**1177-Pos Board B21**

**Ionic Strength Influence on Myofibril Thin Filament State Probed by Troponin Exchange**

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Activation of striated muscle contraction is a cooperative process initiated by calcium binding to troponin C (TnC). This is a solid-phase signal transduction process involving 3 states of thin filament: a blocked (B) state preventing myosin interaction, a closed (C) state allowing weak myosin interaction, and an fully activated open or M state. Here we evaluated the influence of ionic strength on Tn dissociation in relationship to myofibril thin filament state. The data we obtained showed that ionic strength had a significant influence on Tn dissociation with low ionic strength increasing Tn dissociation rate from non-overlap region (B or C state) and high ionic strength increasing Tn dissociation rate from overlap region (M state) of myofibril thin filament in both absence and presence of calcium. When ionic strength was decreased to $\leq$ 50 mM, Tn dissociation from non-overlap region became even faster than overlap region in the absence of calcium, suggesting that actin-tropomyosin in the non-overlap region actually changed from the inhibited B state to the C state at low ionic strength. Also, low ionic strength increased myofibril ATPase activity in the absence of calcium and the calcium-sensitivity of ATPase activity. Based on this and our current studies, we propose a refined kinetic scheme for Tn dissociation from myofibril thin filament, which suggests that the regulatory dissociation of TnI from actin-tropomyosin and association to TnC is not necessarily calcium coupled. Low ionic strength favors the B to C state equilibrium towards the C state (Head et al., Eur. J. Biochem, 227: 694) by favoring TnI dissociation from actin and possibly association with TnC in the absence of calcium. This well explains reported inconsistence between ATPase activity and S1 binding to actin under low ionic strength.

**1178-Pos Board B22**

**Kinetic Modeling Shows that the Apparent Rate of Troponin Dissociation from Myofibrils Probes the B-state to C-state Equilibrium of the Thin Filament**

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The apparent rate of troponin (Tn) dissociation from myofibrils has been used as a method to study thin filament regulation. The rate is dependent upon calcium and strong crossbridges and supports the 3-state model for thin filament regulation (Swartz et al., 2007, J. Mol. Biol. 361:420). Tn dissociation rate is extremely slow so it is not intuitive that such a slow process probes thin filament regulation. To address this, we developed a simple two step kinetic model for Tn dissociation rate, as measured by labeled Tn exchange in myofibrils, and simulated the progress of labeled Tn incorporation into myofibrils and the intensity ratio of the non-overlap to overlap region. TnI's regulatory domain switches from actin-tropomyosin to TnC in the first step and TnT dissociates from actin-tropomyosin in the second step. Step 1 is the transition of the thin filament from the B-state to the C-state, is calcium dependent, and is several orders of magnitude faster than the forward reaction of step 2. By integrating the dimensionless rate equations of the above kinetic model, we have computed the time course of each of the various components. In our numerical simulations, the forward rate constant for step 1 was varied from 2 - 500 s-1 to simulate the pCa 9.0, B-state to pCa 4.0, C-state range. The progress curves for labeled Tn exchange into myofibrils and the derived intensity ratio, assuming a fixed exchange rate in the overlap region, well explain the observed intensity ratio progress curves for labeled Tn exchange into myofibrils. These simulations and experimental observations show that the apparent rate of Tn dissociation probes the B-state to C-state equilibrium of the myofibrillar thin filament.
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**Dynamic Studies Of Truncated Troponin Complex Usin Hdx**

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Troponin is the primary control protein of the cardiac and skeletal muscle contractile apparatus. Troponin is made up of three subunits (TnT, TnI, TnC) and is located on the thin filament together with tropomyosin. The binding of $Ca^{++}$ to TnC induces conformational changes in troponin leading to muscle contraction, via a regulatory mechanism that involves tropomyosin. Neither the transmission of the dis-inhibitory $Ca^{++}$ binding 'signal' from TnC to other thin filament proteins, nor the mechanism of troponin's inhibitory action in the absence of $Ca^{++}$ binding, is well understood. Both NMR and X-ray studies show conformational change in the N-terminal domain of cardiac troponin on binding to $Ca^{++}$. We have expressed full length TnI, TnC and truncated form of TnT (residues, 183-288) and reconstituted into the troponin core domain complex. To address the question of how the dynamic properties of the apo- (i.e., regulatory site apo) and $Ca^{++}$-saturated troponin may facilitate the conformational change and function, native state amide hydrogen/deuterium exchange was used. The experiments were carried out by pepsin digest, HPLC coupled to electrospray FT-MS. The TnT-TnI coiled-coil is the most stable portion of the core domain, i.e., the (hydrogen-bonded) amide H groups from this part of troponin are most protected from exchange with solvent D. Furthermore, all peptides derived from this coiled-coil exchange at similar rates, suggesting that local unfolding was small, and exchange rates reflect the (unfavorable, so slow) global unfolding of the entire coiled-coil. The extent of deuterium incorporation in troponin in the presence/absence of $Ca^{++}$ will be presented, discussed and compared to the crystal structure.

