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Alteration of the enzymatic properties of smooth muscle myosin by a monoclonal antibody against subfragment 2

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A monoclonal antibody against subfragment 2 (S-2) of smooth muscle myosin, designated MM-9, was generated and characterized. MM-9 potently inhibited subfragment 1 (S-1) release by papain proteolysis of myosin, suggesting that the epitope of MM-9 is at or very close to the S-1/S-2 junction. The depression of Ca^{2+}- and Mg^{2+}-ATPase activities of myosin at low ionic strength was significantly reduced by MM-9. MM-9 increased the acto dephosphorylated HMM ATPase activity about 3-fold. On the other hand, the antibody had no effect on the KCl-dependence of viscosity of monomeric myosin. These results suggest that the folding of the myosin rod is not the direct determinant of enzymatic activity, and that the subtle conformational change at the S-1/S-2 junction (head-neck region) plays a critical role in determining enzymatic activities.

Monoclonal antibody; Smooth muscle myosin conformation; Subfragment 2

1. INTRODUCTION

Smooth muscle myosin [1–3] and myosin from several non-muscle sources [3–5] forms a folded structure (10 S) that dramatically alters its hydrodynamic properties. Under certain conditions it undergoes a large conformational change to an extended form (6 S), and this transition is characterized by distinct enzymatic properties [6]. A high concentration of Mg^{2+} favors the 6 S conformation and causes tension development in skinned smooth muscle fibers in the absence of phosphorylation [7]. These findings led to the 'shape-activity' hypothesis that the 10 S–6 S transition could be a component of the regulatory mechanism in smooth muscle myosin. However, it is unlikely that a large conformational change is in fact essential in determining enzymatic activity. Some subtle conformational change in the S-1/S-2 junction may be more important because of the following evidence: (1) the resistance to the proteolysis at the S-1/S-2 junction but not the LMM/HMM junction was markedly altered with the conformational transition of myosin [8,9]; (2) the actin-activated ATPase activity of S-1, which retains a phosphorylatable light chain, is not regulated by phosphorylation while HMM, even though it does not form a folded structure, was regulated by phosphorylation [9]; (3) the change in the digestability at the S-1/S-2 junction, which accompanies the 10 S–6 S transition, also markedly differed between phosphorylated and dephosphorylated HMM [8].

Recently we generated a monoclonal antibody against the 17 kDa light chain of smooth muscle myosin, and it was found that the antibody, which inhibited the conformational change of smooth muscle myosin, markedly activated actin-activated ATPases [10]. Trybus et al. reported that the regulatory light chain deficient myosin was unable to form the folded monomer [11]. Taken together, this evidence suggests the spatial relationship between the heads, including the two light chains, and S-2 at the S-1/S-2 junction would play an important role in the conformational change.

In this paper, we investigated the effect of a monoclonal antibody against S-2 region on the hydrodynamic property and enzymatic activity of smooth muscle myosin. This antibody may provide new insight into the function of S-2, which still remains unsolved.

2. MATERIALS AND METHODS

2.1. *Proteins*

Myosin [12] and MLCK [13] were prepared from turkey gizzard. Calmodulin [14] and F-actin [15] were prepared from bull testes and rabbit, respectively. HMM and S-1 were prepared using *S. aureus* protease as described previously [9].

2.2. *Production of monoclonal antibodies*

The production and characterization of antibodies were done as described previously [10]. MM-9 and MM-6 were grown in ascitic fluid and purified with ammonium sulfate precipitation followed by DEAE-cellulose chromatography [16].

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2.3. *Others*

ATPase activity was measured as described previously [9]. Viscosity was measured with a Cannon-Ubbelohde viscometer with a water flow time of approximately 54 s. Electrophoresis was carried out on a 7.5–20% polyacrylamide gradient slab gel using the discontinuous buffer system of Laemmli [17]. Immunoblotting was carried out according to the method of Towbin et al. [18]. ELISA, electron micrograph (rotary shadowing) of myosin/antibody complex and immunoaffinity column were performed as described previously [10]. Molecular masses were estimated using the following standard: smooth muscle myosin heavy chain (200000), β-galactosidase (116000), phosphorylase *b* (97000), bovine serum albumin (66000), ovalbumin (45000), carbonic anhydrase (29000), trypsin inhibitor (20100), and lysozyme (14300).

