Phytochemistry, 1975, Vol. 14, pp. 1283–1284. Pergamon Press. Printed in England.

PURIFICATION AND PROPERTIES OF AN ACID PROTEASE FROM *PHASEOLUS AUREUS*

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(*Revised Received* 11 *September* 1974)

Key Word Index—*Phaseolus aureus*; mung bean; leguminosae; acid protease.

Abstract—An acid protease has been purified 470-fold from mung bean seedlings. With bovine serum albumin as substrate, the enzyme showed an optimum at pH 3·6 and at 45° or incubation for 1 hr. The enzyme was relatively stable after incubation for 20 hr between pH 3·0 and pH 6·0 but lost activity on incubation at 15° for 15 min at pH 6·8.

INTRODUCTION

Studies on the proteolytic activity associated with germinating seedlings have shown that considerable complexity exists among the proteolytic enzymes [1]. Usually, the endopeptidases and the carboxypeptidases show acidic pH optima [1] but only in a few cases have the enzymes been studied in detail [2–5]. It has been suggested that some of these enzymes with acidic pH optima are part of a lysosomal-like digestive process that takes place in the aleurone grain during germination [4].

RESULTS AND DISCUSSION

An acid protease has been purified 470-fold from mung bean seedlings using ammonium sulphate fractionation and stepwise elution from DEAE-cellulose. The enzyme was obtained with a maximum sp act of 182 units/mg protein; different sp act were obtained from various preparations. It was found that the protease was eluted from DEAE-cellulose by 10 mM sodium phosphate buffer, pH 6·8, containing 50 mM sodium chloride. When this fraction was examined by polyacrylamide gel electrophoresis [6], there was one major component present, together with several other minor components. Attempts to purify the protease further by gel filtration and by gradient elution from DEAE-cellulose led to almost complete loss of the enzyme activity. The properties of the protease were therefore examined using the preparation obtained from the stepwise elution of the DEAE cellulose.

The pH optimum of the protease was *ca* pH 3·6 with bovine serum albumin as substrate. At pH 5 the activity was only 35% of the optimum; when the pH was greater than pH 6 little activity was found. The temperature optimum of the protease for 1 hr incubations was found to be at least 45°; at 55° the activity was only 30% of that at 45°, and at 70° the activity was negligible. The pH stability of the protease was examined by incubating at various pH values for 20 hr at 4°, prior to assay at pH 3·6. At least 90% of the activity remained after incubation between pH 3 and pH 6. Below pH 3 the remaining activity was low. Above pH 6 the amount of activity which remained gradually declined with increasing pH and by pH 8 only 25% of the original activity remained. In view of these results, an attempt was made to use buffers of lower pH during extraction and purification. The amount of activity obtained however was less than that obtained with the pH 6·8 buffers. The temperature stability of the protease under the conditions of the purification was examined. When the protease was incubated for 15 min at pH 6·8, without the addition of any substrate, at temperatures greater than 15° nearly all activity was lost. The effect of urea solutions on the protease was examined by incubating at pH 6·8 for 1 hr at 4°, prior

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to assay at pH 3·6. Activity was reduced by 40% by incubation with 2M urea and completely destroyed by 4 M urea; the activity was not restored by dialysis.

From its properties the mung bean acid protease appears to be very similar to the acid proteases from sorghum [2], lotus [3] and hempseed [4]. The proteolytic activities of extracts from various other species, e.g. barley [7,8], cotton [9], wheat [10], suggest that a similar acid protease may also be responsible in part for the activities observed in these species. A specific acid protease may therefore be widely distributed in higher plants. In certain cases [4,7,9] acid protease activity is associated with the protein bodies. However, this mung bean acid protease has been extracted from whole seedlings and therefore there is no evidence that it is associated with the protein bodies in any way. Purified sorghum protease mainly attacks the α-carboxyl of aspartic acid and glutamic acid residues [11], and an acid protease from azuki seed [12] has been reported to cleave polypeptides after residues which have a hydroxyl group [13]. In the present work performic oxidized insulin was incubated with the enzyme and the new *N*-terminal amino acids released determined by the dansyl method [14]. The results suggested that the protease was an endopeptidase, and were consistent with it having a specificity similar to that of the sorghum acid protease.

EXPERIMENTAL

Materials. Mung bean seeds (2 kg) were soaked for 18 hr in running H_2O and then placed in seed trays in the dark at 25–28° for 3 days.

Purification was performed at 4° and Na Pi buffer, pH 6·8, was used throughout. Seedlings were homogenized in 50 mM buffer (5 l.) and after standing for 1 hr, the homogenate was centrifuged at 5000 *g* for 30 min. The supernatant (4·5 l.) was taken to 80% $(NH_4)_2SO_4$ sat and after standing for 1 hr, the 80% ppt was collected by centrifugation at 12000 *g* for 30 min. The ppt was redissolved in 50 mM buffer (2 l.), with $MnSO_4$ (10 mM), and taken to 20% $(NH_4)_2SO_4$ sat. The 20% ppt was removed by centrifugation at 12000 *g* for 30 min and discarded; the supernatant was taken to 45% $(NH_4)_2SO_4$ sat, the ppt collected by centrifugation at 12000 *g* for 30 min and the supernatant discarded. The ppt was redissolved in 10 mM buffer (30 ml) and dialysed against the same buffer. (3 × 8 l.). After dialysis, the small amount of ppt was removed by centrifugation and the supernatant applied to a col of DEAE-cellulose (7·5 mm × 12 cm) equilibrated in 10 mM buffer. The col was washed with 10 mM buffer (500 ml) and then eluted with 10 mM buffer containing 5, 50, 100 and 150 mM NaCl (250 ml/step). Eluate fractions were taken to 80% $(NH_4)_2SO_4$ sat and the ppt removed by centrifugation. After redissolving in 10% of original vol of 10 mM buffer, fractions which contained proteolytic activity were pooled; when necessary, the pooled material was stored at −20° until used.

Assay of proteolytic activity was by the method in Ref. 2, except that incubations were at 37°. One unit is defined as the amount of enzyme which releases 10 μg of tyrosine equiv per hr under the condition of assay. Sp act refers to the units per mg protein. Protein was determined by the method of Lowry *et al.* [15] using bovine serum albumin as standard.

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