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 states: (1) the equilibrium mixture of chemical states of cardiac myosin subfragment-2 and the effects of a highly lethal form of hypertrophic cardiomyopathy mutation (del930). 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 uncoiling 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 del930 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 TropoI-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 tropoI-I (TnC(A8V)). Hypertrophy is commonly observed in hearts carrying the 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 uncoiling 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 del930 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.

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 the 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 myofibrillar 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 actin-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 associated with E62Q mutant tropomyosin required lower Ca2+ to fully activate troponin-troponin 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 Ca2+-sensitivity toward normal while slightly reducing sliding velocity. Thus, the shift in Ca2+-sensitivity by E62Q and subsequent reversal by S61D parallel the blocked-state phosphorylation-dependent stabilization of the actin-tropomyosin interface. Likewise, a decrease in Ca2+-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