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Polyamine Metabolism in Ripening Tomato Fruit[1]

I. Identification of Metabolites of Putrescine and Spermidine

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ABSTRACT

The metabolism of [1,4-^{14}C]putrescine and [terminal methylene-^{3}H]spermidine was studied in the fruit pericarp (breaker stage) discs of tomato (*Lycopersicon esculentum* Mill.) cv Rutgers, and the metabolites identified by high performance liquid chromatography and gas chromatography-mass spectrometry. The metabolism of both putrescine and spermidine was relatively slow; in 24 hours about 25% of each amine was metabolized. The ^{14}C label from putrescine was incorporated into spermidine, γ-aminobutyric acid (GABA), glutamic acid, and a polar fraction eluting with sugars and organic acids. In the presence of gabaculine, a specific inhibitor of GABA:pyruvate transaminase, the label going into glutamic acid, sugars and organic acids decreased by 80% while that in GABA increased about twofold, indicating that the transamination reaction is probably a major fate of GABA produced from putrescine *in vivo*. [^{3}H]Spermidine was catabolized into putrescine and β-alanine. The conversion of putrescine into GABA, and that of spermidine into putrescine, suggests the presence of polyamine oxidizing enzymes in tomato pericarp tissues. The possible pathways of putrescine and spermidine metabolism are discussed.

The diamine Ptc[2] and the PAs, Spd, and Spm are apparently ubiquitously distributed in plants, and changes in their levels and biosynthesis have been correlated with a variety of plant developmental processes (6, 22, 24).

To determine the physiological role of PAs, it is important to understand how PA levels are regulated in plant tissues. Considerable attention has been given to PA biosynthesis, and the enzymes involved have been well-characterized in certain systems (6, 22, 24). PA breakdown, however, has been studied only in a few cases, and therefore relatively little is known of the metabolic fate of these compounds. It was previously reported that soybean seedlings labeled with [^{14}C]Ptc produced GABA, Glu, Asp, and organic acids (15). Similarly, GABA, Glu, and Asp were also produced from Ptc by cultured radiata pine cotyledons (14). Terano and Suzuki (28, 29) demonstrated the formation of β-alanine from Dap, Spd, and Spm, and that of GABA from Spm, by corn shoots and seedlings, respectively. Also, pea shoots and oat leaves, respectively, were shown to oxidatively metabolize Ptc and Spd to GABA (7).

We have been interested in investigating PA metabolism during tomato (*Lycopersicon esculentum* Mill.) fruit ripening, primarily because ethylene is involved in ripening (4), the functions of ethylene and PAs are antagonistic in senescence, and the two share a common intermediate, *i.e.* *S*-adenosylmethionine for their biosynthesis (24). Earlier, we (5) reported that fruit of the Alcobaca landrace of tomato, which ripen slowly and have prolonged keeping qualities, contain three times as much Ptc as the normal variety at the ripe stage. It was suggested that the enhanced Ptc level in this line may be responsible for the ripening and storage features. Since PA breakdown, in part, can also regulate PA levels, it is possible that the Alcobaca fruit have an altered PA catabolism. However, it has been speculated that in Solanaceous tissues PA levels are regulated by PA biosynthesis and conjugation rather than by PA breakdown and interconversion as amine oxidases have not been detected in such tissues. It was, therefore, of considerable interest to investigate the degradative PA metabolism in tomato pericarp tissues. In this paper, we report on the metabolism of [1,4-^{14}C]Ptc and [terminal methylene-^{3}H]Spd by fruit pericarp discs (breaker stage) of tomato cv Rutgers, and the identification of metabolites by HPLC and GC-MS.

MATERIALS AND METHODS

Plant Material

The seeds of tomato (*Lycopersicon esculentum* Mill.) cv Rutgers were germinated in 5 inch plastic pots in a mixture of peat and vermiculite (1:1 by weight), and seedlings with three to four leaves were transferred to 12 inch pots containing a mixture of prolite, vermiculite, and peat (1:1:2 by weight). Plants were grown in the greenhouse and supplemental lighting was provided by mercury halide lamps for 16 h/d. The day/night temperature was 21 to 24°C/19°C. Fruit at the breaker stage were harvested and used for experiments.

