Arginine and Ornithine Decarboxylases, the Polyamine Biosynthetic Enzymes of Mung Bean Seedlings

Arie Altman, Ra’anan Friedman, and Nitsa Levin

Department of Horticulture, The Hebrew University of Jerusalem, Rehovot 76-100, Israel

ABSTRACT

General properties and relative activities of L-arginine decarboxylase (ADC) (EC 4.1.1.19) and L-ornithine decarboxylase (ODC) (EC 4.1.1.17), two important enzymes in putrescine and polyamine biosynthesis, were investigated in mung bean (Vigna radiata L.) tissues. Both activities increase linearly with increasing concentrations of crude enzyme, but the increase in ADC activity is considerably greater. The decarboxylation reaction is linear for up to 30 to 60 minutes, and both enzymes have a pH optimum of 7.2. α-Difluoromethyl-ornithine inhibits ODC activity of excised roots, while increasing ADC activity.

High specific activity of both enzymes is detected in terminal buds and leaves, while root and hypocotyl activity is low. Different ADC-to-ODC activity ratios are found in various tissues of mung bean plants. Substantial increase in the activity of both enzymes is detected in incubated sections as compared with intact plants. A comparison of several plant species indicates a wide range of ADC-to-ODC activity ratio.

It is suggested that both ADC and ODC are active in plant tissues and that their relative contribution to putrescine biosynthesis is dependent upon the type of tissue and growth process.

The importance of polyamines in various growth and physiological processes in plants has been inferred from both application of exogenous polyamines and from changes in endogenous polyamines and related metabolites (1, 2, 4, 9, 13). Thus, a better elucidation of the role of polyamines in plant physiology warrants a careful examination of the various metabolic steps involved.

ADC (EC 4.1.1.19) and ODC (EC 4.1.1.17) are two key enzymes in the biosynthetic pathway of polyamines. Extensive studies (3, 6, 7, 15) with mammalian cells established ODC as the key enzyme for synthesis of putrescine from L-ornithine, putrescine being metabolized later to spermidine and spermine. In plants, however, ODC seemed to be of less importance, and it has been claimed that putrescine is synthesized from L-arginine by ADC via the intermediate, agmatine (17, 18). This was supported by the relative high ADC activity found in plant tissues in response to several growth stimuli and physiological conditions. Therefore, ADC activity was increased in embryonic cells of Daucus carota (13), in potassium-deficient barley (17), and in soybean seedlings grown on ammonium (11). Similarly, N6-benzyladenine enhanced ADC activity in cucumber cotyledons (20), and ADC was found to be controlled by phytochrome and GAs (8). Other observations indicate, however, that this is not always the case and that both ADC and ODC are active and involved in polyamine biosynthesis (16, 19, 21). In recent studies (10), ODC activity of developing tomato fruits was found to increase dramatically up to the 3rd day after pollination, while ADC activity was very low, and both arginine and ornithine were equally well decarboxylated in tobacco cell cultures (5).

In view of the contradictory results regarding the activity and importance of ADC and ODC in plants, a comparative study of these two polyamine-biosynthetic enzymes was undertaken. We report the kinetics and several properties of ADC and ODC as well as their relative activity in tissues of mung bean seedlings.

MATERIALS AND METHODS

Plant Material. Mung bean (Vigna radiata L.) seeds were germinated and grown in vermiculite in a controlled phytotron (27°C-day and 22°C-night temperature, 16-h photoperiod, 78% RH). One cm subapical sections of epicotyls were harvested from 7- to 8-day-old seedlings and were extracted for determination of enzyme activities. Occasionally, other plant parts were extracted, as mentioned. Epicotyl sections of French bean (Phaseolus vulgaris L. var. Brittle Wax) and cotton (Gossypium hirsutum L. SJ-2), grown for 8 days under the same conditions, were also used in several experiments.

