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Original Article

ALKALINE PROTEASE FROM MAGGOTS: A LIKELY SOURCE OF INDUSTRIAL ENZYME

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Abstract: A protease enzyme was isolated from maggots, developed from chicken and fish remains. The enzyme was purified by gel filtration on sephadex G-75 followed by ion-exchange chromatography using DEAE- cellulose and was partially characterized. Enzymes activity was found to be 1.87 mmol/unit and 13.34 mmol/unit after each purification stage respectively using casein as substrate. Optimum temperature and pH was 45 °C and 11 respectively. The enzyme has a K_m of 0.11 mM and V_{max} of 1.21 mmol/min for casein.

Key words: Alkaline proteases, Maggot, Industrial enzymes

INTRODUCTION

Proteases are the single class of enzymes, which occupy a pivotal position with respect to their application in both physiological and commercial fields [1]. They catalyze the cleavage of peptide bonds in other proteins [1]. Proteases represent one of the largest groups of industrial enzymes and account for about 60 % of the world wide sale of enzymes [2, 3]. Proteases are physiologically necessary for living organisms as such they are ubiquitous being found in a wide diversity of sources such as plants, animals and micro-organisms [4]. Proteases have a large variety of application mainly in the detergent and food industries. Proteases are also envisaged to have extensive application in leather treatment and in several bioremediation processes. All detergent proteases currently used in the market are serine proteases from *Bacillus* strains. Fungal alkaline proteases are advantageous due to the ease of down stream processing to prepare a microbe-free enzyme. An alkaline protease from *Conidiobolus coronatus* was found to be compatible with commercial detergent used in India [5] and retained 43 % of its activity at 50 °C for 50 min. in the presence of Ca^{2+} (25 mM) and glycine (0.001 mM) [6]. The use of enzymes in the leather industry as alternative to chemicals has proved successful in improving leather quality and in reducing environmental pollution [7]. Proteases have been routinely used for various purposes such as cheese making, baking, preparation of soy hydrolysates and meat tenderization. The major application of proteases in the dairy industry is in the manufacture of cheese [2]. The wide diversity and specificity of proteases are used to a great advantage in developing effective therapeutic agents. Oral administration of proteases from *Aspergillus Oryzae* has been used as a digestive aid to correct certain lytic enzymes deficiency syndromes. Alkaline proteases from *Conidiotolus coronatus* were found to be able to replace trypsin in animal cell culture [8].

Maggots are larval forms of housefly, they are whitish in colour and feed on rottening matter [9]. They feed mainly by a process of extracorporeal digestion. They have a pair of mandible and hooks, which they use to assist in locomotion and provide attachment to the

tissue. It has been observed that these hooks are used during feeding to disrupt membranes and thus facilitate the penetration of their proteolytic enzymes [10]. The clinical application of maggots in wound care has been reported [11, 12]. In this article, we describe the isolation along with some biochemical properties of a protease enzyme from maggots.

MATERIALS AND METHODS

Production of maggot: - Fish and chicken remains were collected in a tightly covered plastic container and left for five days. At day five, with the aid of disposable gloves, maggots were collected and put in a phosphate buffer solution (pH 7.8).

Isolation of enzyme: 50 g of maggots was homogenized using mortal and pestle in a little amount of phosphate buffer (pH 7.8), the slurry mixture was filtered using a clean white piece of cloth. The filtrate was then centrifuged at 7000×g for 10 min to obtain a clear filtrate.

The supernatant was subjected to 75 % ammonium sulphate precipitation and left for 24 h, after which it was centrifuged at 7000×g for 10 min and the pellet dissolved using small amount of phosphate buffer (pH 7.8) and dialyzed against distilled water for 48 h with continuous stirring.

Protein determination:- The protein concentration of the dialysate was quantitatively analyzed using Biuret method [13] and Bovine serum albumin as standard.

Enzyme assay: Enzyme assay was performed using modification of Kunitz caseinolytic assay as described by Janssen et al. [14]. 0.5 ml of the enzyme source was added to 2.0 ml of 0.5 % casein. The reaction mixture was incubated at 37 °C and terminated after 30 min by the addition of 3.0 ml of 5 % TCA. The solution was kept at room temperature for 30 min, centrifuged and the supernatant read at 280 nm. One unit of enzymes was defined as an increase of 0.1 in absorbance of TCA soluble casein hydrolysis product.

Purification procedure: 5.0 ml of dialyzed protein sample was carefully layered on pre-swollen sephadex G-75, which was packed carefully in a pharmacia chromatographic column (2 × 37 cm). The column was previously equilibrated with 50 mM Tris buffer pH 7.8 and the protein eluted using the same buffer. A flow rate of 0.2 ml/min was maintained and 4 ml fraction was collected into fifty test tubes. Protein was monitored at 280 nm.

