Cadaverine, an Essential Diamine for the Normal Root Development of Germinating Soybean (Glycine max) Seeds

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ABSTRACT

When the polyamine content of soybean (Glycine max) seeds was examined during the early stages of germination, the major polyamine in the cotyledons was found to be spermidine, followed by spermine; while very low concentrations of cadaverine were found. In the embryonic axes, however, cadaverine was the main polyamine and its content markedly increased 24 hours after the start of germination. When the germination of the seeds was performed in the presence of 1 millimolar a-difluoromethylornithine (DFMO), a marked decrease in the cadaverine content was found, while the other polyamines were not affected. This decrease of the cadaverine content was already noticeable after the first hours of germination. In the presence of DFMO, a pronounced elongation in the roots of the seedlings and a marked decrease in the appearance of secondary roots as compared with controls, was observed. This abnormal rooting of the seedlings caused by DFMO was almost completely reverted by the addition of 1 millimolar cadaverine. The latter also increased the appearance of secondary roots in the seedlings. The decrease in the cadaverine content produced by DFMO could be traced to a strong inhibition of lysine decarboxylase. A temporal correlation between the increase in cadaverine content and the increase in lysine decarboxylase activity was found. Both reached a maximum at the second day of germination. The activity of diamine oxidase, the cadaverine degrading enzyme, started to increase at the third day and reached a maximum between the fourth and fifth day of germination. DFMO increased the activity of diamine oxidase by about 25%. Hence, the large decrease in cadaverine content produced by DFMO has to be attributed to the in vivo suppression of lysine decarboxylase activity. Ornithine decarboxylase activity was also suppressed by DFMO, but putrescine and spermidine contents were not affected, except in the meristematic tissues. The obtained results suggest an important role for cadaverine in the normal rooting process of soybean seedlings.

The polyamines are cationic molecules which are widely distributed in all living cells and which are essential for the processes of cell growth and differentiation (20). The definition of polyamine is usually applied only to a few of the many bases found in plants; namely, PUT (1,4-diaminobutane), cadaverine (1,5-diaminopentane), SPD, and SPM. Polyamines have been shown to be involved in several aspects of plants growth and development such as cell division and embryogenesis (1, 9). Although it is known that polyamines, in particular SPM and SPD, interact directly with anionic, cellular components such as membranes (10) and nucleic acid (11, 15, 20), polyamines may play a more complex role in plants which can be unique to them. PUT, SPD, and SPM are linked by a common biosynthetic pathway which is initiated in the decarboxylation of either ornithine or arginine to give PUT, which is then aminopropylated to SPD and SPM (20). While the decarboxylation of ornithine to PUT by ODC is a pathway common to all living cells, the pathway leading from arginine to agmatine, which is mediated by ADC, and from agmatine to PUT is exclusive of bacteria and plants. Cadaverine is not as widely distributed as PUT and is mainly found in the Leguminosae (19) and in the flowers of Arum lilies (17). It originates in the decarboxylation of lysine through the action of LDC, a pyridoxal-phosphate dependent enzyme (2) which has not been as extensively studied as ODC. The presence of cadaverine in germinating soybean seedlings has been reported (12), as well as its function as a source of H₂O₂ when oxidized by DAO (8). It has therefore been proposed to be a cofactor for uricase activity in soybeans (21) and for the process of root lignification (7).

In this paper we report a function for cadaverine in the root development process of soybean.

MATERIALS AND METHODS

Plant Material

Seeds of soybean (Glycine max, [L] Merr. cv Carcarañá) harvested in 1988 and 1989 in the Province of Córdoba, Argentina, were used in this study. The seeds were surface sterilized with dilute hypochloride solution (50 mg active Cl/mL), thoroughly rinsed with distilled water, and then soaked for 4 h in distilled water. They were germinated in the dark at 27°C on wet filter paper. The seedlings were grown under these conditions for the indicated times up to a maximum of 9 d. When the seeds were germinated in the presence of DFMO, DFMO plus cadaverine, cadaverine, or PUT, they were first soaked for 4 h in water containing 1 mM concentration of the above mentioned compounds, and were then placed on the moistened germination paper prepared with the same 1 mM solution. Samples were taken at intervals throughout germination and the cotyledons and the germinated embryonic axes were manually separated. At longer germination periods (4–6 d) the seedlings were divided into three parts:
the radicle with the secondary roots, the hypocotyl and the hypocotyl hook. The latter is the first part to turn greenish after exposure of the seedlings to light.

