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Interaction of Smooth Muscle Tropomyosin and Smooth Muscle Myosin. Effect on the Properties of Myosin†

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ABSTRACT: Several techniques were used to investigate the possibility that smooth muscle tropomyosin interacts with smooth muscle myosin. These experiments were carried out in the absence of actin. The Mg^{2+}-ATPase activity of myosin was activated by tropomyosin. This was most marked at low ionic strength but also occurred at higher ionic strength with monomeric myosin. For myosin and HMM, the activation of Mg^{2+}-ATPase by tropomyosin was greater at low levels of phosphorylation. There was no detectable effect of tropomyosin on the Mg^{2+}-ATPase activity of S1. The KCl dependence of myosin viscosity was influenced by tropomyosin, and in the presence of tropomyosin, the 6S to 10S transition occurred at lower KCl concentrations. From the viscosity change, an approximate stoichiometry of 1:1 tropomyosin to myosin was estimated. The phosphorylation dependence of viscosity, which reflects the 10S–6S transition, also was altered in the presence of tropomyosin. An interaction between myosin and tropomyosin was detected by fluorescence measurements using tropomyosin labeled with dansyl chloride. These results indicate that an interaction occurs between myosin and tropomyosin. In general, the interaction is favored at low ionic strength and at low levels of phosphorylation. This interaction is not expected to be competitive with the formation of the actin–tropomyosin complex, but the possibility is raised that a direct interaction between myosin and tropomyosin bound to the thin filament could modify contractile properties in smooth muscle.

The dominant regulatory mechanism in smooth muscle involves phosphorylation and dephosphorylation of the 20 000-dalton light chains of myosin, catalyzed by myosin light chain kinase (MLCK)[1] and myosin light chain phosphatase, respectively. Phosphorylation is thought to alter the conformation of myosin and facilitate cross-bridge cycling, and this event is essential for initiation of the contractile response (Hartshorne, 1987). Regulation of skeletal muscle activity is different and utilizes the thin filament based regulatory proteins troponin and tropomyosin. Although the exact function of tropomyosin is not established, it is clearly an essential component of the regulatory system in striated muscle. Troponin is not found in smooth muscle (Hartshorne, 1987), although tropomyosin is present in approximately the same stoichiometry with actin as in skeletal muscle. Thus, there is no obvious role for tropomyosin in smooth muscle

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[1] Abbreviations: MLCK, myosin light chain kinase; HMM, heavy meromyosin; S1, heavy meromyosin subfragment 1; S2, heavy meromyosin subfragment 2; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; dans-tropomyosin, tropomyosin labeled with dansyl chloride.

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function. Regulatory systems associated with thin filaments in smooth muscle have been proposed, namely, leiotonin (Nonomura & Ebashi, 1980) and caldesmon (Sobue et al., 1987). Leiotonin requires tropomyosin for activity (Nonomura & Ebashi, 1980), and caldesmon binds to tropomyosin (Smith et al., 1987; Graceffa, 1987). However, the in vivo concentrations of both leiotonin A (Nonomura & Ebashi, 1980) and caldesmon (Smith et al., 1987; Ngai & Walsh, 1987) are considerably less than tropomyosin, and, assuming a 1:1 stoichiometry, the bulk of tropomyosin must exist as the actin–tropomyosin complex (as opposed to a complex of actin, tropomyosin, and another protein). A similar situation is probably found in many nonmuscle cells.

Despite these uncertainties concerning the function of tropomyosin in smooth muscle, it has been observed in many laboratories, and with proteins from several source tissues, that tropomyosin activates actomyosin ATPase activity (Hartshorne et al., 1977; Sobieszek & Small, 1977; Chacko, 1981; Nag & Seidel, 1983). Under certain conditions, both smooth and skeletal muscle tropomyosins activate ATPase activity (Hartshorne et al., 1977; Chacko, 1981), although some differences between tropomyosins from the two muscle types have been observed (Sobieszek & Small, 1981; Yamaguchi et al., 1984). In addition, both skeletal and smooth muscle actins can form actomyosin hybrids with smooth muscle myosin capable of activation by tropomyosin (Nag & Seidel, 1983).