3. RESULTS AND DISCUSSION

We produced a monoclonal antibody against gizzard smooth muscle myosin, designated MM-9 (IgG_1, k light chain). ELISA and immunoblot data (Fig. 1) suggest that MM-9 recognizes subfragment 2 (S-2) of smooth muscle myosin. Electron micrograph (rotary shadowing) of myosin/MM-9 complex showed that MM-9 binds at or near the head/rod junction (not shown).

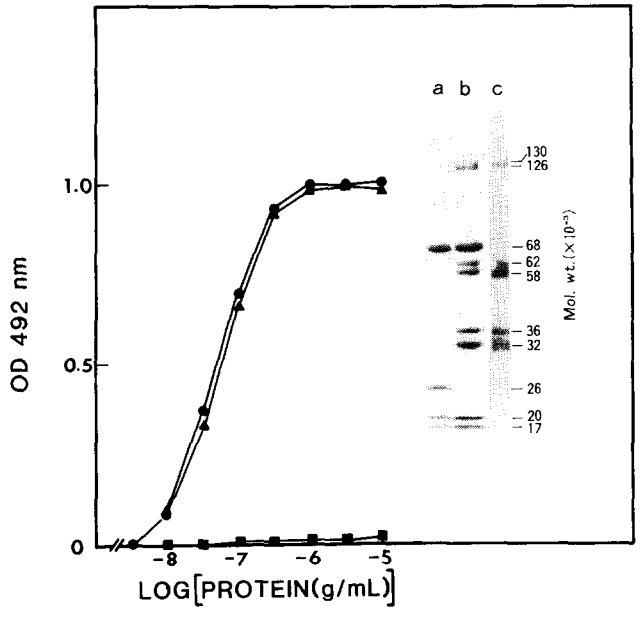

Fig. 1. Identification of the epitope of MM-9. (A) ELISA of MM-9. (●—●) Whole myosin; (■—■) S-1 (94 kDa native S-1 heavy chain is cleaved into 68 kDa and 26 kDa fragments by SAP digestion (see references in [9]); (▲—▲) HMM. 1 μg of protein was precoated on 96 wells. Inset shows the SDS-PAGE and immunoblot of the fractions from MM-9 affinity column. Gizzard smooth muscle myosin digested by SAP was passed through MM-9 affinity column (see section 2). The flow-through fraction was S-1 (lane a) and bound protein was revealed to be HMM plus S-2 fragments (lane b). The latter sample was electrophoresed and transferred to the nitrocellulose membrane. Amido black staining of sample showed 130-, 126-, 68-, 62-, 58-, 36-, 32-kDa peptides and two light chains of 20- and 17-kDa (not shown). Since MM-9 bound to both the 32- and 36-kDa proteins (both bands were referred to as S-2) and both the 58- and 62-kDa proteins (S-2 plus 26-kDa peptide of S-1) (lane c), the epitope of MM-9 is not located at the C-terminus 4-kDa peptide of S-2 (see references in [9]).

Furthermore, MM-9 potently inhibited the S-1 release by papain digestion of smooth muscle myosin, indicating that the epitope of MM-9 is very close to the S-1/S-2 junction (Fig. 2).

Recently we reported a monoclonal antibody against the 17-kDa light chain (MM-2), which inhibits the 6 S

