Plant Physiol. (1988) 87, 463–467
0032-0889/88/87/0463/05/$01.00/0

Interrelationship of Polyamine and Ethylene Biosynthesis during Avocado Fruit Development and Ripening

Received for publication July 28, 1987 and in revised form February 17, 1988

MOSBAH M. KUSHAD*, GEORGE YELENOSKY, AND ROBERT KNIGHT
Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0327 (M.M.K.); *United States Department of Agriculture, Agricultural Research Service, Orlando, Florida 32803* (G.Y.); *and United States Department of Agriculture, Agricultural Research Service, Miami, Florida 33158* (R.K.)

ABSTRACT

Concentrations of polyamines (PA) and the activities of the PA-synthesizing enzymes ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) extracted from the mesocarp tissue of avocado (*Persea americana* Mill, cv 'Simmonds') fruits at different stages of development were compared with DNA content and the activities of 5′-methylthioadenosine (MTA) nucleosidase and 5-methylthioribose (MTR) kinase. Putrescine, spermidine, and spermine were at their peak concentrations during the early stages of fruit development (362, 201, and 165 nanomoles per gram fresh weight, respectively, at 15 days from full bloom), then declined to 30% or less at full maturity. Agmatine showed only a slight change in concentration throughout the fruit development. The activity of ODC, which was low during flowering (8 nmoles per milligram protein per hour), increased more than threefold during the first 2 months then declined at the later stages of fruit development, while ADC activity showed only a slight increase. DNA content followed a similar pattern of change as that of PA and ODC. The decline in DNA and ODC activity suggest a lack of correlation between cell proliferation and PA at the later stages of the avocado fruit development. It is also possible that any cell division which may take place during the latter stages of the fruit development is not sufficient to alter the pattern of PA biosynthesis. MTA nucleosidase and MTR kinase activities increased during the first 15 days of fruit development followed by a slight decline at 60 and 90 days from full bloom. At 120 days (1 month before full maturity) both MTA nucleosidase and MTR kinase activities increased significantly. During maximum ethylene synthesis, MTA nucleosidase and MTR kinase activities were approximately fivefold and eightfold, respectively, higher than during maximum PA synthesis. The data indicate that the MTA molecules produced during PA and ethylene synthesis are actively metabolized to MTR and MTR-1-P, the two intermediates involved in the regeneration of *S*-adenosylmethionine from MTA. The data also suggest that the PA and ethylene biosynthetic pathways are not actively competing for the same substrates at any given stage of the avocado fruit development and ripening.

Plant PA[1] have been associated with growth, development, and senescence (14, 26, 28). PA and PA-synthesizing enzymes were reported to be at their peak activity during rapid cell division and at their lowest activity in mature nondividing tissues (24, 27). However, the exact role of PA in tissue differentiation and development is still not quite clear. Increased PA levels were observed during somatic embryogenesis of carrot and tobacco cell cultures (9, 12), in developing leaves and buds of *Phaseolus vulgaris* (22), in meristematic tissues of potato sprouts (16), and in developing tomato fruits (15). Endogenous PA were also reported to increase under various stress conditions in both fruits and vegetables (21, 28). Exogenous PA were reported to delay senescence of leaf segments (25) and isolated protoplasts (2). Costa and Bagni (8) observed a twofold increase in fruit set when apple trees were sprayed with 1 μM and 10 μM Spm and Put. In tomato and tobacco ovaries the activity of ODC increased while the activity of ADC showed minimal change during rapid cell proliferation period (7, 15, 27). Recently, Slocum and Galston (27) suggested a regulatory role for PA in the postfertilization growth of tobacco ovaries.

Radioactive studies of young and actively dividing fruit tissues have shown that the amino moiety of SAM is directed toward the formation of PA via decarboxylated SAM (26). Spd and Spm are snythesized from Put by subsequent addition of aminopropyl groups from decarboxylated SAM (26). In contrast, mature nondividing fruit tissues shift the metabolism of SAM toward the formation of ACC and therefore ethylene, which is necessary for the induction of fruit ripening (1). MTA, the product of SAM metabolism during PA and ethylene formation, was reported to be recycled back to SAM through MTR, MTR-1-P, 2-keto-4-methylthiobutyric acid, and methionine (1, 17) during ethylene synthesis; however, no information is available on the recycling of the MTA molecules produced during PA synthesis.

