Short Communication

Ethylene Production by Albedo Tissue of Satsuma Mandarin (Citrus unshiu Marc.) Fruit

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ABSTRACT

Isolated albedo tissue of Satsuma mandarin (Citrus unshiu Marcovitch, cv. Owari) fruit produced a large quantity of ethylene during incubation at 26 C in the dark. When sliced, albedo tissue began producing ethylene at an increasing rate until a maximum was reached after incubation for about 30 hours. Aged albedo discs which were capable of producing ethylene, actively converted L-[U-14C]methionine into both ethylene and carbon dioxide. In fresh tissue, almost no measurable conversion of radioactive methionine into ethylene took place. Conversion of labeled L-methionine into ethylene was totally inhibited by the addition of nonradioactive L-methionine or L-ethionine. It appears possible, from these findings, that methionine is a precursor of ethylene in the aged albedo discs. Ethylene synthesis in the aged albedo tissue was markedly reduced in the presence of cycloheximide, suggesting that there may be a rapid turnover of the ethylene-producing system, and that its formation involves protein synthesis. Actinomycin D exerted no effect.

It has been demonstrated by Aharoni (1) that young, unripe oranges evolve a large amount of ethylene after harvest, paralleling a marked increase in respiration. Similar findings have been made by Eaks (3) for young fruits of grapefruit and oranges. Recently, I have also reported that young, immature fruit of Satsuma mandarin produced a great amount of ethylene after harvest (5). The rate of ethylene production by Satsuma mandarin fruit markedly decreased as the fruit developed and increased in weight. When young fruits were cut into small pieces (e.g. quarters or eighths) ethylene was produced at a much greater rate than by the intact fruit (5). Similar results were obtained with isolated albedo tissue. Albedo discs vigorously evolved ethylene, even though the tissue was taken from fruit which did not produce ethylene when intact. Subsequently, albedo has been used for studying the mode of ethylene production in the Satsuma mandarin. Methionine has been shown to be a precursor of ethylene in plants (2, 7). In the present experiments L-[14C]methionine was used to test the efficiency of methionine as a possible precursor of ethylene in the albedo tissue.

MATERIALS AND METHODS

Satsuma mandarin fruit (Citrus unshiu Marcovitch, cv. Owari) were harvested from the orchard during the months from July to November 1975. Ethylene production, respiration, increase in fresh weight, and some properties of the postharvest fruit were described elsewhere (5). Disks of the peel of the fruit were excised with a cork borer 9 mm in diameter. Albedo discs (2-4 mm thick), separated from the flavedo, were placed in a 130-ml Erlenmeyer flask and the flask was sealed with a rubber serum cap. Discs were incubated in the dark at 26 C. After closure for 1 hr, the atmosphere in the flasks was sampled through the serum caps at regular intervals with a plastic syringe, and ethylene concentration was determined. After each sampling, the flasks were flushed with air and then reclosed. Ethylene was measured on a Hitachi gas chromatograph equipped with a hydrogen flame ionization detector and an activated alumina column. Ethylene was produced at an increasing rate, reaching a maximum about 30 hr after the start of incubation.

Similar aged (preincubated) discs, known to be capable of vigorous ethylene production, were used for the following incorporation studies. Twelve albedo discs (1.1-1.6 g) were placed in a 55-ml Erlenmeyer flask on 3 ml of medium containing 2.5 µCi (0.01 µmol) L-[U-14C]methionine obtained from New England Nuclear. The flask was sealed with a rubber serum cap. In the flask were suspended two polypropylene center wells (Kontes Glass Co.), one containing 0.1 ml of 0.25 M mercuric perchlorate solution and the other 0.1 ml of 20% KOH solution, and fitted with filter paper wicks, to absorb evolved ethylene and CO2. The flasks were placed on a shaker at 23 C with a shaking speed of 110 strokes/min. The ethylene and CO2 produced were trapped with an efficiency greater than 80 and 90%, respectively, by this procedure. After an appropriate period of incubation, the filter paper wicks were taken out and put in vials with 10 ml of Bray's solution. The radioactivity was measured in a Horiba liquid scintillation spectrometer. In some experiments, actinomycin D, cycloheximide, L-methionine, d-methionine, or L-ethionine was included in the test solution to examine the effects of these compounds on ethylene production. All chemicals used were obtained commercially except for actinomycin D, which was a generous gift of Merck Sharp & Dohme.

