Ultrastructural Investigations on the Anterior Adductor Muscle of a Brachiopoda, *Lingula unguis*

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**ABSTRACT.** The brachiopoda, *Lingula unguis*, has a pair of anterior adductors located in the center of the shell. Each muscle consists of an opaque and a translucent portion which is constructed of smooth and obliquely-striated muscle respectively.

According to our ultrastructural observations, the opaque portion seems to have two types of cells. They differ only in the diameters of their thick myofilaments. The fine structure of their cell organelles resembles each other. We measured the diameters of the thick myofilaments in each type of cell to distinguish between the two cell types. About 500 measurements of myofilament diameters were made for each type of cell and statistically analyzed. For one type of cell, the distribution of diameters of the thick myofilaments fit a normal distribution curve with a peak at 37–60 nm. The distribution of diameters of the thick myofilaments for the other type fit a curve in which two normal distribution curves having peaks at 37–60 and 75–97 nm respectively partially overlapped. According to these results, we suggest that the opaque portion contains two types of cells, each having a different distribution of thick myofilament sizes.

*Lingula unguis* (Brachiopoda) has a pair of shells, one on the dorsal and one on the ventral side of the body (6). Movement of the shells is regulated by anterior and posterior adductors.

The pair of anterior adductors consists histologically of an opaque and a translucent portion. The histological structures of the adductor resemble those of other bivalved adductors. It is well known that the adductors of bivalves have an opaque and a translucent portion, constructed of smooth muscle and obliquely-striated muscle respectively (4, 5, 10). However, reports on the fine structure of adductors of the brachiopods are few, although physiological (13) and structural (14) studies on *Terebratula trasversa*, were reported.

The thick myofilaments in the smooth muscles of bivalved adductors characteristically appear to be constructed of a core of paramyosin surrounded by polarized myosin (1, 3, 8, 9, 11, 12). Paramyosin has been supposed to have some function in catch-contraction (12).

We studied the ultrastructure of the anterior adductor of this unique material to reveal what kind of smooth muscle cells make up the muscle, and whether they are similar to those of other bivalves.
MATERIALS AND METHODS

*Lingula unguis* is generally distributed from the Japanese Islands (Honsyu, Shikoku, Kyusyu) to the Philippine Islands, to the Indian Ocean. The material for this work was obtained from the Bay of Ariake in Japan. The animals were of the standard size, measuring about 40 mm in length and 18 mm in width.

After the adductors were relaxed enough, wedges were applied to keep the gap in the shells at the width of the relaxed state. Then, the animals were prefixed in a solution containing 1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.3. Prefixed anterior adductors were dissected into several pieces, and fixed again in a newly prepared solution for 90 min. These specimens were rinsed with 0.1 M cacodylate buffer (pH 7.3), and postfixed for 120 min in a solution of 1% Osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) at about 4°C. They were then dehydrated with a series of ethanol solutions (70, 80, 90, 95, 98, 100%) and embedded in epoxy resin after Glycidyl-n-butyl-ether treatment.

Hardened specimens in resin were thin sectioned with glass or diamond knives. The sections were scooped up on grids, and stained with saturated aqueous uranyl acetate and a lead citrate staining solution. They were observed in a JEM 100C type electron microscope, and photographed at a magnification of 2,000–33,000 times.

The diameters of thick myofilaments were measured directly from the screen of a “Profile Projector” on which was mounted the electron microscopic negative and which magnified the negative 50 times. Cross sections of the adductors were photographed at a magnification of 10,000 times. Measurements were made starting from the cell periphery through the central portion of the cell and on to the other side of the cell and repeated on the same cell until about 500 thick myofilament profiles had been measured. Five cells of each type were measured.

RESULTS

The pair of anterior adductors in *L. unguis* is located at the midpoint of the shell. In the central portion of the muscle, connective tissue joins the muscles from the dorsal and ventral shells. We did not look closely at the connective tissue in this investigation.

