Seasonal Variation of Lysozyme Activity in Short-necked Clam*1

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The seasonal variation of lysozyme activity and six principal constituents of the short-necked clam, Tapes philippinarum, collected off the coast of Funabashi City was investigated over a period of 12 months. Lysozyme activity was assayed in the use of Micrococcus lysodeikticus cells as substrate. A high lysozyme activity in the short-necked clams collected in December and a low activity in those collected in April were observed. The lysozyme activity and water content of the whole body were positively correlated. On the other hand, the lysozyme activity and glycogen content, which is closely related to the ponderal index, were negatively correlated. It appears that the lysozyme activity of the short-necked clam increases when the body constituents are physiologically exhausted.

The authors so far have reported on the presence of a lysozyme-like enzyme in cuttlefish ink,1 the distribution of lysozyme (EC 3.2.1.17) in eight species of marine fish,2) the distribution of lysozyme in the body of cephalopoda, namely the pearly nautilus Nautilus macromphalus and the cuttlefish Sepia esculenta,3) and the internal distribution of lysozyme in 11 species of shellfish, as well as purification and some properties of lysozyme in the corbicula Corbiculina leana.4)

One of the roles of lysozyme in fish5-7) and mollusks8,9) has been considered to be that of protection from bacteria which penetrate from the outside. However, this has been presumed on the basis of tissue distribution of lysozyme and its properties, while no report on the role of this enzyme in view of seasonal variation in the activity level has been available.

Thus, in this study, seasonal variations in the activity of lysozyme and the proximate composition in the whole body of the short-necked clam are reported, together with some discussion on the role of this enzyme.

Materials and Methods

Materials

The short-necked clam Tapes philippinarum collected once a month off the coast of Funabashi-shi, Chiba, Japan, was used, and details of this are shown in Table 1.

Preparation of Crude Lysozyme Solution

The whole body, except the outer shell of the short-necked clam, was homogenized with a five-fold volume of 1/15 M phosphate buffer (pH 8.0) and centrifuged at 10,000×g for 20 min. The supernatant was used as a crude enzyme solution.

Measurement of Lysozyme Activity

Lysozyme activity was measured by the bacteriolytic method in the same manner as stated in previous papers.2-4) Firstly, dry cells of Micrococcus lysodeikticus (Sigma) were suspended in 0.2 M phosphate-citrate buffer (pH 4.8) so as to have a transmittance of 15% at a wave length of 550 nm. Next, to 1.0 ml of this buffer solution 0.05 ml of the crude enzyme solution, 0.45 ml of 1/15 M phosphate buffer solution, and 1.5 ml of the cell suspension were added, and the whole was mixed well. The transmittance at 550 nm of the reaction mixture was measured before and after incubation for 10 min at 40°C. The activity of lysozyme was calculated from an increase in transmittance after the reaction and expressed as µg of lysozyme weight/g of body weight. The standard curve was prepared using egg-white lysozyme (Sigma, 57,500 units/mg protein).

Measurement of Proximate Composition of the Body

Assays were performed for water content by...
Table 1. Details of short-necked clam employed for experiment

<table>
<thead>
<tr>
<th>Date</th>
<th>Shell length (mm)*</th>
<th>Shell height (mm)*</th>
<th>Shell thickness (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan. 16</td>
<td>33.6 ± 2.8</td>
<td>24.3 ± 1.8</td>
<td>16.0 ± 1.7</td>
</tr>
<tr>
<td>Feb. 5</td>
<td>30.9 ± 3.3</td>
<td>22.7 ± 2.2</td>
<td>15.8 ± 2.4</td>
</tr>
<tr>
<td>Mar. 13</td>
<td>28.7 ± 1.1</td>
<td>20.7 ± 0.7</td>
<td>15.2 ± 1.9</td>
</tr>
<tr>
<td>Apr. 10</td>
<td>31.1 ± 2.3</td>
<td>21.5 ± 1.5</td>
<td>15.3 ± 1.3</td>
</tr>
<tr>
<td>May 10</td>
<td>32.7 ± 1.8</td>
<td>21.2 ± 1.1</td>
<td>14.9 ± 2.6</td>
</tr>
<tr>
<td>Jun. 15</td>
<td>27.6 ± 0.9</td>
<td>19.2 ± 1.1</td>
<td>15.6 ± 2.8</td>
</tr>
<tr>
<td>Jul. 15</td>
<td>27.5 ± 2.6</td>
<td>19.7 ± 0.8</td>
<td>13.8 ± 0.4</td>
</tr>
<tr>
<td>Aug. 10</td>
<td>29.7 ± 1.3</td>
<td>20.1 ± 1.1</td>
<td>12.8 ± 0.9</td>
</tr>
<tr>
<td>Sep. 11</td>
<td>27.9 ± 1.4</td>
<td>19.0 ± 0.4</td>
<td>12.5 ± 0.8</td>
</tr>
<tr>
<td>Oct. 11</td>
<td>25.0 ± 0.8</td>
<td>17.8 ± 1.0</td>
<td>11.9 ± 0.3</td>
</tr>
<tr>
<td>Nov. 29</td>
<td>30.0 ± 2.0</td>
<td>22.3 ± 3.0</td>
<td>14.0 ± 2.8</td>
</tr>
<tr>
<td>Dec. 11</td>
<td>28.6 ± 1.6</td>
<td>20.0 ± 1.8</td>
<td>13.3 ± 1.0</td>
</tr>
<tr>
<td>Average</td>
<td>29.4 ± 2.3</td>
<td>20.7 ± 1.7</td>
<td>14.3 ± 1.3</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

