Macromolecular Crowding Increases Cross-Bridge Performance via Reduction of ADP Affinity to Acto-Myosin

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We used methods of transient kinetics to determine if crowding agent Ficoll70, added to the reaction solution, changes the rate-limiting step of acto-myosin cycle, to explain the two-fold increase of acto-myosin ATPase activity in the presence of Ficoll70. D.discoideum myosin II S1 and rabbit skeletal actin were used as a model system. We determined kinetic rates of ATP binding to myosin, myosin recovery stroke, acto-myosin binding and dissociation, with and without ADP. Our experiments showed that virtually every kinetic step of the cross-bridge cycle was affected by Ficoll. Addition of Ficoll70 slows the rates of ATP binding and actin binding to myosin, as well as the rate of acto-myosin dissociation. Affinity of ADP to acto-myosin was reduced two-fold in the presence of Ficoll70. ADP release from acto-myosin is the rate limiting step of D.discoideum myosin II ATPase cycle, therefore reduced ADP affinity is the reason of increased acto-myosin ATPase activity in the presence of Ficoll70. Addition of Ficoll70 does not change the rate limiting step of D.discoideum myosin II ATPase cycle.

Macromolecular Crowding Modulates Cross-Bridge Performance

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Cross-bridge kinetics is usually studied in diluted solution that does not reflect in vivo conditions of cell cytoplasm. In cells, myosin and actin work in dense environment of small and large macromolecules, affecting cross-bridge kinetics. We used high molecular weight sucrose polymer Ficoll70 to examine the effect of crowding on kinetics of acto-myosin interaction and cross-bridge cycle. Dictyostelium discoideum myosin II S1 and rabbit skeletal actin were used in the study. We observed two-fold increase of the rate of acto-myosin cycle in the presence of 25% w/v Ficoll70 in the reaction solution. Transient kinetics studies showed that virtually every kinetic step of the cross-bridge cycle was affected by Ficoll70. Ficoll70 decreases ADP affinity to acto-myosin, and therefore increases the overall rate of the cycle, because ADP release is the rate limiting step in D.discoideum myosin II ATPase activity. We conclude that macromolecular crowding modulates cross-bridge performance in cells.

Mutating the SH1 Helix Region of Dictyostelium Myosin II Impairs Motile Activities and Thermal Stability

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Mutations at the SH1 helix region of the myosin II motor domain, such as E706K (Glu-706 to Lys) and R702C (Arg-702 to Cys) have been reported to link to some autosomal-dominant diseases. The SH1 helix region acts as a linker for transmitting the structural changes of ATP-binding site in the catalysis domain to the lever arm. To investigate the effect of the mutation on the actin-myosin motility, we have introduced a corresponding mutation into the SH1 helix of Dictyostelium myosin II (E683K and R686C). The mutations resulted in a decrease in the actin-myosin sliding velocity (65% and 63% of the wild type for E683K and R686C, respectively), a decrease in the thermal stability and the thermal aggregation of the myosin, which might be implicated in the disease process.

A Myosin II FRET-Based Biosensor Expressed in Dictyostelium

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Myosin II is responsible for force-generation in skeletal and cardiac muscle. Understanding the mechanism of force generation is essential for developing novel treatments of muscle pathologies. Here we present a FRET-based biosensor capable of resolving structural states in the motor domain of Dictyostelium myosin II. We fused a green fluorescent protein (GFP) to the N-terminus of myosin subfragment 1 (S1) and incorporated a sequence of four cysteines into the SH1 helix of the upper 50 kDa domain (Sun et al., J Biol Chem, 2006).

This tetracysteine site was then labeled with the biarsenical dye ReAAS as an acceptor for fluorescence resonance energy transfer (FRET). The FRET efficiency depends on the inverse sixth power of the distance between the two probes, as well as their relative orientations, so that small conformational changes of the motor domain are resolvable by nanosecond-resolved measurements of the GFP fluorescent lifetime. We found the labeled apo S1 had a 17% decrease in fluorescence lifetime due to FRET. We then compared nucleotide-bound states of the S1 motor domain with the apo state, finding significant lifetime differences in theADP,Af and ADP,V04 states (analogs of the ADP, P- intermediate state) as compared with apo and ADP-bound GFP-myosin lifetimes.. This result is consistent with crystallographic and tryptophan fluorescence data showing myosin structural state changes with nucleotide binding. We are now pursuing dual tagged GFP-RFP myosin constructs with the goal of resolving myosin structural states in living cells.

The Inhibited, Interacting-Heads Motif Charactizes Myosin II from the Earliest Animals with Muscles

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Electron microscopic studies have shown that the inhibited (relaxed) state of myosin II is characterized by an intramolecular interaction between myosin heads, in both thick filaments and isolated monomers. Interaction inhibits head activity by blocking actin-binding in one head and ATPase activity in the other. Interacting-heads are present in vertebrate smooth muscle and non-muscle monomers, and in thick filaments of invertebrate smooth muscle (flatworms), invertebrate striated muscle (mollusks, arthropods) and vertebrate striated muscle (zebrafish, mouse and human); furthermore, head interactions underlie the off-state in myosins regulated by both Ca2+ binding and RLC phosphorylation. Head-head interaction has thus been conserved through evolution from mollusks and arthropods to humans. Our goal has been to determine how early this self-inhibiting motif arose, by studying the off-state of myosin monomers and/or filaments from primitive species. Filaments from sea anemones (Cnidaria), the most primitive animals with muscles, have the same 14.5-nm repeat as other muscles, but EM images have not yet been reconstructed. However, sea anemone myosin monomers, under relaxing conditions, have the same folded tail and bent-back, interacting-heads motif as myosins from the higher species, further demonstrating the very early origin of this structure. Interestingly, unlike the animals mentioned above, Acanthamoeba myosin monomers under relaxing conditions appear to lack a folded tail or interacting heads. The sequence of this myosin is strikingly different from the myosins IIs of animals, and its regulatory mechanism is quite different, suggesting that self-inhibition by interacting heads evolved after amoebae and animals diverged. Conservation of interacting heads over time suggests that it is a highly successful mechanism for regulating myosin activity. We are now studying the more primitive sea sponges (Porifera), one of the earliest animal groups, which lack muscles.

Detection of Ultrfast Mechanical Transitions in β-Cardiac Myosin using High-Speed Optical Trapping

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The β isoform of myosin II (MYH7) is the primary myosin heavy chain present in both slow skeletal muscle and the cardiac ventricles of large mammals. In cardiac and skeletal muscle, strong binding of myosin to the thin filament plays an important role in the cooperative activation of contraction. Studying the weak-to-strong transition and the initiation of the force-producing power stroke has been difficult using single molecule techniques because standard optical trapping methods require 10-15 ms to detect actin-myosin interactions. This latency is much longer than the expected time from initial actin-myosin interaction to strong binding and force production. In this work we study full-length, tissue-purified, porcine β-cardiac myosin using an ultrafast optical trapping technique that allows the detection of actin-myosin binding events within...