Dynamics of the troponin molecular switch in the thin filament using SDSL-EPR

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Troponin (Tn) is a heterotrimeric protein comprised of TnC, TnI and TnT subunits, which acts as the molecular switch of striated muscle contraction. The proposed mechanism of this calcium dependent regulation is based on static structural models. The Tn complex interacts *in vivo* with both the Tropomyosin and actin constituents of the thin filament. However, current structural models available of the Tn complex were made with experimental data collected in the absence of these two important binding partners. Site-Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR) is a technique that can be used to study protein structure and dynamics. It requires the insertion of cysteine residues into the protein backbone onto which spin labels can be specifically attached. Spin labels provide local environment structural and dynamic information. The advantage of SDSL-EPR over other techniques in the analysis of Tn is that binding partners Tropomyosin and actin can be included in the investigation. Additional advantages are that measurements can be made at physiological conditions allowing the dynamics of the Tn complex to be monitored in both regulation states, i.e. in the presence and absence of Ca^{2+}. A series of single cysteine substitution mutants, labeled with the nitroxide spin label MTSSL (1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl-methanethiosulfonate) were constructed to explore the dynamics of four functionally significant regions of the cardiac TnI subunit. These regions include the TnI structural core (Iso132Cys), the 'primary inhibitory peptide' (Leu144Cys), the 'switch peptide' (Ala151Cys) and the controversial second actin-binding domain termed the 'mobile domain' (Gln175 to Arg186, each substituted to Cys in turn). The current model suggests that there should be significant domain movement observed in the selected regions with the exemption of the structural core. EPR mobility experiments demonstrate these Tn domain movements in the thin filament, as proposed from the static models.