allosteric proteins. While both models fit biochemical data and serve as useful conceptual tools, neither describes the atomic details of the mechanisms responsible for how allosteric proteins switch from one conformational state to another. Motivated by recent advances in high-resolution modeling, we set out to develop a method that predicts a protein's alternative state from a starting crystal structure. This method allowed us to probe the atomic details underlying conformational switching. To test this method, we chose proteins whose conformational change, both large and small, were induced by an external trigger such as ligand binding. We hypothesized that removal of the trigger would destabilize the starting structure, making the alternative, "unbound" state lower in energy, and thus more favorable. Given sufficient conformational sampling, we proposed that lower-energy models would represent the alternative structure. Here we demonstrate a method for predicting alternative structures of allosteric and non-allosteric proteins with an accuracy of 1 Ã... root mean square deviation (RMSD) to the experimentally determined structure. In the process of predicting these alternative states, we generated a large set of conformations that map a potential energy landscape. Conformations within this landscape clustered into near-native structures, suggesting these proteins behave like a two-state system. By dividing the energy landscape into the near-native (starting and alternative) and far-from-native structures, we were able to identify residue pairs that predict the structural transitions in switching between conformational states. The combination of predicting unknown alternative conformational states and identifying important contact pairs that drive these conformational changes presents an important advance in computational modeling of allosteric proteins.

Platform T: Muscle Mechanics & Ultrastructure

903-Plat Force depression in single myofibrils

Venus Joumaa, Walter Herzog

University of Calgary - Human Performance Laboratory, Calgary, AB, Canada.

Introduction

It is well accepted that the steady-state isometric force following shortening of an activated muscle is smaller than the corresponding steady-state force obtained for a purely isometric contraction at the corresponding length. This phenomenon is referred to as force depression. The mechanism underlying force depression is not well understood. However, it has been suggested that a decrease in the number of attached cross-bridges might be responsible for the loss of force after shortening. In this study, we wanted to gain further insight into force depression by testing whether force depression occurs in single myofibrils and whether force depression is associated with a decrease in myofibril's stiffness.

Materials and Methods

Myofibrils (n=11) were activated at an average sarcomere length of 2.8μm and then shortened to an average sarcomere length of 2.4μm. In order to measure the stiffness of activated myofibrils after shortening, a quick stretch-release cycle was imposed to myofibrils before deactivation. Myofibrils were then reactivated at the sarcomere length of 2.4μm in order to obtain the isometric reference force and associated stiffness.

Results and discussion

Shortening of myofibrils produced force depression in all eleven myofibrils of (mean±SEM) 30.9±3.9% of the reference force. Furthermore, there was a decrease in stiffness after shortening of 30.2%. We conclude from these results that force depression is a sarcomeric property and that it is caused exclusively by a decrease in the proportion of attached cross-bridges, rather than a decrease in the force per cross-bridge. The mechanisms responsible for the shortening-induced decrease in the proportion of attached cross-bridges remain unknown. At present, we speculate that shortening causes an increase in the rate of cross-bridge attachment, thereby reducing the duty ratio in a shortening-magnitude and force dependent way.

904-Plat Critical Sarcomere Length Extension And Phase Transition Of Force During Lengthening Of Skeletal Muscle Myofibrils

Dilson E. Rassier

McGill University, Montreal, QC, Canada.

