Characterization of Three Types of Turban Shell *Batillus cornutus* Muscle—Ultrastructure and Protein Composition

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Three types of turban shell *Batillus cornutus* muscle—foot, opercular and visceral—were examined under an electron microscope, and the results discussed in association with their differences in muscle protein composition.

Myofibrils of foot muscle were partially surrounded by a collagen fibril layer of about 1 μm thickness. Diameters of thick filaments fell in a wide range of 22–70 nm (mean value, 50 nm). The myofibrils were found to be crossing each other at right angles. The opercular muscle showed a similar structure, except that the collagen fibril layer was clearly thinner. The visceral muscle which surrounds the viscera was composed of roughly parallel-running myofibrils whose thick filaments had a mean diameter of 50 nm.

Paramyosin contents in the myofibrillar protein were 29–41% through the three types of muscle. The paramyosin to myosin heavy chain ratios were 2.5 and 2.3 in the foot and opercular muscles, respectively, whereas the ratio in the visceral muscle was 1.4.

The collagen to crude protein ratio was 45% in the foot, 34% in the opercular and 5% in the visceral muscle, roughly agreeing with their electron microscopic differences.

The adductor muscle of molluscs (smooth muscle) is composed of huge thick filaments of up to 150 nm diameter, each of which consists of a paramyosin core surrounded by a myosin layer.^{1,2)} Such a type of muscle makes it possible to sustain a large tension with little energy expenditure. This phenomenon, so-called “catch” contraction, has been suggested to be associated with paramyosin,^{3–5)} whose involvement in actomyosin Mg^{2+} -ATPase and superprecipitation seems to be much more complicated than has so far been supposed.^{2,6–8)}

The muscle of gastropods also contains paramyosin. Their opercula, once locked, are difficult to open, reminding us of the catch contraction in bivalves. However, physiological and biochemical studies on catch mechanism have exclusively been carried out with bivalves,^{2,4–10)} and none with gastropods. The muscle of gastropods can macroscopically be classified into three types: foot muscle, opercular (adductor) muscle that attaches

to the operculum, and visceral muscle surrounding the viscera. Takahashi and Tanaka¹¹⁾ reported that the three muscle types of turban shell differ from each other in appearance.

In the present study, the ultrastructures of three types of turban shell muscle were examined under a transmission electron microscope. Their differences thus observed were discussed in association with differences in contents of some protein components.

Materials and Methods

Materials

Live specimens of the turban shell *Batillus cornutus* (average weight, about 400 g) were obtained from the Tokyo Central Wholesale Market, and transported to the Laboratory of Marine Biochemistry. After the shell was removed, the muscle was macroscopically dissected into three types of muscles—the foot, opercular and visceral

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Fig. 1. General appearance of turban shell muscles. Mantle (a), foot (b), opercular (c) and visceral (d) muscles were dissected as indicated in the photographs.

Table 1. Contents of myofibrillar proteins and collagen in the foot, opercular and visceral muscles of turban shell

	Muscle		
	Foot	Opercular	Visceral
Weight percent	25	15	60
Crude protein (A)* ¹	18.2	16.1	17.4
Protein components:			
Myofibrillar protein (B)	4.1	5.0	7.9
Collagen (C)* ²	8.2	5.5	0.9
Paramyosin (D)	1.7	2.0	2.3
Myosin heavy chain (E)	0.68	0.87	1.6
Ratios:			
(B/A) × 100 (%)	23	31	45
(C/A) × 100 (%)	45	34	5
(D/B) × 100 (%)	41	40	29
D/E	2.5	2.3	1.4

*¹ N content × 6.25.

*² Hydroxyproline content × 10.

muscle—along with mantle (Fig. 1). Average weight ratios of foot, opercular and visceral muscles were 25, 15 and 60%, respectively (Table 1).

Electron Microscopy

Each type of turban shell muscle was fixed with 4% glutaraldehyde-containing cacodylate buffer (pH 7.4) at room temperature. During this procedure, each muscle was cut into small pieces (*ca.* 1 × 5 × 5 mm). After about 1 h, the muscle pieces were washed with the above buffer, cut into smaller pieces or strips (*ca.* 0.5 × 0.5 × 5 mm), and were post-fixed with 1% osmium tetroxide at 0°C for about 1 h. The strips were stained *en bloc* with 1% uranyl acetate and then were dehydrated in a graded series of ethanol and acetone, followed by treatment with a mixture of acetone and Poly/Bed 812 resin (Polysciences Inc.) and embedding in the resin. Thin sections of about 100 nm thickness obtained therefrom were stained with a lead citrate reagent according to

Reynolds.¹²⁾ Micrographs were taken in a JEM 100B-4 type transmission electron microscope at an 80 kV accelerating voltage with a 30 μm objective aperture, at magnifications of 4,000 to 10,000.

