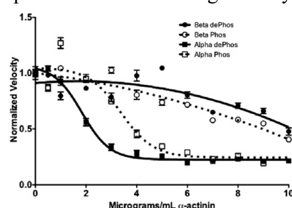
affect of OM on the actin-activated power-stroke. These results show that OM has a direct effect on the power-stroke structural transition that drives force generation in muscle, thus providing structural insight into the mechanism of this new potential therapy for heart failure. Our results also highlight the utility of structure-based time-resolved FRET and EPR assays for the discovery and characterization of allosteric enzyme modulators.

2844-Pos Board B536

Impact of Regulatory Light Chain Phosphorylation on the Stiffness of α and β Myosin

Gerrie P. Farman, **Jeffery R. Moore**.
Boston University, Boston, MA, USA.

It has been demonstrated that the phosphorylation of the RLC can also aid in the attachment of myosin heads to actin. Moreover, it has been demonstrated that phosphorylation of the RLC is altered during heart failure progression. Here we examined the role of the RLC, and its phosphorylation, on the motility of α and β Myosin. We characterized the strain dependent kinetics of both myosins by analyzing the dependence of velocity on load using increasing amounts of α-actinin up to 10 μg/ml. As expected, α-myosin, both phosphorylated and dephosphorylated, had a faster actin sliding velocity than β-myosin over the majority of α-actinin concentrations, while phosphorylation of the β-myosin had no significant impact on the actin sliding velocity. Interestingly, although phosphorylation of α-myosin significantly reduced the unloaded sliding velocity it, increased velocity under loaded conditions (See Figure). These results suggest that the phosphorylation of the RLC acts at the molecular level to stiffen the lever arm of myosin aiding in both myosin head attachment and power stroke.



2845-Pos Board B537

The Effect of Myosin Regulatory Light Chain Phosphorylation on N47K Mutant Myosin Mechanics

Anastasia Karabina[1], Priya Muthu[2], Katarzyna Kazmierczak[2], Danuta Szczesna-Cordary[2], Jeffrey Moore[1].
[1]Boston University, Boston, MA, USA, [2]University of Miami, Miami, FL, USA.

Familial Hypertrophic Cardiomyopathy (FHC) is characterized by left ventricular hypertrophy that can often be preceded by diastolic dysfunction. The clinical presentation of the disease varies from asymptomatic to progressive heart failure to sudden cardiac death. FHC is caused by mutations in genes that encode for all major sarcomeric proteins. There are 12 known FHC-linked mutations in the myosin regulatory light chain (RLC). The RLC mechanically stabilizes the myosin lever arm, which is crucial to myosin's ability to transmit contractile force. Two FHC mutations, N47K and R58Q, located in the RLC Ca^{2+}-Mg^{2+} site have previously been shown to reduce actin filament velocity under load, stemming from a more compliant lever arm (Greenberg et al., PNAS add details 2010). In contrast, phosphorylation of the RLC can impart stiffness to the myosin lever arm. We hypothesized that phosphorylation of the N47K-RLC may mitigate distinct mutation-induced structural and functional abnormalities. To generate mutant β-myosin, native pig RLC was depleted from porcine cardiac myosin heavy chain and reconstituted with mutant N47K or wild-type human RLC. In the work presented here, *in vitro* motility assays were utilized to investigate the effects of RLC phosphorylation on the N47K-RLC mutant phenotype in the presence of an α-actinin frictional load. Consistent with previous findings, myosin bearing the N47K mutation reduced actin sliding velocity compared to WT when incubated with α-actinin, resulting in a 25% reduction in force production. Phosphorylation of N47K mutant myosin increased sliding velocity and restored force production to WT values. These results point to RLC phosphorylation as a potential target to ameliorate the FHC RLC phenotype at the molecular level. Supported by AHA-12PRE11910009 (AK), 10POST3420009 (PM), NIH- HL071778 & HL108343 (DSC) and HL077280 (JM).

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Ventricular Myosin Modifies in Vitro Step-Size When Phosphorylated

Yihua Wang, Katalin Ajtai, Thomas P. Burghardt.
Mayo Clinic, Rochester, MN, USA.

