Participation of Ornithine Decarboxylase in Early Stages of Tomato Fruit Development

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ABSTRACT

The apparent association of ornithine decarboxylase (ODC) with rapid cell proliferation in developing tomato (Lycopersicon esculentum Mill. cv. Pearson ms-35) fruits has been previously described. Further evidence is provided by the use of two ODC inhibitors, a-difluoromethylornithine (a-DFMO) and a-methylornithine (a-MO). Fruit development was inhibited by these inhibitors if applied during the period of intensive cell division. When applied in vitro, the two inhibitors were shown to inhibit the activity of ODC but not that of arginine decarboxylase (ADC). When applied in vivo, a-DFMO, a catalytic irreversible inhibitor, caused 97.1% reduction of ODC activity in the dialyzed extract from the treated ovaries, while it had no effect on ADC. On the other hand, a-MO, a reversible inhibitor, did not reduce the activity of these two enzymes in the dialyzed extracts when applied in vivo. The dialysis procedure probably removed a-MO from the enzyme fraction. Putrescine, the product of both ODC and ADC, alleviated the inhibition of fruit development but did not restore ODC activity to the control level. These results suggest that in the young developing tomato fruit, ODC is the enzyme responsible for the synthesis of putrescine, which is essential for the early stages of fruit development. The reduced activity of ODC elicited by putrescine suggests a mechanism of feedback regulation by enzyme repression or release of an ODC anti-enzyme.

Polyamines are widely distributed in nature, but their precise role in cellular processes is not always fully understood. They are associated with cell proliferation, tissue regeneration, and malignancy (1, 6, 7, 17, 18). Most of the information on the biosynthetic pathways of putrescine, spermidine, and spermine, their regulation, and the possible site of action has been obtained from studies with microorganisms and mammalian cells (1). Such information is lacking for plant systems. Several reports (4, 8, 14, 15, 20–22) do, however, describe the presence of various polyamines in plants and the occurrence of enzymes involved in polyamine biosynthesis, e.g. ODC and ADC. It is commonly accepted that ODC is the enzyme responsible for the production of putrescine in plants and that ODC is of lesser importance (14, 15, 20–22).

We have recently described an apparent association between elevated ODC activity and rapid cell proliferation in two plant systems: tomato ovaries during the first 10 d after pollination and tobacco XD cells growing in suspension culture during the logarithmic phase of growth (9). ADC was also present in tomato ovaries. However, its activity did not change during the logarithmic phase of growth, being one-fourth of this maximal level of ODC (E. Cohen, S. (Malis) Arad, Y. M. Heimer, and Y. Mizrahi, unpublished results). It was, therefore, suggested that in these two plant systems (8, 9), as in mammalian cells (2, 6, 17), ODC is the first enzyme in polyamine biosynthetic pathway.

In the present paper, we provide additional support for our claim that ODC is indeed an essential enzyme for putrescine biosynthesis in developing tomato fruits that putrescine is important for fruit development. For this purpose, we used two inhibitors of ODC—one, a catalytic irreversible inhibitor, a-DFMO, and the other, a reversible inhibitor, a-MO (11, 13, 19).

MATERIALS AND METHODS

Plant Material. The male sterile line of tomato (Lycopersicon esculentum Mill.) cv. Pearson ms-35 was used. Plants were grown in a greenhouse under natural illumination. Flowers were hand pollinated at full anthesis and tagged. Applications of Chemicals. Chemicals were applied to the flowers via the inflorescence stalk in the following manner. The flower stalk was cut transversely up to the middle and then longitudinally towards the flowers. The free end of the cut stalk was inserted into a small test tube, which contained an aqueous solution of the chemical to be tested. The solutions were replenished daily until the fruits were analyzed. This method of application did not allow us to control and estimate the amount of material that penetrated the ovary. As control, only water-treated ovaries were used since they yielded the same results as untouched ovaries.

Enzyme Extraction and Assay. ODC and ADC determinations were performed as follows. For extraction, 1 g (fresh weight) of tissue was ground in an ice-cold mortar in 4 ml of extraction medium consisting of 0.25 M K-phosphate (pH 8), 10 mM DTT, 50 μM EDTA, and 25 μM pyridoxal phosphate. The cold homogenate was clarified by centrifugation at 10,000g for 20 min. The supernatant was dialyzed overnight at 0 to 4°C in a dialysis medium consisting of 0.025 M K-phosphate (pH 8), 1 mM DTT, 50 μM EDTA, and 25 μM pyridoxal phosphate. All the above conditions were found optimal for ADC. ODC activity was somewhat higher under Tris-HCl buffer (8). However, inasmuch as ODC yielded similar results in both buffers, the same extract (in phosphate buffer) was used for both enzymes for reasons of convenience. Enzyme activity was assayed by measuring the decarboxylation of L-[1-14C]ornithine for ODC and of L-[U-14C] arginine for ADC, as previously described (8).