Muscle Protein Composition

The three types of turban shell muscle were fractionated into sarcoplasmic, myofibrillar, alkali-soluble and stroma protein fractions according to the method of Hashimoto *et al.*¹³⁾

Collagen Content

Five grams of each muscle was hydrolyzed with 4 vol. of 6 N HCl at 110°C for 24 h. To 1 ml hydrolyzate was added 15 vol. of 6 N HCl and the mixture was hydrolyzed again under the same conditions. The hydrolyzate thus provided was evaporated to dryness at 45°C, dissolved in water, and was neutralized with 3.5 N NaOH. One half ml of the sample solution thus prepared was pipetted into a 10 $\times$ 100 mm test tube. After adding 1 ml of iso-propanol and 0.5 ml of solution A (1.4% chloramine T in 0.9 M acetate-citrate buffer, pH 6.0), the test tube was allowed to stand for 4 min at room temperature, followed by adding 1.3 ml of solution B (7.5% *p*-dimethylamino-benzaldehyde/80% iso-propanol). The test tube containing the mixture was heated at 60°C for 25 min, and cooled by tap water. The absorbance at 558 nm of the solution was measured, and the concentration of hydroxyproline was determined from the calibration curve obtained by using hydroxyproline standard (Wako, reagent grade).

The collagen content was determined on the assumption that the hydroxyproline content of turban shell collagen was 10.0% by weight.¹⁴⁾

Paramyosin Content

Paramyosin was isolated and weighed from the whole muscle by the method of Johnson *et al.*¹⁵⁾

SDS-Gel Electrophoresis

SDS-gel electrophoresis was carried out according to Weber and Osborn,¹⁶⁾ using 10% polyacrylamide gels. After the run, gels were stained with 0.25% Coomassie brilliant blue R-250 and scanned at 550 nm with a Gelman ACD-18 densitometer.

Protein Concentration

Protein concentration was determined by the micro-Kjeldahl method.

Results and Discussion

Electron Microscopy

Electron micrographs of the three types of turban shell muscle are shown in Figs. 2-4. Interdigitating arrays of thick and thin filaments, which are characteristic of the cross-striated muscle,¹⁷⁾ did not appear in turban shell muscle. This indicates that turban shell muscle belongs to the smooth muscle as do some molluscan muscles.³⁾ The interior of the cell was filled with thick and thin filaments constituting the contractile apparatus.

In Fig. 2a is shown a typical ultrastructure of turban shell foot muscle; *i.e.*, a random arrangement of myofibrils with or without a collagen layer. In this connection, the foot muscle exhibits peristaltic movement.

Diameters of the thick filaments of foot muscle varied widely from 22 to 70 nm with the mean value of 50 nm, suggesting the presence of a large diameter paramyosin core. Fig. 2b shows cross-striation of collagen fibrils with a typical periodicity of about 60 nm.¹⁸⁾ Some myofibrils were surrounded by a collagen layer of about 1 μm thickness.

Sovieszek¹⁹⁾ performed electron microscopic studies on the anterior byssus retractor muscle of *Mytilus edulis*, reporting that the extracellular space was partially filled with collagen fibrils. Some connective tissues of echinoderms such as sea cucumber and sea urchin change the stiffness in response to stimuli. Because of an analogy to catch in bivalves, such a phenomenon is called "connective tissue catch."²⁰⁻²³⁾ However, involvement of collagen fibrils in catch mechanism has not been confirmed at all.

Diameters of the opercular thick filaments varied widely from 24 to 81 nm with a mean value of 60 nm (Fig. 3), suggesting the abundance of paramyosin. The opercular muscle cells were also set up with myofibrils which extended to each other at a right angle (Fig. 3a). However, collagen fibril layers were clearly thinner (about 600 nm) than those of foot muscle (Fig. 3b).

