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Modulation of the Actin-Activated Adenosinetriphosphatase Activity of Myosin by Tropomyosin from Vascular and Gizzard Smooth Muscles†

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ABSTRACT: Tropomyosins from bovine aorta and pulmonary artery exhibit identical electrophoretic patterns in sodium dodecyl sulfate but differ from tropomyosins of either chicken gizzard or rabbit skeletal muscle. Each of the four tropomyosins binds readily to skeletal muscle F-actin as indicated by their sedimentation with actin and by their ability to maximally stimulate or inhibit actin-activated ATPase activity at a molar ratio of one tropomyosin per seven actin monomers. Smooth and skeletal muscle tropomyosins differ in their effects on activity of skeletal myosin or heavy meromyosin (HMM); the former can enhance activity under conditions in which the latter inhibits. Gizzard and arterial tropomyosins are usually equally effective in stimulating ATPase activity of skeletal acto-HMM, but at high concentrations of Mg^{2+} gizzard tropomyosin is more effective, a result that cannot be attributed to differences in the binding of the two tropomyosins to F-actin. The effects of tropomyosin also depend on the type of myosin; tropomyosin enhances activity of gizzard myosin under conditions in which it inhibits that of skeletal myosin. Increasing the pH or the Mg^{2+} concentration can reverse the effect of tropomyosin on actin-stimulated ATPase activity of skeletal HMM from activation to inhibition, but this reversal is not found with gizzard myosin. Activity in the absence of tropomyosin is independent of pH, and the loss of activation with increasing pH is not accompanied by loss of binding of tropomyosin to actin.

In skeletal muscle, tropomyosin plays a direct role in Ca^{2+}-dependent regulation of the ATPase[1] activity of actomyosin, acting together with troponin to inhibit activity in the absence but not in the presence of Ca^{2+} (Ebashi et al., 1969). Many of the structural studies on Ca^{2+}-dependent regulation (Haselgrove, 1972; Huxley, 1972; Taylor & Amos, 1981) indicate that in the absence of Ca^{2+}, tropomyosin moves to a position on the actin filament where it sterically blocks the binding of the myosin head. Recent biochemical findings show that at very low ionic strengths removal of Ca^{2+} produces inhibition of acto-S-1 catalyzed hydrolysis of ATP but does not inhibit the binding of S-1 to regulated actin (Chalovich et al., 1981; cf. Wagner & Giniger, 1981), a finding not readily explained by a simple steric blocking model.

In smooth muscle the role of tropomyosin is less clear. Smooth muscle tropomyosin stimulates rather than inhibits activity (Chacko et al., 1977; Hartshorne et al., 1977; Ebashi et al., 1977; Sobieszek & Small, 1977) and according to most reports plays no direct role in Ca^{2+}-dependent activation of actomyosin ATPase activity, which requires enzymatic phosphorylation of the myosin light chain (Chacko et al., 1977; Gorecka et al., 1976; Sobieszek & Small, 1976). A possible link to Ca^{2+}-dependent regulation in smooth muscle is suggested by the requirement of both Ca^{2+} and tropomyosin for optimal actin-activated ATPase activity with phosphorylated smooth muscle myosin (Chacko et al., 1977; Chacko & Rosenfeld, 1982; Nag & Seidel, 1983), while a report of a similar requirement with unphosphorylated myosin (Ebashi et al., 1977) has not yet been confirmed.

In addition to stimulating the actomyosin ATPase of smooth muscle, tropomyosin activates or potentiates the activity of several other actomyosin systems including those of rabbit skeletal muscle (Bremel et al., 1972; Shigekawa & Tonomura, 1972) and Limulus (Lehman & Szent-Gyorgyi, 1972). The resemblence of the activation by tropomyosin in skeletal and smooth muscle (Bremel et al., 1972; Chacko et al., 1977;

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[1] Abbreviations: ATPase, adenosinetriphosphatase; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; HMM, heavy meromyosin; MOPS, 3-(*N*-morpholino)propanesulfonic acid; S-1, subfragment 1; TM, tropomyosin; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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Hartshorne et al., 1977; Ebashi et al., 1977; Sobieszek & Small, 1977) suggests that similar molecular events underlie activation in the two systems (Sobieszek & Small, 1981; Sobieszek, 1982). On the one hand, tropomyosin from skeletal muscle can stimulate actin-activated ATPase activity of gizzard myosin (Hartshorne et al., 1977), while on the other, gizzard tropomyosin can confer Ca^{2+} sensitivity on skeletal actomyosin in the presence of troponin (Darbrowska et al., 1980), suggesting basic similarities between the two tropomyosins in terms of structure and function.

