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Purification and characterization of trypsin from the digestive system of carp *Catla catla* (Hamilton)

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Abstract

Trypsin was purified from the digestive system of carp *Catla catla* (Hamilton) by ammonium sulfate fractionation, diethylaminoethyl-cellulose column chromatography, and Benzamidine Sepharose 4 fast flow column affinity chromatography. Trypsin was purified 26.2-fold with an 11.1% yield. The purified enzyme was active between pH 7.0 and 9.8, and maximal activity of the enzyme was observed at pH 7.0. Highest activity was found at 40°C. The activity was reduced to 52.84% at 60°C and was completely lost at 70°C. An addition of 2 mM $CaCl_2$ enhanced trypsin activity during the 8-h incubation. The K_m, K_{cat}, and catalytic efficiency values of purified enzyme were 0.062 mM and 19.23/s, and 310.16/s/mM, respectively. The enzyme activity was inhibited by soybean trypsin inhibitor, phenylmethylsulfonylflouride, and N-α-p-tosyl-L-lysine chloromethyl ketone. The molecular mass of the purified enzyme was 20.2 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Mass spectrometry study of purified enzyme gave the peptide sequences LGEHNIAVNEGTEQFIDSVK (MW = 2,027.9568) and HPSYNSRNLDNDIM (MW = 1,692.6952) showing identical sequence with trypsin from various fishes.

Keywords: *Catla catla*, Trypsin, Optimum temperature, Peptide sequence

Background

Fish viscera are a potential source of digestive enzymes, especially digestive proteases. Proteases represent an important class of industrial enzymes, accounting about 50% of the total sale of the enzymes in the world (Souza et al. 2007). Various digestive proteases such as aspartic protease pepsin and serine proteases - trypsin, chymotrypsin, and elastase - are isolated from the fish viscera. Trypsin (EC 3.4.21.4) plays a pivotal role in digestive physiology. This endopeptidase hydrolyzes peptide bonds at the carboxyl end of lysine and arginine residues. Trypsin plays major roles in biological processes including digestion and activation of zymogens (Cao et al. 2000). Proteases have diverse industrial applications such as in detergent, food, pharmaceutical, leather and silk industries (Klomklao et al. 2005). Fish viscera have wide biotechnological potential as a source of digestive enzymes, especially digestive proteases that have high activity