Analysis of Polyamines

The plant material (routinely 5–10 embryonic axes or the other parts derived from the seedlings) was homogenized with cold 5% perchloric acid and kept 1 h on ice and centrifuged. The free polyamines were determined using the dansylation method (17), and 2-methylputrescine was used as an internal standard. The dansylated polyamines were separated on silica gel chromatofoil (Merck) using a two-solvent system to obtain good separations. The first solvent was n-hexane:ethanol, 2:1 (solvent A). In this solvent the dansylated derivatives of 5-aminovaleraldehyde (the oxidation product of cadaverine by DAO), SPD and SPM were separated from the unresolved mixture of the dansyl derivatives of PUT and cadaverine. The lower part of the chromatofoil (6 cm from the starting line) which contained the dansyl derivatives of SPM and SPD was cut away and the rest of the foil (14 cm) was further developed with solvent B (chloroform:triethylamine, 9:1) until the dansyl derivatives of cadaverine and PUT were separated. The dansylated derivatives were visualized by fluorescence and quantified after elution from the foil using ethyl acetate. Their fluorescence was measured at 365 nm excitation and 510 nm emission. Standard concentration curves of the dansylated cadaverine, PUT, SPD, and SPM were simultaneously run for each polyamine analysis. Free polyamines and amino acids were separated on cellulose and silica gel TLC plates using propanol:HCl:water (8:3:1), and ethanol:ammonium hydroxide (7:3), respectively, and detected with ninhydrin reagent or, when radioactive material was used, the bands were eluted and the radioactivity was determined in a liquid scintillation counter.

Enzyme Assays

Diamine Oxidase

Radicle axes or other parts of the seedlings were homogenized with 10 volumes (w/v) of 50 mm phosphate buffer (pH 7.4) in the presence of insoluble PVP. The extracts were filtered through nylon cloth and centrifuged at 20,000g for 15 min. The supernatants were either dialyzed overnight against 20 mm phosphate buffer (pH 7.4), or filtered through a PD10 column (Sephadex G-25 Pharmacia), equilibrated with the above mentioned buffer. These enzyme preparations, free of endogenous polyamines, were routinely used to assay DAO activity. In a typical assay, carried out in a final volume of 260 μL, 100 μL of 50 mm phosphate buffer (pH 7.4) were incubated with 50 μL of the enzymatic extract (50–150 μg of protein), 0.2 μmol of either cadaverine or PUT and 100 μL of 0.1% α-aminobenzaldehyde. The mixture was incubated for 10 min at 37°C. The reaction was then stopped by dilution and the quinazolinium derivatives formed were determined spectrophotometrically at 461 nm (ε = 1.6 × 10⁷ L mol⁻¹ cm⁻¹) in the case of cadaverine and at 431 nm (ε = 1.8 × 10⁵ L mol⁻¹ cm⁻¹) in the case of putrescine. One unit represents the amount of enzyme catalyzing the formation of 1 μmol of 5-aminovaleraldehyde in 10 min.

Lysine, Arginine, and Ornithine Decarboxylases

The embryonic axes of the different parts of the seedlings were homogenized in a chilled mortar, with 50 mm phosphate buffer (pH 8.0) containing 0.5 mm EDTA, 5 mm diethiothreitol (or 10 mm β-mercaptoethanol), 1 mm PMSF, 1 mm pyridoxal-phosphate, and 1% polyethylene glycol (buffer A). The extracts were centrifuged at 20,000g for 30 min and the supernatants were used either before or after filtration through a PD10 column (Pharmacia) equilibrated with buffer A.

The standard incubation mixture for LDC contained, in a final volume of 200 μL: 65 μL of buffer A, 2 mm pyridoxal phosphate, 5 mm diethiothreitol, and 100 μL of enzyme (100–200 μg of protein). The reaction was started by the addition of 1 mm (250,000 dpm) of [U-1⁴C]lysine (282 mCi/mmol).

The standard incubation mixture for the ODC assay was similar to that described above except for the substrate, which was [1-¹⁴C]ornithine (54.3 mCi/mmol). It was added at a final 1 mm concentration (150,000 dpm). When putrescine was isolated, [U-¹⁴C]ornithine (New England Nuclear, 266 mCi/ mmol, 310,000 dpm) was used. ADC activity was assayed under similar conditions except for the buffer which was pH 7.0 and the substrate, which was 1 mm [U-¹⁴C]arginine (325 mCi/mmol, 200,000 dpm).

