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ALLANTOINASE FROM NODULES OF PIGEONPEA (*CAJANUS CAJAN*)

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Key Word Index—*Cajanus cajan*; Leguminosae; pigeonpea; nodules; allantoinase; properties; intermediate metabolites; regulation.

Abstract—Allantoinase was purified about 10-fold from nitrogen fixing root nodules of pigeonpea (*Cajanus cajan*) using $(NH_4)_2SO_4$ fractionation and chromatography on Sephadex G-100. The purified preparation showed a specific activity of 1.73 nkat/mg protein, M_r of 125 000, pH optimum between 7.5 and 7.7 and K_m of 13.3 mM. The enzyme was heat stable up to 70° and metal ions, except Hg^{2+}, had no effect on the enzyme activity. The enzyme was inhibited significantly by reducing agents. Amino acids, ammonium, nitrate, potential precursors of allantoin and a number of other intermediate metabolites of ureide biosynthetic pathway had no effect on enzyme activity. It is suggested that allantoinase is unlikely to regulate the production of ureides in the nodule tissue.

INTRODUCTION

In many of the tropical legumes including pigeonpea, most of the nitrogen fixed in nodules is translocated to the shoot in the form of ureides, allantoin and allantoic acid [1–7]. These ureides are synthesised via a pathway involving *do novo* synthesis of purines followed by oxidation and hydrolysis [8–10]. Xanthine dehydrogenase, uricase and allantoinase are the enzymes involved in the degradative pathway leading to the formation of ureides from purines. The purification and properties of xanthine dehydrogenase have been described from root nodules of navy bean [11] and soybean [12]. Similarly, uricase has also been purified and studied from cowpea [13] and soybean root nodules [14]. However, there are few reports on the properties of nodule allantoinase [15, 16], though this has been studied in a variety of plants including some legumes [17–23]. Here we describe some properties of allantoinase isolated from nodules of pigeonpea.

RESULTS AND DISCUSSION

Developmental pattern and localisation

From a low level at the 15 day stage, allantoinase activity per plant increased steadily up to 90 days and then abruptly to attain a peak value at 105 days after sowing. Thereafter, a decline was observed at the 120 day stage (Fig. 1). The peak in activity coincided with the peak in ureide concentration. As reported earlier for soybean nodules [16], the enzyme in the present case was also found to be localised in the microsomal fraction of the nodules (Table 1).

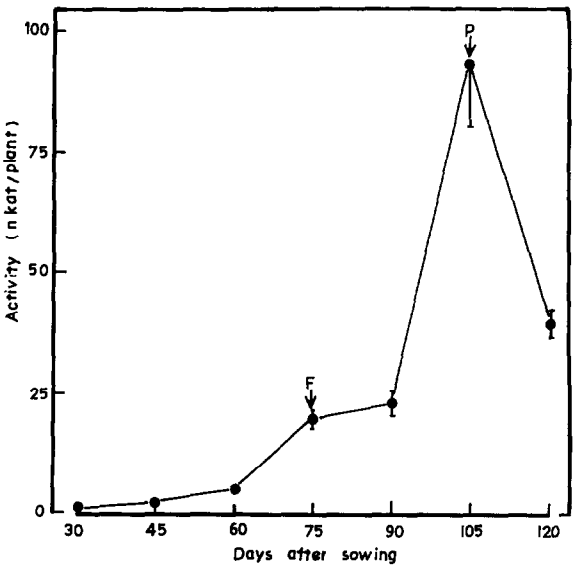

Fig. 1. Allantoinase activity in pigeonpea nodules at different stages of plant growth. F = Flowering, P = pod-filling.

Partial purification

The enzyme allantoinase was purified × 10 with 112% recovery using $(NH_4)_2SO_4$ fractionation and Sephadex G-100 column chromatography. The marginal increase in total activity could be attributed to the removal of a low M_r inhibitory protein after molecular sieving through Sephadex G-100. However, the same could not be proved as the eluate from the Sephadex column failed to inhibit the enzyme activity. The enzyme could not be purified further since it lost activity after elution from a DEAE-cellulose column with Tris–HCl buffer. The soybean shoot enzyme also lost its activity after elution from DEAE-cellulose column [23]. The enzyme was quite stable as it could retain 90% of the original activity after storage at 8° for 3 weeks. From gel permeation data, the estimated M_r was *ca* 125 000. Thomas *et al.* [23] have reported the M_r of allantoinase from soybean leaf to be 50 000.