PC-2

Purification and Characterization of a serine proteinase from the mackerel, *Scomber Japonicus*

Pyo-Jam Park and Se-Kwon Kim
Department of Chemistry, Pukyong National University, Pusan 608-737, Korea

Introduction

The proteinases with collagenolytic activity are divided into two types, metallocollagenases and serine collagnases. metallocollagenases are zine-containing enzymes, requiring calcium for stability. The metallocollagenases have been widely reported from various mammalian tissues, bacteria and snake venome. On the other hand, the serine collagenanases are probably involved in food digestion rather than in morphogenesis. In addition, the enzymes are involved in the production of hormones and pharmacologically active peptides and in various cellar functions such as protein digestion, blood-clotting, fibrinolysis, complement activation and ferilization. These serine collagenases have been found from various sources such as filefish, fermented fish sauce, greenshore crab, Kamchatka crab, Atlantic cod, Antarctic krill, shrimp and catfish. In this study, we report the purification of a serine proteinase with collagenolytic activity from the macerel, *Scomber japonicus*

Materials and Methods

Enzyme assay Collagenolytic activity was measured by the method of Moore and Stein (1954) with a slight modification. A reaction mixture containing 5 mg of collagen type I, 1.0 ml of 50 mM Tris-HCl (pH 7.5) containing 5 mM $CaCl_2$ and 0.1 ml of enzyme solution was typically incubated at 37C for 1 h. The reaction was stopped by adding 0.2 ml of 50% trichloroacetic acid, and after 10 min at room temperature, the solution was centrifuged at 1,800 × g for 20 min at room temperature. The supernatant (0.2 ml) was mixed with 1.0 ml of ninhydrin solution, incubated at 100℃ for 20 min, and then cooled to room temperature. Subsequently, the mixture was

diluted with 5 ml of 50% 1-propanol for absorption measurement at 570 nm. Tris-HCl buffer (50 mM, pH 7.5) was used instead of enzyme solution as a reference. The concentration of hydrolyzed amino acids was determined by a standard curve based on a solution of L-leucine. One unit (U) of enzyme activity is defined as the amount of enzyme required for the hydrolysis of 1 μmole of substrate per h.

Molecular mass determination The molecular mass of enzyme was estimated by gel filtration and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The standard proteins in gel filtration were albumin (66.0 kDa), carbonic anhydrogenase (29.0 kDa), cytochrome C (12.4 kDa) and aprotinin (6.5 kDa). SDS-PAGE was done by the method of Laemmli. (1970) protein bands were stained Coomassie Brilliant Blue R-250. The molecular mass standards were bovine ovalbumin (45.0 kDa), pepsin (34.7 kDa), trypsinogen (24.0 kDa), ß-lactoglobulin (18.4 kDa) and lysozyme (14.3 kDa).

Results

A serine proteinase with collagenolytic activity from internal organs of mackerel was purified using acetone precipitation, ion-exchange chromatography on a DEAE-Sephadex A-50, gel filtration chromatography on a Sephadex G-100, ion-exchange chromatography on DEAE-Sephacel, and gel filtration chromatography on a Sephadex G-75 column. The molecular mass of the purified enzyme was estimated to be 14.8 kDa by gel filtration and SDS-PAGE. The purification and yield were 39.5-fold and 0.1% as compared with those in the starting crude extract. The optimum pH and temperature for the enzyme activity were around pH 7.5 and 55℃ , respectively. The Km and Vmax of the enzyme for collagen Type I were approximately 1.1 mM and 2.343 U, respectively. The purified enzyme was strongly inhibited by Hg^{2+}, Zn^{2+}, PMSF, TLCK, and soybean trypsin inhibitor.

References

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685.

Moore S, Stein W. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* 1954; **211**: 907-913.