134. Actin-linked Regulation in Scallop Striated Muscle

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Introduction. In vertebrate striated muscles, the actin-myosin interaction is regulated by troponin-tropomyosin system dependent on the Ca^{2+} concentration. These regulatory proteins, troponin and tropomyosin, are located on the thin filaments.^{1),2)} In contrast, Ca^{2+} regulation of molluscan actomyosin ATPase is known to be associated with the myosin molecule.^{3),4)} Recently, troponin-like proteins have also been detected in the striated muscle of scallop,^{5),6)} where the myosin-linked system had been regarded as essential.

In the present study, thin filaments were prepared from scallop striated muscle. When they were added to scallop myosin, the Mg^{2+} -ATPase activity was more deeply inhibited in the presence of 10^{-7} M Ca^{2+} and further activated in the presence of 10^{-4} M Ca^{2+} as compared with that of actomyosin reconstituted of scallop myosin and actin. When scallop thin filaments were added to rabbit skeletal myosin, the Mg^{2+} -ATPase activity was inhibited and this inhibition was independent of Ca^{2+} concentrations.

This suggests that scallop striated muscle contains an actin-linked regulatory protein which may be different from the troponin-tropomyosin system.

Materials and method. Scallop actomyosin and myosin were prepared from washed striated muscle according to the procedure described by Szent-Györgyi *et al.*⁷⁾ Rabbit skeletal myosin was prepared according to the method described by Perry⁸⁾ with slight modifications. Rabbit and scallop actin were prepared by the method of Spudich and Watt.⁹⁾ Thin filaments from scallop striated muscle were extracted with 5 mM Tris-HCl (pH 7.6) for 30 min from the acetone dried powder at 0°C in the presence of 0.3 mM ATP. After addition of KCl to 0.1 M, it was centrifuged for 3 h at 30,000 rpm. The resulting pellet was collected, homogenized, and resuspended in 5 mM Tris-HCl (pH 7.6) without ATP. Mg^{2+} -ATPase activities were measured in solutions containing 50 mM KCl, 20 mM Tris-HCl (pH 7.0), 1 mM MgCl_2 and 1 mM ATP with various Ca^{2+} concentrations. Ca^{2+} sensitivities (%) were measured by comparing the ATPase activities in the presence of 0.1 μM Ca^{2+} and 0.1 mM Ca^{2+} by using the following relationship:

$$\text{Ca}^{2+} \text{ sensitivity} = \frac{\text{ATPase}(1 \times 10^{-4} \text{M Ca}^{2+}) - \text{ATPase}(1 \times 10^{-7} \text{M Ca}^{2+})}{\text{ATPase}(1 \times 10^{-4} \text{M Ca}^{2+})} \times 100.$$

The liberated Pi was determined according to Fiske and SubbaRow method with a slight modification.¹¹⁾ The free Ca^{2+} concentration was calculated using the binding constant of Ca^{2+} to EGTA reported by Ogawa.¹²⁾ Disc gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed according to the method of Weber and Osborn.¹⁰⁾ Slab gel electrophoresis was performed according to the method of Porzio and Pearson.¹³⁾ The protein concentration was determined by biuret reaction.¹⁴⁾

Results and discussion. Fig. 1 shows the pattern of polyacrylamide slab gel electrophoresis of purified scallop actin and thin filaments in the presence of

Fig. 1. Polyacrylamide gel electrophoresis of scallop thin filaments and actin in the presence of SDS. Electrophoresis was carried out using 12.5% polyacrylamide gel containing 0.1% SDS. Lane 1, scallop striated thin filaments (30 μ g); Lane 2, scallop striated actin (15 μ g). 47 K, A, TM, 23 K, and 22 K represent 47 K protein, actin, tropomyosin, 23 K protein, and 22 K protein, respectively.

SDS. Thin filaments were composed of 47 K protein, actin, tropomyosin, 23 K and 22 K proteins. Molecular weights were estimated by SDS disc gel electrophoresis. Effects of the thin filaments and actin on Mg^{2+} -ATPase activities of the scallop myosin were investigated (Fig. 2). Mg^{2+} -ATPase activities of reconstituted scallop actomyosin were 0.31 and 0.62 μ mole Pi/mg·min in the presence of 1.1×10^{-7} M and 1.0×10^{-4} M Ca^{2+} , respectively, and the Ca^{2+} sensitivity was 50%. Ca^{2+} sensitivity of natural actomyosin was 88.9%.

When scallop thin filaments were added to scallop myosin, Mg^{2+} -ATPase activity at 1.1×10^{-7} M Ca^{2+} was 0.08 μ mole Pi/mg·min and that at 1.0×10^{-4} M Ca^{2+} was 1.16 μ mole Pi/mg·min. The Ca^{2+} sensitivity became 93.1%, which was even higher than that of natural actomyosin, 88.9%. This low Ca^{2+} sensitivity

Fig. 2. Effects of scallop thin filaments and actin on Mg^{2+} -ATPase activities of scallop striated myosin at various Ca^{2+} concentrations. Reaction mixture contained 1 mM MgCl_2 , 20 mM Tris-HCl buffer (pH 7.0), and 1 mM ATP, at 25°C. ○, 0.1 mg/ml of scallop natural actomyosin; ◐, 0.1 mg/ml of scallop myosin and 0.025 mg/ml scallop actin; ●, 0.1 mg/ml of scallop myosin and 0.03 mg/ml of scallop thin filaments.

Fig. 3. Effects of scallop striated thin filaments and actin on Mg^{2+} -ATPase activity of rabbit skeletal myosin. Reaction mixtures and conditions were the same as those described in the legend to Fig. 2. ○, 0.16 mg/ml of rabbit myosin and 0.04 mg/ml of scallop actin; ●, 0.4 mg/ml of rabbit myosin and 0.13 mg/ml of scallop thin filaments.

of reconstituted actomyosin is probably due to the myosin-linked regulation, because the actomyosin consists of pure actin and myosin.

Fig. 3 shows effects of scallop actin and thin filaments on Mg^{2+} -ATPase activity of rabbit skeletal myosin. The Mg^{2+} -ATPase activity of actomyosin reconstituted of rabbit skeletal myosin and scallop actin was $0.24 \mu\text{mole Pi/mg}\cdot\text{min}$ regardless of Ca^{2+} concentrations. However, the ATPase of skeletal myosin with scallop thin filaments was as low as $0.04 \mu\text{mole Pi/mg}\cdot\text{min}$ under the same condition. Therefore, the function of scallop thin filaments on rabbit skeletal myosin seems simple inhibition irrespective of the Ca^{2+} concentrations, in contrast to the function of troponin-tropomyosin system which depends on Ca^{2+} concentrations.

These results confirm the presence of actin-linked regulatory system as reported by Lehman *et al.*^{5),6),15)} They suggested the presence of troponin-tropomyosin system in scallop striated muscle, but we could not reproduce their results. We could restore the Ca^{2+} sensitivity of myofibril or natural actomyosin by reconstituting the actomyosin system of scallop myosin and thin filaments, whereas the Ca^{2+} sensitivity of reconstituted actomyosin was only one-half that of natural actomyosin (Fig. 2).

Our results have shown the existence in scallop striated muscle of a new actin-linked system different from the troponin-tropomyosin system, in addition to the myosin-linked system. This strongly suggests that the contraction of scallop striated muscle is dually regulated. This seems applicable also to other molluscan muscles. It is therefore likely that the dual system is common among invertebrates.

Further studies are now in progress.

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