Localization and Topography of Antigenic Domains within the Heavy Chain of Smooth Muscle Myosin

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ABSTRACT We have produced and characterized monoclonal antibodies that label antigenic determinants distributed among three distinct, nonoverlapping peptide domains of the 200-kD heavy chain of avian smooth muscle myosin. Mice were immunized with a partially phosphorylated chymotryptic digest of adult turkey gizzard myosin. Hybridoma antibody specificities were determined by solid-phase indirect radioimmunoassay and immunoreplica techniques. Electron microscopy of rotary-shadowed samples was used to directly visualize the topography of individual [antibody·antigen] complexes.

Antibody TGM-1 bound to a 50-kD peptide of subfragment-1 (S-1) previously found to be associated with actin binding and was localized by immunoelectron microscopy to the distal aspect of the myosin head. However, there was no antibody-dependent inhibition of the actin-activated heavy meromyosin ATPase, nor was antibody TGM-1 binding to actin–S-1 complexes inhibited. Antibody TGM-2 detected an epitope of the subfragment-2 (S-2) domain of heavy meromyosin but not the S-2 domain of intact myosin or rod, consistent with recognition of a site exposed by chymotryptic cleavage of the S-2:light meromyosin junction. Localization of TGM-2 to the carboxy-terminus of S-2 was substantiated by immunoelectron microscopy. Antibody TGM-3 recognized an epitope found in the light meromyosin portion of myosin. All three antibodies were specific for avian smooth muscle myosin. Of particular interest is that antibody TGM-1, unlike TGM-3, bound poorly to homogenates of 19-d embryonic smooth muscles. This indicates the expression of different myosin heavy chain epitopes during smooth muscle development.

The structural organization of myosin molecules is conserved throughout evolution and among myosins of disparate tissue origin. A pair of heavy chains comprises both carboxy-terminal α-helical domains that form a coiled-coil "tail" and amino-terminal domains that form two globular "heads." Each head is associated with a regulatory and an essential light chain. Interaction of myosin heads with filamentous actin, activation by actin of the Mg^{2+}-ATPase of the myosin molecule, and functional coupling between ATP hydrolysis and force generation are essential and ubiquitous processes for contraction and other motile events in muscle and non-muscle cells (1). In vertebrate striated muscles, myosin polymorphism is apparent not only for light chains characteristic of specific muscle classes, fiber types, and developmental stages (2–4), but also for myosin heavy chains of skeletal (5–9) and cardiac (10–12) muscle myosin. In contrast, the structural and functional organization and development of myosin in vertebrate smooth muscle have received relatively little study. We have produced a series of monoclonal antibodies (mAb)[1] directed against antigenic determinants (epitopes) of smooth muscle myosin, as probes for such investigations.

We report here results obtained with hybridoma antibodies that recognize three distinct nonoverlapping domains of the heavy chain of avian smooth muscle myosin: heavy meromyosin (HMM) subfragment-1 (S-1), subfragment-2 (S-2),

[1] *Abbreviations used in this paper*: HMM, heavy meromyosin; ^{125}I-F(ab')$_2$, ^{125}I-labeled F(ab')$_2$ fragment of affinity-purified sheep IgG directed against mouse immunoglobulins; LC_{17}, 17-kD myosin light chain; LC_{20}, 20-kD myosin light chain; LMM, light meromyosin; mAb, monoclonal antibody; ^{32}P-LC_{20}, LC_{20} radiophosphorylated by myosin light chain kinase; S-1, HMM subfragment-1; S-2, HMM subfragment-2.

and light meromyosin (LMM). We have studied functional aspects of smooth muscle myosin by assaying for antibody-dependent effects on the actin-activated HMM Mg^{2+}-ATPase and for actin-dependent effects on mAb binding. We have investigated the structure of the smooth muscle myosin heavy chain, not only with its purified peptide domains, immunoblot techniques, and electron microscopy of rotary-shadowed [antibody·antigen] complexes, but also in conjunction with radiophosphorylated myosin light chains (13) as a unique structural probe.

MATERIALS AND METHODS

Materials: Nitrocellulose filtration plates (pore size 0.45 μm, type STHA) were purchased from Millipore Corp. (Bedford, MA), and nitrocellulose membranes (pore size 0.20 μm, type BA83) from Schleicher and Scheull, Inc. (Keene, NH). ^{125}I-labeled F(ab')$_2$ fragment of affinity-purified sheep IgG directed against mouse immunoglobulins [^{125}I-F(ab')$_2$, 8.5 × 10^5 Ci/mol] was purchased from New England Nuclear (Boston, MA). Reagents for sodium dodecyl sulfate ($NaDodSO_4$)-polyacrylamide gel electrophoresis (SDS PAGE) were obtained from Bio-Rad Laboratories (Richmond, CA). Trypsin (3× crystallized, type TRL3), chymotrypsin (3× crystallized, type CDI), and papain (2× crystallized, type PAP) were obtained from Worthington Biochemical Corp. (Freehold, NJ). Initial stocks of P3-X63.Ag8 murine myeloma cells were obtained from Dr. Marshall Nirenberg (National Institutes of Health).

