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LYSINE DECARBOXYLASE IN PLANTS AND ITS INTEGRATION IN QUINOLIZIDINE ALKALOID BIOSYNTHESIS

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Key Word Index—Leguminosae, quinolizidine alkaloids, biosynthesis, lysine decarboxylase, occurrence and distribution

Abstract—In leaves of alkaloid producing *Lupinus polyphyllus* lysine decarboxylase activity is positively correlated with the chlorophyll content during leaf regreening A similar positive correlation was found between the leaf alkaloid content and lysine decarboxylase activity in *L albus* and *L luteus* These results indicate that in lupin leaves lysine decarboxylase is an integrated part of the alkaloid specific biosynthetic sequence Lysine decarboxylase could also be demonstrated in 46 alkaloidal and non-alkaloidal species out of 17 families of higher plants, including cell cultures of seven species

INTRODUCTION

Since lysine is incorporated into the quinolizidine alkaloid skeleton via cadaverine [1], it was suggested that a lysine decarboxylase (EC 4 1 1 18) should catalyse the first step of alkaloid biosynthesis [2, 3] although Hasse [4] and Suzuki [5] had searched in vain for this enzyme

Only recently lysine decarboxylase was detected in two plant species *Lathyrus sativus* [6], and in the quinolizidine alkaloid producing *Lupinus polyphyllus* [7] The enzyme from lupin leaves is localized in the chloroplast together with 17-oxosparteine synthase, the key enzyme of the biosynthesis of tetracyclic quinolizidine alkaloids [8, 9] We therefore suggested that lysine decarboxylase of lupin leaves is an integrated part of the biosynthetic sequence of lupin alkaloids [8–11] The picture is further complicated, however, by the fact that lysine decarboxylase activity is also present in organs of *L polyphyllus* which definitely do not produce alkaloids, e g roots [12] In this communication we provide further evidence that lysine decarboxylase of lupin leaves is involved in alkaloid formation In addition the widespread occurrence of lysine decarboxylase in higher plants and plant cell cultures is shown

RESULTS

Correlation of chlorophyll content with lysine decarboxylase activity in leaves

The alkaloid content of cell suspension cultures of *Lupinus polyphyllus* and *Cytisus scoparius* is positively correlated with greening of the cells [13, 14] A similar correlation was established between greening and the enzyme activities involved in quinolizidine alkaloid biosynthesis [Wink, M and Hartmann, T, unpublished]

This phenomenon can also be followed in intact leaves Plants of *Lupinus polyphyllus* were kept in the dark for 10 days and were subsequently re-exposed to natural light The leaves produced in the dark became green within 5 days During this period the reduced level of alkaloids increased concomitantly with chlorophyll formation In addition a significant positive correlation was established between the degree of greening and lysine decarboxylase activity (Fig 1) The correlation between greening (i e chloroplast differentiation) and enzyme activity is in accordance with the fact that quinolizidine alkaloid biosynthesis takes place in the chloroplast [8, 9]

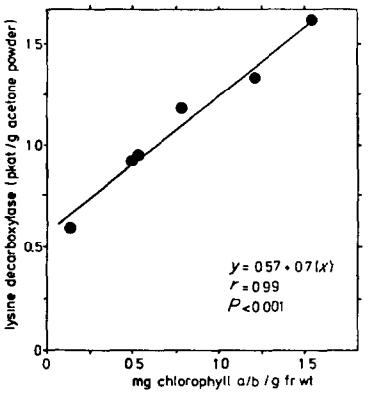

Fig 1 Correlation between chlorophyll content of greening leaves and lysine decarboxylase activity, measured in crude acetone powder preparations of *L polyphyllus* leaflets

Correlation of enzyme activity with alkaloid formation

Some lupin species are of agricultural importance Field populations of *L luteus* and *L albus* near Braunschweig display great variations in the alkaloid content ranging from high alkaloid specimens 'bitter lupins' to specimens with low alkaloid content 'sweet lupins' Thus the population offered an excellent opportunity to study the correlation between alkaloid content and the activity of the alkaloid-specific enzymes