In comparing the contractile proteins from gizzard and arterial smooth muscles we observed that tropomyosin from these two sources differed in their electrophoretic properties and in their effects on ATPase activity. The present studies were carried out to compare the effects of gizzard, arterial and skeletal muscle tropomyosins on activity and to obtain insight into the activation by tropomyosin of ATPase activity in smooth muscle.

Materials and Methods

Tropomyosin was prepared from chicken gizzard and bovine thoracic aorta or main pulmonary artery by extraction from ethanol–ether or acetone powders and was purified by standard procedures (Bailey, 1948; Cummins & Perry, 1973). To prepare the acetone powder, tissues were passed through a meat grinder, in the case of aorta the adventitial layer was first removed, and the minced muscle was washed 3 times with a solution containing 0.1 M KCl, 1 mM $NaHCO_3$, and 10 μM phenylmethanesulfonyl fluoride. The washed tissue was extracted with 2 volumes of a solution containing 0.3 M KCl, 0.15 M potassium phosphate, pH 6.5, 1 mM ATP, 10 μM phenylmethanesulfonyl fluoride, and 0.5 mM dithiothreitol for 16 h at 4 °C. The extract was centrifuged at 10000g for 10 min, and the residue was washed once with water, twice with 0.4% $NaHCO_3$, 4 times with H_2O, and 3 times with chilled acetone and dried at room temperature.

Myosin from rabbit skeletal muscle (Nauss et al., 1969) or chicken gizzard (Sobieszek & Small, 1976) and actin from rabbit skeletal muscle (Spudich & Watt, 1971) were prepared by standard procedures. HMM was prepared from skeletal myosin by digestion with chymotrypsin (Weeds & Pope, 1977). Light chain kinase was prepared from chicken gizzard as described by Aksoy et al. (1976), the preparation being carried out through the isoelectric precipitation step and found to be free of tropomyosin by gel electrophoresis in $NaDodSO_4$. Protein concentrations were determined by the biuret method except for tropomyosin which was determined by using an absorption of 0.24 for a 1 mg/mL solution at 277 nm.

ATPase activity was measured by determination of orthophosphate released from ATP (Fiske & SubbaRow, 1925) or with the use of a pH stat. Unless otherwise noted incubations from 5 to 20 min were carried out at 25 °C in a medium containing 45 mM KCl, 20 mM MOPS, pH 6.8 or 7.5, 0.2 mM EGTA, 2 mM ATP, 0.5 mg/mL rabbit skeletal F-actin, 0.14 mg/mL tropomyosin, and 0.2–0.5 mg/mL myosin or 0.1–0.4 mg/mL HMM with $MgCl_2$ as indicated. The free concentrations of magnesium as indicated by Mg^{2+} and given throughout the text have been calculated as the difference between the total concentration of $MgCl_2$ and the total concentration of ATP. The total magnesium concentrations are given as $[MgCl_2]$.

To measure the binding of tropomyosin to F-actin, rabbit skeletal muscle F-actin was mixed with tropomyosin in the ATPase assay medium and sedimented at 130000g for 2 h, the supernatant fractions were removed, the pellets were resuspended in the solution used for the ATPase assay, and both fractions were subsequently analyzed by gel electrophoresis in $NaDodSO_4$. Polyacrylamide gel electrophoresis was carried out in $NaDodSO_4$ on 12% slab gels with 3% stacking gels by using the discontinous Tris–glycine buffer system (Laemmli, 1970).

