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METHIONINE SYNTHESIS FROM 5-METHYLTHIORIBOSE IN APPLE TISSUE[1]

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The primary fate of 5-methylthioribose in apple tissue is the formation of methionine. Using dual labeled 5-methylthioribose, it was shown that both the CH_3S- group and the ribose portion of 5-methylthioribose were equally incorporated into methionine. Thus, the pathway involves modification of the ribose portion of 5-methylthioribose into the 2-aminobutyrate portion of methionine. This pathway functions to recycle methionine for continued synthesis of ethylene in fruit tissues. The methionine cycle in relation to ethylene biosynthesis is presented.

INTRODUCTION

Ethylene is a plant hormone initiating the ripening of many fruits (1). In the unripe apple, the ethylene production is very low, but increases dramatically at the onset of ripening and is sustained at a very high rate over a period of months. The biosynthetic pathway of ethylene in apple has been established as follows(2): Met[3] $\longrightarrow$ SAM $\longrightarrow$ ACC $\longrightarrow$ C_2H_4. Since the concentration of methionine in apple tissues is quite low, Baur & Yang (3) have suggested that the sulfur atom must be recycled back into methionine to maintain this continuous ethylene production. Subsequently Adams & Yang (2) demonstrated in apple tissue that during ethylene production the CH_3S- group of methionine was released as MTA from SAM with the concomitant formation of

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[3]Abbreviations: Met:methionine; SAM:S-adenosylmethionine; Ade:adenine; ACC:1-aminocyclopropane-1-carboxylic acid; MTA:5'-methylthioadenosine; MTR:5-methylthioribose.

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ACC, which was then degraded into ethylene. The MTA thus released was rapidly hydrolyzed to MTR, the CH_3S- group of which was then recycled as a unit into methionine (4,5). Adams and Yang (5) proposed that MTR donates its CH_3S- group to a 4-carbon acceptor, such as homoserine, to form methionine, while the ribose portion of MTR is split off. Thus, the 2-aminobutyrate portion of methionine would be derived from the 4-carbon acceptor, but not from the ribose portion of MTR.

Recent work by Shapiro & Schlenk (6) with yeast cells, Backlung & Smith (7) with rat liver homogenates, and Shapiro & Barrett (8) with cell-free extracts of *Enterobacter aerogenes* demonstrated that the radioactivity of MTA or MTR uniformly labeled at the ribose unit was recovered in methionine. They suggested that the ribose unit of MTA or MTR furnishes most, if not all, of the carbon chain of methionine. This prompted us to re-examine the fate of MTR in the recycling process of methionine formation in apple tissues. In this communication we report that the primary fate of MTR in apple tissue is the formation of methionine and that both the CH_3S- group and ribose portion of MTR are incorporated into methionine with equal efficiency.

MATERIALS AND METHODS

Plant Material. Apples, *Malus sylvestris* Mill, var. Golden Delicious, were obtained from a local grower at the beginning of the commercial harvest.

Chemicals. [Methyl-^{3}H]MTR was prepared from [methyl-^{3}H]SAM according to Schlenk & Ehninger (9) with modification: [Methyl-^{3}H]SAM (11 Ci/mmol) was first hydrolyzed into MTA in acetate buffer (0.01 M, pH 4.7) at 100 C for 20 minutes, then in 0.2 N HCl at 100 C for another 150 minutes. The reaction mixture was then separated and purified by passing through a Dowex-50 (H^+) column (0.6 X 6 cm). Methionine was added to the reaction mixture during the acid hydrolysis as well as to the effluents from the Dowex-50 column to prevent oxidation of MTR into its sulfoxides.

[Ribose-U-^{14}C]MTR was synthesized from [adenosine-U-^{14}C]MTA by the same procedures. [Adenosine-U-^{14}C]MTA (1.5 μCi/μmol) was synthesized as described elsewhere (6).

The purity of the prepared radioactive MTR was verified by paper chromatography and paper electrophoresis.

