**Communication**

**Inhibition by Ethylene of Polyamine Biosynthetic Enzymes Enhanced Lysine Decarboxylase Activity and Cadaverine Accumulation in Pea Seedlings**

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**ABSTRACT**

Exposing etiolated pea seedlings to ethylene which inhibited the activity of arginine decarboxylase and S-adenosylmethionine decarboxylase caused an increase in the level of cadaverine. The elevated level of cadaverine resulted from an increase in lysine decarboxylase activity in the tissue exposed to ethylene. The hormone did not affect the apparent $K_m$ of the enzyme, but the apparent $V_{max}$ was increased by 96%. While lysine decarboxylase activity in the ethylene-treated plants increased in both the meristematic and the elongation zone tissue, cadaverine accumulation was observed in the latter only. The enhancement by ethylene of the enzyme activity was reversed completely 24 hours after transferring the plants to an ethylene-free atmosphere. It is postulated that the increase in lysine decarboxylase activity, and the consequent accumulation of cadaverine in ethylene-treated plants, is of a compensatory nature as a response to the inhibition of arginine and S-adenosylmethionine decarboxylase activity provoked by ethylene.

Increasing evidence accumulated in recent years suggests that the naturally occurring polyamines, putrescine, spermidine, and spermine, act as modulators of some cellular and physiological processes during plant growth and development (11). Consequently, changes in the level of these polyamines and in the activity of their biosynthetic enzymes have been studied in a variety of plant species at different stages of development (11, 17). Palavan et al. (15) showed that ethephon inhibited the activity of ADC and ODC in pea terminal buds.

We have recently shown (4-6) that ethylene which is known to influence many aspects of plant growth and development (1) inhibited the activity of ADC (7) and SAMDC (13), thereby reducing the rates of formation of the subsequent polyamines. The requirement for putrescine, spermidine, and spermine usually found in eukaryotes can apparently be fulfilled, at least for a short period, by closely related amines like cadaverine or aminopropyl cadaverine (2, 3). In a recent study we have shown (8) that cadaverine was formed in *Pisum sativum* seedlings via a specific LDC activity. In addition, we found a higher level of cadaverine in the nonmeristematic subapical tissue of pea seedlings (8) where cell elongation, differentiation, and active DNA synthesis take place (4, 5).

Because polyamines are required for various plant cellular processes (11), a study was undertaken to follow cadaverine formation in pea seedlings where ADC and SAMDC activity were inhibited by ethylene treatment.

In this communication, we describe a stimulatory effect of ethylene on LDC activity in pea seedlings and accumulation of cadaverine in the elongation zone of the plant.

**MATERIALS AND METHODS**

Pea seeds (*Pisum sativum* L. var 'Kelvedon Wonder') were soaked in water for 6 h and sown in moist vermiculite. Seedlings were grown in the dark at 22°C and 80% RH for 3 to 6 d before use. For ethylene treatment, potted seedlings were placed in 10-L desiccators constantly ventilated with ethylene (50 μL/L) at a flow rate of 100 ml/min and an RH of about 80%.

**Enzyme Extraction.** The tissue for the enzyme extraction was excised from etiolated pea seedlings. The apical meristem in the hook region or the nonmeristematic tissue from the subhook zone were excised and ground in an ice-cold mortar and pestle in 3 volumes of extraction buffer containing 50 mm Tris HCl (pH 8.0), 0.5 mM EDTA, and 5 mM DTT. The homogenate was centrifuged for 15 min at 5,000g at 4°C and the supernatant fraction was used for the enzyme assay and protein determination.

**Assay of LDC Activity.** Lysine decarboxylating activity was assayed by measuring released $^{14}$CO$_2$ 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-[U-$^{14}$C]lysine (317 mCi/mmol), and appropriate amounts of enzyme protein (100–500 μg) in a total volume of 250 μl. The incubation was carried out in test tubes capped with rubber caps fitted with polypropylene center wells containing a paper wick soaked with 0.2 ml of soluene 350 (Packard Inc.). The reaction was initiated by the addition of substrate and carried out in a shaking water bath for 1 h at 45°C. The reaction was terminated by the addition of 0.2 ml of 6 N H$_2$SO$_4$ and incubated for an additional 30 min to release all $^{14}$CO$_2$ from the reaction mixture. The center wells containing the paper wick were transferred to plastic scintillation vials containing 4.5 ml of Aqualuma Plus scintillation liquid (Lumac B.V., Holland), and counted in a Kontron liquid scintillation counter. Blank values were obtained.

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2 Abbreviations: ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; LDC, lysine decarboxylase; PLP, pyridoxal phosphate.
by using boiled or acid-treated extracts. These two nonenzymic controls were always included in the experiments and their values subtracted from those obtained from the enzymic reaction. Activity is expressed as nmol CO$_2$/mg protein·h.