Production of Monoclonal Antibodies: To prepare smooth muscle myosin as immunogen and as antigen for initial screening assays, myosin was purified from adult turkey gizzards (14). Gizzard myosin was phosphorylated by myosin light chain kinase (14) to ~0.5 mol PO_4/mol 20-kD myosin light chain (LC_{20}) and subsequently subjected to limited proteolysis with chymotrypsin (1 mg/100 mg myosin) at 25°C in 500 mM NaCl, 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4. The reaction was stopped after 10 min with the addition of phenylmethylsulfonyl fluoride to 0.5 mM. Female 6-wk-old BALB/c mice were inoculated intraperitoneally at 0 and 8 d with 75 μg of myosin chymotryptic digest in complete Freund's adjuvant, and at 15 d with 75 μg of myosin digest intraveneously without adjuvant. Cell fusion at 18 d and subsequent derivation of hybridoma cell lines (15) were as previously described (16, 17).

Solid-Phase Indirect Radioimmunoassay: Myosin chymotryptic digest (10 μg in 2 μl per well of 0.5 M NaCl, 50 mM HEPES, pH 7.5) was immunobilized by evaporation onto 96-well nitrocellulose filtration plates, reacted sequentially with 50 μl/well of hybridoma-conditioned medium and with 4 nM ^{125}I-F(ab')$_2$. Bound radioactivity was determined by gamma-spectrometry. Hybridoma-conditioned media and ^{125}I-F(ab')$_2$ solutions were centrifuged immediately before use (11,600 *g* for 60 min and 178,000 *g* for 10 min, respectively), which substantially reduced background variance attributed to nonspecifically bound IgG. All results shown [fmol ^{125}I-F(ab')$_2$ specifically bound per well] are corrected both for hybridoma antibody bound to immobilized bovine serum albumin (BSA) and for nonspecifically bound P3-X63.Ag8 IgG [typically 1–1.5% of total ^{125}I-F(ab')$_2$]. Replicates varied by <5%.

Preparation of Tissue Homogenates: Embryonated White Leghorn chick eggs were obtained from Truslow Farms (Chestertown, MD). Portions of 19-d embryonic chick organs were homogenized at 4°C in 5 vol of 40 mM NaCl, 10 mM HEPES, pH 7.5, 1 mM $MgCl_2$, 5 mM EDTA, 1 mM NaN_3, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, with 12 strokes of a motor-driven Potter-Elvehjem homogenizer at the highest setting (Model 5VB-C, Eastern Mixers, Inc., Clinton, CT), adjusted to 0.5 M NaCl, and protein concentration determined (18).

Preparation of Proteins: HMM and LMM were produced from phosphorylated myosin by limited proteolysis with chymotrypsin (1 mg/100 mg myosin) in the presence of ATP and terminated by the addition of 0.5 mM phenylmethylsulfonyl fluoride. HMM then was purified as described by Sellers et al. (19), whereas LMM was purified essentially as described by Margossian and Lowey (20). S-1 and rod were produced by limited digestion of myosin with papain (0.01 U/mg myosin) and were then purified as described by Greene et al. (21) and Margossian and Lowey (20), respectively. S-2 was produced by chymotryptic digestion of myosin rod and purified by the method of Margossian and Lowey (20) with the inclusion of low ionic strength, 10 mM $MgCl_2$ precipitation of contaminating LMM by centrifugation at 40,000 *g* for 20 min. LC_{20} and the 17-kD (LC_{17}) light chains of gizzard myosin were isolated (13), and LC_{20} was radiophosphorylated as described (14). Actin was purified from rabbit skeletal muscle by the method of Spudich and Watt (22) as modified by Eisenberg and Kielley (23). Protein concentrations of myosin subfragments were calculated from absorbance measurements at 280 nm using extinction coefficients reported by Margossian and Lowey (20). Monoclonal IgG was purified by precipitation in 70% $(NH_4)_2SO_4$ followed by ion-exchange chromatography on DEAE-cellulose as described by Parham (24) and was homogeneous by SDS PAGE.

