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Ornithine Decarboxylase and Arginine Decarboxylase Activities in Meristematic Tissues of Tomato and Potato Plants

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ABSTRACT

Ornithine decarboxylase and arginine decarboxylase activities were measured in roots and buds of tomato (*Lycopersicon esculentum* Mill. cv. Pearson ms-35) and potato (*Solanum tuberosum* cv. Desire) plants. In both tomato and potato, the activity of ornithine decarboxylase was the highest at the root tip, decreasing proximally. The same was true for potato buds. In vegetative buds of tomato, the highest activity was found in the youngest leaves. The older the leaf, the lower was orithine decarboxylase activity. Arginine decarboxylase, on the other hand, did not display a similar gradient. These findings are in accordance with the suggestion that in tomato and potato elevated ornithine decarboxylase activity is associated with intense mitotic activity.

Enhanced synthesis of polyamines in animal cells is associated with cell proliferation, cell differentiation, tissue regeneration, malignancy, etc. (2, 13, 16). Various polyamines were also found in plants (1, 11, 14).

In animal cells, the polyamine putrescine is formed from L-ornithine by ODC[3] (2, 12). In plants, however, the accepted notion is that putrescine is formed from arginine by ADC via agmatine (11, 14, 15). Recently, it was shown that elevated ODC activity is associated with rapid cell proliferation in two plant systems, tomato ovaries immediately after pollination and tobacco XD cells growing in suspension culture (8, 9). ADC was also present in tomato ovaries. However, its activity did not change during the logarithmic phase of growth and was only 25% that of the maximal activity of ODC. It was, therefore, expected that elevated ODC activity would be associated with meristematic regions in these plants.

This communication describes the observation that elevated activity of ODC, but not of ADC, is associated with intense mitotic activity taking place in meristematic tissues in roots and vegetative apical buds in tomato and potato.

MATERIALS AND METHODS

Plant Material. In the experiments, we used buds and roots newly developed on cuttings of tomato (*Lycopersicon esculentum* Mill. cv. Pearson ms-35) and on tubers of potato (*Solanum tuberosum* cv. Desire). In the tomato, buds and roots were removed from 2-week-old cuttings, which were grown in aerated half-strength Hoagland solution in a greenhouse under natural light conditions. In the potato, tubers were allowed to bud under 100% RH at 28°C until the buds reached 20 mm. Then the tubers were transferred for 2 weeks to moist vermiculite for rooting, and 10-mm roots were used.

Root Zoning. Ten-mm-long roots were detached and sliced into 2-mm sections, starting from the root tip. Hundred-mg samples of sections of the same zone were used.

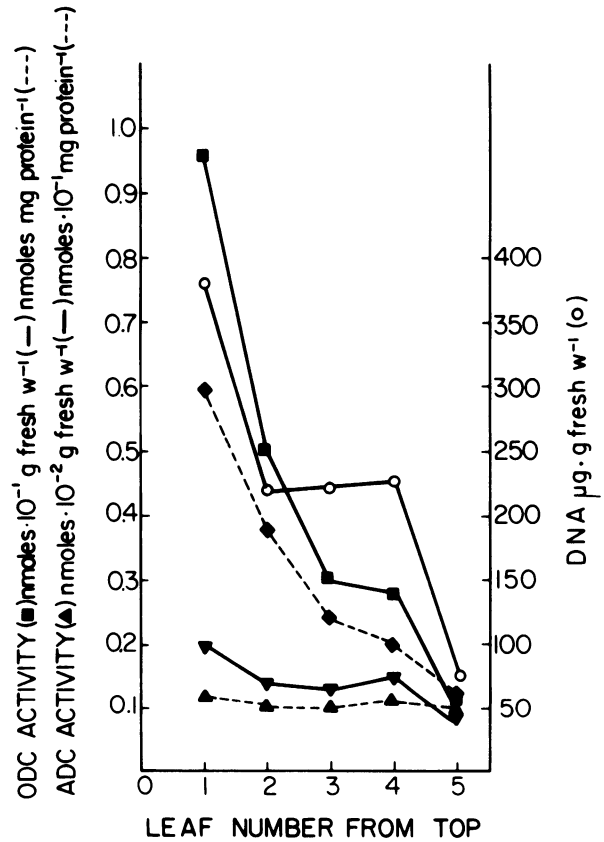

FIG. 1. ODC and ADC activities and DNA content in consecutive leaves of the tomato buds. Numeral 1 is the youngest leaf, while 5 is the oldest one. Reaction mixture contained: (a) 4.2 nmol L-[1-^{14}C]ornithine (59 µCi/µmol) or 4.1 nmol L[U-^{14}C]arginine (342 µCi/µmol) in 0.05 ml H_2O; and (b) 0.25 ml enzyme preparation in 0.025 M phosphate buffer (pH 8.0). Activity was expressed as nmol $^{14}CO_2$ released/h. Each experiment was done at least three times, yielding similar results. The figure represents a typical experiment.

[1] The work was performed in partial fulfillment of the requirements for the PhD thesis of E. C.

