INVESTIGATION OF PROTEASES IN PLANT SEEDS

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SUMMARY: Ten plant seeds were analyzed for protease activities. During the course of screening Cajanus cajan seeds were found more potent for acidic protease activity. The Cajanus cajan protease activity was optimum at pH 2.5 and 5.5 and at temperature 30°C. It is fairly stable up to 40°C. Cajanus cajan seeds protease activity is markedly increased in presence of Ca^{2+}, cysteine and mercaptoethanol but strongly inhibited with EDTA, iodoacetic acid, o-phenanthroline, Hg^{2+} and PMSC.

Key Words: Acid proteases, cajanus cajan seeds.

INTRODUCTION

The proteases are useful in various ways and their applications are increasing at a fantastic rate. Proteases are used in the degumming of silk goods, in the manufacture of liquid glue, in the preparation of cosmetics, in the preparation of detergents, in the meat tenderization, in the preparation of cheese, in medicine preparation and in agriculture as growth promoters (1-3). The major source of these proteases are microorganisms (4) but proteases of plant origin have not been extensively examined. So far studies have been conducted on proteolytic enzymes occurring in plant seeds and beans including wheat (5), barley (6), sorghum (7), corn (8), potato tubers (9), mung beans (10), soybean (11), sunflower seeds (12), rice seeds (13), Moringa oleifera seeds (14) and lotus seeds (15).

Ichishima (16) have reported that most of the plant proteases are neutral or alkaline and there are few acid proteases with a pH optimum at 2-3 are widely distributed in the plant seeds and play some important physiological roles in the metabolism of seed proteins.

This work was under taken to elucidate the properties of Cajanus cajan seed protease in a homogeneous state and its function in seed protein metabolism. The paper describes some properties of crude Cajanus cajan seed protease.

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MATERIALS AND METHODS

Materials

The Cajanus cajan seeds were collected in dry state from the village of Qaim Babar, District Hyderabad, Sindh. All the reagents used were of analytical grade and were used without further purification.

Methods

Enzyme powder preparation

Seeds were crushed in pestle mortar and defatted with diethyl ether. The defatted residue was further crushed in chilled acetone. Acetone was removed by filtration through Whatman No. 1 filter paper and the residue was dried at low temperature (20°C).

Preparation of soluble enzyme

The crude enzyme solution was prepared from above enzyme powder as described previously (14).

Substrate preparation

0.5% solution of Hemoglobin (bovine) and casein were prepared in Universal buffer pH 4.0 and 7.0 respectively during screening of plant seeds for protease activity.

Determination of protein

Protein content of enzyme solution was determined by the method of Lowry *et. al.* (17), with bovine serum albumin as standard.

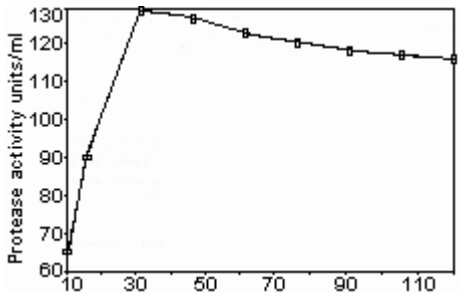
Determination of protease activity

Protease activity was determined as described in earlier

Table 1: Comparison of proteases activities in different plant seeds.

Botanical name	Local Common name	Protein mg/ml	Protease activity Units/ml	
			Acid	Neutral
Cajanus cajan seeds	Matri seeds	1.10	120	40
Erythrina glabrescens	-	1.40	95	72
Glycine max	Soybean	2.76	63	70
Achras sapota seeds	Chicu seeds	2.00	47	23
Melia azadircahta seeds	Neem seeds	2.40	31	04
Mangifera indica seeds	Mango seeds			
	Desi	1.00	31	07
= =	Sindhri	1.30	23	12
= =	Chosa	1.15	05	06
Dolichos lablab seeds	Sem seeds	1.10	23	07
-	Golden seeds	1.00	08	15
Gossypium hirsutum	Cotton seeds	2.30	07	100

Figure 1: Effect of time period on Cajanus cajan acidic protease activity.