However, activation of actin-activated ATPase activity by tropomyosin is not constant and depends on the assay conditions (Seidel et al., 1986). In general, the extent of activation is greater at low free Mg^{2+} concentrations both for myosin (Nag & Seidel, 1983) and for HMM (Yamaguchi et al., 1984; Kaminski & Chacko, 1984; Seidel et al., 1986). Tropomyosin also influences the Ca^{2+} dependence of actin-activated ATPase of myosin (Nag & Seidel, 1983; Ikebe & Hartshorne, 1985a; Seidel et al., 1986) or HMM (Kaminski & Chacko, 1984; Seidel et al., 1986), and in general tropomyosin enhances Ca^{2+} sensitivity at low free Mg^{2+} levels. The conventional way to explain the effects of tropomyosin is that these are all mediated via actin; i.e., the actin–myosin interaction is influenced by the presence of tropomyosin. One reasonable, but unproven, possibility is that tropomyosin influences the conformation of actin and that different conformations of actin (at least two, to correspond to plus and minus tropomyosin) are more or less effective in the activation of myosin ATPase. To rationalize different levels of activation would imply distinct binding states of tropomyosin on the thin filament. Another possibility that has not been considered is that tropomyosin, while bound to the thin filament, i.e., actin, can also interact directly with myosin. Different levels of activation by tropomyosin might then reflect different interactions (e.g., affinity) with myosin. Although there is no direct evidence in favor of this idea, some of the previous data are consistent with the hypothesis. The greater extent of activation by tropomyosin at low Mg^{2+} concentrations is an example. The binding of tropomyosin to actin is dependent on the Mg^{2+} concentration. If the activation of ATPase activity by tropomyosin reflected the affinity of the actin–tropomyosin complex, one would expect more activation at the higher Mg^{2+} levels. This is not observed. Thus, the dependence of activation on the Mg^{2+} concentrations could relfect altered states of myosin rather than changes in the thin filament. Previously it was shown by Ikebe et al. (1984) that lower concentrations of Mg^{2+} in the assay media favor the 10S conformation for phosphorylated gizzard myosin. Our objective in this paper was to determine if myosin–tropomyosin interactions could be detected. To facilitate interpretation of the data, since many of the procedures used were complicated by actin, these assays were carried out in the absence of actin.

Materials and Methods

Myosin, myosin light chain kinase, and tropomyosin were isolated from frozen turkey gizzards. The procedures used were the following: myosin, Ikebe & Hartshorne (1985a); myosin light chain kinase, Ikebe et al. (1987); for tropomyosin, the method of Hartshorne and Mrwa (1981) was used followed by further purification using isoelectric precipitation at pH 5.2 (Merkel et al., 1984) and chromatography on hydroxyapatite (Eisenberg & Kielly, 1974). The tropomyosin used for these studies did not contain any detectable contamination by actin. HMM and S1 were prepared from gizzard myosin following proteolysis with *Staphylococcus aureus* protease (Ikebe & Hartshorne, 1985b). Actin was prepared from rabbit skeletal muscle (Driska & Hartshorne, 1975) and calmodulin from frozen bull testes (Walsh et al., 1983).

Tropomyosin was labeled with dansyl chloride (Sigma Chemical Co.) at 37 °C for 2 h as described by Burtnick and Bhangu (1986). Under these conditions, approximately 15 dansyl groups are incoporated per mole of tropomyosin. Fluorescence intensities were measured by scanning emission spectra using a Farrand Mark I spectrofluorometer at a fixed excitation wavelength, 365 nm.

ATPase activities were measured at 25 °C as described by Ikebe and Hartshorne (1985b). Each point is the mean of three to seven determinations. Assay conditions are given in the figure legends. Phosphorylation assays were carried out as described by Walsh et al. (1983). [γ-^{32}P]ATP was obtained from New England Nuclear. Conditions for phosphorylation of myosin are given in the figure legends. Viscosity was measured at 25 °C in Cannon–Ubbelohde viscometers with water flow times of approximately 25 s. Solvent conditions are given in the figure legends. The viscosity data are expressed as η_{rel} (viscosity of protein solution/viscosity of solvent). With mixtures of myosin and tropomyosin, the contribution of tropomyosin was determined (i.e., flow time of tropomyosin solution minus flow time of solvent) and subtracted from the total flow time of the mixture. At 0.4 mg/mL, η_{rel} tropomyosin was not significantly different at 0.15 and 0.35 M KCl and was approximately 1.06. For the samples of phosphorylated myosin, the phosphorylation level was assayed before and after the viscosity measurements, and the viscosity data were used only if phosphorylation was constant. Electrophoresis was carried out on 7.5–20% polyacrylamide gradient slab gels in the presence of 0.1% $NaDodSO_4$ using the discontinuous buffer system of Laemmli (1970). Other analytical procedures were as described by Walsh et al. (1982).