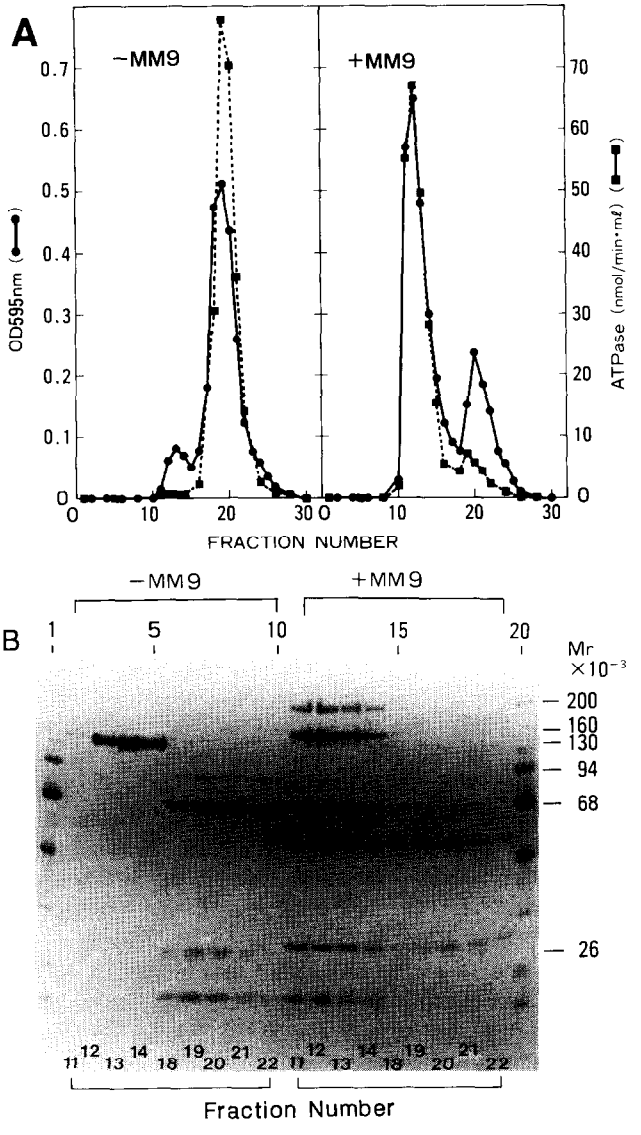

Fig. 2. The effect of MM-9 on S-1 release by papain digestion of smooth muscle myosin. (A) The elution profile of digests of myosin by papain. Myosin (600 μg/ml) in 0.5 M NaCl, 30 mM Tris-HCl, pH 7.5, in the absence or presence of MM-9 (300 μg/ml) was digested by papain (1:40 papain/myosin (w/w)) for 5 min at 25°C, and the reaction was stopped with 5 mM iodoacetic acid. 1 ml of sample was applied to a TSK SW4000 column and eluted at a flow rate of 0.6 ml/fraction. (●—●) Protein concentration determined by a dye binding method [19]; (■—■) Ca^{2+}-ATPase activity of the fractions. Assay conditions for ATPase activity: 0.5 M KCl, 50 mM Tris-HCl, pH 7.5, and 10 mM $CaCl_2$. (B) SDS-PAGE profiles of the fractions. The 1st and 20th lanes show molecular weight standards. The 2nd–10th lanes correspond to fractions 11–14 and 18–22 of left panel of A (minus MM-9), respectively. The 11th–19th lanes correspond to fractions 11–14 and 18–22 of right panel of A (plus MM-9), respectively.

to 10 S conformational change and increases the V_{max} of acto-ATPase activity of dephosphorylated myosin about 10-fold [12]. The epitope of MM-2 was located very near the S-1/S-2 junction (51 ± 25 Å from the head/rod junction) judged from rotary shadowing of electron microscopy. Therefore, it is of interest to examine whether MM-9, which recognizes a part of S-2 of myosin molecule close to the S-1/S-2 junction, affects the 10 S–6 S transition of smooth muscle myosin and ATPase activity. Fig. 3 shows the effects of MM-9 on the KCl dependence of Ca^{2+}- and Mg^{2+}-ATPase activities of smooth muscle myosin. As is shown previously [6], both Ca^{2+}-, and Mg^{2+}-ATPase activities in the absence of the antibody were markedly depressed below 0.3 M KCl. It was shown previously [6] that this was accompanied by the conformational transition of myosin from 6 S to 10 S. In the presence of MM-9, the depression of ATPase activities below 0.3 M KCl was significantly reduced although the activities were still decreased below 0.3 M KCl. The effect of MM-9 on the conformational transition of myosin was monitored by viscosity measurements (Fig. 4). The relative viscosity of myosin was markedly reduced below 0.3 M KCl and it was shown previously [6] that this is due to the change in the conformation from 6 S to 10 S (the folding of the tail portion of the molecule). MM-9 did not affect the viscosity change of myosin (Fig. 4), and therefore this suggests that MM-9 does not inhibit the large conformational change, i.e. the folding of the tail portion of the molecule. We have proposed that some subtle conformational change in the head/rod junction is the determinant of ATPase activity of myosin (see section 1). The effects of MM-9 can be explained as follows. MM-9 binds at the head/rod junction and affects the conformation at the head/rod junction. This does not interfere to form a folded structure (Fig. 4); however, it significantly abolishes the depression of ATPase activity of myosin (Fig. 3). That is, MM-9 altered the conformation at the head/rod junction of myosin and this conformational change was significant enough to alter the ATPase activity of myosin, but not enough to alter the folded structure to the extended one.

