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Purification and characterization of the low molecular weight collagenase from pyloric caeca of tuna, *Katsuwonus pelamis.*

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Introduction

Collagenases are generally defined as enzymes capable of degrading the polypeptide backbone of native collagen under conditions which do not denature the protein. Two types of proteases with collagenolytic activity have been reported and thought to play different physiological functions. Metallo-collagenases, firstly discovered in tadpole tissue explants are zinc-containing enzymes requiring calcium for optimum activity and stability, and These enzymes have been widely studied from various mammalian tissues as well as from bacteria, such as *Bacillus cereus, Clostridium histolyticum, Achromobacter, Vibrio alginolyticus* and *Clostridium perfringens* and snake venoms.

On the other hand, serine collagenolytic proteases, firstly isolated from the hepatopancreas of the fiddler crab, *Uca pugilator,* are probably involved in food digestion rather than in morphogenesis. The collagenolytic serine proteinases have also been reported from greenshore crab *Carcinus maenas* (1), Kamchatka crab *Paralithodes camtschatica,* Atlantic cod *Gadus morhua,* insect *Hypoderma lineatum* , Antarctic krill *Euphasia superba* Dana, midgets of Penaeid shrimps *Penaeus monodon* and pancreas of catfish *Parasilarus asotus* (2). The objectives of this study were to purify a collagenolytic enzyme from the pyloric caeca of tuna, *Katsuwonus pelamis* and to characterize the enzyme with respect to the responses to pH, temperature and inhibitors

Materials and Methods

The pyloric caeca of tuna were provided by Dongwon Industries Co. and stored at -20℃ before use. Reagents for electrophoresis, DEAE-Sephadex A-50, Sephadex G-100, Sephadex G-75, N-P-toluenesulfonyl-L-lysine chloromethyl ketone (TLCK), N-P-toluenesulfonyl-L-phenylalanine chloromethyl ketone (TPCK), 1,10-phenanthroline, phenylmethylsulfonyl fluoride (PMSF), soybean trypsin inhibitor, L-cysteine, ethylenediamine tetraacetic acid (EDTA), collagen (type I) and collagenase (type I) from *Clostridium Histolytium* were products of Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals were of analytical reagent grade.

Collagenolytic activity assay, Collagenolytic activity was measured by modified Moore and

Stein's ninhydrin method; the reaction mixture, containing 5mg collagen (type I), 1ml of 50mM Tris-HCl buffer (pH 7.5) containing 5mM $CaCl_2$ and 0.1ml of enzyme solution, was incubated at 55℃ for 1 hr. The reaction was stopped by adding of 0.2ml of 50% trichloroacetic acid (TCA). After 10 minutes at room temperature, the solution was centrifuged at 1,800×*g*, 20 min. 1ml of ninhydrin solution was added to 0.2ml of supernatant. The mixture was incubated for 20 minutes at 100℃ and then cooled at room temperature. Then 5ml of 50% 1-propanol was added to dilute. After standing for 15 minutes, absorbance was checked with spectrophotometer at 570nm. One unit of specific activity was defined as the 1 μmole of hydrolyzed amino acid produced per milligram of enzyme per hour under the above experimental condition.

Results

A low molecular weight collagenase was isolated from pyloric caeca of tuna, *Katsuwonus pelamis*. The enzyme was purified 30.4 folds with 0.4% yield by precipitation by acetone, gel filteration on Sephadex G-100 column chromatography, ion exchange on DEAE-Sephadex A-50 column chromatography and gel filteration on Sephadex G-75 column chromatography. The molecular weight was estimated 15 kDa by 15% SDS polyacrylamide gel electrophoresis and gel filteration with Sephadex G-75 column chromatography. The optimal collagenolytic activity of the purified collagenase was found at pH 7.5 and 55℃. The enzyme maintained 90% activity at pH 7.5 and 35℃ for 12 hr. The purified enzyme was almost completely inhibited by specific inhibitors of serine protease such as soybean trypsin inhibitor and PMSF, but activated by Ca^{2+}, Mg^{2+}, Ba^{2+} and Mn^{2+}. The Km and Vmax values of the collagenase for collagen (type Ⅰ) were 377 mM and 12,772 U/μmol, respectively. The collagenase was capable of degrading insoluble native collagen substrate (type Ⅰ, Ⅱ, Ⅲ and Ⅴ), and revealed higher activity against collagen (type Ⅰ) than any other protease (*Clostridium histolyticum* collagenase of Type Ⅰ, α-chymotrypsin, trypsin, pronase E, papain and pepsin) at pH 7.5 and 55℃. The patterns of the sequential hydrolysis were showed by thin layer chromatography. N-terminal sequence of purified collagenase was ENADQTKLAF. These results suggest that the purified collagenase from pyloric caeca of tuna is a serine collagenase.

References

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