The incubations were run for 60 min at 37°C with constant shaking. The reaction was stopped by addition of 100 μL 4 M citric acid and the incubations were continued for an additional hour with constant shaking. The ¹⁴CO₂ released was trapped by 50 μL Protosol (New England Nuclear) adsorbed on Whatmann No. 1 filter strips suspended above the reaction tubes, in glass wells identical to the plastic wells of Kontes. Controls were simultaneously run for each assay using boiled enzyme extracts which were incubated and assayed as described above. Formation of the diamines from the labeled amino acids were confirmed using TLC (see above). One unit is defined as the enzyme catalyzing the release of 1 nmol of ¹⁴CO₂/60 min.

Localization of DAO in Germinating Soybean Seedlings

Extracellular solutions from hypocotyls grown in the dark were obtained according to the method of Terry and Bonner (22). Hypocotyl sections (2 cm long) were cut 1 cm below the apical bud and placed in distilled water for 1 h at 25°C. The sections were then packed vertically in the barrel of a 20 mL plastic syringe and were thoroughly washed with cold distilled water using a circulating pump. The sections were then infiltrated under vacuum with a 50 mm potassium phosphate buffer (pH 7.4) for 2 min and centrifuged at 3,000 rpm for 5 min. This extraction procedure was repeated for three times and the pooled extracts were used for enzyme and protein determinations. The sections were then homogenized as described above for the assay of DAO. The activity of the DAO in the extracellular extracts and in the homogenates was determined as described above. Glucose-6-P dehydrogenase activity was used as a marker to detect cytoplasmic contami-
nation of the extracellular extracts. It was assayed as described by Kornberg and Horecker (12).

Protein Determinations

Proteins were determined by the method of Bradford (4) using bovine serum albumin as a standard. In every experiment the enzymatic activities were the mean of at least three separate estimations of protein on separate samples that agreed within 5%.

RESULTS

Distribution and Dynamics of Free Polyamines during the Early Germination Stages of Soybean Seeds

Cotyledons and embryonic axes were analyzed to evaluate the content in free polyamines and the dynamics of their free pools during the early germination stages of soybean seeds. Cotyledons were separated into two groups, those adjacent to the embryonic axes (a, inset to Fig. 1) and those further removed from the embryonic axes (b, inset to Fig. 1). Special care was taken to avoid contamination of the cotyledons with embryonic axes material. Free polyamine content in the cotyledons and in the embryonic axes were measured after seed imbibition in water for time intervals from 10 min to 120 h (Fig. 1, A and B). The PUT, SPD, and SPM contents of both cotyledon parts (a and b, inset to Fig. 1) was always found to be very similar (Fig. 1, A and B). Cadaverine concentration in part a of the cotyledons was, however, thrice the concentration found in part b. The major polyamine found in the cotyledones was SPD while the content of cadaverine was low (Fig. 1, A and B). SPD and SPM contents decreased 1 h after the start of germination by about 25%, and the content of SPM in part b of the cotyledon continued to decrease until it reached 50% of its original concentration 3 h after the start of germination.

The changes in the content of polyamines of the embryonic axes that took place in the seeds after the start of germination are shown in Figure 1, C and D. Putrescine content was found to be low during the first 18 h and then started to increase (Fig. 1C). Cadaverine content was higher than that of putrescine and also increased with germination time, measured either on an axes basis (Fig. 1C), or on a fresh weight basis (Fig. 1C, numbers in parentheses). SPM concentration in the embryonic axes was low and was not found to change with germination time when expressed on an axis basis. Its content decreased, however, when expressed on a fresh weight basis (data not shown). SPD content, when expressed on an axis basis, remained constant during the first 12 h of germination and then started to increase (Fig. 1D). However, when expressed on a fresh weight basis it was found to be high at the start of germination, to reach a maximum 30 h later, and then to decrease (Fig. 1D, numbers in parentheses).

The dry weight of the embryonic axes increased very little during the first 30 h of germination. Hence only cadaverine content was found to increase in absolute values during the course of the 120 h of germination (independent of the way the data are expressed), while the contents of SPD and PUT decreased in absolute values after the first 30 h of germination.