Western Blots: Peptides were resolved by SDS PAGE, performed as described by Laemmli (25) or as modified by Matsudaira and Burgess (26). Electrophoretic transfer of peptides to nitrocellulose was performed according to Towbin et al. (27) at 10 V for 16 h at 4°C in 25 mM Tris-HCl, 192 mM glycine, 20% methanol (pH 7.5). Polypeptides of tissue homogenates were transferred in the presence of 0.1% SDS to ensure transfer of the 200-kD myosin heavy chain. Each nitrocellulose replica was reacted sequentially for 2 h at 4°C with hybridoma-conditioned medium or purified monoclonal IgG (5 μg/ml) and with 0.2 nM ^{125}I-F(ab')$_2$. The distribution of specifically bound monoclonal IgG was determined by autoradiography at −70°C employing Kodak X-Omat AR film and X-Omatic fine intensifying screens. No binding of ^{125}I-F(ab')$_2$ to peptides of purified myosin fragments or of tissue homogenates was detected in the presence of equivalent concentrations of parental P3-X63.Ag8 myeloma IgG or normal mouse IgG.

Low-angle Platinum Shadowing of [mAb·HMM] Complexes: Adult turkey gizzard myosin or HMM at 120 μg/ml in 0.5 M ammonium acetate was incubated with 150 μg/ml of DE52-purified monoclonal IgG for 16 h at 4°C, and diluted to 60 μg/ml of antigen in 50% glycerol immediately before spraying onto freshly cleaved mica. Low-angle rotary shadowing with platinum and subsequent carbon-coating of metal replicas was performed in a Balzers vacuum evaporator (Balzers, Hudson, NH) by the method of Tyler and Branton (28). A Zeiss EM-10 electron microscope (Carl Zeiss, Inc., Thornwood, NY) was employed at 60-kV accelerating voltage.

RESULTS

Specificity for Avian Smooth Muscle Myosin: Purified Myosins

63 of 173 hybridoma colonies assayed 14–24 d after cell fusion produced detectable antibody directed against a chymotryptic digest of partially phosphorylated adult turkey gizzard myosin. We report results obtained with three hybridoma cell lines, each recloned three times at limiting dilution to ensure homogeneity and adapted to growth in roller bottles yielding ~150 mg/liter of monoclonal immunoglobulin. The binding specificity of each mAb was first determined in the presence of 10^{-9}–10^{-5} g/well of myosin purified from adult turkey gizzard, chicken pectoralis, or calf aorta (Fig. 1). Antibodies TGM-1 and TGM-3, under the conditions shown, recognized intact turkey gizzard myosin but neither avian skeletal muscle myosin nor mammalian smooth muscle myosin. Antibody was comparably bound to myosin purified from gizzards of adult turkey versus adult chicken (data not shown). mAb TGM-2, which recognized an epitope of digested gizzard myosin in the screening assay, did not, however,

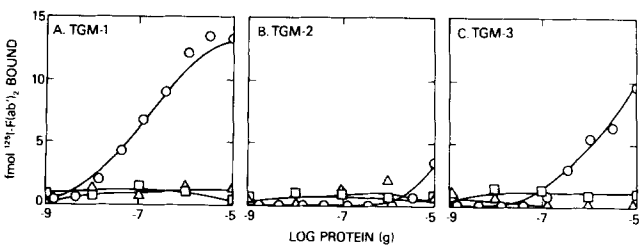

FIGURE 1 Specificity of mAbs for avian smooth muscle myosin. Myosin purified from adult turkey gizzard (○), chick pectoralis (□), or calf aorta (△) was immobilized by evaporation onto 96-well nitrocellulose plates (10^{-9}–10^{-5} g/well) and reacted with hybridoma antibodies by indirect radioimmunoassay. Results [fmol ^{125}I-F(ab')$_2$ specifically bound per well], corrected as discussed in Materials and Methods, are shown for three monoclonal antibodies that bind the chymotryptic gizzard myosin digest used as immunogen: (*A*) TGM-1, (*B*) TGM-2, and (*C*) TGM-3.

bind to intact myosin. None of the antibodies tested bound to rabbit skeletal muscle HMM or to nonmuscle myosin purified from chicken intestinal brush border.

Tissue Homogenates

To determine the tissue specificity of epitopes recognized by antibodies TGM-1 and TGM-3, SDS-denatured homogenates of 19-d chicken embryo tissues were analyzed by indirect radioimmunoassay (Fig. 2). Antibody TGM-3 bound to homogenates of 19-d embryonic chicken gizzard, aorta, and intestine, but not to cardiac ventricle, pectoralis, or cerebellum. The binding of mAb TGM-3 was specific for the 200-kD smooth muscle myosin heavy chain as demonstrated in the accompanying Western blot (Fig. 3). In contrast, using antibody TMG-1, little binding to myosin in homogenates of 19-d embryonic chicken smooth muscle was detected by indirect radioimmunassay (Fig. 2). Results demonstrating binding of each antibody in the presence of purified adult turkey gizzard myosin are included for comparison. Thus, antibody TGM-1 may identify a region of smooth muscle myosin that undergoes developmental regulation and is preferentially expressed in the adult. None of the antibodies bound SDS-denatured homogenates of uterus, stomach, cardiac ventricle, pectoralis, cerebellum, or spleen of adult rat (data not shown), indicating specificity for avian smooth muscle myosin.

