Bivalve Tropomyosins Exhibit Strong Inhibition of Actomyosin Mg-ATPase and High Viscosity

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Tropomyosins were isolated from striated and smooth adductor muscles of akazara scallop *Chlamys nipponensis akazara* and ezo-giant scallop *Patinopecten yessoensis* and smooth adductor muscle of surf clam *Spisula sachalinensis*. These bivalve tropomyosins inhibited Mg-ATPase activity of rabbit reconstituted actomyosin to 15% of original activity, *i.e.*, approximately 1.4 times stronger than rabbit and carp tropomyosins did at a weight ratio of 2:1 for F-actin: tropomyosin. On the other hand, the relative viscosities in the absence of KCl were 3.5–6 times higher than those of rabbit and carp tropomyosins. The intrinsic viscosities were, however, comparable to that of rabbit tropomyosin, *i.e.*, 0.5 dl/g for bivalve tropomyosins and 0.4 dl/g for rabbit tropomyosin. These viscosities suggest that the bivalve tropomyosins tend to form a highly polymerized form compared with rabbit tropomyosin. Circular dichroism spectra and amino acid compositions of the bivalve tropomyosins are similar to one another and also to rabbit and carp tropomyosins. C-terminal three amino acids of bivalve tropomyosins were sequenced as -Ala-Gly-Tyr (scallops) and -Gly-Tyr-Thr (surf clam), which are quite different from -Thr-Ser-Ile and Thr-Ser-Leu of rabbit α - and β -tropomyosin, respectively.

Key words: bivalve, tropomyosin, inhibition of Mg-ATPase, viscosity

Tropomyosin is a well-investigated regulatory protein in muscle and non-muscle cells.^{1–7)} It is a highly α -helical, rod-shaped dimeric protein forming a coiled-coil structure and binds along the grooves of F-actin filaments. In striated muscles and some of smooth muscles, tropomyosin plays a regulatory role for contraction together with troponin.^{7–10)} Up to now a number of tropomyosin isoforms have been isolated from various vertebrate and invertebrate muscles and the molecular heterogeneity and tissue specificity have been extensively investigated.^{2,6,11–15)}

On the other hand, we have isolated tropomyosin and troponin from striated and smooth adductor muscles of both akazara scallop and ezo-giant scallop in which a myosin-linked regulatory system exists. We have also shown that those tropomyosins strongly inhibit Mg-ATPase activity of rabbit reconstituted actomyosin.^{9,10,16,17)} It is considered that scallop troponin can confer Ca^{2+} -sensitivity to actomyosin in such a manner as increasing the repressed activity by tropomyosin in the presence of Ca^{2+} rather than decreasing the activity in its absence as seen in vertebrate regulatory systems. A similar conferring manner of Ca^{2+} -sensitivity has also been reported in ascidian smooth muscle tropomyosin-troponin.⁸⁾ Thus, the strong inhibitory effect of tropomyosin on actomyosin Mg-ATPase seems to be a characteristic property of invertebrate tropomyosins.

In the present paper, we show that several bivalve tropomyosins also exhibit strong inhibitory effects on rabbit actomyosin Mg-ATPase activity, in addition to extremely high specific viscosity.

Materials and Methods

Tropomyosin was prepared from striated and smooth adductor muscles of akazara scallop and ezo-giant scallop and smooth adductor muscle of surf clam. Preparation was achieved by the methods described by Endo and Obinata⁸⁾ and Ojima and Nishita⁹⁾. Briefly, crude tropomyosin was extracted with 0.4 M LiCl at pH 5.0 from the myofibrils and purified by repeating ammonium sulfate fractionation at 35–45% saturation in the presence of 0.4 M LiCl. Rabbit and carp tropomyosins were isolated from white skeletal muscle and dorsal muscle, respectively, by the method used for the scallop tropomyosins except that the ammonium sulfate fractionation was achieved at 45–55% saturation. Rabbit myosin and actin were prepared by the methods of Perry¹⁸⁾ and Spudich and Watt,¹⁹⁾ respectively. Actomyosin was reconstituted by mixing the myosin and actin in a weight ratio of 2:1. SDS-PAGE was performed by the method of Porzio and Pearson.²⁰⁾ The gel was stained with 0.2% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, and background of the gel was destained with 10% acetic acid and 5% methanol. Mg-ATPase activity of actomyosin was measured in a solution containing 50 mM KCl, 20 mM Tris maleate (pH 6.8), 2 mM MgCl_2 , 0.1 mg/ml rabbit myosin, 0.05 mg/ml rabbit F-actin, and various concentrations of tropomyosin. The inorganic phosphate liberated by ATP hydrolysis was determined by the method of Youngburg

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Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Mr, molecular weight.

