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

Roni F. Rayes[1], Arthur T. Coulton[2], Michael A. Geeves[2], Piotr G. Fajer[1].
[1]Florida State University, Tallahassee, FL, USA, [2]University of Kent, Canterbury, United Kingdom.

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.

1932-Plat

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

Amal W. Mudalige, Sherwin S. Lehrer.
Boston Biomedical Research Institute, Watertown, MA, USA.

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).

1933-Plat

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

Nicky M. Boontje[1], Daphne Merkus[2], Vincent J. de Beer[2], Giulia Mearini[3], Lucie Carrier[3], Lori A. Walker[4], Ger J.M. Stienen[1], Dirk J. Duncker[2], Jolanda van der Velden[1].
[1]Institute for Cardiovascular Research VU University Medical Center, Amsterdam, Netherlands, [2]Erasmus Medical Center, Experimental Cardiology, Rotterdam, Netherlands, [3]Institute of Experimental and Clinical Pharmacology and Toxicology, Hamburg, Germany, [4]Department of Medicine, Section of Cardiology, Denver, CO, USA.

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

Danielle M. Paul[1], William Lehman[2], Alnoor Pirani[2], Roger Craig[3], Larry S. Tobacman[4], John M. Squire[5], Edward P. Morris[1].
[1]Institute of Cancer Research, London, United Kingdom, [2]Boston University School of Medicine, Boston, MA, USA, [3]University of Massachusetts, Worcester, MA, USA, [4]University of Illinois at Chicago, Chicago, IL, USA, [5]University of Bristol, Bristol, United Kingdom.

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

Zhiling Zhang, Steven Mottl, J.-P. Jin.
NorthShore University HealthSystem and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.

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

C98S) in a cardiac TnI core structure (McTnI-ND_{29}-Cys) did not affect the COOH-terminal conformation of TnI and preserved binding to TnT and TnC. McTnI-ND_{29}-Cys purified from bacterial culture was fluorescently labeled with the Alexa Fluor 532 dye and used to reconstitute troponin complex. After verifying the ratio of fluorophore to protein conjugation by spectrophotometer and SDS-PAGE, Ca^{2+}-titrations were performed for fluorescence intensity and polarization changes. The results demonstrated Ca^{2+} regulated conformational/environmental changes as well as flexibility change in the COOH terminus of TnI. Further experiments are performed to measure the Ca^{2+}-induced structural changes in reconstituted myofilaments to understand the function of TnI COOH terminal domain in calcium-regulation of muscle contraction.

1936-Plat

Cardiomyopathy Causing Mutations Stabilize an Intermediate State of Thin Filaments

Mohit C. Mathur[1], Tomoyoshi Kobayashi[2], Joseph M. Chalovich[1].
[1]Brody School of Medicine, East Carolina University, Greenville, NC, USA, [2]University of Illinois at Chicago, Chicago, IL, USA.

Congenital cardiomyopathies are initiated by changes in ATP hydrolysis and result in hypertrophy, fibrosis, and myofibrillar disarray. We studied the mechanism by which mutations in troponin and tropomyosin change ATPase rates and have linked several mutants to inappropriate switching between the inactive and active states of the actin thin filament. We have shown that troponin I mutants mimicking protein kinase C phosphorylation stabilize the inactive state of actin filaments whereas the Δ14 TnT mutant stabilizes the active state. We have now shown that two mutations on troponin I, R146G and R146W, which cause cardiomyopathy produce complex effects on ATPase activity. These TnI mutations produced increased ATPase rates in the absence of calcium and decreased rates in the presence of calcium compared to wild type. These differences were maintained at high actin concentrations. Saturating concentrations of the activator NEM-S1 equalized the rates of both the mutants and wild type. The NEM-S1 data rule out alterations in rate constants of transitions (i.e. product release) along the active pathway. The results from the R146G and R146W mutants have implications for the function of the 3 structural states of regulated actin that have been observed. That is, the results can be explained most readily if the mutants stabilize an intermediate state in both calcium and EGTA with an activity between that of the inactive and active states. In the past we have assumed that the intermediate state had properties identical to the inactive state. Our current data show that while the intermediate more closely resembles the inactive state it has unique properties. Our present results, as well as previous results, indicate that inappropriate stabilization of any state of regulated actin can result in cardiac dysfunction.

Platform AI: Protein Dynamics I

1937-Plat

Probing Conformational Motion of Serpin by Time-Resolved and Single Molecule Fluorescence

Chin Wing Ko[1], Wenhua Zhou[1], Richard J. Marsh[2], Daven A. Armoogum[2], Nick Nicolaou[2], Angus J. Bain[2], Zhenquan Wei[3], Aiwu Zhou[3], **Liming Ying**[1].
[1]Imperial College London, London, United Kingdom, [2]University College London, London, United Kingdom, [3]University of Cambridge and Cambridge Institute for Medical Research, Cambridge, United Kingdom.