Fractions within the highest enzyme activity peak were pooled together and purified further on DEAE-cellulose, which was carefully packed in a column (2.5 × 25 cm) and previously equilibrated using Tris buffer (pH 7.8). The protein was eluted using Tris buffer in a linear gradient of increasing NaCl concentration from 0 to 0.3 M. A flow rate of 0.6 ml/min was maintained and the absorbance was monitored at 280 nm. Fractions showing highest enzyme activity were pooled, concentrated and characterized.

Determination of K_m and V_{max}:- The K_m and the V_{max} of the enzyme for casein was calculated using the Michaelis-menten equation. The enzyme was reacted with varying concentrations of substrate (casein) in a reaction volume of 2.2 ml incubated for 30 min at 37 °C and terminated using 3.0 ml of 5 % TCA.

Effect of temperature: The effect of temperature on the enzyme activity was done at a temperature range of between 15- 55 °C with an interval of 5 °C. Modified Kunitz caseinolytic assay method as described for enzyme assay was used.

Effect of pH: This was carried out following the method described above but now varying the pH of the reaction mixture between a range of 2.0 – 11.0 with an interval of 1.0 using 0.1 M NaOH or 1.0 M HCl as found appropriate.

RESULTS AND DISCUSSION

The cost of enzyme production is a major obstacle in the successful application of proteases in industries. Proteases are unique class of enzymes, since they are of immense physiological as well as commercial importance. In this study, a protease enzyme was isolated from maggots developed from chicken and fish remains. The elution profile of the protein extract on sephadex G-75 gave two peaks with peak I showing a higher protease activity (Fig. 1). Further purification of pooled fractions of peak I on DEAE-cellulose gave two peaks with the major peak showing more protease activity (Fig. 2). The enzyme kinetics study reveals a V_{max} of 1.21 mmol/min and a K_m of

