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Structural Changes Accompanying Phosphorylation of Tarantula Muscle Myosin Filaments

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Abstract. Electron microscopy has been used to study the structural changes that occur in the myosin filaments of tarantula striated muscle when they are phosphorylated. Myosin filaments in muscle homogenates maintained in relaxing conditions (ATP, EGTA) are found to have nonphosphorylated regulatory light chains as shown by urea/glycerol gel electrophoresis and [32P]phosphate autoradiography. Negative staining reveals an ordered, helical arrangement of crossbridges in these filaments, in which the heads from axially neighboring myosin molecules appear to interact with each other. When the free Ca²⁺ concentration in a homogenate is raised to 10⁻⁴ M, or when a Ca²⁺-active myosin light chain kinase is added at low Ca²⁺ (10⁻⁵ M), the regulatory light chains of myosin become rapidly phosphorylated. Phosphorylation is accompanied by potentiation of the actin activation of the myosin Mg-ATPase activity and by loss of order of the helical crossbridge arrangement characteristic of the relaxed filament.

We suggest that in the relaxed state, when the regulatory light chains are not phosphorylated, the myosin heads are held down on the filament backbone by head-head interactions or by interactions of the heads with the filament backbone. Phosphorylation of the light chains may alter these interactions so that the crossbridges become more loosely associated with the filament backbone giving rise to the observed changes and facilitating crossbridge interaction with actin.

Contraction in motile systems is controlled by Ca²⁺-dependent regulatory protein switches associated with the myosin or actin filaments. At low Ca²⁺ levels, actin–myosin interaction is inhibited and actin-activated myosin ATPase activity is low, whereas at high Ca²⁺ levels, inhibition of actin–myosin interaction is removed and the myosin ATPase is activated by actin. In the case of myosin-linked regulation, which occurs in many muscle and nonmuscle cells, Ca²⁺ regulation is mediated by small polypeptide chains (regulatory light chains) that are present on the myosin heads (Kendrick-Jones et al., 1976; Kendrick-Jones and Scholey, 1981). Two mechanisms of myosin-linked regulation have been described. In one, exemplified by scallop striated muscle, activation of the myosin MgATPase by actin requires binding of Ca²⁺ to the myosin heads (Kendrick-Jones et al., 1970; Szent-Györgyi et al., 1973). In the other, the myosin filaments are switched "on" (enabled to interact with actin) by Ca²⁺-induced phosphorylation of the regulatory light chains. This mechanism occurs in vertebrate smooth muscle and nonmuscle cells (Adelstein and Eisenberg, 1980; Kendrick-Jones and Scholey, 1981; Small and Sobieszek, 1980; Watanabe, 1985) and has also been reported to operate in the striated muscle of the horseshoe crab, Limulus (Sellers, 1981).

The structural changes that occur in myosin filaments when they are switched "on" (but before their interaction with actin) are poorly understood. The problem is difficult to study by X-ray diffraction of contracting muscle since when thick and thin filaments overlap, the effects of interaction of the crossbridges with actin will be superimposed on the changes in the thick filaments themselves. However, preservation of the three-dimensional crossbridge array in isolated negatively stained relaxed thick filaments (e.g., Kensler and Levine, 1982; Vibert and Craig, 1983; Crowther et al., 1985) provides a starting point for observing such changes by electron microscopy. Our most detailed knowledge of thick filament structure in the "off" (relaxed) state has come from three-dimensional reconstruction of negatively stained filaments from the leg muscles of the tarantula. Relaxed tarantula thick filaments have a helically ordered arrangement of crossbridges in which the heads of individual myosin molecules appear to point in opposite directions along the filament such that interactions occur between the heads of axially adjacent myosin molecules (Padrón et al., 1984; Crowther et al., 1985). On the basis of this structure we suggested (Crowther et al., 1985) that head–head interaction or interaction of the heads with the backbone locked the crossbridges close to the thick filament backbone, helping to inhibit interaction with actin and to maintain the "off" state. Here we test the hypothesis that switching "on" of tarantula thick filaments

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phorylated (or thiohosphorylated) using 30 μg/ml calmodulin, 25 μg/ml MLCK, and 2.5 mM MgATP (or 1 mM MgATPγ-S) at 25°C for 15 min. Since the filament suspensions used in the electron microscopic studies contained high levels of endogenous calmodulin and MLCK, phosphorylation was carried out in these experiments by the addition of Ca2+ (0.1 mM final concentration of free Ca2+) to the filaments in relaxing medium followed by incubation at 25°C for 5–30 min. Alternatively, these filament suspensions were phosphorylated in relaxing medium (absence of Ca2+) by the addition of trypsin-treated Ca2+-insensitive MLCK (50 μg/ml) followed by incubation at 25°C for 5–30 min.

