Inhibition of protease activity by the components from scallop shells

YUN CHUN LIU, KOHJI UCHIYAMA, NAOMI NATSUI, AND YASUSHI HASEGAWA

Muroran Institute of Technology, Department of Applied Chemistry, Muroran, Hokkaido 050-0071, Japan
(hasegawa@mmm.muroran-it.ac.jp).

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INTRODUCTION

Scallop is one of the major marine products industry in Hokkaido (Japan). As the result, the scallop shells of about 300 thousands ton per a year are generated as industrial wastes.

The shells are composed of two kinds of CaCO_3 polymorphs of prismatic layer and nacreous layer. The nacreous layer has been used as Chinese medicine for keeping skin moist, counteracting the poison, and making the spirit stable. The nacreous layer is also used as a cosmetic in Japan. However, only few studies about the bioactive substances in the shell have been reported.¹⁾ In this study, we investigated biological activities of components which was extracted from scallop shells.

MATERIALS AND METHODS

Extraction of scallop shell components

Shells were isolated from a scallop *Patinopecten yessoensis* and the shells were cleanly brushed to remove an adhered materials, and then crushed to a powder. The powdered shells 200g were dialyzed against 1L of 5% acetic acid to be allowed to decalcify absolutely. This was followed by exhaustive dialysis against 1L of deionized water to remove acetic acid. After dialysis, the water-soluble fraction was obtained by centrifugation at 16,000xg for 20 min at room temperature. The water-soluble fraction was concentrated and loaded onto a Sephacryl S-200

gel filtration column (0.5 x 35cm) previously equilibrated with deionized water, and the fractions of 1mL were collected. Each fraction was employed as the scallop shell extract for protease inhibitory assay.

Measurement of protease activities

Elastase activity was measured by the method of Ito *et al.*²⁾ The reaction mixture was 50 mM Hepes-NaOH (pH7.5) and 0.3 mM butoxycarbonyl-alanine-4-nitrophenol as substrate in the presence and the absence of the scallop shell extract. After 1 μg of pancreas elastase was added to the reaction mixture, the color development was measured at 405nm.

Trypsin activity was measured in a solution containing 12.5 mM Tris-HCl (pH8.0) and 0.0625 mg/mL α -N-benzoyl-DL-arginine-*p*-nitroanilide in the absence and the presence of the scallop shell extract.³⁾ The reaction was started by adding trypsin to be 0.5 mg/mL, and the optical density at 405nm was measured.

RESULTS

The scallop shell extract after gel filtration inhibited elastase activity in a dose-dependent manner (Fig. 1A), and the activity was inhibited to about 80% at the protein concentration of 0.06mg/ml. When the elastase activities were measured in solutions containing various concentrations of substrate in the presence and the absence of the scallop shell extract,

Fig. 1. Inhibition of elastase and trypsin activities.

(A) Elastase activity was measured as described in MATERIALS and METHODS. The reactions were performed in the absence (●) and the presence of 0.06 mg/mL (□), 0.03 mg/mL (▲), 0.015 mg/mL (△), and 0.006 mg/mL (○) of the scallop shell extract.

(B) Trypsin activity was measured in a solution containing 12.5 mM Tris-HCl (pH8.0) and 0.0625 mg/mL α -N-benzoyl-DL-arginine-*p*-nitroanilide in the absence (●) and the presence of 0.087 mg/mL (▲), 0.05 mg/mL (△), and 0.025 mg/mL (○) of the scallop shell extract.

double reciprocal plots of elastase activities indicated non-competitive inhibition (data not shown). In trypsin inhibitory assay, the activity was also detected in several fractions. The inhibition was about 70% at the protein concentration of 0.087 mg/mL (Fig. 1B). However, any fractions did not exhibit the inhibitory activity for α -chymotrypsin. On the other hand, several fractions enhanced activities for

α -chymotrypsin (data not shown), but not for elastase and trypsin. These results suggest that the scallop shells contain the bioactive substances which inhibit or activate serine protease.

DISCUSSION

Proteases are involved in blood coagulation and pathogenicity of a variety of human infectious agents. Elastase is responsible for the abnormal turnover of the elastin associated with the development of pulmonary emphysema and rheumatoid arthritis. The components of scallop shells may be of value in diseases.

The shell organic matrix constitutes of proteins, polysaccharides, lipids, and so on. Analysis of the water-soluble fraction by SDS-PAGE revealed at least three kinds of proteins with molecular masses of 90kDa, 20kDa, and 17kDa (data not shown). The existence of saccharide in the water-soluble fraction was also found by orcinol-H₂SO₄ method. However, it is unclear whether bioactive substances are proteins or saccharides or the other components. It will be necessary to isolate and identify these substances in future.

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