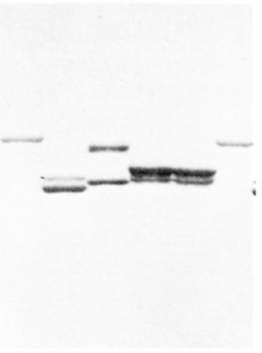

FIGURE 1: Polyacrylamide gel electrophoresis of purified tropomyosin in $NaDodSO_4$: (A and F) actin, (B) skeletal muscle tropomyosin, (C) gizzard tropomyosin, (D) pulmonary artery tropomyosin, and (E) aorta tropomyosin. Each channel contained 5 μg of actin or 20 μg of tropomyosin.

Results

Gel Electrophoresis of Tropomyosins from Bovine Arteries. Tropomyosins from bovine aorta or pulmonary artery exhibit two bands on gel electrophoresis in $NaDodSO_4$, the slower moving β chain having greater staining intensity (Figure 1). These two tropomyosins cannot be distinguished by migration rates or relative staining intensities of their polypeptide chains, but they differ from those of rabbit skeletal muscle where the α chain predominates (Cummins & Perry, 1973) and from gizzard where both chains have about equal intensities (Cummins & Perry, 1974). On electrophoresis of tropomyosin mixtures only the α chain of arterial tropomyosin and the β chain of skeletal tropomyosin appear to comigrate.

The possibility that differences between the arterial tropomyosins and those of gizzard and skeletal muscle might be the result of proteolysis during preparation was tested by mixing purified tropomyosins with homogenates of the corresponding arterial muscle or with extracts prepared from the muscles by using solutions containing 0.1 M NaCl. These mixtures were incubated for 18 h at 4 °C, and subsequent gel electrophoresis in $NaDodSO_4$ revealed no change in either the rates of migration or the relative staining intensities of the two tropomyosin bands. We also observed no change in the electrophoretic patterns of any of the tropomyosins after reduction and reaction with iodoacetamide.

Effects of Tropomyosins on the Actin-Activated ATPase Activity of Gizzard and Skeletal Myosins. The actin-activated ATPase activity of gizzard myosin is enhanced equally by each of the four tropomyosins (Figure 2), reaching the same maximal activities at tropomyosin to actin ratios of 1/7. Activities with smooth muscle tropomyosins increase almost linearly with concentration, while activity with skeletal tropomyosin shows some indication of a sigmoidal behavior (cf. Hartshorne et al., 1977). In contrast, the activity of skeletal myosin is inhibited by skeletal tropomyosin and enhanced by gizzard and arterial tropomyosins (Table I). Thus, the qualitative response of activity to tropomyosin depends not only on the source of tropomyosin but also on the source of the myosin.

Effects of Tropomyosin on Activity of Skeletal HMM. A significant difference between the effects of gizzard and arterial

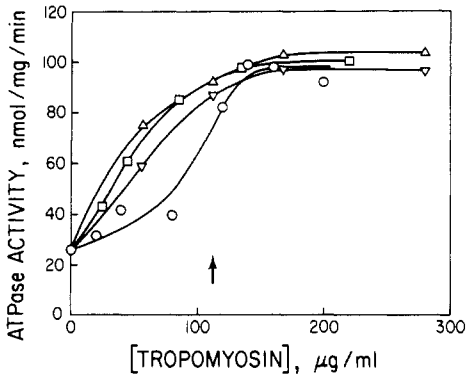

FIGURE 2: Effect of tropomyosin on ATPase activity of gizzard myosin in the presence of F-actin. Assays were carried out for 20 min at 25 °C in a medium containing 45 mM NaCl, 10 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.4 mM dithiothreitol, 2 mM ATP, 20 mM Tris, pH 7.5, 0.5 mg/mL myosin, 0.5 mg/mL F-actin, and 85 μg/mL light chain kinase. Arrow indicates 1/7 molar ratio of tropomyosin to actin. (○) Skeletal muscle tropomyosin; (□) gizzard tropomyosin; (△) aorta tropomyosin; (▽) pulmonary tropomyosin.

