J Plant Growth Regul (1983) 2:177–184

Journal of
Plant
Growth
Regulation

Polyamine Titer and Biosynthetic Enzymes During Tuber Formation of *Helianthus tuberosus*

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Received May 19, 1983; accepted July 29, 1983

Abstract. During the formation of *Helianthus tuberosus* tubers the activities of arginine decarboxylase (ADC) and S-adenosylmethionine decarboxylase (SAMDC), examined in medullary parenchyma cells, increase with the increase in weight of the tuber. The ornithine decarboxylase (ODC) activity is about 100-fold less with respect to ADC activity, and it was detected only during the deceleration phase of the growth curve. Spermidine and spermine content are strictly related to the SAMDC activity and tuber growth. The increase of ADC and SAMDC activity is directly related to cell extension and increase in weight. The limited area of cell division in parenchyma tissue found during the first stage of tuber formation could justify the low ODC activity. The data suggest that ADC affects mainly growth processes, while ODC seems to be preferentially related to cell division.

The tuber of *Helianthus tuberosus* has been extensively studied in relation to different physiological phenomena occurring during dormancy and break of dormancy (see, e.g., Courduroux 1967, Courduroux et al. 1972, Serafini-Fracassini et al. 1980, Bagni et al. 1981a). The fast reaction of cells of homogeneous medullary parenchyma of tuber represents one of the best models from which to study growth and its regulation by using different growth substances and specific inhibitors (Bagni et al. 1981b, Bagni et al. 1983). However, growth at the natural or artificial break of dormancy induced by an exogenous hormone is conditioned by all the events occurring during tuber formation, particularly those influenced by environmental conditions, as demonstrated, e.g., by the

levels of polyamines in different years, one class of plant growth substances studied in our laboratory (Cocucci and Bagni 1968, Bagni et al. 1980). In a previous work, the ribosomes and their qualitative (monosome-polysome) variations and quantitative variations over a whole vegetative period of the tuber of *Helianthus tuberosus* were investigated. In particular during flowering, tuber polysomes disappeared almost completely and rRNA decreased in comparison with the level present at the beginning of tuber formation. After flowering, there was a new synthesis of monosomes and polysomes until the onset of dormancy (Bagni et al. 1972).

After the levels of aliphatic polyamines during dormancy had been studied, their importance in the induction of natural or artificial break of dormancy and their effect on the different macromolecular syntheses during the cell cycle (Bagni et al. 1980, Serafini-Fracassini et al. 1980), in the present paper we have investigated the levels of polyamines and the activitiy of ODC, ADC, and SAMDC during tuber formation and up to dormancy.

Materials and Methods

Plant Material

Helianthus tuberosus L. (Jerusalem artichoke) cv. OB1 was grown by vegetative reproduction in the Botanical Garden of Bologna University for several years. Three plants corresponding to 100 tubers were harvested for each of eight samples, and the fresh weight of single tubers for the preparation of a growth curve was determined. Ten g of medullary parenchyma from each sample was utilized to determine polyamine and protein contents and enzyme activities. The first sampling (September 13), consisting of stolons before the onset of growth, was utilized entirely.

Polyamine Analysis

Polyamines were extracted, separated, and detected by the method of direct dansylation described by Smith and Best (1977), using TLC precoated plates of silicagel 60 with concentrating zone (Merck). Ethylacetate-cyclohexane 2:3 (v/v) was used as the solvent. Spots were scraped from the plates, extracted in pure acetone on a Vortex mixer, and centrifuged; fluorescence was measured using a Jasco FP-550 spectrofluorimeter (emission 505.5 nm, excitation 360 nm) and the results compared with dansylated standards.

pH-Optimum of ADC and ODC Activities

In preliminary experiments, the pH-optimum requirements for ADC and ODC in crude extracts from slices of *Helianthus* tuber activated for 12 h, as described by Bagni et al. (1983), were determined.

