Chitosan is a β-1,4-linked glucosamine polymer. It is usually obtained by artificial deacetylation of chitin, a polymer of N-acetyl-β-d-glucosamine, with a concentrated NaOH solution. Recently, as the medical uses of chitosan, its derivatives or its partially degraded oligosaccharides have been developed, the demands are growing for chitosanases needed for mild degradation of chitosan.

Chitosanases (EC 3.2.1.99), a new class of enzymes that hydrolyze chitosan, are widely distributed in nature and are produced by many microorganisms, including fungi, bacteria and actinomycetes. There are many investigations about purification of chitosanases from some microbial sources, such as Pseudomonas sp. H-14, Myxobacter AL-1, Bacillus R-4, Streptomyces sp. no. 6, Penicillium isoladicum, Bacillus sp. 99–5, Streptomyces griseus, Bacillus sp. no. 7-M, and Bacillus circulans MH-K1. However, there have been few reports on that of marine vertebrates and invertebrates. This paper presents the possible existence of chitosanase-like activity in sea urchin ovary.

Sea urchins Hemicentrotus pulcherrimus, Strongylocentrotus nudus and purple sea urchin Anthocidaris crassispina were collected in Senzaki Bay, Nagato City, Yamaguchi Prefecture, Japan, cooled in ice and transported to the laboratory. They were stored at –85°C until use. The ovaries were removed from each sea urchin. These were not contaminated by any microorganisms. These were homogenized with 50 mM sodium phosphate buffer (pH 7.0) containing 4 mM 2-mercaptoethanol (2-ME), 1 mM ethylenediaminetetraacetic acid (EDTA), and 2 mM phenylmethylsulfonyl fluoride (PMSF). The homogenates were centrifuged at 230000 g for 30 min, and then the supernatants were dialyzed against 10 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA and 4 mM 2-ME. The dialysates were used as the crude enzyme solution.

The enzyme activity was measured by the method of Price and Storck using colloidal chitosan as a substrate. An enzyme preparation was added to 0.5 mg of colloidal chitosan in a final volume of 0.5 mL at pH 7.2. The assay of enzymic activity was carried out with a spectrophotometer by monitoring the decrease in absorbance at 520 nm at 25°C. One unit of chitosanase was defined as decrease in absorbance of 0.001 measured for 5 min after addition of enzyme solution. Sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli using 12.5% gel.

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The distribution of chitosanase activity in the ovaries of three species of sea urchin is shown in Fig. 1. As a result, H. pulcherrimus and S. nudus exhibited no activity. In contrast, A. crassispina showed activity and its value was 10.8 U. So the crude enzyme solution prepared from A. crassispina was treated with solid ammonium sulfate to 0–60%, 60–80% and over 80% saturation. The precipitates obtained from each fraction were dissolved in a minimal volume of 10 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA and 4 mM 2-ME. The activity of each fraction was: 1.2 U for 0–60%, 30.4 U for 60–80%, 0 U for over 80% fractions. To identify the existence of chitosanase-like enzyme, SDS-PAGE was performed using 12.5 gel. The under 60% and 60–80% fractions showed the main protein band with about 34 kDa (Fig. 2). Moreover, several protein bands were observed over about 50 kDa in these fractions. It was not found whether this enzyme was the protein band with about 34 kDa or not. No protein band was observed in over 80% fraction. These results indicate that chitosanase-like enzyme possibly exists in the ovary of purple sea urchin. The molecular weights of the previously reported enzymes are: Pseudomonas sp. H-14 (35 kDa), Streptomyces sp.
No.6 (26 kDa), Bacillus sp. No.7-M (41 kDa), and Bacillus circulans MH-K1 (32 kDa). This report is, to my knowledge, the first on the enzyme from an organism other than microorganism. Further work is aimed at isolating this enzyme and examining the physicochemical properties.

REFERENCES