The effects of MM-9 on the actin-activated Mg^{2+}-ATPase activity for dephosphorylated HMM were examined (Fig. 5). V_{max} of the actin-activated ATPase activity was increased 3.2 times and K_{actin} was decreased 2 times by MM-9. Recently, we reported a monoclonal antibody aginst the 17-kDa light chain (MM-2) which inhibits the formation of a folded struc-

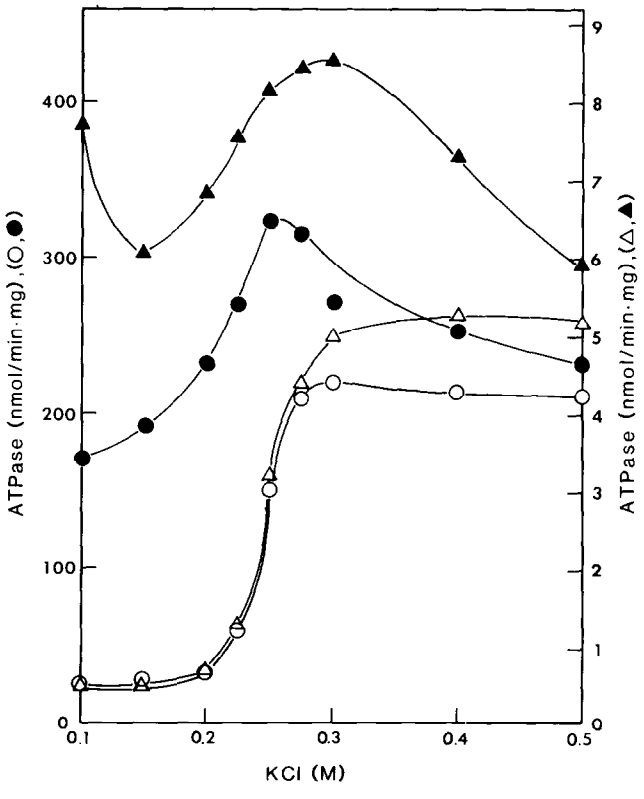

Fig. 3. The effect of MM-9 on KCl dependence of ATPase activities of myosin. Ca^{2+}-ATPase activity (○,●) and Mg^{2+}-ATPase activity (△,▲). Assay conditions: dephosphorylated myosin (120 μg/ml), 50 mM Tris-HCl, pH 7.5, and 10 mM $CaCl_2$ or 1 mM $MgCl_2$ in the absence of (○,△) or presence (●,▲) of MM-9 (60 μg/ml).