The level of phosphorylation of the "regulatory" light chains in the filament preparations assayed under various conditions (see Fig. 2) was monitored by the change in their mobility on 40% glycerol/urea PAGE. Aliquots of the filament suspensions were taken at fixed time points, mixed 1:1 with SDS sample buffer, boiled, and at the end of the assay (i.e., within approximately 30 min) run on gels, together with the control samples and the following molecular mass standards: 200 kD, myosin heavy chains; 94 kD, phosphorylase b; 68 kD, BSA; 42 kD, actin; 25 kD, rabbit skeletal muscle myosin LC1; light chain; 19 kD, rabbit myosin "regulatory" LC2, light chain.

Both types of gels were stained with 0.5% Coomassie Blue (PAGE Blue 83) in 50% methanol, 10% acetic acid (the glycogen/urea gels take longer to stain/desain and are less sensitive than the SDS gels). They were rapidly destained and dried, and if [32P]γ-ATP (5 μCi/μmol) had also been used (to monitor phosphorylation and demonstrate that the change in mobility was due to light chain phosphorylation), then the gels were autoradiographed in cassettes fitted with calcium/tungstate/phosphor screens (Cronex Lightning Plus; Dupont Co., Wilmington, DE) at −70°C using pre-fogged film to increase sensitivity. We are thus able to detect faint traces of kinase activity in our myosin preparations.

The actin-activated MgATPase activities of the myosin preparations were routinely measured at 25°C by the pH-stat assay procedure (Kendrick-Jones et al., 1976), but were also assayed using the isotopic [32P]phosphate procedure (Scholery et al., 1981).

Electron Microscopy

The compositions of the solutions used to prepare the filament suspensions for electron microscopy and for measuring their level of light chain phosphorylation were as follows.

**Relaxing Medium.** 0.1 M NaCl, 8 mM MgCl2, 5 mM ATP, 1 mM EGTA, 3 mM NaN3, 10 mM NaF, pH 7.0.

**Activating Medium.** Relaxing medium + 1.1 mM CaCl2, pH 7.0.

**Relaxing Rinse.** 0.1 M Na acetate (Ac), 2 mM MgAc, 1 mM ATP, 0.2 mM EGTA, 3 mM NaN3, 2 mM imidazole, pH 7.0.

**Activating Rinse.** Relaxing rinse + 0.3 mM CaCl2, pH 7.0.

Filament homogenates were prepared from chemically skinned tarantula muscles as described by Crowther et al. (1985), with the following modifications: (a) The concentration of EGTA in the relaxing medium was 1 (not 0.5) mM. (b) The precaution of using a cocktail of proteolytic enzyme inhibitors was found to be unnecessary for good structural preservation and was omitted. (c) Muscles were homogenized in a Polytron homogenizer. Muscles, stored in relaxing medium on ice, were used within 3 d of removal from the animal, and once homogenized, experiments were completed on them within about 2 h to minimize structural deterioration and uncontrolled phosphorylation, which occurred with longer storage.

Negative staining with 6–10 drops of 1% (wt/vol) uranyl acetate was carried out at room temperature, as described by Crowther et al. (1985), on filament suspensions in which the state of phosphorylation of the regulatory light chains was monitored as described above. Aliquots of the filament suspensions were removed for electron microscopy at the same time points as those for the phosphorylation assays and were diluted 20-fold into, and kept in, activating medium (or relaxing medium for "relaxed" controls) at 0°C.