**1180-Pos Board B24**

**Host-guest Study of the Hypothesis that Different Tropomyosin Quasi-repeats Have Different Effects on Myosin**

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Tropomyosin is semi-modular protein, comprised of an elongated coiled coil that stretches along the thin filament and weakly interacts with multiple actin monomers. Despite the weakness of these interactions, they accomplish potent regulatory effects. In muscle thin filaments, tropomyosin increases myosin S1-actin binding affinity ~4-fold (either + or - ADP.) Deletional studies suggest (but do not prove) that this average effect of the entirety of muscle tropomyosin varies among the seven quasi-repeating regions of the molecule. To test this hypothesis, different quasi-repeats of alpha-striated muscle tropomyosin were inserted into *Saccharomyces cerevisiae* tropomyosin isoform TPM1, swapping out a region of the yeast isoform potentially well suited for such maneuvers. Importantly, TPM1 does not greatly affect myosin-actin binding. Also, its residues 70-107 span one actin, are an internal repeat region, and have heptad phase staggered at each end. A series of chimeric tropomyosins was generated by replacing these 38 residues of TPM1 with heptad phase- and length-corresponding sections of muscle tropomyosin. Five of 7 such chimeric tropomyosins bound poorly to actin and to actin-S1, despite coiled-coil folding. Chimeras containing muscle tropomyosin residues 74-111 and 228-265 bound tightly to actin, and thus could be studied for functional effects of the residue substitutions. When myosin S1 was added, it increased chimera 74-111 binding to actin and decreased chimera 228-265 association with actin. None of the chimeric tropomyosins altered the actin-activated ATPase rate of skeletal muscle S1. Effects on S1-ADP binding to actin are under examination. The results support the concept that the effects of muscle tropomyosin on myosin-actin interactions are not homogeneous along the seven-actin spanning length of this regulatory protein.

**1181-Pos Board B25**

**The Orientation of the H1 and H2 Helices of Troponin I in Ventricular Trabeculae**

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Contraction of cardiac muscle is triggered and modulated by structural changes in the heterotrimeric troponin complex (TnC, TnI and TnT) on the actin filaments. The changes in conformation of the N-terminal lobe of TnC induced by $Ca^{2+}$ binding are well understood, but the structural basis of the downstream signaling pathway via TnI and TnT to tropomyosin and actin is much less clear, as is that of the modulation of contractility by TnI phosphorylation. To address these questions we are investigating the orientation of the TnI H1 helix (residues 43-79, human cardiac TnI; hcTnI) and H2 helix (residues 93-135) of the troponin core domain (Takeda *et al.*, *Nature* 424: 35, 2003) in a cardiac muscle cell using bifunctional fluorescent probes (Corrie *et al.*, *Nature* 400:425, 1999). The hcTnI cysteines 80 and 97 were replaced by serines, and pairs of cysteines were introduced for covalent attachment of bifunctional sulphorhodamine (BSR) at surface-accessible positions 60 and 67 or 119 and 126. Full-length troponin complex was formed from these modified hcTnIs in combination with cysteine-null hcTnC and hcTnT. The cysteine pair on hcTnI in each complex was labeled with BSR, and the hcTnI-BSRs were characterized by HPLC and mass spectroscopy. The labeled troponin complex will be exchanged into skinned trabeculae from rat ventricle for determination of the orientation of the H1 and H2 helices with respect to the thin filament axis by polarized fluorescence (Dale *et al.*, *Biophys. J.* 76:1606, 1999; Julien *et al.*, *Biophys. J.* 93:1008, 2007).
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