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# CHANGES IN THE FORMS OF INVERTASE DURING GERMINATION OF MUNG BEAN SEEDS

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**Key Word Index** – *Vigna radiata*; Leguminosae; mung bean; invertase; seed.

**Abstract**—High levels of 'alkaline' invertase activity occur in dormant mung bean seeds. During germination this activity decreases rapidly and is replaced by high 'acid' invertase activity. Cycloheximide prevented the formation of the latter activity and also inhibited germination. It is suggested that *de novo* synthesis of 'acid' invertase occurs during germination. Both enzymes bind to concanavalin A and, hence, are presumed to be glycoproteins. Affinity-purified enzyme samples show similar ratios of 'acid' and 'alkaline' invertase activities to the crude preparations indicating that specific enzyme inhibitors or activators are probably not involved in controlling the activities *in vivo*.

## INTRODUCTION

Sacher *et al.* [1] have reported that two invertases can be extracted from sugar cane internodes, one with a pH optimum at 5.5 and the other at 7.6, termed 'acid' invertase and 'alkaline' invertase, respectively. The 'alkaline' enzyme was confined to mature tissue, whereas actively growing tissue was characterized by a high activity of the 'acid' enzyme. The data found in the literature support the role of the invertases in the regulation of sucrose metabolism [2, 3]. The 'acid' enzyme directs sucrose to hexoses in tissues where demand for monosaccharides is high, and the 'alkaline' enzyme hydrolyses sucrose in cells of storage organs. In addition, soluble and cell wall-bound invertases have also been identified [2]. This study was undertaken to examine the changes in the activities of 'alkaline' and 'acid' invertases in mung beans during germination.

## RESULTS AND DISCUSSION

Binding of 'acid' and 'alkaline' invertases to cell-wall components had been reported earlier [4–9], hence extraction of the seeds was carried out routinely with 0.1 M sodium chloride to solubilize all of the invertase activity in the tissues [4, 5]. No further activity was extracted with 0.5 M sodium chloride.

The pH profiles of invertase activity in extracts prepared from resting mung bean seeds and seeds at different stages of germination are shown in Fig. 1. This shows that the level of 'alkaline' activity, which is high in resting seeds, declines rapidly on germination and is replaced by 'acid' activity which equates with the theory that the latter enzyme is required by developing tissues to convert sucrose to free hexoses (see also refs [2, 3]). A decline in 'alkaline' invertase with a concomitant build-up of 'acid' invertase activity during germination may be due to one or more of the following processes: (a) degradation of the 'alkaline' enzyme accompanied by *de novo* synthesis of 'acid' enzyme, (b) conversion of 'alkaline' enzyme into the 'acid' enzyme, (c) removal of specific inhibitors of 'acid' enzyme accompanied by the formation of inhibitors of 'alkaline' enzyme or (d) removal of activators of the 'alkaline' enzyme followed by formation of activators of 'acid' enzyme.