Results

As an example of the variable activation by tropomyosin, the Mg^{2+} dependence of actin-activated ATPase activity of phosphorylated (2 mol of P/mol of myosin) and dephosphorylated gizzard myosin are shown in Figure 1. The activation of ATPase activity on increasing Mg^{2+} levels in the absence of tropomyosin has been shown previously (Ikebe et al., 1984). For both phosphorylated and dephosphorylated myosin, the presence of tropomyosin enhances activity, and this is found only at the lower Mg^{2+} concentrations (Figure 1B). With dephosphorylated myosin, maximum activation is found at approximately 8 mM $MgCl_2$ (total), and for phosphorylated myosin, the extent of activation declines progressively at concentrations higher than 1 mM $MgCl_2$. Activation in absolute terms (i.e., difference in specific activities plus and minus tropomyosin) is greater for phosphorylated myosin but in

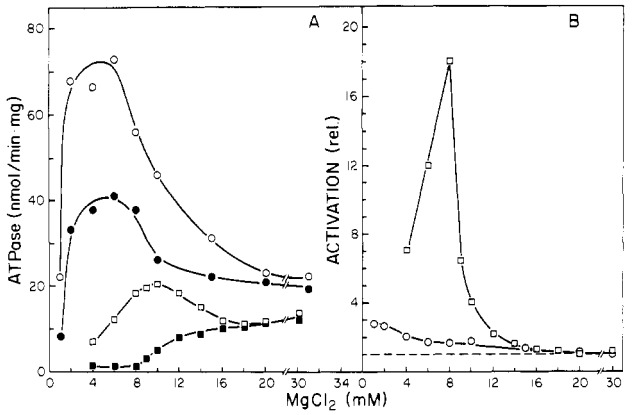

FIGURE 1: Mg^{2+} concentration dependence of actin-activated ATPase activity of phosphorylated and dephosphorylated myosin in the presence and absence of tropomyosin. Assay conditions: 2 mg/mL myosin (4.3 μM), 1.26 mg/mL actin (30 μM), 75 mM KCl, 30 mM Tris-HCl (pH 7.5), 1 mM [γ-^{32}P]ATP, and 1 mM EGTA. Myosin was phosphorylated in the presence of 5 μg/mL MLCK, 17 μg/mL calmodulin, and 1×10^{-5} M $CaCl_2$ at 25 °C for 10 min. Phosphorylation level was 1.93 ± 0.021 mol of P/mol of myosin (n = 4). ATPase assay started by the addition of actin and EGTA. The dephosphorylated myosin was treated similarly but in the absence of MLCK and calmodulin. Phosphorylated myosin (○, ●); dephosphorylated myosin (□, ■). Presence of tropomyosin (0.4 mg/mL, 5.9 μM) indicated by open symbols. (A) Actin-activated ATPase activities; (B) relative activation by tropomyosin (i.e., ATPase activity in the presence of tropomyosin/ATPase activity in the absence of tropomyosin).