Feeding Experiment. Plugs (1 cm in diameter and 1.5 cm in length) were cut from an apple with cork borer and blade. Each plug was vacuum infiltrated (10) with 50 μl solution containing 3 μmol K_2HPO_4, 0.5 μmol methionine and the desired concentration of radioactive MTR. After incubation, plugs were homogenized and extracted with 80% EtOH. After concentration, the combined extracts were passed through a Dowex-50 (H^+) column and the column was eluted with 2N NH_4OH. The metabolites were separated by paper chromatography using 1-butanol:acetic acid:water (4:1:1.5, v/v/v) as developing solvent and by paper electrophoresis at pH 2.2 (10% acetic acid) or at pH 10.5 (0.1 M Na-borate)

and the radioactivity was monitored by a Packard radiochromatogram scanner. Radioactive CO_2 produced from the apple plugs was first absorbed into 0.2 ml of 20% KOH, from which the CO_2 was released by lactic acid and reabsorbed into 0.5 ml of ethanolamine-ethoxyethanol mixture (1:1, v/v). The mixture was counted by a scintillation counter. Radioactive ethylene produced was absorbed into 0.2 ml of 0.25 M $Hg(ClO_4)_2$ which was counted by a scintillation counter.

For the dual label experiment [methyl-^{3}H]MTR and [ribose-U-^{14}C]MTR were mixed to give a $^3H/^{14}C$ ratio (cpm) of 1.10, before feeding to the plugs. After chromatography, radioactivity in regions corresponding to methionine were cut from the paper, eluted with 50% EtOH, and the $^3H/^{14}C$ ratio was determined by a Beckman liquid scintillation counter. The counting efficiencies for ^3H and ^{14}C were 80 and 30%, respectively.

RESULTS AND DISCUSSION

After incubating apple plugs with [ribose-U-^{14}C]MTR, the metabolites eluted from the cation exchange resin column showed only one major radioactive peak in the paper chromatogram and paper electrophoretograms at pH 2.2 and pH 10.5. This radioactive compound co-migrated, both in paper chromatography and electrophoresis, with authentic methionine. For further characterization, the radioactive material was mixed with unlabeled methionine and was subjected to oxidation with 2% H_2O_2. The R_f value of the radioactive compound, originally 0.45, changed to 0.15 and the compound co-chromatographed with methionine sulfoxide. When this oxidized radioactive compound was subsequently reduced with 1% mercaptoethanol at 100 C for 1 h, the R_f value shifted back to 0.45 and the reduced compound again co-chromatographed with methionine. These oxidized and subsequently reduced metabolites also co-migrated in the paper electrophoresis with authentic methionine sulfoxide and methionine respectively.

Chromatographic analysis of the crude extract before ion exchanging revealed that after 6.5 h incubation with [ribose-U-^{14}C]MTR, 10% of the total radioactivity was in methionine and 86% in MTR; after 18 h the radioactivity in methionine increased to 30% while the radioactivity in MTR decreased to 54% (Fig. 1). The lower percentage of conversion of labeled MTR to methionine observed in this study as compared to that observed by Adams & Yang (2), is probably due to the lower specific radioactivity of MTR used in this investigation.

Previous studies have established that in the conversion of MTR to methionine in apple tissue, the methyl group and the sulfur atom were incorporated into methionine. In order to quantitate the relative incorporation of