Labeling with Radioactive Polyamines

The fruit were washed and surface sterilized with 95% ethanol. In a sterile hood, pericarp discs (diameter 20 mm) were then cut with a cork borer and placed in sterile Petri

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[2] Abbreviations: Ptc, putrescine; PA(s), polyamine(s); Spd, spermidine; Spm, spermine; GABA, γ-aminobutyric acid; Dap, 1,3-diaminopropane; AG, aminoguanidine; GCU, gabaculine (3-amino 2, 3-dihydrobenzoic acid); MDL-72521, N^1-methyl-N^2-(buta-2,3-dienyl)butane-1,4-diamine.

dishes containing moistened filter paper. The Petri dishes with the pericarp discs were kept overnight in an incubator maintained at 20 to 22°C and illuminated by fluorescent tubes at an intensity of 50 to 60 $\mu E\ s^{-1}\ m^{-2}$. The following day, the discs were treated either with 0.54 μCi [1,4-^{14}C]Ptc (107 mCi/mmol) or 5 μCi [terminal methylene-^{3}H]Spd (29.7 Ci/mmol), by placing the radioactive material (5 μL in each case) as a drop in the center of the discs. Five microliters of 0.01 N KOH were also added to each disc to neutralize the HCl present in the radioactive PA solutions. The treated discs were further incubated for 24 h, and smaller discs (diameter 16–17 mm) were centrally cut from the 20 mm discs and frozen at −80°C until further analysis.

In some experiments, the inhibitors AG, GCU, and MDL-72521 were used to probe the metabolism of Ptc, GABA, and Spd, respectively.

Extraction and Purification of Polyamines and Their Metabolites

All glassware was silanized (Aquasil, Pierce, Rockford, IL). The pericarp discs were thoroughly rinsed with distilled water, homogenized in cold 0.2 N perchloric acid (4 mL/disc), and the extracts kept on ice for 1 h. The extracts were then centrifuged, and the supernatant dansylated. To the supernatant, 4 mL saturated sodium carbonate and 8 mL dansyl (dimethylaminonaphthalene-1-sulfonyl) chloride (7 mg/mL, freshly prepared) were added, and the mixture incubated in a thermal block at 60°C for 1 h. The mixture was partitioned twice with toluene. The toluene phase was evaporated *in vacuo* and the residue dissolved in 1 mL HPLC-grade methanol. The aqueous phase was evaporated to 2 mL, and the sodium carbonate precipitated with acetone. The acetone fraction was dried *in vacuo* and suspended in 2 mL water. Both samples were filtered through a nylon 66 membrane (pore size 0.22 μm; Rainin, Woburn, MA) prior to HPLC.

HPLC

Organic Phase (Dansyl PAs)

The HPLC equipment used consisted of two Altex model 110A solvent metering pumps controlled by the Altex 420 microprocessor programmer. The danysl PAs were injected onto an analytical C_{18} (5 μm spherical particle, Spherisorb, Norwalk, CT) HPLC column (4.6 × 250 mm). The column was eluted with a methanol to water (v/v) solvent gradient (30–60% over 10 min and 60–100% over 37 min) at a flow rate of 1 mL min^{-1}. The column was washed with 100% methanol for 5 min, and reequilibrated at 30% for 10 to 15 min before the injection of next sample. The column eluate was passed through an on-line radioactivity flow monitor with the data recorded as counts per each 12 s interval, and the fractions containing the radioactive PAs were collected separately. The identity of the PAs was determined by mixing the radioactive peaks with a mixture of danysl PAs and running the resulting mixture on HPLC using the same column and solvent gradient. The peaks were detected by an attached fluorescence spectrophotometer as described in Dibble *et al.* (5).

Aqueous Phase

The aqueous samples were also injected on a C_{18} column. The solvents and gradient were modified from Weiner and Tishbee (30). The solvents were buffers A (50 mM ammonium acetate [pH 6.3], 12.5 % acetonitrile, and 5% isopropanol) and B (50 mM ammonium acetate [pH 6.5], 50% acetonitrile, and 1% isopropanol). The flow rate was 0.6 mL/min. The gradient was as follows: 0% buffer B for 15 min, 0 to 30% buffer B over 10 min and holding it at 30% for 5 min, 30 to 50% buffer B over 10 min and isocratic at 50% for 5 min, 50 to 100% buffer B over 5 min and held at 100% for 10 min. The radioactive peaks were collected as before. Aliquots of all the peaks were subjected to a second HPLC coupled with fluorescence detector, with or without a mixture of dansyl amino acids. The relatively nonpolar peaks were dried and prepared for GC-MS, and the polar peaks were subjected to anion exchange chromatography as described below.