Histologically, an opaque portion was found on the front side of the adductor and a translucent portion on the rear side. The two portions showed a different pattern by Masson-Goldner staining (Fig. 1). These observations suggested that each portion of the adductor is constructed of a different type or types of muscle cells. When we studied the fine structure of each portion by electron microscopy, we observed that one portion was constructed of smooth and the other of obliquely-striated muscle cells. The muscle cells of the two portions usually bordered but did not intermingle with each other (Fig. 2).

The ultrastructural characteristics of the two portions of the adductor are described individually in the following sections.

**Opaque portion.** The smooth muscle cells in this portion were filled with myofilaments. Cell organelles such as mitochondria or sarcoplasmic reticular (S. R) systems were located in the peripheral regions of the cell (Fig. 3). In longitudinal sections of the cells, the thick myofilaments appeared to have cross striations at intervals of 36 nm, which is characteristic of paramyosin (Fig. 4). When we studied cross sections of the cells, we noticed that there were two types of cells, each having thick
myofilaments differing in size form those of the other cell type (Fig. 3). Similar differences were found in longitudinal sections (Fig. 4). Therefore, we suggest that the opaque portion consists of two types of cells, each having thick myofilaments differing in diameter from those of the other. To classify the cell types, we measured the diameters of the thick myofilaments. About 500 thick myofilament profiles were measured per cell. Their diameters were classed by size using a 7.5 nm interval, and the frequency (%) of thick myofilaments in each sizes class was calculated. The results are shown in a histogram in which the horizontal axis indicates the class size and the vertical axis, the relative frequency of each class (Fig. 7-A, B). Then, we tested to see if the results fit a normal distribution. if the data in the histogram fit a normal distribution curve, the highest point of the curve should indicate the average diameter of the thick myofilaments in the cell. We calculated the average diameter of the thick myofilaments in each type of cells by this method. A normal curve was obtained by the following statistical analysis. The frequencies for each class were plotted on normal probability paper and a line drawn through the points. If the diameters measured belong to one normal distribution group, the points should show a linear distribution. If they consist of more than one normal distribution group, the line may show one or more points of inflection, which equal one less in number than the number of groups (Cassie’s plot, 1 inflection, 2 groups).

In the A type cell which contains thinner myofilaments, the plot of diameter versus frequency give a line on the normal probability graph (Fig. 7-D, a). These findings suggest that the diameters of thick myofilaments in A type cells are distributed on a normal distribution curve. We then calculated the best fit normal distribution curve for this histogram (Fig. 7-A), and compared, using the $\chi^2$-test, the values of the actual measurements to the theoretical values from the best fit curve. By the $\chi^2$-test, the curve fit with a 90% probability. Thus, the average thick myofilament diameter in A type cells appears to be about 50 nm.

In the B type cell which contained thicker myofilaments, a Cassie’s plot was made of the diameter versus frequency as was done for A type cells. The plots of this data had a point of inflection. From these results, we suggest that the distribution of data in this histogram consists of two normal distribution curves. After calculating the two best fit normal distribution curves, we graphed the best fit curve synthesizing the two normal distribution curves on the histogram (Fig. 7-B). Then the $\chi^2$-test was
used to compare the values of the actual measurements with those from the theoretical best fit curve. By the $\chi^2$-test, the curve fit with an 80% probability. Thus, B type cells appear to be composed of two kinds of thick myofilaments, with average diameters of the two represented about 50 and 80 nm respectively.

We measured five sets of thick myofilament diameter from A and B type cells. From these results, we computed five sets of average diameters by the statistical analysis just described, and the results are presented in Table 1. The table shows that A type cells have thick myofilaments of about 37-60 nm in diameter and B type cells have two kinds of thick myofilaments of about 37-60 nm and 75-97 nm in diameter respectively.

**Translucent portion.** The translucent portion of the adductor is composed of
obliquely-striated muscle cells (C type cell) in which the cell organelles are located in the peripheral regions of the cell (Fig. 5).

