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Ca^{2+}-Dependent Inhibition of Actin-Activated Myosin ATPase Activity by S100C (S100A11), a Novel Member of the S100 Protein Family

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S100C (S100A11, calgizzarin) inhibits the actin-activated myosin Mg^{2+}-ATPase activity of smooth muscle in a dose-dependent manner: its half-maximal effect occurs at a S100C/actin molar ratio of 0.05 and its maximal effect occurs at a ratio of 0.20. Furthermore, S100C was found to bind to actin with a stoichiometry of 1:6–7 in the presence of Ca^{2+}, with an affinity of 1×10^{-6} M determined by cosedimentation assays. Other Ca^{2+}-binding proteins such as S100A1, S100A2, S100B, and calmodulin did not inhibit actin-activated myosin Mg^{2+}-ATPase activity. Calmodulin, S100A1, and S100B reversed the inhibitory effect of calponin in a Ca^{2+}-dependent manner, S100A2 had no effect, and S100C had additional inhibitory effects. The results suggest that S100C might be involved in the regulation of actin-activated myosin Mg^{2+}-ATPase activity through its Ca^{2+}-dependent interaction with actin filaments.

Recently, we purified S100C (S100A11, calgizzarin), a novel Ca^{2+}-binding protein that consists of two subunits and cloned and sequenced its cDNA (1). Several functions of S100C have been suggested, including binding to cytoskeletal components at physiological concentrations of Ca^{2+} (2–4). In this study, we found that S100C is abundant in smooth muscle and is a Ca^{2+}-dependent actin binding protein. Further, S100C inhibits actin-activated myosin Mg^{2+}-ATPase activity by binding to actin only in the presence of Ca^{2+}. Our results suggest that S100C is a Ca^{2+}-dependent actin binding protein and is involved in the Ca^{2+}-dependent regulation of actin filaments.

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MATERIALS AND METHODS

Materials. The radiolabel [γ-^{32}P]ATP was purchased from Amersham Corp. (U.S.A.). Dithiothreitol (DTT) was purchased from Sigma (U.S.A.). All other chemicals were of the highest analytical grade commercially available.

Preparation of proteins. The protein S100C (S100A11) was purified from porcine kidney by the previously reported procedure (2). The following proteins were purified by previously described methods: F-actin from rabbit skeletal muscle (5), phalloidin-stabilized actin in the presence 30 mM NaCl and 1 mM $MgCl_2$ (6), calmodulin from frozen bovine brain (7), and S100L (S100A2), thiophosphorylated smooth muscle myosin, and calponin (8–10).

Assay of actin binding. Cosedimentation assays were used to study the binding of S100C to F-actin. The S100C and F-actin were incubated for 30 min at 25°C in a solution containing 25 mM Tris-HCl (pH 7.5), 70 mM KCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mM DTT. The samples were centrifuged at 100,000 × g for 1 h to sediment F-actin and its associated proteins. The pellets and supernatants were separated by 15% SDS-PAGE. The gels were stained with Coomassie Brilliant Blue R-250 and the fragments distributed in the pellets and supernatants were quantified by densitometric scanning. Determination of a dissociation constant (K_d) was also carried out from the cosedimentation assay.

Assay of Mg^{2+}-ATPase activity. The actin-activated myosin Mg^{2+}-ATPase activity was determined under the following conditions: 10 mM F-actin, 0.85 mM thiophosphorylated myosin, and S100C or another Ca^{2+}-binding protein were mixed in buffer C [25 mM Tris-HCl (pH 7.5), 70 mM KCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$ or 1 mM EGTA, and 1 mM DTT] to a final volume of 100 ml. The reactions were started by the addition of 1 mM [γ-^{32}P] ATP. After incubation for 10 min at 25°C, the reaction was stopped with 10% perchloric acid. The radioactivity of inorganic phosphate was quantitated in a liquid scintillation counter.

Other techniques. The concentrations of protein were measured according to previously reported methods (11).

