Peptide Modulation of Myosin Coiled Coil Stability Monitored with Optical and Force Spectroscopy
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The structural impact of hypertrophic cardiomyopathy mutations on the myosin subfragment-2 are investigated including the coiled coil’s susceptibility to force and alpha-helical content. The gravitational force spectrometer can measure the reversible unfolding of the myosin coiled coil under piconewton and subpiconewton forces. This exquisite sensitivity has enabled the measurement of the four transitions associated with (1) calcium binding of cardiac myosin subfragment-2 and the effects of a highly lethal form of hypertrophic cardiomyopathy mutation (delE930). Because each one of the two synthetic peptides of the coiled coil are covalently attached to opposing surfaces before forming the coiled coil, it is possible to investigate heterodimers as well as homodimers containing hypertrophic cardiomyopathy mutations. Initial results indicate that an order of magnitude more force is required to unfold the wild type sequence compared to the homodimer while the heterodimer needs only about twice as much force as the homodimer to unfold. The shapes of the reversible force distance curves are similar in all three conditions. Furthermore, the addition of a short positively charged poly-D-lysine peptide that can bind to the negatively charged glutamate side chains on the peptides strongly increased the amount of required force for unfolding myosin. Similar results were obtained by optical spectroscopy of the alpha-helical content of myosin synthetic peptides. Based on changes in peptide bond absorbance between the random coil and alpha helix, it is estimated that the delE930 mutation decreased alpha helical content relative to wild type. The addition of poly-D-lysine peptide increased the alpha helical content of both wild type and mutant peptides. These results suggest that it might be possible to use targeted synthetic peptides to reverse some structural effects of certain hypertrophic cardiomyopathy mutations.

Enhanced Troponin-I Binding Explains the Functional Changes Produced by the Hypertrophic Cardiomyopathy A8V Mutation of Cardiac Troponin-C
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Our modeling supports a simple molecular mechanism underlying functional changes in hearts that carry the hypertrophic cardiomyopathy A8V mutation of troponin-C (TnC\textsuperscript{A8V}). Hypertrophy is commonly observed in hearts carrying sarcomeric protein mutations that render the regulatory mechanism more sensitive to Ca\textsuperscript{2+}. Indeed, cardiac muscle fibers reconstituted with TnC\textsuperscript{A8V} require ~2.3 fold less [Ca\textsuperscript{2+}]\textsubscript{35} to achieve 50% maximum tension compared with controls. In the tertiary structure of TnC\textsubscript{c} alanine 8 is a conserved residue located in the N-helix situated between the EF hand that binds Ca\textsuperscript{2+} and the hydrophilic patch that binds the switch peptide of troponin-I (Tn\textsubscript{I}). In binding assays, Ca\textsuperscript{2+} and Tn\textsubscript{P} each have higher affinity for TnC\textsuperscript{A8V}, i.e. ~1.7 and ~1.6 fold higher respectively compared with control. But, because the largest difference in Ca\textsuperscript{2+} affinity is measured only when Tn\textsubscript{I} is reconstituted with the other thin filament proteins and myosin heads, we model the mutation as having no direct effect on Ca\textsuperscript{2+} coordination. Given the affinity for Tn\textsubscript{P} as the only adjustable parameter, we obtain the best fits of the tension data if affinity for Tn\textsubscript{P} is made ~1.5 fold higher for TnC\textsuperscript{A8V} compared with control, which coincides with the binding measurement. We also report reconstituted myofilament ATPase assays that support the hypothesis of stronger peptide binding when the Ca\textsuperscript{2+} concentration is high, although the predicted enhancement at low Ca\textsuperscript{2+} was absent or too small to measure. Possible subtle effects of the mutation on Ca\textsuperscript{2+} coordination notwithstanding, results of modeling suggest that the functional phenotype of the A8V mutation is a consequence of altered interaction between TnI\textsuperscript{P} and TnC\textsuperscript{A8V}. Supported by NIH HL103840 (JRP)