In this paper, we report on the fate of the MTA molecules and the kinetic changes in MTA nucleosidase and MTR kinase in relationship to PA and ethylene biosynthesis. We have also included a thorough examination of the relationship between PA and ethylene biosynthetic pathways. In addition, since avocado fruit growth was reported to differ from other fruit in that cell division is the major factor contributing to the increase in fruit size, in early as well as later stages of fruit development (23, 29), we had anticipated that polyamines will remain relatively high throughout the avocado fruit development. However, the present data demonstrate that any cell division which may occur during the later stages of the avocado fruit growth is not sufficient to maintain high levels of polyamines.

MATERIALS AND METHODS

Plant Materials. Avocado fruit (*Persea americana* cv Simmonds) were grown at the USDA Subtropical Horticulture Station, Miami, FL.

[1] Abbreviations: PA, polyamines; Spm, spermine; Put, putrescine; ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAM, *S*-adenosylmethionine; MTA, 5′-methylthioadenosine; MTR, 5-methylthioribose; MTR-1-P, 5-methylthioribose-1-phosphate; DAPI, 2′6′-diamidino-2-phenylindole; agm, Agmatine; ACC, 1-aminocyclopropane-1-carboxylic acid.

Chemicals. L-[U-^{14}C]ornithine (270 mCi/mmol) and L-[U-^{14}C]-arginine (336 mCi/mmol) were purchased from Amersham Corp.;[2] L-arginine,L-ornithine, DTT, pyridoxal-5′-phosphate, and Agm from Sigma; Spm tetrahydrochloride, Spd trihydrochloride, and Put from Calbiochem; benzoyl chloride and acetonitrile from Baker.

Preparation of Cell Free Extracts. For ODC activity, fruit tissues were homogenized in 0.1 M Hepes buffer (pH 7.8) containing 2 mM DTT, 1 mM pyridoxal-5′-phosphate, and 20 mM Na_2-EDTA at 0.1 g fresh weight/ml. For ADC activity, fruit tissues were homogenized in 0.2 M K-phosphate buffer (pH 7.2), 2 mM DTT, 1 mM pyridoxal-5′-phosphate, and 20 mM Na_2-EDTA at 0.1 g fresh weight/ml buffer. The homogenates were centrifuged for 20 min at 20,000*g* and the supernatants were used as the cell free extracts. Protein concentrations were determined according to Bradford (6).

Polyamine Analysis. PA concentrations were monitored throughout fruit development. Three fruit from each stage of development were randomly selected and a total of 2.0 g fresh weight of tissue was blended in 10 ml of 5% perchloric acid in a polytron homogenizer (Brinkman Instruments). The homogenates were centrifuged at 20,000*g* for 20 min, and the supernatant was saved, while the pellet was rehydrolyzed with the same volume of PCA to remove most of the PCA soluble conjugated PA. The two fractions were pooled and assayed for total PA (free and conjugated) according to a previously described procedure (13, 19) with some modifications. To 500 μl of the extract, 2 ml of 2 N NaOH and 5 μl benzoyl chloride were added, and the mixture was vortexed for 10 s and incubated at 30°C for 20 min. The reaction was terminated by adding 2 ml of saturated NaCl and was extracted with 4 ml chilled diethyl ether. The ether fraction was collected and centrifuged at 3,000*g* for 5 min at 2°C, and a 2 ml aliquot was dried in a vacuum oven. The benzoylated PA were resuspended in 200 μl acetonitrile, and a 20 μl sample was injected into a Waters HPLC system equipped with a C-18 reverse phase column (30 cm × 4 mm), a UV detector set at 254 nm, and a mobile phase of acetonitrile. Put, Spd, and Spm were chromatographed using 50% acetonitrile in an isocratic elution, while Agm was separated using a linear gradient of 50 to 100% acetonitrile.