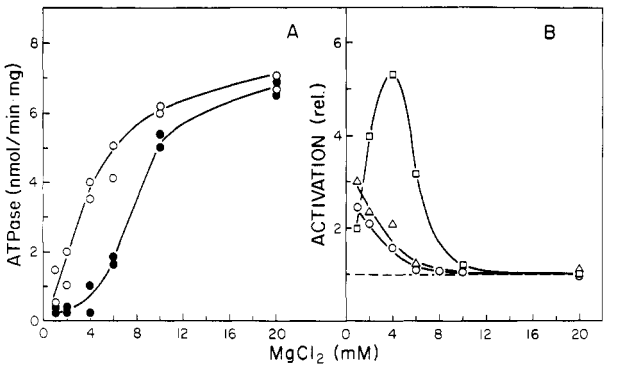

FIGURE 2: Mg^{2+} concentration dependence of ATPase activity of dephosphorylated myosin in the presence and absence of tropomyosin. Assay conditions: 0.5 mg/mL myosin (1 μM), 0.1 mg/mL tropomyosin (1.5 μM), if required, 75 mM KCl, 1 mM [γ-^{32}P]ATP, 1 mM EGTA, and 30 mM Tris-HCl (pH 7.5). (A) Mg^{2+}-ATPase of dephosphorylated myosin in the presence (○) and absence (●) of tropomyosin; (B) relative activation by tropomyosin for dephosphorylated myosin (□), partially phosphorylated myosin, 1.04 ± 0.045 (n = 5) mol of P/mol of myosin (△), and phosphorylated myosin, 1.95 ± 0.02 (n = 5) mol of P/mol of myosin (○).

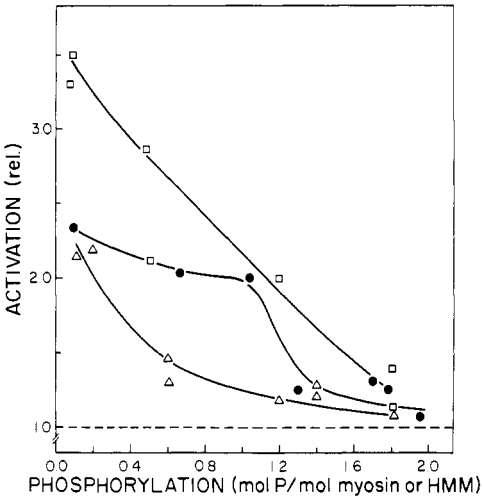

FIGURE 3: Relative activation by tropomyosin of myosin and HMM Mg^{2+}-ATPase activity. Myosin (2 mg/mL) was phosphorylated at different concentrations of MLCK (0.1–20 μg/mL) and 17 μg/mL calmodulin for 10 min in 1 mM $MgCl_2$, 1 mM [γ-^{32}P]ATP, 1×10^{-5} M $CaCl_2$, 0.2 M KCl, and 30 mM Tris-HCl (pH 7.5) in the presence and absence of 0.4 mg/mL tropomyosin. EGTA was added to 1 mM, aliquots were removed to determine phosphorylation levels, and ATPase activity was monitored for 10 min. For HMM (0.1 mg/mL), a similar sequence was followed. The conditions were the same except for either 1 or 10 mM $MgCl_2$, 20 mM KCl, and 0.07 mg/mL tropomyosin. Myosin (●), HMM in 1 mM $MgCl_2$ (□) or 10 mM $MgCl_2$ (△).

relative terms (activity plus tropomyosin/activity minus tropomyosin) is more pronounced for dephosphorylated myosin.

To investigate whether tropomyosin can interact directly with myosin, a number of procedures were tried. Those procedures that would indicate a relatively tight complex formation were unsuccessful, and these included sedimentation velocity experiments with monomeric myosin at different KCl concentrations, gel filtration chromatography at various KCl concentrations, and sedimentation of filamentous myosin (i.e., low ionic strength) in the presence of tropomyosin and tropomyosin affinity columns. Thus, it is unlikely that tropomyosin forms a "high-affinity" complex with myosin, i.e., similar to the tropomyosin–actin complex, and any interaction that might occur must be of lower affinity. To investigate whether such complexes could be detected, several additional properties of myosin were measured in the presence and absence of tropomyosin.

