Trypsins from Yellowfin Tuna (*Thunnus albacores*) Spleen: Purification and Characterization

°Sappasith Klomklao[1, 3], Soottawat Benjakul[1], Wonnop Visessanguan[2],
Hideki Kishimura[3], Benjamin K. Simpson[4] and Hiroki Saeki[3]

[1]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Thailand.
[2]National Center for Genetic Engineering and Biotechnology, Thailand.
[3]Research Faculty of Fisheries Sciences, Hokkaido University, Hokkaido, Japan
[4]Department of Food Science and Agricultural Chemistry, McGill University, Canada.

Keywords: Trypsin; Purification; Spleen; Tuna

Objective: Fish viscera produced during the processing is a potential source of enzymes such as proteinases that may have some unique properties for industrial applications. Among fish industries, tuna including yellowfin, skipjack, tonggol has become the important species for canning worldwide and a large amount of viscera is generated during processing. Based on our previous study, yellowfin tuna spleen contained high proteolytic activity, which was identified as a trypsin-like serine proteinase. However, no information regarding the molecular and biochemical properties of trypsin from yellowfin tuna spleen has been reported. The objectives of this study were to purify and to characterize the trypsin from yellowfin tuna spleen.

Methods: Trypsins from yellowfin tuna spleen were purified with a series of chromatographies including Sephacryl S-200, Sephadex G-50 and DEAE-cellulose. Trypsin activity was measured by the method of Hummel (1959) using TAME as a substrate. One unit of enzyme activity was defined as the amount causing an increase of 1.0 in absorbance at 247 nm per min. Protein concentration was measured by the method of Lowry et al. (1951). Activity assay for temperature profile study was carried out at pH 8.0 at various temperatures (20-80 °C). The pH profile was studied over the pH range of 4.0-11.0 at 30 °C. Thermal stability of the enzyme was evaluated by incubating the enzyme at pH 8.0 for 15 min at temperature ranging from 20 to 80 °C. For pH stability study, the residual activity after incubation at various pHs (4.0-11.0) for 30 min at 30 °C was determined. The effect of inhibitors on trypsin activity was determined by incubating trypsins with an equal volume of inhibitor solution to obtain the final concentration designated and the remaining activity was measured. N-terminal amino acid sequence of 20 amino acid residues was also studied. SDS-PAGE was performed using 12.5% running and 4% stacking gel according to the method of Laemmli (1970). Native –PAGE was also run in the same manner with SDS-PAGE but all denaturants were left out.

Results: Two trypsins, A and B, were isolated from yellowfin tuna spleen, and purified to 70.6- and 91.5-fold with the yields of 2.8 and 15.6%, respectively. The apparent molecular mass of both enzymes were 24 kDa by SDS-PAGE. On native-PAGE, both trypsin A and B showed a single band. Trypsin A and trypsin B displayed optimal temperature of 55 °C and 65 °C, respectively, and had the same optimal pH of 8.5 using TAME as a substrate. Both enzymes were stable to heat treatment up to 50 °C, and were stable at pH 6.0-11.0 for 30 min at 30 °C. Both trypsin A and trypsin B were inhibited effectively by soybean trypsin inhibitor, TLCK and partially inhibited by EDTA, but was not inhibited by E-64, N-ethylmaleimide, iodoacetic acid, TPCK and pepstatin A. The N-terminal amino acid sequences of trypsin A, IVGGYECQAHSQPHQVSLNA, and trypsin B, IVGGYECQAHSQPPQVSLNA, were homologous to those of trypsin from other fish species.

Acknowledgments: Financial support from Thailand Research Fund under the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0216/2546) to Sappasith Klomklao.