RESULTS AND DISCUSSION

Distribution of S100C (S100A11) in muscles. Recently, we purified a novel Ca^{2+}-binding protein with a molecular weight of 25,000 by Ca^{2+}-dependent dye-affinity chromatography and Ca^{2+}-dependent interaction with cytoskeleton (2). We detected S100C in various porcine muscles by Western blotting analysis with

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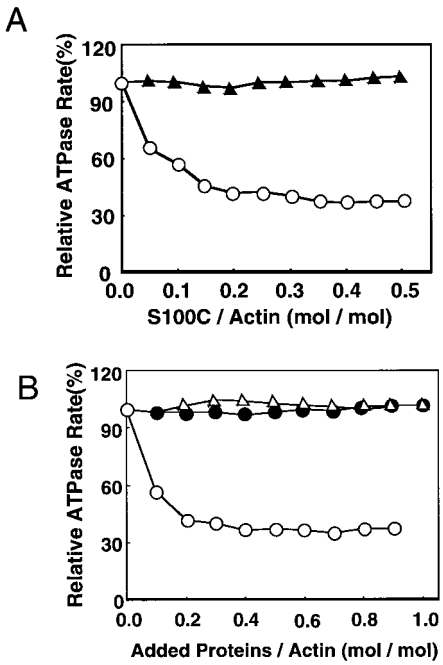

FIG. 1. (A) Inhibition of actin-activated myosin Mg^{2+}-ATPase activity by S100C (S100A11) protein. Actin-activated myosin Mg^{2+}-ATPase activity was measured in the presence (○) and the absence (▲) of Ca^{2+}. (B) Effect of Ca^{2+}-binding proteins on actin-activated myosin Mg^{2+}-ATPase activity in the presence of Ca^{2+}. 0–10 mM S100C (○), S100A2 protein (△), and calmodulin (●). The assay conditions are described under Materials and Methods. Protein concentrations were 10 mM F-actin and 0.85 mM thiophosphorylated myosin. For comparison, each ATPase rates are shown relative to the rate in the absence of Ca^{2+}-binding protein.

purified polyclonal antibody against S100C. S100C was present in various smooth muscle and most abundant in stomach smooth muscle (1.4 ± 0.1 mg/mg soluble protein, approximately 3 μM).

Inhibition of actin-activated myosin Mg^{2+}-ATPase activity by S100C. We examined the effect of S100C on smooth muscle actin-activated myosin Mg^{2+}-ATPase activity. Figure 1A shows that S100C inhibits actin-activated myosin Mg^{2+}-ATPase activity in a concentration-dependent and Ca^{2+}-dependent manner with a half-maximal effect at an S100C/actin molar ratio of 0.05. Maximum inhibition of actin-activated myosin Mg^{2+}-ATPase activity occurred when the ratio of S100C to actin monomers was approximately 0.20.

We found that S100C interacts not only with annexins as previously reported (2, 4), but also with actin in a Ca^{2+}-dependent manner. Moreover, our results show that S100C interacts with actin and not with tropomyosin(data not shown). The extents of inhibition by S100C on actin-activated myosin Mg^{2+}-ATPase activity and of S100C binding to actin filaments are not influenced by the use of phalloidin-stabilized actin which does not depolymerize (12). This suggests that the mechanism of inhibition of actin-activated myosin Mg^{2+}-ATPase activity by S100C may be the result of direct regulation of actin filaments and not of depolymerization.

Binding of S100C to actin. We examined the actin-binding activity of S100C at a wide range of concentrations. S100C bind to actin filament in a dose-dependent manner. Saturation was achieved at a ratio of one S100C protein to six or seven actin monomers. The dissociation constant (K_d) was 1×10^{-6} M. These finding are in agreement with our results of the actin-activated myosin Mg^{2+}-ATPase activity assays.