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Since phosphorylation has a high $Q_0$, this should have essentially halted further phosphorylation until the grids were made, generally within 30 min. Immediately before staining, the grid was washed with ~10 drops of relaxing rinse or activating rinse. All micrographs are of filaments suspended in unbroken films of stain over holes in the carbon substrate (Vibert and Craig, 1982, 1983). Micrographs were recorded with normal electron doses, at a nominal magnification of 26,000X, on a JEOL 100CX electron microscope operated at 80 kV with an anticontamination cold finger.

Results

Thick Filaments in Relaxing Medium

Muscle homogenates, containing suspensions of thick and thin filaments, were incubated in relaxing medium at 25°C and after 5, 10, and 30 min, drops were removed for electron microscopy and aliquots taken to determine the level of light chain phosphorylation. To avoid the deleterious effects of staining thick filaments in the presence of Cl⁻ ions (Crowther et al. 1985), grids were rinsed for 5-10 s with 6-10 drops of relaxing rinse (in which the major anion is acetate) immediately before staining. The majority of thick filaments observed showed a clear helical organization of crossbridges (Fig. 1) as reported previously (Levine et al., 1983; Crowther et al., 1985). The diameter of the filaments to the outer edges of the crossbridges was ~36 nm. The appearance of the helical structure was independent of the length of time (up to 30 min) of incubation in relaxing medium, and gel electrophoresis demonstrated that the light chains remained as shown in Fig. 2 (samples 1-3), where we believe they are mainly nonphosphorylated. Even under these relaxing conditions, some thick filaments appeared to have thin filaments attached to them.

The filaments were tested for any direct effect of Ca²⁺ on their structure by rinsing for 5-10 s with 6-10 drops of activating rinse (relaxing rinse + 10⁻⁴ M free [Ca²⁺]) immediately before staining (cf., Vibert and Craig, 1985). Filaments showed a similar ordered, helical structure to that seen at low Ca²⁺ levels (Fig. 1 c).

Thick Filaments in Activating Medium

Filament suspensions were incubated at 25°C in activating medium (containing 10⁻⁴ M free Ca²⁺) and after 5, 10, and 30 min, aliquots were removed for determining the extent of light chain phosphorylation and simultaneously for examining thick filament structure by negative staining. Grids were washed with activating rinse immediately before staining. From the earliest time point (5 min), thick filaments incubated in activating medium had completely phosphorylated light chains (Fig. 2, samples 7-9). They also looked clearly different in structure from those in relaxing medium: the ordered helical structure of the thick filaments was generally lost (Fig. 3) and the crossbridges sometimes appeared clumped or to project further from the filament backbone (Fig. 3 c).

The disorder was not due to the slight (0.15 U) drop in pH that occurred on addition of Ca²⁺ to the relaxing medium, since relaxed filaments rinsed with relaxing rinse at this lower pH still showed helical crossbridge order. The disorder produced by activation was not reversed by rinsing with relaxing (low Ca²⁺) rinse before staining (Fig. 3 c).

To prevent any reversal of phosphorylation by endogenous phosphatase, filaments were also incubated in activating

Figure 1. Relaxed tarantula thick filaments (light chains nonphosphorylated) negatively stained with 1% uranyl acetate. Filaments were incubated in relaxing medium for 10 (a), 30 (b), and 15 (c) min before rinsing on the grid with relaxing rinse (a and b) or activating rinse (c). The appearance of helical order is enhanced by viewing the filaments obliquely along their length or by turning them horizontally. In all electron micrographs in this and other figures the bare zone of the filament (not always visible) is towards the bottom. Bar, 0.2 µm.
Light chain phosphorylation of tarantula filament suspensions monitored by electrophoresis on 10% acrylamide–40% glycerol/Tris-glycine buffer gels. Filament suspensions were incubated at 25°C in relaxing medium (MgATP, EGTA) for 5, 10, and 30 min (samples 1, 2, and 3); in relaxing medium plus 50 µg/ml trypsin-treated (Ca2+-insensitive) MLCK for 5, 10, and 30 min (samples 4, 5, and 6); and in activating medium (0.1 mM free [Ca2+]) for 5, 10, and 30 min (samples 7, 8, and 9). Aliquots of the filament suspensions were taken for electron microscopy at the same time points as for electrophoresis. Gel samples were prepared rapidly and the gels run as soon as possible as described in Materials and Methods (to prevent any possible change that may occur on storage). Shown is a typical dried Coomassie-stained gel run immediately at the end of the experiment. Non P denotes the positions of the regulatory light chains in relaxing medium (presumed to be mainly nonphosphorylated), and P marks their position after incubation in activation buffer and endogeneous kinase or with Ca2+-insensitive kinase (phosphorylated).

