perturbations were used. Experiments were performed in identical solutions with 0.2M ionic strength at pH 7.00. The concentration of MgATP was varied to detect kinetic constants of the ATP binding step 1 (K_1: dissociation constant), the cross-bridge detachment step 2 (k_2, k_{-2}: rate constants), and the ATP cleavage step 3 (k_3, k_{-3}). By following the fast rate constant at 20°C, we found in myofibrils: k_2/K_1=1.0∈ $\mu M^{-1}s^{-1}$, K_1=0.3mM, k_2=300s^{-1}, and $k_{-2} \approx 0$; in fibers: k_2/K_1=0.23∈ $\mu M^{-1}s^{-1}$, K_1=1.58mM, k_2=363s^{-1}, k_{-2}=180s^{-1}. From these results we conclude that (1) ATP binding is ~5X stronger in myofibrils than in fibers, (2) cross-bridge detachment rate is just about the same, and (3) its reversal step is almost absent in myofibrils, but it is finite in fibers. Consequently, we found a good agreement in the results obtained from myofibrils and fibers, indicating that phase 2 of tension transients from step analysis in fibers (Huxley and Simmons, 1971) represents the cross-bridge detachment step. We also studied actin-myosin cross-linked myofibrils and found no difference, indicating that cross-linking does not significantly modify steps 1-3 kinetics. We further studied the Pi effect in myofibrils, and found that Pi is a competitive inhibitor of MgATP with the inhibitory dissociation constant of 7-8mM. To deduce the kinetic constants of the ATP cleavage step, we measured the slower rate constant in fluorescence in myofibrils and found that k_3+k_{-3}=10.7s^{-1} at 4°C. From the Pi burst experiments using radioactive ATP, we found that K_3=6.1 at 4°C. From these, k_3=9.2s^{-1} and k_{-3}=1.50s^{-1} were deduced.

747-Pos

Myosin ATP Turnover Rate: A Mechanism Involved in Thermogenesis in Resting Skeletal Muscle Fibers

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Thermogenesis by resting muscle varies with conditions and plays an active role in homeostasis of body weight. The low metabolic rate of living resting muscles requires that ATP turnover by myosin be inhibited relative to the purified protein in vitro. This inhibition has not been previously seen in in vitro systems. We used quantitative epifluorescence microscopy of fluorescent nucleotides to measure single nucleotide turnovers in relaxed permeable skeletal muscle fibers. We observed two lifetimes for nucleotide release by myosin, a fast component with a lifetime of 0.2- 0.3 minutes, similar to that of purified myosin, and a slower component with a lifetime of 3.8 ± 0.4 minutes. We define the latter component to be the ''super relaxed state''. The fraction of myosins in the super relaxed state was decreased at lower temperatures, by substituting GTP for ATP or by increased levels of myosin phosphorylation. All of these conditions have also been shown to cause increased disorder in the structure of the thick filament. We propose a model in which the structure of the thick filament modulates the nucleotide turnover rates of myosin in relaxed fibers. Modulation of the relative populations of the super relaxed and conventional relaxed states would have a profound effect on muscle thermogenesis, with the capacity to significantly alter whole body metabolic rate. The mechanism proposed provides a new target for therapeutics with the potential to treat obesity or help in controlling high blood sugar levels.

748-Pos

Structural Impact Of Myosin Methionine Oxidation

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We have examined the structural and functional consequences of methionine (Met) oxidation in Dictyostelium (Dicty) myosin II using a three-pronged approach that includes Met mutagenesis, site-directed spectroscopy, and molecular dynamics simulations. Protein oxidation by reactive oxygen species (ROS) is a critical element of cell function, but in the context of oxidative stress, has been implicated in disease progression and biological aging. Our goal is to bridge our understanding of protein oxidation and muscle dysfunction with molecular-level insights into actomyosin interaction. A Cys-lite version of Dicty myosin II serves as our model system for examining site-specific Met oxidation. Peroxide treatment to mimic oxidative stress induced a two-fold decline in Vmax and KATPase for actin-activation, consistent with the decline in actomyosin interaction observed for biologically aged or peroxide-treated skeletal myosin. We tested the oxidation sensitivity of previously characterized myosin labeling sites in the force-producing region and actin-binding interface and found that spin label mobility and distance measurements in the actin-binding cleft are particularly sensitive to Met oxidation, but only in the presence of actin. Moreover, we conclude that the oxidation-induced structural change in myosin includes a redistribution of structural states involved in the weak to strong actin-binding transition, the step associated with muscle force production. Site-specific Met substitutions combined with functional measurements have allowed us to pinpoint which Met is responsible for the observed structural change. Lastly, we will examine Met oxidation in silico to gain mechanistic knowledge of how residue-specific oxidation translates into changes in both local and global myosin structural dynamics. We expect that our results will be applicable to the many biological and pharmaceutical contexts in which a detailed understanding of protein oxidation, function and structure relationships are sought. This work is supported by the NIH training grant ''Functional Proteomics of Aging'' (T32AG029796).