Effect of other Ca^{2+}-binding proteins on actin-activated myosin Mg^{2+}-ATPase activity. Calmodulin and S100A2 proteins did not inhibit actin-activated myosin Mg^{2+}-ATPase activity (Fig. 1B). The proteins S100A1 and S100B also did not exhibit any effects (data not shown). These results suggest that the inhibitory effects of S100C on the actin-activated myosin Mg^{2+}-ATPase activity is specific. Calmodulin, S100A1, and S100B reversed the inhibitory effect of calponin in a Ca^{2+}-dependent manner, S100A2 had no effect, and S100C had additional inhibitory effects (Fig. 2). These results suggest that the binding site of S100C on actin may be different from that of calponin.

Effect of Ca^{2+}-concentration on inhibition of the actin-activated myosin Mg^{2+}-ATPase activity. We examined the effect of Ca^{2+}-concentration on inhibition of the actin-activated Mg^{2+}-ATPase activity. The apparent half-maximal concentration of Ca^{2+} was 2×10^{-5} M (Fig. 3). We found that S100C protein contents increased when exposed to hypoxia (unpublished data). Furthermore, recent investigation showed that hypoxic relaxation occurs at constant levels of myosin light chain phosphorylation (13). These results suggest that S100C may be involved in relaxation without change in

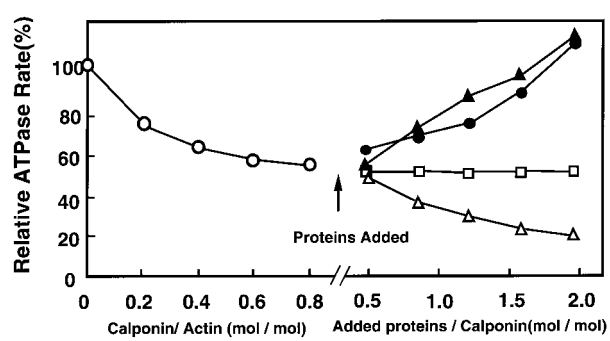

FIG. 2. Effect of Ca^{2+}-binding proteins on the calponin-induced inhibition of actin-activated myosin Mg^{2+}-ATPase activity. Thiophosphorylated myosin (1.2 mM), actin (6 mM), calponin (0–5 mM), and Ca^{2+}-binding proteins S100A1 (▲), S100A2 (□), S100C(S100A11) (△) and Calmodulin (●) (2.5–10 mM) were incubated at 30°C for 30 min in buffer C [25 mM Tris-HCl (pH 7.5), 70 mM KCl, 10 mM $MgCl_2$, and 1 mM $CaCl_2$] to a final volume of 100 ml. The reactions were started by the addition of 1 mM [γ-^{32}P] ATP.

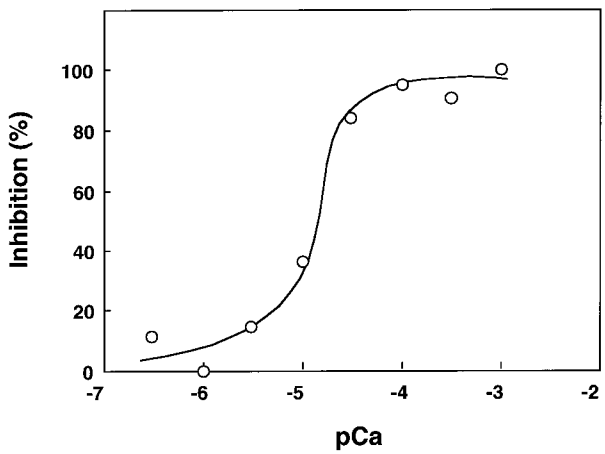

FIG. 3. Effect of Ca^{2+} concentration on inhibition of actin-activated myosin Mg^{2+}-ATPase activity by S100C (S100A11) protein. Conditions for actin-activated myosin Mg^{2+}-ATPase activity are described in Fig. 1, except for the free Ca^{2+} concentrations (pCa) indicated.

myosin light chain phosphorylation such as a hypoxic relaxation.

Because the functional properties of S100C are clearly different from those of previously identified Ca^{2+}-binding proteins and actin-binding proteins, S100C appears to play a role in the regulation of actomyosin Mg^{2+}-ATPase activity through a unique Ca^{2+}-dependent interaction with actin filaments.

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