A range of pH 7.1 to 8.9 Tris buffer was used. ADC activity showed a maximum at pH 8.3 and decreased sharply above and below pH 8.3. This pH-

optimum is in accordance with the enzyme from *Lathyrus sativus* seedlings, which had an optimum of pH 8.5 (Suresh and Adiga 1977), with *Chlorella,* which showed a maximum at pH 8.5 (Cohen et al. 1983), and with the biosynthetic enzyme from *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae,* which showed an optimum at pH 8.25 (Kallio et al. 1981).

ODC activity showed a maximum at pH 8.7 and decreased rapidly with decreasing pH. Only *Escherichia coli* showed a similar pH-optimum for ODC (8.25 in Tris-HCl buffer) (Kallio et al. 1981).

Extraction and ADC and ODC Assays

Tuber tissue was homogenized in an Omni Mixer at 180 rpm in the assay buffer containing 50 μM ethylenediaminetetracetic acid; 25 μM pyridoxal phosphate; 2.5 mM 1,4-dithiothreitol; 25 mM TRIS–HCL, pH 8.3 for the ADC assay, and pH 8.7 for the ODC assay. It was then centrifuged at 20,000 x *g* for 10 min at 0°C. ODC activity was determined by incubating 0.5 ml of the supernatant, previously filtered through filter paper, with 14.8 KBq in 20 μl of L-[1-^{14}C]ornithine (1.93 GBq/mmol). The ADC activity was determined in the same manner except for the labeled compound, which in this case was 14.8 KBq in 20 μl of L-[U-^{14}C]arginine (12.43 GBq/mmol). The test tubes containing the reaction mixture were capped with special rubber stoppers fitted with center wells, each containing 0.2 ml of Protosol (NEN). The reaction was allowed to proceed for 2 h at 37°C in a water bath and was terminated by the injection of 0.2 ml of 6% perchloric acid. After an additional 60 min of shaking, the Protosol was removed from the center well and placed in a scintillation counter (Isocap/300, Nuclear Chicago).

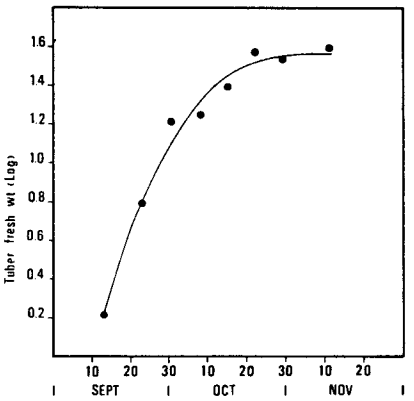
In preliminary experiments, the enzyme activities were linear with increasing concentrations of crude enzyme and with time of incubation up to 120 min. No differences were found in ODC and ADC activity in the assay with carrier added at 1 mM with respect to the assay without carrier; this is probably due to the large pool of endogenous amino acids in the crude extract. The ODC activity in the precipitate after 20,000 x *g* centrifugation determined after sonication showed that only 30% of the activity was found there.

Higher efficiency in capturing CO_2 was obtained with Protosol with respect to Hyamine. Each sample was repeated five times. Data are presented as the mean and standard errors were calculated. Proteins in the supernatant crude enzyme were determined by the method of Lowry et al. (1951) after precipitation with 7.5% trichloroacetic acid and solubilization in 1N NaOH.

Extraction and SAMDC Assay

SAMDC activity was assayed following the method of Suresh and Adiga (1977). Tuber tissue was homogenized in an Omni Mixer at 180 rpm in the assay buffer containing 5 mM 1,4-dithiothreitol; 5 mM Mg^{++}; 0.1 mM pyridoxal phosphate; 0.2 M Tris-HCl pH 7.6, and then centrifuged at 20,000 × *g* for 10 min at 0°C.

