

C98S) in a cardiac TnI core structure (McTnI-ND₂₉-Cys) did not affect the COOH-terminal conformation of TnI and preserved binding to TnT and TnC. McTnI-ND₂₉-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, Ca²⁺-titrations were performed for fluorescence intensity and polarization changes. The results demonstrated Ca²⁺ regulated conformational/environmental changes as well as flexibility change in the COOH terminus of TnI. Further experiments are performed to measure the Ca²⁺-induced structural changes in reconstituted myofilaments to understand the function of TnI COOH terminal domain in calcium-regulation of muscle contraction.

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Cardiomyopathy Causing Mutations Stabilize an Intermediate State of Thin Filaments

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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 $\Delta 14$ 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

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

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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 relies 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 polymerization process. Preliminary results will be presented.