Ca^{2+}-sensitivity are therefore consistent with the hypothesis that cross-bridges play a key role in cardiac thin filament activation. Supported by: NIH HL63974, GM07592, AHA 0615164B.

1931-Plat

Dynamics of Bi-Functional Labeled Tropomyosin in Muscle Ghost Fiber Monitored by Saturation Transfer EPR

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Tropomyosin (Tm), an alpha-helical coiled-coil protein, is a key regulatory protein in muscle contraction. To date, little is known about the extent of Tm flexibility and the role of Tm dynamics in muscle regulation. In this work, the flexibility of two different regions of Tm was assessed using Saturation Transfer Electron Paramagnetic Resonance (ST EPR). In order to fully immobilize the spin probe on the surface of Tm we used a bi-functional spin label attached to i, i+4 positions of the coiled-coil obtained by cysteine mutagenesis. We have used conventional EPR and ST-EPR to detect wide range of dynamics from the very slow (millisecond) motions to fast sub-nanosecond modes. The labeled Tm mutants were reconstituted into ''ghost muscle fibers'' from which the myosin filaments and intrinsic regulatory proteins (tropomyosin, troponin) were removed.

ST-EPR of the two mid-region mutants Tm H153C/D157C and Tm G188C/E192C as well as the C-terminus mutant Tm A268C/E272 gave a correlation time of 10.5 us $\pm$ 4.5 us, 42.5 us $\pm$ 27.5 us, and 42.5 us $\pm$ 27.5 us respectively (using H''/H and L''/L ratios of V'_2 spectra). The difference in correlation time between the different di-mutants is an indication of the differential flexibility of the Tm protein. The study of the N-terminus (L13C/N17C) di-mutant will give us an additional understanding of Tm flexibility. Finally the introduction of Troponin complex (Tn) as well as S1 head of myosin under high and low calcium concentrations will give a complete picture of the dynamics of Tm in muscle regulation.

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C-terminal Region Of Troponin I Interacts Near Residue 146 Of Tropomyosin In A Ca^{2+} Dependent Manner

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Force generation in striated muscle is initiated by Ca^{2+} binding to troponin C in the actin-tropomyosin-troponin (actinTmTn) thin filament. Potter & Gergely, (*Biochemistry* 1974); have suggested that the inhibitory subunit of Tn, troponin I (TnI) interacts with Tm as well as actin to inhibit contraction in the absence of Ca^{2+}. Zhou et. al., and Geeves et. al., (*Biochemistry* 2000) proposed that this interaction involves a specific site on Tm. Last year (Mudalige, Tao and Lehrer, 52nd annual meeting of Biophysical Society 2008) we reported the formation of a Ca^{2+}-dependent cross-link between a benzophenone-maleimide label at Tm residue 146 and TnI (Tm*146-TnI).

To determine the cross-linking site of TnI with Tm*146, we purified the photochemically cross-linked complex, Tm*146-TnI from uncross-linked proteins using HPLC, and SDS gels and subjected the selected Tm*146-TnI band to in-gel tryptic digestion .

From the comparison of MALDI-TOF spectra of tryptic peptides of in-gel digested Tm*146, TnI and Tm*146-TnI, a new peptide of MW 2601.2 Da was identified. Two possible TnI tryptic peptides which contains the Tm 143-154 tryptic peptide and probe with similar MW were identified: 1) peptide 157-163 (MW 2602.4 Da); 2) peptide 176-182 Met oxidized, (MW 2600.3 Da). A cross-link in either of thee peptides supports the recently published image reconstructions which show the C-terminal domain of TnI interacting with both actin and Tm across the actin filament away from the bulk of the Tn complex (Galinska_Pakoczy et al, JMB, 2008). Our identification of the cross-linked residue on TnI (in progress), will further localize Tn on the actinTm muscle thin filament in the absence of Ca^{2+} (Supported by NIH HL 22461).

