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THE EFFECT OF METYRAPONE ON CYTOKININ ([8-^{14}C]BENZYLAMINOPURINE) METABOLISM IN MATURE GREEN TOMATO PERICARP

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SUMMARY: Cytokinin, [8-^{14}C]Benzylaminopurine, metabolism in tomato pericarp was followed during a 3 h period utilizing thin layer chromatography and visualization by fluorography. Fluorography indicated the formation of at least 7 metabolites during 3 h. Cytokinin metabolism was reduced by approximately 40% in 3 h by the presence of 250μM metyrapone, an inhibitor of cytochrome P-450 related enzyme systems. In the presence of metyrapone, the number of radioactive metabolites on the thin layer plate was reduced from 7 to 4 and 2 of these were unique to the metyrapone-treated sample. These data suggest the initial step in benzylaminopurine metabolism in tomato pericarp may be mediated by a cytochrome P-450 related enzyme system which is altered in the presence of metyrapone.

Cytokinins are a group of N^6 substituted purine analogs which have been shown to be involved in growth (1) and senescence (2) of plants and/or fruits of plants.

Cytokinins have been implicated in the ripening characteristics of normal and mutant tomato varieties (3-6). In addition, the metabolism of cytokinins in tomatoes has been shown to be complex, forming metabolites as a result of side-chain modifications, side-chain cleavage, ribosylation phosphorylation and deamination (7-9).

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Abbreviations: BA, N^6-Benzylaminopurine; [8-^{14}C]BA, [8-^{14}C]Benzylaminopurine; MES, (2-[N-Morpholino]ethanesulfonic acid).

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Recent work by Chen and Leisner (7) indicated the involvement of Cytochrome P-450 related microsomal enzyme systems in the metabolism of cytokinins by cauliflower. Metyrapone, a potent cytochrome P-450 microsomal enzyme inhibitor (10-12), was shown to inhibit side-chain hydroxylation of the cytokinin isopentenyladenine by cauliflower microsomal preparations.

Metyrapone is an inhibitor of at least two classes of cytochrome P-450 enzymes, those being 11-Beta-hydroxylase and most phenobarbitol-inducible forms. These inducible enzymes are responsible for the oxidation of lipophilic compounds such as drugs, steroids and polycyclic aromatic hydrocarbons in animal systems. This communication describes the effect of metyrapone on the metabolism of [8-^{14}C]Benzylaminopurine in mature green tomato pericarp.

MATERIALS AND METHODS

Materials

Tomatoes (Lycopersicon esculentum Mill. Ohio "CR-6") were obtained from the Ohio Agricultural Research and Development Center, Wooster, OH. Fruit were rinsed with distilled water and stored at room temperature and prevailing light conditions until the desired degree of ripeness was obtained (13).

N^6-Benzylaminopurine (BA), 2-[N-Morpholino]ethanesulfonic acid (MES) and 12-methyl-1,2-di-3-pyridyl-1-propanone(metyrapone) were obtained from Sigma Chemical Co., St.Louis, MO. All solvents used were reagent grade. En^{3}Hance Spray (surface autoradiography enhancer) was obtained from New England Nuclear. Radiochemically labeled cytokinin [8-^{14}C]Benzylaminopurine (54mCi/mmole) ([8-^{14}C]BA) was obtained from Amersham, Arlington Heights, IL. The [8-^{14}C]BA was subjected to thin-layer chromatography with basic, neutral and acidic solvent systems and scanned for radioactivity. Radiochemical purity was greater than 98%.

Plant Tissue Preparation

Mature green tomatoes were cut into 8 sections. The seeds and locules were removed. The remaining pericarp was cut into uniform 0.5cm square subsections. A sample (10gm) of pericarp subsections was placed into each of two beakers. A solution containing the compounds of interest was added and the beaker

was placed in a vacuum chamber and aspirated for a total of 10 min. with a vacuum release at 5 min. The two solutions (12.5ml) both contained 50μM BA in an 0.05M MES buffer (pH 6.8) containing 5.57μCi (0.446μCi/ml) of [8-^{14}C]BA. The control solution contained no metyrapone and the sample solution concentration was 250μM with respect to metyrapone.

