Free Polyamine Contents and Decarboxylase Activities during Tomato Development and Ripening

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The levels of free polyamines (PAs) and the activities of arginine decarboxylase (ADC), ornithine decarboxylase (ODC), and *S*-adenosylmethionine decarboxylase (SAMDC) were measured in pericarp tissues of tomato fruit (*Lycopersicon esculentum* cv. Indalo) during development and ripening. Putrescine (Put) was the predominant PA during the early stages of growth (less than 1 g weight), while contents of spermidine (Spd) and spermine (Spm) were lower. The PA concentration clearly increased at the early stage of development, reaching a peak in fruits weighing 10 mg. ADC, ODC, and SAMDC activities also all peaked in fruits with 10 mg of fresh weight. The initial increase in PA levels occurred concomitantly with a rise in these decarboxylases. During ripening, levels of Spd declined and no pronounced changes in Put occurred. Given that these variations did not reflect decarboxylase activities, which increased slightly at the pink stage, PA catabolism or conjugation might be involved in the ripening of this tomato variety.

Keywords: *Fruit development; fruit ripening; Lycopersicon esculentum; putrescine; spermidine; spermine; postharvest*

INTRODUCTION

Polyamines (PAs) are involved in several facets of plant development and are particularly associated with continued cell division and the prevention of senescence (Slocum et al., 1984). The main polyamine biosynthetic enzymes in plants are ornithine decarboxylase (ODC), arginine decarboxylase (ADC), and *S*-adenosylmethionine decarboxylase (SAMDC). Putrescine (Put) is synthesized by either ADC or ODC, and its relative importance varies in different systems. SAMDC is related to spermidine (Spd) and spermine (Spm) biosynthesis. High polyamine biosynthetic enzyme activity characterizes rapidly proliferating tissues (Smith, 1985).

Fruiting and endogenous PA contents are closely related, and PA levels seem to be involved in fruit development (Kakkar and Rai, 1993). In 1982, Cohen et al. described an association between elevated ODC activity and rapid cell proliferation in tomato ovaries (*Lycopersicon esculentum* Mill. cv. Pearson ms-35) during the first 10 days after pollination. In this variety, further evidence of this association was provided by the use of ODC inhibitors (Cohen et al., 1982). Conversely, in developing F121 tomato fruit most of the PAs were found as conjugates during cell division. The concentration of PAs was very low after cell division and during ripening, the concentration of the free forms being higher than that of the other fractions (Egea-Cortines et al., 1993).

Ripening starts after the fruit reaches its final size and is triggered and modulated by ethylene. It involves the formation of specific compounds (proteins, pigments, flavor components, etc.) and the degradation of chlorophyll, cell walls, and alkaloids. The ripening process ends with the senescence of the organ (Brady, 1987). In pericarp from normal ripening tomato cultivars (Pik Red, Alcobaca-Red, Rutgers), ADC and ODC decrease throughout the final stages of fruit maturation and ripening, the levels of soluble and wall bound polyamine conjugates are very low or undetectable, and Put and Spd levels decline between the immature and mature green stages, remaining low thereafter. The Spm content is always very low (Saftner and Baldi, 1990; Rastogi and Davies, 1991). Recently, a translational or posttranslational regulation of ADC gene expression has been described during tomato fruit ripening (Rastogi et al., 1993).

SAMDC activity has been studied in the mature floral organs of a tomato mutant with abnormal stamens and naked ovules (Rastogi and Kaur-Sawhney, 1990), but it has never been reported in tomato fruits. Moreover, levels of PAs, ADC, ODC, and SAMDC have not been recorded simultaneously through both development and ripening in any tomato line. This paper describes the changes in decarboxylase activities and free PAs levels during tomato development and ripening.

MATERIALS AND METHODS

Seedlings of tomato (*Lycopersicon esculentum* cv. Indalo) were transplanted into loamy clay soil in a greenhouse located in Seville. Plants were grown under natural irradiance and receiving a water delivery rate of 315−380 L m^{-2} $month^{-1}$. The day/night temperatures were 35.8/15.3 °C. Fruits at various stages of development and ripening were harvested, being classified according to their weights (inmature tomatoes) or graded as described by Ryall and Lipton (1972) for ripening tomatoes: immature green, mature green, breaker, turning, pink, light red, and red. The number of tomatoes per each development stage (randomly collected from different plants) decreased with fruit growth: about 300 fruits were taken at ≤0.01 g of fresh weight stage, 60 fruits at 0.1 g of fresh weight and decreasing progressively until at 30 tomatoes per sample at the highest size. Each group of collected fruits was divided in three homogeneous samples. Fruits of each sample were sliced, and pieces from cut pericarp were destined half to PA analysis and half to decarboxylases assays. Data presented in figures and tables are the mean of three replicates.