When skeletal muscle is stretched while activated, force increases in two phases. The purposes of this study were to evaluate the critical stretch for attaining the force transition in individual sarcomeres, and to test the hypothesis that stretch forces are produced largely by pre-powerstroke cross-bridges. Rabbit psoas myofibrils were attached between a pair of microfabricated cantilevers and a glass needle connected to a motor arm, activated, and stretched (~5% sarcomere length) at velocities ranging between 0.25 to 5.5 $\mu m \cdot sec^{-1}$ (~0.10 to 2.4 $L_o \bullet sec^{-1}$). Sarcomere length showed significantly dispersion at rest and activation (maximal dispersions of 35.4 nm and 76.0 nm, respectively), which increased at the transition point (84.2 nm) and reached the maximum at the end and just following stretch (131.9 nm and 159.6 nm, respectively). When stretch was performed at 0.5 $\mu m \cdot sec^{-1}$, the transition between the two phases occurred at an average critical stretch (SLc) of 7.0 ± 0.6 nm/half sarcomere, but the critical stretch varied considerably among sarcomeres (from 2 to 14 nm/half sarcomere). The force attained at the critical stretch (Fc) was 1.69 ± 0.24 times the isometric force produced before stretch. Stretch velocity did not affect SLc, but the F_c increased with increasing velocities up to 2.0 μm/s. 2,3-Butanedione monoxime (BDM), which biases cross-bridges into pre-powerstroke states, did not significantly change the SLc (8.5 ± 0.3 nm/half sarcomere). BDM decreased the isometric force to 21.45 ± 9.22% of the isometric force, but it increased the relative F_c to 2.21 ± 0.34 times the isometric force (at 0.5 $\mu m \bullet sec^{-1}$), suggesting that pre-powerstroke cross-bridges contribute to the stretch forces.

905-Plat Atomic Force Microscopy Reveals Details of Myofibril Architecture

Patrick Lang, Anika Grützner, Wolfgang A. Linke

University of Muenster, Muenster, Germany.

Atomic Force Microscopy (AFM) was used to study the surface topology of isolated myofibrils in physiological salt buffer. Topographical images were recorded from myofibrils of rabbit skeletal muscles (psoas and soleus) and human non-failing hearts. Z-discs, M-bands, A-bands and I-bands were clearly observed and myosin filaments were readily detectable on the myofibrillar surface. The measured lateral inter-filament spacing correlated well with previous estimates from X-ray diffraction studies. The most frequently detected values of thick-to-thick filament spacing were 48, 81, and 120 nm, translating into a $d_{1.0}$ spacing of 40.3 nm. Since the lateral thick-filament spacing has been suggested to determine the sarcomere-length dependence of active force generation in muscle (the molecular basis of the Frank-Starling law in heart), AFM analyses of the thick filament-lattice spacing may provide new insights into this phenomenon at the level of the single myofibril.

AFM was also applied to visualize the binding of an antibody to a myofibrillar protein. Antibodies directed against various immunoglobulin domains of the giant elastic protein titin appeared at the predicted epitope location in the I-band region, suggesting the method can be explored as a novel tool to study sarcomere ultrastructure under physiological buffer conditions at high spatial resolution.

906-Plat Studies of the Conformation of A-band Titin

Larissa Tskhovrebova[1], G Nasir Khan[1], Andrew Baron[1], J GÃ¼nter Grossmann[2], John Trinick[1]

[1] *Leeds University, Leeds, United Kingdom*
[2] *STFC Daresbury Laboratory, Warrington, United Kingdom.*

Titin plays important roles in muscle assembly, intracellular signalling and passive mechanical properties. The titin molecule is >1 micron long and is formed by a single ~3 MDa polypeptide folded into a chain of ~300 immunoglobulin (Ig) and fibronectin (Fn3) domains. *In situ* more than half the molecule is bound to the thick (myosin) filament and in this region the domains are arranged in periodic patterns or 'super-repeats', which correlate with the arrangement of the other thick filament proteins, myosin and C-protein. The large super-repeat contains 11 Ig and Fn domains and is repeated eleven times; thus the entire large super-repeat region contains 121 domains and is ~0.5 micron long. To determine the conformation in this region, we have expressed a set of two- and three-domain overlapping constructs spanning a single super-repeat from human titin. We have studied the structure and self-association of these by circular dichroism, analytical centrifugation and synchrotron X-ray solution scattering. Circular dichroism showed that the constructs contain mainly β-structure and no α-helix, consistent with the expectation for Ig- and Fn3-like domains. Analytical ultracentrifugation indicates that the constructs are elongated and have little tendency to self-associate at physiological ionic strength. Low resolution models of the constructs obtained by fitting the X-ray scattering curves suggest that they are also slightly twisted along their long axes.

