hydrophobic. The deletion of the period 6 increased force beyond that at saturating [Ca^{2+}], indicating that the period 6 is related to Ca^{2+} regulation. It was further found that Tms with an increased number of periods with coincident Ala clusters invariably increased force. Because the local destabilization with an Ala cluster facilitates the Tm-actin interaction, these results can be interpreted to mean that WT Tm allosterically affects actin, which in turn increases the actomyosin interaction to result in larger force. The interaction between actin and Tm can be either ionic, hydrophobic, or both. Our report demonstrates the significance of Tm's modulatory effect on the actomyosin interaction.

1944-Plat

Low Temperature Dynamic Mapping Reveals Unexpected Order and Disorder in Troponin

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Troponin is a pivotal regulatory protein that binds Ca2+ reversibly to act as muscle contraction's on-off switch. To understand troponin function, the dynamic behavior of the Ca2+-saturated cardiac troponin core domain was mapped in detail at 10 °C, using H/D exchange-mass spectrometry. The low temperature conditions of the present study greatly enhanced the dynamic map compared to previous work. Approximately 70% of assessable peptide bond hydrogens were protected from exchange sufficiently for dynamic measurement. This allowed the first characterization by this method of many regions of regulatory importance. Most of TnI's COOH-terminus was protected from H/D exchange, implying an intrinsically folded structure. This region is critical to troponin's inhibitory function, and has been implicated in thin filament activation. Other new findings include unprotected behavior, suggesting high mobility, for the residues linking the two domains of TnC, as well as for the inhibitory peptide residues preceding the TnI switch helix. These data indicate that, in solution, the regulatory sub-domain of cardiac troponin is mobile relative to the remainder of troponin. Relatively dynamic properties were observed for the interacting TnI switch helix and TnC NH2-domain, contrasting with stable, highly protected properties for the interacting TnI helix 1 and TnC COOH-domain. Overall, exchange protection via protein folding was relatively weak or for a majority of peptide bond hydrogens. Several regions of TnT and TnI were unfolded even at low temperature, suggesting intrinsic disorder. Finally, change in temperature prominently altered local folding stability, suggesting that troponin is an unusually mobile protein under physiological conditions.

1945-Plat

Determining the Effects of Different Cross-Bridge States on the Rate of Calcium Dissociation from Troponin C Mutants in Rabbit Ventricular Myofibrils

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The molecular events required for thin filament activation and deactivation in cardiac muscle are generally thought to be in rapid equilibrium. Stopped-flow measurements obtained with reconstituted thin filaments suggest that the rate of Ca2+ dissociation from cardiac TnC can be modulated by myosin S1 binding to actin and is not always rapid, but approaches the rate of mechanical relaxation at 15oC. To further probe the effects that myosin contributes to thin filament deactivation, we have utilized rabbit ventricular myofibrils as a more physiological biochemical system to examine the kinetics of Ca2+ dissociation from TnC. Incorporating a fluorescent TnC (C35S, C84S, T53C) labeled with IANBD into the myofibrils allows us to follow both the rates of Ca2+ dissociation from TnC, as well as what we think is cross-bridge (CB) detachment, through a change in IANBD fluorescence from TnC. However, CB detachment is only reported by the wild type TnC construct when there is no Ca2+ on the thin filament, while in the presence of ATP, Ca2+ dissociation is no longer observed. CB detachment can be observed in the presence of Ca2+ when Ca2+ binding to TnC is inactivated by D65A TnC. Additionally, the rate of Ca2+ dissociation from TnC can be observed when CBs are detached (presence of ATP) by the Ca2+ sensitizing mutations V44Q or L48Q TnC, but not when Ca2+ binding is desensitized by D73N TnC. Thus, weak and strong binding states of the thick filament differentially influence those of the thin filament within the cardiac myofibril, and are not necessarily in rapid equilibrium.

1946-Plat

Kinetic Mechanism of Ca^{2+}-Induced Conformational Changes of Skeletal Muscle Troponin I in Rabbit Psoas Myofibrils