Polyamine Analysis. Free polyamine analysis was performed as already described (Smith and Davies, 1987) with some modifications. Pericarp tissue was homogenized in an ice-cold potter in 5% $HClO_4$ (0.1 g of tissue/mL of acid). Hexanediamine at 0.05 μmol/g of fresh weight tissue was added to the extracts as an internal standard. After 1 h on ice, the homogenates were centrifuged at 26890*g* for 30 min at 4 °C. The supernatants were derivatized immediately for polyamine analysis: 0.1 mL aliquots of the supernatant were

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added to 0.2 mL of saturated Na_2CO_3 and 0.4 mL of dansyl chloride in acetone (5 mg/mL) in a 3 mL tapered amber reaction vial. The mixture was incubated in a water bath at 60 °C for 1 h in the dark. Excess dansyl chloride was then removed by adding 0.1 mL of proline in H_2O (0.1 g/mL) to the mixture. The mixture was sonicated at room temperature for 4 min in the dark. The polyamines were then extracted with 0.5 mL of ethyl acetate and vortexed vigorously for 30 s. The organic phase was dried completely under N_2. The polyamine residue was dissolved in 0.3−0.1 mL of 60% acetonitrile, depending on the sample, filtered through Lida (Kenosha, WI) PTFE filters (0.2 μm pore), and assayed inmediately. HPLC analysis of dansylated PAs was done with a LDC analytical liquid chromatograph (Riviera Beach, FL). The solvent system consisted of a water−acetonitrile gradient increasing from 60 to 100% acetonitrile in 23 min at a flow rate of 0.6 mL/min. Dansylated samples (5 μL) were eluted at 37 °C through a reversed-phase Spherisorb column (Sugelabor, C_{18}, 25 × 0.46 cm, 3 μm particle size) and detected by an on-line 821-FP-fluorescence detector (Jasco, Tokyo, Japan; excitation wavelength, 325 nm and emission wavelength, 490 nm). The peak areas were recorded on 3390 A integrator (Hewlett-Packard, Avondale, PA). For quantification, a relative calibration procedure was used (Smith and Davies, 1985).

Enzyme Extraction and Assay. ODC, ADC, and SAMDC were determined using a modification of several procedures (Mizrahi and Heimer, 1982; Cohen et al., 1982; Rastogi and Kaur-Sawhney, 1990; Rastogi and Davies, 1991). For extraction, 0.5−1 g (fresh weight) of pericarp tissue was ground in an Omnimixer in 10 mL of extraction medium consisting of 0.1 M sodium phosphate (pH 7), 1 mM DTT, 5 mM EDTA, and 0.1 mM pyridoxal phosphate. The cold homogenate was clarified by centrifugation at 26890*g* for 20 min at 0−4 °C. All the above conditions were found optimal for assaying the three activities simultaneously in only one extract by measuring the decarboxylation of L-[1-^{14}C]ornithine, L-[1-^{14}C]arginine, and *S*-adenosyl-L-[*carboxy*-^{14}C]methionine for ODC, ADC, and SAMDC, respectively. For the ADC assay, the reaction mixture consisted of 190 μL of crude enzyme preparation and 10 μL of 108 μCi/mL DL-[1-^{14}C] arginine (46 mCi/mmol; Dositek, Orsay, France) diluted 1:5 in 10 mM unlabeled L-arginine. For ODC and SAMDC assays, 10 μL of 100 μCi/mL DL-[1-^{14}C]ornithine (58.4 mCi/mmol; NEN, Dreieich, Germany) diluted 1:5 in 50 mM unlabeled L-ornithine and 10 μL of 20 μCi/mL *S*-adenosyl-L-[*carboxy*-^{14}C] methionine (47.8 mCi/mmol; NEN) were used respectively. The incubation was carried out at 37 °C for 45 min in small glass vials with rubber stoppers pierced by a pin on which a piece of filter paper impregnated with 15 μL of 4 N potassium hydroxide was suspended; the reaction was stopped by injection of 0.2 mL of 10% $HClO_4$. The vials were opened after a further 45 min and the filter papers placed in scintillation vials. Blanks containing boiled enzyme extracts were used. The soluble protein was assayed according to Bradford (1976). Activity is expressed as pmol of $^{14}CO_2$ released h^{-1} (mg of protein)$^{-1}$.