Fig. 1. Seasonal variations of lysozyme activity (A), contents of water (B), crude protein (C), crude lipid (D), glycogen (E), and ash (F), and condition factor (G) of the short-necked clam. Each value denotes the mean for 6 different samples with standard deviation indicated by a vertical bar.
the atmospheric thermal drying method, for crude protein by the Kjeldahl method, for crude lipid by extraction with chloroform-methanol mixture, for ash by direct ashing (500–600°C), and for glycogen by Haines' ferricianide titration of glucose yield after acid hydrolysis. Meanwhile, the condition factor was expressed as: body weight (shelled)/body weight (whole) × 100.

Results

Seasonal Variation of Lysozyme Activity

The activity of lysozyme varied markedly with the seasons (Fig. 1-A). The lowest value of 65.6 µg/g in April increased gradually to 157.9 µg/g in July, decreased again in August to 89.4 µg/g, and was enhanced to the highest level of 160.7 µg/g in December. The annual mean value of activity was 119.5 ± 29.6 µg/g.

Seasonal Variation of Proximate Composition

The water content showed a similar trend to the lysozyme activity, with rapid reduction from March to the lowest value of 81.2% in May (Fig. 1-B). After a gradual increase in July it decreased again to 83.3% in August. Another increase was shown in September to the highest level of 91.2% in December.

The content of crude protein showed its lowest value of 5.18% in January (Fig. 1-C). After a gradual increase it decreased again in June and July. From August it increased again to its highest level in October, while it decreased in November and December.

The content of crude lipid showed similar variations to those of crude protein, starting to increase from 1.02% in April to 1.20% in May (Fig. 1-D). After another reduction and increase in August to its highest level of 1.35%, it decreased again gradually.

The content of glycogen was low from January to March, but rapidly increased from April onward, arriving at its highest level of 1.60% in June (Fig. 1-E). After a rapid reduction in July and an increase in August, it remained low from September onward.

The content of ash reached its highest level in March, and after remaining comparatively stable for a while, it decreased in September to its lowest level of 1.58% (Fig. 1-F).

The condition factor decreased from January to March, but thereafter it increased to its highest level of 48.7 in May (Fig. 1-G). It again showed a reduction up to July, but increased in August to 46.5. Thereafter it gradually decreased again to its lowest level 38.8 in November. Meanwhile, the annual mean value of these components obtained in this study virtually corresponded with...
that stated in the Japan Standard Tables of Food Composition.14

Correlation between Lysozyme Activity and Proximate Composition

As shown in Fig. 2, the activity of lysozyme was correlated with the water content at \( r = 0.72 \). Further, negative correlations of \( r = -0.67 \) and \( r = -0.70 \) were observed between the lysozyme activity and the glycogen content as well as the condition factor, respectively.

Discussion

It has been assumed that one role of lysozyme in bivalves is to protect them from bacteria penetrating from the outside.8,9 Mochizuki and Matsumiya13 reported that the comparatively higher activity in pearly nautilus was observed in the digestive system, blood vessels and mantle, suggesting the protective performance of this enzyme form bacteria inside and outside of the body.

In this study, it was shown that the lysozyme activity of the short-neched clam, together with the proximate composition of its body, varied markedly with the seasons. The lysozyme activity of the short-neched clam decreased rapidly in April when the constituents including crude protein, crude lipid, and glycogen were enriched to increase the condition factor, but was enhanced in July when such constituents were consumed to reduce the condition factor.

Further, a positive correlation was noted between the lysozyme activity and the water content, while a negative correlation was noted between the lysozyme activity and glycogen content as well as the condition factor. These observations that the lysozyme in the short-neched clam increases its activity in the season when the body constituents are consumed may support our previous suggestion that lysozyme protects bivalves from bacteria inside and outside of their bodies.

References