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Impaired Myofilament Contractility in Post-infarct Remodeled Myocardium is Restored upon β-Adrenergic Stimulation

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Previously we have shown that *in vivo* cardiac responsiveness to exercise-induced increases in noradrenaline was blunted in pigs with a myocardial infarction (MI), consistent with defects in β-adrenergic signaling. Here we tested the hypothesis that the blunted increase in pump function with exercise after MI is due to reduced myofilament responsiveness, and is prevented by β-blocker therapy. In pigs with a MI induced by ligation of the left circumflex coronary artery, β-blocker therapy (bisoprolol, MI+β) was initiated on the first day after MI. Myofilament force measurements and protein analysis were performed in left ventricular subendocardial biopsies taken at baseline, and upon dobutamine stimulation 3 weeks after MI or sham (n=6). Isometric force was measured in single permeabilized cardiomyocytes. At baseline, maximal force (Fmax) was lower in MI compared to sham, while Ca^{2+}-sensitivity (pCa_{50}) was higher (both $P<0.05$). Passive force (Fpas) did not differ. Fmax did not change upon dobutamine in sham, while it markedly increased in MI. Moreover, the dobutamine-induced decrease in pCa_{50} was larger in MI than in sham. Beta-blockers prevented baseline myofilament dysfunction, reduced Fpas and enhanced the responsiveness to β-AR stimulation illustrated by a large change in pCa_{50} upon dobutamine. Baseline phosphorylation of β-adrenergic target proteins (myosin binding protein C and troponin I) was not altered in MI, while the dobutamine-induced increase in troponin I phosphorylation was less in MI compared to sham and MI+β. Dobutamine enhanced myosin light chain 2 phosphorylation solely in sham. In conclusion, acute β-adrenoceptor stimulation largely restores baseline myofilament dysfunction despite attenuation of β-adrenergic-mediated troponin I phosphorylation. Myofilament dysfunction in remodelled myocardium and its reversal by β-blockers is not a direct consequence of reduced PKA-mediated phosphorylation, and does not contribute to the blunted in vivo response to β-adrenoceptor stimulation.

1934-Plat

Reference Free Single Particle Analysis Of Reconstituted Thin Filaments

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A detailed three-dimensional structure of the muscle thin filament is required in order to understand its regulation. To this end we have applied a reference free single particle analysis approach to electron microscope images of negatively stained reconstituted thin filaments from skeletal actin and cardiac tropomyosin and troponin. The filaments were prepared in a low Ca^{2+} buffer. For image analysis the filaments were segmented into ~800Å long particles centred on the troponin complex. Density attributable to troponin and tropomyosin is readily identifiable in the two-dimensional class averages and the three-dimensional reconstruction. The data have previously been analysed using a model-based single particle method (Pirani *et al.*, 2005, 2006). Our non-model based approach and novel strand averaging procedure has enabled us to quantify directly the stagger or axial rise between adjacent troponin complexes (~27.7Å). Comparison with our previous analysis of native thin filaments indicates that reconstituted filaments assemble with the same arrangement of troponin as in vivo, *viz* .in register on both helical strands with a ~40 nm repeat. This indicates that troponin and tropomyosin can organise themselves on actin filaments without requiring any other sarcomeric proteins.

Pirani A., Vinogradova M.V., Curmi P.M., King W.A., Fletterick R.J., Craig R., Tobacman L.S., Xu C., Hatch V., Lehman W. 2006. An atomic model of the thin filament in the relaxed and Ca^{2+}-activated States. J Mol Biol 357(3):707-17.

Pirani A., Xu C., Hatch V., Craig R., Tobacman L.S., Lehman W. 2005. Single particle analysis of relaxed and activated muscle thin filaments. J Mol Biol 346(3):761-72.

1935-Plat

Calcium-Regulated Conformational Changes in the COOH-terminus of Troponin I

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The troponin complex plays an essential role in the calcium regulation of skeletal and cardiac muscle contractions. Of the three subunits of troponin (TnC, TnI and TnT), TnI is the inhibitory subunit that responds to the binding of Ca^{2+} to TnC during the activation of contraction. The COOH-terminal region of TnI is a highly conserved structure implying a fundamental function. Previous studies using reconstituted troponin or myofilaments suggested that the COOH-terminal domain of TnI undergoes epitopic and positional changes in the presence or absence of calcium. Here we tested the calcium-induced conformational changes in the COOH-terminal region of TnI by engineering a unique Cys at the COOH terminus of TnI for the addition of a reporting label. Monoclonal antibody epitope analysis and protein binding assays indicated that this modification and the replacement of two internal Cys residues (C81I and