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Partial Purification of Acidic protease from the Mung Bean (*Phaseolus aureus* roxb.) Seeds

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Abstract

The purpose of the research was to study the extraction, purification and partial characterization of the protease from seeds of mung bean. The enzyme was purified in a 2-step procedure involving ammonium sulphate at 90% saturation, desalting by dialysis; and ion exchange chromatography on a column containing DEAE-cellulose. The activity of protease was higher at pH 6. The optimum temperature of the enzyme was 40 ˚C. The enzyme was show to have a higher specific activity (153 U/mg) and purified to 28 fold.

الخلاصة

ان الغرض من هذه الدراسة هو استخلاص انزيم اليوريز من بذور نبات الماش وتنقية وتوصيف جزئياً. نقـي الانـزيم بخطوتين: اولاً الترسيب بكبريتات الامونيوم بنسبة اشباع 90% والثانية طريقة التبـادل الايـوني باسـتخدام المبـادل DEAE-cellulose. اظهر الانزيم اعلى فعالية عند الرقم الهيدروجيني 6. كما لوحظ ان اقصى فعالية للانزيم كانت عند درجة حرارة 40 م. اظهر الانزيم فعالية نوعية تقريباً 153 وحدة/ملغم بعدد مرات تنقية تقدر بـ 28 مرة.

Introduction

Protease execute a large variety of function and have important biotechnological applications. Proteases are used in the degumming of solk goods, in the manufacture of liquid glue, in the preparation of cosmetics, in preparation of detergents, in the meat tenderization, in the preparationof cheese, in pharmacentical industry and in agriculture as growth promoters (Fox & Morrissey, 1980; Ward, 1985; Gupta *et al*., 2000). The major source of these proteases are microorganisms (Prescott & Dunn, 1959) but protease of plant origin have not been extensively examined. So for studies have been conducted on proteolytic enzymes occurring in plant seeds and beans including wheat (Skupin & Warchalewski, 1071) barley (Bunger, 1973) soybean (Weil *et al*., 1966) and *Dolichos lablab* L. (Ramahrishna & Rao, 2005).

Protease are crucial for living cells and play a role in plant cell adaptation to environments conditions. Oxidative stress produced oxidized proteins which are selectively degraded by protease (Pona *et al*., 2006).

This present work describes the partial purification and characterization of protease from seeds of mung bean.

Materials & Methods

Plant material

Dry seeds of mung bean (*Phaseolus aureus*) were collected from local market.

Protease preparation

Dry seeds of mung bean were ground into powder in a coffee grinder. 20 gm of powder were mixed with 100 ml of cold phosphate buffer (0.1 M, pH=6). The homogenate was filtered through two layers of cheese cloth and the filtrate was centrifuged at 3000 rpm for 15 min to get clear supernatant.

Estimation of proteins

Protein content was estimated by the method of Lowry (1951).

Protease assay

The protease activity was spectrophotomertically according to (Kuntiz, 1947). 0.1 ml of enzyme extract was added with 1.9 ml of phosphate buffer (pH 6) dissolved albumin and incubated for 20 minute at 25 ˚C. Then added 3 ml of TCA (5%) and centrifuged 5000 rpm for 15 minute and absorbance by spectrophotometer at 280 nm.

Purification of protease

Ammonium sulphate precipitation

The clear supernatant was fractionated with 90% ammonium sulphate. All subsequent steps were carried out at 4 ˚C. the resulting precipitate was collected by centrifugation at 3000 rpm. The precipitate was dissolved in 5 ml phosphate buffer (pH 6) and dialyzed against the same buffer and used for ion exchange chromatography.

Ion exchange chromatography

The enzyme obtained from the above step was loaded on to a DEAE cellulose column (2.2 cm×20 cm). The buffer (phosphate buffer) was used for preliminary washing of unbound proteins. The bound enzyme was then eluted with a stepwise of NaCl (0.1-1 M) in phosphate buffer (pH 6). Fraction of 3 ml were collected.