907-Plat Electron Microscopic Recording of Cross-bridge Preparatory Stroke in Living Muscle Thick Filaments Using the Gass Environmental Chamber

Haruo Sugi[1], Hiroyoshi Minoda[2], Yuri Inayoshi[2], Fumiaki Yumoto[3], Takuya Miyakawa[3], Masaru Tanokura[3]

[1] *Teikyo University, Niizashi, Saitamaken, Japan*
[2] *Tokyo University of Agriculture and Technology, Kodairashi, Tokyo, Japan*
[3] *University of Tokyo, Bunkyo-ku, Tokyo, Japan.*

The most straightforward way to investigate the cross-bridge movement producing myofilament sliding in muscle is to record ATP-induced cross-bridge movement in living muscle thick filaments, using the gas environmental chamber with which biomolecules are kept in wet, living state in the electron microscope. Large bipolar thick filaments were prepared by slowly polymerizing rabbit skeletal muscle myosin, and the cross-bridges were position-marked with gold particles(diameter, 20nm). To avoid electron beam damage to the specimen, total incident electron dose was limited below 10^{-5}C/cm^2. ATP application to the specimen was made iontophoretically. The specimen (magnification, 10,000X) was recorded on the imaging plate (exposure time, 100ms). Methods to determine the cross-bridge position were identical with those of Sugi *et al.* (PNAS,*94*,4378,1997). Without ATP application, the cross-bridge position did not change appreciably, indicating that, despite thermal motion, the time-averaged cross-bridge position remained almost unchanged. In response to applied ATP, the cross-bridges moved by 5-8nm parallel to the filament axis in a reversible manner. At both sides of the filament bare region, across which the cross-bridge polarity is reversed, the cross-bridges were found to move away from, but not towards, the bare region. Since the experimental system does not contain thin filaments, the above finding constitutes the first direct demonstration of the cross-bridge preparatory stroke, having the amplitude identical with, and the direction opposite to, those of the cross-bridge power stroke. We emphasize that our work opens a new research field, in which structural changes of living biomolecules associated with their function can be studied electron microscopically.

908-Plat Phylogenic and Functional Analysis of the Myosin Light Chain Amino Terminal Extensions

David W. Maughan[1], Mark S. Miller[1], Felipe N. Soto-Adames[1], Yuan Wang[1], Jeff Robbins[2], Jim O. Vigoreaux[1]

[1] *University of Vermont, Burlington, VT, USA*
[2] *University of Cincinnati, Cincinnati, OH, USA.*

The myosin light chains in many species have amino-terminal extensions with similar amino acid motifs, including lysine clusters near the terminus that are involved in thin filament binding, and repetitive proline-alanine sequences in the bridging arm. Parsimony analysis of amino acid sequences obtained from the EST database in GenBank (www.ncbi.nlm.nih.gov/dbEST) indicates that N-termi-

nal extensions (NTE) evolved independently in several branches of two major metazoan lineages, Protostomes and Deuterostomes. In some Protostomes (insects and flatworms) the regulatory light chains (RLC) have the NTE; in Deuterostomes (chordates and other phyla) only the essential light chains (ELC) of vertebrates have the NTE. Mechanical studies of insect (*Drosophila*) indirect flight muscle and vertebrate (mouse) myocardial strips, conducted at *in vivo* lattice spacing, indicate the NTE modifies cross-bridge kinetics. In flies, genetic deletion of amino acids 2–46 of the RLC NTE reduces the frequency of optimum cross-bridge oscillatory power. The reduced frequency can be modeled as a decrease in forward rate constant of the power stroke, resulting in a reduced number of force-generating myosin heads. In mice, genetic deletion of amino acids 5–14 of the ELC NTE increases the characteristic frequency of cross-bridge work production but reduces isometric tension. The frequency increase can be modeled as an increase in reverse rate constant of the power stroke, resulting in a reduction in both the number of myosin heads bound and generated force, thereby leading to a slightly hypertrophic heart as a compensatory response to reduced force. Both RLC and ELC phenotypes suggest the primary role of the NTE is to promote the formation of force-producing cross-bridges. The absence of structural abnormalities in the mutants is consistent with the emergence of the extensions being associated with fine tuning of the contraction kinetics.

909-Plat A Population Of Smooth Muscle Myosin With One Head Phosphorylated Has Half The Mechanical Activity Of Doubly Phosphorylated Myosin

Sam Walcott, Patricia M. Fagnant, Kathleen M. Trybus, David M. Warshaw

University of Vermont, Burlington, VT, USA.