The activities of lysine decarboxylase and 17-oxosparteine synthase in crude enzyme preparations of leaflets were determined and related to the respective alkaloid

content The results are summarized in Fig 2(A–D) In both preparations derived from acetone powders and isolated chloroplasts, a significant positive correlation could be established between enzyme activity and leaf alkaloid content This means that the low alkaloid accumulation in 'sweet lupins' may be caused by low enzyme activities and thus a reduced rate of alkaloid synthesis A similar conclusion has been reached by Nowacki [15] who studied the genetics of 'sweet lupins' Low alkaloid accumulation was explained as a genetic block in enzyme synthesis

Distribution of lysine decarboxylase in a legume

In *Lupinus polyphyllus* 17-oxosparteine synthase is restricted to the leaves, whereas lysine decarboxylase is present in the leaves but also in all the other organs, such as stems, roots and pods [12] In this study we have analysed lysine decarboxylase activity in two other alkaloid-producing legumes and one non-alkaloidal plant (Table 1) Lysine decarboxylase was found in all plant parts studied and reached relatively high values in leaves and roots, irrespective of alkaloid formation in the plant

Table 1 Intraspecific distribution of lysine decarboxylase in *Lupinus luteus*, *Baptisia australis* and *Phaseolus vulgaris*

Species	Decarboxylase activity (pkat/g acetone powder)				
	Leaflets	Stems	Roots	Pods	Flowers
Lupinus luteus	10	05	06	06	17
Baptisia australis	24	38	21	10	nd
Phaseolus vulgaris	11	08	14	nd	04

n d , Not determined

Enzymic activity was assayed in acetone powder extracts following the formation of [^{14}C]cadaverine from [^{14}C]L-lysine under standard conditions

Distribution of lysine decarboxylase in higher plants

Having found lysine decarboxylase in plant parts which are not involved in quinolizidine alkaloid biosynthesis [12], we decided to screen lysine decarboxylase activity in leaves of legume species which produce quinolizidine alkaloids in comparison to non-alkaloidal legumes and