Subfragments of Smooth Muscle Myosin Heavy Chain

Binding of each antibody was determined in the presence of purified intact adult turkey gizzard myosin or its purified subunits by indirect radioimmunoassay (Fig. 4) and by Western blot (Fig. 5). mAb TGM-1 identified intact myosin, HMM, and S-1, but not S-2, rod, or LMM. mAb TGM-3 recognized intact myosin, rod, and LMM, but not HMM, S-1, or S-2. Thus, the TGM-1 epitope was localized to S-1, and the TGM-3 epitope to LMM.

mAb TGM-2, which did not bind to intact myosin, bound to HMM and S-2 but not S-1, indicating that a free carboxy-terminus of S-2 must be present for recognition. To confirm this localization of the TGM-2 binding site, HMM was digested with papain, and myosin rod was digested with chymotrypsin (Fig. 6, *A* and *E*). The resultant peptides were analyzed by Western blot. mAb TGM-2 bound to undigested HMM, to a number of intermediate fragments, and to S-2 produced in a time-dependent manner from the carboxy-terminus of HMM (Fig. 6*C*). Whereas no binding to undigested rod or its intermediate fragments was detected, mAb TGM-2 did bind to S-2 cleaved from the amino-terminus of

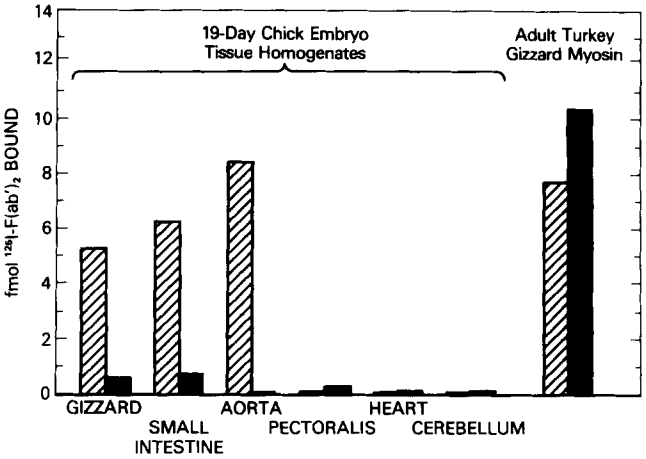

FIGURE 2 Specificity of mAbs for embryonic chick smooth muscles. SDS-solubilized homogenates of 19-d embryonic chick gizzard, aorta, intestine, cadiac ventricle, pectoralis, or cerebellum were characterized by indirect radioimmunoassay (10 μg tissue protein or purified adult turkey gizzard myosin per well) as above, using mAb TGM-1 (■) or TGM-3 (▨). Results shown are fmol ^{125}I-F(ab')$_2$ specifically bound per well.

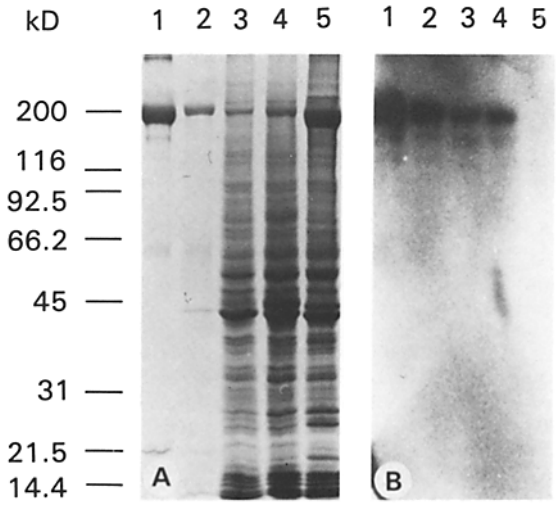

FIGURE 3 Identification of smooth muscle myosin heavy chain in tissue homogenates. Samples (10 μg tissue protein or 3 μg purified myosin per lane) were subjected to SDS PAGE (5–20% acrylamide gradient): Lanes: (*1*) adult turkey gizzard myosin, (*2*) embryonic chick gizzard myosin, (*3*) embryonic chick gizzard, (*4*) embryonic chick intestine, and (*5*) embryonic chick pectoralis. Panel *A* shows the Coomassie Blue-stained gel; panel *B* is an autoradiogram of a nitrocellulose replica reacted sequentially with 5 μg/ml of monoclonal TGM-3 IgG and with 0.2 nM ^{125}I-F(ab')$_2$.