Polyamine Distribution in Germinated Soybean Seedlings

When the distribution of free polyamines was analyzed in 6-d germinated soybean seedlings, cadaverine was found to be mainly concentrated in the roots and the hypocotyls, while its concentration in the hypocotyl hooks was very low (Fig. 2). PUT concentration was highest in the roots and lowest in the hypocotyls. SPM content was low in all three tissues, but lowest in the hypocotyls. SPD content was also lowest in the hypocotyls, but high in the hypocotyl hooks and roots (Fig. 2). In the latter a high SPD/PUT ratio was found, a characteristic feature of a fast-growing, meristematic zone. A similar

Figure 1. Polyamine contents in cotyledons and embryonic axes of soybean seeds as a function of germination time. The dotted lines represent the polyamine content in segment a of the cotyledons, located near the embryonic axes (see inset to A). The polyamine content was expressed per g/fresh weight in the cotyledons (A and B) and per five axes in the embryonic axes (C and D). The data represent the average of four independent experiments which varied less than 10% of the mean. PUT (O) and cadaverine (A) in the cotyledons (A) and embryonic axes (C); SPD (*) and spermine (O) in the cotyledons (B) and embryonic axes (D).

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high ratio was found in the apical part (tip section) of the roots of 7-d germinated soybean seedlings. Polyamine distribution was measured in the root of the latter by slicing 1 cm segments from the root apex up to the start of the secondary roots. At the apex and up to 3 cm further away cadaverine content was low (600 nmol/g fresh weight) and then increased. PUT content was low up to 5 cm (300 nmol/g fresh weight) while SPM and SPD contents were significantly higher at the tip section (which contains the meristematic zone) and then strongly decreased when approaching the secondary roots (Fig. 3). Similar results have been found in corn roots (6).

**Effect of DFMO on Root Development and Polyamine Content of Germinating Soybean Seeds**

When soybean seeds were germinated in the presence of DFMO, a significant effect was observed on root elongation. Roots were strongly elongated and secondary roots were scanty or missing. The effect was dependent on DFMO concentration (Fig. 4). When the polyamine content of the embryonic axes was analyzed 24 and 48 h after the start of germination in the presence of DFMO a very strong decrease in the content of cadaverine was found (Fig. 5, A and B). Cadaverine content was already low 4 h after the start of germination in the presence of DFMO (data not shown), and stayed that way up to the ninth day of germination. The content of the other polyamines in the embryonic axes was not affected by DFMO when expressed either per axes (Fig. 5, A and B), or per fresh weight (Fig. 5, C and D).

To establish a possible link between the decrease in cadaverine content and the physiological effect observed in the seedlings under the effect of DFMO, cadaverine (1 mM) was added together with DFMO (1 mM). The addition of the diamine almost completely reversed the effect of the DFMO on the root development, as shown in 5-d seedlings (Fig. 6, A–C).
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contents weight (C and C) contents inhibitory the addition of about 40% in the different seedling tissues, as compared to controls (Fig. 7). The fact that exogenously added cadaverine was not reflected in a strong increase in the intracellular levels of this diamine could be attributed to a degradation of the externally added cadaverine before it entered the plant cells. It is well known that, in the Leguminosae, cadaverine (and PUT) are oxidized by a DAO (19). We found that in the soybean hypocotyl DAO activity was mainly localized in the cell walls. When the hypocotyl sections were extracted as described in "Materials and Methods," about 70% of the DAO activity was detected in the extracellular fluid. The specific activity of the enzyme in the latter was 500-fold higher than that of the total extract. No cadaverine or G-6-P dehydrogenase activity was detected in this extracellular fluid (data not shown).

When the effect of DFMO on the polyamine content of the root tips was examined, it was found that in this meristematic tissues SPD and PUT contents were also reduced (Fig. 7, roots, inset).

Effect of DFMO on the Activities of DAO, LDC, and ODC in Germinating Soybean Seedlings

Although DFMO is a well known ODC inhibitor, its inhibition of the cadaverine content in the germinating soybean seedlings suggested that it also either inhibited LDC or increased the activity of DAO. This latter possibility was explored by measuring the oxidase activity and cadaverine content in 2- and 5-d germinating seedlings treated with DFMO. Hypocotyl, hypocotyl hooks, and roots were used in the 5-d seedlings, while after 2 d germination, the total seedlings were used. DAO activities were found to increase by about 20% in the 5-d DFMO treated seedlings. No major changes were found in the 2-d germinated seedlings (Table I). Clearly, there was no correspondence between the decrease in

Figure 5. Effect of DFMO on the polyamine contents of 24 h (A and C) and 48 h (B and D) germinated soybean seedlings. The polyamine contents are expressed per embryonic axes (A and B) and per fresh weight (C and D). Error bars are ± se (n = 4).