Attempts to reverse the phosphorylation, by addition of a phosphatase isolated from chicken gizzard muscle (Kendrick-Jones et al., 1983) were unsuccessful as judged by urea/glycerol gel electrophoresis and electron microscopy: only a small amount of light chain dephosphorylation was observed and no significant decrease in crossbridge disorder was seen. In addition, prolonged incubation of the filament preparations, even in EGTA, slowly renders the kinase Ca2+- insensitive, presumably due to proteolytic cleavage (Adelstein et al., 1981), and thus leads to a steady increase in kinase activity and to a decrease in dephosphorylation.

Thick filaments were sometimes observed attached to thick filaments under activating conditions. Crossbridges at various angles appeared to link thick and thin filaments (Fig. 4), possibly representing interactions between actively sliding filaments that were trapped by uranyl acetate staining.

**Thick Filaments in Relaxing Medium Containing Desensitized Myosin Light Chain Kinase**

The regulatory light chains can also be phosphorylated in relaxing medium, i.e., at low Ca2+ levels, by the addition of trypsin-treated gizzard MLCK, which does not require Ca2+ for its activity (Fig. 2, samples 4–6). Filaments phosphorylated at low [Ca2+] in this way were applied to a grid and washed with relaxing rinse. They showed loss of helical ordering of the crossbridges not obviously distinguishable from that occurring at high [Ca2+] (Fig. 5). The structure was not obviously altered by subsequent exposure to high [Ca2+] (rinsing with activating rinse; Fig. 5 c). These results suggest that phosphorylation of the regulatory chains, rather than [Ca2+] per se, is the factor responsible for disordering of the crossbridge array.
Biochemical Studies of Tarantula Thick Filaments

To establish the polypeptide composition of tarantula thick filaments and to demonstrate that the changes observed on the urea/glycerol gels are due to myosin light chain phosphorylation, tarantula myosin was isolated and purified. Gel analysis of the purified myosin and of the crude filament homogenates used for electron microscopy revealed the following. (a) The myosin contains two types of light chains of 26 and 19 kD (Fig. 6 C); corresponding bands are seen on SDS gels of the filament homogenates (Fig. 6 E). (b) In both the purified myosin and the filament homogenates, only the
regulatory (26 kD) light chain is phosphorylated by the endogenous and the purified gizzard kinases (Fig. 6, C-F). (c) Purified myosin preparations run on glycerol/urea gel electrophoresis showed two bands in the expected position of the regulatory light chain (Fig. 6 A). When the myosin was phosphorylated, the upper band disappeared and a new band appeared with higher mobility than the lower one (Fig. 6 A); the latter two bands both incorporated [\(^{32}\)P]phosphate (Fig. 6 B). Filament homogenates gave exactly the same result (Figs. 2 and 6 E). We presume, therefore, that these bands must be the regulatory light chains and that the change in mobility must be due to phosphorylation. The two bands are present in a roughly constant ratio in the filament and myosin preparations and their extent of [\(^{32}\)P]phosphate incorporation is about the same (Fig. 6 B). The simplest interpretation of these results is that the light chains are originally non-phosphorylated and that both become singly phosphorylated at about the same rate. Thus, tarantula myosin appears to contain two phosphorylatable regulatory light chain species which differ slightly in net charge, but have the same molecular weight (26 kD). This is different from the situation in Limulus striated muscle (Sellers, 1981).

The interaction of the purified tarantula myosin with purified rabbit actin, as measured by the actin-activated myosin MgATPase activity, did not appear to be controlled by Ca\(^{2+}\), but phosphorylation caused a significant potentiation of the ATPase activity (Table I).