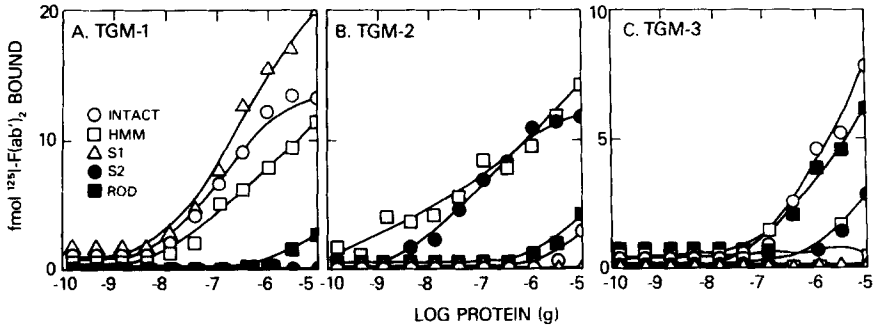

FIGURE 4 Specificity of mAbs for identified domains of smooth muscle myosin heavy chain by indirect radioimmunoassay. Adult turkey gizzard myosin and its purified proteolytic subunits were analyzed by indirect radioimmunoassay (10^{-10}–10^{-5} g/well) using mAbs TGM-1 (*A*), TGM-2 (*B*), or TGM-3 (C). Results shown are fmol ^{125}I-F(ab')$_2$ specifically bound per well. The symbols are identified in panel *A*.

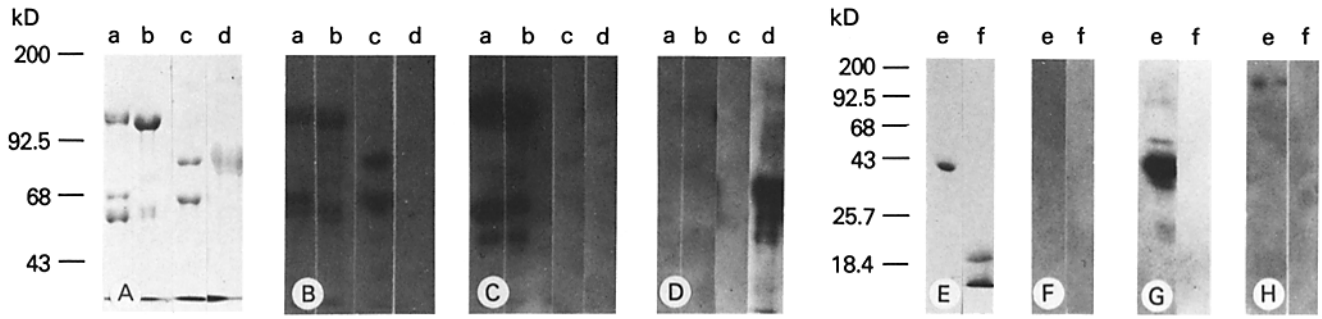

FIGURE 5 Specificity of mAbs for identified domains of smooth muscle myosin heavy chain by Western blot. Purified subunits of adult turkey gizzard myosin (1 μg/lane) were subjected to SDS PAGE (panels A–D, 7.5% acrylamide; E–H, 12.5% acrylamide). Lanes: (*a*) HMM, (*b*) HMM produced by chymotryptic cleavage of myosin in the presence of ATP, (*c*) S-1, (*d*) LMM, (*e*) S-2, and (*f*) mixed LC_{20} and LC_{17}. Panel *E* shows the Coomassie Blue–stained gel; B–D and F–H are autoradiograms of nitrocellulose replicas using mAbs TGM-1 (*B* and *F*), TGM-2 (*C* and *G*), or TGM-3 (*D* and *H*). The heavy chain of both 130-kD HMM and 97-kD S-1 undergo secondary proteolysis at a site ~70 kD from the amino-terminus (see Fig. 11). This gives rise to the additional bands seen below the main heavy chain bands in the HMM and S-1 preparations. (See reference 13 for a discussion of the digestion patterns.)

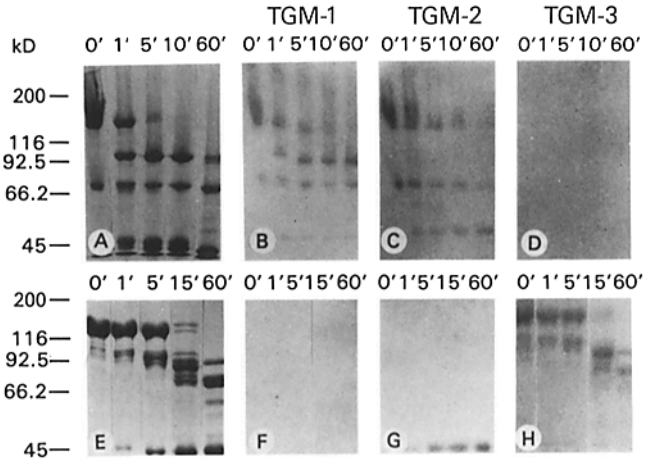

FIGURE 6 mAb TGM-2 recognizes HMM, S-2 derived from either HMM or from rod, but not intact rod. (*A–D*) HMM was digested with papain (0.01 U/mg HMM) and (*E–H*) rod with chymotrypsin (1 mg/100 mg rod) at 25°C for the times shown. Peptides (1 μg/lane) were separated by SDS PAGE (12.5% acrylamide). *A* and *E* show the Coomassie Blue–stained gel; *B–D* and *F–H* are autoradiograms of nitrocellulose replicas with mAbs TGM-1 (*B* and *F*), TGM-2 (*C* and *G*), or TGM-3 (*D* and *H*).

