Elevated Ionic Strength Diminishes Force/Cross-Bridge and the Number of Force-Generating Cross-Bridges
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Ionic strength (IS) is an important parameter to govern the inter- and intra-molecular interactions. In muscle, it has been known that an increase in IS lowers Ca2+-induced detachment tension, however, its molecular mechanism is not well understood. Our aim was to determine whether force/cross-bridge or the number of force-generating cross-bridges changes with IS. Stiffness during rigor was studied on single fibers from rabbit psoas, which showed that there was no effect of IS, demonstrating that in-series compliance is not affected by IS. This observation indicates that stiffness of thick filament, thin filament, myosin head, and actomyosin interface are not affected by IS. Sinusoidal analyses were performed during Ca2+ activation, and the effects of ATP, phosphate (P_i), and ADP on three rate constants were studied at IS ranging 150mM-300mM to characterize elementary steps of the cross-bridge cycle. Both ATP binding (K1) and ADP binding (K0) increased to 2x, and the Pi binding (K5) decreased to 1/2 when IS was increased from 150mM to 300mM. The effect of P_i can be explained by the electrostatic interaction with the Pi binding site on myosin. The effect on ATP/ADP can be attributed to improved stereoscopic and hydrophobic interaction with nucleotide binding site. The increase in IS increased cross-bridge detachment steps (k2 and k4), indicating that electrostatic counteracts these steps. However, IS did not affect attachment steps (k-2 and k4). Consequently, the equilibrium constant of the detachment step (k2) increased to 2x, and the force generation step (k4) decreased to 0.7x. These effects together diminished the number of force-generating cross-bridges by ~10%. Because associated decrease of tension was ~40%, the major effect of IS is a decrease in force/cross-bridge, but also a decrease in the number of force-generating cross-bridge occurs.

Formation of the Motor Protein-Photochromic ADP Analogue-Fluorometal Ternary Complex and Photo-Reversible Transition along the Steps in ATPase Cycle
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In the presence of Mg2+-ADP, myosin forms stable ternary complexes with phosphate analogues, fluoroberyllate (BeF₄⁻), fluorooxalate (AlF₄⁻) and orthovanadate (V₅), each of which may mimic different transient state along ATPase cycle. It is known that kinesin also forms similar ternary complexes. Our previous studies on the photoaffinity-labelling using photoactive ADP analogue, 19F-labeled ADP analogue, revealed that there is some variation in the myosin-nucleotide contacts at the nucleotide base among the ternary complexes. Previously, we have incorporated photochromic molecular architecture, azobenzene derivative into the functional region of ATP driven motor proteins and succeeded to control activities and conformation reversibly by photochromic irradiation. Aim of this study is to induce transition between the different steps in ATPase cycle of motor proteins utilizing photoisomerization on the motor protein-photochromic ADP analogue-fluorometal ternary complexes. In this study, three types of photochromic ATP analogues composed of azobenzene, spiroyn and fulgimide derivatives were synthesized. The photo-responsive interaction of the photochromic ATP analogues with myosin and kinesin were examined. Photo-dependent conformational changes of the myosin motor domain were monitored by X-ray small angle scattering. And the photo-reversible changes in the velocity of the microtubule gliding for kinesin in the presence of the photochromic ATP analogues were also observed. In the presence of fluorometal, BeF₄⁻, photochromic ADP analogues were trapped within the ATPase site of kinesin and myosin resulted in formation of ternary complexes. The conformational changes of the complexes induced by photo irradiation were studied.

Backbone Orientation and Distance Measurements in Myosin II: Applications of High-Resolution EPR using a Bifunctional Spin Label
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We have used electron paramagnetic resonance (EPR) of a bifunctional spin label (BSL) to obtain high-resolution measurements of individual structural elements within the myosin II catalytic domain (CD). Two complementary EPR techniques were employed to measure protein orientation (conventional EPR) and inter-spin distances (dipolar electron-electron resonance, DEER). The use of BSL greatly enhances the resolution of EPR, by virtue of its strongly immobilized and stereoselective bifunctional attachment to the protein backbone at two engineered Cys residues. Crucially, both techniques utilized here permit the elucidation of myosin structure while in complex with actin, generating relevant constraints for the refinement of actomyosin structural models. In the current work, Dictyostelium myosin II was used as our model system. We measured nucleotide-dependent structural transitions of three key helices within the myosin CD. Three double-Cys sites were engineered, with Cys pairs used to measure myosin orientation relative to oriented actin. BSL on a construct with two pairs was used to measure interprobe distances. The effect of ADP binding was clearly detected by EPR, and subsequently modeled using the orientation and distance measurements as constraints. We find that the structural change induced by ADP in the actin-bound myosin CD is clearly different from that predicted from actin-free crystal structures. This work was funded by grants from NIH (R01 AR82961, T32 AR07612, P30 AR507220).

Reloading Injury, Chronic Recovery, and Fiber Type Adaptation of Mouse Soleus Muscle after Four Weeks of Hind Limb Suspension
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Disuse and unloading rapidly result in skeletal muscle atrophy, fiber type switch and limited contraction-induced muscle injury. The recovery from those conditions has broad physiological and medical significances. To understand the recovery process and the molecular adaptations in muscle reloading, mouse soleus muscles were studied after 4 weeks of hind limb-suspension and reloading for 3, 7, 15, 30, 45 and 60 days. The results showed that 4 weeks of unloading produced significant muscle atrophy and reductions of contractile force and fatigue tolerance, accompanied with...