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Effects of Disease Causing Point Mutations on Actin's Resilience

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We have used spectroscopic probes to determine the structural dynamics of actin, as affected by point mutations that are known to cause Duchenne muscular dystrophy (DMD). DMD is caused by mutations within the cytoskeletal protein dystrophin. The present study focuses on single amino acid replacements in the N-terminal actin-binding domain (DysABD1), which result in muscular dystrophies with a wide spectrum of severities. Though these point mutations occur in ABD1, the affinity of dystrophin for actin is not substantially perturbed (Henderson et al., PNAS 2010). However, affinity is only one facet of dystrophin's interaction with actin. We have previously shown, using transient phosphorescence anisotropy (TPA), that binding of dystrophin to actin increases its resilience, and we hypothesize that this cytoskeletal resilience is needed to act as a mechanical buffer during contractions. Previous results have shown a correlation between the TPA-measured resilience and the physiological measures of muscle mechanical function in mice (Lin et al., J. Mol Biol 2012). In this project, we expand this area of research by testing several known disease-causing point mutations in DysABD1 and evaluate their effects on actin's resilience. We hypothesize that while affinity is not significantly affected, mutant full length dystrophin loses its natural ability to increase the resilience in actin as determined from TPA. Our findings will provide insight into the pathophysiology of these disease-causing mutations - why some mutations are more devastating than others, and why a single amino acid change can have a large impact on dystrophin's function.

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Large Scale Opening of the N Terminal Actin-Binding Domain of Dystrophin Detected by Dipolar Electron-Electron Resonance (DEER)

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We have used dipolar electron-electron resonance (DEER) to observe the structural dynamics of the calponin homology (CH) domains within the N-terminal actin binding domain (ABD1) of dystrophin, in order to determine the conformation of the ABD1 while free in solution and while actin-bound. Loss of functional dystrophin causes Duchenne (DMD) and Becker (BMD) muscular dystrophies, prompting a need for structural information about dystrophin to aid in engineering gene therapy constructs. We have previously shown that the ABD1 of dystrophin's autosomal homologue utrophin opens upon actin binding via an induced fit mechanism, suggesting a similar mechanism for the dystrophin ABD1. Site-directed spin labeling and DEER were used to measure the distance between labels on the two CH domains, in the presence or absence of actin and at varying ionic strength. DEER spectra showed that like utrophin, the actin-bound dystrophin ABD1 occupied a single well-ordered conformation in which the CH domains were much more open than when the ABD1 is free in solution. Unlike utrophin, the dystrophin ABD1 was much more disordered when free in solution, an effect that was exacerbated by increasing the ionic strength. We conclude that, similarly to utrophin, the dystrophin ABD1 undergoes a closed-to-open transition upon binding to actin. In contrast to utrophin, the dystrophin ABD1 is much more disordered when not bound to actin, suggesting that dystrophin is intrinsically less stable than utrophin. These results provide the needed structural foundation for studying disease-causing mutations in ABD1, and they provide a rationale for using utrophin in place of dystrophin in DMD therapy.

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Structural Dynamics of Actin during Active Interaction with Myosin depends on the Isoform of the Essential Light Chain

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Muscle contraction results from cyclic interaction of myosin and actin, driven by ATP hydrolysis, where the actomyosin complex undergoes transitions between weakly and strongly bound structural states. Rabbit skeletal muscle myosin has two isoforms of the essential light chain (ELC), designated A1 and A2. These isoforms have significant effects on myosin's interaction with actin, resulting in higher catalytic efficiency for A1 but higher in vitro motility and muscle shortening velocity for A2. In order to understand the structural basis of these differences, we have used time-resolved phosphorescence anisotropy (TPA) to investigate the effects of ELC isoforms on the microsecond dynamics of actin during interaction with skeletal muscle myosin in the absence and presence of saturating ATP. Actin was labeled with a phosphorescent probe at C374, and the myosin head (S1) was separated into isoenzymes containing A1 (S1A1) and A2 (S1A2) by ion-exchange chromatography. As previously reported, S1A1 exhibited substantially lower ATPase activity at saturating actin but substantially higher apparent actin affinity, resulting in higher catalytic efficiency. In the absence of ATP, each isoenzyme increased actin's final anisotropy similarly, indicating similar restriction of the amplitude of intrafilament rotational motions in the strong-binding (rigor) state of actomyosin. In contrast, in the presence of saturating ATP, the final anisotropy of actin was substantially higher during interaction with S1A1 than with S1A2. Thus, S1A1 was more effective in restricting actin dynamics during the active interaction of actin and myosin, suggesting greater population of the strong-binding state of actomyosin. We conclude that ELC of skeletal muscle myosin modulates strong-to-weak structural transitions during the actomyosin ATPase cycle in an isoform-dependent manner, and thus modulates the contractile function of actomyosin.

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A Small Chemical Mimicking Actin Binding to Myosin

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We synthesized TRA, a potent inhibitor to skeletal myosin, and its analogues, and investigated the effect of TRA on function and structure of skeletal myosin. ATPase assays using TRA and its analogues showed that long alkyl chain and carboxyl group of the TRA structure was indispensable for the inhibitory effect and the interaction with myosin structure. TRA activated the Mg^{2+}-ATPase activity and decreased the amount of M.ADP.Pi formation of myosin. Myosin head did not bind actin filament in the presence of TRA. Fluorescence measurements and circular dichroism data reflected that specific structural change of myosin heads occurred while the myosin Mg^{2+}-ATPase activity was activated in the presence of TRA. All these results support the idea that TRA binds myosin heads and induce the actin bound state of myosin heads. TRA may be a useful chemical to mimic the actin bound state of muscle myosin. We also studied a possible binding site of TRA on the myosin structure by using computational modeling.

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High-Resolution EPR of a Bifunctional Spin Label Reveals Structural Transitions within Myosin's Catalytic Domain

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We present a method for obtaining high-resolution information on protein backbone structure and dynamics using electron paramagnetic resonance (EPR) of a bifunctional spin label (BSL) and molecular modeling. Two complementary EPR techniques were employed to measure protein orientation (conventional EPR) and intra-protein distances (dipolar electron-electron resonance, DEER). BSL attaches at Cys positions i and i+4 on a helix, greatly reducing probe mobility relative to the peptide backbone, compared to monofunctional labels. Accurate modeling of BSL provides the coordinates required to directly relate spectroscopic data to backbone structure (both orientation and distance), and dynamics (rotational motion). In the current work, the motor protein *Dictyostelium* myosin II was used to demonstrate this approach. We measured nucleotide-dependent structural transitions of two key helices within the myosin catalytic domain (CD). Two double-Cys sites were engineered, with one Cys pair located on the relay helix, and the other on a stable helix in the upper 50kD domain. BSL on a construct with one of these pairs was used to measure myosin orientation relative to oriented actin. BSL on a construct with two pairs was used to measure interprobe distances. The effect of ADP binding on both orientation and distance was clearly detected with BSL, but not with a monofunctional label. The significance of this work is twofold: (1) A structural transition in the relay helix upon ADP binding was clearly defined with high resolution. (2) BSL spectra demonstrate superior resolution, compared to monofunctional spin labels, making it possible to directly translate spectroscopic data to protein structure and dynamics.

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Cardiac F-Actin has Two Functional States

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Previously, in mouse cardiac myofibrils, we showed that (i) strong cross-bridges (XBs) prevent the exchange of cTn within the A-band and (ii) ATP-induced release of strong XB allowed nearly uniform cTn exchange within the thin filament. Here, we examined the mechanism for XB-dependent