First, the Mg^{2+} dependence of myosin ATPase activity was assayed in the presence and absence of tropomyosin at low ionic strength (75 mM KCl). Shown in Figure 2A are the results for dephosphorylated myosin. As the Mg^{2+} concentration is increased, the ATPase activity also increases. Activation by tropomyosin is apparent but only at the lower levels of $MgCl_2$. Maximum activation (expressed as relative activation) is obtained at approximately 4 mM $MgCl_2$ (Figure 2B). Both partially phosphorylated (1.04 mol of P/mol of myosin) and myosin phosphorylated at both serine-19 residues (1.95 mol of P/mol of myosin) are activated by tropomyosin (Figure 2B), and, as with the actin-activated ATPase, activation decreases above 1 mM $MgCl_2$. Thus, the patterns of activation by tropomyosin are similar for myosin in the presence and absence of actin, although the extent of activation is less in the absence of actin, and for dephosphorylated myosin the Mg^{2+} dependence of activation is altered (i.e., maxima at 8 and 4 mM $MgCl_2$; cf. Figures 1B and 2B).

The Mg^{2+}-ATPase of monomeric myosin (at 0.2 M KCl) also is activated by tropomyosin. In Figure 3 is shown the extent of activation as a function of phosphorylation (conditions given in the figure legend). Activation by tropomyosin is higher at lower levels of phosphorylation. At this ionic strength, phosphorylation of both serine-19 residues converts 10S to the 6S conformation; thus, it is inferred that activation by tropomyosin is greater for the 10S conformation.

In addition, the Mg^{2+}-ATPase activity of HMM is activated by tropomyosin. Shown in Figure 3 are the phosphorylation dependencies of activation for HMM in 1 and 10 mM $MgCl_2$. Activation is greater for the lower $MgCl_2$ concentration and in both cases is more pronounced at lower phosphorylation levels. These results are consistent with the idea that activation by tropomyosin is more effective with the 8.6–9.0S conformation of HMM (i.e., that conformation of HMM analogous to the 10S myosin conformation; Suzuki et al., 1985; Ikebe et al., 1988).

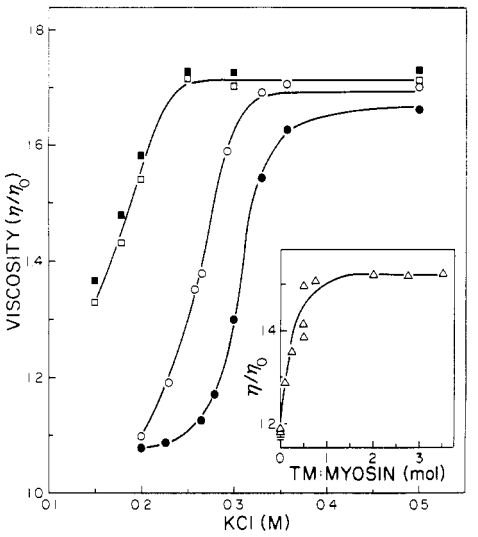

FIGURE 4: KCl concentration dependence of viscosity of dephosphorylated and phosphorylated myosin in the presence and absence of tropomyosin. Conditions: 2 mg/mL myosin, 0.4 mg/mL tropomyosin, if required, 1 mM $MgCl_2$, 1 mM [γ-^{32}P]ATP, 1 mM EGTA, and 30 mM Tris-HCl (pH 7.5). Phosphorylation to 1.94 ± 0.02 (n = 10) mol of P/mol of myosin was achieved by preincubation at 25 °C for 10 min with 5 μg/mL MLCK, 17 μg/mL calmodulin, and 1×10^{-5} M $CaCl_2$ (other conditions as given). Phosphorylation was stopped by the addition of EGTA to 1 mM. Dephosphorylated myosin plus (○) and minus tropomyosin (●). Phosphorylated myosin plus (□) and minus tropomyosin (■). Inset: Relative viscosity of dephosphorylated myosin in 0.28 M KCl and different levels of tropomyosin; other conditions the same.

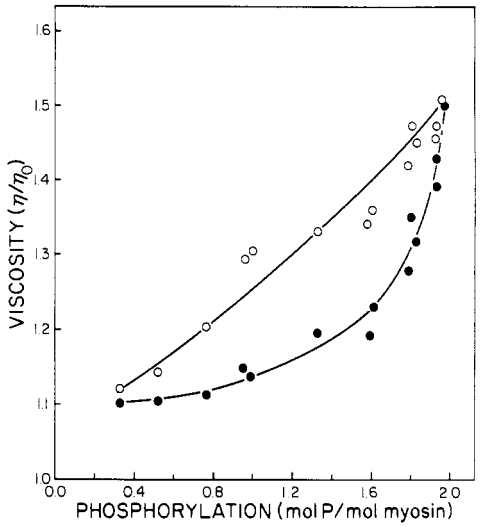

FIGURE 5: Phosphorylation dependence of relative viscosity of myosin in the presence and absence of tropomyosin. Conditions as in Figure 4, except KCl was constant at 0.2 M. Different phosphorylation levels obtained by preincubation with different concentrations of MLCK (as in Figure 3). Myosin plus (○) and minus tropomyosin (●).