Anion Exchange Chromatography of the Polar Peaks from the Aqueous Fraction

The polar peaks were pooled, and the pH was adjusted to 7.9 to ionize the acids. The sample was then loaded onto a 15 × 1 cm glass column packed with Dowex 1-X2 anion exchange resin (chloride form, Bio-Rad). The column was first washed with water and then with 1 N HCl. Two fractions (neutral and acidic) were collected, dried *in vacuo*, and prepared for GC-MS.

GC-MS Analysis

GC-MS analyses were done with a Hewlett Packard 5890A gas chromatograph connected to a 5970B mass selective detector and equipped with an HP 9133 computer. Samples (3–5 μL) were injected in split mode (50–1) onto a bonded methyl silicone capillary column (HP-1, 25 m × 0.2 mm). The carrier gas (helium) flow rate was about 30 cm s^{-1}.

Ten to 20 μL each of samples to be analyzed by GC-MS were transferred to 100 μL glass tubes held in larger capped glass vials, and taken to dryness under N_2 and then *in vacuo* over P_2O_5. For methylation of amino acids, the sample was dissolved in 10 μL acetonitrile followed by addition of 10 μL methylating agent (Methyl-8, Dimethyl acetal; Pierce, Rockford, IL), and the reaction mixture heated at 100°C for 20 min. Three to 5 μL of derivatized sample was injected. For trimethylsilylation, the residue in the glass tubes was solubilized in 10 μL pure, dry pyridine and then an equal volume of Sylon BFT trimethylsilylating reagent (bistrifluoroacetamide + 1% trimethylchlorosilane; Supelco, Bellafonte, PA) was added. The reaction mixture was heated at 60°C for 1 h in a heating block. Two to 4 μL of the trimethylsilyl derivative was injected. After injection, the operating temperature conditions were as follows. Methyl derivative of dansyl GABA: initial temperature 180°C held for 1 min, 30°C min^{-1} to 240°C, 10°C min^{-1} to 300°C and held at 300°C for 5 min. For methyl derivatives of dansyl glutamic acid and dansyl β-alanine, the temperature conditions were similar to those for methylated dansyl GABA except that the initial temperature was 110°C (isothermal for 4 min) and the final temperature, *i.e.* 300°C, was held for 10 min. For GABA and β-alanine,

the ions were scanned from 100 to 500 mass units resulting in 1.07 scans/s, and those for glutamic acid from 50 to 500 producing 0.95 scans s^{-1}. Trimethylsilyl derivatives: isothermal at 110°C for 4 min, 4°C min^{-1} to 200°C, 10°C min^{-1} to 250°C and held for 1 min; ions were scanned from 10 to 500 mass units resulting in 0.87 scans/s. In all cases, the temperature of the injector was 250°C, and that of the detector and tranfer line 300°C. The ionizing energy was 70 eV for all runs.

Chemicals

Hydrochlorides of PAs, dansyl chloride, AG and GCU were obtained from Sigma Chemical Co. MDL-72521 was a gift from Merrell Dow Research Institute (Cincinnati, OH). [^{14}C] Ptc and [^{3}H]Spd were purchased from RPI Corp. (Mount Prospect, IL) and NEN Research products (Wilmington, DE), respectively.

RESULTS

Identification of Metabolites of PAs

The fruit pericarp discs were treated with [^{14}C]Ptc and [^{3}H] Spd, and the uptake of label after 24 h was 75 and 80%, respectively, as determined by radioactivity present in the 0.2 N perchloric acid extracts. The extracts, after dansylation, were partitioned with toluene, and about 75 to 80% of the radioactivity partitioned into the toluene phase containing the dansyl PAs, and the rest remained in the aqueous phase. Both the toluene phase and the water phase were dried and injected on an analytical C_{18} column.

