

A CHLOROPLAST-LOCALIZED LYSINE DECARBOXYLASE OF *LUPINUS POLYPHYLLUS*

The first enzyme in the biosynthetic pathway of quinolizidine alkaloids

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1. Introduction

It is well established from *in vivo* tracer experiments that tetracyclic quinolizidine alkaloids, such as sparteine and lupanine, derive from lysine via cadaverine [1,2]. The key enzyme which synthesizes the tetracyclic quinolizidine skeleton from cadaverine in a pyruvate-dependent transamination process, was demonstrated in crude preparations from cell suspension cultures of *Lupinus polyphyllus* [3,4]. This enzyme (17-oxosparteine synthase) was then localized in *L. polyphyllus* leaf chloroplasts which contain the enzymes of the biosynthetic sequence leading from cadaverine via 17-oxosparteine to lupanine, the main alkaloid of this species (fig.1) [5].

Since chloroplasts are the site of lysine formation as well [6,7], the enzymes of lysine biosynthesis and those of the biosynthesis of lysine-derived lupin alkaloids are localized in the same subcellular compartment. The two pathways are linked by lysine decarboxylase which had been sought [8,9], without success, in relation to lupin alkaloid biosynthesis.

Here we describe some properties of the lysine decarboxylase, isolated from *L. polyphyllus* leaf chloroplasts, and discuss some of the implications of the results in respect to lupin alkaloid biosynthesis in chloroplasts.

2. Materials and methods

2.1. Chloroplast isolation and enzyme extraction

Chloroplasts were isolated from leaves of non-flowering *Lupinus polyphyllus* plants grown in a

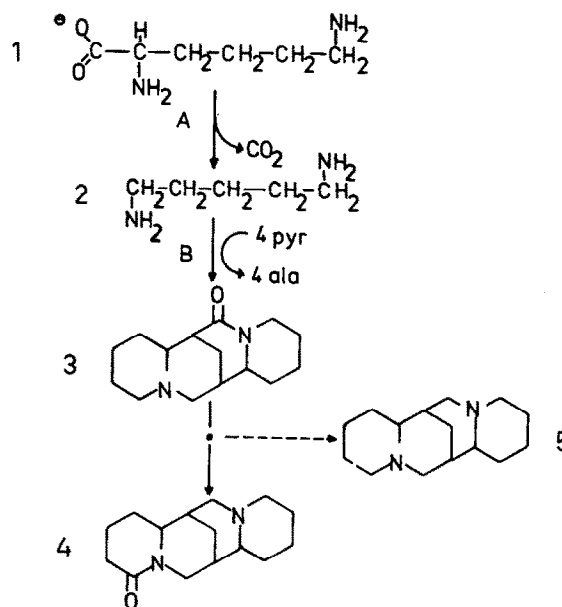


Fig.1. Biosynthetic pathway of lupanine in *Lupinus polyphyllus* chloroplasts: (1) lysine, (2) cadaverine, (3) 17-oxosparteine, (4) lupanine, (5) sparteine; (A) lysine decarboxylase, (B) 17-oxosparteine synthase.

greenhouse. The centrifugation procedure was described in [5]. The isolation buffer had the following composition: 600 mM mannitol, 10 mM pyrophosphate (pH 7.8), 1 mM DTE, 10 mM DIECA. The chloroplasts obtained from 30 g lupin leaves were suspended in hypotonic 0.1 M pyrophosphate buffer (pH 8.0) containing 1 mM DTE and 10 mM DIECA and ground with glass beads (0.1 mm diam.). The resulting homogenate (final vol. 10 ml) was centrifuged at $12\,000 \times g$ for 10 min. The supernatant was employed in the enzyme assays.

Abbreviations: DTE, dithioerythritol; DIECA, diethyldithiocarbamate; PLP, pyridoxal-5'-phosphate