DNA Quantification. DNA content was quantified by using DAPI, which binds specifically to the A-T base pairs of the DNA to form a fluorescent complex. Fruit tissues were extracted in 10 mM tris buffer (pH 7.0) containing 10 mM Na_2-EDTA, 2 M NaCl, and 3% (w/w) polyvinylpyrrolidone at 0.1 g fresh weight/5 ml buffer using a polytron homogenizer set at medium speed for 20 s. The tissue extract was centrifuged at 27,000*g* for 20 min, the supernatant was extracted with 1.5 volume chloroform, and assayed for DNA content according to Baer *et al.* (4).

Enzyme Assays. ODC and ADC activities were assayed according to Slocum *et al.* (27), except that phenethylamine was used instead of KOH to trap $^{14}CO_2$. The activities of MTA nucleosidase and MTR kinase were assayed according to previously described procedures (10, 11). The standard reaction mixture for MTA nucleosidase assay contained in a total volume of 0.2 ml, 0.1 M Hepes buffer (pH 7.8), 10% (v/v) glycerol, 0.1 mM Na_2-EDTA, 3 mM DTT, 100 μM 5′-[$^{14}CH_3$]-MTA (3.5×10^7 cpm/μmol, 0.5 μmol/ml), and enough protein to allow for approximately 20% substrate consumption. The reaction mixture was incubated at 30°C for 1 h then terminated by adding 0.6 ml chilled 95% ethanol. The resulting precipitate was removed by centrifugation in a Beckman microfuge set at about 5000*g* for 4 min. A 400 μl fraction of the supernatant was applied to a Dowex 50W × 4 (H^+) 100 to 200 mesh column, and the product of the enzyme reaction (MTR) was eluted off the column with a total of 6 ml distilled water. To avoid quenching, each 3 ml water fraction was counted separately, and then all the fractions were pooled. Radioactivity was determined in a LKB model 1217 Rackbeta using toluene/Triton X-100 (2:1 v/v) and a fluor. MTR kinase activity was determined according to Ferro *et al.* (10).

ACC and Ethylene Determination. ACC and ethylene levels were monitored throughout the course of fruit ripening. Unripe mature avocado fruits were placed in a chamber equipped with flow-through humidified air at 200 ml/min. Ethylene was determined daily by collecting a 1 ml air sample from the outlet of the air flow through the chamber and injecting it into a GC (Hewlett-Packard model 5890A) equipped with a flame ionization detector. After ethylene sampling, three fruits were randomly selected, and a total of 2.0 g tissue was weighed and blended in 5 ml chilled 9% TCA. The homogenate was centrifuged at 20,000*g* for 20 min, and the supernatant was passed through a Dowex 50W × 4 (H^+) column. ACC was eluted from the column with 2 N NH_4OH. After vacuum concentration at 50°C, the eluates were assayed for ACC according to Lizada and Yang (20).

RESULTS

Changes in DNA Content during Fruit Development. Avocado fruits have higher DNA levels during the early stages than during the later stages of development (Fig. 1). DNA content reached approximately 620 μg/g fresh weight at 15 d from full bloom then sharply declined to 200 μg/g fresh weight at full maturity. These results indicate that 'Simmonds' avocado fruits are undergoing active cell division during the first 2 months of fruit development, while very little cell division is taking place at the later stages.