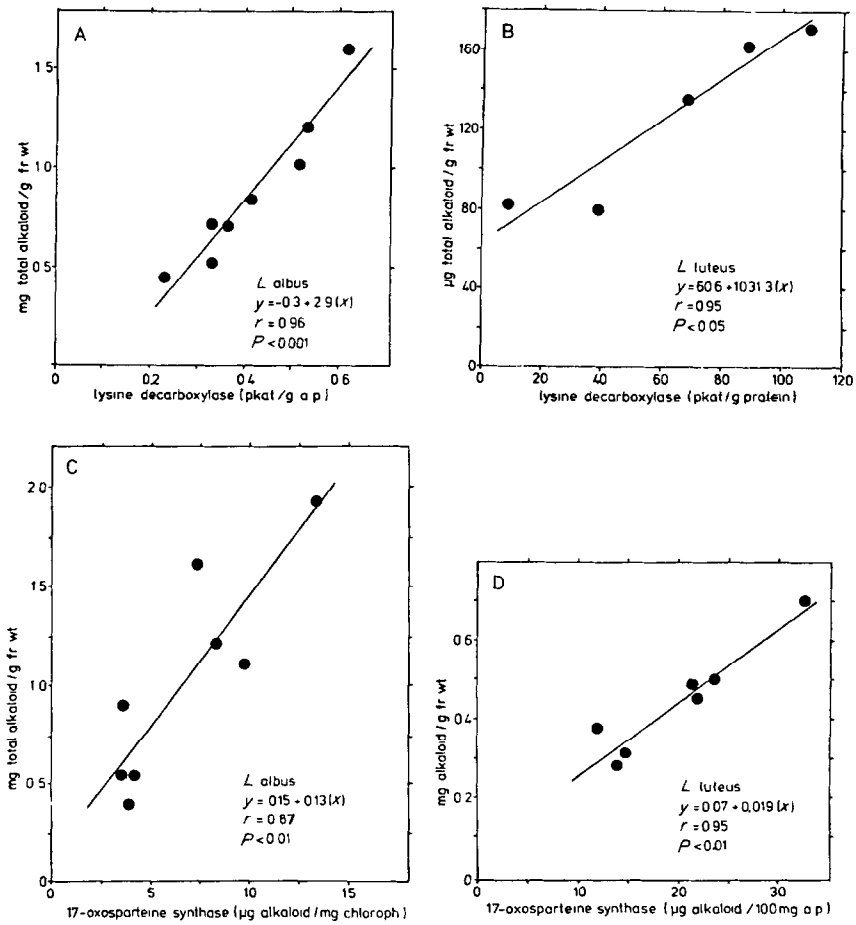

Fig 2 Correlation between enzyme activity and alkaloid content of *Lupinus* ssp leaflets A, Lysine decarboxylase activity in acetone powder preparations from eight *L albus* plants (field population) B, Lysine decarboxylase activity in chloroplast isolations of five *L luteus* plants (greenhouse population) C, 17-Oxosparteine synthase activity in chloroplast isolation of eight *L albus* plants (field population) D, 17-Oxosparteine synthase activity in acetone powder preparations of seven *L luteus* plants (field population) a p , Acetone powder

species of other families As expected, lysine decarboxylase activity could be demonstrated in the leaves of all 18 species studied which produce quinolizidine alkaloids (Table 2A) Surprisingly lysine decarboxylase activity was also detectable in crude leaf extracts of 11 legume species which do not produce quinolizidine alkaloids (Table 2B), and in 17 species from 16 other families (Table 2C)

In addition cell cultures were included in the screening (Table 2D) In cell suspension cultures of *Lupinus polyphyllus* and *Cytisus scoparius*, which accumulate relatively small amounts of alkaloids [14], lysine decarboxylase was detectable and reached activities which are similar to those of the respective plants producing alkaloids in high yield Lysine decarboxylase activity could also be demonstrated in cell suspension and callus cultures of five other plant species which do not produce quinolizidine alkaloids

Evaluating the data summarized in Table 2 we have to consider that the values were obtained from an enzyme of comparably low activity and were determined in crude enzyme preparations Therefore the data have to be interpreted with some caution as far as the absolute figures are concerned Since in each case the lysine decarboxylation was followed by determination of the cadaverine produced, the assay is highly specific Interference by diamine oxidase (EC 1 4 3 6), which is present in most legume species, could be excluded since the respective reaction product 2-carboxy-Δ1-piperideine behaves differently in the TLC system used Furthermore diethyldithiocarbamate, which inhibits legume diamine oxidase completely [24] but does not influence lysine decarboxylase activity, was included in many enzyme assays

DISCUSSION

In the leaves of quinolizidine alkaloid-producing legumes, where alkaloid formation is localized in the chloroplast [8–12], lysine decarboxylase activity is closely correlated with both alkaloid accumulation and leaf greening, i e chloroplast development Furthermore, the activity of lysine decarboxylase in legume leaves is of the same order of magnitude as that of 17-oxosparteine synthase [9], the key enzyme of quinolizidine alkaloid biosynthesis, which shows the same correlation as given for the decarboxylase This strongly supports the view that lysine decarboxylase activity in leaves of quinolizidine alkaloid producing legumes is an integrated part of the alkaloid-specific pathway

Although the physiological significance of lysine decarboxylase in the leaves of alkaloid-producing legumes is quite obvious, the question of its function remains for the non-alkaloid-producing organs and the various other species The results presented here suggest that lysine decarboxylase activity occurs universally in higher plants In many respects this situation resembles that of L-alanine aldehyde aminotransferase, an enzyme which catalyses the formation of aliphatic amines, [16–19] and which is specifically involved in the biosynthesis of hemlock alkaloids [20, 21] Alanine aldehyde aminotransferase occurs ubiquitously in higher plants irrespective of its function in amine formation [17] The enzyme isolated from amine-containing and amine-free plants has identical kinetic properties and is localized in the mitochondria [19] In *Conium maculatum* the transaminase catalyses the formation of γ-coniceine, a specific intermediate in the hemlock alkaloid biosynthesis In this plant two isoenzymes of the transaminase exist, one localized in mitochondria and the other in the chloroplasts It was assumed that the chloroplast enzyme is the alkaloid-specific transaminase [22]

