Native frog Tm was cross-linked with DTNB at Cys 190. Tryptic cleavage patterns of this cross-linked species were compared to a control Tm in which the Cys 190 groups were maintained reduced state using DTT. The course of tryptic cleavage of the cross-linked molecule was altered compared to the fully reduced molecule. Proteolysis of the cross-linked molecule produced new tryptic fragments seen in a reduced SDS gel at 30kDa and 20kDa. Trypsin cleaves reduced tropomyosin at Arg 133 to produce two new tryptic fragments (17kDa and 15kDa). The observation that cross-linked Tm exhibits a change in the cleavage rate, and affects the cleavage pattern, demonstrates a long-range effect of the cross-link at Cys 190 on the site of trypsin cleavage, Arg 133. This finding adds evidence to the idea that ''flexibility'' is associated with a locally fluctuating region in the middle of the molecule which has implications on our understanding of how Tm moves on the actin surface, and provides insight into understanding how single point mutations in Tm may result in dysfunction and disease associated with various myopathies.

1814-Pos

Tropomyosin Pseudo-Phosphorylation and Muscle Kinetics

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Tropomyosin contains a phosphorylation site at Ser-283 located within the head-to-tail overlap region that regulates muscle contraction. Previously we demonstrated that recombinant wild-type (Tm-WT) and pseudo-phosphorylated (Tm-S283D) alpha tropomyosin, expressed and purified from insect cells, exhibit regulated ATPase activity and troponin T binding similar to that reported for non-phosphorylated and phosphorylated tropomyosin (pTm) purified from muscle. We further demonstrated transgenic mice expressing the pseudo-phosphorylated tropomyosin (Tm-S283D Tg) exhibit increased mortality and decreased rate of relaxation in the work performing heart preparation. These changes occur in the absence of altered steady state maximal force or calcium-sensitive force development in skinned papillary bundles. In light of these findings, we sought to investigate the effect of pTm on the kinetics of cardiac muscle function. Using an extraction/replacement protocol, we measured force kinetics in a myofibril preparation. Results demonstrate no significant difference between myofibrils replaced with Tm-S283D or Tm-WT in maximal force, the rate of force activation, or the rate of force redevelopment, implying pTm does not play a role in altering muscle activation or cycling kinetics. To investigate if pTm affects the kinetics of thin filament inactivation we measured the rate of calcium disassociation from troponin C. Results demonstrate thin filaments reconstituted with Tm-S283D decreased the rate of calcium disassociation from troponin C consistent with the previously observed relaxation impairment. Finally, to determine the effects of pTm on systemic alterations in cardiovascular performance we measured heart function in Tm-S283D Tg mice by echocardiography. Results demonstrate Tm-S283D Tg mice exhibit trends towards impaired cardiac contractility compared to non-transgenic mice including decreased peak systolic velocity and ejection fraction. Overall, these findings demonstrate tropomyosin phosphorylation contributes to the regulation of cardiac dynamics; however, the precise role of pTm in the development of cardiac dysfunction remains elusive.

1815-Pos

Studies of the Mid Region of Tropomyosin Using an Incorporated Tryptophan Analogue

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Striated muscle contraction is controlled by highly sensitive alterations in thin filament conformation that involve a Ca2+-dependent interaction between troponin I (TnI) and actin-tropomyosin (Tm). To study these interactions, 5-OH Tryptophan (OHW) was incorporated into striated muscle alpha-tropomyosin. A Single Trp tropomyosin mutant, Q135W, was generated and expressed in Trp auxotrophs. OHW incorporation was 76%. Thin filaments containing Q135W retained excellent function: 12-fold Ca2+ regulation in myosin S1-thin filament MgATPase assays. Fluorescence emission titrations with increasing amounts of Ca2+ were performed (emission max 338nm) in presence of thin filaments comprised of cardiac troponin, F-actin, and Q135W tropomyosin. Similar titrations using troponin, actin, and wt Trp-free tropomyosin produced low fluorescence (ex wavelength 312 nm). Ca2+ decreased the OHW fluorescence of Q135W-containing thin filaments by 7.0 +/− 0.4 %. This transition occurred with K = 1.8 +/− 0.3 uM −1, consistent with the affinity of the cardiac thin filament regulatory calcium site on TnC. OHW Q135W tropomyosin is a novel tool for monitoring thin filament behavior, in particular for detecting Ca2+ binding to troponin. Troponin has been viewed as binding to the C-terminus of tropomyosin, which does not include the mid-tropomyosin residue 135 that is found in the current study to be Ca2+-sensitive. However, the findings may be related to recent results, suggesting that the TnI C-terminus of troponin bridges from one actin strand to the other, and contacts tropomyosin near residue 146. Galinska-Rakoczy et al, JMB 2008. Mudalige et al, JMB2009. Finally, human cardiac troponins with defective inhibitory activity due to alterations in the TnI inhibitory region have been generated. They are currently under study using this tropomyosin.

