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Recycling of 5′-Methylthioadenosine-Ribose Carbon Atoms into Methionine in Tomato Tissue in Relation to Ethylene Production

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ABSTRACT

The ribose moiety of 5′-methylthioadenosine (MTA) is metabolized to form the four-carbon unit (2-aminobutyrate) of methionine in tomato tissue (*Lycopersicon esculentum* Mill., cv. Pik Red). When [U-^{14}C-adenosine] MTA was administered to tomato tissue slices, label was recovered in 5-methylthioribose (MTR), methionine, 1-aminocyclopropane-1-carboxylic acid (ACC), C_2H_4 and other unidentified compounds. However, when [U-^{14}C-ribose]MTR was administered, radioactivities were recovered in methionine, ACC and C_2H_4, but not MTA. This suggests that C_2H_4 formed in tomato pericarp tissue may be derived from the ribose portion of MTA via MTR, methionine and ACC. The conversion of MTR to methionine is not inhibited by aminoethoxyvinylglycine (AVG), but is O_2 dependent. These data present a new salvage pathway for methionine biosynthesis which may be important in relation to polyamine and ethylene biosynthesis in tomato tissue.

Methionine serves as a precursor of ethylene in model systems as well as in fruits and other plant tissues (10). In the conversion of methionine to ethylene in model systems, and also to some extent in tissues, C-1 is converted to CO_2, C-2 to formic acid, and C-3, 4 to ethylene (10). The sulfur atom and its related methyl group appear to be retained in the tissue (18). Adams and Yang (1) examined the fate of the CH_3S-group of methionine during its conversion to ethylene in apple tissue and found that it is converted into MTA[1] and MTR via SAM. The CH_3S-group of MTA was effectively recycled back as a unit to reform the CH_3S-group of methionine via MTR. They also postulated that MTR donates its methylthio group to a four-carbon acceptor to reform methionine. The metabolic fate of the ribose part of the MTA molecule in plant tissue remained obscure.

Shapiro and Schlenk (17) noted that [U-^{14}C-adenosine]MTA was converted into SAM by yeast cells. The U-^{14}C-labeled pentose of [U-^{14}C-adenosine]MTA was present in the four-carbon chain of the methionine part of the sulfonium compound but none of the label of the pentose was found in the ribose portion of the SAM molecule. Shapiro and Barrett (16) further demonstrated that the ribose moiety of MTR furnished part, if not all, of the four carbon chain of methionine. The ribose part as well as the methyl group of MTR contributed to the structure of methionine in cell-free extracts of *E. aerogenes*. Backlund and Smith (4) also reported the formation of methionine from MTA in cell-free homogenates of rat liver. The CH_3S-group and carbons from the ribose portion of MTA were also incorporated into methionine.

Because the metabolic fate of the ribose portion of MTA in plant tissue remained unclear, the present study was undertaken to determine the fate of the ribose part of MTA in tomato tissue, to see if it relates to what has been found in yeast, bacteria and rat liver.

MATERIALS AND METHODS

Plant Materials. Tomato fruits (*Lycopersicon esculentum*, Mill., cv. Pik Red) in the breaker stage grown at Beltsville, MD, were used in the experiments.

Chemicals. Methionine was purchased from J. T. Baker, Phillipsburg, NJ. AVG was a gift from A. Stempel of the Research Division, Hoffman LaRoche. [^{14}C-methyl]SAM was purchased from Amersham. [U-^{14}C]ATP was obtained from New England Nuclear. [U-^{14}C-adenosine]SAM was synthesized from L-methionine and [U-^{14}C]ATP using a partially purified methionine adenosyltransferase prepared from baker's yeast (4, 7). [^{14}C-methyl] MTA and [U-^{14}C-adenosine]MTA were prepared from [^{14}C-methyl]SAM and [U-^{14}C-adenosine]SAM, respectively (15), and were further purified by TLC on silica gel using chloroform: methanol:water (65:25:4, v/v/v) as a solvent system (8). [U-^{14}C-ribose]MTR was prepared by hydrolysis of [U-^{14}C-adenosine] MTA in 0.01 N HCl or by MTA nucleosidase prepared from apple suspension cells (unpublished data) and purified by ion exchange with Dowex 50-H^+ to remove adenine. Unlabeled MTR was similarly prepared from MTA.