The solution remaining after vacuum-infusion was discarded and the infused pericarp was rinsed twice with double-distilled deionized water. One gram of pericarp was placed in a test tube and extracted with 1.5ml of cold (-60°) methanol by homogenization for 15 sec. utilizing a Polytron Homogenizer PT 10-35 equipped with a PT 10-ST Probe (Brinkman Instrument, Westbury, N.Y.). The initial extract was designated "0" time. The remaining pericarp was incubated at room temperature (23°). One gram samples were removed at 30 min. increments and extracted as described above. The homogenates were centrifuged at 1300xg for 15 min., after which the pellet was discarded. The extract was dried under a steady flow of nitrogen. Ten μl of an 0.1N NaOH solution was added to dissolve the residue which was then brought to a final volume of 0.2ml by the addition of methanol:chloroform (1:1,v/v). A thin-layer plate (20 x 20cm silica gel G No.06-600A Fisher Scientific Co., Pittsburgh, PA.) was spotted with 4μl of this solution. The thin-layer plates were developed utilizing a butan-1-ol/conc. NH_4OH (sp gr 0.9)/water (18:14:5), v/v/v) solvent system. The developed plates were allowed to dry at room temperature.

Fluorography

The dried plates were sprayed with En^{3}Hance autoradiography enhancer. The plates were allowed to expose x-ray film (Kodak X:OMAT AR No. XAR-2) for 7 days at -20C. The x-ray film was developed by standard methods resulting in the fluorogram. The spots on the fluorograms were scanned utilizing a soft laser scanning densitometer Model SL-504-XL (Biomed Instruments, Inc.), Fullerton, CA. 92631).

RESULTS AND DISCUSSION

Fluorography results (Fig.1) clearly showed the metabolism of [8-^{14}C]BA in tomato pericarp to be rapid and complex, forming at least 7 major metabolites during 3 h. The "0" time sample, which required 12 min. from initial infusion to extraction, indicated approximately 25% of the [8-^{14}C]BA had already been metabolized in the non-metyrapone treated sample (Fig. 1). Metyrapone (250μM) reduced the "0" time metabolism of [8-^{14}C]BA to approximately 10%. In addition metyrapone (250μM) inhibited [8-^{14}C]BA metabolism by 40% in tomato pericarp after 3 h. (Fig.2) compared to the non-metyrapone

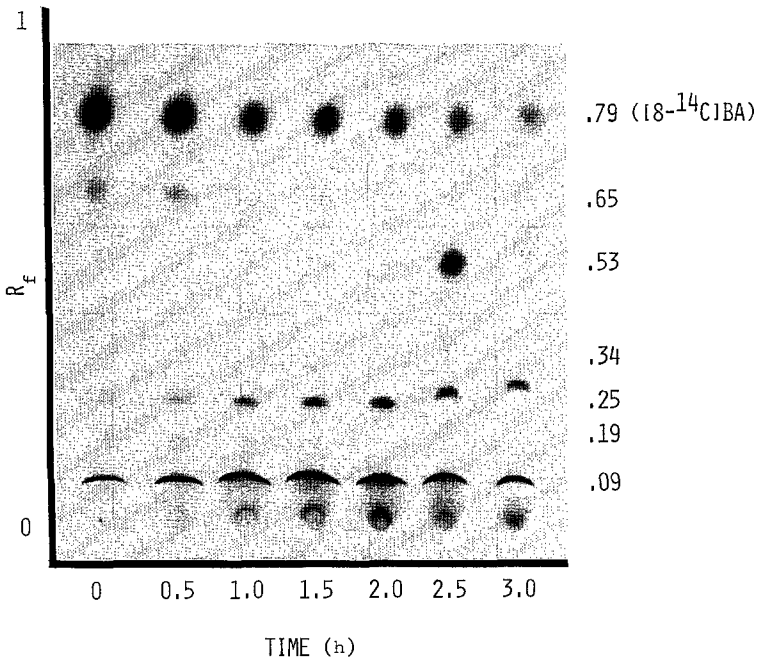

Figure 1. Fluorogram of [8-^{14}C]Benzylaminopurine metabolites isolated from mature green tomato (Lycopersicon esculentum Mill. "Ohio CR-6") pericarp.

treated sample (Fig.1). These data demonstrate metyrapone to be an effective inhibitor of [8-^{14}C]BA metabolism in tomato pericarp.

