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5-METHYLTHIORIBOSE KINASE ACTIVITY IN PLANTS*

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Summary. The presence of a previously unidentified plant enzyme, 5-methylthioribose kinase, has been demonstrated to exist in cell-free extracts from several fruit tissues. The enzyme catalyzes the ATP-dependent phosphorylation of 5-methylthioribose to 5-methylthioribose-1-phosphate. Enzyme activity has been found in avocado, pear, apple, strawberry and tomato tissues. The significance of the presence of this enzyme in relation to ethylene biosynthesis is discussed.

INTRODUCTION

Ethylene is a plant hormone regulating many aspects of growth, development and senescence in higher plants (1). The identification of S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) as precursors of ethylene (2-4) has led to the elucidation of the following metabolic sequence for the biosynthesis of ethylene:

$$\text{Methionine} \rightarrow \text{SAM} \rightarrow \text{ACC} \rightarrow \text{ethylene} + \text{MTA}$$

ACC synthase catalyzes the conversion of SAM to ACC and 5'-methylthioadenosine (MTA) (4). Various moieties of MTA have been shown to be recycled back into methionine (5-7). The first step in this recycling pathway is the degradation of MTA. In most microorganisms and plants, MTA is degraded by a nucleosidic cleavage via MTA nucleosidase to 5-methylthioribose (MTR) and adenine, whereas in animal tissue a phosphorylytic cleavage results in the formation of 5-methylthioribose-1-phosphate (MTR-1-P) and adenine (8).

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Abbreviations: MTA, 5'-Methylthioadenosine; SAM, S-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; MTR, 5-methylthioribose; MTR-1-P, 5-methylthioribose-1-phosphate.

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Regardless of whether the direct product of MTA degradation is MTR or MTR-1-P, MTA has been shown to be recycled back into methionine. It has been proposed that in order for this recycling to occur, MTR-1-P must first be synthesized (9). In Enterobacter aerogenes (9), a new enzyme, MTR kinase, was identified and found to catalyze the ATP-dependent phosphorylation of MTR to MTR-1-P. In this communication, we report for the first time the presence of MTR kinase activity in tissue from higher plants.

MATERIALS AND METHODS

[$^{14}CH_3$]MTR was prepared from [$^{14}CH_3$]MTA which was obtained by the acid hydrolysis of [$^{14}CH_3$]SAM, (Amersham, 58 mCi/mmol) (10). [$^{14}CH_3$]MTR-1-P was prepared and purified as described by Ferro et al. (11) from the reaction mixture of an MTA phosphorylase assay utilizing a partially purified enzyme from guinea pig liver. Unripe mature avocado fruit was purchased from a local store, tomato and strawberry fruits were grown in a greenhouse, whereas apples and pears were harvested from the Hood River Experiment Station, Hood River, Oregon. Cell extracts were prepared by homogenization of the tissues suspended in buffer consisting of 0.2 M potassium phosphate (pH 7.2), 1% polyvinylpyrrolidone, 1% Triton-X100, and 3 mM dithiothreitol. The homogenate was passed through four layers of cheese cloth and centrifuged at 20,000 x g for 20 min. The supernatant fluid was utilized as a source of enzyme. The avocado extract was further purified by ammonium sulfate precipitation. Powdered $(NH_4)_2SO_4$ was added to 40% saturation and the precipitate obtained after centrifugation (14,000 x g) was resuspended in buffer. This step resulted in a 4-fold purification of the enzyme and this preparation was utilized in those experiments designed to identify reaction products.

The radiochemical assay used to measure MTR kinase activity (9) measures the conversion of MTR to MTR-1-P by chromatographic separation of the reactants and products on Dowex 1-X8 columns. For product identification, descending paper chromatography was performed using Whatman No. 3 paper. The solvent system was 2-butanol=acetone:acetic acid:H_2O (70:70:20:40). Ultraviolet-absorbing substances were detected with a Mineralight lamp, and sulfur-containing compounds were observed by spraying the chromatograms with potassium iodoplatinate. Radioscans of paper chromatograms were performed with a Packard radiochromatogram scanner. Alkaline phosphatase treatment was performed by incubation with 0.10 unit of calf intestine alkaline phosphatase (Sigma) for 30 min at 30°C in 0.05 M Tris-HCL, pH 8.0.