Protein determination. Protein content was determined in the soluble extract by the Bio Rad Coomassie Blue method (Biorad

1 The work was performed in partial fulfillment of the requirements for the PhD thesis of E. C.
2 To whom correspondence should be addressed.
3 Abbreviations: ODC, ornithine decarboxylase; ADC, arginine decarboxylase; α-DFMO, α-difluoromethylornithine; α-MO, α-methylornithine.
development. For the derived from Mizrahi, temperature (Table 12, 16), seem incorrect. The first pollinated rather and in the protein the inhibitor to 10 applied tissue, fruit. The putrescine biosynthesis always statistically significant (Tables the product of ODC, whose biosynthesis is presumably inhibited by α-DFMO, could reverse this inhibition (Table I).

Putrescine alone, when applied at the time of pollination, enhanced fruit development to a small extent, which was not always statistically significant (Tables I and III).

An ADC-catalyzed pathway is claimed to be the prevalent route for putrescine biosynthesis in plant cells (14, 21, 22). However, in the early stages of tomato fruit development, when fruit is at the logarithmic phase of growth, ADC activity was much lower (25%) than that of ODC (E. Cohen, S. (Malis) Arad, Y. M. Heimer, and Y. Mizzahi, et al., unpublished results). In addition, in dialyzed extracts derived from α-DFMO-treated tomato ovaries, whose development was largely inhibited, ODC activity amounted to about 2% to 3% that of untreated ones, on both fresh weight and protein basis, while ADC activity remained unchanged (Table II). When the inhibitor was incorporated in the reaction mixture of the enzymic assay of control ovaries, it inhibited the activity of ODC to the extent of its inhibition in vivo but not that of ADC. These results are in keeping with the assumption that α-DFMO inhibits ODC activity in tomato ovaries as it does to ODC in mammalian systems (11, 13). Putrescine, although able to reverse the inhibition of fruit development, did not restore ODC activity to the level of the untreated control (Table II). Obviously, putrescine circumvented ODC inhibition and allowed normal fruit development. ADC, although unaffected by α-DFMO, could not substitute for the inhibited ODC. Thus, it can be concluded that the inhibition of fruit development by α-DFMO is related to the inhibition of ODC-catalyzed putrescine biosynthesis in tomato fruit.

α-MO, a reversible inhibitor of ODC, inhibited tomato fruit development to an extent similar to that of α-DFMO (Table III), the inhibition being alleviated by putrescine. However, the activity of the extractable ODC measured after dialysis was not lower than that of the untreated control fruits, although α-MO strongly inhibited enzymic activity when added to the test tube. This difference between the in vivo and in vitro effect of α-MO may lie in the reversible nature of its action. Once the inhibitor is removed by dialysis, the normal enzymic activity is restored. ADC activity did not decrease by α-MO, when added either in vivo or in vitro. Here also, as in Table I, putrescine given alone enhanced fruit development.

A noteworthy observation is that putrescine itself caused reduction in the extractable activity of both ODC and ADC (Tables II, III). The lower degree of inhibition exerted by putrescine in the experiment presented in Table III compared with that in Table II may be due to a possible smaller penetration of the chemical in this experiment because of technical limitations, as mentioned in “Materials and Methods.” We suggest that the low levels of enzyme activity exerted by putrescine represent some kind of regulation by the end product and are not due to the presence of low-mol-wt endogenous inhibitors, inasmuch as, if present, these inhibitors would have been removed by the dialysis. Whether the regulation acts via repression of enzyme synthesis or a release of ODC antienzyme, similar to that found in mammalian cells (5), remains to be investigated.

**CONCLUSIONS**

The following conclusions may be drawn from the data presented above: (a) putrescine seems to play a role during the first

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**Table 1. Effect of α-DFMO and Putrescine on Tomato Fruit Fresh Weight When Given Immediately, 1 Day, or 10 Days after Pollination**

The data represent one typical experiment out of three experiments, which yielded similar results. Each treatment group consisted of at least 20 fruits.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>0/5</th>
<th>0/10</th>
<th>1/6</th>
<th>10/17</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (H2O)</td>
<td>42.09 (100)y</td>
<td>1017.4 (100)z</td>
<td>130.0 (100)z</td>
<td>6493 (100)z</td>
</tr>
<tr>
<td>α-DFMO (1 mm)</td>
<td>12.52 (29.7)y</td>
<td>178.5 (17.5)y</td>
<td>29.2 (22.5)y</td>
<td>6345 (97.7)z</td>
</tr>
<tr>
<td>Putrescine (1 mm)</td>
<td>48.78 (115.9)z</td>
<td>1245.6 (122.4)z</td>
<td>116.0 (89.2)z</td>
<td>7281 (112.1)z</td>
</tr>
<tr>
<td>α-DFMO (1 mm) + putrescine (1 mm)</td>
<td>39.94 (94.5)z</td>
<td>1047.2 (102.9)z</td>
<td>94.4 (72.6)z</td>
<td>6197 (95.4)z</td>
</tr>
</tbody>
</table>

* The concentrations of the chemicals refer to the concentration of the solution applied.