Effect of pH on crude enzyme activity

The activity of the crude protease was measured at different pH values. The pH was adjusted using the following buffers (0.1 M): phosphate buffer (3-7), Tris-HCl buffer (8-10).

Optimum temperature

The effect of temperature on protease activity obtained at different temperature values (20-60 ˚C). After that optimum temperature was determined.

Results & discussion

Ammonium sulphate fractionation. In the present study, the most widely used ammonium sulphate fractionation (25, 50, 75 and 90% w/v, saturation) was carried out directly with crude extract. Considerable amount of the enzyme was recovered in precipitate obtained by fractionating with 90% ammonium sulphate supernatant Fig. (2). This suggested that the protease was either of extremely small molecular or that it was protected by an essentially polar shield such as associated carbohydrate (Shepard & Moore, 1978).

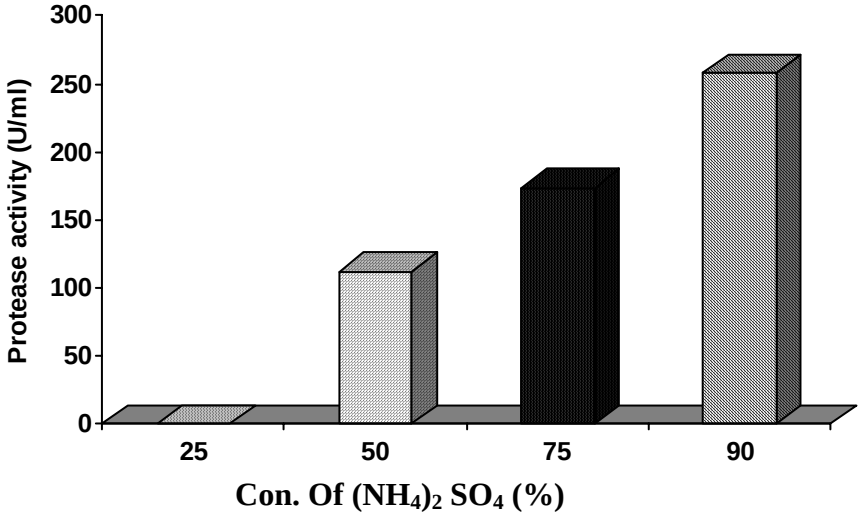

Figure (1): Effect of different concentration of $(NH_4)_2 SO_4$ on protease activity.

Ion exchange chromatograpgy

The results obtained for the partial purification of protease from mung bean seeds extract summarized in Table (1) $(NH_4)_2SO_4$ (90 % saturation) fractionation gave rise to 2.5 fold. The yield increased to about 46%. Ion exchange of the second step by DEAE-cellulose the enzyme was purified with yield of 28% and 8 fold. The purified enzyme displayed a specific activity 153.36 U. mg^{-1}. Similar or even lower yields are common for acidic proteases from other germinating seeds. Such as GP-HO (4.5%) from horse gram seedlings (Rajeswari, 1997), acidic protease (15%) from germinating winged-bean seeds (Usha & Singh, 1996) and EP-1(1.6%)from barley seedling and GA3-induced cysteine protease (3.38%) from barley aleurone layers (Koehlar & Ho, 1988).

Table (1): Summary of purification steps of acid protease from mung bean seeds.

Purification step	Protein con. (mg/ml)	Activity (U/ml)	Specific activity (U/mg)	Total activity (U)	Purification (fold)	Yield
Crude extract	27.85	1.44	19.34	2785	1	100
(NH4)2SO4 Fractionation	258.16	5.26	49.07	1290.8	2.5	46
DEAE-cellulose	155	1.01	153.36	775	8	28

pH optimum

For the determination of the pH optimum different buffer (3-10) were used. The highest protease activity was found to be at pH 6 Fig. (2). The enzyme functions best within a certain pH range when the pH changes, the active site progressively distorts and affects enzyme function. The protease at high pH values complete loss of activity figure (2).