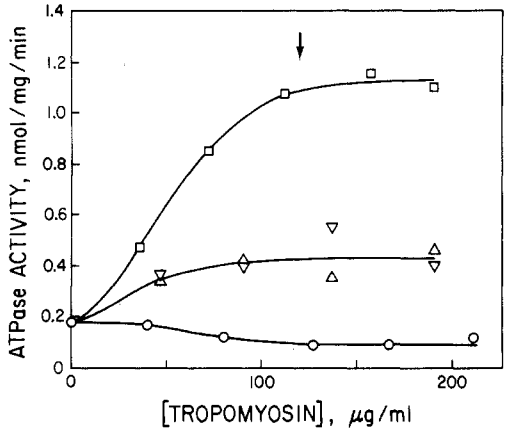

FIGURE 3: Effect of tropomyosin on ATPase activity of acto-HMM. Assays were carried out as described under Materials and Methods for 5 min at 30 °C in a medium containing 10 mM $MgCl_2$, 20 mM MOPS, pH 6.8, 0.1 mg/mL HMM, and 0.5 mg/mL F-actin. Arrow indicates 1/7 molar ratio of tropomyosin to actin. Symbols as indicated in the legend to Figure 2.

Table I: Effects of Tropomyosin on Actin-Activated ATPase Activity of Skeletal Muscle Myosin[a]

	P_i (μmol mg^{-1} min^{-1})	
	3 mM $MgCl_2$	10 mM $MgCl_2$
no TM	0.25	0.076
gizzard TM	0.72	0.125
aorta TM	0.87	0.122
pulmonary TM	0.78	0.091
skeletal TM	0.31	0.036

[a] ATPase assays were carried out in suspensions containing 45 mM KCl, 20 mM MOPS, pH 6.8, 3 or 10 mM $MgCl_2$, 0.2 mM EGTA, 2 mM ATP, 0.2 mg/mL myosin, 0.5 mg/mL F-actin, and 0.14 mg/mL tropomyosin. Activity in the absence of actin with 10 mM $MgCl_2$ was less than 0.01 μmol mg^{-1} min^{-1}.

tropomyosins is observed with skeletal HMM at 10 mM $MgCl_2$ (Figure 3), the former producing a much greater activation than the latter. This does not reflect a difference in the dependence of activity on tropomyosin concentration, since activity with each tropomyosin reaches a plateau at a 1/7 molar ratio with little or no further enhancement, indicating that weaker binding of the arterial protein cannot account for the smaller increase in activity (Figure 3). This conclusion is supported by direct binding studies described below.

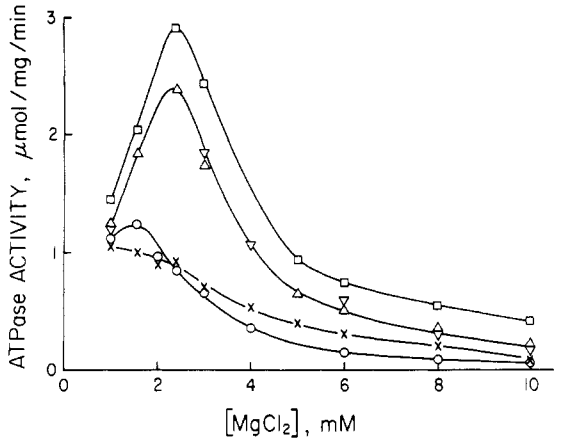

FIGURE 4: Dependence on $MgCl_2$ of the effects of tropomyosin on ATPase activity of acto-HMM. Assays were carried out as described under Materials and Methods for 5 or 10 min at pH 6.8 and 30 °C by using 0.1 mg/mL HMM, 0.5 mg/mL F-actin, and 0.14 mg/mL tropomyosin. The abscissa indicates the total concentration of $MgCl_2$ added. (×) No tropomyosin; (○) skeletal tropomyosin; (□) gizzard tropomyosin; (△) aorta tropomyosin; (▽) pulmonary tropomyosin.