Figure 6. Reversion of the DFMO effect by cadaverine. Soybean seedlings were germinated 5 d under control conditions (A), in the presence of DFMO (B), in the presence of DFMO plus cadaverine (C), and in the presence of cadaverine (D). The DFMO and cadaverine were used at a 1 mm concentrations. The seedlings are typical of those obtained in at least 20 independent experiments.
therefore investigated. Germination period ranging from 2 h up to 3.5 days after the start of germination (Fig. 8). Two hours after the start of germination the activity of LDC was low (3.40 ± 0.40 units/g fresh weight) but it was strongly increased by 1 d after germination and reached a maximum at the second day. When germination took place in the presence of DFMO, LDC activity was almost entirely suppressed (Fig. 8). At the fifth day of germination the decarboxylase activity was found to be higher in the roots than in the hypocotyls, but was entirely suppressed in both when the seedlings were germinated in the presence of DFMO (inset, Fig. 8).

A proposal has been made that ODC could also decarboxylate lysine (14), and many literature reports seem to support the idea that DFMO is specific for ODC (1, 3). Therefore, ODC activity was determined in seedlings germinated in the presence and absence of DFMO during the time period mentioned above. It was found that ODC activity was much higher than LDC activity at the start (2 h) (20.0 ± 1.0 units/g fresh weight) and also reached a maximum at the second day of germination (Fig. 8). As in the case of LDC, ODC activity was much higher in the roots than in the hypocotyls (Fig. 8, inset).

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<th>Table II. Effect of Increasing Concentrations of DFMO on the in Vitro Activities of LDC and ODC from Soybean Seedlings</th>
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<td>The enzymes were obtained from 2-d-old germinated seedlings and the incubations were performed as described in &quot;Materials and Methods.&quot; DFMO was added at the start of the incubations. The activities of the controls taken as 100% were: 32.0 ± 1.5 units/mg protein for the LDC and 7.5 ± 0.5 units/mg of protein for ODC. The results are the mean ± SE of three independent experiments.</td>
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The effect of DFMO on the synthesis of cadaverine was therefore investigated. LDC activity was measured in seedlings germinated in the presence and absence of DFMO during a time period ranging from 2 h up to 3.5 days after the start of

cadaverine content and the increase in DAO activity in the DFMO treated soybean seedlings.

The effect of DFMO on the synthesis of cadaverine was measured in seedlings germinated in the presence and absence of DFMO during a time period ranging from 2 h up to 3.5 days after the start of germination (Fig. 8). Two hours after the start of germination the activity of LDC was low (3.40 ± 0.40 units/g fresh weight) but it was strongly increased by 1 d after germination and reached a maximum at the second day. When germination took place in the presence of DFMO, LDC activity was almost entirely suppressed (Fig. 8). At the fifth day of germination the decarboxylase activity was found to be higher in the roots than in the hypocotyls, but was entirely suppressed in both when the seedlings were germinated in the presence of DFMO (inset, Fig. 8).

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<td>The activities and cadaverine contents were determined as described in &quot;Materials and Methods.&quot; Each value is the mean ± SE of three experiments in duplicate.</td>
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Figure 8. LDC (▲) and ODC (●) activities as a function of germination time. Soybean seedlings grown in the presence (---) and absence (— —) of DFMO were used to prepare the enzyme. The activities are expressed as a function of protein content (——) and as a function of fresh weights (- - -). Values are the mean ± se of three experiments performed in duplicate. The inset represent the activities, expressed in units/mg protein, of LDC and ODC in the absence and presence of DFMO, determined in the hypocotyls and roots of 5-d germinated seedlings.