**Discussion**

In this paper we have shown that phosphorylation of the regulatory light chains modulates the actin activation of tarantula myosin ATPase and that paralleling this there is a change in the arrangement of the myosin crossbridges from a helically ordered to a disordered state.
MgATPase Activity of Tarantula Myosin (AM-MgATPase) changed. either 0.1 mM EGTA (-Ca²⁺) or 0.1 mM EGTA + 0.15 mM CaCl₂ potentiate the force at submaximal Ca²⁺ levels (Persechini et al., 1985). The situation is different from vertebrate striated muscle where, although light chain phosphorylation occurs though it does have a pronounced potentiating effect (Table 1). This situation is similar to that in vertebrate striated muscles where, although light chain phosphorylation occurs both in vivo and in vitro, there is no direct evidence that it is involved in regulating myosin interaction with actin (Morgan et al., 1976; Manning and Stull, 1979; Pemrick, 1980; Barsotti and Butler, 1984). However, it may, for example, potentiate the force at submaximal Ca²⁺ levels (Persechini et al., 1985). The situation is different from vertebrate smooth muscle, where light chain phosphorylation is essential for myosin regulation (Adelstein and Eisenberg, 1980).

Sellers (1981) demonstrated that myosin isolated from the striated (telson) muscles of Limulus (the horseshoe crab), a closely related arthropod, is regulated by light chain phosphorylation. This raises the possibility that the lack of such an effect in tarantula myosin is due to some “modification” of the myosin during preparation. Although we cannot exclude this possibility, we think that it is unlikely for the following reasons. (a) Four different myosin preparations gave basically the same results. (b) The myosin preparative procedure is extremely rapid and does not use extreme conditions. DTT and proteolytic inhibitors were included and there was no sign of proteolytic degradation of either heavy chain or light chain. Gizzard myosin was prepared by the same procedure in parallel, and was shown to be regulated by light chain phosphorylation. (c) The light chains appear to be mainly nonphosphorylated when the myosins are prepared and can be phosphorylated to greater than 90% by endogenous kinase or gizzard MLCK. Myosins prepared by the same or similar procedures (e.g., Limulus myosin [Sellers, 1981], thymus myosin [Scholey et al., 1982], gizzard myosin [Kendrick-Jones et al., 1983], rabbit skeletal muscle myosin [Kendrick-Jones, J., unpublished observations]) are all non-phosphorylated (>90%) when prepared. (d) The myosins have actin-activated MgATPase activities which are in the expected range for a striated muscle myosin (cf., vertebrate striated muscle myosin; Morgan et al., 1976) and which are remarkably consistent between the different myosin preparations. (e) The myofibrils at the initial stage of myosin preparation are Ca²⁺ regulated and therefore one presumes the regulatory proteins are intact and unmodified.

We therefore conclude that in tarantula striated muscle, myosin light chain phosphorylation is not the “primary” regulatory mechanism, but may play a role in facilitating myosin crossbridge interaction with actin.

### Table 1. Effect of Phosphorylation on the Actin-activated MgATPase Activity of Tarantula Myosin (AM-MgATPase)

<table>
<thead>
<tr>
<th>AM-MgATPase</th>
<th>-Ca²⁺</th>
<th>+Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarantula Myosin</td>
<td>0.012 ± 0.005</td>
<td>0.021 ± 0.010</td>
</tr>
<tr>
<td>Nonphosphorylated myosin alone</td>
<td>0.226 ± 0.031</td>
<td>0.276 ± 0.037</td>
</tr>
<tr>
<td>Thiophosphorylated myosin + actin-tropomyosin</td>
<td>0.017 ± 0.009</td>
<td>0.052 ± 0.021</td>
</tr>
<tr>
<td>Thiophosphorylated myosin + actin-tropomyosin</td>
<td>0.576 ± 0.068</td>
<td>0.585 ± 0.048</td>
</tr>
</tbody>
</table>

Effect of Light Chain Phosphorylation on Tarantula Myosin

Tarantula thick filaments contain phosphorylatable regulatory light chains of 26 kD. Under relaxing conditions these light chains appear to be nonphosphorylated, and become rapidly phosphorylated when Ca²⁺ is added to activate the endogenous kinase or when trypsin-treated gizzard kinase is added in the absence of Ca²⁺ (Figs. 2 and 6).