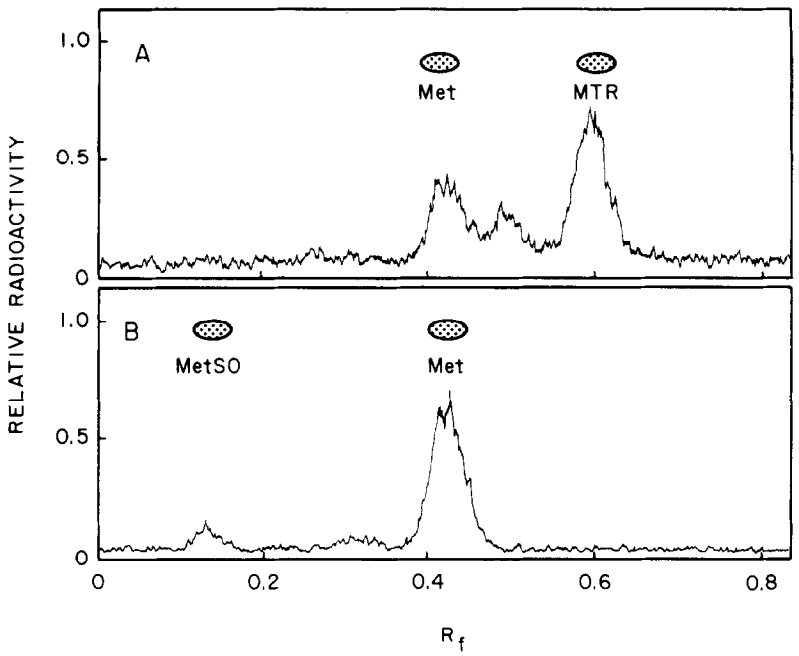

Fig. 1. Radiochromatogram scans of ethanol extracts of apple plugs which were infiltrated with 71 nCi [ribose-U-^{14}C]MTR (0.75 μCi/μmol) and incubated for 18 h. (A) Crude extract before passing through Dowex-50 (H^+) column. (B) Extract after passing through Dowex-50 (H^+) column.

CH_3S- group and ribose portion of MTR into methionine, a dual labeling experiment using [methyl-^{3}H]MTR and [ribose-U-^{14}C]MTR was performed. Following incubation, extraction, ion exchange resin fractionation, and paper chromatography, the methionine produced was eluted from the paper chromatogram, and the radioactivity was assayed by a scintillation counter. The $^3H/^{14}C$ ratio for precursor MTR was 1.10, and for methionine isolated after 6.5 and 18 h incubation was 1.35 and 1.29 respectively. If the pathway for methionine synthesis involves modification of the 5-carbon ribose portion of MTR into 4-carbon 2-aminobutyrate portion of methionine with the CH_3S- group remaining intact, the expected ratio for methionine should be 1.37 (1.10 X 5/4). Thus, the observed ratios of 1.35 and 1.29 for dually labeled methionine recovered after 6.5 and 18 h incubation (Table I) were very close to the predicted value of 1.37 expected for the salvage pathway in which the CH_3S- group and the ribose portion of MTR are incorporated together into methionine.

If unlabeled methionine was left out from the feeding solution, an increase in radioactive ethylene and a decrease in radioactive methionine were

Table I. $^{3}H/^{14}C$ ratio in methionine isolated from apple plugs which were fed with [methyl-^{3}H, ribose-U-^{14}C]MTR and incubated for 6.5 or 18 h.

Compound	^{3}H	^{14}C	$^{3}H/^{14}C$
	cpm	cpm	
MTR	21180	19177	1.10
Methionine (6.5 h)	4560	3379	1.35
Methionine (18 h)	7359	5700	1.29

Each apple plug was fed with 0.29 μCi [methyl-^{3}H, ribose-U-^{14}C]MTR (3.49 μCi/μmol) and 0.5 μmol unlabeled methionine.

observed (data not shown). This is expected because unlabeled methionine reduces the specific radioactivity of methionine pool in the tissues, and hence reduces the conversion of labeled methionine into ethylene.

When methionine is metabolized to ethylene, the C_1 carbon atom of methionine becomes CO_2 while carbon atoms C_3 and C_4 become C_2H_4. CO_2 produced from this conversion should have only half of the radioactivity of C_2H_4. In this investigation, the high radioactivity found in CO_2, 19 to 20 times higher than in C_2H_4, indicates that CO_2 is largely generated from biochemical reactions other than the conversion of methionine to ethylene. The biochemical steps involved in this conversion of MTR to methionine are not known. The conversion of MTR to methionine probably involves dehydration, oxidation, reduction, decarboxylation and transamination. If the conversion involves decarboxylation, the expected ratio of the radioactive methionine to readioactive CO_2 should be 4 after feeding the tissues with [ribose-U-^{14}C]MTR. In this investigation, the observed ratios were 3.0 and 1.9, respectively, after 6.5 and 18 h incubation (Table II). It is possible that substantial portion of the radioactive CO_2 might have been derived from the decarboxylation step. It has been proposed that the first step in this conversion involves the formation of MTR-1-phosphate (7,8). However, this intermediate was not detected in this apple system.