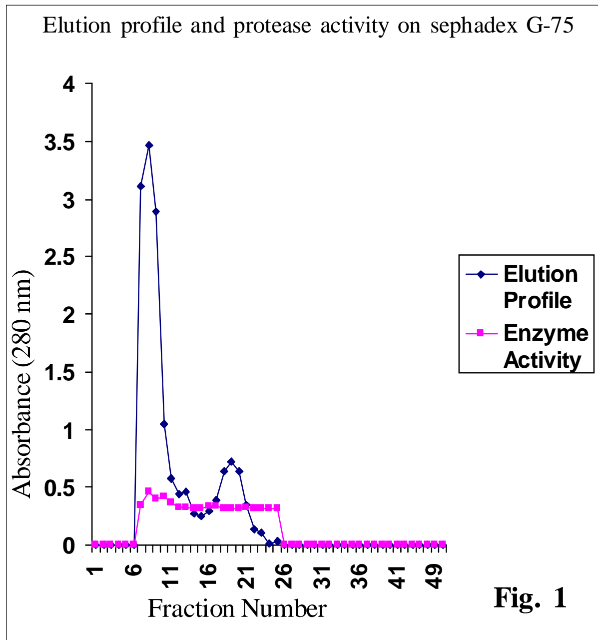

Fig. 1

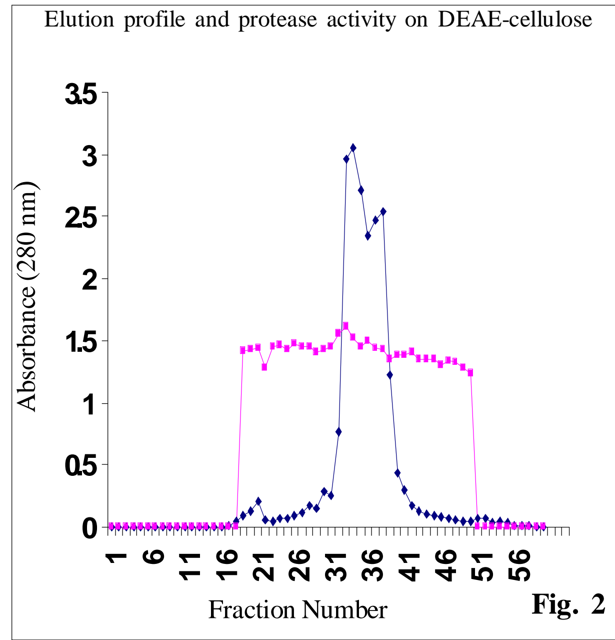

Fig. 2

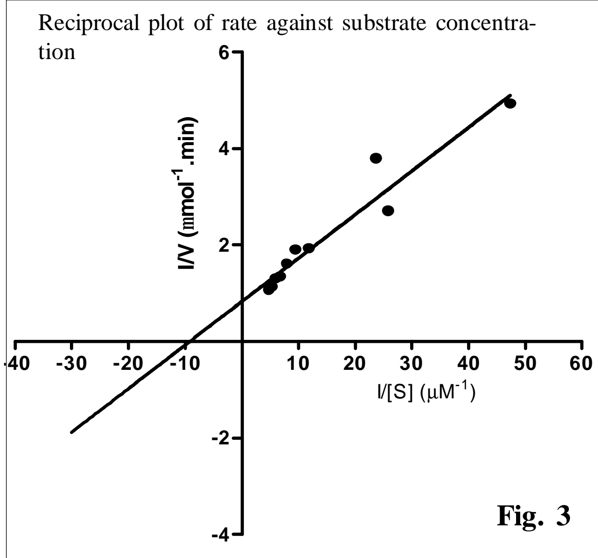

Fig. 3

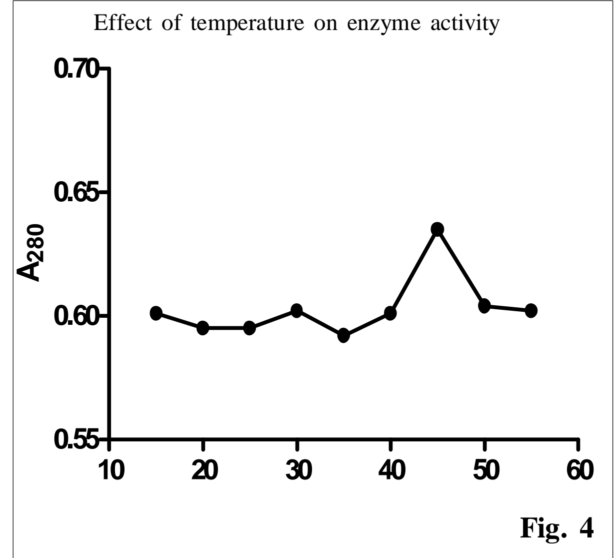

Fig. 4

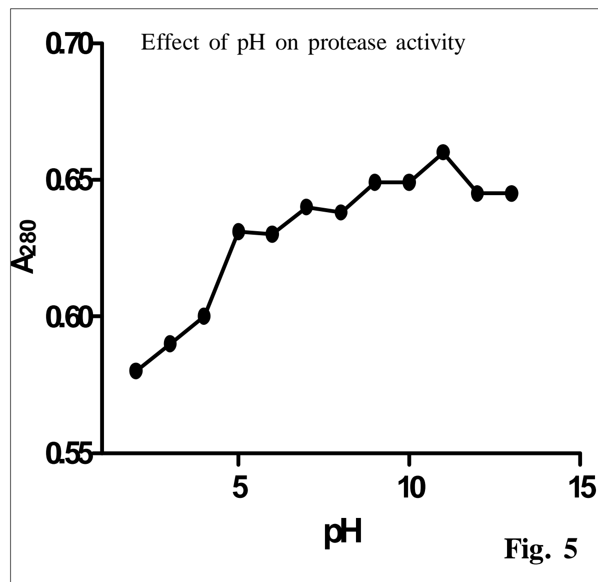

Fig. 5

0.11 mM. This indicates that the enzyme has affinity for casein as a substrate (Fig. 3). Anwar and Saleemuddin [15], in trying to assess further the suitability of the protease from *S. obliqua* for its use in laundering detergent carried out an experiment in which various proteins were used as substrate and it was found that the protease was able to cleave all the proteins tested but casein was the most preferred substrate [15]. The enzyme from maggot was found to have a temperature optimum of 45^{0}C (Fig.4) which implies that it is a little bit heat stable and also active at alkaline pH of 11 (Fig. 5) making it an alkaline protease. Work on the potential application of alkaline protease in bio-formulations has been intensified [15]. An industrially important alkaline protease, alcalase, isolated from a selective strain of *Bacillus*, has been shown to be useful as a catalyst for the resolution of *N*-protected amino acids having unusual side chains [16. The major component of this enzyme is subtillisin, which is a serine protease and is widely used as a detergent additive [16. The detergent industry has now emerged as the single major consumer of several other hydrolytic enzymes, including proteases acting in the alkaline pH range. The major use of detergent compatible proteases is in laundry detergent formulation [17,18].

CONCLUSION

The cost of enzyme production is the major obstacle in the successful application of proteases in industry. It is the understanding of this that prompted us into finding a possible use for maggots as a source of industrial enzymes. This study has therefore, demonstrated the presence of an alkaline protease enzyme in maggot which could be exploited commercially.

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