Serpin (serine protease inhibitor) is a structural prototype for the study of the molecular mechanism of many diseases due to the conformational instability which leads to protein aggregation. The inhibitory function of serpin relys on a flexible loop undertaking a striking conformational transition, but this property also leaves serpin at risk of polymerization. We have investigated the conformational dynamics of the reaction center loop (RCL) of the plasminogen activator inhibitor-1 (PAI-1) by time resolved fluorescence spectroscopy. The RCL becomes more solvent exposed and exhibits faster rotation when PAI-1 interacts with an octapeptide which blocks the loop insertion pathway, indicating that the RCL is well displaced from the protein surface. A heterogeneous population model with three rotational correlation times has been developed to account for the ''dip and rise'' observed in fluorescence anisotropy decays. We have also employed single molecule FRET to probe the conformational change of serpin under different environment and the early stage of its ploymerization process. Preliminary results will be presented.

1938-Plat

Evolution of enzyme fold: Linking protein dynamics and catalysis

Pratul K. Agarwal.
Oak Ridge National Laboratory, Oak RIdge, TN, USA.

Enzymes are dynamic molecules. In the past, enzymes have been viewed as static entities and their high catalytic power has been explained on the basis of direct structural interactions between the enzyme and the substrate. Recent evidence has linked protein dynamics to catalytic efficiency of enzymes. Further, motions in hydration-shell/bulk solvent have been shown to impact protein motions, therefore, function.

Theoretical and computational studies of protein dynamics linked to enzyme catalysis will be discussed. Investigations of cyclophilin A and dihydrofolate reductase have lead to the discovery of networks of protein vibrations promoting catalysis. Results indicate that the reaction promoting dynamics in these enzymes is conserved across several species. Moreover, we have characterized the protein dynamics of a diverse super-family of dinucleotide binding enzymes. These enzymes share very low sequence similarity and have different structural features. The results show that the reaction promoting dynamics is remarkably similar in this enzyme super-family.

1939-Plat

Function And Activity Of Von Willebrand Factor Is Regulated By A Hierarchy Of Mechanical Forces

Carsten Baldauf[1], Tobias Obser[2], Antje Pieconka[3], Sonja Schneppenheim[3], Ulrich Budde[3], Reinhard Schneppenheim[2], Frauke Graeter[1].
[1]CAS-MPG PICB, Shanghai, China, [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]AescuLabor Hamburg, Coagulation Lab, Hamburg, Germany.

The von Willebrand factor (VWF) is a shear-flow sensitive multimeric protein. Under normal flow conditions VWF is in a globular state, it unfolds at high shear rates and is activated for adhesion at the blood vessel wall [Schneider et al. 2007 PNAS p7899]. The elongation of multimeric VWF results in a force pulling along the VWF length axis. Based on a model of the VWF A domain organization, we performed force probe molecular dynamics simulations. We reveal the basis of two force-sensing VWF functions, and test the results by experiments. Our results indicate a competition between VWF A2 domain and glycoprotein Ib (GPIb) for the same binding site of the VWF A1 domain. When the stretching force along VWF reaches a critical point, the A1 A2 interaction is lost. The domains remain connected by a linker that gives space for GPIb to bind to the A1 domain. We thus suggest a force-dependent platelet binding to VWF as mediated by GPIb, which is experimentally testable and represents an alternative mechanism to recently published studies [Chen et al. 2008 Biophys J p1303; Lou et al. 2008 PNAS p13847].

We show how proteolysis of the VWF is activated under shear conditions. The specific proteolytic site is buried in the VWF A2 domain [Sutherland et al. 2004 J Mol Model p259]. At extreme forces as present in high molecular weight VWF multimers, the A2 domain C terminus unfolds until the ADAMTS13 cleavage site is uncovered. Introducing a disulfide bond by mutagensis prevents VWF cleavage. This explains the size regulation of VWF by ADAMTS13: larger multimers involve higher pulling forces and therefore higher unfolding rates under shear flow. Larger VWF is cleaved faster, preventing blood clots and thrombosis [in preparation].

1940-Plat

Experimental Confirmation of an NtrC Transition Pathway Predicted by Targeted MD

Janice Velos, Ming Lei, Ce Feng Liu, Phillip Steindel, Dorothee Kern.
Brandeis University, Waltham, MA, USA.

The infinitely short lifetime of transition states makes characterization extremely difficult. We have used a combination of molecular dynamics and experimental approaches to determine two important rate-limiting interactions involved in the allosteric transition of a signaling protein. Targeted MD simulations of the receiver domain of NtrC (Nitrogen Regulatory Protein C) were used to predict interactions that are important in stabilizing the transition state between the known inactive and active structures of the protein. Mutations were made to test these predictions and the rate of exchange between the two substates were measured by 15N-CPMG relaxation dispersion experiments. The results verify the importance of these key interactions in the transition pathway of NtrC. This work shows that targeted molecular dynamics together with experimental validation can be an invaluable tool at elucidating the structure and rate-limiting interactions of conformational transitions.

1941-Plat

Real-time 3D Tracking of Structural Transitions in Adenylate Kinase by Thermal Noise Imaging

Yunhsiang Hsu, Vijay Rana, Arnd Pralle.
SUNY Buffalo, Amherst, NY, USA.

Proteins have to be flexible enough to support turn-over rates up to hundreds per second, yet stable enough to maintain their three-dimensional structure over hours and days. As result of thermal excitation they fluctuate between structural conformations. We measured thermally excited structural fluctuations in the Adenylate Kinase using a site-specifically attached nanoparticle