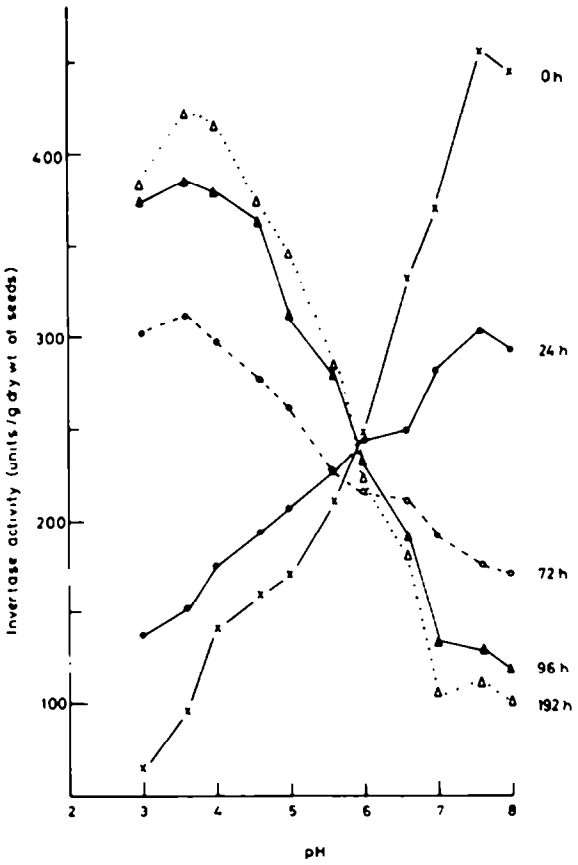


Fig. 1. Invertase activity–pH profiles of enzyme extracts prepared from mung bean seeds at different times (shown on the right) of germination.

The effect of cycloheximide on the levels of the two invertases during germination are shown in Table 1. This

Table 1. Levels of 'acid' and 'alkaline' invertase in mung bean extracts under different conditions of germination*

| Germination time (hr) | Germination medium | Total protein (mg × $10^{-2}$) | Total enzyme units | |
|---|---|---|---|---|
| | | | 'Alkaline' | 'Acid' |
| 0 | Water | 19.7 | 3660 | 1220 |
| 24 | | 19.2 | 2450 | 1560 |
| 72 | | 21.9 | 1430 | 2220 |
| 96 | | 22.7 | 1020 | 2880 |
| 192 | | 25.0 | 8900 | 2970 |
| 0 | Cycloheximide | 20.6 | 3680 | 1020 |
| 24 | (10 μg/ml) | 17.6 | 300 | 240 |
| 72 | | 15.0 | 260 | 240 |
| 96 | | 21.0 | 200 | 280 |
| 192 | | 19.0 | 180 | 190 |

*8 g seeds for germination time were used.

translation inhibitor strongly interfered with the development of 'acid' invertase activities and also led to a rapid loss of the 'alkaline' activity. It is conceivable that synthesis of alkaline invertase can continue during the early stages of germination but that degradation of the enzyme, involving proteases laid down in the maturing seed, is the more important process. The presence of cycloheximide would inhibit protein synthesis and, hence, could account for the observed rapid loss of 'alkaline' activity. In the case of the 'acid' invertase activity it can be similarly postulated that the normal rapid rise on germination is a result of *de novo* enzyme synthesis which can be inhibited by cycloheximide.

No evidence for the presence of specific endogenous inhibitors or activators of the 'alkaline' and 'acid' invertases which might control enzyme levels during mung bean germination could be obtained by protein fractionation. Invertase activities from both dormant and 192 hr germinated seeds were found to be totally adsorbed on to Con A-Sepharose columns, suggesting that both the 'acid' and 'alkaline' enzymes are glycoproteins. On elution of the enzymes from the columns with methyl α-D-mannoside the 'alkaline'/'acid' invertase activity ratio in the eluate fractions was comparable with that of the corresponding crude seed extracts (see Fig. 2). Considerable purification (40–90 fold) of the enzymes was achieved by this affinity chromatography procedure.