and Youngburg.²¹⁾ The viscosity of tropomyosin was measured with Ostwald's viscometer (flow time of the solvent was 29 s). Circular dichroism spectrum was measured by using a Jasco J-600 spectropolarimeter and a cuvette with a 1 mm path length. The α -helical content was estimated with the following equation:²²⁾

$$\text{helix(\%)} = \frac{[\theta]_{222} + 3,000}{39,000} \times 100.$$

Amino acid composition was analyzed by using a Hitachi 655-A high performance liquid chromatograph equipped with Wakosil-PTC column (4 × 200 mm). The amino acid sequence of the C-terminal region was determined by the digestion of carboxypeptidase Y followed by the analysis of released amino acids. Protein concentration was determined by the biuret method using bovine serum albumin Fraction V as a standard protein.²³⁾

Results and Discussion

SDS-PAGE of Bivalve Tropomyosin

Figure 1 shows the SDS-gel electrophoretic patterns of seven species of tropomyosin. Striated adductor tropomyosins from both akazara and ezo-giant scallops showed a single band of around Mr 35,000, while smooth adductor tropomyosins showed two subunit bands with Mr 35,000 and 36,000. The surf clam smooth muscle tropomyosin showed a single band with Mr 34,000. These results coincide with the previous reports.^{11,14,24)} Thus, two isoforms and one isoform with respect to the mobility on SDS-PAGE were observed in scallop smooth and striated muscle tropomyosins, respectively,^{14,24)} while no polymor-

Fig. 1. SDS-polyacrylamide gel electrophoresis of various tropomyosins.

Electrophoresis was carried out in 10% polyacrylamide slab gel containing 0.1% SDS according to Porzio and Pearson.²⁰⁾ Ak-st, akazara scallop striated adductor Tm; Ak-sm, akazara scallop smooth adductor Tm; Ez-st, ezo-giant scallop striated adductor Tm; Ez-sm, ezo-giant scallop smooth adductor Tm; Surf, surf clam smooth adductor Tm; Rab, rabbit skeletal Tm; Carp, carp dorsal muscle Tm. The approximate molecular weights are shown in the right-hand side of the figure.

phism was seen in surf clam tropomyosin.¹¹⁾ The rabbit and carp tropomyosins showed a doublet and singlet band, respectively, as reported previously.^{7,25,26)}

Effects of Bivalve Tropomyosins on Actomyosin Mg-ATPase Activity

We have shown that tropomyosin from scallop striated and smooth muscles strongly inhibits Mg-ATPase activity of rabbit reconstituted actomyosin, and the scallop tropomyosin confers Ca^{2+} -sensitivity to the actomyosin-tropomyosin Mg-ATPase activity by removing the inhibition of tropomyosin in the presence of Ca^{2+} .^{9,10,16,17)} Therefore, we examined the inhibitory effect of bivalve tropomyosins on the actomyosin Mg-ATPase activity and compared with those of rabbit and carp tropomyosins. As shown in Fig. 2, all the bivalve tropomyosins tested inhibited approximately 85% of original Mg-ATPase activity, while the rabbit and carp tropomyosins inhibited 60%. Thus, those tropomyosins appear to be classified into two types, *i.e.*, one showing stronger inhibition (bivalve tropomyosins) and the other showing weaker inhibition (rabbit and carp tropomyosins). The physiological significance of the strong inhibition of the bivalve tropomyosins is obscure. However, it has been reported that superprecipitation activity and its Ca^{2+} -sensitivity of ezo-giant scallop striated adductor actomyosin are improved by the presence of scallop tropomyosin.²⁴⁾ The effects of coexistence of myosin-linked regulatory system on the function of bivalve tropomyosins are now under investigation.

Fig. 2. Effect of tropomyosin on the Mg-ATPase activity of rabbit reconstituted actomyosin.

Mg-ATPase activity was measured as described in "Materials and Methods". $\circ$ and $\bullet$, akazara scallop striated and smooth adductor Tms, respectively; $\triangle$ and $\blacktriangle$, ezo-giant scallop striated and smooth adductor Tms, respectively; $\square$, surf clam smooth adductor Tm; ∇ , rabbit Tm; $\blacktriangledown$, carp Tm. Activity of actomyosin in the absence of Tm was $0.098 \mu\text{mol Pi/min} \cdot \text{mg}$ and represented as 100% relative activity on the ordinate.

Viscosity of Bivalve Tropomyosins

The head-to-tail polymerization ability of tropomyosin has been considered as a crucially important function for the Ca^{2+} -regulation of troponin-tropomyosin.^{7,27,28)} Since the polymerization of tropomyosin is enhanced under lower ionic conditions, the viscosity becomes higher concomitantly with decrease in salt concentration. As shown in Fig. 3, the relative viscosity steeply increased with decrease in KCl concentration below 0.05 M. In addition, the extent of increase in viscosity of bivalve tropomyosins was higher than those of rabbit and carp tropomyosins. The relative viscosities of striated and smooth muscle tropomyosins of akazara and ezo-giant scallops were about 6 times higher than those of rabbit and carp tropomyosins in the absence of KCl, and that of surf clam Tm was about 3.5 times higher than those of rabbit and carp tropomyosins. The reduced viscosities of bivalve tropomyosins were plotted against concentration along with those of rabbit and carp (Fig. 4). Accordingly, the intrinsic viscosities of the bivalve tropomyosins were estimated to be 0.5 dl/g, slightly higher than those of rabbit and carp tropomyosins of 0.4 dl/g. These results strongly suggest that the bivalve tropomyosins have markedly higher polymerizability than vertebrate tropomyosins at lower salt concentrations, though their molecular dimensions appear to be similar.