Feeding Experiments. Tomato pericarp tissue slices 0.7 cm wide and 2.5 cm long, were cut from tomato fruit with a scalpel and were quickly rinsed with 2% (w/v) KCl and blotted dry with a paper towel. The desired labeled substrates were infused by a vacuum infiltration technique previously described (5). For incubation in air or nitrogen atmospheres, tissue was prepared as above and incubated in a 12-ml plastic syringe as described by Adams and Yang (1).

$^{14}C_2H_4$ Determination. After flushing with air, the syringes were sealed. Gas samples were taken periodically from the incubation syringe with a gas-tight hypodermic syringe. Radioactive C_2H_4 was absorbed in 0.5 ml of cold 0.25 M $Hg(ClO_4)_2$ for 3 h. Then, 10 ml of Aquasol scintillation fluid was added and radioactivity was assayed in a liquid scintillation counter.

Identification of Radioactive Metabolites. After incubation, tissues were quickly rinsed with 2% KCl and distilled H_2O to remove excess radioactive MTA or MTR which adhered to the tissue surface and were then homogenized and extracted with ice-cold 80% ethanol, centrifuged (1,000*g* for 10 min), and the pellet was reextracted twice with an additional aliquot of 70% ethanol. The combined supernatants were concentrated *in vacuo* at 40°C.

[1] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; Ad, adenine; AVG, aminoethoxyvinylglycine (2-amino-4-(2′-aminoethoxy) *trans*-3-butenoic acid); FMN, flavin mononucleotide; MTA, 5′-methylthioadenosine or 5′-*S*-methyl-5′-thioadenosine; MTR, 5-methylthioribose or 5-*S*-methyl-5-thioribose; SAM, *S*-adenosylmethionine; SAP, *S*-adenosyl-3-methylthiopropylamine.

The radioactive metabolites, such as ACC, methionine, MTA, and MTR were identified by paper co-chromatography and co-electrophoresis with authentic compounds (1, 2). Paper chromatography was carried out in 1-butanol:acetic acid:water (4:1:5, v/v/v). Paper electrophoresis for separation of MTA and MTR was performed at pH 2.2 in 10% acetic acid. The chromatograms were scanned for radioactivity with a Baird-Atomic radiochromatogram scanner equipped with a digital integrator. The regions corresponding to ACC, methionine, MTA, and MTR were cut from the chromatograms and the materials were eluted from the paper with 50% ethanol and concentrated *in vacuo* at 40°C. Identification of radioactive ACC from tomato tissue fed with [U-^{14}C-adenosine] MTA was carried out by degrading the labeled ACC to C_2H_4 by the NaOCl-Hg reaction (12). To determine the efficiency of the degradation of ACC to C_2H_4, 100 nmol unlabeled ACC was added to the labeled ACC and the percent recovery was calculated from the C_2H_4 produced after the degradation. Labeled C_2H_4 formed from labeled ACC was absorbed in 0.5 ml cold 0.25 M $Hg(ClO_4)_2$ for 3 h and counted as described above.

Methionine and its sulfoxide were identified by co-chromatography with authentic compounds in 1-butanol:acetic acid:H_2O (4:1:5, v/v/v). Radioactive methionine was oxidized to the methionine sulfoxide by reaction with 2% H_2O_2 at room temperature for 3 h, MTR was oxidized to the sulfoxide of MTR by treating with 0.05% dimethylsulfoxide in 3 N HCl at 100°C for 5 min (11). MTA and MTR were separated by paper electrophoresis at pH 2.2 in 10% acetic acid (1). MTA on the chromatogram was viewed under UV light (254 nm). The spot for MTR was visualized by reaction with an aniline-phosphoric acid solution. Spots for methionine and methionine sulfoxide were observed after spraying with 0.2% ninhydrin (9).