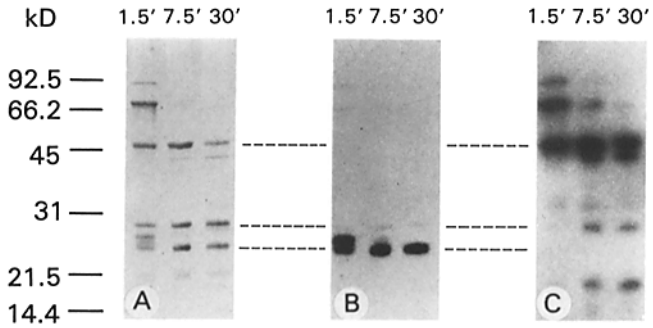

FIGURE 7 Identification of distinct S-1 domains by ^{32}P-LC_{20} and mAb TGM-1. Gizzard myosin S-1 was digested with trypsin (1 mg/100 mg S-1) at 25°C for the times shown, and peptides (1 μg/lane) were resolved by SDS PAGE (12.5% acrylamide). (*A*) Coomassie Blue–stained gel; (*B*) autoradiogram after overlay with ^{32}P-LC_{20} as described in detail elsewhere (13); (*C*) autoradiogram of a nitrocellulose replica using mAb TGM-1.

rod (Fig. 6*G*). Thus, [^{125}I-F(ab′)$_2$·TGM-2·S-2] complexes were not formed when the S-2:LMM junction was intact, and we postulate that the TGM-2 binding site is at or near the S-2:LMM junction. mAb TMG-1 (Fig. 6, *B* and *F*) and TGM-3 (Fig. 6, *D* and *H*) identified distinct families of proteolytic peptides derived only from HMM or from rod, respectively, and are shown for comparison.

The globular S-1 head produced after digestion of myosin with papain retains the actin-binding and actin-activated Mg^{2+}-ATPase activity of the intact myosin molecule (29). Controlled proteolysis of skeletal myosin S-1 with trypsin (30–32) results in formation of an amino-terminal 25-kD adenine nucleotide–binding fragment, a 50-kD central fragment containing one of two actin cross-linking sites, and a carboxy-terminal 20-kD fragment that contains the second actin cross-linking site, the S-1:S-2 junction, and a site of probable association with myosin light chains (13, 33, 34). Smooth muscle myosin S-1 similarly digested with trypsin results in homologous fragments of apparent molecular weight 29-, 50-, and 26-kD (references 13 and 35; Fig. 7*A*). The Western blot employing mAb TGM-1 localizes the TGM-1 epitope (Fig. 7*C*) to the 50-kD tryptic fragment; the antibody bound neither to the amino-terminal 29-kD fragment, nor to the carboxy-terminal 26-kD fragment that binds radiophosphorylated 20-kD light chains of gizzard myosin (see reference 13 and Fig. 7*B*). The 28-kD band detected in the immunoblot only at 7.5 and 30 min-digestion does not correspond with the 29-kD fragment, as shown in the 1.5-min lanes.

After incubation of gizzard HMM or S-1 with rabbit skeletal muscle F-actin in the absence of ATP, such that all actin-binding sites were occupied, as determined by airfuge sedimentation of [S-1·actin] complexes, little or no inhibition of mAb TGM-1 binding was detected (Fig. 8). Phosphorylated gizzard HMM was incubated with mAb TGM-1 and then assayed for actin-activated Mg^{2+}-ATPase activity. Little or no inhibition was detected under the conditions tested. In contrast, incubation of HMM with mAb TGM-2 resulted in a slight increase in the actin-activated Mg^{2+}-ATPase activity (see Table I).

Topography of mAb Epitopes

Fig. 9 shows rotary-shadowed specimens of HMM and HMM reacted with TGM-1 and TGM-2. HMM molecules

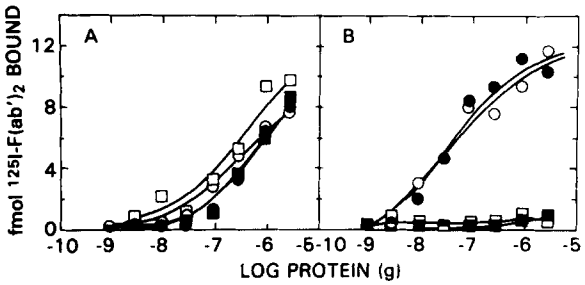

FIGURE 8 Binding of mAb directed against myosin S-1 and HMM in the presence and absence of actin. Turkey gizzard HMM (circles) or myosin S-1 (squares) was incubated at 25°C for 30 min in the presence (solid symbols) or absence (open symbols) of rabbit skeletal muscle F-actin, as previously described (19). The proteins were immobilized on nitrocellulose for solid-phase indirect radioimmunoassay (see Materials and Methods) at the antigen concentration indicated. Results shown are fmol ^{125}I-F(ab')$_2$ specifically bound per well. (*A*) Reaction of HMM and S-1 with TGM-1. (*B*) Reaction of HMM and S-1 with TGM-2.