Quality evaluations were performed at two opposite sites around the equator of fruits in 10 tomatoes per sample and three samples per ripening group.

Firmness. Resistance of the flesh to deformation was measured with a Zwick 3300 densimeter (Zwick Gmbh & Co., Ulm, Germany) with a 5 mm diameter disk (force required to depress the disk 2.4 mm into the fruits), and the result expressed in N/cm^2.

Color. The color of the fruits was determined using the *L*a*b** color spacing system with a CR200 Minolta chromameter (Minolta, Osaka, Japan) with an 8 mm diameter measuring aperture, diffuse illumination, C light source and an angle of vision of 0°.

RESULTS AND DISCUSSION

The levels of the three PAs, Put, Spd and Spm, increased during the initial stages of Indalo tomato fruit development (Figure 1), with a peak at 10 mg of fresh weight (1800, 600, and 500 nmol/g FW, respectively).

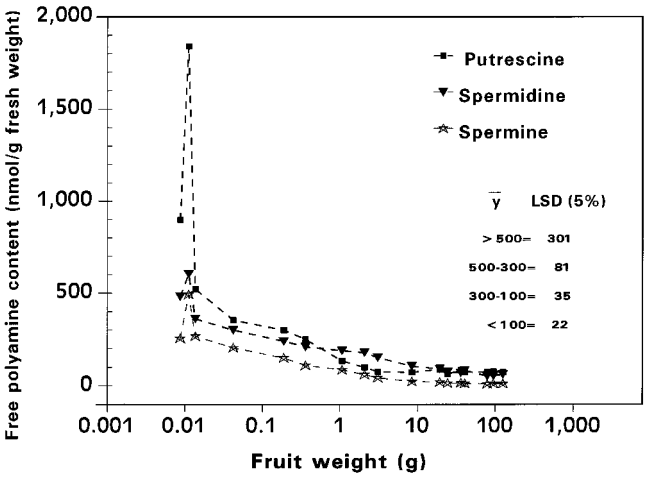

Figure 1. Free polyamine contents during tomato fruit development. Least significant differences at $P \leq 0.05$ were calculated for the different mean values of free polyamine contents.

Put was consistently the major polyamine in ≤0.1 g fruits, whereas for F121 tomato, Spd showed a higher level than the other PAs (Egea-Cortines et al., 1993). The concentration of free PAs declined sharply after the peak and continued decreasing throughout development up to ripening, reaching 75, 60, and 10 nmol/g FW for Put, Spd, and Spm, respectively. In the F121 tomato, pollination induced an increase in the concentration of free Spd and Spm (Egea-Cortines et al., 1993). Previously, it has been demonstrated that in apple, pear, and Rutgers tomato free PA levels decrease during fruit development (Biasi et al., 1988; Toumadje and Richardson, 1988; Saftner and Baldi, 1990). Egea-Cortines et al. (1993) also found very low contents of PAs after cell division in the F121 tomato pericarp. In this context it may be noted that growth resulting from cell expansion is not sensitive to PA biosynthesis inhibitors in several systems. As Egea-Cortines and Mizrahi (1991) suggested, PAs might have a specific role in cell division and not act as cofactors of general cell processes.

The changes in the levels of the three decarboxylase activities assayed, ADC, ODC, and SAMDC, paralleled those of the PA levels (Figure 2): The highest levels were reached practically at the beginning of fruit development, when there is very intense cell division (Heimer et al., 1979). Maxima coincided with the 10 mg of fresh weight stage. At this point ODC and SAMDC showed similar specific activities [2200 and 2100 pmol of $^{14}CO_2$ h^{-1} (mg of protein)$^{-1}$, respectively], while ADC was much lower [1300 pmol of $^{14}CO_2$ h^{-1} (mg of protein)$^{-1}$]. Subsequently, the activities decreased very sharply to values lower than 500 pmol of $^{14}CO_2$ h^{-1} (mg of protein)$^{-1}$ and maintained this low level until the fruit reached the mature weight. ODC activity was higher than that of ADC from the beginning of fruit development until the developmental stage corresponding to 1 g of fruit weight. From this point, growth is largely a result of cell enlargement and both activities showed very similar low values. These results agree with those obtained for ODC activity in other tomato varieties (Heimer et al., 1979; Mizrahi and Heimer, 1982; Cohen et al., 1982; Apelbaum, 1990).