Determination of Polyamine Levels during Fruit Development. HPLC analysis of PA titers during avocado fruit development from full bloom (0 month) to full development (5 months from full bloom) showed significant differences in their concentrations (Fig. 2). Put concentration during the early stages of fruit de-

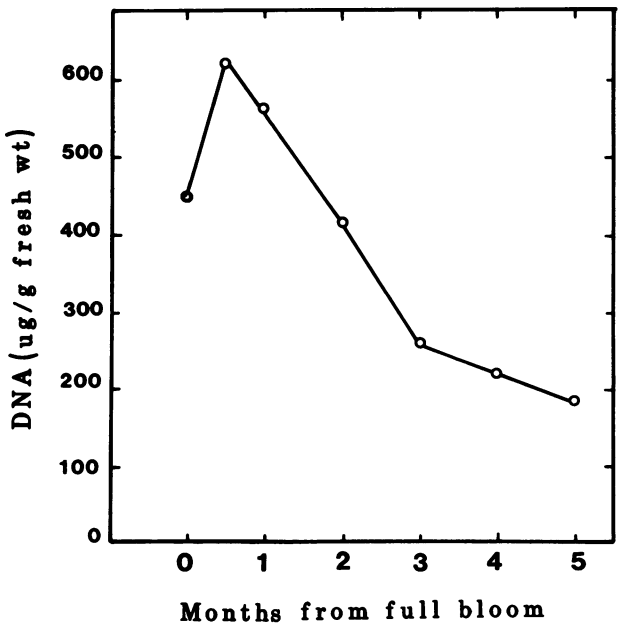

FIG. 1. DNA content of 'Simmonds' avocado fruits at different developmental stages. Results are an average of duplicate measurements.

[2] Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

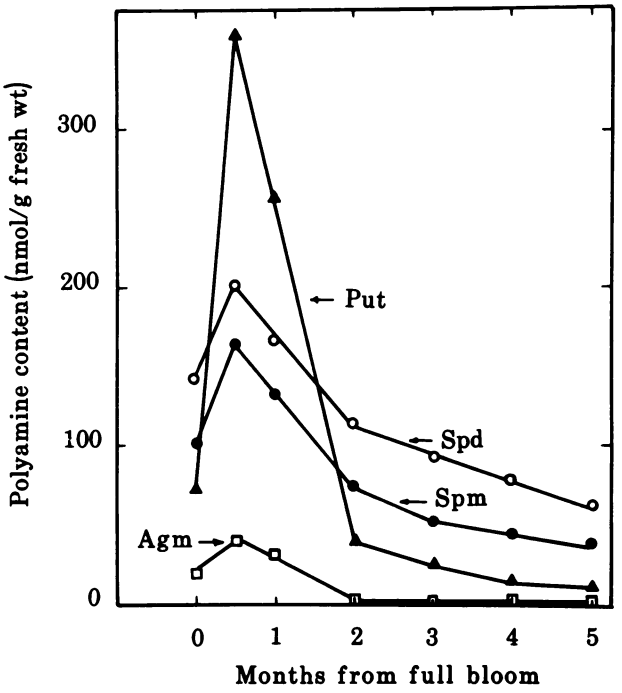

FIG. 2. Changes in total polyamine titers during avocado fruit development. (▲), putrescine; (○), spermidine; (●), spermine; (□), agmatine. Results are an average of duplicate measurements.

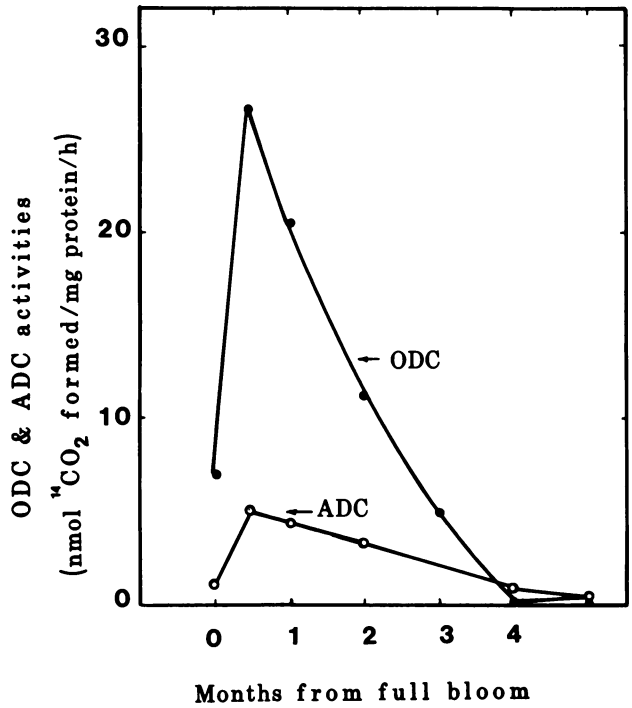

FIG. 3. Changes in ODC (●) and ADC (○) activity during fruit development. Results are an average of duplicate measurements.