There was no detectable effect of tropomyosin on the Mg^{2+}-ATPase activity of S1, both at high (0.3 M KCl) and at low (75 mM KCl) ionic strength. Related to this is the finding that the Mg^{2+}-ATPase of acto–S1 (S1 prepared both with papain and with *Staph. aureus* protease) is not activated by tropomyosin (M. Ikebe, unpublished observation).

Another technique that was used to investigate a possible interaction between tropomyosin and myosin was viscosity. The transition from 6 S to 10 S that occurs on decreasing ionic strength is monitored conveniently by viscosity (Ikebe et al., 1983), and thus the ionic strength dependence of viscosity was measured for phosphorylated and dephosphorylated myosin in the presence and absence of tropomyosin (the contribution of tropomyosin to viscosity was subtracted from the myosin plus tropomyosin mixtures; see Materials and Methods). For dephosphorylated myosin in the absence of tropomyosin, the conversion of 6 S to 10 S occurs as the KCl concentration is reduced below 0.35 M (Figure 4). In the presence of tropomyosin, this transition is shifted to lower KCl concentrations. Thus, within the appropriate range of ionic strengths, the addition of tropomyosin to dephosphorylated myosin increases viscosity, and this is consistent with a partial transition toward the 6S conformation. For phosphorylated myosin, tropomyosin had no detectable effect on the viscosity–KCl concentration profile.

To determine an approximate stoichiometry for the tropomyosin–myosin interaction, the viscosity of dephosphorylated myosin was measured at 0.28 M KCl with varying amounts of tropomyosin. These data are shown in the inset of Figure 4. The increase in viscosity is optimum at approximately 1 mol of tropomyosin/mol of myosin.

The effect of tropomyosin on the phosphorylation dependence of viscosity was measured at 0.2 M KCl, and the results are given in Figure 5. At this ionic strength, phosphorylation drives the transition from 10 S to 6 S, and this is reflected by an increase in viscosity. As shown previously (Ikebe et al., 1983), the increase occurs when the second site of phosphorylation is filled. In the presence of tropomyosin, the dependence of viscosity on phosphorylation is altered and approaches a linear relationship.

Table I: Enhancement of Fluorescence Emission of Dans-tropomyosin and Myosin[a]

conditions[b]	% enhancement
DeP-myosin, 1 mM ATP, 1 mM $MgCl_2$, 0.28 M KCl	5 ± 1 (n = 4)
DeP-myosin, 1 mM ATP, 1 mM $MgCl_2$, 0.2 M KCl	20 ± 1.5 (n = 6)
P-myosin, 1 mM ATP, 1 mM $MgCl_2$, 0.075 M KCl	14 (n = 2)
DeP-myosin, 1 mM ATP, 6 mM $MgCl_2$, 0.075 M KCl	51 ± 8.5 (n = 4)

[a] Enhancement calculated as the percentage increase of fluorescence at the emission maximum for a mixture of dans-tropomyosin plus myosin compared to the emission maximum of dans-tropomyosin alone. [b] Abbreviations: DeP-myosin, dephosphorylated myosin; P- myosin, phosphorylated myosin. Myosin (1 mg/mL) and dans-tropomyosin (0.3 mg/mL) concentrations were constant. Other solvent conditions, 30 mM Tris-HCl (pH 7.5).