ADC appears to be the primary enzyme for Put synthesis in nondividing mature tissues and in plant tissues subjected to environmental stress (Evans and Malmberg, 1989). Nevertheless, in avocado and tomato, a correlation exists between high ADC activity and rapid

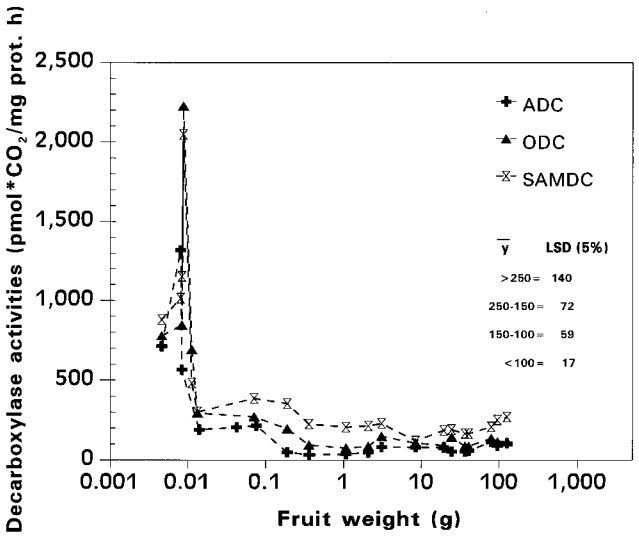

Figure 2. Decarboxylase activities during tomato fruit development. Least significant differences at $P \leq 0.05$ were calculated for the different mean values of decarboxylase activities.

cell proliferation in the early stages of fruit growth. During development of these fruits, the pattern of change in ADC activity resembled that of ODC (Kushad et al., 1988; Heimer et al., 1979). Moreover, in the Rutgers tomato, which also contains the diamine cadaverine, a relatively high ODC activity is prevalent in young fruits compared with ADC and lysine decarboxylase, an enzyme related to cadaverine biosynthesis (Apelbaum, 1990). The emphasis of all these references is on the activities of ADC and ODC, both involved in Put biosynthesis. Nevertheless, SAMDC activity, whose product, decarboxylated *S*-adenosylmethionine (dSAM), serves as an aminopropyl donor in the synthesis of both Spd and Spm, has not been followed through development and ripening in any tomato line. The results of our study demonstrate that not only ODC and ADC activities but also that of SAMDC are correlated with changes in PA levels during growth associated with cell division. These enhanced decarboxylase activities in lower weight fruits may account for the high PA concentrations present in this period. As far as we know, this is the first work describing the involvement of SAMDC, together with ADC and ODC, in tomato fruit development.

The elevated level of decarboxylase activities in young tomatoes may reflect a need for high levels of PAs for the intensive synthesis of DNA, RNA, and protein typical of mitotic cells. PAs have been shown to have stimulatory effects on DNA methylation, and RNA and protein synthesis (Slocum et al., 1984), and DNA synthesis seems to be PA dependent (Egea-Cortines and Mizrahi, 1991). PAs appear to promote the negative supercoiling of intracellular DNA necessary for DNA replication (Altman et al., 1982), stabilize double-helicoidal structures (DNA, stems and loops in rRNA and mRNA, tRNA conformation) (Heby and Persson, 1990), and control the formation and maintenance of functional polysomes and the binding of aminoacyl tRNAs to ribosomes (Slocum et al., 1984; Tabor and Tabor, 1984; Pegg, 1986).

During fruit ripening the polyamine levels were low as compared to the cell division stages (Table 1). There was no pronounced change or difference in Put levels (60−80 nmol/g of fresh weight). The levels of Spd declined during ripening from 60 nmol/g of fresh weight at the mature green stage to 37 nmol/g of fresh weight at the mature red stage. Lower and slightly decreasing contents of Spm were also observed. The Put and Spd concentrations found in this study are similar to those described for other normal-ripening tomato fruits, such as Rutgers, Pik Red, and Alcobaca-Red. However, Spm levels were higher in the present study as compared with the barely detectable levels found in the other tomato varieties (Dibble et al., 1988; Saftner and Baldi, 1990). Casas et al. (1990) suggested that the decrease of Spd and Spm may be a marker for the beginning of ripening in the tomato Lorena, but our results show that Spd decreased when fruits changed from breaker to turning stages, and for Spm, the levels diminished later on the ripening process (from pink to light red tomatoes).