TABLE I

Actin-activated HMM ATPase in the Presence of mAbs Directed against HMM

	0.02*	0.05	0.10
mAb	Percentage of control of actin-activated MgATPase activity		
TGM-1	86	117	110
TGM-2	192	171	185

Phosphorylated gizzard HMM (0.02–0.1 μM) was incubated at 37°C for 60 min with monoclonal IgG (0.33 μM) purified by DEAE-cellulose chromatography, then assayed for actin-activated Mg^{2+}-ATPase activity as previously described (14). P3-X63.Ag8 IgG was used as control. The control HMM ATPase activity was 0.35 μmol P_i released/min per mg.

* Values in heading represent μM HMM.

(Fig. 9*B*) and [mAb·HMM] complexes (Fig. 9, *C* and *D*) were examined, and the topographic localization of bound IgG was determined. mAb TGM-1 mapped specifically to S-1. Two forms of the [TGM-1·S-1] complex were detected (Fig. 9*C*): one antibody attached to the lateral aspect of a single S-1 head, or one antibody attached between the two S-1 heads of one HMM molecule (intramolecular cross-linking). Immunoglobulin was present at approximately twofold excess. However, the proportion of nonreactive parental P3-X63.Ag8 IgG and hybrid molecules in the reaction mixture was not determined. Univalent [TGM-1·S-1] complexes were essentially symmetric with respect to the long axis of the bound S-1 head, and thus were not analyzed for evidence of rotational freedom of the head as was detected using mAbs directed against the amino-terminal 25-kD fragment of skeletal myosin S-1 (9).

mAb TGM-2, which in peptide binding studies bound S-2 only in the absence of an intact S-2:LMM junction, was mapped specifically to S-2 and was further localized to the carboxy-terminus of S-2 (Fig. 8*D*). The α-helical portion of HMM did not extend beyond bound antibody molecules. Thus, localization of mAb TGM-1 to S-1 and TGM-2 to the carboxy-terminus of S-2, based on indirect radioimmunoassay and Western blot techniques, was substantiated by the topography of individual [mAb·HMM] complexes determined by immunoelectron microscopy.

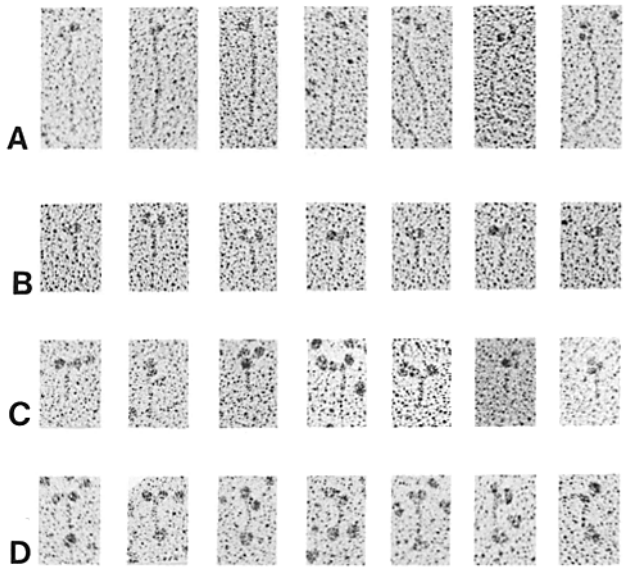

FIGURE 9 Low-angle rotary shadowing of [mAb·HMM] complexes with platinum. Gallery of individual unlabeled myosin (*A*) or HMM molecules (*B*) for reference. (*C*) HMM molecules treated with anti-S-1 TGM-1, labeled on the distal aspect of a single S-1 head, or labeled symmetrically between two heads. (*D*) HMM molecules treated with anti-S-2 TGM-2, labeled at the carboxy-terminus of S-2. ×125,000.

