The role of myosin light chain phosphorylation in the regulation of contractile activity

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Muscular contraction and various types of non-muscle cell motility are generated by interactions between myosin and actin. We are interested in the role of the myosin regulatory light chains in regulating such interactions in vertebrate smooth muscle and nonmuscle cells. It is now generally accepted that in these cells, actin–myosin interactions are initiated by a calcium- and calmodulin-dependent kinase which phosphorylates the myosin 20000 Mₘ regulatory light chains (Adelstein & Eisenberg, 1980). It has further been shown that phosphorylation of the regulatory light chains of smooth muscle and nonmuscle myosins also initiates the assembly of these myosins into filaments in vitro (Suzuki et al., 1978; Scholey et al., 1980).

We have concentrated our studies on the effects of phosphorylation of the 20000 Mₘ light chains of myosins isolated from a vertebrate smooth muscle (chicken gizzard) and a non-muscle cell (bovine thymus). These studies on the two types of myosins have been run in parallel to confirm the effects of light chain phosphorylation. The myosins were rapidly prepared to a high level of purity, in high yield and in a non-phosphorylated state. We consistently observed that the interaction of these myosins with actin as measured by the actin-activated Mg²⁺-ATPase activity of the myosins was dependent on the level of phosphorylation of the light chains; for example, in the nonphosphorylated form, the myosins' Mg²⁺-ATPase activity were not activated by actin, whereas when the light chains were phosphorylated, the Mg²⁺-ATPase activity of these myosins were activated approx. 8–10-fold by actin. The light chains thus act as inhibitors of myosin interaction with actin and phosphorylation relieves this inhibition, i.e. the basis of regulation exerted by these light chains is therefore repression–derepression.

Using a variety of techniques, i.e. turbidity measurements, quantitative high speed centrifugation, electron microscopy and dark-field light microscopy, we observed that the stability of thymus and gizzard myosin filaments at approximately physiological conditions was dependent on the level of light chain phosphorylation. In 0.1–0.15 M-NaCl, pH 7.0, approximately stoichiometric levels of Mg²⁺-ATP disassembled these nonphosphorylated myosin filaments into species with sedimentation coefficients of approx. 11 S (myosin monomer in high salt sediments at 65%). It was originally suggested that the 11 S myosin species was a dimer (Suzuki et al., 1978) and might be the antiparallel dimer which had been suggested as the building block for smooth muscle and nonmuscle myosin filaments (Hinsen et al., 1978). It has now been shown however that the 11 S smooth muscle myosin species is monomeric and has a folded conformation (Suzuki et al., 1982; Trybus et al., 1982). We found by rotary shadowing that the 11 S molecules of both gizzard and thymus myosins were folded twice, at the two hinge points approximately one-third and two-thirds of the way along the length of the tail, forming relatively compact structures. The key feature seems to be the binding of a portion of the myosin tail (near the second or distal hinge) to the neck region of the myosin heads, possibly near where the regulatory light chains are thought to be located. When the 20000 Mₘ regulatory light chains were phosphorylated by light chain kinase, calmodulin and Ca²⁺, the myosin tails unfolded to form the conventional extended monomers which were then able to assemble into filaments.

These results demonstrate that in vitro under physiological conditions of ionic strength and Mg²⁺-ATP concentrations, non-phosphorylated vertebrate non-muscle and smooth-muscle myosins are folded monomeric molecules which are prevented by their folded structure from assembling into filaments. Phosphorylation of the 20000 Mₘ light chains not only initiates myosin cross-bridge interaction with actin but also causes the molecules to unfold, producing the extended state which can readily assemble into filaments and thus in combination with the actin filaments form the required 'tension-generating structures'. It remains to be established whether these results have any relevance to the state of myosin assembly in living vertebrate nonmuscle and smooth muscle cells. In nonmuscle cells, where many motile activities occur in different places and at different times during the cell's life cycle, a transient assembly/disassembly of myosin filaments may be an essential requirement for these activities. In living vertebrate smooth muscles, however, it is difficult to envisage the physiological advantage of regulating the assembly/disassembly of myosin filaments during repeated cycles of contraction/relaxation.