The activity of ODC was greater than that of ADC, mainly from the initial stages of fruit ripening to the development of light red fruits (Table 1). Rastogi and Davies (1991) made a similar observation in the Rutgers tomato. The activities of ADC, ODC, and SAMDC did not reflect the changes in PA levels: From low levels, they rose a little in the pink fruit and fell again in the mature red fruit to levels similar to those present in the immature green fruit. The lack of correlation between decarboxylase activities and PA levels in ripening Indalo fruit suggests changes in PA conjugation or catabolism rather than synthesis. A report on the metabolism of [1,4-^{14}C]Put and [*terminal methylene*-^{3}H]-Spd strongly suggested the possibility that PA oxidizing enzymes are present in tomato tissues, although their activities were not measured in vitro (Rastogi and Davies, 1990). This metabolism was analyzed in the fruit pericarp disks of Rutgers tomato at four different stages of ripening (immature green, mature green, breaker, ripe). The rate decreased with ripening, but

Table 1. Free Polyamine Contents and Decarboxylase Activities during Tomato Fruit Ripening[a]

ripening stage	L^* [b]	chroma[c]	hue angle[d]	firmness	Put[e]	Spd[e]	Spm[e]	ADC[f]	ODC[f]	SAMDC[f]
immature green	64.8 ± 1.54	31.6 ± 1.73	90.3 ± 4.32	56.8 ± 0.26	71 ± 1.71	53.7 ± 0.96	9.1 ± 0.42	146 ± 5.5	124 ± 10.6	365 ± 10.7
mature green	59.3 ± 0.89	29.5 ± 1.30	95.4 ± 3.28	38.6 ± 0.15	70 ± 3.17	58.8 ± 1.47	9.4 ± 0.18	148 ± 2.3	190 ± 9.2	257 ± 11.1
breaker	56.4 ± 0.63	29.7 ± 0.89	91.6 ± 2.48	34.5 ± 0.42	76 ± 3.16	57.0 ± 1.84	9.6 ± 0.13	173 ± 6.7	250 ± 4.8	300 ± 0.4
turning	54.0 ± 0.61	34.0 ± 1.73	75.7 ± 1.30	28.0 ± 0.16	78 ± 2.95	52.0 ± 1.05	9.4 ± 0.02	203 ± 15.7	295 ± 12.8	410 ± 18.6
pink	51.2 ± 0.72	35.6 ± 0.80	71.3 ± 1.66	26.3 ± 0.14	79 ± 4.28	48.3 ± 2.92	9.2 ± 0.29	228 ± 18.0	328 ± 9.7	518 ± 14.6
light red	44.2 ± 0.43	36.5 ± 0.92	55.1 ± 2.70	18.4 ± 0.09	60 ± 4.91	47.4 ± 1.73	8.5 ± 0.04	219 ± 19.6	202 ± 3.2	340 ± 9.2
red	42.5 ± 0.24	35.5 ± 0.68	49.9 ± 0.72	17.4 ± 0.06	67 ± 5.15	37.2 ± 0.86	7.6 ± 0.04	144 ± 9.5	100 ± 15.3	160 ± 9.2
mature red	40.5 ± 0.42	38.8 ± 1.18	41.3 ± 0.61	15.5 ± 0.10	76 ± 6.38	37.4 ± 1.55	7.8 ± 0.01	120 ± 3.5	78 ± 9.6	151 ± 10.0

[a] Data presented as mean values ± SE. [b] L^*, from the $L^*a^*b^*$ color spacing system. [c] Chroma, $[(a^*)^2 + (b^*)^2]^{1/2}$. [d] Hue angle, $\tan^{-1}(b^*/a^*)$. [e] In nmol/g FW. [f] In pmol of $^{14}CO_2$ h^{-1} (mg of protein)$^{-1}$.

other ripening stages (turning, pink, light red, red) were not characterized (Rastogi and Davies, 1991).

Concerning the role of SAMDC, this activity is a critical branch point between biosynthesis of polyamines and ethylene, because its substrate, SAM, is also the precursor of ethylene. Ethylene and polyamines are known to have opposite effects on fruit ripening and senescence (Kakkar and Rai, 1993). Although changes in ethylene concentrations in this fruit were not determined, it has been shown in other varieties that the climacteric rise in ethylene production takes place at the pink and light red stages (Saftner and Baldi, 1990). Thus, on the basis of these examples, it is not unreasonable to argue that the synthesis of PAs (decarboxylases activities) and ethylene in this tomato may be noncompetitive. In avocado, these two pathways do not actively compete for the same substrates at any given stage of fruit development and ripening (Kushad et al., 1988).

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