The SAMDC activity was determined in the same manner as ADC and ODC



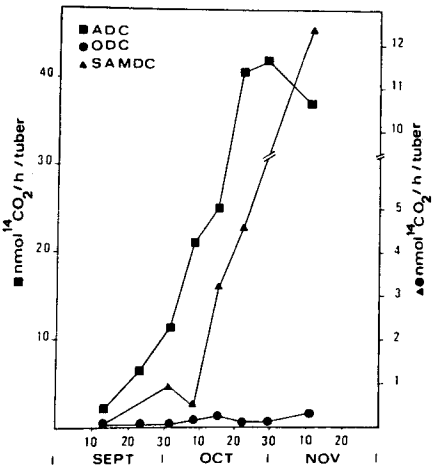


Fig. 1. Growth curve of tuber formation of *Helianthus tuberosus*. **Fig. 2.** Arginine, ornithine, and S-adenosylmethionine decarboxylase activity during tuber formation of *Helianthus tuberosus*. The standard errors were omitted because their values were too small for the scales used.

activity except for the labeled compound, which in this case was 9.25 KBq in 20 µl of S-adenosyl-L-[carboxyl-^{14}C]methionine (2.22 GBq/mmol). Each sample was repeated five times. Data are presented as the means and standard errors were calculated.

In preliminary experiments, the assay buffer was controlled, and about 2-fold higher efficiency in capturing CO_2 was obtained with the method of Suresh and Adiga (1977) with respect to the method of Suzuki and Hirosawa (1980), who used Tris-HCl pH 8.6 in the assay buffer.

Results

Fig. 1 shows the growth curve of tuber formation in 1982. It was not possible to reveal any correlation between tuber growth and flowering because of three different thunderstorms during the first period of tuber growth (September 1–20) that broke many flower buds and young stems supporting flower buds.

Therefore in 1982 there was no decrease in tuber growth rate corresponding to the flowering period noted in previous years in the same cultivar (Bagni et al. 1972). If we express the data as nmoles of $^{14}CO_2$/h/tuber, considering all the tuber as medullary parenchyma cells without cortex and cambial zones, the activity of ADC is present until the first stage of tuber formation, while ODC can be detected later, showing a very low peak of activity and only during the deceleration phase of growth.

The ADC activity is about 100-fold higher than ODC activity during tuber formation. The activities of ADC and SAMDC increase rapidly with the increase of tuber growth (Figs. 1, 2). A similar pattern, but more pronounced, is shown if we refer the data of ODC and SAMDC activities to mg protein or to g fresh weight; the pattern of ADC seems to be unrelated to the growth curve during the deceleration-stationary phase.

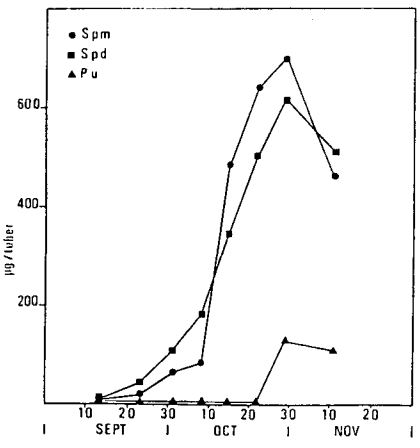


Fig. 3. Spermine (Spm), spermidine (Spd) and putrescine (Pu) contents during tuber formation of *Helianthus tuberosus*. The standard errors were omitted because their values were too small for the scales used.

The presence of putrescine in traces during the greater part of tuber formation is probably due to its utilization for spermidine and spermine synthesis, as shown by their increasing concentration during the end of the exponential-deceleration phase (Fig. 3) as well as by increasing activities of ADC and SAMDC.

Discussion

Recent papers show that in many plant systems the increase in growth occurs parallel to the enhancement of ODC and/or ADC activity (Heimer et al. 1979, Montague et al. 1979, Bagni et al. 1983, Cohen et al. 1983, Kaur-Sawhney et al. 1982). SAMDC shows a similar trend in some cases.

