Scallop striated tropomyosin prepared by a new method

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Abstract: A large amount of tropomyosin was quickly prepared from scallop striated muscle by the method combining ammonium sulfate fractionation in the presence of Mg^{2+} -ATP and subsequent dialysis. The yield of tropomyosin by this method was about four times higher than that by conventional method. The tropomyosin thus prepared showed essentially the same activity as ordinary preparation, e.g., it depressed the Mg^{2+} -ATPase activity of rabbit skeletal actomyosin by 25%, whereas no effect on that of reconstituted scallop actomyosin was observed. This new method for preparing tropomyosin characterized by its simplicity and rapidity is expected to be applicable to different kinds of muscle.

Key words: New preparation method; tropomyosin; scallop striated myosin; Mg^{2+} -ATPase activity; Ca^{2+} concentration; reconstituted actomyosin.

Introduction. The contraction of vertebrate skeletal muscle is regulated by changes that take place in the thin filament. These changes are initiated by the binding of Ca^{2+} to the troponin complex which is located at intervals of approximately 40 nm along the thin filament.^{1,2)} The mechanism of regulation is believed to involve a change in the position occupied by tropomyosin in the actin long-pitch helical grooves.³⁾

In contrast, Ca^{2+} regulation of molluscan actomyosin ATPase is known to be associated with the myosin molecule.⁴⁾ Recently, troponin has also been detected in the striated muscle of scallop,⁵⁻⁷⁾ where the myosin-linked system had been regarded as essential.

In a previous paper,⁵⁾ we prepared thin filaments 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 highly 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 a little different from the troponin-tropomyosin system. Although Ca^{2+} sensitivity is conferred on the actomyosin system only by the complete troponin-tropomyosin complex, tropomyosin alone has interesting effects on the actomyosin ATPase activity.

In this study, we established a new simple and rapid preparation method of tropomyosin from scallop striated muscle. The purity of tropomyosin prepared according to this method was essentially as same as that obtained by the method of Bailey⁸⁾ and the yield was much higher than that by the latter method.

Materials and method. Scallop actomyosin was prepared from washed striated muscle with the modification of the procedure described by Szent-Györgyi *et al.*^{4,10)} Rabbit skeletal myosin was prepared according to the method described by Perry¹¹⁾ with slight modifications. Rabbit and scallop actin were prepared by the method of Spudich and Watt.¹²⁾ Thin filaments of 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, 10 mM MOPS (pH 6.8), 3 mM $MgCl_2$ and 2 mM ATP in the presence of 0.1 μ M Ca^{2+} or 0.1 mM Ca^{2+} .

The liberated Pi was determined according to

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Table I. The average yields of tropomyosin from muscles

	TM prepared from scallop NAM (numbers of preparation)	TM prepared with the method of Bailey (numbers of preparation)
Scallop striated muscle	0.160% (5)	0.039% (5)
Rabbit skeletal muscle	0.180% (4)	0.030% (4)

Fig. 1. SDS-PAGE pattern of fraction obtained during the preparation of scallop tropomyosin. Electrophoresis was carried out using 10% polyacrylamide gel containing in 0.1% SDS. Lane 1, scallop striated natural actomyosin; Lane 2, supernatant obtained by 0.4 saturated $(\text{NH}_4)_2\text{SO}_4$; Lane 3, the fraction precipitated between 0.4–0.55 saturated $(\text{NH}_4)_2\text{SO}_4$; Lane 4, the supernatant (tropomyosin) after the precipitate between 0.4–0.55 saturated $(\text{NH}_4)_2\text{SO}_4$ was dialyzed in 2 mM MOPS (pH 7.0), diluted in the distilled water and centrifuged; Lane 5, tropomyosin (tropomyosin prepared according to the method described in the legend of lane 4 was concentrated with $(\text{NH}_4)_2\text{SO}_4$ of 0.55 saturation, dialyzed in 5 mM MOPS buffer (pH 7.0) and centrifuged. The supernatant is tropomyosin).

Fig. 2. Hydroxylapatite column chromatography of scallop tropomyosin. 30.8 mg tropomyosin was dialyzed against 5 mM phosphate buffer (pH 7.0) applied to a hydroxylapatite column (1.3×2.2 cm) equilibrated with the same buffer solution and eluted by using a linear gradient formed by phosphate buffer (pH 7.0) between 5 mM and 250 mM. Phosphate buffer concentration is shown as a dotted line. Each fraction is 3.05 ml.