Smooth muscle myosin is fully active when the regulatory light chains on each of its two heads are phosphorylated (2P), and fully inhibited and in a folded conformation when both heads are unphosphorylated (0P). The inhibited state involves an asymmetric interaction between the two heads. Is phosphorylation of a single head (1P) sufficient to disrupt the inactive conformation and allow both the phosphorylated and unphosphorylated heads to be mechanically active? Using smooth muscle heavy-meromyosin (HMM) engineered to be exclusively 1P, we compared the mechanical activity of 1P to 2P HMM. We determined actin filament velocity in the motility assay under limiting myosin conditions, where velocity depends on the number of myosin molecules interacting with the actin filament. Then, using the optical trap, we compared force-velocity relationships for small ensembles of 1P and 2P HMM. In both assays, the 1P was slower ($p<0.001$) than the 2P HMM at equal concentrations. However, by doubling the concentration of 1P HMM, the 1P mechanical activity equaled that of the 2P HMM. To test whether both heads in the 1P HMM are partially active, we performed motility at saturating myosin concentrations and measured step-size and attachment time for single molecules in the optical trap. The 1P HMM had velocity (0.6 um/s), step-sizes (11 nm), and attachment times at 10 uM ATP (90 ms) indistinguishable from the 2P HMM, indicating that the active heads in the two preparations have similar mechanics and kinetics. We conclude that a population of 1P HMM has half the mechanical activity of 2P HMM, a result that could be explained either by an equilibrium between fully active and fully inhibited molecules, or by each molecule having only one mechanically active head.

910-Plat An In Vitro Model System For Determining Regulatory Mechanisms Of Smooth Muscle Mechanics

Feng Hong[1], David P. Wilson[2], Michael P. Walsh[3], Christine R. Cremo[1], Josh E. Baker[1]

[1] *University of Nevada School of Medicine, Reno, NV, USA*
[2] *University of Adelaide, Adelaide, Australia*
[3] *University of Calgary, Calgary, AB, Canada.*

The goal of this work is to develop an in vitro, regulated smooth muscle system for studying the mechanisms by which smooth muscle mechanics are modulated. Here we have developed a reconstituted, Ca2+-sensitive smooth muscle system incorporating physiological ratios (at least 20:1) of smooth muscle myosin (SMM) to myosin light chain kinase (MLCK) on a nitrocellulose-coated coverslip. We show that Ca2+ activates actin motion with a similar pCa observed in vivo. Using a novel approach we show that SMM is phosphorylated on the coverslip, but only in the presence of Ca2+. Phosphorylation was inhibited in the presence of wortmannin, an MLCK inhibitor. Trifluoroperazine (calmodulin [CaM] antagonist) treatment abolished Ca2+-dependent actin motion, which could be reconstituted on the coverslip by adding exogenous MLCK and CaM, but not either protein individually. Pre-treatment of the SMM with a peptide constituting the actin-binding domain of MLCK (which should dissociate MLCK if bound to the minute amount of actin present in the system) did not inhibit Ca2+-activated motion. This suggests that MLCK and CaM together form a tightly-bound, Ca2+-independent complex with SMM, not actin, and act as a functional complex that can be Ca2+-activated to phosphorylate SMM. We provide evidence that the MLCK:CaM:Ca2+ complex phosphorylates one myosin and then diffuses to other myosins to effect further phosphorylation, thus explaining the observed motion at the high ratio of SMM to MLCK.

Platform U: Biotechnology & Bioengineering

911-Plat Spatially Arranged Nonadhesive Surface Domains for Differentially Controlled Cell Adhesion

Peter Krsko[1], Herbert M. Geller[1], Matthew R. Libera[2]

[1] *National Institutes of Health, Bethesda, MD, USA*
[2] *Stevens Institute of Technology, Hoboken, NJ, USA.*

Surface-patterned nanoscopic poly(ethylene glycol) [PEG] hydrogels can promote a wide range of cell responses. In contrast to conventional approaches of direct surface patterning and immobilization of specific adhesion-promoting molecules, the electron-beam patterning of PEG is a relatively simple and flexible technique for fabrication of nanoscopic hydrogels with defined dimensions and crosslink density. Here, we engineered surfaces that are selec-