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domain. Myosin-7a unfolds at either high ionic strength or in the absence of ATP, revealing a clearly recognizable motor domain, the lever arm and some features of the tail region. C-terminal truncations were made to determine which portions of the multi-domained tail are necessary for the regulation. Removal of the last 99 amino acids which are highly conserved in all myosin-7a molecules and form a subdomain (termed MyTH7) of the FERM domain, or mutation of two conserved amino acids in this region, is sufficient to prevent folding of the molecule in the presence of ATP and activates the enzymatic activity. A construct consisting of the second FERM domain binds actin in an ATP-insensitive manner with a Kd of 30μM which is similar to the KATPase value for the full length molecule. We propose that at low actin concentration myosin-7a is folded and inactive, but at high actin concentrations such exists in actin bundles, it binds first via its tail binding site which then frees the motor domain to functionally interact with actin.

18-Symp

The Ups and Downs of Smooth Muscle Myosin Regulation

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Since discovery that regulatory light chain (RLC) phosphorylation was the primary method of regulation of smooth and non-muscle myosin II, the structure of the phosphorylated and dephosphorylated forms has been a goal only partially realized. Regulation in both smooth muscle and the related scallop striated muscle myosin requires a 2-headed myosin species; single headed species are unregulated. Thus head to head interactions are key to achieving the inhibited state. A folded conformation of full length myosin, generally referred to as the 10S conformation was discovered early by conventional electron microscopy and in 1999, a structural explanation for the head-head interactions was obtained from 2-D arrays of dephosphorylated smooth muscle heavy meromyosin formed on lipid monolayers and imaged in 3-D by cryoEM. The structure was later confirmed by 2-D arrays of the 10S conformation of whole myosin. While this structure explained inhibition of the solubilized form of regulated myosins, it had not been observed in filaments. Surprisingly, the first observation of smooth muscle myosin-like head-head interactions in a thick filament was obtained from tarantula striated muscle, not from smooth muscle myosin. Even more surprising was the observation of a similar conformation in relaxed cardiac muscle thick filaments. Thus, these head-head interactions observed first in smooth muscle HMM, appear to be ubiquitous in relaxed muscle although still not confirmed for thick filaments in relaxed smooth muscle. Still to be determined is an explanation of the factors that can lead to solubilization and the location of the N-terminus of the RLC, whose location and structure has yet to be revealed, in the phosphorylated and dephosphorylated state. Theoretical modeling has provided possible explanations for several factors that affect regulation but has not yet yielded a coherent theory. Supported by NIAMSD.

19-Symp

Switching Gears with Myosin Binding Protein-C

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Myosin binding protein-C (MyBP-C) is a thick-filament protein in vertebrate sarcomeres that limits cross-bridge cycling kinetics and reduces myocyte power output. However, the mechanisms by which MyBP-C influences cross-bridge kinetics are not well understood. The goal of the present study was to investigate the ability of the first 4 N-terminal domains (C0-C1-motif-C2) of cardiac (c) MyBP-C to affect actomyosin interactions and interact with actin. Here we show that recombinant proteins containing the C1 and motif domains increased Ca^{2+} sensitivity of tension and increased rates of tension redevelopment (k_{tr}) at submaximal [Ca^{2+}] in permeabilized rat trabeculae. Proteins containing these domains also biphasically activated then inhibited Ca^{2+}-activated ATPase rates of heavy meromyosin and myosin S1 in solution. Cosedimentation binding assays demonstrated saturable binding of the 4 N-terminal domains to F-actin at a 1:1 molar ratio (Kd ~ 10μM). However, more than one interaction site was indicated by turbidity and electron microscope analyses that showed actin bundling in the presence of recombinant proteins. Phosphorylation of the motif or increasing pH reduced binding to a 1:2 molar ratio and abolished actin bundling. Phosphorylation reduced but did not eliminate effects of recombinant proteins to increase Ca^{2+} sensitivity of tension and k_{tr} at submaximal [Ca^{2+}] in permeabilized trabeculae. Together these results suggest that the N-terminus of cMyBP-C interacts with F-actin through multiple distinct sites, at least one site is modulated by electrostatic charge interactions, and that functional effects of the N-terminus of MyBP-C are mediated in part by phosphorylation independent mechanisms. Supported by NIH HL080367.

Platform A: Protein Conformation

20-Plat

Active Unfolding of Collagen is not Required for Collagenolysis to Occur in Solution

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A number of disorders such as tumor metastasis, arthritis, and atherosclerotic heart disease are related to excessive collagen degradation. Therefore methods that further our understanding of collagenolysis are of particular interest. However, as the collagenase active site is too small to accommodate the triple-helical structure of collagen and the scissile bond within collagen is not accessible to the collagenase catalytic site, the precise molecular mechanism of collagenolysis is unclear. Prior experiments have been interpreted to mean that collagenases actively unfold collagen, in a process that requires intact full length collagenase - which is typically a multi-domain protein, containing both a catalytic and hemopexin-like domain - leading to exposure of the scissile bond. Here we demonstrate that collagen types I and III can be degraded by the catalytic domain alone of either MMP1 or MMP8 at temperatures far below the melting temperature of collagen. These data argue that active unwinding of collagen is not required for collagenolysis to occur in solution. Molecular simulations further suggest that normal thermal fluctuations in the structure of the triple-helical structure of collagen lead to the protein sampling states where the scissile bond is relatively exposed and hence accessible to collagenase active site. Taken together, these data suggest that collagen degradation is the result of the interaction of preformed locally unfolded states of collagen and collagenases, rather than a mechanism that involves active collagenase-mediated unfolding.

21-Plat

Lipid Bilayer Coated Gold Nanoparticles Provide Insight Into Proteins' Conformational Changes

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While nanoparticles have been studied and used for many years and their unique chemical and physical properties have been extensively characterized, nanoparticles are now increasingly used to investigate biological systems. Nanoparticles can be formed on a submicron scale and do not interfere with normal biological processes, thus can be used in an *in vivo* system, allowing researchers to gain insight into its inner workings. The use of nanoparticles has also proven to be quite flexible, and has provided an indispensable tool in the advancement of drug delivery, tissue engineering, and detection of biomolecules. We have previously studied conformational changes occuring within proteins upon binding to phospholipid model membranes, particularly the tumor suppressor protein PTEN. To gain a more in depth characterization of these protein conformation changes, we have developed a technique in which we have fabricated 50nm gold nanoparticles coated with an asymmetric lipid bilayer, in a manner that allows us to control the identity of the lipids making up the outer leaflet. Using Raman spectroscopy, we will take advantage of the surface enhanced Raman spectroscopy (SERS) effect, which will increase PTEN band intensities binding of the protein to the lipid covered nanoparticle. Systematic mutation of tryptophan residues within the protein will allow us to probe the binding induced conformational changes in the vicinity of the tryptophan. While SERS has been used previously to study protein conformational changes, proteins were in these cases directly immobilized onto the nanoparticles. In contrast, our novel approach immobilizes the lipids on the nanoparticles and the protein can freely interact with the target lipid. While we plan to use these methods to examine PTEN, further development of this technique will allow for better study of conformational changes in a myriad of interfacial enzymes.

22-Plat

Extended conformations in alanine peptides

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Spectroscopic evidence for the presence of local order in unfolded proteins, including polyproline II (PII) structure, now appears incontrovertible. The data supporting this order relies on analysis of short chain peptides. The dimensions of unfolded chains nevertheless conform to random coils. We have re-examined the dimensional properties of short chains using paramagnetic proton spin relaxation measurements to evaluate intermediate range distances (r)