FMN Model System. The extracted methionine was degraded to CO_2, HCOOH, and C_2H_4 in the FMN-light degradation reaction (19). The reaction mixture consisted of 0.14 μCi [^{14}C]methionine, 1.0 μmol FMN and 50.0 μmol phosphate buffer (pH 8.4), made to a final volume of 1.3 ml in a 25-ml Erlenmeyer flask. The [^{14}C]methionine was recovered from tomato tissue after infiltration and incubation for 6 h with [U-^{14}C-adenosine]MTA. The flasks containing the reaction mixture without FMN were flushed with N_2 and closed with a serum cap. The reaction was started by injecting FMN through the serum cap. The flasks were incubated at room temperature (26°C) for 1 h at a light intensity of 350 ft-c (fluorescent lamp). $^{14}C_2H_4$ was absorbed in 0.5 ml cold 0.25 M $Hg(ClO_4)_2$. $^{14}CO_2$ was absorbed in 0.5 ml ethanolamine:2-ethoxyethanol mixture (1:1, v/v). After determination of $^{14}C_2H_4$ and $^{14}CO_2$ from the reaction mixture, the flasks were kept ice cold and a solution of 2 ml 3 M phosphate buffer (pH 2.5) and 1.0 g of $HgCl_2$ were added to convert HCOOH to CO_2 (14). A vial containing 0.2 ml 2 N NaOH was placed in each flask. The flasks were then fitted with serum caps, evacuated and heated slowly and held at 80°C in a water bath for 1 h. $^{14}CO_2$ was absorbed in 2 N NaOH and was released from NaOH by injecting 0.2 ml 2 N H_2SO_4 and was reabsorbed in 0.5 ml ethanolamine:2-ethoxyethanol mixture (1:1, v/v) and counted in a liquid scintillation counter as described above. Additional details of methodology are given in the legends.

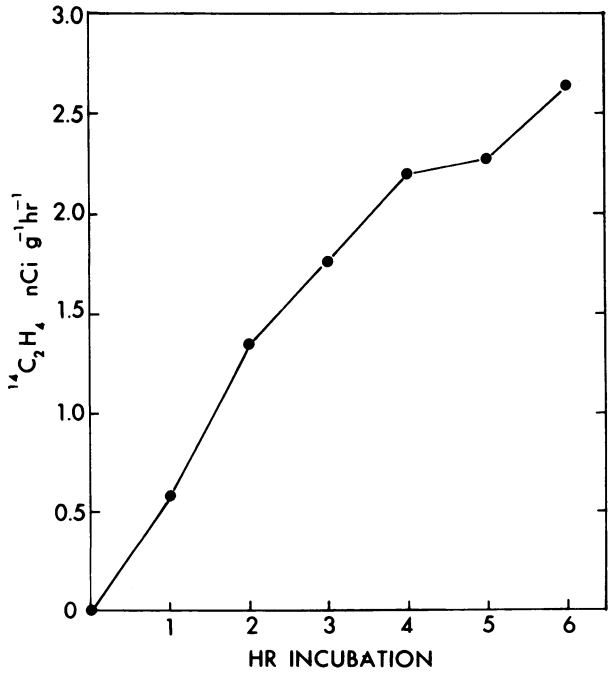

FIG. 1. Incorporation of radioactivity into ethylene by tomato pericarp slices (2 g) infiltrated with 1.5 μCi [U-^{14}C-adenosine]MTA. The specific radioactivity of MTA was 41.0 μCi/μmol.

RESULTS AND DISCUSSION

Conversion of [U-^{14}C-adenosine]MTA into $^{14}C_2H_4$ in Tomato Tissue. The rate of $^{14}C_2H_4$ production by the tissue after infiltration with 1.5 μCi [U-^{14}C-adenosine]MTA increased with time during the 6 h incubation period (Fig. 1). The radioactivity in $^{14}C_2H_4$ must be derived from labeled MTA indicating that tomato tissue is capable of converting [U-^{14}C-adenosine]MTA into $^{14}C_2H_4$.