A good correlation can be shown between SAMDC, spermine, and spermidine levels and *Helianthus* tuber formation (Figs. 1–3) during the deceleration phases. The SAMDC activity and polyamine accumulation exhibit a pattern similar to the rRNA accumulation previously studied in the same conditions (Bagni et al. 1972). In *Helianthus* tuber, in spite of the high endogenous level of arginine, the main nitrogen storage substance, and the high ADC/ODC ratio in dormant tuber, ODC appears to be very rapidly induced when cell division in the tissue is stimulated by auxin (Bagni et al. 1983). This shows a generally high correlation between meristematic activity, polyamine content, and activity of polyamine biosynthesis (Serafini-Fracassini et al. 1980). However, during tuber formation and until dormancy, ADC is the main enzyme for putrescine biosynthesis, and its level seems to correlate with tuber growth (Figs. 1, 2). We do not consider the slight increases of ODC during the deceleration phase of tuber growth to be particularly relevant, because this activity is about 100-fold less than ADC activity (Fig. 2).

A possible explanation involves the fact that only during the first stage of tuber formation is there an active cell division in a limited area of medullary parenchyma, between September 13 and 23, immediately followed by cell extension, as has been verified with microscopic sections (see Table 7). Further-

Table 1. Surface area, % of meristematic area, and % dry weight of medullary parenchyma during *Helianthus* tuber formation.

Sample	μm^2/cell[a]	% of meristematic area	% dry weight
September 13	8643 a[b]	20	17
September 23	10697 b	0[c]	23
October 1	10398 b	0	23
October 8	10000 b	0	23.4
November 11	9500 b	0	24.2

[a] Average calculated on 100 cells.
[b] The difference between the values indicated by the same letter is not significant at the 5% level.
[c] Tracheid differentiation.

more, the increase in dry weight (about 30% between September 13 and November 11), could partly be accounted for by a great synthesis of storage substances, notably arginine and glutamine (Bagni et al. 1980). We have not taken into consideration the increase in weight due to the activity of the two cambial zones of the tuber, which were not utilized in our experiments.

The low ODC activity could be explained by a low and limited cell division in the parenchyma tissue examined (see Table 1). Recently Dai and Galston (1981) reported that ADC activity in buds and epicotyls of etiolated Alaska pea seedlings was altered in opposite directions following conversion of phytochrome from Pr to Pfr; therefore, in some specific cases, ADC activity cannot be related to growth processes. Also, in fourth internodes of Progress pea seedlings, GA_3, which is known to induce a dramatic stem elongation, induced ADC and inhibited ODC activity (Dai et al. 1982).

ADC activity greater than ODC activity has also been measured in epicotyls of Mung and French bean and cotton (Altman et al. 1982). In oat leaf cells and other Gramineae exposed to osmotic stress, ADC activity increased parallel to putrescine, whereas ODC remained unchanged (Flores and Galston 1982). Similar responses were previously observed under potassium deficiency in oat (Smith 1963, 1979) and upon acid treatment in barley (Smith and Sinclair 1967).

Greater ODC than ADC activity was found in many plant systems in active cell multiplication, such as tomato ovaries after pollination, tobacco cells in suspension (Heimer et al. 1979), parenchyma cells of *Helianthus* tuber after break of dormancy (Bagni et al. 1983), and sprouting potato tuber (Kaur-Sawhney et al. 1982). In animal systems ODC is considered the key enzyme for cell multiplication and growth (see review by Heby 1981). In other plants, however, ADC was predominant, such as in embryogenic cells of *Daucus carota* during cell proliferation (Montague et al. 1979). We have not taken into consideration seed germination because of the difficulty in separating cell division from cell enlargement. Undoubtedly many factors in addition to tissue and/or plant specificity can affect the activities of ADC and ODC and their regulation in plants.

In conclusion, our present results and those of papers cited in the discussion

could suggest that in plants ODC is the key enzyme preferentially involved in cell division, while ADC is involved in the growth processes mainly for cell extension and increase in dry weight, in addition to phytochrome control, osmotic and acid stress, and potassium deficiency.

Our results and the few data available on SAMDC activity (Kaur-Sawhney et al. 1982) suggest a good correlation with growth processes.

Acknowledgment. This research was supported by funds from Ministero della Pubblica Istruzione, Italy.

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