AMINOACID METABOLISM IN PLANTS:

THE BIOSYNTHESIS OF β-CYANOALANINE AND ASPARAGINE IN LUPINS

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University.

Michael Lever

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1. SUMMARY

β-Cyanoalanine synthase in etiolated seedlings of *Lupinus angustifolius* is found mainly in the mitochondrial fraction of the cotyledons and stems. In seedlings developing at 25 °C in the dark it reaches a maximal concentration after 5 days, at the same time as asparagine accumulation is most rapid and other physiological changes occur. However, maximal ability to assimilate HCN gas to asparagine develops after 3 to 4 days, before asparagine accumulation begins.

A partial purification of lupin β-cyanoalanine synthase is described and its distinction from cysteine synthase confirmed.

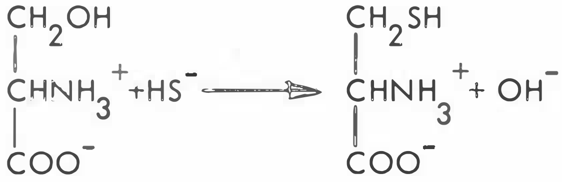
Carbon-14 labelled substrates were supplied to etiolated seedlings of lupin in order to identify precursors of asparagine. Four carbon acids related to the TCA cycle were readily converted to asparagine *in vivo* but not *in vitro*; the carbon skeleton of aspartate is retained in asparagine. Comparison of the distribution of label in the carbon skeletons of aspartate and asparagine from plants supplied carboxyl labelled fumarate shows a separation of the aspartate pool for asparagine biosynthesis from most cell aspartate. Metabolites that could be expected to give rise to cyanide and β-cyanoalanine are relatively ineffective as asparagine precursors.

Thus the β-cyanoalanine pathway is not of major importance in asparagine biosynthesis in lupins.

II. INTRODUCTION

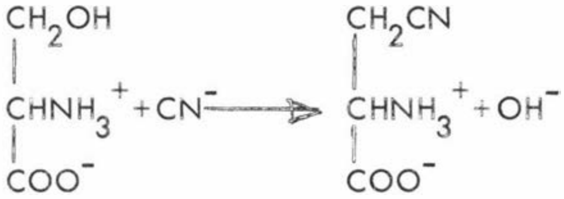
When Blumenthal-Goldschmidt et al. (1963) and Tschiersch (1963) observed that $H^{14}CN$ supplied to higher plants was converted into asparagine by way of β- cyanoalanine, serine providing the other three carbon atoms, the possibility suggested itself that the initial reaction of this pathway was catalysed by a relatively unspecific cysteine synthase, possibly the same as that described by Bruggemann <u>et al</u>. (1962)

(i) cysteine synthase



$$\begin{array}{l}\mathrm{CH_2OH}\\ |\\ \mathrm{CHNH_3}^+\\ |\\ \mathrm{COO^-}\end{array} + \mathrm{HS^-} \longrightarrow \begin{array}{l}\mathrm{CH_2SH}\\ |\\ \mathrm{CHNH_3}^+\\ |\\ \mathrm{COO^-}\end{array} + \mathrm{OH^-}$$

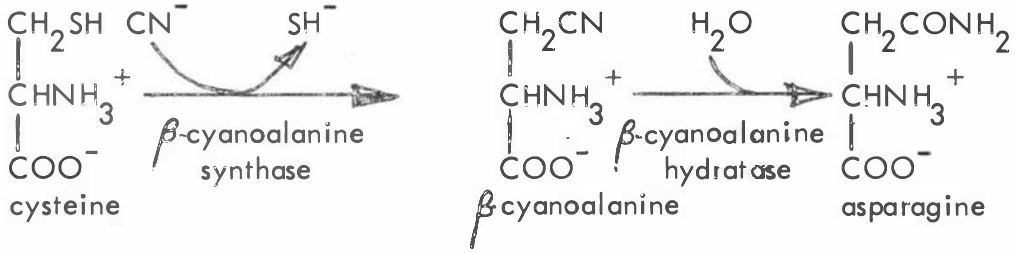
(ii) β-cyanoalanine synthase



$$\begin{array}{l}\mathrm{CH_2OH}\\ |\\ \mathrm{CHNH_3}^+\\ |\\ \mathrm{COO^-}\end{array} + \mathrm{CN^-} \longrightarrow \begin{array}{l}\mathrm{CH_2CN}\\ |\\ \mathrm{CHNH_3}^+\\ |\\ \mathrm{COO^-}\end{array} + \mathrm{OH^-}$$

Alternatively, these observations may be a clue to a solution of the vexed question of asparagine biosynthesis in plants. Described here is work intended to evaluate the metabolic role of these processes.

During the course of this work much relevant information has been published. Floss <u>et.al</u>. (1965) studied cell-free systems containing β-cyanoalanine synthase, and found that cysteine could replace serine as a substrate. Hendrickson and Conn (1969) have recently described a partial purification of β-cyanoalanine synthase from lupins, and they corroborate the evidence described here that the lupin enzyme will utilise cysteine as substrate but not serine. Meanwhile, doubt has been thrown on the significance of the enzyme described by Bruggemann *et al.*, since the metabolically important substrate for cysteine synthase in higher plants now appears to be O-acetyl serine (Giovanelli and Mudd, 1967; Thompson and Moore, 1968). Hendrickson and Conn found that lupin β-cyanoalanine synthase will utilise O-acetyl serine as substrate at 5% of the rate that it will utilise cysteine. In addition an O-acetyl serine dependent cysteine synthase was found in their plant material, localised in a different subcellular fraction. Fowden and Bell (1965) have investigated the distribution of β-cyanoalanine hydratase, which converts β-cyanoalanine to asparagine in legume species, and found that it is absent in those that accumulate β-cyanoalanine and γ-glutamyl-β-cyanoalanine. Thus it is established that a wide variety of higher plants possess two enzymes, β-cyanoalanine synthase and β-cyanoalanine hydratase, that catalyse what will be called in this thesis the "β-cyanoalanine pathway".



This information, in conjunction with the earlier results described here, makes it most improbable that the enzymes of the β-cyanoalanine pathway are involved in cysteine biosynthesis in plants. The emphasis in this work was thus directed more towards elucidating the relationship between cyanide assimilation and asparagine biosynthesis in etiolated *Lupinus angustifolius* seedlings; these plants accumulate up to 30% of their dry weight as asparagine, and also readily assimilate cyanide. The development of β-cyanoalanine synthase activity was studied as a function of plant development, and compared with the development of the ability to assimilate exogenous cyanide and with the accumulation of asparagine. Radio-active substrates were supplied to intact plants to study which were the preferred substrates for asparagine biosynthesis, and to look for possible precursors of cyanide; attempts were made to locate the asparagine synthetase described by Webster and Varner (1955 a & b) but not found by other workers (Meister, 1962; Lees et al., 1968). The results suggest that the major pathway of asparagine synthesis is from four carbon dicarboxylic acids, and not from cyanide; but an isolation of an asparagine synthetase was not achieved.

III. METHODS AND RESULTS