Metabolism of [U-^{14}C-adenosine]MTA and Identification of MTR, Methionine, and ACC as Metabolites of [U-^{14}C-adenosine] MTA in Tomato Tissue. After chromatography of the tissue extracts, two of the radioactive peaks were identified by co-chromatography, oxidation, and degradation as ACC (R_F 0.42) and methionine (R_F 0.50) (Fig. 2). However, MTA and MTR have the same mobility in this chromatography system and appeared as a single peak at R_F 0.69. They were subsequently separated by paper electrophoresis (1) and found to consist of 60% of MTR. Positive identification of the MTR was provided by the following: (a) the radioactive material co-chromatographed with authentic MTR; (b) after oxidation with dimethylsulfoxide, the radioactive material co-chromatographed with the sulfoxide of authentic MTR at R_F 0.35: (c) the radioactive material showed no charge and did not move on paper electrophoresis at pH 2.2. These data provided evidence for the formation of MTR from MTA in tomato tissue. Adams and Yang (1) also showed conversion of MTA to MTR in apple tissue. The radioactive metabolite with an R_F 0.50 was identified as methionine by co-chromatography with authentic methionine on paper chromatography and also by co-chromatography with authentic methionine sulfoxide (R_F 0.17) after oxidation with 2% H_2O_2 at room temperature for 3 h. This suggests that MTA is converted into both MTR and methionine by tomato tissue. The radioactive spot at R_F 0.42 was identified as ACC by co-chromatography with authentic ACC, and by its ability to yield labeled C_2H_4 in the chemical degradation reaction of Lizada and Yang (12). Since the sequence for the biosynthesis of ethylene has been established as: methionine → SAM → ACC → C_2H_4 (2), it is assumed that labeled ACC was formed from labeled methionine. Additional peaks of radioactivity in Figure 2 are presently unidentified. Figures 1 and 2 indicate that C_2H_4 is produced in tomato tissue from the ribose portion of MTA via MTR, methionine, and ACC.

Metabolism of [U-^{14}C-ribose]MTR in Tomato Tissues. Labeled C_2H_4 was also produced after feeding tomato tissue with [U-^{14}C-ribose]MTR (Fig. 3). The amount of radioactivity recovered in C_2H_4 was approximately the same as in Figure 1 where about twice as much MTA radioactivity was used. This further proves that all the $^{14}C_2H_4$ derives from the ribose moiety of the molecule. Incorporation of label from [U-^{14}C-ribose]MTR into methionine and ACC by tomato pericarp slices incubated for 6 h is shown in

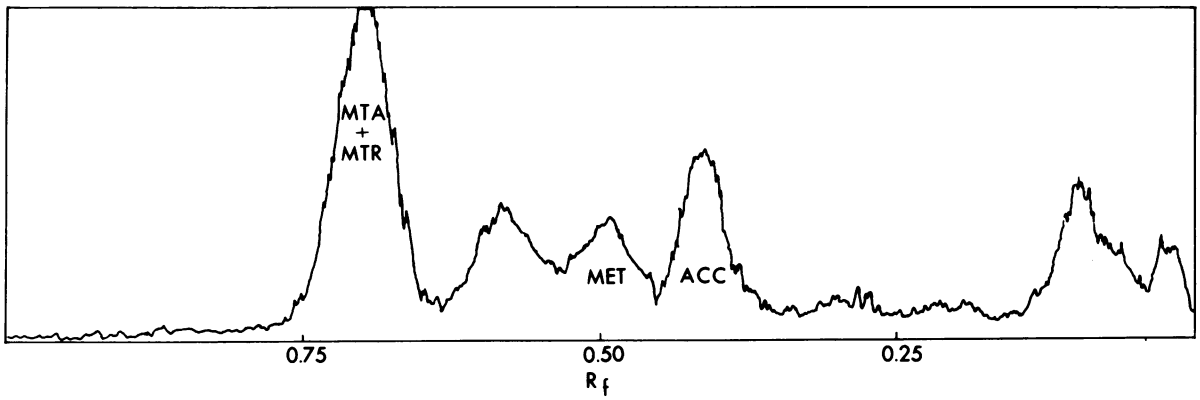

FIG. 2. Radiochromatogram scan of ethanol extract prepared from tomato pericarp tissue (2 g) infiltrated with 1.5 μCi of [U-^{14}C-adenosine]MTA and incubated for 6 h. The specific radioactivity of MTA was 41.0 μCi/μmol. The extract was chromatographed on paper in 1-butanol:acetic acid:H_2O (4:1:5, v/v/v).