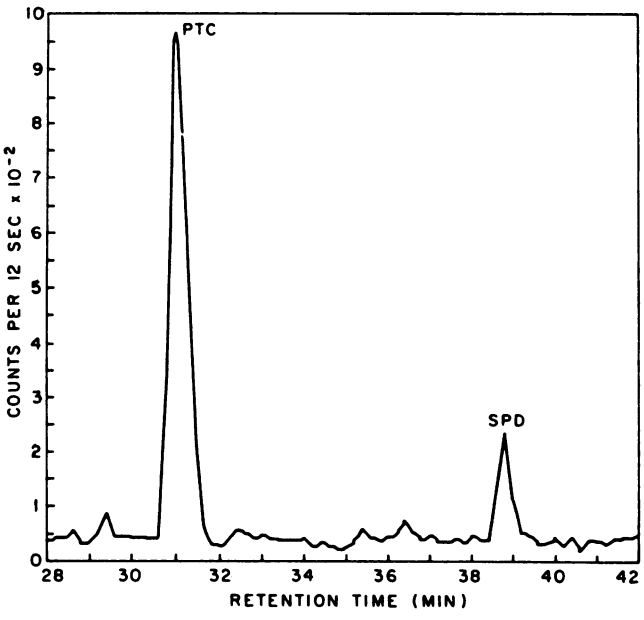

Figure 1. C_{18} HPLC trace of the metabolites of [^{14}C]Ptc partitioning, into the toluene phase, following dansylation and extraction with toluene.

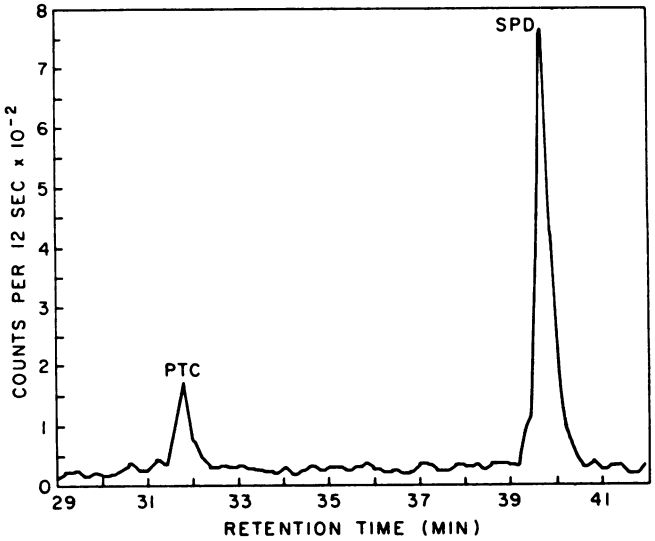

Figure 2. C_{18} HPLC trace of the metabolites of [^{3}H]Spd partitioning into the toluene phase, following dansylation and extraction with toluene.

Analysis of the Toluene Phase

The HPLC profiles of the metabolites of [^{14}C]Ptc and [^{3}H] Spd partitioning in toluene are shown in Figures 1 and 2, respectively. In both samples, two radioactive peaks each were detected. In the case of [^{14}C]Ptc, a large, relatively more polar peak (31.0 min), and a small, relatively less polar peak (38.8 min) were present (Fig. 1). However, [^{3}H]Spd produced a small, relatively more polar peak (31.8 min), and a large, relatively less polar peak (39.6 min) (Fig. 2). The relatively polar and nonpolar peaks, by comparison of their elution times with those of the standards, were identified as Ptc and Spd, respectively. The identity of all the radioactive peaks was further determined by subjecting them to a second HPLC run, either alone or after mixing them with dansyl PA (Dap, Ptc, Spd, and Spm) standards, and the peaks detected using a fluorescence detector. The peaks indeed cochromatograped with Ptc and Spd (HPLC scans not shown). Thus, Ptc was converted to Spd, and Spd was retroconverted to Ptc.

Analysis of the Aqueous Phase

The HPLC of the metabolites of [^{14}C]Ptc and [^{3}H]Spd partitioning into water produced radioactive scans shown in Figures 3 and 4, respectively. Since the PAs are known to be converted into amino acids (GABA, Glu, Asp, and β-alanine), and the dansyl amino acids remain in the water phase following dansylation and partitioning with toluene, the acetate buffer to acetonitrile solvent system used for HPLC was specifically devised to resolve dansyl amino acids on a C_{18} column.

The aqueous fraction containing the Ptc metabolites showed several radioactive peaks; three polar peaks (4.8, 6.6, and 8.2 min) and two relatively less polar peaks (18.8 and 55.0 min) (Fig. 3). In the water fraction containing the Spd metabolites, only one radioactive peak was present (49.6 min). All the radioactive peaks were separately mixed with a mixture

