Studies on Protease from Aspergillus Aculeatus.

Elegbe, Isaac Anuolowapo

P.h.D. Microbiology

Department of Microbiology

Obafemi Awolowo University, Ile Ife, Nigeria

1985.

Abstract:

Aspergillus aculeatus associated with the black tongue disease was able to grow in liquid synthetic medium. Growth was maximum on the fifth day of incubation. Growth was optimum at pH 6.5 and 35 °c. The best carbon source for growth was glucose while among the nitrogen sources used, optimum growth occurred on tryptone. During growth of A. aculeatus in liquid synthetic medium, proteins were released and proteolytic activity was detected. Proteolytic activity was optimum when the sole carbon and nitrogen sources were galactose (or inositol) and peptone respectively.

The proteins were separated into three peaks of absorption by gel permeation chromatography, and only one of the components exhibited proteolytic activity. The molecular weight of this protease from its elution volume on Sephadex G-100 was approximately 28,500 Daltons. Ion exchange chromatography resolved only one peak of absorption with a purification fold of approximately 309.5, a specific activity of 2.7 x 10' units/mg protein, and a Km of approximately 0.26 mg/ml for the hydrolysis of casein.

The partially purified enzyme was able to hydrolyse casein, lactalbumin, egg albumin and human haemoglobin. Optimum activity of the enzyme occurred at pH 6. 5 and 40 °C. Activity of the enzyme was stimulated by low concentrations of Cu^{++}, Ba^{++}, Ca^{++}, Mg^{++} and K^{+} but inhibited by Mn^{++}, Zn +, Fe +, $_{Hg}$++, iodoacetic acid and ethylene diamine tetraacetic acid. The enzyme was highly susceptible to heat, losing all its activity within 2 minutes at 70 °C. Intraperitoneal inoculation of white rats (Fisher's breed) with the crude and the partially purified enzyme caused marked histological changes resulting in cellular degeneration and local necrosis of three major organs (liver, kidney and spleen).
The partially purified enzyme was more effective than the crude enzyme, and the changes produced by both enzymes were more pronounced in the male than in the female rats.

Keywords: Black tongue disease / incubation / protein/ gel permeation chromatography/ proteolytic activity/ enzymes/ cellular degeneration/ intraperitoneal inoculation

Supervisor: P.O. Olutiola.

183p