ORNITHINE DECARBOXYLASE ACTIVITY IN RAPIDLY PROLIFERATING PLANT CELLS

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1. Introduction

Polyamines are widely distributed in nature, but their precise role in cellular processes is not fully understood. Polyamines are associated with cell proliferation, tissue regeneration and malignancy [1–3]. Most of the information on the biosynthetic pathways of putrescine, spermine and spermidine, their regulation and possible sites of action has been obtained from studies with micro-organisms or mammalian cells. In mammalian cells, putrescine is synthesized from L-ornithine by L-ornithine decarboxylase (ODC EC 4.11.17) [2]. In plants, however, it is commonly accepted that putrescine is formed from L-arginine by L-arginine decarboxylase (ADC EC 4.11.19), via the intermediate agmatine. The activity of ODC in plants is usually found to be much lower than that of ADC [4–7], and ODC has therefore been claimed to be of little significance in the formation of putrescine in plants. We felt that in plant cells such an essential biosynthetic pathway of putrescine should not differ from that in mammalian cells and therefore decided to study ODC activity in plant systems.

We chose to search for ODC activity in two plant systems of rapidly proliferating cells – the XD cell line of tobacco in suspension culture and tomato ovaries before and after pollination. After subculture of XD cells there is usually a lag period of a few days, after which the culture enters an exponential phase, lasting for several generations [8]. The culture then enters a stationary phase. In tomato ovaries before pollination there is some cell division; after pollination there is very intense cell division, which lasts for 7 to 10 days [9]. We therefore felt that these two systems were particularly suitable for the study of ODC in plant cells.

2. Materials and methods

XD tobacco cells were grown as previously described on nitrogen-free M-ID medium supplemented with casamino acids (Difco, USA), 1.5 g/litre, as a nitrogen source [10]. Every 3 weeks the cells were subcultured into fresh medium. Tomato plants (*Lycopersicon esculentum* Mill F_1 hybrid, line 367234) were grown in a glasshouse under natural lighting conditions. Plants were trained to one stem. Flowers were hand pollinated at full anthesis and tagged. ODC activity was determined in ovaries and in developing and fully ripened fruits. Other parts of the plant, such as sepals and mature leaves, served as controls.

ODC was extracted and assayed as previously described [11], with some modifications. Tris–HCl (pH 8.0), 0.25 M and 0.1 M, was used as the extraction buffer for tomato tissues and tobacco cells, respectively. The concentration of dithiothreitol (DTT) was increased to 10 mM. Tobacco cells were homogenized in a Teflon-glass homogenizer. Tomato tissues were pulverized with a mortar and pestle. The homogenate was clarified by centrifugation at 10 000 × *g* for 20 min. The supernatant was then fractionated with ammonium sulphate. The fraction precipitated between 0–50% saturation of ammonium sulphate was collected, redissolved in the extraction buffer and dialysed for 18 h against

0.025 M Tris–HCl (pH 8.0) containing 1 mM DTT, 50 μM EDTA and 25 μM pyridoxal phosphate.

The stoichiometry of the decarboxylation of L-ornithine and the formation of putrescine was investigated with L-[1-^{14}C]ornithine and DL-[5-^{14}C]-ornithine, respectively, as the substrates. Putrescine was separated from ornithine by means of two separation systems: (a) the dansylated compounds in the reaction mixture were separated on a silica gel G thin-layer chromatography plate, with ethyl acetate/cyclohexane 2 : 3 as the solvent, and (b) the hydrochloride derivatives of the compounds in the reaction mixture were separated on What No. 1 paper by descending chromatography with methanol/pyridine/H_2O/acetic acid (6 : 6 : 4 : 1). ADC was investigated in a similar manner, with L-[U-^{14}C]arginine as the substrate. Activity is expressed as nmoles CO_2 released per hour. Protein was determined by the method of Lowry et al. [12].

3. Results and discussion

The growth of the XD culture and the ODC activity in the cells over a period of 16 days are shown in fig.1. There is a lag period of 2 or 3 days before the culture enters the exponential phase of growth during which the increase in fresh weight correlates well with the increase in cell number [8]. Between the 12th and 14th days the increase of fresh weight ceases, and the culture enters the stationary phase. The protein content of the cells begins to increase immediately after subculture and levels off on the 8th day. ODC activity also begins to increase after subculture, even before cell division takes place; it reaches a maximum between the 4th and 6th days and then declines. The peak activity is 30-fold higher than that at zero time when expressed on a gram fresh weight basis and 10-fold higher when expressed per mg of protein. The pattern of change in ADC activity resembles that of ODC, but the value of ADC activity is one tenth to one fourth of that of ODC activity.