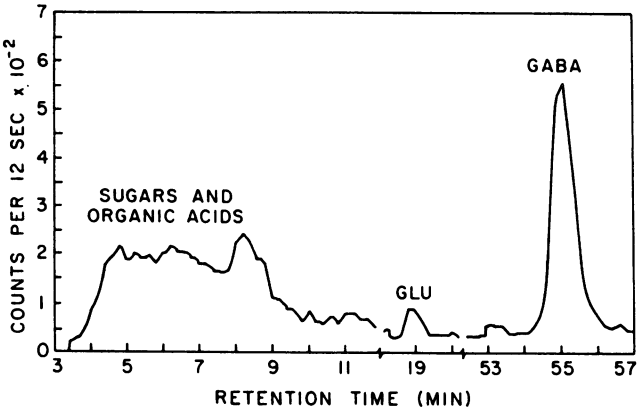

Figure 3. C_{18} HPLC trace of [^{14}C]Ptc metabolites separating in the water phase, following dansylation and extraction with toluene.

of dansyl amino acids, injected onto HPLC and the peaks visualized fluorometrically. The polar radioactive peaks resulting from Ptc metabolism were not fluorescent, indicating that they were not dansylated and were unlikely to be amino acids.

The two relatively less polar Ptc metabolites and the Spd metabolite were fluorescent and coeluted with Glu and GABA, and β-alanine, respectively. These three fractions for unequivocal identification were injected on GC but, except dansyl GABA, none of the others were volatile enough. The above three radioactive peaks and the dansylated standards of GABA, Glu, and β-alanine were then methylated and subjected to GC-MS, and the principal ion fragments and their normalized intensities are summarized in Table I. A comparison of the spectra of derivatized standards and plant samples showed essentially identical fragmentation patterns. Molecular ions were observed in all cases. Dansyl GABA (M^+ 336) was methylated at carboxyl position (M^+, 350), whereas dansyl Glu (M^+, 380) was methylated at three different positions: carboxyl and amino positions, and carboxyl group of the side chain (M^+, 422). Dansyl β-alanine (M^+, 322) was

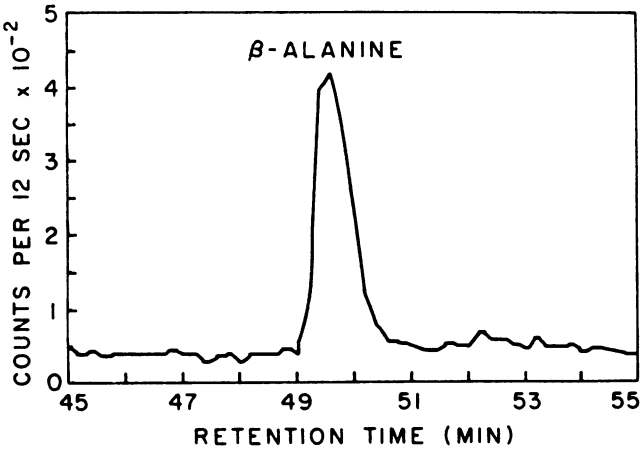

Figure 4. C_{18} HPLC trace of the [^{3}H]Spd metabolite partitioning in the water phase, following dansylation and extraction with toluene.

methylated either at the carboxyl position (M^+, 336) or both at the carboxyl as well as amino group (M^+, 350). Another large peak appeared in all spectra at m/z 170 or 171 (the 100% peak) (Table I). This ion resulted from the cleavage of the bond between the sulfonyl group and the naphthalene ring of dansyl chloride, and usually serves as a marker indicating the fragmentation of dansyl derivatives. The GC-MS analyses, thus, unambiguously proved that the relatively less polar peaks from [^{14}C]Ptc metabolism were Glu and GABA, and that from [^{3}H]Spd was β-alanine.

Since Ptc can be metabolized via GABA to succinic acid (7) which, in turn, could be converted into other organic acids through Krebs cycle, the polar radioactive metabolites of [^{14}C] Ptc were tested for a carboxyl moeity. Derivatization with Methyl-8 showed no peaks on the GC. To determine if there were any acids present in these fractions, the fractions were pooled, pH adjusted to 7.9, and subjected to anion exchange chromatography. Neutral and acidic (eluted with HCl) fractions were collected, and were found to have 80% and 20% radioactivity, respectively. These fractions were trimethylsilylated and analyzed by GC-MS. The acidic fraction showed several peaks (GC retention times from 5–15 min), and algorithmic matching indicated that these peaks were probably organic acids. The neutral fraction also contained many peaks on GC (retention times ranging from 20–30 min) and their spectra very closely matched those of various sugars (spectra not shown).