Table II: Dependence of ATPase Activity of Skeletal Muscle Acto-HMM on the Concentration of Mg^{2+} at High pH[a]

$[MgCl_2]$ (mM)	P_i (μmol mg^{-1} min^{-1})		
	no TM	pulmonary TM	skeletal TM
1	0.85	0.93	0.44
2	0.65	0.71	0.28
3	0.53	0.51	0.26
4	0.48	0.35	0.22
6	0.38	0.25	0.16

[a] Activity was measured in a pH stat at pH 7.85 in a solution containing 45 mM KCl, 2 mM ATP, 0.2 mM EGTA, 0.29 μM HMM, 12 μM F-actin, and 2 μM tropomyosin. The time course of ATP hydrolysis was followed with the pH stat, and the hydrolytic rates were plotted against the concentration of ATP remaining at various times, calculated from the volume of titrant added. There is a concomitant increase in the concentrations of ADP and P_i as the ATP concentration decreases.

Dependence of the Effects of Tropomyosin on Mg^{2+} and pH. Gizzard and arterial tropomyosins stimulates the ATPase activity of acto-HMM about equally well at low concentrations of $MgCl_2$ but differ at higher concentrations, where the gizzard protein produces a 3-fold greater increase (Figure 4). Skeletal tropomyosin produces only a slight increase in activity when the $MgCl_2$ concentration is less than that of ATP but becomes inhibitory with $MgCl_2$ in excess.

The extent of activation by smooth muscle tropomyosin decreases with increasing pH, the stimulation seen at pH 6.5 being lost at 7.5 (Figure 5A) and activity with skeletal tropomyosin being lower but following the same trend. Over this range, activity in the absence of tropomyosin is independent of pH. At an appropriate value of pH or $[Mg^{2+}]$, increasing the other variable reverses the effect of tropomyosin from activation to inhibition. This is illustrated with increasing pH in Figure 5B, and upon increasing $[Mg^{2+}]$ at a constant pH of 7.1, the gizzard protein increases activity from 0.22 to 0.32 μmol mg^{-1} min^{-1} with 4 mM Mg^{2+} but decreases it from 0.13 to 0.10 with 6 mM Mg^{2+}. This type of reversal is also seen with skeletal tropomyosin but at lower Mg^{2+} concentrations (Figure 4). However, it is seen only with skeletal myosin or HMM (Table II), with gizzard myosin activation only being found (Figure 2).

Activation at Low Concentrations of ATP. Activation by tropomyosin can be induced by nucleotide-free S-1 as reflected

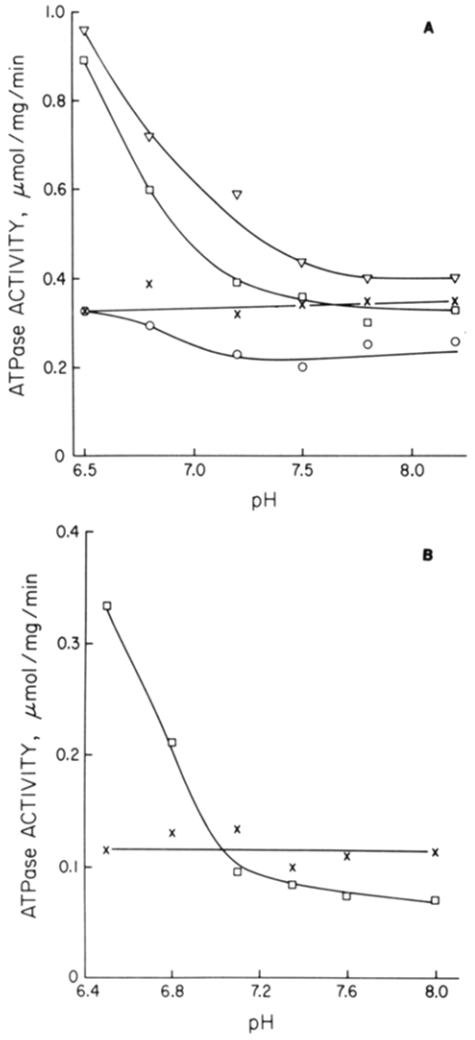

FIGURE 5: Dependence on pH of the effects of tropomyosin on ATPase activity of acto-HMM. Assays were carried out as described under Materials and Methods for 5 min at 25 °C with 30 mM KCl and (A) 3 mM $MgCl_2$ and 0.1 mg/mL HMM or (B) 8 mM $MgCl_2$ and 0.3 mg/mL HMM. Symbols as indicated in the legend to Figure 4.