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[3] Abbreviations: ODC, ornithine decarboxylase; ADC, arginine decarboxylase.

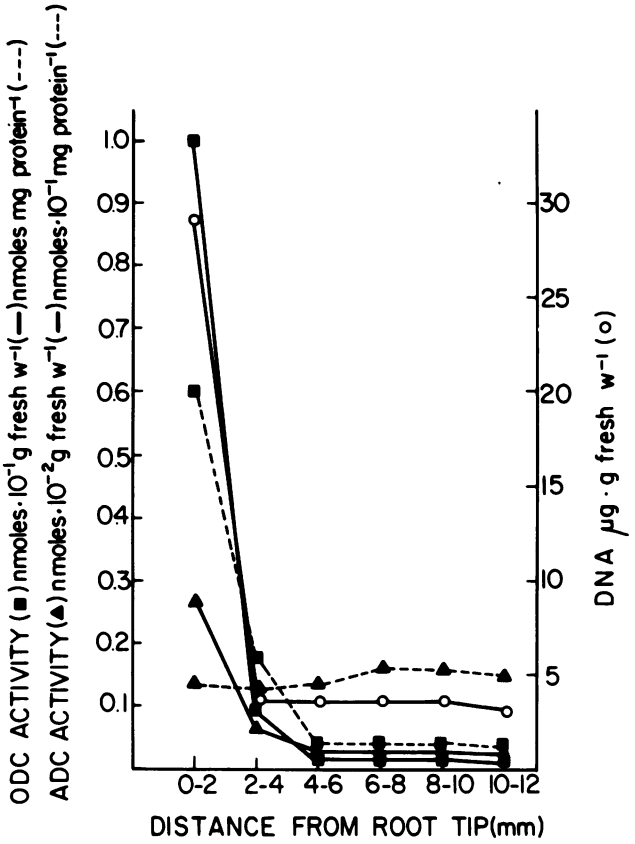

FIG. 2. ODC and ADC activities and DNA content in consecutive zones along the tomato root tip. Details of assay are given in Figure 1.

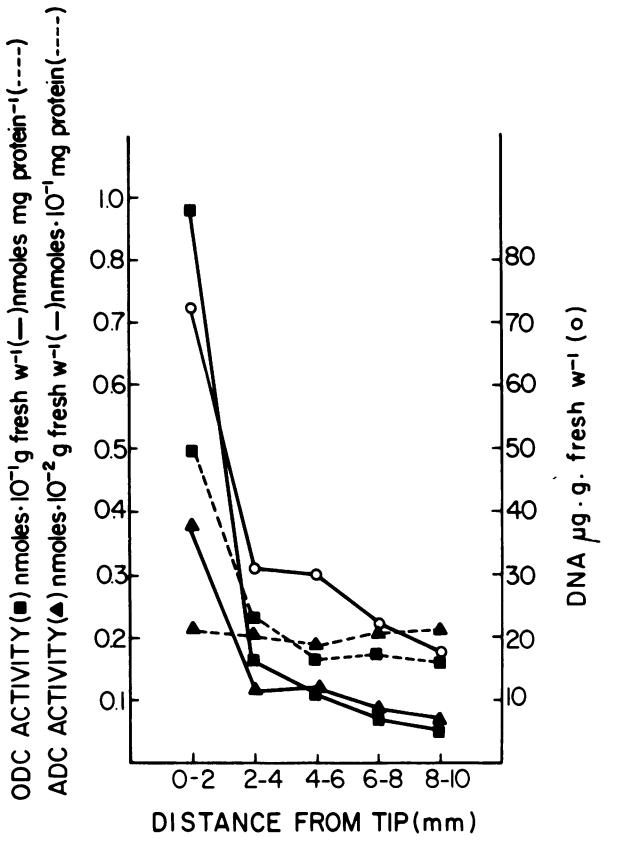

FIG. 3. ODC and ADC activities and DNA content in consecutive sections along the growing potato buds. Details of assay are given in Figure 1.

Tomato Bud Zoning. Leaf samples of 250 mg were collected from buds containing five leaves, the youngest being 1 to 2 mm in length. Each sample was made of the same leaves, numbered from the top, *i.e.* sample number 1 contained the youngest leaf, in which cell division still takes place (17).

Potato Bud Zoning. Twenty-mm-long buds were detached and sliced into 1-mm sections. Section number 1 was the bud tip. Five sections were removed from each bud, and 200-mg samples were used.

Assays. Enzyme activity and DNA content were determined in a 200- to 300-mg fresh weight tissue samples of both roots and shoots in the two plants studied. ODC and ADC were extracted from the various tissues and measured as already described (9) with some modifications. The extraction medium consisted of 0.25 M K-phosphate (pH 8.0), 25 µM pyridoxal phosphate, and 10 mM DTT. The samples were pulverized in an ice-cold mortar. The homogenate was centrifuged at 10,000*g* for 20 min at 0 to 4°C. The supernatant, dialyzed for 18 h against 0.025 M K-phosphate (pH 8.0), containing EDTA and DTT as in the extraction buffer, served as the enzyme preparation. All the above conditions were found optimal for ADC. ODC activity was somewhat higher under Tris-HCl buffer (8). However, since ODC yielded similar results in both buffers, the same extract (in phosphate buffer) was used for both enzymes for reasons of convenience. The precipitate was kept for DNA analysis since the supernatant did not contain measurable amounts of DNA. L-[1-^{14}C]Ornithine and L-[U-^{14}C]arginine served as the substrates for ODC and ADC, respectively. Activity is expressed as nmol CO_2 released/h on both fresh weight and protein basis. Protein and DNA were determined according to Bradford (5) and Giles *et al.* (7), respectively.