1816-Pos

Loss of Function in β-Tropomyosin (TPM2) Mutants Causing Nemaline Myopathy or Cap Disease

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Nemaline myopathy (NM) and cap disease (CD) are two congenital skeletal muscle myopathies characterized by general muscle weakness with early neonatal onset. They are both clinically and genetically heterogeneous, but at the histological level they present characteristic pathology: nemaline rod-like bodies within myofibers generating from the Z-discs for NM and cap-like structures at cell periphery underneath the sarcolemma for CD. Recently several different mutations on the TPM2 gene, coding for the -tropomyosin (-Tm) isoform expressed in skeletal muscles (especially slow-twitch type 1 fibers), have been reported to cause either NM or CD. In this study six -Tm mutants connected either to NM (K7, E117K, Q147P) or to CD (E41K, K49, E139) and the wild type form have been expressed in a baculovirus/sf9 system. This system, being based on eukaryotic cell cultures, permits the N-terminal acetylation of -Tm, which has been shown to be important for its linear head-to-tail polymerisation. Thin filaments reconstituted with these recombinant proteins have been tested with the *in vitro* motility assay finding a considerable loss of function for each of them. K7, Q147P, K49 and E139 showed, through indirect observations, a reduced affinity to actin, possibly explained by destabilising the Tm homodimer itself or important actin-Tm binding sites. With all mutants the Ca^{2+}-induced increase in sliding speed was greatly reduced, indicating an altered TroponinT-Tm interaction, but thin filaments Ca^{2+} switching was unaltered. The three NM mutants showed a reduced Ca^{2+}-sensitivity, whereas two of the CD mutants showed an increase. E41K had a different phenotype and seemed to strongly inhibit filament motility. A well-defined scenario is taking shape and further studies could directly correlate these findings with the etiology of the diseases.

1817-Pos

An Evolutionary Structural Bioinformatics Analysis of the Actin Binding Protein, Tropomyosin

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Tropomyosin is a two-chained, α-helical coiled-coil protein that associates end-to-end to form a continuous strand along actin filaments and regulates the functions and stability of actin. Mutations in tropomyosin cause skeletal and cardiomyopathies. We have carried out a phylogenetic analysis of tropomyosin to identify its conserved residues and to elucidate their importance for binding actin. Actin is a highly conserved protein and our hypothesis is that the actin binding sites of tropomyosin have been conserved through evolution to retain its actin-binding function. Phylogenetic trees of 60 coding sequences were constructed from tropomyosin genes from 19 species within the phyla Chordata, Hemichordata and Echinoderm. The rates of substitution (ω) at amino acid sites (codons) in the protein sequence were calculated to identify the most conserved sites (lowest ω) using CODEML in PAML 4.1. A total of 103 out of 284 residues were identified as highly conserved ($\omega \leq 0.015$), of which 24 are in *a* and *d* positions of the heptad repeat involved in the hydrophobic coiled coil interface, 38 are in potential interhelical *e-g* position salt bridges important for folding of the coiled coil, and 41 are in *b*, *c*, or *f* surface positions available for binding to other proteins, such as actin. The conserved residues at the *b*, *c*, and *f* surface positions were selected for initial mutagenesis studies with mutations to alanine. Preliminary actin binding co-sedimentation assays carried out for three tropomyosin mutants showed a 2- to 3-fold decrease in actin-binding affinity compared to the wild-type protein. Further analysis of mutations at other conserved surface positions and their effect on actin-binding affinity and stability of the mutant proteins are in progress. *Supported by Muscle Dystrophy Association.*