In order to make sure that we were indeed measuring the two different enzymatic activities, LDC and ODC, and that cadaverine was not being originated by the action of ODC on lysine, both decarboxylases were isolated from 2-d germinated seedlings and were assayed in vitro on 14C-enriched substrates, at pH 8.8, the optimum pH found for both enzymes of the soybean seedlings. LDC was assayed with 1 mm [U-14C]lysine in the presence of 10 mm ornithine. The 14CO₂ release from the [14C]lysine was not inhibited by the presence of ornithine, while in the presence of 10 mm unlabeled lysine, 50% of the 14CO₂ release from the labeled lysine was inhibited. On the other hand, ODC activity was not affected when 10 mm lysine was added to an assay solution which contained 1 mm [14C] ornithine, while the 14CO₂ release was decreased by 50% when 10 mm unlabeled ornithine was added. Hence, although LDC and ODC have not been separated, their activities could be differentiated in the in vitro assays. Additional evidence that we were dealing with two different enzymatic activities was provided by the fact that increasing concentrations of DFMO affected differently the in vitro activities of both (Table II). Therefore, it could be concluded that DFMO inhibits LDC activity from soybean seedlings.

**DISCUSSION**

The analysis of polyamine distribution in the cotyledon and in the embryonic axes of germinating soybean seedlings showed that cadaverine concentration was very low in the cotyledon, but was high in the embryonic axes. Since it has been shown that cadaverine is originated in lysine in these tissues (13) it is conceivable that the amino acid is liberated in the cotyledon from protein material during germination and then diffuses or is transported toward the embryonic tissues where it is decarboxylated to cadaverine. The activity of LDC was found to be located in the embryonic axes. These results lend support to the idea that cadaverine may be involved in processes of cell division and proliferation in the germinating soybean seedlings. The content of cadaverine in the developing seedlings increases with germination time, again suggesting a central role for this diamine in this process. No clear relation was found for the dynamics of the other polyamines and the germination process in soybeans. As was the case with other germinating seedlings (1), SPM and SPD contents were also found to increase in the meristematic seedling zones such as the root tips and the hypocotyl hooks.
The decrease in the cadaverine content in soybean seedlings germinated in the presence of DFMO produced striking morphological changes in their rooting (Figs. 4 and 6B), which were prevented by the simultaneous addition of cadaverine (Fig. 6C). The addition of cadaverine alone increased the appearance of secondary roots (Fig. 6D). Root elongation produced by the absence of cadaverine could be attributed to the fact that under the action of the DAO, hydrogen peroxide is formed during the oxidation of cadaverine. The H₂O₂ could then act as a cosubstrate of the peroxidases in the processes of lignification and wall stiffening (7, 8). In the absence of cadaverine the above mentioned processes will be hindered and abnormal root elongation will take place. In support for this explanation we (this paper), and others (8), found that the DAO in the seedling hypocotyls was mainly localized in the cell walls. The formation of hydrogen peroxide during the oxidation of cadaverine has also been found to be correlated with the uricase activity detected in soybean roots and hypocotyls (21). Cadaverine seems, therefore, to be essential for the normal root development in soybean seedlings.

The addition of DFMO to germinating seedlings affected only to a slight extent the contents of PUT and SPD during the first 24 h of germination (Fig. 2A). Since DFMO suppressed ODC activity in the soybean seedlings (Fig. 8), PUT is probably originated in the latter by the alternative ADC pathway. We found that this decarboxylase was present in germinating soybean seedlings and was not inhibited by DFMO (data not shown). In the meristematic tissue (Fig. 7, hypocotyl hooks), DFMO was a more efficient inhibitor of PUT content suggesting that in these tissues the ODC pathway was more active; similar results have been reported for corn seedlings (16). The effect of DFMO on the rooting process of the soybean seedlings was found to be very pronounced (see above). This contrasts with the lack of effect of DFMO, even when used at higher concentrations on plant cell cultures (2). The difference may be the special role that cadaverine plays in the germinating soybean seedlings. In the latter we found that DFMO strongly inhibited LDC activity and therefore the formation of cadaverine. We established a temporal correlation between the content in cadaverine and LDC activity in the germinating seedlings. The inhibition of LDC by DFMO could be due either to a decrease in the content of this enzyme, or to an irreversible inhibition of its activity. It is well known (5) that at equilibrium conditions under which the classical Michaelis-Menten theory of enzyme mechanism is valid, the enzymes distinguish between two substrates with an accuracy of 1:20. This could explain the inhibition of LDC by an ODC specific inhibitor. The exact function of cadaverine (or a product of its metabolism) in normal soybean root development has yet to be elucidated.

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LITERATURE CITED


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