2.2. Assay procedures

Lysine decarboxylase activity was assayed either by measuring the $^{14}\text{CO}_2$ or the $[^{14}\text{C}]$ cadaverine produced from $[^{14}\text{C}]$ lysine. The standard assay mixture (total vol. 1 ml) consisted of the following: enzyme solution (~ 0.25 mg protein); 0.1 M pyrophosphate (pH 8.0); 0.1 mM PLP; 1 mM DTE; 10 mM DIECA; 0.1–2 mM L-lysine containing 1.25–5 μCi L- $[^{14}\text{C}]$ lysine (Amersham/Buchler). The incubations were carried out under N_2 for 5 h at 37°C and continuous shaking. $^{14}\text{CO}_2$ release was measured using Warburg flasks according to [6]. For the cadaverine assays the incubations were performed in 20 ml plastic vials; the reaction was terminated with 0.1 ml 3 N HCl and aliquots with added unlabelled lysine and cadaverine (200 μl) were subjected to thin-layer chromatography on silica plates (Merck 60F-254) using the solvent system 96% ethanol/25% NH_3 (70/35). The respective zones of cadaverine (R_F 0.11) and lysine (R_F 0.38) were localized by radio-scanning or ninhydrin reaction. The cadaverine zone was scraped off the plate, eluted with 4 ml acidified 50% methanol; 2 ml aliquots were added to 15 ml dioxan-based scintillation cocktail and the radioactivity was measured in a Betasint BF-5000 scintillation counter (Berthold/Friesseke).

Chlorophyll was determined according to [10] and protein by the biuret method [11].

2.3. Paper electrophoresis

Cadaverine and lysine were separated by paper electrophoresis (DC-electrophoresis, DESAGA) on Schleicher and Schüll paper 2043a in 0.5 M sodium citrate buffer (pH 3.5). Conditions: 35 V/cm, 30 min. Detection: ninhydrin or radioscanning.

3. Results

3.1. Identification of cadaverine as the product of lysine decarboxylase

When isolated lupin leaf chloroplasts were incubated with $[^{14}\text{C}]$ lysine two major radioactive compounds could be detected by thin-layer chromatography of the reaction mixture (fig.2). The predominant peak (2) was identified as lysine, the second had the same R_F value as cadaverine (1). Its identity with cadaverine was further confirmed: A reaction mixture was alkalized and extracted with ether or methylene chloride. The extracts were dried over anhydrous

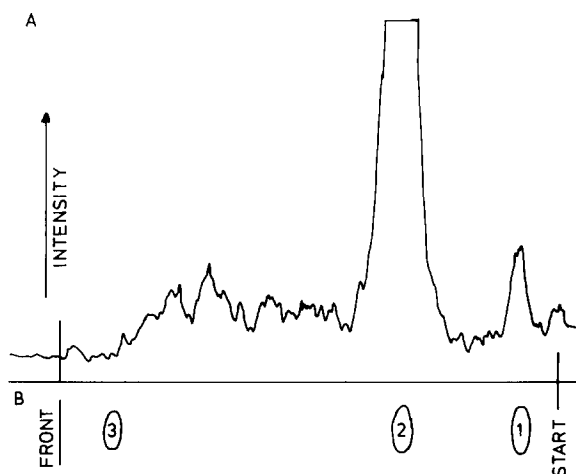


Fig.2. Thin-layer chromatographic identification of $[^{14}\text{C}]$ -cadaverine produced in an incubation mixture of isolated chloroplasts with $[^{14}\text{C}]$ lysine. Incubation was done under standard conditions with 0.08 mM $[^{14}\text{C}]$ lysine (2.5 μCi) for 6 h. (A) Radioactive spots detected by radio-scanning. (B) Co-chromatographed cadaverine (1) and lysine (2), visualized with ninhydrin, and sparteine (3), detected with Dragendorff's reagent.

Na_2SO_4 , evaporated and rechromatographed. The only major radioactive spot was found to be identical with reference cadaverine (R_F value and autoradiography). The spot was eluted, mixed with unlabelled cadaverine and subjected to paper electrophoresis. Radioactivity migrated together with the added cadaverine.

Concomitant determinations of $[^{14}\text{C}]$ cadaverine production and $^{14}\text{CO}_2$ release, revealed a stoichiometric relationship but the $^{14}\text{CO}_2$ values showed greater variations than the respective cadaverine values. Therefore, all subsequent enzymatic assays were performed on the basis of cadaverine determinations.

3.2. Some properties of lysine decarboxylase

About 70–80% of total decarboxylase activity could be solubilized from isolated chloroplasts by mechanical grinding in hypotonic buffers. Addition of detergents like Triton X-100 increased the efficiency of extraction to ~ 85 –90%. For the preparation of soluble enzyme the first method was preferred because less lipids and lipoproteins were solubilized.