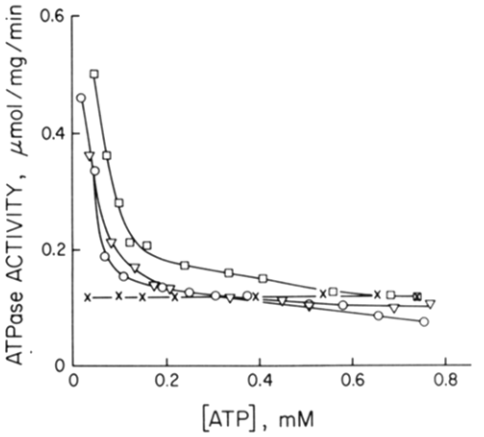

FIGURE 6: Dependence of the ATPase activity of acto-HMM on the extent of hydrolysis of ATP with and without tropomyosin. Activity was measured in a pH stat under conditions described under Materials and Methods at pH 7.85 and 20 °C with 4.5 mM $MgCl_2$, 50 mM NaCl, 0.35 mg/mL HMM, 0.2 mg/mL F-actin, and 0.056 mg/mL tropomyosin. Symbols as indicated in the legend to Figure 4.

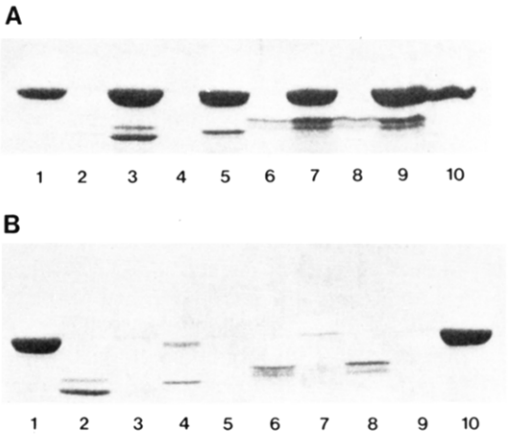

FIGURE 7: Binding of tropomyosin to F-actin in the presence of 10 mM $MgCl_2$ and 2 mM ATP. Binding was measured under conditions described in the legend to Figure 3 with 0.5 mg/mL F-actin and 0.14 mg/mL tropomyosin without HMM, by sedimentation and analysis of supernatant and pellet fractions by gel electrophoresis in $NaDodSO_4$, as described under Materials and Methods. Pellets were resuspended in half the volume of the supernatant fraction, and 30 μL of each sample was applied to 0.7 mm thick gels. (A) Pellets; (B) supernatant fractions. (1) F-Actin, not sedimented; (2) skeletal tropomyosin; (3) skeletal tropomyosin plus F-actin; (4) gizzard tropomyosin; (5) gizzard tropomyosin plus F-actin; (6) pulmonary tropomyosin; (7) pulmonary tropomyosin plus F-actin; (8) aorta tropomyosin; (9) aorta tropomyosin plus F-actin; (10) F-actin, not sedimented.

in an increase in activity at low concentrations of ATP (Bremel et al., 1972), gizzard tropomyosin producing the same effect as skeletal tropomyosin (Sobieszek, 1982). Experiments were carried out to compare the three tropomyosins at low ATP concentrations, in part to determine whether their effects measured with 2 mM ATP might involve depletion of substrate. Above 0.2 mM ATP, all three tropmyosins have little or no effect on ATPase activity of skeletal HMM, and there is very little dependence on ATP concentration, while below 0.2 mM all three produce substantial activation (Figure 6). That the rates of ATP hydrolysis remain constant with time, with and without gizzard tropomyosin, is consistent with this conclusion.

Binding of Tropomyosins to F-Actin. These experiments were designed to determine whether differences among the effects of various tropomyosins on activity, specifically the much larger stimulation by gizzard than by arterial tropomyosin (Figure 3), could be attributed to differences in binding to actin. Virtually all tropomyosin sedimented with F-actin under these conditions (Figure 7A). The small amount of arterial tropomyosin, probably not more than 10%, that did not sediment and a small amount, probably less than 20% that sedimented without actin might lead to an overestimate of the amount bound, but even if 30% of the total were not bound, the difference in binding could not account for the 3-fold difference in activity (Figure 3). Although these experiments were carried out without HMM, an experiment done in the presence of HMM produced the same results.

