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Cadaverine formation by specific lysine decarboxylation in *Pisum sativum* seedlings *

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Abstract

Cadaverine was found to be formed in Pisum sativum seedlings via a specific lysine decarboxylation pathway as revealed by specific inhibitor studies. Lysine decarboxylation activity was recorded in the meristems and non-meristematic tissue of the shoots and the roots. In the shoot elongation zone, the specific activity was double that in the other tissues and cadaverine level was 90-fold higher. The results presented in this study suggest possible regulatory control by polyamines of lysine decarboxylase activity in Pisum sativum seedlings.

Abbreviations: LDC, lysine decarboxylase; α-DFMO, DL-α-difluoromethyl ornithine; α-DFMA, DL-α-difluoromethyl arginine; PLP, pyridoxal phosphate; DTT, dithiothreitol.

Introduction

Increasing attention has been given to polyamines and their biosynthetic enzymes because of their involvement in the regulation of growth and cell division in almost every biological system studied so far (Galston 1983, Tabor and Tabor 1984). Recently, evidence has been presented indicating that requirements for putrescine, spermidine and spermine, usually found in eukaryotes, can apparently be fulfilled at least for a short period by closely related amines, such as cadaverine or aminopropyl cadaverine (Alhonen-Hongisto and Janne 1980). Unlike for the other polyamines, little research has been done concerning cadaverine biosynthesis in higher organisms. In bacteria, cadaverine arises from lysine via lysine decarboxylase activity through a pathway different from that of putrescine biosynthesis (Goldenberg 1980, Wertheimer and Leifer 1983). In animal tissues, evidence was presented indicating that lysine decarboxylation is catalyzed by ornithine decarboxylase (Pegg and McGill 1979, Persson 1977). However, Alhonen-Hongisto and Janne (1980) claimed that this is not the case, since cadaverine was formed in the presence of DL-α-difluoromethyl ornithine, a specific inhibitor for ornithine decarboxylase. In plants, Ramakrishna and Adiga (1976) have demonstrated that homoarginine and lysine decarboxylating activities in Lathyrus sativus are associated with the same protein fraction. Hasse et al. (1967) demonstrated lysine decarboxylation and cadaverine formation in pea seedlings, but no information regarding the nature of this reaction was provided. The level of cadaverine in seedlings of various plant species has been determined (Lin 1984, Villanueva et al. 1978). However, its distribution in meristematic and non-meristematic tissues was not studied.

In this communication we present evidence that in etiolated pea seedlings, lysine is decarboxylated specifically to produce cadaverine, and that cadaverine is accumulated in the shoot subapical non-meristematic tissue.

Materials and Methods

Pea seedlings (Pisum sativum L. var. 'Kelvedon Wonder') were grown for six days in the dark in moist vermiculite. The apical or subapical sections (Apelbaum and Burg 1972) were excised and ground in 3 volumes of extraction buffer containing 50 mM Tris HCl pH 8.0, 0.5 mM EDTA and 5 mM DTT, centrifuged for 15 min at 5,000xg at 4°C and the supernatant fraction was used for the enzyme assay. Lysine decarboxylating activity was assayed by measuring $^{14}CO_2$ liberated from U-[^{14}C] lysine. The reaction mixture contained: 10 mM Tris-HCl, pH 8.0, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PLP, 5 mM lysine, 0.2 μCi L-[^{14}C(U)] lysine (sp. act. 317 mCi/mmole), and enzyme (0.1-0.5 mg prot.) in a total volume of 250 μl. The reaction was carried out in a shaking water bath for 1 h at 45°C, and terminated by the addition of 0.2 ml of 6 N H_2SO_4, $^{14}CO_2$ was trapped and determined as described by Icekson et al. (1985).

In some cases, at the end of the incubation period, the polyamines were extracted from the reaction mixture, dansylated, chromatographed on a silica gel-coated plate and the labeled amine product was identified by autoradiography.

Polyamines were determined following the danysla-tion procedure of Dai et al. (1982). The dansylated polyamines were measured in an Aminco-Bowman spectrofluorimeter, with an excitation wavelength at 350 nm and emission at 495 nm.

The data presented are from single experiments representative of three or four experiments. Each measurement was done in duplicate.

Results and Discussion

Lysine decarboxylase (L-lysine carboxy-lyase, EC.4.1.1.18) activity in extracts from etiolated

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P. sativum seedlings was found to be linear with time up to 90 min and with a protein concentration up to 0.5 mg in the apical meristematic tissue and up to 0.2 mg in the subapical non-meristematic tissue (not shown). The optimal conditions for the enzyme activity were found to be pH 8.0 and 45°C. The enzyme follows Michaelis-Menten kinetics; the Km^{app} value for lysine, calculated from a Lineweaver-Burk plot (not shown), was approximately 20 mM, and the V^{app}_{max} obtained was 519 nmole·mg $prot^{-1}·h^{-1}$. Hence, lysine decarboxylase from P. sativum seedlings showed characteristics different from those described in other plants and in terms of catalytic efficiency it appears to be more active, by several orders of magnitude, than that from other sources (Ramakrishna and Adiga 1978, Suresh and Adiga 1977). Lysine decarboxylating activity from P. sativum showed no dependency on added metal ion; Mg^{2+} and Mn^{2+} had no effect on the enzyme activity, while Fe^{2+} reduced it by 40%. Only Ca^{2+} had a slight (15-20%) stimulatory effect (not shown). These results contradict those reported for the enzymes from L. sativus and L. polyphyllus, which exhibit an absolute requirement for Fe^{2+} or Mn^{2+} (Ramakrishna and Adiga 1976, Hartmann et al. 1980).