O, immediately after pollination; 1, 24 h after pollination.

* Figures in parentheses represent percent of control.

* The difference between values marked 'z' and those marked 'y' in the same column is statistically significant at 99.9% level.

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Table II. Effect of α-DFMO and Putrescine, Given at the Time of Pollination, on Fresh Weight and the Activity of Extractable ODC and ADC in Tomato Fruits 5 Days after Pollination

Reaction mixture contained 4.2 nmol of L-(14C)-ornithine (59 μCi/μmol) or 4.1 nmol of L-(14C)-arginine (342 μCi/μmol) in 0.05 ml H2O and 0.25 ml enzyme preparation in 0.025 m phosphate buffer (pH 8.0). The data represent one typical experiment out of three experiments, which yielded the same results. For fresh weight, 20 ovaries were used in each experiment; for enzyme activity, 4 to 10 ovaries were used to give 200 to 300 mg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg. Fresh Wt (5 d after pollination)</th>
<th>ODC Activity</th>
<th>ADC Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/fruit</td>
<td>nmol 14CO2 released g⁻¹ fresh wt h⁻¹</td>
<td>nmol 14CO2 released g⁻¹ protein h⁻¹</td>
</tr>
<tr>
<td>Control (H2O)</td>
<td>118 (100)x</td>
<td>35.13 (100)</td>
<td>6.60 (100)</td>
</tr>
<tr>
<td>α-DFMO (1 mM)</td>
<td>37 (31)y</td>
<td>1.02 (2.9)</td>
<td>0.16 (2.0)</td>
</tr>
<tr>
<td>Putrescine (1 mM)</td>
<td>110 (93)z</td>
<td>5.87 (16.7)</td>
<td>0.86 (13)</td>
</tr>
<tr>
<td>α-DFMO (1 mM) + putrescine (1 mM)</td>
<td>92 (77)z</td>
<td>4.50 (12.9)</td>
<td>0.46 (7.0)</td>
</tr>
<tr>
<td>Control (H2O) + α-DFMO (0.1 mM) in assay³</td>
<td>0.97 (2.8)</td>
<td>0.18 (2.7)</td>
<td>10.12 (92)</td>
</tr>
</tbody>
</table>

* Figures in parentheses represent percent of control.

b The difference between values marked 'x' and those marked 'y' in the same column is statistically significant at 99.9% level.

α-DFMO was added to the dialyzed extract of control ovaries to a final concentration of 0.1 mM.

Table III. Effect of α-MO and Putrescine, Given at the Time of Pollination, on Fresh Weight and the Activity of Extractable ODC and ADC in Tomato Fruits 5 Days after Pollination

Details of assay are the same as in Table II. The data represent one typical experiment out of three experiments, which yielded the same results. For fresh weight, 20 ovaries were used in each experiment; for enzyme activity, 4 to 10 ovaries were used to give 200 to 300 mg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg. Fruit Fresh Wt (5 d after pollination)</th>
<th>ODC Activity</th>
<th>ADC Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/fruit</td>
<td>nmol 14CO2 released g⁻¹ fresh wt h⁻¹</td>
<td>nmol 14CO2 released mg⁻¹ protein h⁻¹</td>
</tr>
<tr>
<td>Control (H2O)</td>
<td>69.40 (100)x</td>
<td>52.16 (100)</td>
<td>4.51 (100)</td>
</tr>
<tr>
<td>α-MO (10 mM)</td>
<td>38.40 (55)y</td>
<td>44.46 (86)</td>
<td>3.86 (86)</td>
</tr>
<tr>
<td>Putrescine (1 mM)</td>
<td>84.60 (121)z</td>
<td>24.77 (47.5)</td>
<td>2.12 (47)</td>
</tr>
<tr>
<td>α-MO (10 mM) ± putrescine (1 mM)</td>
<td>72.20 (104)z</td>
<td>20.60 (39.5)</td>
<td>1.72 (38)</td>
</tr>
<tr>
<td>Control (H2O) + α-MO (1 mM²) in assay³</td>
<td>8.3 (15.9)</td>
<td>0.72 (15.9)</td>
<td>21.83 (98.4)</td>
</tr>
</tbody>
</table>

* Figures in parentheses represent percent of control.

b The difference between values marked 'x' and those marked 'y' in the same column is statistically significant at 99.9% level.

α-MO was added to the dialyzed extract of control ovaries to a final concentration of 1 mM.

10 d of tomato fruit development; (b) ODC is an essential enzyme for putrescine biosynthesis during the early stage of development since ADC cannot substitute for the α-DFMO- or α-MO-inhibited ODC; (c) ODC in the developing tomato fruit is subjected to some kind of feedback regulation by its product, putrescine.

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