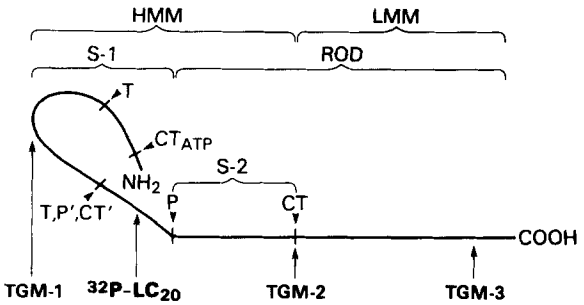

FIGURE 10 Schematic diagram indicating the organization of smooth muscle myosin heavy chain domains. Subunits generated after limited proteolysis with papain (*P*), yielding S-1 and rod, or with chymotrypsin (*CT*), yielding HMM and LMM, are labeled above the figure. *T*, principal sites of tryptic cleavage within S-1 and rod. Secondary sites of cleavage within S-1 by papain (*P'*) or chymotrypsin (*CT'*) are also shown, as well as an amino-terminal S-1 site of ATP-dependent chymotryptic digestion (CT_{ATP}). For clarity, only one heavy chain is illustrated, and subfragment lengths are not to scale. The topographic specificity of each mAb is indicated below the figure; the ^{32}P-LC_{20} binding site is labeled for comparison.

DISCUSSION

We have produced and characterized monoclonal antibodies that label epitopes distributed among three distinct, nonoverlapping identified peptide domains of the 200-kD heavy chain of avian smooth muscle myosin. Though turkey gizzard myosin shares similar native structure and domain organization with myosin not only from mammalian smooth muscle but also from striated muscle, the antibodies reported here exhibit specificity for avian smooth muscle myosin alone and do not cross-react with purified myosin from calf aorta, chicken pectoralis, or with avian nonmuscle myosin(s). Localization of the smooth muscle myosin epitopes (Fig. 10)

was established by indirect radioimmunoassay employing purified myosin subunits as well as by Western blot analysis of these subunits or of fragments generated by further proteolysis. Assignment of mAb TGM-1 to S-1 and TGM-2 to the carboxy-terminus of S-2 based on peptide mapping is additionally supported by the topography of [mAb·HMM] complexes visualized by electron microscopy after low-angle rotary shadowing with platinum.

mAb TGM-1 recognizes a 50-kD tryptic peptide of S-1 but not the amino-terminal 20-kD fragment or carboxy-terminal 26-kD LC_{20}-binding fragment. However, actin fails to inhibit TGM-1 binding, and conversely, antibody fails to inhibit actin-activated Mg^{2+}-ATPase of HMM. Thus, we have detected no functional interaction between mAb TGM-1 and the actin-binding site within the 50-kD peptide.

mAb TGM-2 recognizes an epitope of S-2 that is created by chymotryptic cleavage of the S-2:LMM junction. Thus, TGM-2 recognizes HMM and S-2 but not intact myosin or myosin rod. Analogous results have been reported for polyclonal antibodies produced in rabbits immunized with S-1 derived from chicken pectoralis myosin that precipitate S-1 but not intact myosin (36). We postulate, therefore, that the antibody-combining site is located at or near the carboxy-terminus of S-2 and that [TGM-2·S-2] complexes are not formed when the S-2:LMM junction is intact. This is confirmed by immunoelectron microscopy.

Evidence for myosin heavy chain polymorphism has been well established for a variety of vertebrate striated muscles by biochemical (6, 37), immunologic (5, 9, 10), and molecular genetic (8, 11, 12) investigations: synthesis of myosin heavy chains in both cardiac and skeletal muscle is encoded and directed by a multigene family (11) resulting in the orderly sequential expression of a series of myosin heavy chains. In contrast, the structural and functional properties of myosin in developing smooth muscles have received little study. Katoh and Kubo identified a 23-kD myosin light chain of 9-d embryonic chick gizzard, progressively replaced by LC_{17}, but stated that myosin heavy chains from embryonic and adult gizzard were immunologically identical by Ouchterlony immunodiffusion and quantitative immunoprecipitation (38). In the present report mAb TGM-1, directed against an epitope of S-1 detected in the myosin heavy chain of adult turkey gizzard, bound poorly to the myosin heavy chain of embryonic chick smooth muscles. Thus, myosin heavy chains from embryonic and adult gizzard were found to be distinct; these results contradict the previous report and indicate developmental regulation of this site within the 50-kD tryptic peptide of S-1, whether by serial gene expression or by post-translational events (39).

By employing low-angle platinum shadowing, we have localized the 50-kD tryptic peptide of S-1, which occupies the mid-portion of the S-1 linear amino acid sequence, to the distal aspect of the myosin head. This finding should be considered in the context of previous reports that mAbs that map to the amino-terminal fragment of S-1 localize to either the upper or lower surface of the head (9) and is consistent with the proposal by Mornet et al. (30) regarding the skewed distribution of mass in rabbit skeletal myosin S-1. These observations thus provide the first direct evidence for the topographic organization of the myosin heavy chain within the smooth muscle myosin head and furnish additional evidence that homologous structural organization of domains is conserved even in the presence of significant antigenic diversity among myosins of disparate origin.

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