Fiske-SubbaRow method with a slight modification.¹³⁾ The free Ca^{2+} concentration was calculated using the binding constant of Ca^{2+} to EGTA reported by Ogawa.¹⁴⁾ Slab gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli.¹⁵⁾ The concentration of scallop tropomyosin was estimated by ultraviolet spectrophotometry using the given extinction coefficient (in units of mg/ml), rabbit skeletal tropomyosin, 0.24 at 277 nm.¹⁶⁾

Results and discussion. The preparation of tropomyosin from natural actomyosin: Adductor striated muscle obtained from living scallop, *Patinopecten yessoensis*, in the Sea of Okhotsk. The yield of natural actomyosin (NAM) was 4.87% of wet muscle. The mixture of tropomyosin and myosin was fractionated by ammonium sulfate between 0.40 to 0.55 saturation in the presence of 7 mM MgCl_2 , 5 mM ATP, and 20 mM phosphate buffer (pH 7.0). The mixture of the precipitated myosin and tropomyosin was dialyzed against 2 mM MOPS buffer (pH 7.0) for about 3 hrs and the dialyzate

was diluted in 5 vol. of cold water and centrifuged. The supernatant was the crude tropomyosin and the precipitate was myosin. Tropomyosin was precipitated with ammonium sulfate of 0.55 saturation, dialyzed in 5 mM MOPS buffer (pH 7.0) and centrifuged. Fig. 1 shows SDS-PAGE patterns in the course of the preparation of scallop tropomyosin. It is shown that tropomyosin (Fig. 1, lane 5) contains minor components, 43 kDa (actin) and 18 kDa (myosin light chain). The yields of scallop tropomyosins prepared according to two kinds of methods, the method of Bailey and a new method described in this paper are summarized in Table I. The yields of rabbit skeletal tropomyosin are also listed in the same Table. The yield of scallop tropomyosin prepared from NAM was 0.160%, about 4 times as high as that of tropomyosin prepared according to the method of Bailey. Similar results were obtained from rabbit skeletal tropomyosin. Tropomyosin was dialyzed against 5 mM phosphate buffer (pH 7.0) and purified by linear phosphate buffer (pH 7.0) gradient on hydroxylapatite col-

Fig. 3. SDS-PAGE patterns of fractions obtained during purification of scallop tropomyosin. Electrophoresis was carried out using 10% polyacrylamide gel containing 0.1% SDS. Lane 1, scallop striated muscle tropomyosin; Lane 2–4 were fraction numbers 10, 15 and 16 eluted by hydroxylapatite column chromatography, respectively.

umn (Fig. 2). Fig. 3 shows SDS-PAGE patterns of fractions obtained by the column chromatography. Two of fractions was shown homogeneous, its molecular weight being estimated at 34 kDa.

Effects of scallop purified tropomyosin on Mg^{2+} -ATPase activities of actomyosin reconstituted from scallop myosin and actin were investigated (Fig. 4). The Mg^{2+} -ATPase activities of reconstituted scallop actomyosin were 0.40μ mole Pi / mg · min and 0.80μ mole Pi / mg · min in the presence of 1.1×10^{-7} M and 1.0×10^{-4} M Ca^{2+} , respectively. When scallop tropomyosin was added to reconstituted scallop actomyosin, Mg^{2+} -ATPase activities did not show any changes.

Fig. 5 shows effects of rabbit purified tropomyosin on Mg^{2+} -ATPase activity of actomyosin reconstituted from skeletal myosin and actin. The Mg^{2+} -ATPase activity of reconstituted rabbit skeletal actomyosin was 0.21μ mole Pi / mg · min regardless of Ca^{2+} concentrations. When scallop tropomyosin was added, the ATPase activity of skeletal actomyosin was 0.16μ mole Pi / mg · min and showed 25% inhibition compared with that of reconstituted rabbit actomyosin. When rabbit skeletal purified tropomyosin in place of scallop tropomyosin was added to reconstituted rabbit actomyosin, 25% inhibition of Mg^{2+} -ATPase activity was observed and showed the same inhibition as that in the case of scallop tropomyosin. It is well known that rabbit skeletal tropomyosin inhibits the Mg^{2+} -ATPase activity of reconstituted rabbit actomyosin.^{17),18)} The inhibitory effects of scallop tropomyosin on Mg^{2+} -ATPase activity of reconstituted rabbit actomyosin are also reported,^{19),20)} but the effect of scallop tropomyosin on Mg^{2+} -ATPase activity of reconstituted scallop actomyosin shown in Fig. 4 is the first finding.

Fig. 4. Effects of scallop tropomyosin on Mg^{2+} -ATPase activities of scallop reconstituted actomyosin at various Ca^{2+} concentrations. Reaction mixture contained 3 mM $MgCl_2$, 10 mM MOPS buffer (pH 6.8), and 2 mM ATP at 25 °C. ○, 0.1 mg/ml scallop myosin and 0.035 mg/ml scallop actin; ●, 0.1 mg/ml scallop myosin and 0.035 mg/ml scallop actin and 0.010 mg/ml scallop tropomyosin.

Fig. 5. Effects of scallop tropomyosin on Mg^{2+} -ATPase activity of reconstituted rabbit skeletal actomyosin. Reaction mixtures and conditions were the same as those described in the legend to Fig. 4. ○, 0.20 mg/ml rabbit myosin and 0.07 mg/ml rabbit actin; ●, 0.20 mg/ml rabbit myosin, 0.07 mg/ml rabbit actin and 0.02 mg/ml scallop tropomyosin; ■, 0.20 mg/ml rabbit myosin, 0.07 mg/ml rabbit actin and 0.02 mg/ml rabbit tropomyosin.

The preparation of tropomyosin from scallop striated muscle according to this new method takes one day, whereas the procedure described by Bailey⁸⁾ takes at least 4 days. We expect that this new method for preparing tropomyosin will be widely used because of its simplicity and rapidity.

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