The ATPase results suggest that in our myosin preparations, light chain phosphorylation is not an essential requirement for the actin-activated myosin MgATPase activity, although it does have a pronounced potentiating effect (Table I). This situation is similar to that in vertebrate striated muscles where, although light chain phosphorylation occurs both in vivo and in vitro, there is no direct evidence that it is involved in regulating myosin interaction with actin (Morgan et al., 1976; Manning and Stull, 1979; Pemrick, 1980; Barsotti and Butler, 1984). However, it may, for example, potentiate the force at submaximal Ca²⁺ levels (Persechini et al., 1985). The situation is different from vertebrate smooth muscle, where light chain phosphorylation is essential for myosin regulation (Adelstein and Eisenberg, 1980).

Sellers (1981) demonstrated that myosin isolated from the striated (telson) muscles of Limulus (the horseshoe crab), a closely related arthropod, is regulated by light chain phosphorylation. This raises the possibility that the lack of such an effect in tarantula myosin is due to some “modification” of the myosin during preparation. Although we cannot exclude this possibility, we think that it is unlikely for the following reasons. (a) Four different myosin preparations gave basically the same results. (b) The myosin preparative procedure is extremely rapid and does not use extreme conditions. DTT and proteolytic inhibitors were included and there was no sign of proteolytic degradation of either heavy chain or light chain. Gizzard myosin was prepared by the same procedure in parallel, and was shown to be regulated by light chain phosphorylation. (c) The light chains appear to be mainly nonphosphorylated when the myosins are prepared and can be phosphorylated to greater than 90% by endogenous kinase or gizzard MLCK. Myosins prepared by the same or similar procedures (e.g., Limulus myosin [Sellers, 1981], thymus myosin [Scholey et al., 1982], gizzard myosin [Kendrick-Jones et al., 1983], rabbit skeletal muscle myosin [Kendrick-Jones, J., unpublished observations]) are all non-phosphorylated (>90%) when prepared. (d) The myosins have actin-activated MgATPase activities which are in the expected range for a striated muscle myosin (cf., vertebrate striated muscle myosin; Morgan et al., 1976) and which are remarkably consistent between the different myosin preparations. (e) The myofibrils at the initial stage of myosin preparation are Ca²⁺ regulated and therefore one presumes the regulatory proteins are intact and unmodified.

We therefore conclude that in tarantula striated muscle, myosin light chain phosphorylation is not the “primary” regulatory mechanism, but may play a role in facilitating myosin crossbridge interaction with actin.

Structural Changes Associated with Phosphorylation of the Myosin Light Chains

Relaxed (nonphosphorylated) tarantula filaments have a well-ordered helical array of crossbridges (Fig. 1) and show strong first and fourth layer lines and third and sixth order meridional reflections, indexing on a repeat of 43 nm, by optical diffraction (Crowther et al., 1985; Levine et al., 1983). The similarity of these patterns to X-ray diffraction patterns of whole relaxed tarantula muscle (Wray, 1982) suggests that the native relaxed state is well preserved by negative staining and that these filaments provide a good basis for observing any structural changes that may be induced by phosphorylation.

When relaxed filaments were incubated in activating medium, the regulatory light chains were rapidly phosphorylated and concomitantly the thick filaments equally rapidly lost their crossbridge order. There was no obvious shortening or dissolution of the myosin filaments on phosphorylation as might have been expected by comparison with studies of Limulus filaments (Brann et al., 1979; Levine and Kessler, 1985; Levine, 1986). Although we were unable to reverse phosphorylation to any significant extent owing to the lack of a purified phosphatase having a sufficiently high activity to overcome the high level of endogenous myosin kinase activity in these filament preparations, we believe that the disordering we observed is a specific result of phosphorylation for the following reasons. (a) The structural changes correlate in time with light chain phosphorylation-induced by Ca²⁺. (b) Light chain phosphorylation induced at low Ca²⁺ concentrations by the addition of a Ca²⁺-insensitive kinase also correlates with a transition to disorder of the crossbridge array (Fig. 5). Thus Ca²⁺ is unnecessary for the disordering and Ca²⁺-activated proteases are also unlikely to be its cause. Since tarantula muscle appears to have actin-linked regulation (data not shown; see also Lehman and Szent-Györgyi, 1975), the changes observed at low Ca²⁺ also imply that crossbridge disordering is not simply a result of the cyclic interactions of crossbridges with actin that
may occur in filament homogenates in high Ca²⁺ (activating) conditions. The gradual phosphorylation that occurs when a filament preparation stands in low Ca²⁺ (relaxing) medium for several hours probably results from a gradual loss of Ca²⁺ sensitivity (and thus activation) of endogenous kinase and is probably responsible for the gradual loss of helical order of filaments left to stand for such periods. (c) Disorder is not a direct result of Ca²⁺ binding since rinsing a relaxed preparation of filaments on the grid with activating rinse did not alter the structure of the filaments (cf., scallop filaments, which are disordered by such a Ca²⁺ rinse; Vibert and Craig, 1985). Similar rinsing of a preparation which had first been phosphorylated at low Ca²⁺ caused no further loss of order.