As a final means of assessing the putative tropomyosin–myosin interaction, the fluorescence emission spectra of tropomyosin labeled with dansyl chloride, i.e., dans-tropomyosin, were monitored in the presence and absence of myosin. This procedure was used previously to demonstrate complex formation of tropomyosin with F-actin (Burtnick & Bhangu, 1986). The myosin and dans-tropomyosin concentrations were kept constant at 1 and 0.3 mg/mL, respectively, and the emission spectrum was scanned under a variety of conditions. Under all of the conditions tested, myosin caused an enhancement of fluorescence and a slight blue shift of the emission maximum wavelength (about 5 nm). However, the fluorescence enhancement varied and depended on the conditions employed. The results are summarized in Table I. For monomeric myosin (first two sets of conditions), the fluorescence enhancement is highest under those conditions where the concentration of the 10S species is highest, i.e., dephosphorylated myosin and 0.2 M KCl. However, the largest enhancement of fluorescence is observed with filamentous dephosphorylated myosin (last set of conditions). The addition of bovine serum albumin to 1 mg/mL (used as a control for

nonspecific effects) caused a slight reduction (approximately 5%) of the emission intensity.

Discussion

Several different techniques have been used to demonstrate that smooth muscle tropomyosin can interact with smooth muscle myosin. Although it is difficult to compare the results from each technique, since each is subject to different experimental biases, it appears that with monomeric myosin, interaction is most noticeable with dephosphorylated myosin under conditions that favor the 10S conformation. A similar situation was found for HMM in the 8.6–9.0S conformation. This is observed for the activation of ATPase activity and for the viscosity and fluorescence data. With phosphorylated monomeric myosin, interaction is indicated by the fluorescence measurements but not by the viscosity data. Presumably, each method is sensitive to and reflects different parameters. At lower ionic strengths (where phosphorylated myosin is filamentous), the ATPase assays and fluorescence data indicate an interaction with phosphorylated myosin. The detection of the tropomyosin–myosin complex relied on the modification of some of the properties of smooth muscle myosin. Tropomyosin altered Mg^{2+}-ATPase activity of myosin and HMM, modified the viscosity of dephosphorylated myosin, and altered the viscosity pattern for the transition from dephosphorylated to phosphorylated myosin. Thus, it is important to emphasize that the direct interaction of the two proteins can influence the biological profile of myosin.

These results raise some questions: (1) What are the effects of the interaction? The viscosity data are consistent with the idea that tropomyosin facilitates the 10S to 6S transition and affects that part of the transition that is critical in modifying ATPase activity, possibly the flexibility of the head–neck junction (Ikebe et al., 1988). Tropomyosin changes the relationship between phosphorylation and conformational change (i.e., viscosity; this study) and alters the phosphorylation dependence of actin-activated ATPase activity (Merkel et al., 1984). Our interpretation of the nonlinear relationships observed in the absence of tropomyosin is that these reflect cooperative interactions between the two myosin heads (Persechini & Hartshorne, 1981; Ikebe et al., 1983). Activation of ATPase activity by tropomyosin, therefore, may be due to the partial cancellation of the negative cooperativity thought to exist in the smooth muscle myosin molecule. (2) Can the interaction occur in the presence of actin? This is obviously a critical point. The tropomyosin–myosin interaction is not suggested to be competitive with the formation of the actin–tropomyosin complex. Clearly, the binding of tropomyosin to actin is of a higher affinity, but the interesting point is whether the tropomyosin bound to F-actin can also interact with myosin. Our results indicate that this possibility should be considered. The position of tropomyosin relative to S1 has not been unambiguously assigned, but from three-dimensional image analyses of skeletal muscle thin filaments decorated with S1, one possibility for the tropomyosin location is in proximity to the actin–S1 binding site [for discussions of various models, see Toyoshima and Wakabayashi (1985) and El-Saleh et al. (1986)]. The cross-linking results of Tao and Lamkin (1984) would also indicate this. From our data, the similarity in patterns of activation of ATPase activity in the presence and absence of actin would suggest a similar mechanism, i.e., that activation in both instances is due to an interaction of tropomyosin with myosin. The effect of tropomyosin on the phosphorylation dependence of conformational change and actin-activated ATPase might also reflect the same underlying mechanism. In order to maximize the possibility of interaction between myosin and tropomyosin, it would also follow that thin filaments saturated with tropomyosin (i.e., a stoichiometry of 1:7 for tropomyosin/actin) would most effectively activate Mg^{2+}-ATPase activity of myosin, and this has been observed by Miyata and Chacko (1986). The lack of activation of ATPase activity by tropomyosin of S1 alone and acto–S1 indicates that the S1–tropomyosin interaction does not occur. However, if activation of the actin-activated ATPase is due entirely to effects on actin, then an enhancement of acto–S1 ATPase activity by tropomyosin might still be expected.