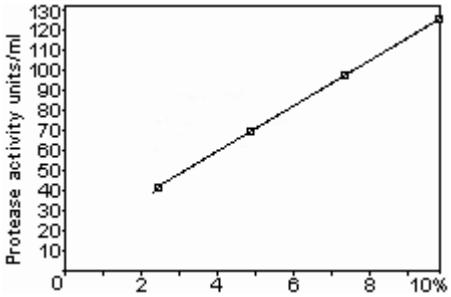
reports (18,19).

One unit of the protease activity was defined as the amount of enzyme that liberated 1 ug of tyrosine under the standard assay conditions.

RESULTS AND DISCUSSION

First of all the presence of protease activity in plant seeds extract was ascertained by using acid treated casein and hemoglobin as substrate. From the results presented in Table 1, it is evident that acidic protease activity is higher than neutral protease activity in Erythrina glabrescent seeds, Melia azadirachta seeds, Achras sapota seeds, Cajanus cajan seeds, Mangifera indica seeds and Dolichos lablab seeds but in some cases neutral protease is higher than acidic protease activity in Gossypium hirsutum seeds, Glycine max L. seeds and golden seeds. Results shown in Table 1 indicate that acidic protease activity is much higher in Cajanus cajan seeds in comparison to other plant seeds and hence it was selected for further work.

Figure 2: Effect of enzyme concentration on Cajanus cajan protease activity.



The protease activity of Cajanus cajan seeds was observed to be increasing with respect to time up to 30 minutes and then slightly declined as shown in Figure 1. The decrease in enzyme activity after 30 minutes, may be suggested due to self digestion (11). Work is in progress at present for purification of protease, to determine whether self digestion is actually observed with all fractions or whether one fraction hydrolyzes another.

The effect of enzyme concentration and substrate concentration on protease activity of Cajanus cajan seeds were studied and results are depicted in Figures 2 and 3. It is observed that the rate of reaction increases proportionally to enzyme and substrate concentration. In subsequent experiments 10% enzyme concentration (10% crude enzyme solution of Cajanus cajan seeds) and 0.5% Hemoglobin was used as substrate.

The pH activity curve of crude enzyme of Cajanus cajan seeds in the hydrolysis of Hemoglobin is shown in

Figure 3: Effect of substrate concentration on Cajanus cajan protease activity.

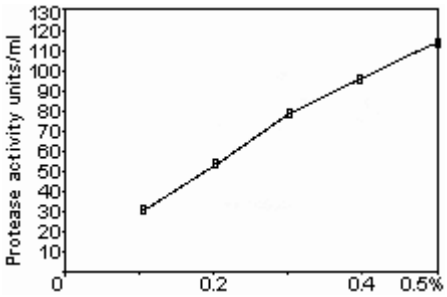


Figure 4: Effect of pH on Cajanus cajan protease activity.

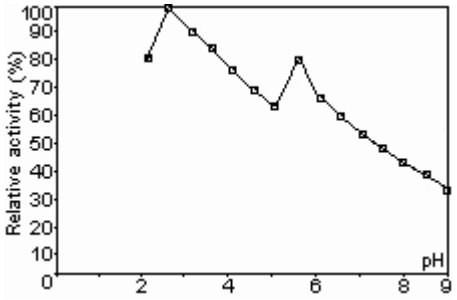


Table 2: Effect of chemicals on acidic protease activity of Cajanus cajan seeds.

Chemicals	Conc. (mM)	Activity Units/ml	% Relative Activity*	% Activation/ (Inhibition)
Control	5	120	100	-
Ca^{2+}	5	187.27	156.06	56.06
Cysteine	5	240.00	200.00	100.00
Mercaptoethanol	5	245.00	204.06	104.06
EDTA	5	58.18	48.48	(51.52)
o-phenanthroline	5	68.09	57.57	(42.43)
PMSC	5	58.18	48.48	(51.52)
Iodoacetic acid	5	32.00	26.66	(73.34)
Hg^{2+}	5	27.27	22.72	(77.28)
Mn^{2+}	5	76.36	63.83	(36.37)
Zn^{2+}	5	81.18	67.65	(32.35)
Co^{2+}	5	87.27	72.73	(27.27)

PMSC: Phenylmethanesulfonylchloride.