Loss of relaxed order has been observed in other species of myosin filaments when they are activated. Levine (1986) has recently reported a loss of helical order in Limulus myosin filaments when the regulatory light chains are phosphorylated. Filament bundles formed from purified vertebrate smooth muscle myosin lose their crossbridge periodicity when they are phosphorylated (Ikebe and Ogihara, 1982). In scallop filaments, activation occurs not by phosphorylation but by direct binding of Ca²⁺ to the myosin heads. The periodic arrangement of crossbridges characteristic of relaxed filaments is lost on exposure to activating levels of Ca²⁺ (Vibert and Craig, 1985). These observations suggest that, although the regulatory switch may be either binding of Ca²⁺ to the myosin heads or phosphorylation of the regulatory light chains in the myosin heads, the structural effects are similar.

The structural differences we observe between relaxed and phosphorylated filaments suggest that the crossbridges are held in a well ordered array in relaxed filaments by interactions between heads of neighboring molecules (Crowther et al., 1985) or by binding of the heads to the backbone, and that when the filaments are phosphorylated these interactions are weakened so that the crossbridges are free to adopt a wide variety of configurations. Equatorial X-ray diffraction patterns of demembranated tarantula muscle are consistent with an average 6-nm movement of the crossbridges away from the filament backbone after thiophosphorylation (Sosa et al., 1986a, b; Panté, 1986; Panté et al., 1986a, b), further supporting the view that the crossbridges are more weakly bound to the filament in the phosphorylated state. In the intact muscle, this loosening may facilitate the interaction of crossbridges with actin filaments during contraction.

In the scallop, removal of the regulatory light chains also causes loss of crossbridge order, suggesting that these light chains are essential in binding the heads to the filament (Vibert and Craig, 1985). If the light chains also have such a role in tarantula, then it is not surprising that phosphorylation of this critical region of the myosin head can have such a potent effect on crossbridge binding to the filament.

The binding of myosin heads to the filament also appears to be weakened by increased salt concentration (Wray et al., 1974; Levine et al., 1986), lowering of ATP or Mg²⁺ levels (Haselgrove, 1975; Barnett and Thomas, 1984; Persechini and Rowe, 1984; Pinset-Härström, 1985; Vibert and Craig, 1985; Levine et al., 1986), and by changes in pH (Levine et al., 1986) suggesting that ionic interactions are important in stabilizing the crossbridge array and that small changes in the charge on the crossbridges are sufficient to release them from the filament backbone.

Related changes in structure have been observed in isolated myosin molecules from vertebrate smooth and nonmuscle cells. In low salt conditions (in the presence of ATP and Mg²⁺, and with light chains dephosphorylated), these molecules generally have a folded structure, where the myosin tail bends back up and binds to the heads and the heads point down towards the tail (Trybus et al., 1982; Onishi and Wakabayashi, 1982; Craig et al., 1983). If the salt level is increased, the light chains are phosphorylated, or ATP is removed, the equilibrium moves in the direction of tail extension and random orientations of the heads (Onishi et al., 1983; Craig et al., 1983; Trybus and Lowey, 1984; Suzuki et al., 1985, 1986). These changes are analogous to those we have observed if we assume that in the filaments, the heads of myosin molecules are bound to the backbone through interaction with tails of different molecules (due to the staggered arrangement of molecules in the filament shaft), while in isolated molecules the interactions are intramolecular.

We would like to dedicate this paper to the memory of Marcus Kress.

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