In Fig. 4 are shown the electron micrographs of visceral muscle. Diameters of the thick filaments differed most widely from 10 to 84 nm with a mean value of 50 nm. This value was equal to that of foot muscle, suggesting a high paramyosin content again. Few myofibrils of visceral muscle were surrounded by a collagen layer whose thickness was about 300 nm (photograph not shown). Noteworthy enough is that the visceral myofibrils,

(a)

(b)

Fig. 2. Electron micrographs of the foot muscle of turban shell, showing the random array of myofibrils (a) and the presence of collagen fibrils crossing the myofibrils at a right angle (b). The bar represents 1 μ m. Abbreviations used: Tn, thin filament; Tk, thick filament; Cf, collagen fibril.

unlike foot and opercular ones, were oriented in almost the same direction with few obliquely-running myofibrils (Fig. 4).

Collagen Content

The collagen contents in crude protein of foot, opercular and visceral muscles were 45, 34 and

(a)

(b)

Fig. 3. Electron micrographs of the opercular muscle of turban shell, showing the random arrangement of several myofibrils (a) and the cross section of myofibrils surrounded by the layer of collagen fibrils (b). The bar represents 1 μm . Abbreviations are as in Fig. 2.

5%, respectively (Table 1), correlating well with their electron micrographical features. The collagen contents in foot (45%) and opercular muscles (34%) were remarkably higher than that

of rabbit skeletal muscle (2–10%).²⁴⁾ Incidentally, the collagen content of the mantle was as high as 33%. In this connection, the chain composition of turban shell foot muscle collagen,¹⁴⁾ ($\alpha 1$)₃, has

Fig. 4. Electron micrograph of the visceral muscle of turban shell, showing the cross section of roughly parallel-running myofibrils. The bar represents 1 μ m. Abbreviations are as in Fig. 2.

Table 2. Protein composition of the foot, opercular and visceral muscles of turban shell (mg N/g wet tissue)

Muscle type	Non-protein N	Protein N* ¹			
		SP	MF	AS	ST
Foot	3.65	4.02 (11.8)* ²	9.67 (36.3)	2.68 (10.0)	10.3 (38.9)
Opercular	3.70	3.16 (14.7)	9.79 (45.4)	2.70 (12.5)	5.90 (27.4)
Visceral	5.59	4.09 (18.7)	14.62 (66.7)	2.20 (10.0)	1.00 (4.6)

*¹ SP, Sarcoplasmic; MF, myofibrillar; AS, alkali-soluble; ST, stroma.

*² Numbers in parentheses represent percentage distribution.

recently been demonstrated to be identical to those of the collagens from other tissues.*

Muscle Protein Composition

Protein compositions of foot, opercular and visceral muscles are shown in Table 2. The order of stroma contents of the three muscles is reminiscent of that of the collagen contents. Their stroma protein contents were in negative correlation with their myofibrillar protein contents.

Contents of Myofibrillar Protein Components

Crude protein contents of foot, opercular and

visceral muscles were about 18, 16 and 17%, respectively (Table 1). The myofibrillar to crude protein ratio was highest in visceral muscle (45%). These values were significantly lower than those of rabbit and fish (60–70%) skeletal muscle.^{25,28)}

Finally, the myofibrillar proteins from the three types of turban shell muscle were analyzed by SDS-polyacrylamide gel electrophoresis. Densitometric tracings of the stained gels are shown in Fig. 5. The paramyosin to total myofibrillar protein ratios were estimated to be about 41, 40 and 29% for foot, opercular and visceral muscles, respectively, on the assumption that the staining intensity per unit weight was the same through all

* S. Kimura, unpublished data.

Fig. 5. SDS-gel electrophoretic patterns of myofibrillar proteins from foot (a), opercular (b) and visceral (c) muscle in 10% polyacrylamide gels. Abbreviations used: MHC, myosin heavy chain; PM, paramyosin; AC, actin; TM, tropomyosin.

the protein components. It was noteworthy that the three types of muscle did not differ from each other in paramyosin content significantly.

However, the paramyosin to myosin heavy chain weight ratios were calculated to be 2.5 and 2.3 for the foot and opercular muscles, respectively,

which were clearly higher than that of visceral muscle, 1.4. It follows from their molar ratios that the foot and opercular muscles of turban shell belong to Class III (paramyosin-rich type), while the visceral muscle belongs to Class II (intermediate type).²⁷⁻³¹⁾ The muscle of Class

III generally shows an extremely high value for the maximum active tension, which is correlated with enormously increased filament diameter.²⁷⁻³¹⁾ Since thick filaments of a large diameter were observed in the visceral muscle as described above, this muscle could also belong to Class III.

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