velopment was lower than Spd and Spm but higher than Agm. When the fruits started to increase in size, however, Put concentration increased to more than 5-fold the original concentration at full bloom. Spd increased 1.6-fold, Spm increased 1.4-fold, while Agm increased only 0.8-fold. After 2 months of fruit development, the Put level dropped to below that of Spd and Spm. When fruits reached full development, PA concentrations returned to approximately the same level at full bloom. During fruit development, PA titers (Fig. 2) followed a pattern similar to that of DNA, providing further evidence for increased PA synthesis during active cell division. PCA-insoluble conjugated polyamines were not included in our assay because they have been reported to constitute less than 10% of the total PA (27).

Changes in ODC and ADC Activities. PA biosynthesis during avocado fruit development was also examined by assaying for ODC and ADC activities (Fig. 3). The changes in ODC and ADC activities were similar to the changes in DNA and PA titers. During the first few days of fruit growth, both ODC and ADC activities increased sharply until they reached their peak of 26 and 5 nmol/mg protein/h respectively at 15 d. The activities of both enzymes declined at the later stages of fruit development. At full fruit development, only trace amounts of ODC and ADC activities remained (Fig. 3).

During peak activity ODC was about fivefold higher than ADC, confirming earlier findings (7, 27) that the main pathway for Put synthesis during active cell division is through ornithine rather than Agm.

Changes in MTA Nucleosidase and MTR Kinase Activities during Avocado Fruit Development and Ripening. MTA is produced in stoichiometric amounts as a result of the transfer of an aminopropyl group from decarboxylated-SAM to Spd and Spm (26). Examination of MTA nucleosidase activity showed approximately 50% increase during the first 15 d after full bloom (Table I). The activity remained unchanged until the fruits reached full maturity after which it increased to more than threefold.

Table I. *Changes in MTA-Nucleosidase and MTR-Kinase Activities during Avocado Fruit Development*

Development Index	MTA Nucleosidase	MTR Kinase
d from full bloom	*pmol/mg protein/min*	
0	306.8[a]	28.3
15	468.3	100.1
30	450.7	100.0
60	427.9	91.8
90	443.7	91.0
120	814.0	327.0
150	743.6	364.0

[a] Data represent the mean of two replicates.

MTR kinase activity was 28.3 pmol/mg protein/min at full bloom, then increased to more than threefold at 15 d (Table I). At 120 d, MTR kinase activity increased to 327 pmol/mg protein/min, and at 150 d the activity was 364 pmol/mg protein/min.

MTA was also reported to be produced in a stoichiometric amount during fruit ripening as a result of the metabolism of SAM to ACC (2). Avocado fruits held for 3 d at 20°C showed the highest MTA nucleosidase activity (1600 pmol/mg protein/min) (Fig. 4). The activity then sharply declined as the fruits reached advanced stages of ripening.

MTR kinase exhibited a similar pattern of change as MTA nucleosidase (Fig. 4). MTR kinase activity steadily increased to a maximum level of approximately 870 pmol/mg protein/min after the fruits were held at 20°C for 3 d (Fig. 4). The activity then declined to approximately the same level prior to the onset of the ripening process. Figure 4 also indicates that in all the stages of avocado fruit ripening MTA nucleosidase was twice as active as MTR kinase. Due to the toxic nature of MTA, plant tissues tend to quickly metabolize MTA to MTR.

