Myosin Regulatory Light Chain Phosphorylation and the Production of Functionally Significant Changes in Myosin Head Arrangement on Striated Muscle Thick Filaments

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Previous studies demonstrated that thick filaments isolated from dually regulated striated muscles of tarantula (Craig et al., 1987) and *Limulus* (Levine et al., 1991) respond to incubation with myosin light chain kinase (MLCK), under phosphorylating conditions, by losing the helically ordered arrangement of myosin heads characteristic of the relaxed state. In spider muscle, a shift in the ration of the I_{10}/I_{11} equatorial reflections is also claimed to result from phosphorylation of myosin regulatory light chains (MRLCs) (Padron et al., 1991). Although such a shift is not seen in *Limulus* fibers, MRLC phosphorylation is necessary for thin filament movement by *Limulus* myosin in in vitro motility assays (Wang et al., 1993).

Phosphorylation of MRLC regulates vertebrate smooth muscle and is also a sequela of activation in vertebrate (including mammalian) striated muscles. In rabbit psoas, MRLC phosphorylation causes potentiation of tension development at low levels of calcium (Stull et al., 1990). Conditions that decrease the lattice spacing show that such potentiation of tension is proportional to the distance between thick and thin filaments, and is greatest in fibers with rest-length sarcomeres (Yang et al., 1992). This suggests that MRLC phosphorylation affects the mobility of the myosin heads, allowing them to move away from their relaxed position against the filament backbones and approach the thin filaments (such an effect is less significant at decreased lattice spacing, when the myosin heads are already close to actin). Most recently, we have examined the effect of phosphorylation of MRLCs on the structure of thick filaments separated from rabbit psoas to determine whether they behave in a fashion similar to thick filaments from dually regulated chelicerate arthropod muscles. We incubate filament samples with endogenous MLCK, under conditions of partial and full phosphorylation, using both calcium-dependent and -independent enzyme preparations. Control incubations include: exclusion of calcium from the medium with the calcium-dependent enzyme; introduction of calcium without calmodulin or enzyme; and substitution of an unrelated protein (bovine serum albumin) for the calcium-independent enzyme. Dephosphorylation (using endogenous phosphatase) of some experimental samples is also performed. All incubations are performed at 23°C on samples that are adsorbed to the surface of carbon films on electron microscope grids. Grids are negatively stained and examined and photographed in the electron microscope. Diffraction patterns are obtained from images of filaments on the EM negatives to demonstrate the presence and/or absence of the relaxed helical order of myosin heads and any changes that may occur as a result of MRLC phosphorylation.

Rabbit thick filaments incubated with calcium-dependent MRLC + calmodulin at a pCa of 5.0 for 30 s or 5.8 for 3 min, or with calcium-independent MLCK for 10 min, all appear disordered. In the micrographs, myosin heads extend away from the filaments' backbones for varying distances; diffraction patterns show no evidence of myosin layer lines. Incubations at pCa 5.8 or with calcium-independent MLCK for shorter times produce filaments that appear to be partially disordered: occasional short stretches along some of the filaments show a few features of the helical pattern characteristic of the relaxed state. In all control preparations, thick filaments that are well separated from thin filaments retain the relaxed, near-helical arrangement of myosin heads, and strong myosin layer lines are present in the transforms. Incubation of previously phosphorylated, and thus disordered, thick filaments with endogenous phosphatase restores their relaxed, ordered structure. The myosin helical repeat is present on the filaments, and myosin layer lines reappear in the diffraction patterns. Thick filaments separated from skinned fibers after in situ removal of their MRLCs appear the most disordered: heads extend very far from the filaments' backbones and often appear clumped. X-ray diffraction studies of these phenomena, in skinned fibers, is underway.

Given that MRLC phosphorylation affects the structure of thick filaments separated from both invertebrate dually regulated muscles and thin filament-regulated mammalian striated muscles in a similar fashion, we are exploring the possible relationship of this structural change to phosphorylation-induced alteration of charge in the N-terminal region of the regulatory light chains and/or to the species of myosin·ATP intermediate most heavily populating the active sites.

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