From the above results it is clear that a dramatic

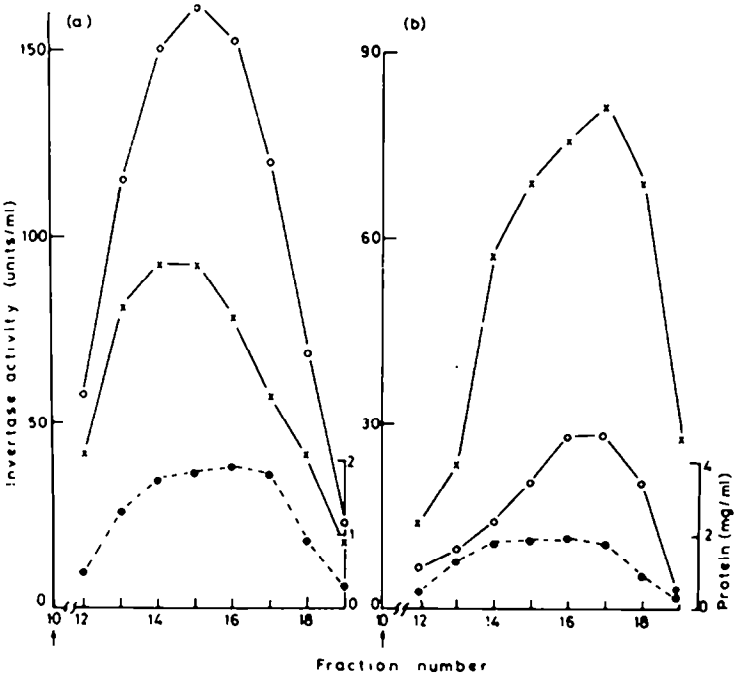


Fig. 2. Elution of Con A-Sepharose-bound invertase activity with 0.2 M methyl α-D-mannoside solution (see arrow). Enzyme extracts used were from (a) dormant and (b) 192 hr germinated mung bean seeds. ○ —— ○, 'alkaline' invertase; × —— ×, 'acid' invertase; ● —— ●, protein.

increase in the 'acid' invertase level occurs in germinating mung bean seeds. This enzyme is probably newly synthesized and then utilized to metabolize the sucrose reserves for the developing tissues. As such, the synthesis of 'acid' invertase may be a key step in the developmental process and relate to seed viability and seedling vigour.

## EXPERIMENTAL

*Enzyme extraction.* Dormant mung bean (*Vigna radiata*) seeds (8 g) were washed with $H_2O$, then allowed to germinate at 25° in petri dishes lined with moist glass wool. For the effects of cycloheximide on germination, the glass wool linings were soaked with this soln (10 μg/ml).

The seeds were harvested at various times, homogenized in 0.1 M NaCl soln (2 ml/g seed) in a pre-cooled (at 4°) pestle and mortar. The slurry was then centrifuged at 20000 *g* for 20 min and the supernatant was used as crude enzyme extract.

*Invertase assay.* Invertase activity was assayed by mixing 100 μl of the enzyme extract with a mixture containing 0.5 M sucrose soln (50 μl) in $H_2O$ and the respective buffer (100 μl). NaOAc buffer (0.1 M) of pH 3–5.6 and Na phosphate buffer (0.1 M) of pH 6–8 were used. The assay mixtures were incubated at 30° for 1 hr and subsequently stopped by adding 0.2 M dibasic potassium phosphate (300 μl) followed by heating at 100° for 3 min. After cooling, the liberated glucose was reacted with 2 ml of glucose oxidase reagent (Tris buffer, pH 7 containing 250 mg/l. glucose oxidase, 25 mg/l. peroxidase and 100 mg/l. *o*-dianisidine. HCl). This was incubated at 30° for 1 hr and the reaction stopped with 9 M $H_2SO_4$ (2 ml). The absorbance of the resultant soln was measured at 540 nm.

The glucose oxidase method was used for the assay, compared to the measurement of reducing power, mainly because less than stoichiometric amounts of free fructose are often known to be liberated by invertases. This is generally due to transfructosylation reaction catalysed by the same enzyme [10].

One unit of enzyme activity is defined as the amount which liberates one nmol of glucose/min under the given conditions. Specific activity is expressed as enzyme units/mg protein. Protein was determined by the method of ref. [11].

*Concanavalin A-Sepharose chromatography.* A 2.5 cm × 6 cm packed column was prepared and equilibrated with 0.1 M Na phosphate buffer, pH 6. A 1 ml enzyme sample predialysed against the column buffer was applied and eluted (30 ml/hr) using the same buffer until $A_{280}$ of the eluate was < 0.01. The entire enzymic activity was adsorbed. The enzyme activity was eluted with aq. methyl α-D-mannoside soln (0.2 M) and 3 ml fractions were collected. These were assayed for enzymic activities and protein content.

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