Steady-State Predictions from a Compact Cooperative Kinetic Model of Cardiac Sarcomere Dynamics
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A compact model of cardiac sarcomere dynamics is useful when developing integrated models of whole ventricular function. Kinetic models can be simplified by separating slower from faster processes. The representation of faster processes can then be reduced to their equivalent equilibrium relationships. Analysis of cooperativity within a cardiac sarcomere is also aided by dealing with equilibria, which can be analyzed using statistical thermodynamics. These principles guided the formulation of a compact kinetic model of cardiac sarcomere dynamics. In this scheme there were three slower processes: (1) reversible transitions between weak and strong crossbridges, (2) detachment of strong crossbridges, (3) dissociation of calcium from regulatory units (RUs) having a strong crossbridge. In the overall kinetic scheme, an individual RU could exist in 6 states: 3 states of crossbridge binding (none, weak, strong) interacting with 2 states of calcium binding (yes, no). Exchanges between the 4 states without strong crossbridges occurred by rapid equilibria. Thus, in the reduced model only 3 kinetic states remained: (1) strong crossbridges attached to RUs where calcium was bound, (2) strong crossbridges remaining attached even after calcium dissociation, (3) the equilibrium mixture of the 4 remaining states. Since strong crossbridges appear to act independently, rate constants between these 3 states were considered independent of neighboring RUs. However, cooperativity between neighboring RUs exerted major impacts on the mixture of 4 states interacting rapidly as equilibria. Applying the linear Ising Model of statistical thermodynamics, the grand partition function characterizing interactions between neighboring RUs included activating effects from strong crossbridges as well as free calcium ions. This model predicted steady-state relationships between force and pCa (given as Hill plots) that reproduced the experimentally observed bend toward less cooperative slope near half activation.

Serine 61 Phosphorylation Rescues Cardiomyopathic Effects of Tropomyosin Mutation
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Point mutations found on myofilibrillar proteins have been shown to affect muscle contractility and lead to cardiomyopathies and skeletal muscle disease syndromes of varying severity. Over 30 mutations localize to residues on the acto-tropomyosin interface and modify the actin-tropomyosin energy landscape, influence tropomyosin positioning on actin, and perturb allosteric cooperative between actin, tropomyosin, troponin and myosin. Here, energy landscape computation, in combination with known actin-tropomyosin sequence and structural information was used prospectively to identify potential effects of post-translational modifications generated to rescue regulatory imbalances. For instance, our interaction energy calculations show that HCM-associated E62Q tropomyosin mutation weakens residue-residue specific actin-tropomyosin binding. We then predicted that phosphorylation of neighboring S61 would rescue the deficit, which was corroborated by further energy landscape determination. To validate these in silico results experimentally, the sliding velocity of tropomyosin-troponin decorated thin filaments was measured as a function of added calcium for thin filaments containing the E62Q, phosphomimetic S61D and E62Q-S61D mutant tropomyosins. In vitro motility assays showed actin-based sliding velocity for actin-tropomyosin in the E62Q mutant requires lower Ca\textsuperscript{2+} to fully activate troponin-tropomyosin regulated mutant filaments when compared to activation of wild-type filaments (as expected from earlier reports based on acto-S1 ATPase work). In contrast, the double mutant E62Q-S61D restores Ca\textsuperscript{2+}-sensitivity toward normal while slightly reducing sliding velocity. Thus, the shift in Ca\textsuperscript{2+} -sensitivity by E62Q and subsequent reversal by S61D parallel the blocked-state phosphorylation-dependent stabilization of tropomyosin-tropomyosin interactions. Therefore, a decrease in Ca\textsuperscript{2+} -sensitivity produced by the single mutant S61D alone likely resulted from blocked site stabilization. Hence, shifts observed in pCa50 for the mutant tropomyosins could be accurately predicted by in silico calculation of