Like the alanine aldehyde aminotransferase, lysine decarboxylase is present in plant tissues at very low levels, up to three orders of magnitude lower than those of enzymes of primary metabolism, such as diaminopimelate decarboxylase, the final enzyme in chloroplast-localized lysine biosynthesis [9] At present we cannot exclude the possibility that the chloroplast enzyme differs from the enzyme from roots or from non-alkaloidal species Therefore further investigations are needed in order to understand the role of lysine decarboxylase in higher plants

Concerning alanine aldehyde aminotransferase it has been suggested that the enzyme is expressed at very low levels even in the absence of any metabolic function (see ref [19] for detailed discussion) Such 'sleeping enzyme activities' may be one basis for the evolution of specific pathways of secondary metabolism The integration of ubiquitous lysine decarboxylase into the biosynthetic pathway of quinolizidine alkaloids may be another example supporting this concept

EXPERIMENTAL

Plant material Most plants were taken from their natural habitats and from agricultural areas (*Lupinus albus, L luteus*) in the vicinity of Braunschweig, or were grown in the Botanical Garden (Braunschweig) Leaf samples for the preparation of Me_2CO powders or isolation of chloroplasts were collected from flowering specimens

Plant cell cultures Cell cultures were kept at 25° and 70% r h under continuous illumination Cell suspension cultures of *Lupinus polyphyllus, Conium maculatum, Symphytum officinale* and *Cytisus scoparius* were isolated in our laboratory and kept according to refs [13, 23] Cell cultures of *Chenopodium rubrum* were originally obtained from Dr Harms (FAL Braunschweig), cultures of *Atropa belladonna* from Professor Neumann (Gießen) and cultures of *Ruta graveolens* from Professor Czygan (Wurzburg)

Isolation of chloroplasts According to ref [8] Prior to chloroplast isolation, leaflets of *Lupinus* were stored at 4° in the dark for 3 hr *Ca* 20 g of leaflets were cut into 200 ml of ice-cold isolation buffer (0 6 M D-mannitol, 10 mM diethyldithiocarbamate, 1 mM DTE, 10 mM pyrophosphate, pH 7 8) and homogenized in a Waring blendor The resulting brei was filtered through two layers of cotton wool and gauze The cleared homogenate was centrifuged at 3000 *g* for 10 min The resulting pellet was suspended in isolation buffer and re-centrifuged at 3000 *g* for 10 min The pellet was suspended in 20 ml hypotonic NaPi buffer (0 1 M, pH 7 9) and used in the enzyme assays

Assays for enzyme activity Lysine decarboxylase activity was prepared and assayed according to ref [7], 100 mg Me_2CO powder was dissolved in 4 ml 0 1 M pyrophosphate buffer (pH 8) containing 3 mM DTE and a drop of Triton X-100 After stirring for 15 min in an ice-bath, the soln was centrifuged at 12 000 *g* for 10 min The supernatant (1 ml) was supplemented with 2 5 mM $FeSO_4$, 0 1 mM pyridoxal-5'-phosphate and 100 μM [U-^{14}C] L-lysine (1 25 μCi) (total vol 1 2 ml) The soln was incubated at 37° and the reaction terminated after 5 hr by adding 100 μl 3 M HCl Aliquots of 200 μl were separated on TLC plates (Si gel 60F-254, Merck) using the solvent mixture 96% EtOH–25% NH_3 (70 35) Lysine and cadaverine were detected with ninhydrin The cadaverine zone was scraped off the plate, eluted with 4 ml 50% MeOH containing one drop of 3 M HCl, 2 ml aliquots were