**Measurement of Polyamines.** The tissue was extracted in 5% cold HClO$_4$, 100 mg/ml. The homogenates were kept in the cold for 1 h and centrifuged for 20 min at 4°C. The supernatant was used for polyamine determination following the dansylation procedure of Seiler and Weichmann (16). The dansylated products were separated on thin-layer plates (Whatman, LKGD) as described by Dai et al. (10). The dansylated polyamines were measured in an Amino-Bowman spectrofluorimeter, with an excitation wavelength at 350 nm and emission at 495 nm.

Protein content in the crude enzyme was estimated by the method of Bradford (9) using BSA as a standard.

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

**RESULTS AND DISCUSSION**

Upon exposure to ethylene, etiolated *Pisum sativum* seedlings, like other dicotyledonous plants, displayed growth inhibition which resulted from inhibition of DNA synthesis, cell division, and cell elongation (4, 5). In addition, inhibition of the activity of ADC and SAMDC, two major enzymes in the polyamine biosynthetic pathway, was observed (7, 13). However, as can be seen in Table I, the activity of lysine decarboxylase, which gives rise to cadaverine formation (8), was rather increased by the ethylene treatment. Exposure for 18 h to 50 μL/L ethylene resulted in a 63% increase in LDC activity in the apical portion of the seedling, where cell division takes place (4). This increase in LDC activity was more pronounced in the subapical region, where only cell elongation occurs; a 3-fold increase over the control.

It appears that the ethylene treatment altered the kinetic parameters of LDC. The apparent $K_m$ value determined from a Line-weaver-Burk plot (Fig. 1) for the ethylene-treated enzyme was 25 mM, which is similar to that of control (22.5 mM). However, the apparent $V_{max}$ of the ethylene-treated enzyme was elevated and almost doubled: 1017 nmol CO$_2$/mg protein·h, as compared with 519 nmol CO$_2$/mg protein·h in the control enzyme. Premixing of the extracts from control and ethylene-treated plants resulted in merely additive results. Hence, ruling out the possibility that the stimulation by ethylene of LDC activity in etiolated pea seedlings was a consequence of the appearance of a free activator.

Ethylene must be continuously present in the plant's environment in order to maintain elevated LDC activity in etiolated pea seedlings. Upon transferring the ethylene-treated seedlings to an ethylene-free atmosphere, LDC activity was reduced. Complete restoration from the ethylene-induced enzyme activity was observed within 24 h, attaining a level similar to that of control (data not shown).

As cadaverine was found to be a product of a specific lysine decarboxylation in etiolated pea seedlings (8), the level of this diamine in ethylene-treated seedlings was examined. A 40% increase in the level of cadaverine was recorded in the apical subapical region (Table I). However, considerable accumulation of cadaverine was recorded in the elongation zone of ethylene-treated plants, reaching a 5-fold increase over control within 18 h of the treatment (Table I). Hence, it appears that in nonmeristematic tissue following ethylene treatment an increased LDC activity is associated with a marked enhancement in the production of cadaverine.

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 the etiolated seedlings, DNA synthesis is prevalent (4), and cell expansion, xylogenesis, and fiber lignification occur (6). The fact that cadaverine accumulates in the subapical region of the seedlings could suggest a possible involvement of this diamine in the indicated growth and development processes. It was suggested (14) that there are two main paths for utilization of lysine: one for cadaverine synthesis and the other for newly synthesized proteins. When polyamines are present in normal levels, lysine is channeled into the synthesis of proteins, whereas under polyamine starvation some of the lysine might be decarboxylated to yield cadaverine. In Ehrlich ascites carcinoma cells, it was shown that cadaverine accumulates when polyamine biosynthesis is blocked (2, 3), whereas in bacteria cells cadaverine

![Fig. 1. Line-weaver-Burk plot of lysine decarboxylase activity from *P. sativum* seedlings. The subapical region from control (——) or ethylene-treated (-----) seedlings was excised and the enzyme activity was determined as described in "Materials and Methods."](https://www.plantphysiol.org/Content/18661183)}
was found to fulfill the polyamine requirement during a temporary polyamine shortage (12).

Ethylene was found markedly to reduce ADC (7) and SAMDC (13) activity, thereby restricting the rate of newly formed polyamines. However, polyamines are required for various cellular processes during growth and development (11) and are used as a defense mechanism against low pH in controlling intercellular CO₂ (12). Therefore, it could be postulated that the increase in LDC activity and the subsequent accumulation of cadaverine in the tissue of ethylene-treated Pisum sativum is of a compensatory nature, as a response to the inhibition of ADC and SAMDC activity caused by ethylene.

The effect hitherto described for ethylene on LDC activity and the accumulation of cadaverine in the subapical tissue of ethylene-treated Pisum sativum seedlings warrant further studies in order to understand the relationships among ethylene, cadaverine and other polyamines in the various physiological processes of plant tissues.

LITERATURE CITED