Cardiac and skeletal muscle myosins have the central role in contraction transducing ATP free energy into the mechanical work of moving actin in transduction/mechanical coupling. Inheritable cardiomyopathies are more frequently linked to myosin mutations than other sarcomeric proteins. Hereditary skeletal myopathies linked to myosin are less common. They lead to muscle weakness or affect myosin isoforms expressed during development leading to arthrogryposis syndromes. Myosin has a motor domain containing ATP and actin binding sites and light chains stabilized lever-arm that undergoes rotation impelling bound actin. The lever-arm converts torque generated in the motor into linear displacement (step-size). Relative myosin and actin filament sliding is modeled in vitro with a motility assay quantitating actin filament translation over a myosin coated surface. A novel quantum dot super-resolution in vitro motility assay confirmed a 5 nm step-size for fast skeletal myosin while β cardiac myosin (βMys) had multiple unitary steps, most frequently 5 and 8 nm, and a rare 3 nm displacement. The myosin lever-arm is stabilized by bound essential and regulatory light chains (ELC and RLC). RLC phosphorylation at S15 is linked to modified lever-arm mechanical characteristics contributing to disease and to myosin filament based contraction regulation. We have studied the effect of RLC phosphorylation on the step-size of porcine βMys. Phosphorylated βMys has ~85% of the myosin phosphorylated. We find RLC phosphorylation causing the distribution of longest step increasing from 37% to 71%. This dramatic re-distribution of step-sizes provides significant gain in average step-size. The results indicate a mechanism for contraction regulation by step-size adaptation using post-translational modification of the myosin filament via RLC phosphorylation. Research supported by R01AR049277, R01HL095572 and the Mayo Foundation.

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A13T Mutation in the Regulatory Light Chain Associated with Cardiac Hypertrophy Imposes Differences in Kinetics of Healthy and Diseased Ventricles

Janhavi Nagwekar[1], Divya Duggal[1], Krishna Midde[2], Priya Muthu[3], W. Huang[3], Rafal Fudala[1], Ignacy Gryczynski[1], Zygmunt Gryczynski[1], Danuta Szczesna-Cordary[3], Julian Borejdo[1].
[1]University of North Texas Health Science Center (UNTHSC), Fort Worth, TX, USA, [2]University of San Diego, San Diego, CA, USA, [3]Miller School of Medicine, University of Miami, Miami, FL, USA.

The effect of A13T (alanine to threonine) hypertrophic cardiomyopathy mutation in the myosin regulatory light chain (RLC) was examined in working ex-vivo myofibrils from the hearts of transgenic (Tg) mice. Myofibrillar actin was labeled with a fluorescent dye. A small volume within the I-band (?1 fL), containing on average 3-4 actin molecules, was observed by confocal microscopy. The myofibrils were cross-linked with EDC [1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide] to prevent shortening during muscle contraction. Working myosin cross-bridges cause actin to undergo the cyclic fluctuations of orientation, which were measured by recording the polarization of fluorescent light emitted by the actin-bound fluorophore. The autocorrelation function of fluctuations of polarized fluorescence contains information about the kinetics of motion, which were found to be very different for left vs. right ventricles. The center of distribution of orientations of transition dipoles during contraction were very different for the Wild Type (WT) and mutated (MUT) ventricles, but their skewness and kurtosis were the same. The distribution of orientations measured in contracting WT myofibrils could be fitted by at least two Gaussians reflecting a pre- and post power stroke states of the myosin cross-bridges. However, the distribution of MUT myofibrils showed only one Gaussian relationship suggesting that the hypertrophic phenotype associated with the A13T-RLC mutation might be characterized by a loss of the pre-power stroke state.

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The K104E Mutation of the Myosin Regulatory Light Chain Alters Kinetics and Distribution of Orientations of Cross-Bridges in Transgenic Cardiac Myofibrils

Divya Duggal[1], Janhavi Nagwekar[1], Ryan Rich[1], W. Huang[2], Krishna Midde[1], Rafal Fudala[1], Ignacy Gryczynski[1], D. Szczesna-Cordary[2], Julian Borejdo[1].
[1]UNT Health Science Center, Fort Worth, TX, USA, [2]University of Miami, Miller School of Medicine, Miami, FL, USA.

Cross-bridge (XB) kinetics and the degree of order were examined in contracting myofibrils from the ex-vivo left ventricles of transgenic (Tg) mice expressing Familial Hypertrophic Cardiomyopathy (FHC) Regulatory Light Chain (RLC) mutation K104E. Since the kinetics and degree of order are best studied when an individual XB makes a significant contribution to the