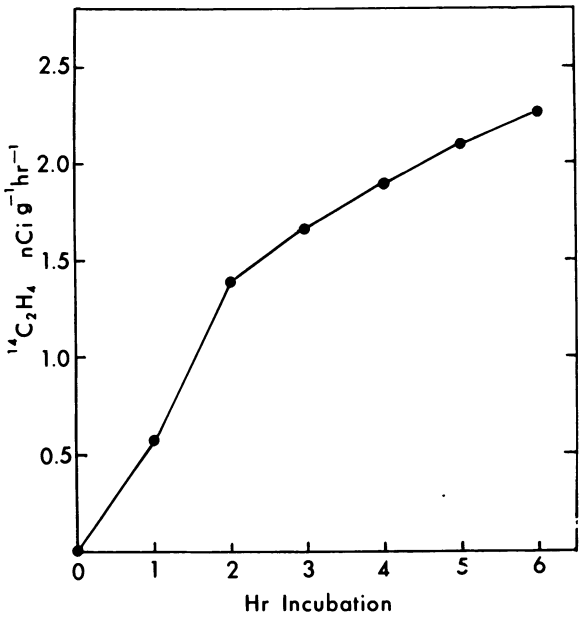

FIG. 3. Incorporation of radioactivity into ethylene by tomato pericarp slices (2 g) infiltrated with 0.70 μCi [U-^{14}C-ribose]MTR. The specific radioactivity of MTR was 41.0 μCi/μmol.

Table I. *Incorporation of 0.7 μCi [U-^{14}C-ribose]MTR into Methionine and ACC by Tomato Pericarp Tissue (2 g) Incubated 6 Hours*

The specific radioactivity of MTR was 41.0 μCi/μmol.

Compound	Total Radioactivity in the Tissue[a]
	%
ACC	24.8
Methionine	12.6
MTR	38.9
Total identified	76.3

[a] Residual activity was in unidentified peaks and the insoluble fraction.

Table I. This radioactive peak at R_F 0.69 of the extracted tissue fed [U-^{14}C-ribose]MTR was found to contain only MTR as determined by paper electrophoresis. Chemical identification of the radioactive materials, methionine and ACC, were as described as above. The radioactivity in ACC and methionine were found to be 24.8 and 12.6%, respectively, of the total radioactivity in the tissue. Radioactivity retained in MTR was 38.9%. The additional

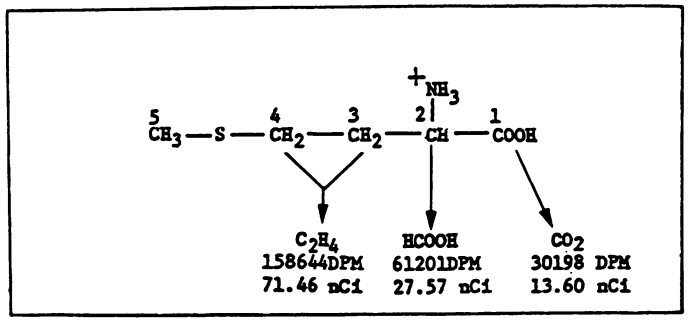

FIG. 4. Production of ethylene, formic acid, and CO_2 from methionine in FMN-light degradation system. Radioactive methionine (0.14 μCi) was recovered from tomato tissue after infiltration with [U-^{14}C-adenosine] MTA. The specific radioactivity of [U-^{14}C-adenosine]MTA was 41.0 μCi/μmol.

Table II. *Effect of AVG on the Conversion of MTA to MTR and Methionine by Tomato Tissue*

Recovery of radioactive MTR and methionine from tomato tissue (1 g) extracts after infiltration with 0.1 μmol methionine and 0.5 μCi [^{14}C-methyl]MTA ± AVG (10 nmol). Plugs were incubated for 3 h and the specific radioactivity of [^{14}C-methyl]MTA was 57.0 μCi/μmol.

Sample	Radioactivity		
	MTA	MTR	Methionine
		nCi[a]	
Control	84.4	119.1	90.6
AVG	82.4	122.6	91.3

[a] Total radioactivities retained in the tissues after 3 h incubation were 0.40 and 0.41 μCi for control and AVG, respectively. Residual activity was in unidentified peaks and the insoluble fraction.

23.6% of the radioactivity was retained in unidentified peak and the insoluble fraction. The present data establish that the ribose portion of MTR is converted into C_2H_4 via methionine and ACC by tomato tissue.