RESULTS

The rate of ethylene production by freshly prepared albedo discs was very low, but increased markedly during incubation in the dark at 26 C (Fig. 1). It reached a maximum after about 30 hr, then declined gradually or remained steady. The rise in the rate of ethylene production seemed faster in young fruit than in more mature fruit. In albedo discs aged (preincubated) for 18 hr, radioactive label from methionine was extensively incorporated into both ethylene and CO2. The incorporation of radioactivity into ethylene, starting after a 40-min lag period, continued linearly for about 3 hr (Fig. 2). No incorporation of 14C from uniformly labeled L-leucine into ethylene was observed, although it was converted substantially into CO2. In contrast to aged tissue, freshly prepared albedo discs produced very little ethylene and incorporated very little label from methionine into ethylene (Fig. 3). Incorporation of label from methionine into ethylene in the aged albedo discs was prevented by 5 mM unlabeled L-methionine or by 5 mM L-ethionine (Fig. 4). D-Methio-
September or later produced no measurable quantities of ethylene (5). However, excised albedo tissue evolved a great amount of ethylene during incubation, even if the fruit from which it was excised was incapable of producing ethylene. This may be due to wound-induced ethylene production. Imaseki et al. (6) reported that sweet potato root tissue produced ethylene in response to cutting injury. Riov et al. (10) found that flavedo discs evinced increasing ethylene production upon incubation. In the case of Satsuma mandarin fruit, aged flavedo tissue showed a similar increase in ethylene production, but albedo tissue had a far greater productive capacity. It is of interest to ask what kind of substance or condition is responsible for the induction of ethylene evolution in the albedo tissue in response to wounding. It is well known that wounding causes an activation of plant metabolism, particularly an increase in some enzyme activities. The possible formation of a triggering substance in the wound tissue, or by a wound reaction, remains obscure (4). Methionine has been shown to be a precursor of ethylene in plants (2, 7, 11, 12). From the results obtained above it is most

**DISCUSSION**

Young immature fruit of Satsuma mandarin produced a great amount of ethylene after harvest. The rate of ethylene production decreased as fruit growth progressed. The fruit harvested in

![Fig. 1. Effect of incubation time on the rate of ethylene production by albedo tissues prepared from fruit harvested from August to November.](image)

![Fig. 2. Incorporation of $^{14}$C from methionine or leucine into ethylene and CO$_2$. Albedo discs aged for 18 hr were incubated in a medium containing 2.5 $\mu$Ci of L-$[^{14}$C]methionine or 2.5 $\mu$Ci of L-$[^{14}$C]leucine. Ethylene and CO$_2$ evolved were trapped and their radioactivities were measured in a liquid scintillation spectrometer.](image)

![Fig. 3. Incorporation of $^{14}$C from methionine into ethylene by fresh albedo tissue or aged (18 hr) albedo tissue. For methods see Fig. 2.](image)

![Fig. 4. Effects of unlabeled L-methionine, L-ethionine, and D-methionine on the incorporation of $[^{14}$C]methionine into ethylene. Albedo discs aged for 18 hr were incubated in a medium containing 2.5 $\mu$Ci of L-$[^{14}$C]methionine for 2.5 hr in the absence (control) or in the presence of 5 mM L-methionine, 5 mM L-ethionine, or 5 mM D-methionine.](image)
Fig. 5. Effects of cycloheximide and actinomycin D on the incorporation of $^{14}$C of methionine into ethylene. Albedo discs aged for 17 hr were incubated with labeled methionine in the absence (control) or in the presence of 72 $\mu$M cycloheximide or 80 $\mu$M actinomycin D.

likely that the precursor of ethylene in the aged albedo tissue is also methionine. In the present experiments, uniformly labeled $[^{14}]$C methionine was used. In future studies, specifically labeled $[^{14}]$C methionine will be applied and inhibitors specific for methionine metabolism (8, 9) will be used.

Ethylene evolution in the aged albedo discs was markedly reduced in the presence of cycloheximide, but not actinomycin D, which suggests that there may be a rapid turnover of the ethylene-producing system, and that its formation requires protein synthesis. The lack of effect of actinomycin D may indicate that mRNA, once formed, is stable in the aged albedo tissue.

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LITERATURE CITED