Changes in ACC and Ethylene during Fruit Ripening. Changes

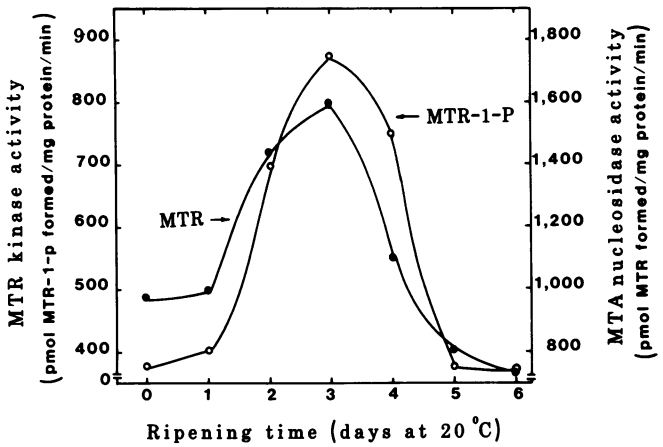

FIG. 4. Changes in MTA nucleosidase activity as represented by the amount of MTR formed (●) upon incubation of tissue extracts at 30°C for 1 h and MTR kinase activity as represented by the amount of MTR-1-P formed (○) upon incubation of tissue extracts at 30°C for 2 h. Results are an average of duplicate measurements.

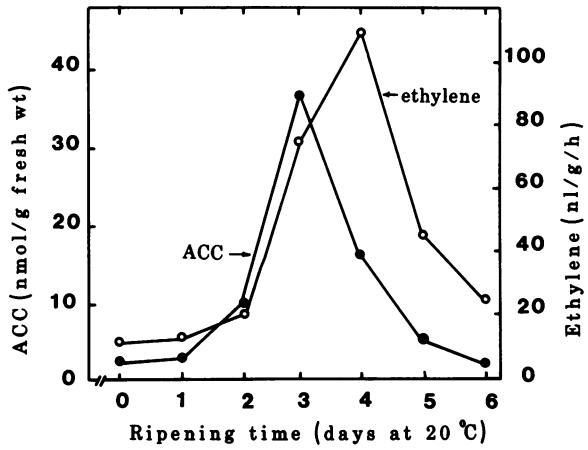

FIG. 5. Changes in ACC and ethylene levels during fruit ripening. (●), ACC; (○), ethylene. Results are an average of triplicate samples.

in the levels of ACC and ethylene during avocado fruit ripening are shown in Figure 5. Trace amounts of ACC and ethylene were detected during the first 2 d of fruit ripening at 20°C. At 3 d ACC reached its maximum level of about 36 nmol/g fresh weight. The ACC concentrations then declined to as low as 2 nmol/g fresh weight at the overripe stage. Ethylene synthesis was observed earlier than ACC synthesis (Fig. 5). Ethylene production, which reached its maximum level of approximately 110 nl/g/h at 4 d of ripening, declined to about 22 nl/g/h at the overripe stage (Fig. 5).

DISCUSSION

Most fruits undergo intensive cell division during the first few weeks postfertilization, after which cell division ceases and the fruits continue to increase in size by cell enlargement (22). Avocado fruit cells are unique, since they have been reported to continue dividing as long as the fruits remain attached to the tree (23, 29). Schroeder (23) showed that after avocado fruits reach approximately half their optimum size, a combination of cell enlargement and cell division are responsible for their continued growth. Valmayor (29) reported that cell division in avocado continues to increase the cell number within the tissue at all the stages of development even after the fruits reach full maturity. The concentration of PA titers in tomato and tobacco ovaries were reported to be higher during phases of active cell division, mainly the first 2 to 3 weeks of fruit growth. Based on the previous observations, we had anticipated that the pattern of PA titers in avocado would continue to be relatively high throughout the fruit development and therefore *S*-adenosylmethionine, which is a substrate for both ethylene and PA synthesis, would be directed toward PA and not ethylene synthesis. Examination of the PA titers during the development of 'Simmonds' avocado, however, showed a similar pattern of change as has previously been reported in tomato and tobacco ovaries (7, 27). Similarly, 'Simmonds' avocado exhibited higher DNA and ODC during the early stages than the later stages of fruit development. Based on these observations, 'Simmonds' avocado appears to have normal growth pattern of active cell division during the early stages followed by cell enlargement at the later stages of fruit development. In addition, any cell division which may take place during the later stages of fruit development is not sufficient to maintain high levels of polyamines.