Results

Cell free extracts of avocado incubated with 5-[$^{14}CH_3$]methylthioribose for 6 hr at 30°C did not catalyze the formation of any products as revealed by radiochromatographic analysis (Fig. 1A). The addition of [$^{14}CH_3$]MTR and 1 mM ATP to the cell extract, however, resulted in the conversion of [$^{14}CH_3$]MTR to two radioactive compounds which migrated at R_f values of 0.17 and 0.26,

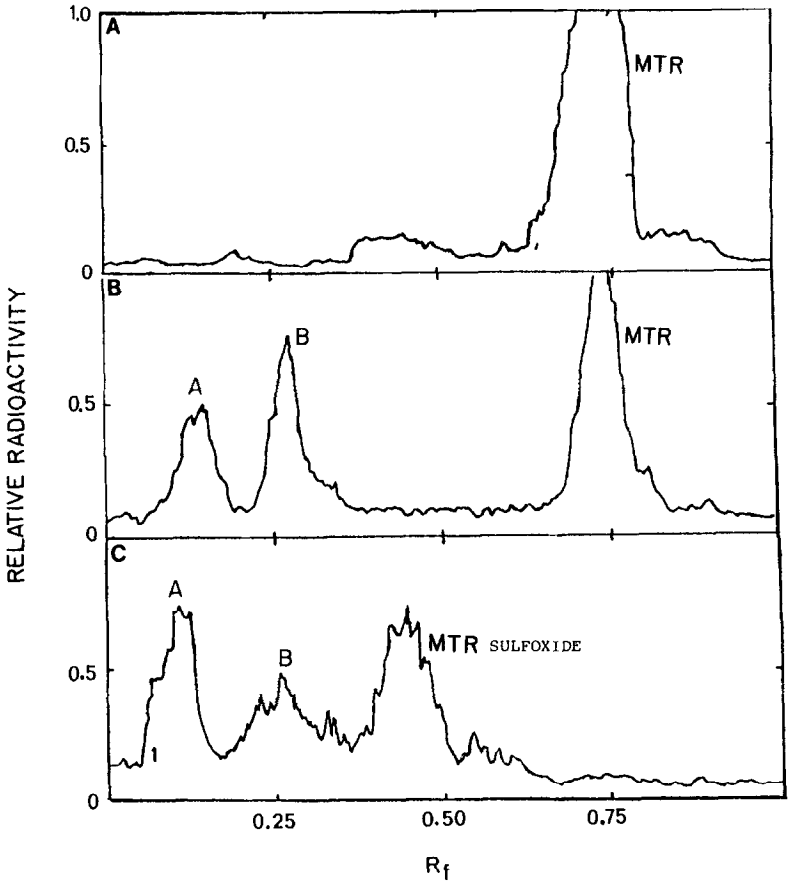

Fig. 1. Radiochromatogram scans of cell free extracts of avocado fruit incubated with 0.1 mM 5-[$^{14}CH_3$]MTR (3 mCi/mole) for 6 h. (A) Crude extract incubated with 5-[$^{14}CH_3$]MTR; (B) crude extract incubated with 5-[$^{14}CH_3$]MTR and 1 mM ATP; (C) peroxide oxidation of the reaction mixture consisting of crude extract, 5-[$^{15}CH_3$]MTR and ATP.

compounds A and B, respectively (Fig. 1B). The formation of compounds A and B were both dependent on the presence of enzyme protein and ATP, and the disappearance of [$^{14}CH_3$]MTR (R_f = 0.72) was proportional to the amount of compounds A and B recovered. Compound B co-migrated with authentic [$^{14}CH_3$]MTR-1-P (R_f = 0.26). Exposure of authentic [$^{14}CH_3$]MTR to 0.1% H_2O_2 resulted in the conversion of [$^{14}CH_3$]MTR to [$^{14}CH_3$]MTR sulfoxide (R_f = 0.45), whereas, the oxidation of the isolated [$^{14}CH_3$]MTR-1-P (compound B) resulted in its conversion to compound A (R_f = 0.17) upon subsequent chromatography. Peroxide oxidation of the radioactive reaction mixture resulted in the

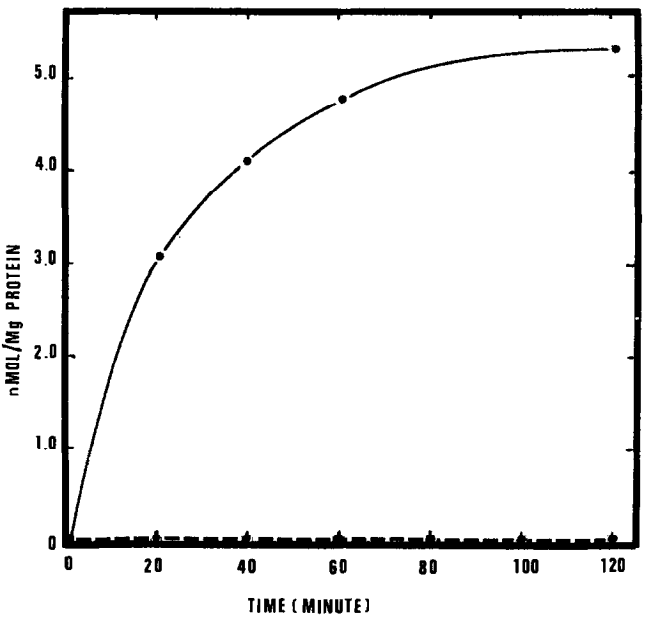

Fig. 2. MTR kinase activity over a 2 h period in avocado tissue.(●-●) activity in the presence of 1 mM ATP,(●---●)activity in the absence of ATP.