* Expressed as % of the activity with no addition of chemicals.

Figure 5: Effect of temperature on Cajanus cajan seeds protease activity.

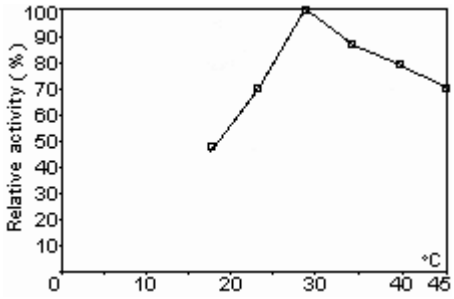


Figure 4. The pH profile (Figure 4) showed two main peaks one at pH 2.5 and the other at pH 5.5. A very similar situation was also reported by Scalet *et. al.* (20) and Ragster *et. al.* (21) for the crude extract of alfalfa leaves and Soybean leaves respectively. It was decided to study further on protease activity at pH 2.5.

The effect of temperature on protease activity from Cajanus cajan seeds was studied and results are compiled in Figure 5. The temperature profile indicates that the optimum temperature for enzyme reaction is 35°C.

The effect of pH stability of the crude enzyme of Cajanus cajan seeds was also studied and results are shown in Figure 6. The protease enzyme was stable to change of pH in the range of 3.0 to 6.0 at 30°C for 20 minutes. Below pH 2.0 and above pH 6.0, the enzyme significantly lost its activity.

The thermo stability curve of the protease activity at pH 2.5 is shown in Figure 7. The enzyme was fairly stable up to 40°C but destabilized with increasing temperature and was completely inactive at 80°C.

The results of the effect of activators and inhibitors on protease activity of Cajanus cajan seeds are summarized in Table 2. The stimulation of activity in presence of cysteine and mercaptoethanol indicates that Cajanus cajan seeds acid protease possess cysteine at active site or near to active site. The inhibitory effect of Hg^{2+} Iodoacetic acid and PMSC supported this assumption. Several authors have reported cysteine protease from various plant sources (11,20,21).

In order to obtain information about the primary specificity of acid protease of Cajanus cajan seeds against various substrates were examined at pH 2.5 and temperature 30°C. It is observed from Figure 8 that the enzyme preparation effectively degraded bovine albumin than other proteins. The order of effective degradation was as follows: albumin> hemoglobin> peptone> acid hydrolyzed casein

Figure 6: Effect of pH stability on Ca- janus cajan seeds protease activity.

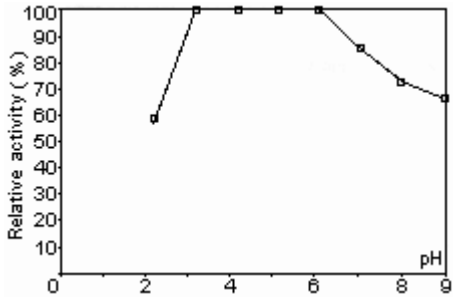
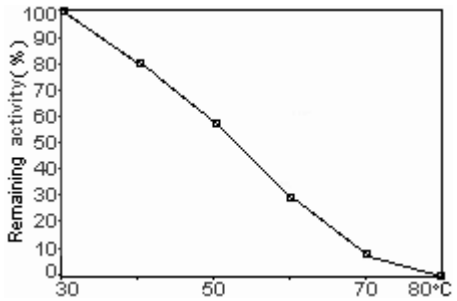


Figure 7: Effect of heat stability on Cajanus cajan seeds protease activity.



and gelatin. Further work of purification and specificity is under investigation and will be reported later on.