Table I. *Relative Intensities (%) of the Principal Ion Fragments (m/z) in the Mass Spectra of Methylated Dansyl Derivatives of GABA, Glutamic Acid, and β-Alanine Copurifying on HPLC with Radiolabeled Metabolites Produced from the Metabolism of [^{14}C] Putrescine (GABA and Glutamic Acid) or [^{3}H]Spermidine (β-Alanine) in the Pericarp Discs of Tomato (cv Rutgers)*

Dansyl Derivative	Principal Ion Fragments (m/z)						
	350(M^+)[a]	318	172	171	170	168	154
	% relative intensity[b]						
GABA standard	29	17	14	100	39	27	13
GABA from pericarp	35	16	15	100	46	26	16
	m/z						
	422(M^+)[c]	331	172	171	170	168	154
	% relative intensity						
Glutamic acid standard	37	47	12	97	100	20	15
Glutamic acid from pericarp	41	51	13	99	100	21	18
	m/z						
	350(M^+)[d]	336(M^+)[a]	172	171	170	168	154
	% relative intensity						
β-Alanine standard	12	24	12	100	37	19	12
β-Alanine from pericarp	12	17	13	100	41	18	11

[a] Monomethylated derivative. [b] Related to m/z 170 or 171 (dimethylaminonaphthalene) = 100%. [c] Trimethylated derivative. [d] Dimethylated derivative.

Table II. *Radioactivity (%) Present in Different Metabolites Produced in the Pericarp Discs of Tomato (cv Rutgers), following a 24 h Treatment with 0.5 μCi [^{14}C]Putrescine*

Values presented are the means ± SE. $n = 2$.

Putrescine	Spermidine	GABA	Glutamate	Sugars and Organic Acids
77.72 ± 0.23	8.83 ± 0.56	4.94 ± 0.42	1.35 ± 0.24	7.11 ± 0.04

Percent Incorporation of Polyamines into Different Metabolites

The proportion of label from [^{14}C]Ptc being incorporated into different metabolites by tomato pericarp is shown in Table II. In 24 h, about 22% Ptc was metabolized; about 9% was converted to Spd and the rest was catabolized into GABA, glutamic acid, and sugars and organic acids (Table II). Incorporation of [^{14}C]Ptc into Spm was not detected.

The conversion of Ptc into GABA suggests that a pathway involving a diamine oxidase may be operative in the tomato pericarp tissue: diamine oxidase produces γ-aminobutyraldehyde (which spontaneously interconverts into pyrroline) which upon further oxidation results in GABA (7). This possibility was tested by application of AG, an inhibitor of diamine oxidase (3, 7, 21). Treatment with AG produced a small but significant inhibition of Ptc breakdown with a concomitant reduction in the formation of GABA, Glu, sugars, and organic acids (Table III).

Whether Glu, sugars, and organic acids were derived directly from Ptc, or whether GABA was an intermediate in their production was probed by using GCU (an irreversible inhibitor of GABA aminotransferase; 7, 8, 12). In the presence of GCU, the GABA fraction accumulated twice as much radioactivity as in its absence, with a concomitant decrease by several-fold in the label appearing in Glu, and sugar and organic acid fractions (Table IV).

The metabolism of [^{3}H]Spd by pericarp tissue of tomato is shown in Table V. Like [^{14}C]Ptc, [^{3}H]Spd also was relatively slowly metabolized, and after 24 h 73% of the label was present in the Spd fraction. The remainder of the [^{3}H]Spd was metabolized into Ptc and β-alanine to the extent of 9 and 18%, respectively (Table V). As with Ptc metabolism, no Spm was apparent. Because in animal tissues Spd is retroconverted to Ptc via acetylation and subsequent oxidation by a PA oxidase, the effect of MDL-72521 (an enzyme-activated, irreversible inhibitor of PA oxidases which degrade acetyl PAs; 18, 21) was studied to determine if Spd metabolism in the pericarp tissues involved acetylation. The inhibitor was ineffective in preventing any Spd breakdown (Table V).

DISCUSSION

The metabolism of [^{14}C]Ptc and [^{3}H]Spd was investigated in tomato pericarp tissue. The metabolism of both Ptc and Spd was slow, with about 25% of the PAs being metabolized in 24 h. The label from [^{14}C]Ptc was incorporated into Spd, GABA, Glu, sugars, and organic acids (Table II), whereas Spd was metabolized into Ptc and β-alanine (Table V). GABA, Glu, β-alanine, sugars, and organic acids were identified by GC-MS.