Tomato fruits start to grow from day zero (flower at full anthesis) and reach their maximal size at the ripening stage (fig.2). During the first 10 days of development, both the fresh weight and the protein content in the soluble fraction containing the ODC increase exponentially, in a manner resembling that

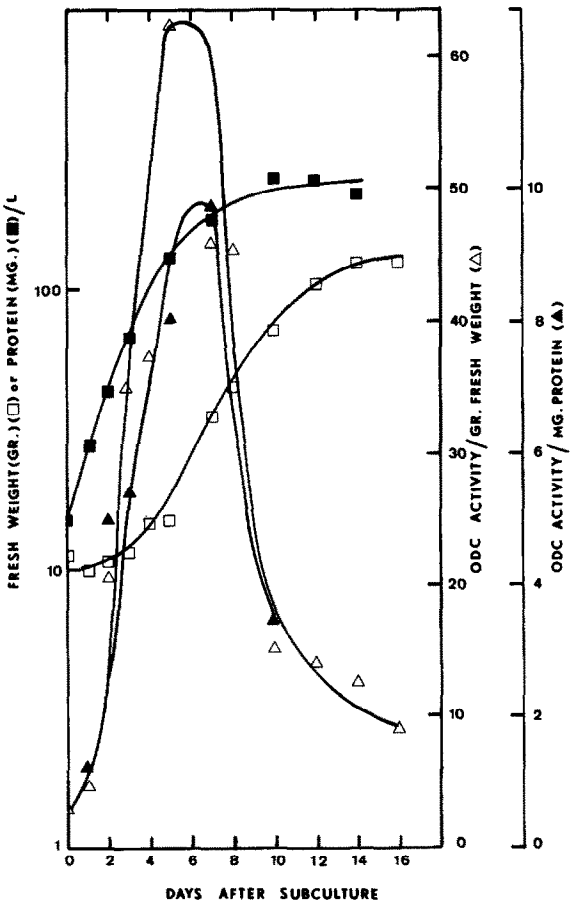

Fig.1. Growth of XD tobacco cells in suspension culture and ODC activity during the growth period. Cells of a 3-week-old culture were subcultured into fresh medium. Fresh weight, protein content in the soluble fraction and the ODC activity of the cells were determined. Fresh weight (g/litre) (□); protein (mg/litre) (■); ODC activity/fresh weight (△); ODC activity/mg protein (▲).

of a cell culture (fig.2). The increase in fresh weight and protein content levels off at the ripening stage. ODC activity (on both a protein and a fresh weight basis) increases rapidly and reaches a peak on the 3rd day after pollination at the same time as maximal cell division activity occurs in the setting fruit [9]. In the other stages of fruit development, during which growth is largely a result of cell enlargement, ODC activity is at least 37 times lower than the peak value. The ODC activity in other plant organs and in old non-pollinated ovaries is very low (table 1). The

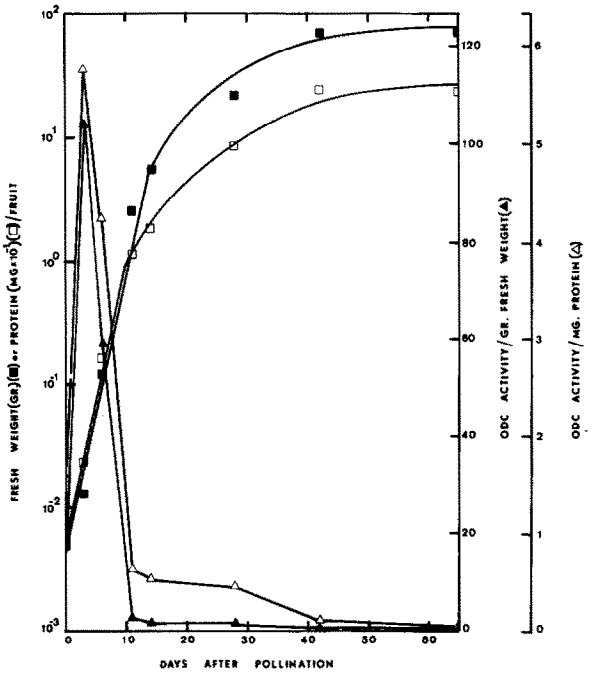

Fig.2. Development of tomato fruits and ODC activity in the ovaries before pollination and in the developing fruits. Fresh weight, protein content and the ODC activity of the ovaries or fruits were determined. Fresh weight (g/ovary or fruit) (■); protein (mg/ovary or fruit) (□); ODC activity/g fresh weight (▲); ODC activity/mg protein (△).

activity of ADC determined in developing fruits 4 days after pollination is 5% of that of ODC.

With ODC from XD cells, the yield of putrescine from L-ornithine was found to be 90% of the theoretical amount.

It is tempting to hypothesize that the increase in ODC activity shortly before or at the onset of cell division in both XD cells and tomato ovaries is, in fact, associated with the cell division. It should be stressed that the ODC activity is relatively low in mature fully expanded tomato leaves (with metabolically active but non-dividing cells) and in resting tobacco cells as well as in fully ripe tomato fruits. The apparent association of elevated ODC activity with cell division is in accordance with that observed in mammalian cells and micro-organisms [1–3]. The results of this study are in contrast with reports in the literature claiming that in plants the main route for putrescine production is via ADC. The failure of other investigators to observe high ODC activities in their experimental systems [4–7] can be attributed to various factors: the use of different plant species or of tissues in which intensive cell division does not take place or the employment of a different methodology for extracting the enzyme.

Table 1
ODC activity in various tissues of the tomato plant

Tissue	ODC activity	
	nmol/g fresh weight	nmol/mg protein
Flowers at full anthesis	19.1	1.6
Old non-pollinated ovaries	5.1	0.7
Sepals from flowers	12.3	0.7
Sepals from old fruits	1.3	0.3
Fully expanded mature leaves	1.9	0.1

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