The thick myofilaments were gathered into units of about 50–60. In longitudinal section, the array of dense bodies was not so regular, but these units ran parallel to the long axis of the cell. We measured the diameters of about 500 thick myofilaments and calculated the average diameter of the thick myofilaments using the same procedures as was used for the smooth muscle cells. The measured diameter values were classed by size using a 5 nm interval and the relative frequency of thick myofilaments in each class was calculated. The results are shown in a histogram in which the horizontal axis indicates the class size and the vertical axis, the relative frequency of each class (Fig. 7-C). We then computed the average diameter of the thick myofilaments in the cell by the same statistical methods and procedures as previously described. Cassie's plot gave a linear distribution (Fig. 7-D, c), and the best fit normal distribution curve was added to the histogram (Fig. 7-C). The $\chi^2$-test was used to compare the actual measured values with those form the theoretical curve. By the $\chi^2$-test, the curve fit with a 90% probability. From the normal curve, the average diameter of thick myofilaments in a C type cell was computed to be about 22.5 nm in diameter.

We measured five sets of thick myofilament diameters form cells from this portion. We added the values obtained by statistical analysis of these results into Table 1. From these measurements, the average diameter of thick myofilaments in C type cells in the portion appears to be about 22.5 nm.

**DISCUSSION**

It is well known that the thick myofilaments in the adductor muscle cells of bivalves contain a paramyosin core. The presence of the core of paramyosin is indicated by striations on the thick myofilaments (1, 3, 7, 9, 11, 12). These smooth muscle cells can go into a catch state, and the relationship between catch-contraction and paramyosin has been discussed (12). Similar striations were observed on the thick myofilaments of the smooth muscle cells of the anterior adductor of *L.*
unguis. The thick myofilaments in bivalved adductors usually measure about 60-
120 nm in diameter (7), and the diameters of the ones seen in this study were within
this range. Therefore, we suggest that the anterior adductor may perform catch
contractions. Adductors of the brachiopoda, Terebratalia, have been shown to
contract by a similar mode of catch-contraction (13).

We believe that the smooth muscle cells in the adductor can be classified into two
types determined by the diameters of their thick myofilaments. Since the two types
of cells have similar intracellular structures and can only be distinguished by the
diameters of their thick myofilaments, one might suspect that the same cell might
give rise to a B type cell cross section in one part of the cell and at a different level,
an A type cell cross section, as the thick myofilaments all become tapered. However,
the differences in diameters were observed not only in cross section, but also in
longitudinal sections. Thus, two types of cells can actually be classified. Although
we did not measure the lengths of the thick myofilaments, the results from the
statistical analysis on the diameter of the thick myofilaments also support the idea
that there are two types of cells. Therefore, it is reasonable to suppose that the
opaque portion of the anterior adductor of L. unguis has two types of smooth
muscle cells.

In contrast to A type cell which contain only thinner sized thick myofilaments, B
type cell contain two kinds of thick myofilaments. This difference in the composi-
tion of thick myofilaments may indicate a difference in function between A and B
type cells. Thicker sized thick myofilaments were found only in B type cells, and
thicker sized thick myofilaments may contain much more paramyosin than thinner
ones. If the quantity of paramyosin in thick myofilaments influences the function of
the thick myofilaments, that is, has some relationship to catch-contraction, B type
cells may produce stronger catch-contractions than A type cells. The opaque
portion of the adductor is constructed of 87.6% B type cells, therefore the adductor
muscle may produce strong catch-contractions. William et al. (1982) reported on
the fine structure of the adductor and diductor of Terebratalia. In their report, they
suggested that the adductor is composed of smooth muscle cells having thicker sized
thick myofilaments (about 108 nm in diameter), and that the diductor is constructed
of thinner sized ones (33–70 nm in diameter). However, they did not suggest that
each muscle is constructed of several types of muscle cells, each containing different
sized thick myofilaments. Thus, our investigations may be the first report on the
various cell types which make up the adductor in brachiopoda.

The translucent portion of the adductor was constructed of obliquely-striated
muscle cells. This portion obviously bordered the opaque portion. The translucent
portion is small in size compared with those of other bivalves. The small size of this
portion of the adductor may be correlate to the slow contraction of this material,
since the translucent portion of the adductor is generally known to produce quick
contractions.

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