Exogenously applied PA have been reported to inhibit ethylene synthesis (3). However, the low level of PA at the later stages of the avocado fruit development (Fig. 2) suggests that PA are not solely responsible for the inability of these fruits to produce ethylene and ripen while still attached to the tree. Also, the lack of any substantial ethylene production during maximum PA synthesis and *vice versa* indicate that the two pathways are not actively competing for their mutual substrates at any given stage of the fruit development.

In carrots and sweet peas, ADC has been reported as the primary enzyme for PA synthesis (12). In tomato and tobacco ovaries, ODC has been reported as the primary enzyme (7, 27), while in young leaves from several plant species, no correlation was observed between ADC or ODC activities and the total PA content (5). Our results indicate that in a partially purified avocado extract from the early stages of fruit growth, ODC activity is up to 7 times greater than ADC activity, while at full maturity both ODC and ADC activities declined considerably (Fig. 3). These results suggest that the main pathway for PA synthesis during the early stages of fruit growth is through ornithine, while at the later stages PA synthesis is negligible. In contrast, Winer *et al.* (30) reported higher levels of ADC activity in 'Fuerte' avocadoes during fruit development and ripening. They attributed the high ADC activity to the elimination of an unidentified inhibitor by dialysis and heat inactivation. In our experiments, however, we were not able to increase ADC activity by dialysis or heat inactivation.

Avocadoes, unlike tomatoes (18), exhibited a relatively lower MTA nucleosidase activity during the early stages than the later stages of fruit development (Table I), which suggests the following: (a) the MTA recycling pathway is less active during PA synthesis than during ethylene synthesis, (b) the activity of MTA nucleosidase during PA and ethylene synthesis is sufficient to accommodate the amount of MTA produced, and/or (c) the MTA produced during PA biosynthesis may be stored for further metabolism during ethylene synthesis. Efforts to detect an increase in MTA level during PA biosynthesis were not successful (data not shown). Nevertheless, the increase in MTA nucleosidase and MTR kinase activities during PA synthesis indicate that MTA is being recycled to SAM.

MTA is also produced in a stoichiometric amount during ethylene synthesis (1). The MTA produced during PA synthesis and ethylene synthesis is recycled through methionine to generate more SAM. MTA nucleosidase activity during avocado fruit ripening followed a similar pattern as ACC and ethylene. Mature fruits lost very little MTA nucleosidase and MTR kinase activities

even when kept in storage for up to 8 months (data not shown), which suggests that the recycling of MTA to SAM is necessary for continuous ethylene synthesis. At peak activity, MTA nucleosidase was 5 times greater during ethylene synthesis than during PA synthesis, an indication that the MTA recycling pathway is significantly more active during ethylene than during PA synthesis.