disappearance of [$^{14}CH_3$]MTR, the appearance of a new peak at R_f 0.45 ([$^{14}CH_3$]MTR sulfoxide), a reduction in the [$^{14}CH_3$]MTR-1-P peak and an increase in compound A (Fig. 1C). Reduction of compound A with 6.7 mM dithiothreitol resulted in the shifting of the R_f value back to 0.26 which again co-migrated with [$^{14}CH_3$]MTR-1-P. By analogy with MTR and MTR sulfoxide, the compound (compound A) formed as a result of peroxide treatment of MTR-1-P appears to be the sulfoxide (MTR-1-P sulfoxide). In addition, treatment of either compound A or compound B with alkaline phosphatase in the presence of 10 mM dithiothreitol resulted in the formation of MTR. On the basis of these results, it is suggested that cell-free extracts of avocado contain MTR kinase, which catalyzes the phosphorylation of MTR to MTR-1-P. The MTR-1-P formed, in turn, is partially oxidized by the crude reaction mixture to MTR-1-P sulfoxide.

The enzyme was partially purified from a cell-free extract of avocado by ammonium sulfate fractionation. MTR kinase activity, as determined by ion-exchange chromatography (9), was completely dependent upon the presence of ATP

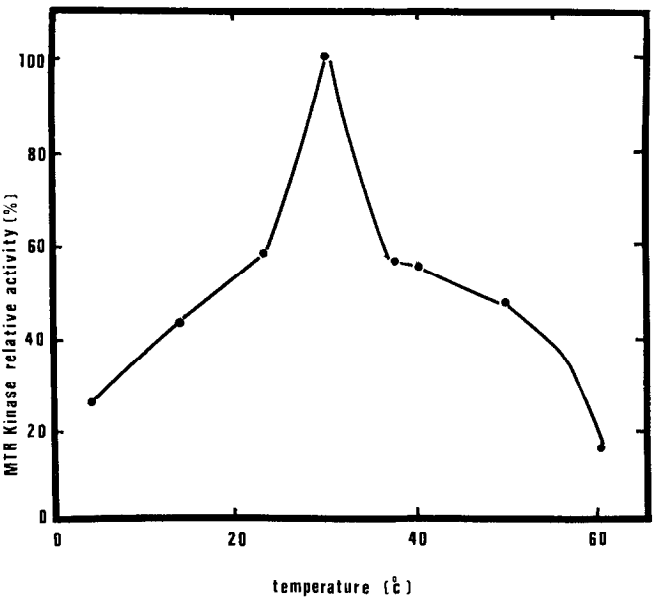

Fig. 3. Effect of temperature of incubation on MTR kinase activity. Controls were run at each temperature.

over a 2 hr incubation period (Fig. 2). The temperature optimum of the enzyme was 30°C (Fig. 3) and the enzyme activity was linear with protein up to about 400 µg protein per assay.

Several cell-free extracts of fruits, in addition to avocado fruit, were also assayed for MTR kinase activity (Table 1). Of the extracts assayed, avocado contained the highest enzyme activity (810 pmole/mg protein/min); this activity is very similar to the specific activity found in crude extracts from E. aerogenes (9). Following the avocado, in descending order of activity, were extracts prepared from pear, strawberry, apple, and tomato.

DISCUSSION

In this study, MTR kinase activity has been demonstrated to exist in cell-free extracts from several fruit tissue. This is the first report of the enzymatic synthesis of MTR-1-P in plant tissues. Therefore, as in E. aerogenes, MTR-1-P is synthesized in a two step reaction from MTA:

1) MTA → MTR + adenine

2) MTR + ATP → MTR-1-P + ADP

Table 1. MTR kinase activity in several fruit cell free extracts.

Fruit Type	pmol MTR-1-P formed/mg protein/min
Avocado	810
'd'Anjou' pear	680
Strawberry (red)	230
Golden delicious apple	140
Tomato (red)	110

This is in contrast to mammalian tissue where MTR-1-P is synthesized directly from MTA via MTA phosphorylase.

The primary fate of MTR in plant tissue appears to be its conversion to methionine; both the methyl group and the sulfur atom have been shown to be incorporated into methonine (2,12). Recently, Yung et al. (13) showed that in apple tissue, like yeast (5), rat liver (6) and E. aerogenes (7), the ribose portion of MTR is also incorporated into methionine. In E. aerogenes (9), it has been suggested that the conversion of MTR to MTR-1-P via MTR kinase may be the first step in this recycling pathway. More recently, MTR-1-P, but not MTR has been shown to be an intermediate in the recycling of MTA back to methionine in rat liver cell extract (14). Since rat liver apparently lacks MTR kinase activity, this demonstrates that only the phosphorylated sugar is recycled in this tissue. These observations suggest that MTR-1-P, formed via MTR kinase in plant tissue, may be an essential intermediate in the biosynthesis of methionine from MTR. Studies investigating this possibility are now in progress.

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