Extraction and Assay of ADC and ODC. Sections were harvested, weighed, and ground in a prechilled mortar with a pestle (250–600 mg fresh weight/2 ml medium). Extraction medium consisted of 10 mM phosphate buffer (pH 7.2), 0.1 mM DTT, 1 mM pyridoxal-5'-phosphate, and 20 mM Na-EDTA. The extracts were centrifuged at 12,000g for 20 min, and the supernatant, hereafter referred to as crude enzyme, was used immediately. The enzymic reaction was started by adding 100 μl crude enzyme to a test tube containing 150 μl of the extraction medium and 50 μl DL-[1-14C]arginine hydrochloride (Commissariat a l’Energie Atomique Gif-sur-Yvette, France, 0.125 μCi, 20 mCi/mmol) or L-[1-14C]ornithine hydrochloride (Amersham, 0.125 μCi, 59 mCi/mmol) for ADC or ODC assay, respectively. The test tubes were then capped with rubber stoppers fitted with plastic center wells (Kontes Glass Co., NJ) containing 0.2 ml of Soluen-100 (Packard) on a Whatman No. 1 paper wick. The reaction was allowed to proceed for 60 min at 37°C in a shaking water bath and was terminated by injection of 0.2 ml 4% (v/v) HClO4 into the reaction medium. After an additional 30-min shaking, the center wells were removed and placed in vials with 10 ml toluene-PPO-POP scintillation mixture. Counting efficiency was about 80%, and blank values were obtained using boiled crude enzyme. The standard procedure was adopted following extensive preliminary experiments, using several compositions of the extraction and reaction media and modified assay procedures. Two to three separate extractions per treatment were used, each assayed in duplicate, and all experiments were repeated 2 to 4 times. ADC and ODC activities are presented as pmol 14CO2 per g fresh weight or mg protein.

Protein Determination. Protein in the extracts was determined...
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Fig. 1. ADC and ODC activities of mung bean epicotyls as a function of crude enzyme concentration.

Fig. 2. ADC and ODC activities of mung bean epicotyls as a function of reaction time.

Fig. 3. Effect of pH on ADC and ODC activities of mung bean hypocotyls.

Table I. Effect of α-Difluoromethyl-Ornithine on ADC and ODC Activity of Excised Mung Bean Roots

<table>
<thead>
<tr>
<th></th>
<th>ADC</th>
<th>ODC</th>
<th>ADC</th>
<th>ODC</th>
<th>ADC/O DC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol CO₂·mg protein(^{-1}) h(^{-1})</td>
<td>%</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>981</td>
<td>527</td>
<td>100</td>
<td>100</td>
<td>1.86</td>
</tr>
<tr>
<td>α-difluoromethyl-ornithine, 1 mm</td>
<td>1,556</td>
<td>239</td>
<td>159</td>
<td>45</td>
<td>6.51</td>
</tr>
<tr>
<td>0.1 mm</td>
<td>1,200</td>
<td>165</td>
<td>122</td>
<td>31</td>
<td>7.27</td>
</tr>
</tbody>
</table>

Table II. Distribution of ADC and ODC Activity in Mung Bean Seedlings

<table>
<thead>
<tr>
<th></th>
<th>ADC</th>
<th>ODC</th>
<th>ADC</th>
<th>ODC</th>
<th>ADC/O DC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol CO₂·g fresh (\text{wt}^{-1}) h(^{-1})</td>
<td>pmol CO₂·mg protein(^{-1}) h(^{-1})</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal bud</td>
<td>1,252</td>
<td>408</td>
<td>1,475</td>
<td>483</td>
<td>3.05</td>
</tr>
<tr>
<td>Leaves</td>
<td>1,404</td>
<td>503</td>
<td>1,465</td>
<td>524</td>
<td>2.80</td>
</tr>
<tr>
<td>Epicotyl</td>
<td>400</td>
<td>96</td>
<td>2,484</td>
<td>582</td>
<td>4.27</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>47</td>
<td>16</td>
<td>554</td>
<td>179</td>
<td>3.09</td>
</tr>
<tr>
<td>Roots</td>
<td>92</td>
<td>15</td>
<td>354</td>
<td>62</td>
<td>4.71</td>
</tr>
</tbody>
</table>