LITERATURE CITED

1. ADAMS DO, SF YANG 1979 Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76: 170–174
2. ALTMAN A, R KAUR-SAWHNEY, AW GALSTON 1977 Stabilization of oat leaf protoplasts through polyamine-mediated inhibition of senescence. Plant Physiol 60: 570–574
3. APELBAUM A, AC BURGOON, JD ANDERSON, M LIEBERMAN, R BEN-ARIE, AK MATTOO 1981 Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts. Plant Physiol 68: 453–456
4. BAER GR, SP MEYER, WT MOLIN, LE SCHRADER 1982 A simple and sensitive DNA assay for plant extracts. Plant Physiol 70: 999–1003
5. BIRECKA H, AJ BITONTI, PP MCCANN 1985 Activities of arginine and ornithine decarboxylases in various plant species. Plant Physiol 79: 515–519
6. BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal Biochem 72: 248–254
7. COHEN E, SM ARAD, YM HEIMER, Y MIZRAHI 1982 Participation of ornithine decarboxylase in early stages of tomato fruit development. Plant Physiol 70: 540–543
8. COSTA G, N BAGNI 1983 Effects of polyamines on fruit set of apple. HortScience 18: 59–61
9. FEIRER RP, G MIGNON, JD LITVAY 1984 Embryogenesis in the wild carrot. Science 223: 1433–1435
10. FERRO AJ, A BARRETT, SK SHAPIRO 1978 5-methylthioribose kinase, a new enzyme involved in the formation of methionine from 5-methylthioribose. J Biol Chem 253: 6021–6025
11. FERRO AJ, A BARRETT, SK SHAPIRO 1976 Kinetic properties and the effects of substrate analogues on 5′-methylthioadenosine nucleosidase from *Escherichia coli*. Biochem Biophys Acta 438: 487–494
12. FIENBERG AA, JH CHOI, WP LUBICH, ZR SUNG 1984 Developmental regulation of polyamine metabolism in growth and differentiation of carrot culture. Planta 162: 532–539
13. FLORES HE, AW GALSTON 1982 Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol 69: 701–706
14. GALSTON AW 1983 Polyamines as modulators of plant development. BioScience 33: 382–388
15. HEIMER YM, Y MIZRAHI, U BACHRACH 1979 Ornithine decarboxylase activity in rapidly proliferating plant cells. FEBS Lett 104: 146–148
16. KAUR-SAWHNEY R, LM SHIH, AW GALSTON 1982 Relation of polyamine biosynthesis to the initiation of sprouting in potato tuber. Plant Physiol 69: 411–415
17. KUSHAD MM, DG RICHARDSON, AJ FERRO 1983 Intermediates in the recycling of 5-methylthioribose to methionine in fruits. Plant Physiol 73: 257–261
18. KUSHAD MM, DG RICHARDSON, AJ FERRO 1985 5′-methylthioadenosine nucleosidase and 5-methylthioribose kinase activities and ethylene production during tomato fruit development and ripening. Plant Physiol 79: 525–529
19. LIN PP 1984 Polyamine metabolism and its relation to response of the aleuron layers of barley seeds to gibberellic acid. Plant Physiol 74: 975–983
20. LIZADA MC, SF YANG 1979 A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100: 140–145
21. MCDONALD R, MM KUSHAD 1986 Accumulation of putrescine during chilling injury of fruits. Plant Physiol 82: 324–326
22. PALAVAN N, AW GALSTON 1982 Polyamine biosynthesis and titer during various developmental stages of *Phaseolus vulgaris*. Physiol Plant 55: 438–444
23. SCHROEDER CA 1953 Growth and development of the avocado fruit. Calif Avocado Soc Yearbook 42: 114–118
24. SEN K, MM CHOUDHURI, B GHOSH 1981 Changes in polyamine contents during development and germination or rice seeds. Phytochemistry 20: 631–633
25. SHIH LM, R KAUR-SAWHNEY, J FUHRER, S SAMANTA, AW GALSTON 1982 Effect of exogenous 1,3-diaminopropane and spermidine on senescence of oat leaves. Plant Physiol 70: 1592–1596
26. SLOCUM RD, R KAUR-SAWHNEY, AW GALSTON 1984 The physiology and biochemistry of polyamines in plants. Arch Biochem Biophys 235: 283–303
27. SLOCUM RD, AW GALSTON 1985 Changes in polyamine biosynthesis associated with post-fertilization growth and development in tobacco ovary tissues. Plant Physiol 79: 336–343
28. SMITH TA 1985 Polyamines. Annu Rev Plant Physiol 36: 117–143
29. VALMYOR RV 1967 Cellular development of the avocado from blossom to maturity. Philipp Agric 50: 907–967
30. WINER L, C VINKLER, A APELBAUM 1984 Partial purification and characterization of arginine decarboxylase from avocado fruit, a thermostable enzyme. Plant Physiol 76: 233–237