749-Pos

Novel Approach Applied to IVMA to Study the Modulation of the Actomyosin Interaction by MgATP In Fast Skeletal Muscle

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In this study we used an ''in vitro motility assay'' (IVMA) approach to investigate the effect of the variation of [MgATP] in determining the number and the sliding velocity (Vf) of actin filaments moved by fast skeletal myosin. Vf was studied on type 2B HMM from rats at 25C°, 100mM ionic strength and at varius [MgATP], [MgADP] and [Pi]. We designed a new experimental set-up to perform experiments at lower ionic strength and in buffers that had no interference with the ionic environment, in order to mimic physiological condition. This set-up allowed a complete and chemical speciation of the solutions opening the possibility to perform an accurate thermodynamic study. Therefore, along with kinetic measurements also quantitative thermodynamics measurements were carried obtaining the $\in''G$ of MgATP hydrolysistaking into account pH and [Mg^{2+}]. We correlate the thermodynamics property of the system to Vf and to the number of sliding actin filaments which were assessed by a purpose-designed software. Preliminary results indicate: **a)** no straightly correlation between values of $\in''G_{ATP}$and the velocity of actin filaments, **b)** an increase in thenumberof sliding actin filaments at low [MgATP] and no changes when the ratio [MgATP] / [HMM] was kept constant **c)** a decrease in the velocity of actin filaments at [Pi]=30mM**.** More investigations are required to confirm the unexpected results that indicate a complex role of MgATP and its metabolites in the modulation of actomyosin interaction.

750-Pos

The Effects of Head-Head Interactions on Myosin-Based Actin Sliding Velocities

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Myosin generates force with its weak-to-strong actin binding transition and senses force through a strain-dependent step closely associated with ADP release. We have developed both analytical and computational models of the cooperative interplay between these force-generating and force-sensing biochemical transitions. These models make several novel predictions for unloaded shortening muscle, such as rate constants, k_D, for ADP release that are [ADP]- and [ATP]-dependent. The model also predicts that the acceleration of k_{-D} is associated with a dissipation of interhead strain. To test these model predictions, we use an in vitro motility assay to determine the effects of [ADP] and [ATP] on actin sliding velocities, V, and to determine changes in interhead strain by measuring the rate of actin filament breaking. Our results show a non-hyperbolic nucleotide-dependence of V and a nucleotide-dependence of the rate of actin filament breaking that are both consistent with our cooperative model.

751-Pos

Single Molecule Stepping and Structural Dynamics of Myosin X

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Myosin X is an unconventional myosin motor protein with puzzling motility properties that are under debate. We studied the motility, angular motions and stepping of myosin X in vitro using single molecule fluorescence of rhodamine bound to the lever arm calmodulins (CaMs) and quantum dots on the CaMs and at the C-terminus of the heavy chain. Myosin X walks processively both on single actin filaments and actin bundles. The average step size, measured by FIONA, is 34 nm, supporting the postulate that an α-helical domain extends the lever arm beyond the binding region of myosin X for its three CaMs per head. The step size and velocity are smaller on actin bundles than individual filaments, suggesting that myosin X often steps onto neighboring actins in a bundle. Alternating larger and smaller steps with FIONA and alternating axial angles of the lever arm measured with polTIRF imply that myosin X steps in a hand-over-hand manner. Single molecule 3-dimensional (3D) tracking by Parallax of quantum dot-labeled myosin X on actin filaments and bundles suspended above the coverslip, by flow over ridges or by dielectrophoresis