Verification of Formation of 2-Aminobutyrate Portion of Methionine from Ribose Carbons of MTA. In order to verify that the 2-aminobutyrate portion of methionine derives from the ribose carbons of MTA, 1.5 μCi [U-^{14}C-adenosine]MTA was infiltrated into pericarp tissue of a breaker tomato along with 0.1 μmol methionine and incubated for 6 h. The inclusion of unlabeled

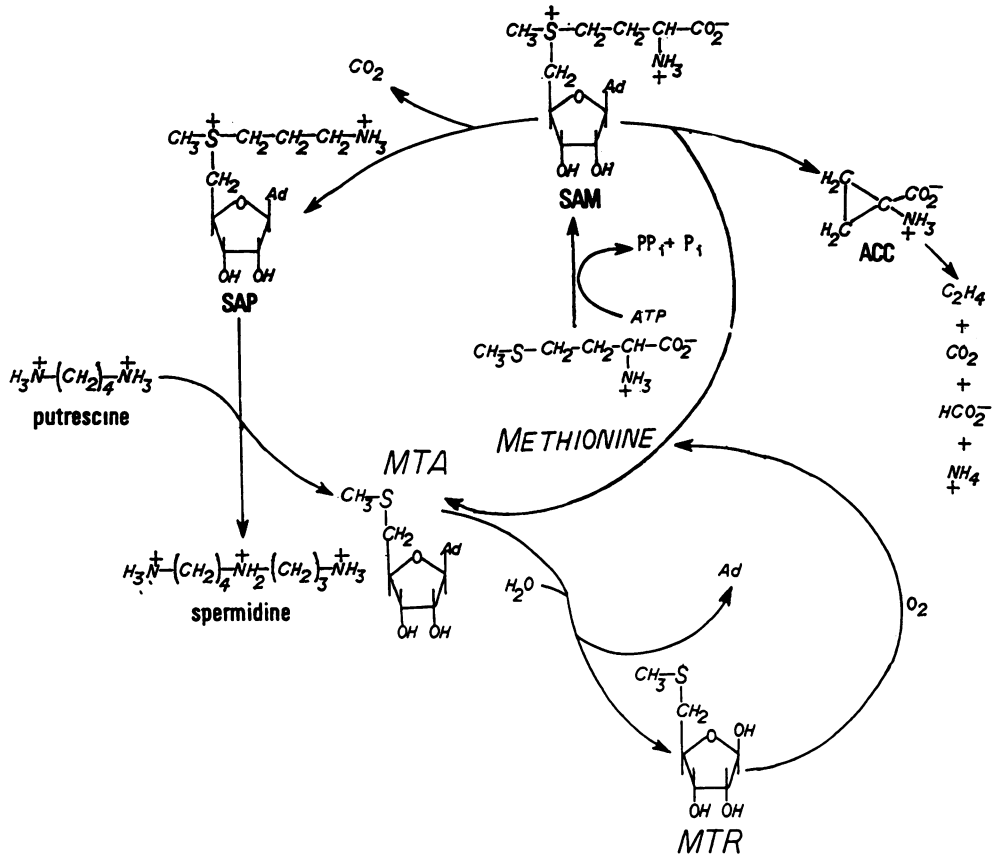

FIG. 5. A proposed scheme of methionine biosynthesis in relation to polyamine and ethylene biosynthesis in tomato tissue.

Table III. *Effect of Nitrogen on the Conversion of [^{14}C-methyl]MTA to MTR and Methionine by Tomato Tissue*

Each plug (1 g) of tomato tissue was infiltrated with 0.1 μmol methionine and 0.5 μCi of [^{14}C-methyl]MTA and incubated in air or in N_2 for 3 h. The specific radioactivity of [^{14}C-methyl]MTA was 57.0 μCi/μmol.

