UDOMLUCK THITIRUKPANICH : PURIFICATION AND CHARACTERIZATION OF NEUTRAL PROTEASE FROM BACILLUS SUBTILIS TISTR 25. THESIS ADVISOR : ASSOC. PROF. PIAMSOOK PONGSAWASDI, Ph.D., 90 PP., ISBN 974-579-436-8

Neutral Protease from Bacillus subtilis TISTR 25 was purified 4.6 fold by using ammonium sulfate precipitation, CM-cellulose and Sephadex G-75 colum chromatography, respectively. The enzyme was highly purified and showed one band on polyacrylamide gel electrophoresis. The purified enzyme was proved to be a monomer with a molecular weight of 37,000 by SDS polyacrylamide gel electrophoresis.

The optimum pH and the optimum temperature were 7 and 50 °C. There was no esterase activity. Michaelis constant(K_m) for casein, hemoglobin and azocoll were 0.04, 0.06 mM and 10 mg/ml, respectively. Comparative studies with Thermolysin indicated that the purified enzyme has lower affinity but greater maximum velocity (Vmax) for these 3 substrates. The enzyme showed similar specificity with Thermolysin towards synthetic substrates. They hydrolysed peptide bonds at the amino end of bulky amino acids such as Leucine, Phenylalanine and Arginine.

The zinc content of neutral protease from B. subtilis TISTR 25 was approximately 1.5 atom/molecule enzyme. The enzyme was inactivated by chelating agents EDTA and Phenanthroline. Eighty percent of the enzyme was inactivated by 1 mM EDTA. The inactive enzyme could be better reactivated by the addition of 1 mM, Zn^{++}, Mn^{++} or Co^{++} than Ca^{++}, Fe^{++} or Mg^{++}.