Fluorography (Fig.2) indicated the number of [8-^{14}C]BA metabolites and the percent of [8-^{14}C]BA metabolized in the presence of metyrapone during 3 h was less than observed in the non-metyrapone treated sample (Fig.1) during the same time period. In addition to inhibiting [8-^{14}C]BA metabolism, metyrapone altered the metabolic pattern which resulted in unique metabolites (R_f .15 and .58; Fig.2) which were not observed in the non-metyrapone treated sample (Fig.1).

The [8-^{14}C]BA metabolites (R_f's .09, .19, .25, .53 and .65; Fig.1) which formed in the non-metyrapone treated sample never appeared in the metyrapone treated sample (Fig.2) even after 3 h incubation suggesting a direct effect by metyrapone

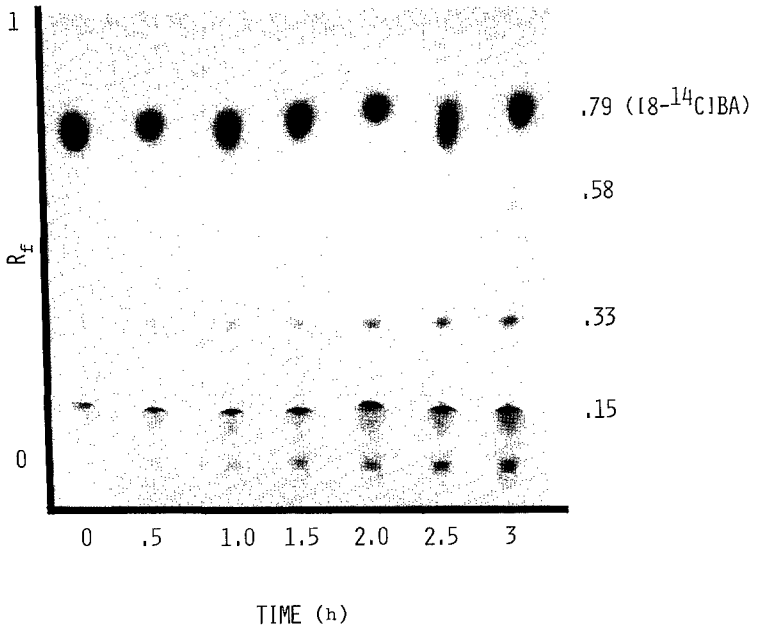

Figure 2. Fluorogram of [8-^{14}C]Benzylaminopurine metabolites formed in the presence of 250μM metyrapone and isolated from mature green tomato (Lycopersicon esculentum Mill. "Ohio CR-6") pericarp.

on the formation of these metabolites. The [8-^{14}C]BA metabolites (R_f .15 and .58) which appeared in the metyrapone treated sample only (Fig.2), may also be cytochrome P-450 mediated but this cannot be unequivocally determined from this experiment.

The structures of the metabolites remain to be elucidated. Metyrapone has been shown to inhibit the hydroxylation of isopentenyladenine in microsomal preparations from cauliflower (7). This mechanism may not be operative in the system described here because we have used a different tissue, studied the metabolism in vivo and utilized a different cytokinin. Some of the results presented here could be explained by a hydroxylation similar to that observed in the cauliflower microsomal preparations (7) but clearly, the in vivo tomato system is more complex and the

formation of unique metabolites in the presence of metyrapone was not seen in cauliflower microsomal preparations.

The relationship of these findings to the role of cytokinins in plant growth or senescence activities remains to be elucidated.

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