Sample	Radioactivity		
	MTA	MTR	Methionine
		nCi[a]	
Air	52.5	98.5	136.7
Nitrogen	48.6	240.0	0.0

[a] Total radioactivities retained in the tissues after 3 h incubation were 0.37 and 0.39 μCi for air and N_2, respectively. Residual activity was in unidentified peaks and the insoluble fraction.

methionine was intended to minimize the further metabolism of labeled methionine. The recovered radioactive methionine (0.14 μCi) from the tissue extract was degraded by the FMN-light reaction system (19). In the FMN-light reaction system, carbon 1 of methionine gives rise to CO_2, carbon 2 to HCOOH, and carbons 3 and 4 are incorporated into ethylene. As shown in Figure 4 radioactivities were found in all these reaction products. These results indicate that the 2-aminobutyrate portion of methionine was derived from the ribose portion of MTA. The specific steps involved in the pathway from MTR to methionine, however, are still unclear. Carbon 1 of the recovered methionine shows much lower activity than the other 3 carbons. This may be due to absorption of some of the CO_2 in the phosphate buffer (pH 8.4) or during synthesis of the parent compound, this carbon may initially have had lower radioactivity.

Shapiro and Schlenk (17) previously demonstrated the conversion of [U-^{14}C-adenosine]MTA into the amino acid part of SAM by yeast cells. They postulated that the recycling of the pentose into the four-carbon chain of methionine might be less effective than that of the other structural units and perhaps only two- or three-carbons were retrieved in the sulfonium compound. In the present study, we have verified that the ribose portion of MTA contributes to the four-carbon chain of methionine probably as a unit in tomato tissue. Backlund and Smith (4) have also shown that the ribose portion of MTA was modified to form the 2-aminobutyrate portion of methionine in the cell-free homogenates of rat liver.

Effect of AVG and Nitrogen on the Conversion of MTA to Methionine by Tomato Tissue. AVG is an irreversible inhibitor of pyridoxal phosphate-dependent enzymes (13) and has been shown to be a powerful inhibitor of ACC synthase (6, 20). It has been shown that the conversion of SAM into ACC is sensitive to AVG inhibition. The conversion of ACC to ethylene, however, is unaffected by AVG (2). Table II illustrates that the conversion of MTA to methionine via MTR is not influenced by 10 nmol AVG. Our unpublished data indicated that 10 nmol AVG is effective in inhibiting ethylene biosynthesis in "breaker" tomato tissue. Inasmuch as the conversion of MTA to methionine is not inhibited by AVG, these results suggest that the conversion of MTA → MTR → methionine is not mediated by pyridoxal phosphate.

Tissues fed with [^{14}C-methyl]MTA incubated in air produced both MTR and methionine, but tissues incubated in nitrogen produced only MTR but not methionine (Table III). These results indicate that the conversion of MTA to MTR is not O_2-dependent, but the conversion of MTR to methionine requires O_2. Adams and Yang (1) showed that under the nitrogen atmospheres, methionine could be converted to SAM which was in turn metabolized to ACC, MTA, and MTR. Our present study demonstrated that whereas the conversion of MTA to MTR does not require O_2,

the conversion of MTR to methionine can not occur without the presence of O_2.

Collectively, the data presented here show that C_2H_4 is produced in tomato pericarp tissue from the ribose portion of MTA via MTR, methionine and ACC. By feeding [U-^{14}C-adenosine]MTA to tomato tissue and by degrading recovered methionine by the FMN-light reaction system, we were able to verify that the 4-carbon unit (2-aminobutyrate) of methionine is derived from the ribose carbons of MTA. Methionine is activated at the sulfur atom to form SAM which serves as a common precursor for both ethylene and polyamines and yields the same product, MTA, upon metabolism of SAM (3). Based on recent studies and the data presented here, a new salvage pathway for methionine biosynthesis in relation to ethylene production and polyamine biosynthesis in tomato tissue is summarized in Figure 5. The CH_3-S-group of MTA (1) as well as the 4-carbon unit of the ribose portion of MTA can be recycled back to reform the methylthio group and 2-aminobutyrate portion of methionine. This appears to be an important salvage pathway for methionine biosynthesis and may be the best utilization of MTA which is a byproduct of SAM metabolism in plant tissue. The conversion of MTR to methionine is not inhibited by AVG, and is O_2-dependent. This salvage pathway for methionine biosynthesis may take place continuously during the production of ethylene and the biosynthesis of polyamines in tomato tissue. The specific steps involving the conversion of MTR to methionine still remain unclear and warrant further investigation.

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