Table 2 Distribution of lysine decarboxylase in plant leaves and tissue cultures

Species		Lysine decarboxylase activity (pkat/g acetone powder	Total leaf alkaloid content (μg/g fr wt)
(A) Quinolizidine-producing Leguminosae			
Baptisia australis		24	115
Cytisus beanii		10	55
canariensis		11	400
purpureus		12	30
scoparius		09	100
Genista anglica		05	20
hispanica		12	10
lydia		04	600
pilosa		04	10
sagittalis		07	500
tinctoria		04	30
Laburnum alpinum		08	100
anagyroides		07	10
Lupinus albus		04	900
luteus		12	500
polyphyllus		08	250
Sophora japonica		12	30
tetraptera		11	1700
(B) Quinolizidine-free Leguminosae			
Astragalus cicer		05	—
glycyphyllus		05	—
Galega officinalis		11	—
Glycine max		08	—
Glycyrrhiza echinata		08	—
Medicago sativa		07	—
Melilotus albus		05	—
Phaseolus vulgaris		11	—
Pisum sativum		13	—
Robinia pseudoacacia		06	—
Vicia faba		05	—
(C) Non-Leguminosae			
Levisticum officinale	Apiaceae	17	—
Arum maculatum	Araceae	04	—
Calla palustris	Araceae	71	—
Asarum europaeum	Aristolochiaceae	48	—
Senecio fuchsii	Asteraceae	06	—
Saponaria officinalis	Caryophyllaceae	05	—
Anchusa italica	Boraginaceae	16	—
Spinacea oleracea	Chenopodiaceae	04	—
Sedum acre	Crassulaceae	13	—
Menyanthes trifoliata	Gentianaceae	111	—
Mentha rotundifolia	Lamiaceae	23	—
Malva silvestris	Malvaceae	11	—
Trollius europaeus	Ranunculaceae	22	—
Sanguisorba officinalis	Rosaceae	16	—
Dictamnus albus	Rutaceae	15	—
Nicotiana tabacum	Solanaceae	10	—
Valeriana sambucifolia	Valerianaceae	136	—
(D) Cell cultures			
Suspension cultures			
Conium maculatum	Apiaceae	04	—
Lupinus polyphyllus	Leguminosae	16	—
Cytisus scoparius	Leguminosae	08	—
Symphytum officinale	Boraginaceae	08	—
Chenopodium rubrum	Chenopodiaceae	09	—
Callus cultures			
Atropa belladonna	Solanaceae	08	—
Ruta graveolens	Rutaceae	12	—

Enzymic activity was assayed in acetone powder extracts following the formation of [^{14}C]cadaverine from [^{14}C]L-lysine under standard conditions

added to a dioxan-scintillation cocktail and analysed in a BF-5000 liquid scintillation counter (Berthold–Friesecke)

17-Oxosparteine synthase activity was prepared and assayed according to ref [24], 20 mg Me_2CO powder was dissolved in 4 ml 0 1 M NaPi buffer (pH 8) The assay contained 1 mM DTE, 10 mM diethyldithiocarbamate, 500 μM pyridoxal-5'-phosphate, 10 mM pyruvate and 10 mM cadaverine The soln was incubated anaerobically in the dark at 30° The reaction was terminated by adding 1 ml 3 M trichloroacetic acid After centrifugation (10 min at 15000 g) an aliquot of the supernatant was used for photometric alkaloid determination Appropriate blanks without added cadaverine and pyruvate were used as controls

Alkaloid extraction and determination *Ca* 3 g of plant material was homogenized in 40 ml 0 5 M HCl (Waring blendor) and left standing at room temp for 1 hr The homogenate was centrifuged at 17000 g for 10 min To 5 ml of the supernatant 1 ml 3 M trichloracetic acid was added and the ppt was removed by centrifugation Aliquots of 2 ml clear supernatant were mixed with 50 μl KI_3 soln and measured photometrically at 830 nm [12] Sparteine (Roth, Karlsruhe) was used for calibration

Chlorophyll Determined according to Arnon [25] *Protein* according to the Biuret method

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