A similar experiment, carried out with gizzard and pulmonary tropomyosins in the presence of 3 mM $MgCl_2$ at pH 7.8, conditions where neither tropomyosin affects activity (Figure 5A), shows that both tropomyosins bind essentially completely to F-actin (Figure 8). A small amount of actin remained in the supernatant fractions (lanes 1 and 2, Figure 8B), migrating at the same position as the slower moving chain of gizzard tropomyosin.

Discussion

The present results indicate the existence of two electrophoretically distinct types of smooth muscle tropomyosin, from chicken gizzard and bovine arteries, which also differ from skeletal tropomyosin. The differences in electrophoretic be-

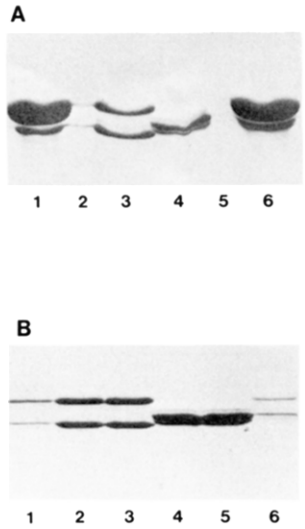

FIGURE 8: Binding of tropomyosin to F-actin in the presence of 3 mM $MgCl_2$ and 2 mM ATP. Binding was measured under conditions described in the legend to Figure 5A with 0.5 mg/mL F-actin and 0.14 mg/mL tropomyosin without HMM, by sedimentatin and gel electrophoresis as described under Materials and Methods. Pellets were resuspended in the same volume as the supernatant fraction, and 25 μL of each sample was applied to 0.7 mm thick gels. (A) Pellets; (B) supernatant fractions. (1) F-Actin plus gizzard tropomyosin; (2) gizzard tropomyosin; (3) gizzard tropomyosin, not sedimented; (4) pulmonary tropomyosin, not sedimented; (5) pulmonary tropomyosin; (6) F-actin plus pulmonary tropomyosin.

havior of gizzard and arterial tropomyosins cannot be attributed to proteolytic fragmentation or oxidation, nor is it likely that they reflect differences in the state of phosphorylation since phosphorylated and dephosphorylated forms of tropomyosin are not readily separable by electrophoresis in Na-$DodSO_4$ (Mak et al., 1978; Montarras et al., 1982). Tropomyosins from gizzard and uterus differ in electrophoretic mobility, the latter exhibiting a single band migrating in the region of the faster moving gizzard band (Cummins & Perry, 1974). Since these electrophoretic measurements were carried out under different conditions than used in our experiments, the possibility that uterine and arterial tropomyosins might have similar or identical electrophoretic mobilities cannot be excluded.

Other differences between the protein components of vascular and nonvascular smooth muscles have also been reported. β and γ forms of actin have been found in nonvascular smooth muscles (Rubenstein & Spudich, 1977), while vascular muscles contain a variant of α-actin (Gabbiani et al., 1981; Frank & Warren, 1981). Amino acid sequences of actins from chicken gizzard and bovine aorta differ by three amino acid residues, all in the N-terminal region (Vandekerckhove & Weber, 1978, 1979). Intermediate filaments in nonvascular smooth muscle contain desmin as the predominant protein while vimentin predominates in at least some vascular muscles (Gabbiani et al., 1981). However, comparison of different smooth muscles using antibodies against these two proteins shows that a substantial number of blood vessels contain both proteins (Gabbiani et al., 1981; Berner et al., 1981).