Circular Dichroism of Bivalve Tropomyosins

The tropomyosin molecule is known to be formed from almost a complete α -helix structure.^{7,22)} As shown in Fig. 5, the circular dichroism spectra of all the tropomyosins tested were indistinguishable from one another. Their α -helical contents were estimated to be about 85% from the

molecular ellipticity at 222 nm. No difference in α -helical content was found among the tropomyosins examined.

Amino Acid Compositions of the Bivalve Tropomyosins

Amino acid compositions of tropomyosins were deter-

Fig. 4. Reduced viscosity of tropomyosin as a function of protein concentration.

Viscosity was measured as in Fig. 3, except that the KCl concentration was fixed to 200 mM and the protein concentration was varied. $\circ$, akazara scallop striated adductor Tm; $\triangle$, ezo-giant scallop striated adductor Tm; $\blacktriangle$, surf clam smooth adductor Tm; ∇ , rabbit Tm.

Fig. 3. Relative viscosity of tropomyosin as a function of KCl concentration.

The relative viscosity was determined at 20°C in an Ostwald type viscometer with the outflow time of 29.0 s for solvent. The concentration of Tm was 1.5 mg/ml in 20 mM Tris maleate (pH 7.0) and various concentrations of KCl. The symbols used are the same as in Fig. 2.

Fig. 5. CD-spectrum of tropomyosin.

The CD-spectrum was measured in a solution containing 0.1 M KCl, 10 mM Tris-HCl (pH 7.5), 1 mg/ml Tm. The spectra for Tms of akazara scallop striated adductor (Ak-st) and rabbit (Rab) are shown as representatives since the profiles of the other Tms were practically identical.

Table 1. Amino acid composition of various tropomyosins (mol%)

Amino acid	Ak-st	Ak-sm	Ez-st	Ez-sm	Surf	Rab	Carp
Asx* ¹	13.2	13.6	13.9	14.0	11.2	10.8	11.1
Thr	7.1	5.0	7.1	4.1	5.6	1.9	3.5
Ser	3.3	3.7	3.9	3.4	5.1	4.5	3.8
Glx* ²	26.0	26.0	24.7	26.8	22.6	26.7	25.9
Gly	1.8	2.0	1.7	2.0	1.8	1.6	1.9
Ala	11.3	12.9	11.5	11.9	10.3	12.7	12.4
Val	4.0	3.6	4.4	4.3	5.7	3.1	3.1
Met	1.1	0.5	1.5	0.6	1.4	1.3	2.3
Ile	2.3	3.5	2.8	3.7	4.4	4.8	3.6
Leu	10.8	11.5	10.6	11.5	11.7	11.3	11.3
Tyr	1.1	1.3	1.2	0.4	2.6	1.4	1.5
Phe	1.8	1.5	1.3	1.9	0.6	0.9	0.9
Lys	10.1	7.9	9.9	8.9	10.6	13.4	13.0
His	0.4	0.4	0.0	0.2	0.0	0.6	0.8
Arg	6.1	6.6	5.5	6.3	6.4	5.0	4.9
Pro	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Abbreviations are the same as in Fig. 1.

*¹ Asp + Asn. *² Glu + Gln.

mined (Table 1). All tropomyosins tested showed fairly similar compositions, *i.e.*, rich in Asx, Glx, Ala, Leu, and Lys, but no Pro. The absence of Pro is a common feature of tropomyosins.²⁹⁻³³ However, some differences were seen, for example, the bivalve tropomyosins contained more Thr and less Lys^{26,29,30} than rabbit and carp tropomyosins.

Amino Acid Sequence of C-terminal Region of Bivalve Tropomyosins

In rabbit skeletal tropomyosin, the C-terminal 9 amino acids have been shown to be involved in the head-to-tail interaction with the N-terminal region.^{7,27,28} We analyzed the C-terminal amino acid sequences of the bivalve tropomyosins by carboxypeptidase Y digestion. As a result, the sequences of three amino acids were determined as -Ala-Gly-Tyr for tropomyosins of akazara and ezo-giant scallop striated and smooth adductor muscles, and as -Gly-Tyr-Thr for tropomyosins of surf clam smooth adductor and foot muscles. These sequences are quite different from those of rabbit α - and β -tropomyosins (-Thr-Ser-Ile and -Thr-Ser-Leu, respectively^{31,32}). Next, we attempted to determine the N-terminal sequences of the molluscan tropomyosin by the Edman method. However, no phenylthiohydantoin amino acid was obtained probably due to the blocking of the N-termini. Although little information about the N-terminal sequence of bivalve tropomyosins is available now, the N-terminal region of vertebrate tropomyosin has been reported to be fairly conserved among various species in contrast to the divergence in the C-terminal region.³³ In order to reveal the reason for the high viscosity of bivalve tropomyosins, detailed studies on the primary structures and polymerizabilities are necessary.

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