanine R238A/E459A mutation eliminated this salt-bridge. There was little resulting change in the conformation of switch 2 (0.55Å r.m.s. deviation, Cα-Cα, a.a. D454-L495) and the crucial hydrogen bond distance between the backbone amide of G457 and the γ-phosphate oxygen of ATP increased from 1.9Å to only 2.0Å in the mutated structure. However, the modeled catalytic water rapidly escaped from the catalytic pocket in the mutated myosin. Thus the simulations suggest that the closed-switch 2 structure is stabilized by a number of interactions in addition to the salt-bridge. The function of the salt-bridge is to serve as a lid to sequester water in the catalytic pocket.

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Analysis Of The Interaction Of The Nucleotide Base With The Myosin Catalytic Pocket And The Effect On Substrate Efficacy

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Myosin has been shown it to be a promiscuous enzyme that can utilize a number nucleotide and non-nucleoside triphosphate substrates. However, the chemomechanical efficacy of the alternative substrates varies widely. In particular, the ability of ''nanalog'' substrates (an amino-ethyl or -propyl linker and a substituted phenyl ring in lieu of the ribose and the nucleotide base) to completely decouple substrate hydrolysis from motility clearly indicates that the correct interaction of myosin with the ribose and base is essential for myosin function. As a first step to understand better the myosin-substrate interaction and how interactions other than with the triphosphates are essential for motility, we have used molecular dynamics simulations of the Dictyostelium ADP●BeFx x-ray structure to investigate the interactions of myosin with ATP, CTP (effective substrate) and GTP (poor substrate) docked at the active site. The simulations with ATP suggest a ''troika'' of conserved amino acids lining the nucleotide site that form a cyclical chain of nucleotide-protein hydrogen bonding interactions: ATP (N6) → Y135 → Y116 → N188 → ATP (N7). In the simulations, substitution of CTP at the active site maintains this coordination. Substitution of GTP at the active site perturbs the interaction. Thus the simulations suggest a set of crucial protein-substrate interactions that must be maintained for an effective substrate. The modeling has predictive power. ''In silico'' mutation experiments suggest that the mutation Y135K preserves troika-mediated pattern with GTP at the active site, but destroys it with ATP at the active site. The prediction would be that the Y135K mutation would make myosin a more effective GTPase and a less effective ATPase.

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Myosin II Trapped In A Weak Actin-binding State Through A Chemical Crosslink Across The Actin-Binding Cleft

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We have trapped *Dictyostelium* myosin II in a weak actin-binding conformation by chemically crosslinking two engineered Cys across the actin-binding cleft using a bifunctional spin label (BSL). With sites in the lower and upper 50 kDa domains, the crosslink restricts the conformation of the actin-binding cleft. The crosslinking reaction was monitored by electron paramagnetic resonance (EPR) based on the spin label immobilization that occurs upon reaction of both Cys. The EPR spectrum of crosslinked myosin is sensitive to structural changes induced by both nucleotide- and actin-binding. Functional assays demonstrate that crosslinking partially impairs actin binding and actin-activation but has negligible effects on basal ATPase activity. We propose that crosslinked myosin is trapped in a weak actin-binding structure in which phosphate release is inhibited by the presence of actin. This conformation presumably binds actin weakly but cannot transition to the ''closed'' cleft structure that is populated with strong actin-binding (Klein et al, 2008, *PNAS* 105:12867-72). The weak actin-binding structure has proven difficult to characterize because of its transient nature; BSL-crosslinked myosin provides a stable model system for analysis of structural dynamics. We are using EPR to analyze the orientation of BSL-crosslinked myosin attached to actin in skinned muscle fibers, and we are using nucleotide probes to investigate the coupling between the actin-binding cleft and the nucleotide-binding pocket. This work is complementary to a study in which BSL was used to crosslink SH1 (C707) and SH2 (C697) in the force-generating domain of myosin, producing a stable complex that bound weakly to actin with slow orientational disorder (Thompson et al., 2008, *Biophys. J.*, in press). This work was supported by grants from NIH (AR32961, AR07612) and the Minnesota Supercomputing Institute.

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Analysis of Conformation of Skeletal Muscle Myosin Cross-linked by pPDM Using FRET

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Previously biochemical studies have demonstrated that the highly reactive cysteine residues SH1 and SH2 can be crosslinked by variety of bifunctional reagents with different spans (3-14 Å) in the presence of nucleotides, suggesting that the region is highly flexible. The SH1-SH2 region is believed to play a key role in the conformational changes that occur in the myosin head during the force generation coupled to ATP hydrolysis. We have previously shown that the HMM, which SH1-SH2 was crosslinked by p-Phenylene-dimaleimide (pPDM) in the presence of ADP, have a novel conformation using quick freeze deep etch electron microscopy (QFDE-EM). We have also demonstrated that conformational change of the myosin motor domain during ATP hydrolysis can be monitored by measuring the FRET using fluorescent ATP analogue NBD-ATP. In the present study, we analyzed the conformation of the myosin crosslinked by pPDM using the FRET between the ATP binding site and the A1 essential light chain (ELC) and compared with the 3D structure models of ATPase intermediates derived from electron microscopic analysis. We prepared the skeletal muscle myosin subfragment-1 (S1), which ELC was labeled by 6-bromoacetyl-2-dimethylaminonaphthalene (BD) at the Cys 177. And fluorescent ADP analogue NBD-ADP was trapped in the ATPase site of S1 labeled by BD. The FRET efficiency was estimated by measuring the change of fluorescence intensity of BD comparing with control BD-S1. FRET efficiency of pPDM-S1-NBD-ADP was apparently different from other nucleotides and nucleotide analogues bound states.