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REFERENCES

1. Fox PF and Morrissey PA : Industrial and Clinical Enzymology, Ed by LJ Vitale, V Simon, Pergamon Press Oxford, 61:43, 1980.

2. Ward OP : Comprehensive Biotechnology, Pergamon Press Oxford, 3:789, 1985.

3. Graham DY : In Enzymes as Drugs, Ed by JS Holcenberg, J Roberts, Wiley New York, p 331, 1981.

4. Prescott SC and Dunn CC : Industrial Microbiology; McGraw Hill Book Co, New York, 1959.

5. Skupin J and Warchalewski J : Isolation and properties of protease A from wheat grain. J Sci Food Agric, 22:11-15, 1971.

6. Bunger WC : Multiple forms of acidic endopeptidase from germinated barley. Plant Physiol, 51:1015-1021, 1973.

7. Garg GK and Virupaksha TK : Acid protease from germinated sorghum 1. Purification and characterization of the enzyme. Eur J Biochem, 17:4-12, 1970.

8. Abe M, Arai S, Fujimaki M : Purification and characterization of a protease occurring in endosperm of germinating corn. Agric Biol Chem, 41:893-899, 1977.

9. Kitamura N and Muruyama Y: Cysteine endopeptidase activity in sprouting potato tubers. Agric Biol Chem, 49:1591-1597, 1985.

10. Baumgartner B and Chrispeels MJ : Purification and characterization of vicilin peptidohydrolase, the major endopeptidase in the cotyledons of mung bean. Eur J Biochem, 77:223-233, 1977.

11. Weil J, Pinsky A, Grossman S : The proteases of soybean. Cereal Chem, 43:392-399, 1966.

12. Walde P, Luisi PL, Palmieri S : Proteolytic activity in sunflower seeds (Helianthus annulus L). J Agric food Chem, 32:322-329, 1984.

13. Arai S, Hosoyama H, Abe K : Gibberellin induced cysteine proteinase occurring in germinating rice seeds and its specificity for digesting oxidized insulin B-chain. Agric Biol Chem, 52:2957-2959, 1988.

14. Dahot MU, Ali SA, Memon AR : Proteolytic enzymes of Moringa oleifera seeds. J Pharm Pb Univ Lhr, 6:1-9, 1985.

15. Shinano S and Fukushima K : Studies on Lotus seed protease Part II. Purification and some properties. Agric Biol Chem, 33:1236-1243, 1969.

16. Ichishima E : J Ferm Assoc Japan, 22:393, 1964.

17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ : Protein measurement with folin phenol reagent. J Biol Chem, 193:265-275, 1951.

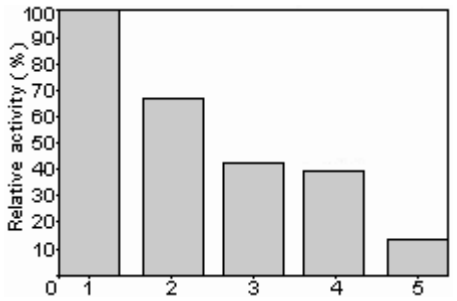
18. Dahot MU : Studies on proteolytic enzyme Part 1. Characteristics of protease synthesis by Penicillium expansum. Pak J Sci Ind Res, 30:194-196, 1987.

19. Dahot MU : Effect of pretreated rice husk on the production of protease by Penicillium expansum. Proc Natl Chem Conf, 2:109-113, 1990.

20. Scalet M, Alpi A, Picciarelli P : Proteolytic activities in Alfalfa (Medicago sativa L.) leaves. J Plant Physiol, 116:133-145, 1984.

21. Ragster L and Chrispeels MJ : Hemoglobin digesting acid proteinases in soybean leaves. Plant Physiol, 67:110-114, 1981.

Figure 8: Effect of substrate specificity on Cajanus cajan seeds protease activity. 1. Albumin, 2. Heamoglobin, 3. Peptone, 4. Acid hydrolyzed casein, 5. Gelatin.



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