The conversion of Ptc to GABA has been previously shown in a few other plant systems: soybean seedlings (15), pea shoots (7), the Ptc utilizing mutant of tobacco (8), and excised cotyledons of *Pinus radiata* (14). In animal (20) and microbial (16) cells, the common pathway of Ptc to GABA conversion involves a two-step oxidation via a diamine oxidase, forming γ-aminobutyraldehyde, and an NAD dependent pyrroline dehydrogenase, which oxidizes the latter to GABA (27). Flores and Filner (7) demonstrated that such a two-step pathway of Ptc oxidation is also operative in pea shoots.

Production of GABA from Ptc in the fruit pericarp of tomato suggests the presence of a diamine oxidase. However, labeled pyrroline was not detected in the tomato tissues. It is quite possible that pyrroline was produced but rapidly converted to GABA. Aminoguanidine, which inhibits Ptc breakdown in some plants (3, 7) and animals (21), produced a small inhibition of Ptc breakdown in the pericarp tissue (Table III). It is likely that either AG did not fully get to the active site or the tomato diamine oxidase is somewhat different from that found in other plants.

In the Ptc utilizing mutant cell line of tobacco which efficiently converts Ptc to GABA, diamine oxidase activity was not detected (8), and the authors suggested that probably there is another pathway for GABA production from Ptc, involving either the acetyl or cinnamoyl derivatives. A degradative pathway from Ptc to GABA, comprising acetylation of Ptc and subsequent oxidation of acetyl Ptc by a monoamine oxidase was shown to be present in mammalian brain (19), and it can be speculated that such a pathway may also be operative in plants. To the best of our knowledge, however, acetyl PAs have not been reported in plants, but their levels may be too low to be detected. The possibility of the involvement of other Ptc derivatives, such as cinnamoyl derivatives which are widely distributed in plants (24), in Ptc oxidation cannot be ruled out. In tomato fruit, however, soluble PA conjugates are in extremely small amounts (R Rastogi, PJ

Table III. *Radioactivity (%) Present in Different Metabolites Produced in the Pericarp Discs of Tomato (cv Rutgers), following a 24 h Treatment with 0.5 μCi [^{14}C]Putrescine with and without Aminoguanidine (40 μL, 0.1 M; an Inhibitor of Diamine Oxidase)*

Values presented are the means ± SE. $n = 2$.

Treatment	Putrescine	Spermidine	GABA + Glutamate + Sugars & Organic Acids
		% radioactivity	
Control	69.00 ± 0.19	6.85 ± 0.20	24.45 ± 0.32
Aminoguanidine	74.55 ± 3.59	6.70 ± 0.54	18.76 ± 3.06

Table IV. *Radioactivity (%) Present in Different Metabolites Produced in the Pericarp Discs of Tomato (cv Rutgers), following a 24 h Treatment with 0.5 μCi [^{14}C]Putrescine with and without Gabaculine (20 μL, 1 M; an Inhibitor of GABA:Pyruvate Transaminase)*

Values presented are the means ± SE. $n = 2$.

Treatment	Putrescine	Spermidine	GABA	Glutamate	Sugars & Organic Acids
			% radioactivity		
Control	69.00 ± 0.19	6.85 ± 0.20	9.09 ± 1.23	0.64 ± 0.17	14.72 ± 1.72
Gabaculine	74.92 ± 0.47	5.98 ± 0.70	16.29 ± 0.22	0.19 ± 0.03	2.60 ± 0.25

Davies, unpublished data), and acid hydrolysis of the perchloric acid extracts of [^{14}C]Ptc treated tissue also did not reveal any radioactive conjugates (data not presented).

Another pathway for the formation of GABA from Ptc involving a diamine transaminase has been documented in some microorganisms (16). Ptc, by transamination to α-ketoglutarate (producing Glu), is converted to γ-aminobutyraldehyde which upon oxidation produces GABA. Since, transaminases are widely distributed in plants (9), and a cadaverine-pyruvate transaminase has been found in lupin (24), it is not unreasonable to speculate that specific diamine transaminases may also exist in tomato and other plants, which, either alone or along with diamine oxidases, can produce GABA from Ptc.

In tomato pericarp discs, the label from Ptc also appeared in Glu and a nonamino acid fraction containing sugars and organic acids (Table II). Pine cotyledons were found to convert Ptc to Glu and Asp together with GABA (14), and in soybean seedlings radioactivity from Ptc was recovered in Asp, Glu, and organic acids (15). In fish brain, label from Ptc was also found in a nonamino acid fraction in which acidic and neutral compounds (glucose, lactic acid, pyruvic acid, etc.) were eluted from ion exchange column (20). The above described studies on Ptc metabolism in Pine cotyledons and soybean seedlings do not, however, provide any evidence as to whether Asp and Glu were derived directly from Ptc, or whether GABA was an intermediate in their formation.