Enzyme activity was found to be linear with time for almost 5 h. Employing both orthophosphate and pyrophosphate buffers, lysine decarboxylase was only

Table 1

Effect of various compounds on lysine decarboxylase activity

Assay	Relative activity (%)
Complete	100
-PLP	64
-DTE	62
-DIECA	81
-DTE, DIECA	33
+ 10 mM Semicarbazide	34
+ 10 mM Hydroxylamine	33
+ 10 mM KCN	80
+ 10 mM EDTA	95
+ 2.5 mM CaCl ₂	63
+ 2.5 mM MgCl ₂	110
+ 2.5 mM FeSO ₄	244
+ 2.5 mM FeCl ₃	81
+ 5 mM Sparteine/17-oxosparteine	91

In addition to the standard assay procedure, the enzyme extracts were purified with Amberlite XAD-4 [13] prior to incubation to remove low molecular weight compounds, such as alkaloids and lipids. To test the effects of DTE and DIECA both compounds were also omitted from the isolation buffer. Enzyme activity was determined by the cadaverine assay

active at alkaline pH values with maximal activity at pH 8.0.

Under standard assay conditions an app. K_m value of 0.76 mM was found for lysine, as calculated from Hanes plots [12]; the regression line reads $Y = 0.15 + 0.19(S)$; $r = 0.98$; $P < 0.001$.

The effects of some inhibitors, potential cofactors and protecting agents on lysine decarboxylase activity is summarized in table 1. Like the majority of amino acid decarboxylases, the enzyme is dependent on PLP and thus sensitive to inhibition by carbonyl reagents. The protective influence of DIECA and DTE may be due to the inhibition of phenolases and peroxidases. DIECA inhibits diamine oxidase totally [3]. Maximum activity was obtained in the presence of Fe^{2+} . Addition of alkaloids such as sparteine or 17-oxosparteine were not inhibitory. Furthermore, removal of endogenous alkaloids, which are always present in soluble enzyme preparations, by addition of the polystyrene resin Amberlite XAD-4, revealed no activation of decarboxylase activity.

Under optimized standard assay conditions, which include incubation with DTE, DIECA, PLP, Fe^{2+} and substrate saturation (2 mM L-lysine) spec. act. 1.4 pkat/mg protein was obtained. On the basis of total

chlorophyll content of the chloroplast preparation the activity of the soluble enzyme was 0.9 pkat/mg chl.

4. Discussion

There is only one report of a plant lysine decarboxylase, from *Lathyrus sativus* [4]. This enzyme which decarboxylates both homoarginine and lysine shows similar properties to the lupin chloroplast enzyme in regard to pH optimum, substrate K_m , Fe^{2+} -activation and low activity.

Chloroplast lysine decarboxylase is the first specific enzyme in the biosynthetic pathway of lupin alkaloids and thus a potential site for metabolic regulation. Since lupin alkaloids display no effects on enzyme activity, control of lysine decarboxylase by feedback inhibition, often proposed for plant secondary metabolism [15], seems to be unlikely. It might be speculated, however, that substrate flow into alkaloid synthesis is controlled by the enzyme activity, e.g., the number of lysine decarboxylase molecules present in a chloroplast; for instance the activities of chloroplast localized starch biosynthetic enzymes are ~3 orders higher [16]. The low activity of plant amino acid aldehyde transaminase, an enzyme responsible for the formation of monoamines [17,18] may represent a similar case. In contrast to this one step reaction, however, a coordinate action of several enzymes appears to be a prerequisite for the regulation of an enzymatic multistep sequence such as lupin alkaloid synthesis. This assumption is supported by several lines of evidence in studies employing lupin cell suspension cultures [3,4,19,20] and isolated lupin chloroplasts [5].

The optimal conditions found for chloroplast lysine decarboxylase such as the alkaline pH optimum, the K_m value of the enzyme, its partial solubility and the total enzyme activity per cell resemble the parameters found for 17-oxosparteine synthase, the next enzyme in the biosynthetic sequence [3,4]. Coordinated action of the two enzymes would explain why free cadaverine is almost undetectable in vivo [1]. Upon feeding of [^{14}C]lysine to *L. luteus* and *Sarothamnus scoparius* plants, which convert lysine into quinolizidine alkaloids, free [^{14}C]cadaverine was only detectable when cold cadaverine was added in excess [21]. Thus biosynthesis of lupin alkaloids in chloroplasts appears to proceed via low enzyme activities in a highly coordinated and channelled process.

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