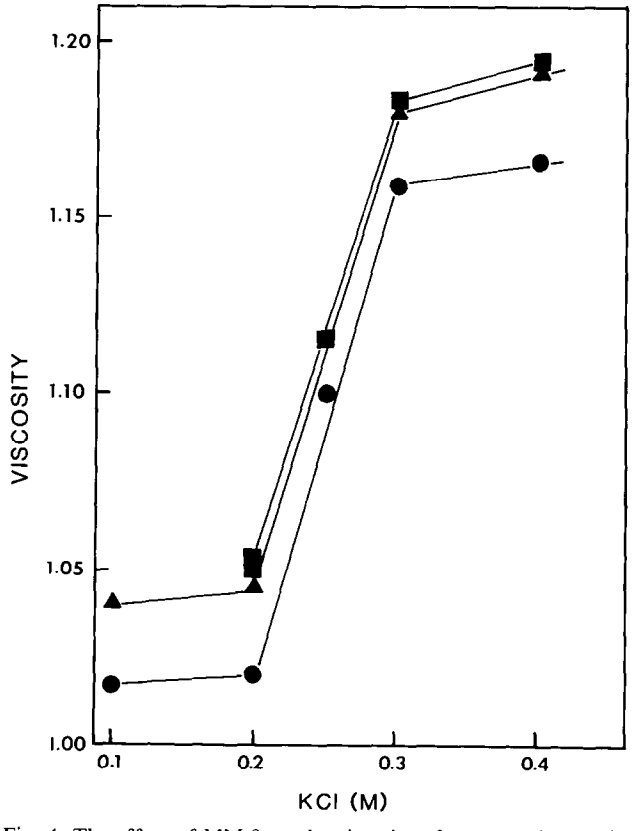

Fig. 4. The effect of MM-9 on the viscosity of monomeric myosin. Viscosity of myosin was measured in the solution containing myosin (0.5 mg/ml), various concentrations of KCl, 30 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM ATP at 23°C. Control (●); MM-9 (0.25 mg/ml, ■); MM-6 (anti-S-1, 0.3 mg/ml, ▲). Values are the mean of 4 measurements.

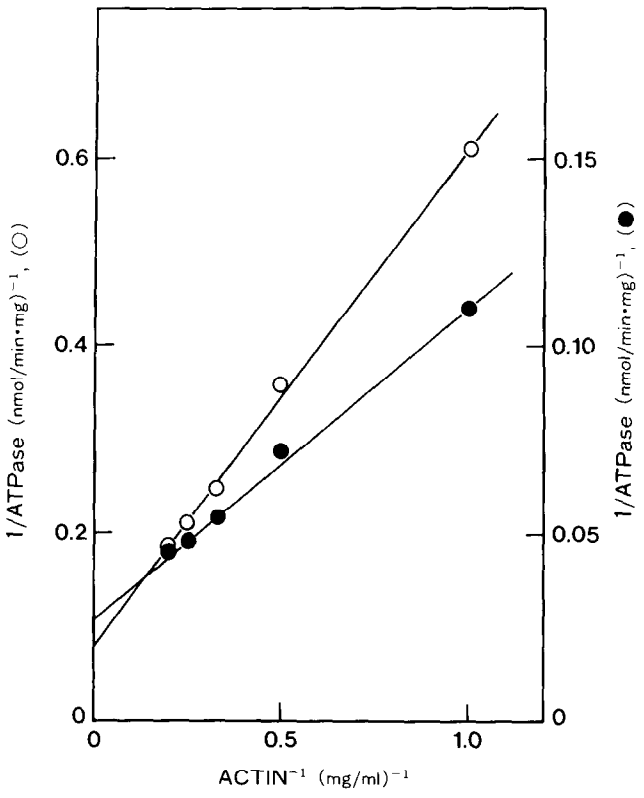

Fig. 5. The effect of MM-9 on the actin-activated Mg^{2+}-ATPase activity of dephosphorylated HMM. Assay conditions: 100 μg/ml, 50 mM KCl, 30 mM Tris-HCl, pH 7.5, 1 mM EGTA and 1 mM [γ-^{32}P]ATP. The reaction was started by the addition of actin (○) or actin plus 2 molar excess of MM-9 (●). The scales of the ordinate are different for (○) and (●). The ATPase activity of myosin alone was subtracted from the total activity.

ture (10 S) and increases the V_{max} of actin-activated ATPase activity of dephosphorylated myosin and HMM about 15–20 times [10]. Although the activation observed using MM-9 was significant, the activation was not as dramatic as MM-2. The change in the conformation of myosin by MM-9 is probably not sufficient to achieve the active conformation for the following reason. The inhibition of the depression of the Ca^{2+}- and Mg^{2+}-ATPase activity of myosin MM-9 at below 0.3 M KCl was not as complete as by MM-2 (Fig. 3). The activation of the actin-activated ATPase activity by MM-9 was not as large as by MM-2 (Fig. 5). MM-9 does not inhibit the formation of a folded structure (Fig. 4). Although the activity change was not complete since the ATPase activities were significantly affected by MM-9, but the global change in the conformation (10 S–6 S) was not, it can be concluded that the global change in the conformation is not the direct determinant of ATPase activity. A more subtle change in the conformation at the head/rod junction is likely to determine the ATPase activity and this subsequently induces the large change in the myosin conformation and forms 10 S and 6 S structures.

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