RESULTS AND DISCUSSION

In roots and vegetative buds, the meristematic regions are localized within the first 1 to 2 mm from the tip. This zone is characterized by its intense mitotic activity (17). Zones at increasing distances from the tips are characterized by cell expansion and differentiation and very little mitotic activity (17). Intense cell division also occurs in very young leaves (17). This type of distinctive mitotic zonation makes young leaves, root tips, and vegetative buds suitable model systems for testing our hypothesis that high ODC activity is expected in highly active mitotic zones.

Figure 1 presents ODC and ADC activity in tomato leaves with increasing leaf age as related to DNA content. The older the leaf, the lower was DNA content per g fresh weight, *e.g.* 72 µg/g in the fifth leaf from the top as compared with 377.2 µg/g in the first leaf from the top. Accordingly, 9- to 4-fold decrease in ODC activity was measured in the fifth leaf as compared with the first leaf on fresh weight and protein basis, respectively. ADC activity, however, was found to decrease only slightly when expressed on fresh weight basis, while no change was evident when calculated on protein basis. In tomato roots, the same trend was evident, *i.e.* from the root tip proximally, both DNA content (on fresh weight basis) and extractable ODC activity (on both fresh weight and protein basis) decreased sharply (Fig. 2). In contrast, ADC activity was found to change only slightly when calculated on fresh weight basis, being unchanged on protein basis (Fig. 2).

In potato buds (Fig. 3) and roots (Fig. 4) too, ODC activity was correlated with zones of intense mitotic activity, being the highest at the extreme 2 mm, where DNA content was the highest. ADC, on the other hand, decreased only slightly in cells removed from the tip, the decrease being evident only when the activity was expressed per fresh weight unit but not per protein unit (Figs. 3 and 4).

The elevated level of ODC in the meristematic regions may reflect a need for high levels of polyamines. This need may arise from the intensive synthesis of DNA, RNA, and protein typical of

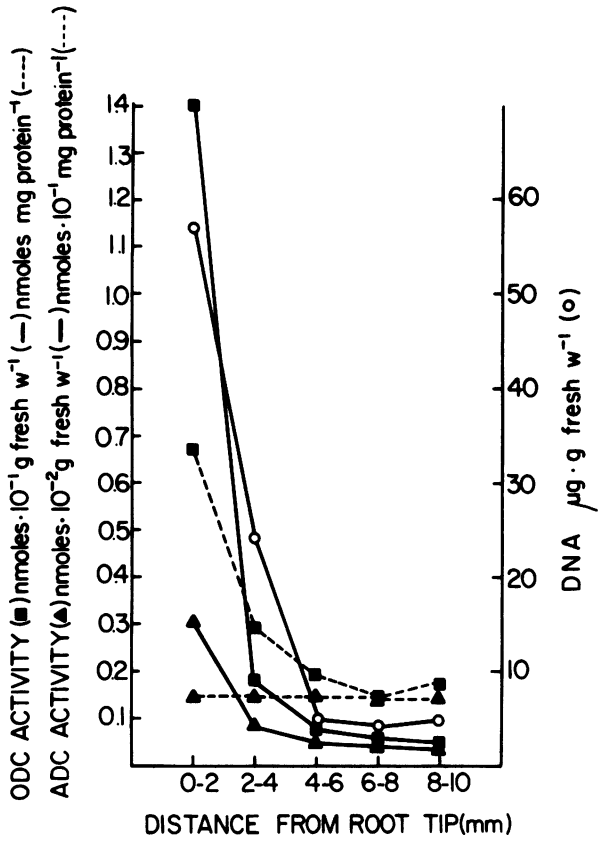

FIG. 4. ODC and ADC activities and DNA content in consecutive sections along the potato root tip. Details of assay are given in Figure 1.

mitotic cell. Such an association has already been pointed out (3, 4, 6, 10). The occurrence of high ODC activity in mitotic zones demonstrates that in some plants, as in animals, ODC plays an important role in polyamine synthesis associated with cell division. The existence of ADC in the same tissues raises the possibility that these two enzymes have different roles. ODC may be very active when large amounts of polyamines are required, *e.g.* when cells proliferate, while ADC is possibly active in biosynthesis of polyamines required for growth by expansion and differentiation or in synthesis of alkaloids from putrescine (14). The two enzymes, and also their products, may be compartmentized separately within the cells. Localization of these enzymes as well as the metabolism of the various polyamines will be the focus of future studies.

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