The recycling of the CH_3S- group for the synthesis of methionine in relation to ethylene synthesis in apple tissue is diagrammed in Fig. 2. In this cycle the CH_3S- group of methionine is recycled via MTA and MTR, while the

Table II. Radioactivity incorporation of [ribose-U-^{14}C]MTR into C_2H_4, CO_2 and methionine.

Incubation time	C_2H_4	CO_2	Methionine	Methionine/CO_2
h	nCi	nCi	nCi	
6.5	0.16	3.3	9.9	3.0
18	0.40	7.7	14.6	1.9

Each apple plug was fed with 71 nCi of [ribose-U-^{14}C]MTR (0.75 μCi/μmol) and 0.5 μmol unlabeled methionine.

4-carbon moiety of methionine is converted into ACC, which is then degraded to ethylene. The 4-carbon moiety of methionine is ultimately replenished from the

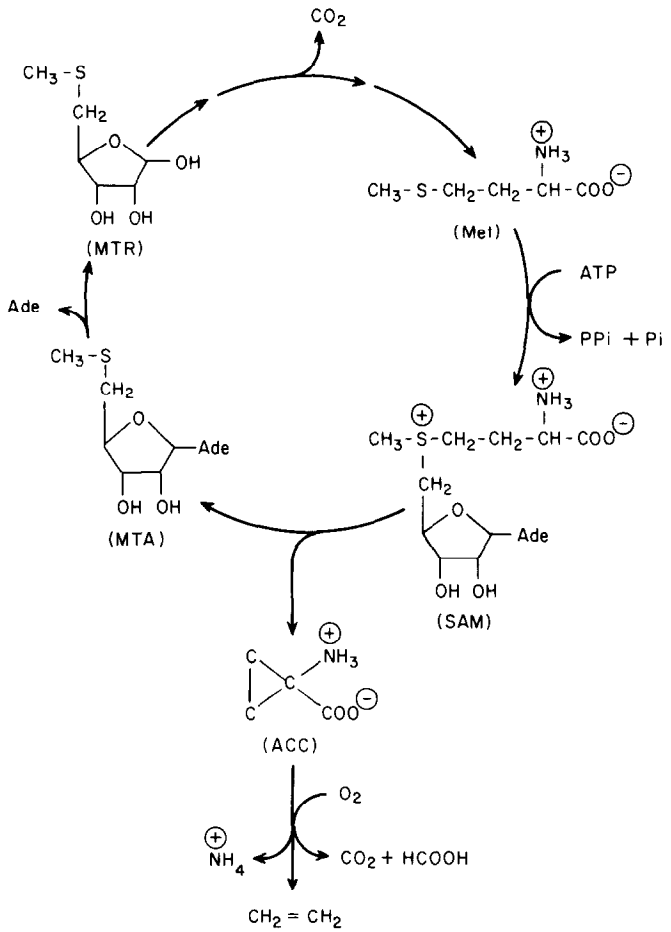

Fig. 2. Methionine cycle in relation to ethylene biosynthesis in apple fruit. The conversion of methionine to ACC and MTA via SAM is unique to ethylene biosynthesis (2) and has been recently reviewed (11). The metabolism of CH_3S- group of MTA to methionine via MTR has been previously reported (4,5).

ribose moiety of ATP. Thus, the overall result of this cycle is the conversion of ATP to phosphate, pyrophosphate, Ade and ACC, while the CH_3S- group is conserved by recycling. This cycle is particularly important in those plant tissues which produce ethylene at high rates but contain very low concentration of endogenous methionine. It should be noted that this pathway merely maintains a methionine supply for ethylene biosynthesis, but does not result in a net increase in methionine synthesis. When methionine is utilized for other biochemical reactions, such as protein synthesis, levels of methionine will decrease unless methionine is replenished from other routes, such as cystathionase pathway.

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