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The kinetics of Ca^{2+}-controlled conformational changes of the inhibitory subunit (sTnI) of the heterotrimeric skeletal troponin complex were determined by rapid stopped-flow at 10 °C. Conformational changes were probed by fluorescent labelling of sTnI at Cys134 located in between the second actin binding site of sTnI that interacts with actin-tropomyosin at low [Ca^{2+}] and the switch peptide of sTnI that interacts with sTnC at high [Ca^{2+}]. The kinetics was analysed for the sTn-complex in isolation and after its incorporation into rabbit psoas myofibrils. The rapid increase of [Ca^{2+}] to pCa 4.5 induced biphasic fluorescence transients with similar rate constants of $k^{+Ca}{}_{1.phase}$ ~800 s^{-1} and $k^{+Ca}{}_{2.phase}$ ~100 s^{-1} in both preparations. Incorporation changed the polarity of the faster phase but not the polarity of the slower phase. Rapid reduction of [Ca^{2+}] resulted in a monophasic fluorescence change whose rate constant k^{-Ca} was 1.5 s^{-1} for isolated and 12 s^{-1} for incorporated sTn-complex. Thus, incorporation of the sTn-complex into the sarcomere increases the off-rate ~8-fold while leaving the on-rate unaffected. The values of $k^{+Ca}{}_{2.phase}$ and k^{-Ca} determined in our myofibrils experiments are very similar to the values of k_{ON} and k_{OFF} reported by Brenner & Chalovich (Biophys J. 1999 77:2692-708). They observed monophasic kinetics for sTnI labelled at Cys134 incorporated into skinned rabbit psoas fibers. In contrast to our experiments, they triggered sTnI-kinetics mechanically, i.e. by rapidly changing the number of force-generating cross-bridges. The faster conformational change in sTnI that is only observed in our Ca^{2+}-triggered experiments is therefore likely associated with the Ca^{2+}-binding process while the slower phase reports the conformational change of sTnI involved in force regulation. Supported by the Center of Molecular Medicine Cologne (CMMC-A6) and the DFG (SFB612-A2).

1947-Plat

Mutations that Alter cTnC Ca^{2+} Binding Affect Interactions with cTnI and Cardiomyocyte Contraction

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We are using a series of cardiac TnC (cTnC) variants with increased (L48Q) or decreased (L57Q or I61Q) Ca2+ binding affinity (in solution) that alter Ca2+ regulation of force development in skinned myocardium to study 1) molecular mechanisms of the thin filament Ca2+ signaling pathway, and 2) their influence on intracellular Ca2+ metabolism. Solution binding studies indicated increased affinity of the cTnC variants for cTnI in the order L48Q > wild type (WT) > L57Q > I61Q. We previously reported for stopped flow experiments that L48Q cTnC-Tn slows calcium dissociation (by about 4 fold compared to WT), and L57Q and I61Q increases the rate of release. Ca2+ dissociation from reconstituted thin filaments containing cTnC variants was increased in all cases, with L48Q again slowing release and I61Q increasing calcium release compared to WT. Previously, our molecular dynamics (MD) simulations suggested increased Ca2+ and cTnI affinity of L48Q vs. WT cTnC resulting from increased mobility of the B-helix, greater exposure of the hydrophobic patch and stronger interactions of Ca2+ with some of the coordinating residues at Ca2+ binding site II. We now report 'lifting' of B helix of L48Q cTnC(1-89) in apo states simulations. There were no similar movements with L57Q and I61Q simulations. Adenoviral mediated expression of cTnC variants in quiescent cultured adult rat cardiomyocytes resulted in shortening rate and magnitude in the order I61Q > L57Q > WT > L48Q. Ca2+ transients (Fura-2) were not affected by L48Q, but reduced with L57Q or I61Q cTnC. Together these data suggest that cTnC variants with altered Ca2+ binding affinity can influence interactions with cTnI and alter myocardial Ca2+ metabolism. HL091368, HL65497 (MR), AHA-09PRE2090056 (DW).

1948-Plat

A Phosphomimetic Mutation of TnI Partially Rescues a Disease Causing Mutation of TnT

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Cardiomyopathy causing mutations of troponin and mutations that simulate phosphorylation of troponin can alter the distribution of states of actin-tropomyosin-troponin. The active state of actin filaments containing the Δ14 TnT mutant is stabilized whereas filaments containing the S45E TnI mutation are more difficult to activate. We show that actin filaments containing both [[Unsupported Character -]]Δ14 TnT and S45E TnI were somewhat normalized. However, because the Δ14 TnT mutation was far from the norm its effects dominated those of the S45E TnI mutation. ATPase activities of actin filaments at high Ca^{2+} followed the pattern: Δ14 > double mutant > wild type > S45E. In EGTA the activities were: Δ14 > double mutant > wild type ≥ S45E. At high concentrations of the activator, NEM-S1 all types of actin filaments had about the same rate. The rate of transition from the active state to the inactive state was measured using acrylodan labeled cardiac tropomyosin. Following rapid dissociation of S1 with ATP, wild type filaments exhibited a rapid decrease in fluorescence (as the intermediate state population increased) followed