It is known that GABA can be transaminated either to pyruvate or α-ketoglutarate to produce β-alanine or Glu, and the succinic semialdehyde produced as a result is further oxidized to succinic acid (25). Succinic acid is subsequently incorporated into Krebs cycle intermediates, some of which can go on to produce Glu and Asp, and sugars (10). This indeed appears to be the case in tomato pericarp tissue with respect to breakdown of GABA produced *in vivo* from [^{14}C] Ptc, since GCU treatment resulted in a significant increase in radioactivity going into the GABA fraction, and a simultaneous decrease in the radioactivity recovered in Glu, and in the sugar and organic acid fraction (Table IV). Thus, it appears that in the tomato fruit, Ptc is metabolized to Spd and GABA. The latter is then further metabolized to Glu, and various sugars and organic acids. In oat and pea tissues, GCU treatment also increased the label in GABA, while radioactivity in a spot on TLC corresponding to succinic acid disappeared (7).

Because Glu can also be derived from α-ketoglutarate by transamination of Ptc (16) or GABA (25), it can be argued that radioactive Glu produced from Ptc metabolism in tomato pericarp was derived from transamination rather than from organic acids resulting from GABA degradation. It is, however, unlikely since the radioactive Ptc used in this study was 1,4-^{14}C-labeled, and Glu produced as a result of Ptc transamination would not be radioactive.

The tomato pericarp tissues metabolized [^{3}H]Spd to Ptc and β-alanine. Formation of Ptc from Spd was also briefly reported in barley seedlings (23), *Helianthus tuberosus* tubers (2), and tobacco epidermal tissue (1). The conversion of Spd to Ptc is of wide occurrence in animals (via acetylation), and has also been found in bacteria and fungi (by direct oxidation). In animal tissues, Spd is first acetylated by N^{1}-acetyl transferase, followed by oxidation by a PA oxidase producing Ptc and acetylaminopropanal (18, 26). The latter can be further oxidized to β-alanine. Further, it was shown that acetylated derivatives of PAs are the true physiological substrates for these PA oxidases. In contrast, in microbes, *e.g. Psuedomonas* (17), *Candida* (11), and *Penicillium* (13), PA oxidases which directly attack the secondary amino groups producing Ptc and aminopropanal have been found. Interestingly, the PA oxidase from *Candida* showed a preference for acetyl Spd, suggesting that acetylation might have preceded oxidation, whereas for the PA oxidase from *Penicillium* acetyl Spd was a poor substrate. In the tomato fruit, however, it appears that a PA oxidase, similar in nature to that found in bacteria and fungi, may be present since the application of MDL-72521, a specific inhibitor of animal PA oxidases had no significant effect on spermidine oxidation (Table V). In plants, PA oxidases have been detected in the members of the Gramineae, and they oxidize Spd and Spm to give pyrroline and aminopropylpyrroline, respectively, together with Dap (24).

The significance of PA catabolism is not clear. One possible role may be in the regulation of cellular PA levels. The conversion of PAs into amino acids, organic acids, and sugars

Table V. *Radioactivity (%) Present in Different Metabolites Produced in the Pericarp Discs of Tomato (cv Rutgers), following a 24 h Treatment with 5 μCi [^{3}H]Spermidine with and without MDL-72521 (20 μL, 1 M; an Inhibitor of a Polyamine Oxidase which Degrades Acetyl Polyamines)*

Values presented are the means ± SE. $n = 2$.

Treatment	Spermidine	Putrescine	β-Alanine
		% radioactivity	
Control	73.12 ± 2.96	9.13 ± 0.37	17.75 ± 2.59
MDL-72521	69.94 ± 2.80	11.10 ± 1.57	18.96 ± 1.23

may be one of the ways the plant ensures C and N recycling. The conversion of Spd to Ptc is perhaps a mechanism of salvaging the Ptc portion of the PA molecule under conditions where it is necessary to reduce PA levels. It is also possible that the metabolites of PAs, rather than PAs themselves, are responsible for the observed effects of PAs on growth and development.

In conclusion, this study has shown that Ptc and Spd are metabolized in tomato pericarp tissues, and strongly suggests the possibility that PA oxidizing enzymes are present in tomato tissues, although their activities were not measured *in vitro*. Further, in the tomato pericarp, as in animals and microorganisms, Spd can be retroconverted to Ptc, and this pathway may be of more common occurrence in plants than previously thought.

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