Table 1 Effect of putative substrates and specific inhibitors on $^{14}CO_2$ released from [^{14}C]-labeled lysine

Addition (mM)		$^{14}CO_2$ released from ^{14}C-lysine	
		DPM / mg prot·h	% of control
none		7615	100
L-homoarginine	(20)	7460	98
L-ornithine		8790	115
L-lysine	(20)	4070	53
α-DFMO	(10)	9080	119
α-DFMA	(10)	8145	106

The standard assay mix contained 5 mM lysine. Putative substrates were added to the reaction mixture before the incubation. The specific inhibitors were preincubated for 15 min with the enzyme prior to the addition of the substrate.

To ensure that the $^{14}CO_2$ evolution from lysine was not due to a non-specific peroxidase activity (Hartmann et al. 1980), a crude extract from the subapical region of the etiolated seedlings was incubated with ^{14}[C]lysine. This assay produced in a stoichiometric fashion labeled compounds that, upon dansylation, co-chromatographed with mono- or bidansyl cadaverine (not shown).

In order to study substrate specificity, the enzyme was incubated with various putative substrates, and their capacity to serve as substrates, as can be inferred by the reduction in $^{14}CO_2$ release from [^{14}C]-lysine, was determined. As seen in Table 1, lysine was the only substrate for the detected decarboxylation activity. Ornithine and homoarginine did not compete with lysine as substrates.

The specific inhibitors DL-α-difluoromethyl arginine (α-DFMA) and DL-α-difluoromethyl ornithine (α-DFMO) for arginine and ornithine decarboxylases, respectively (Kallio et al. 1980), had no inhibitory effect on lysine decarboxylase from P. sativum. This observation rules out any possible contribution of arginine decarboxylase or ornithine decarboxylase activity to the decarboxylation observed.

Our results, showing that the formation of cadaverine from lysine in P. sativum is catalyzed solely by lysine decarboxylase, are compatible with those of Alhonen-Hongisto and Janne (1980), who studied cadaverine formation in Ehrlich ascites carcinoma cells.

All the polyamines tested elicited a concentration-dependent suppression effect on P. sativum lysine decarboxylase activity (Table 2). However, no clear trend as to their inhibitory potency is apparent: at 0.1 mM, all the polyamines had little inhibitory effect; at 1 mM, putrescine and spermine were the most potent inhibitors. At 10 mM, spermidine and the reaction end-product, cadaverine, were more potent inhibitors than the other polyamines. The inhibition of P. sativum LDC activity by polyamines may indicate a possible control mechanism by endogenous polyamine. This notion gains more support from the findings that LDC activity is enhanced in bacteria (Wertheimer and Leifer 1983) or mammalian polyamine-depleted cells (Alhonen-Hongisto and Janne 1980).

Table 2 Effect of various polyamines added to the assay mixture on LDC activity

Polyamine added	(mM)	LDC activity	
		nmole CO_2 / mg prot·h	% of control
none		141	100
putrescine	(0.1)	98	70
	(1.0)	48	34
	(10.0)	34	24
spermidine	(0.1)	100	71
	(1.0)	76	54
	(10.0)	13	9
spermine	(0.1)	86	61
	(1.0)	43	30
	(10.0)	28	20
cadaverine	(0.1)	95	67
	(1.0)	87	60
	(10.0)	16	12

Table 3 depicts the distribution of LDC specific activity and cadaverine level in the various parts of P. sativum seedlings. It appears that this activity is found in the meristematic and non-meristematic tissue of the shoot and the root. However, the specific activity of LDC in the shoot subapex was found to be twice as high as in the other parts of the seedlings analyzed. Specific activity of lysine decarboxylase in the apical meristem of light-grown P. sativum seedlings was 66 nmole CO_2·mg $prot^{-1}·h^{-1}$ (not shown), which is similar to that found in the apical meristem of etiolated seedlings.

Table 3 Distribution of LDC activity and cadaverine levels in different parts of the etiolated pea seedlings

	LDC activity	Cadaverine
	nmole CO_2 / mg prot.h	nmole/g f.wt
Shoot apex	75	7
Shoot subapex	164	620
Root apex	70	-
Root subapex	72	-

A most striking finding is the accumulation of cadaverine in the shoot subapical non-meristematic tissue, which is about 90-fold higher than in the apical meristem (Table 3). In Ehrlich ascites carcinoma cells it was shown that cadaverine accumulates when polyamine biosynthesis is blocked (Alhonen-Hongisto and Janne 1980), whereas in bacteria cells cadaverine was found to fulfill the polyamine requirement during a temporary polyamine shortage. Whether cadaverine has a particular and specific role in the growth and development processes in P. sativum or other plants is not known. Although no cell division takes place in the subapical region of etiolated P. sativum, DNA synthesis is prevalent (Apelbaum and Burg 1972), and cell expansion, xylogenesis and fiber lignification take place in this region (Apelbaum et al. 1972). The fact that cadaverine accumulates in the subapical region of the seedlings may suggest the possible involvement of this diamine in the indicated growth and development processes.

The properties hitherto described for lysine decarboxylase activity and the accumulation of cadaverine in the subapical tissue of etiolated P. sativum seedlings warrant further studies in order to understand the role of cadaverine and other polyamines in the different physiological processes in plant tissues.

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