The final point that should be raised is whether the tropomyosin–myosin interaction is peculiar to smooth muscle or occurs in other cells, notably striated muscle. At the moment, this question cannot be answered. The situation with striated muscle is more complex because of the presence of troponin, and a number of models have been proposed to describe the role of tropomyosin in the regulatory process [reviewed in El-Saleh et al. (1986)]. In addition, skeletal muscle (rabbit) and smooth muscle (gizzard) tropomyosins have slightly different properties as well as differences in sequence (Lau et al., 1985; Sanders & Smillie, 1985). In general, gizzard tropomyosin has a greater tendency to activate ATPase activity than rabbit tropomyosin (Sobieszek & Small, 1981; Lehrer & Morris, 1984) although both tropomyosins will show inhibition and activation of activity depending on the S1 concentration (Lehrer & Morris, 1982, 1984). Also, skeletal muscle myosin does not show the same conformational transitions (i.e., 10 S to 6 S) as smooth muscle myosin, and the effects of phosphorylation are less pronounced. Thus, there are many differences between the two systems, but it is nevertheless an intriguing possibility that the interaction of myosin and tropomyosin in striated muscle may modify enzymatic activity and provide an additional flexibility for the regulatory mechanism.

Registry No. ATPase, 9000-83-3.

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Glycation of Calmodulin: Chemistry and Structural and Functional Consequences†

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ABSTRACT: In the presence of Ca^{2+} and glucose, calmodulin incorporates 2.5 mol of glucose/mol of protein. In the absence of Ca^{2+}, only 1.5 mol of glucose is incorporated per mole of calmodulin. Glycation of calmodulin is associated with variable reductions in its capacity to activate three Ca^{2+}/calmodulin-dependent brain target enzyme systems, including adenylyl cyclase, phosphodiesterase, and protein kinase. In addition, glycated calmodulin exhibits a 54% reduction in its Ca^{2+} binding capacity. Isolated CNBr cleavage fragments of glycated calmodulin suggest that glycation follows a nonspecific pattern in that each of seven available lysines is susceptible to modification. A limit observed on the extent of glycation appears related to the accompanying increase in negative charge on the protein. Glycation results in minimal structural rearrangements in calmodulin, and the Ca^{2+}-induced increase in α-helix content and radius of gyration is the same for glycated and unmodified calmodulin. Since glycated calmodulin's Ca^{2+} binding capacity is reduced, this implies that the Ca^{2+}-induced conformational changes in calmodulin do not require all four Ca^{2+} binding sites to be occupied. Examination of the lysine positions in calmodulin suggests that Ca^{2+} binding to domains II and IV is sufficient to induce these changes. The functional consequences of calmodulin glycation therefore cannot be attributed to inhibition of these conformational changes. An alternative explanation is that the inhibition arises from interference at the target enzyme binding site by bound glucose. While glycation shows minimal structural effects, a large pH dependence is observed for the α-helix content of unmodified calmodulin. It is suggested that pH, as well as ionic strength and Ca^{2+} concentration, may be important in stabilizing the crystal form of the protein.

Since the earliest descriptions of nonenzymatic glycation, there has been interest in the possibility that this covalent modification of protein lysine residues and N-terminal amino moieties might alter the function, regulation, or recognition of the affected proteins (Brownlee & Cerami, 1981; Bunn, 1981; Brownlee et al., 1984). The glycation reaction proceeds through a Schiff base intermediate which undergoes Amadori rearrangement to a stable ketoamine. There is an accompanying increase in the net negative charge of the recipient protein (Bitensky et al., 1988). Because the rate of protein glycation is a function of glucose concentration, the reaction occurs more rapidly in the diabetic milieu and might contribute to the long-term histopathological complications of diabetes mellitus (Vlassara et al., 1986). Several proteins have been

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