High concentrations of S-1 favor the potentiated state; i.e., tropomyosin tends to activate at high ratios of S-1 to actin and inhibit at low ratios (Bremel et al., 1972; Murray et al., 1981, 1982; Sobieszek, 1982; Lehrer & Morris, 1982). This had originally been interpreted in terms of the steric blocking model (Haselgrove, 1972; Huxley, 1972), in which S-1 displaces tropomyosin from an inhibitory position on the actin filament (Bremel et al., 1972). However, displacement of tropomyosin, by itself, may not fully account for the activation, as suggested by a 2-fold increase in maximal velocity of ATPase activity of skeletal acto-S-1 or acto-HMM, produced by skeletal (Murray et al., 1982) or gizzard tropomyosin (Sobieszek, 1982). The potentiated state in the skeletal system is also accompanied by increased binding constants for S-1 and the S-1–ADP complex, although the extent to which this might involve movement of tropomyosin is not certain (Murray et al., 1982). The fact that the activity of gizzard myosin can be activated by tropomyosin while that of skeletal myosin is inhibited suggests that the former is more effective than the latter in producing the potentiated state. This may be directly related to the fact that gizzard S-1 has a greater affinity for actin than does skeletal S-1, at ionic strengths of 0.05 M or greater (Greene et al., 1983), which have been used in the present experiments. At lower ionic strengths, gizzard HMM or S-1 bind less strongly than the corresponding skeletal muscle proteins (Ikebe et al., 1981; Greene et al., 1983).

Two distinct states of the actin–tropomyosin complex having different affinities for S-1 have been assumed (Hill et al., 1980) to explain the cooperativity in the binding of S-1 to actin (Greene & Eisenberg, 1980); the state having higher affinity presumably corresponds to the potentiated state (Murray et al., 1982). A more detailed version of this model including some of the steps in the enzymatic hydrolysis of ATP (Hill et al., 1981) has been shown to simulate the sigmoidal dependence of ATPase activity on S-1 concentration (Lehrer & Morris, 1982). At least two states of the actin–tropomyosin complex producing different maximal ATPase activities of S-1 seem to be necessary to explain the ability of tropomyosin to activate or to inhibit (Murray et al., 1982; Lehrer & Morris, 1982). The present results, particularly the fact that tropomyosin remains bound to actin whether it activates, inhibits, or has no effect on activity, seem to be readily interpretable in terms of two such states, characterized by different values of V_{max} (Murray et al., 1982; Sobieszek, 1982) and different affinities for S-1 (Murray et al., 1982; Greene & Eisenberg, 1980).

The effects of tropomyosin on ATPase activity and on the binding of tropomyosin to F-actin are known to depend on the concentration of $MgCl_2$ (Eaton et al., 1975; Wegner, 1979; Yang et al., 1979). In the absence of KCl, Mg^{2+} concentrations approaching 5 mM are necessary for inhibition of acto-S-1 ATPase activity by skeletal tropomyosin and for the binding of tropomyosin to F-actin (Eaton et al., 1975; Wegner, 1979; Yang et al., 1979). Smooth muscle tropomyosins can activate the ATPase activity of acto-HMM at concentrations of $MgCl_2$ equal to or less than that of ATP (Figure 4; Nag & Seidel, 1983), but activation at these low concentrations may be in part attributable to the presence of 50 mM NaCl in the assay medium, which reduces the concentration of Mg^{2+} required for tropomyosin binding (Eaton et al., 1975; Yang et al., 1979). Increasing the concentration of $MgCl_2$—or the pH as discussed below—tends to reverse the effect to tropomyosin from stimulation to inhibition, as previously observed with cardiac tropomyosin (Leger et al., 1979). The present results indicate that higher Mg^{2+} concentrations are required for inhibition of ATPase activity than for activation.

The response of ATPase activity to increases in pH is similar to the response to increases in the Mg^{2+} concentration. The reversal of the effect of tropomyosin from activation to inhibition with increasing pH is of particular interest in that it occurs over a range of pH in which activity without tropomyosin does not change. This reversal cannot involve dissociation of tropomyosin from actin, since tropomyosin binds strongly throughout the entire pH range, but it could result

from changes in the affinity of the myosin head for actin, changes in the rate of one or more steps in ATP hydrolysis, or a shift in an equilibrium between two states of the actin–tropomyosin complex. Although there is no direct evidence permitting a choice among these alternatives, the last possibility would provide a straightforward explanation for the fact that activity depends on pH only in the presence of tropomyosin. The situation upon increasing the concentration of Mg^{2+} presumably is more complex because Mg^{2+} decreases activity in the absence as well as in the presence of tropomyosin.

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