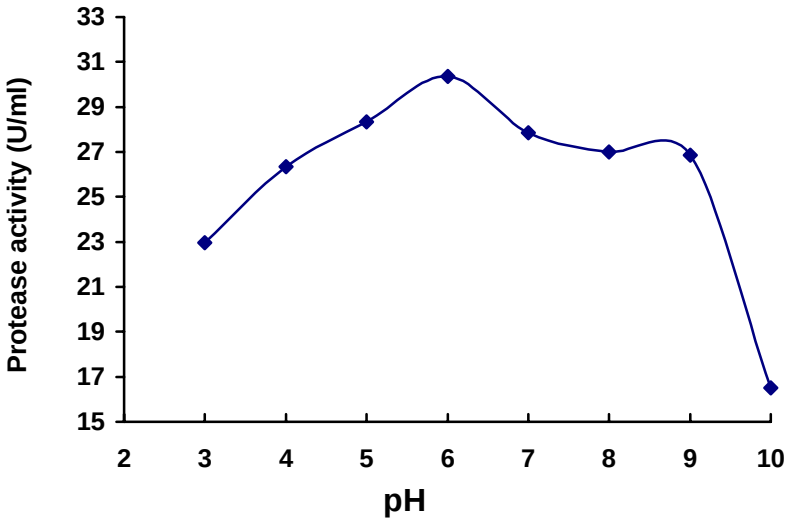

Figure (2): Effect of pH on protease activity (U/ml).

Optimum temperature

The Optimum temperature for protease activity was measured at different temperature at 20-60 ˚C. The substrate and pure enzyme were incubated for 10 min at various temperatures from 20˚C to 60˚C, after spectrophotometric measurement for 5min was carried out at 37˚C. the highest activity was observed as 40˚C (Fig-3).

The optimum temperature we determined was similar to that given in previous studies

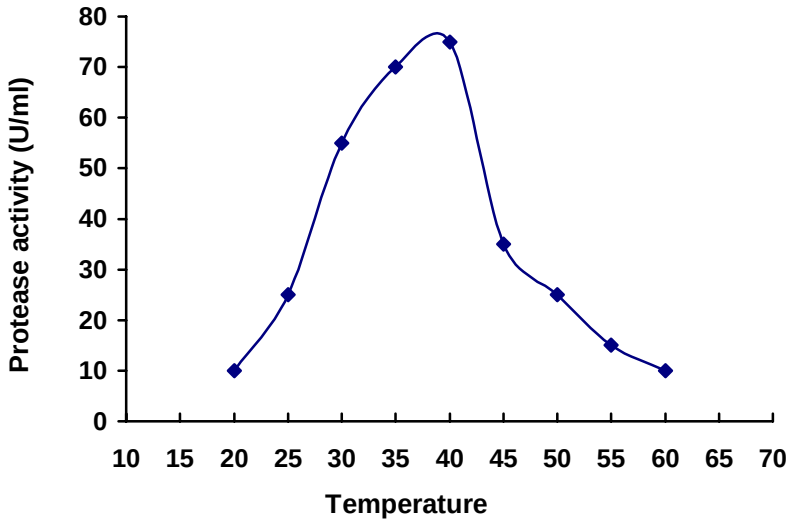

Figure (3): Optimum temperature for the activity of protease for mung bean seeds.

such as cotton seeds (Mezhlum'yan *et al.*, 1986) and *Lupinus angustifolins* (Shepard and Moore, 1978). The chemical reaction speed up as temperature is increased, so, in general, catalysis will increase at higher temperature. However, each enzyme has a temperature optimum, and beyond this point the enzymes functional shape is lost (denatured). The protease in this study lost the activity at 60 ˚C figure (3).

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