RESULTS

General Properties. The linearity of ADC and ODC activity, with increasing concentrations of crude enzyme, is depicted in Figure 1. The greater increase in ADC activity of mung bean epicotyls resulted in an increasing ADC-to-ODC ratio, when expressed both on fresh weight basis and as specific activity. ADC and ODC activity proceeded at a linear rate for up to 30 min and 60 min, respectively (Fig. 2). All assays were, therefore, run for 30 to 60 min. Optimum pH for both enzymes was 7.2 (Fig. 3). ADC evincing a greater decline in activity at both lower and higher pH values. Partial characterization of the enzymic system of mung bean was done by analyzing the effect of α-difluoromethyl-ornithine, an enzyme-activated irreversible inhibitor of ODC (Table I). Treatment of excised roots with 0.1 mm and 1 mm α-difluoromethyl-ornithine resulted in 55 to 69% inhibition of ODC activity and in a considerable increase in ADC activity.

Relative Activities in Various Tissues. The distribution of ADC and ODC activity in mung bean seedlings shows a decreasing gradient from the terminal bud to the roots (Table II). The ADC and ODC activity of leaves was relatively high, and the lower protein content of the subapical epicotyl section was manifested in higher specific activity values. ADC-to-ODC activity ratio was especially high in epicotyl and roots. A comparison of ADC and ODC activity in intact seedlings and in sections incubated in buffer for 24 h (Table III) indicates a considerable increase of enzyme activity as a result of excision and incubation. Both ADC and ODC activity increased equally in incubated leaf sections,
while a greater increase in ODC activity was especially evident in epicotyl and root sections. The relative activity of the two polyamine biosynthetic enzymes was further investigated in three different plant species (Table IV). Cotton hypocotyls had the lowest ADC activity of the three species which were assayed, while no ODC activity could be detected in French bean hypocotyls. These differences in activity are reflected in the ADC-to-ODC ratio.

**DISCUSSION**

It has been claimed that L-arginine decarboxylation is the main pathway for putrescine biosynthesis in plants (17, 18). However, the simultaneous activity of ADC and ODC in mung bean plants and the similarity in their enzymic properties (Figs. 1–3) indicate that no exclusive pathway for putrescine formation should be considered a priori. Rather, the synthesis of putrescine and related polyamines may involve both ADC and ODC as well as other enzymes (Fig. 4). Because both ADC and ODC can be rate-limiting enzymes in putrescine formation, factors which control their relative activity are of special importance for polyamine biosynthesis and effects in plants. Thus, ADC and ODC activity can be regulated by: (a) concentration and availability of arginine and ornithine; (b) interconversion of arginine and ornithine and the degree of their metabolism other than to putrescine; (c) activation, half-life, and compartmentation of ADC and ODC; and (d) de novo enzyme synthesis in response to specific hormonal and physiological stimuli. The tissue-specific activation of ADC or ODC (e.g., a markedly higher ADC-to-ODC ratio in roots, Table II), the differential stimulation of decarboxylation activity in response to excision and incubation, (e.g., ADC increasing more than ADC, Table III), and the considerable difference in ADC and ODC activity in various plant species (Table IV) all may be related to the above-mentioned factors. The possible modulation of ADC and ODC is partially evidenced by the observation that blockage of the ODC route results in the activation of the alternative ADC route (Table I). This is in accordance with earlier observations on multiple pathways for putrescine biosynthesis in *Escherichia coli* (14). ODC inhibition by α-difluoromethyl-ornithine did not affect ADC activity of tobacco cell cultures (5). In addition, arginine and ornithine are equally well incorporated into putrescine, spermidine, and spermine (R. Friedman, A. Altman, U. Bachrach, unpublished data).

Other investigations, which show activation of ADC and/or ODC in response to several growth stimuli (8, 10, 13, 19, 20) as well as the specific activation of ODC in salt-stressed roots (R. Friedman, A. Altman, U. Bachrach unpublished data), support our conclusion that the relative contribution of ADC and ODC to putrescine biosynthesis is dependent on the type of tissue and on the specific growth process. The elucidation of several possible control mechanisms will be reported in a subsequent paper.

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