Short Communication

Ethylene-stimulated Synthesis of Ribosomes, Ribonucleic Acid, and Protein in Developing Fig Fruits

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Ethylene profoundly stimulates the growth and ripening of fig fruits if applied during the second half of growth period II, the slow growth phase of the characteristic double sigmoid growth curve (7, 9). Although ethylene-treated fruits ripened 2 to 3 weeks early, within 6 days from inception of treatment, they were indistinguishable from ripe, untreated fruits (7). Moreover, ethylene induced a typical respiratory climacteric rise identical to that associated with normal ripening and the initiation of growth period III (7).

Recently, Hulme et al. (4) have demonstrated an ethylene-stimulated synthesis of unspecified types of RNA in apple tissue slices in concert with climacteric phenomena. Our intent in this study was to use the dramatic response of fig fruits to ethylene along with available analytical techniques to discern the temporal aspects of stimulated RNA synthesis and the forms of RNA being synthesized.

MATERIALS AND METHODS

Fig (Ficus carica L. cv. Mission) fruits were sampled periodically during growth period II and following ethylene treatments (7). Ethylene (5 µl/liter in an air mixture with a flow rate of 100 ml/min) was applied to fruits still on the tree (in vivo treatment) using the procedure of Marei and Crane (7). In vitro experiments consisted of the application of ethylene to fruit slices during a 6-hr incubation period by bubbling an ethylene/air mixture (5 µl/liter, flow rate of 25 ml/min) through the incubation medium. As an alternative method, ethylene was provided via the decomposition of Ethephon (2-chloroethylphosphonic acid) incorporated into the medium at a concentration of 10 µl/liter.

To determine the rates of ribosome, RNA, and protein synthesis, tissue slices were incubated with uridine-5-3H (specific radioactivity 8 c/mmole, Schwarz BioResearch) or L-phenylalanine-4-3H (specific radioactivity 5 µc/mmole, Schwarz BioResearch). Procedures for the incubation of tissues, the isolation of ribosomes and of RNA, and the use of SDG analysis and MAK column chromatography were those of Ku and Romani (6), with slight modifications detailed elsewhere (8).

The distribution of radioactivity in SDG was assayed by collecting sequential 6-drop fractions onto Whatman No. 3 filter paper discs (23-mm diameter) which were subsequently processed for radioactive counting according to Bollum's method (1). The RNA-containing effluent from MAK columns was collected in 4-ml fractions, enriched with 2 absorbancy units of yeast RNA, denatured with trichloroacetic acid, precipitated on Millipore membrane filter discs, and monitored for radioactivity.

Soluble proteins were obtained from fig fruit slices, after incubation with phenylalanine-4-3H, by freezing the tissue and thoroughly grinding it with an equal portion (g/ml) of buffered medium (50 mM potassium phosphate buffer, pH 6.8; 1 mM dithiothreitol; 0.25 mM sucrose; and 1% [w/v] polyvinylpyrrolidone) in a mortar with the addition of sufficient liquid nitrogen to keep all materials frozen. The mixture was then thawed, shaken vigorously for 5 min at 5°C, and centrifuged at 20,000g for 20 min to remove cellular debris. Methods described by Kruh (5) for trichloroacetic acid precipitation and the determination of radioactivity were used with minor modifications (N. Marei and R. Romani, in preparation). The Lowry method as modified by Miller (10) was used for the quantitative estimation of protein.

The specific radioactivity in fractions of the SDG and MAK absorbancy profiles was calculated as total cpm/total A260 units, whereas that of protein was determined as total cpm/mg protein.

RESULTS AND DISCUSSION

Ethylene and Ethephon applied in vitro to fig fruit slices did not alter the sedimentation profile of ribosomes on SDG nor the profiles of nucleic acids resolved by MAK column chromatography (Figs. 1 and 2). However, the treatments significantly enhanced the incorporation of uridine-5-3H into ribosomes (Fig. 1, Table I) and sRNA (Fig. 2, Table I). Although the stimulating effect of ethylene applied in vitro was evident in tissue slices prepared from fruits in all three growth stages, the effect was much more pronounced during the second half of period II.

The rates of synthesis of ribosomes and of RNA and protein remained relatively constant in untreated fruits throughout period II (Fig. 3). Ethylene applied in vivo during the second half of period II (as exemplified by the treatments of July 20 and August 1, Fig. 3) increased the rates of ribosomal, sRNA, rRNA, and protein synthesis in the first 24 hr of treatment (Fig. 3). At the next sampling, after 48 hr of continuous exposure to ethylene, the rates of synthesis of ribosomes and both RNA fractions had fallen below those of the untreated fruits, and the rates continued to decline until the fruits ripened 4 to 5 days later (Fig. 3). In contrast, the levels of protein synthesis remained elevated for at least 48 hr, and was significantly higher than that of the controls until approximately the 5th day of continuous exposure to ethylene (Fig. 3).

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2 Abbreviations: SDG: sucrose density gradient; MAK: methylated albumin kieselguhr.
with an increase in protein synthesis (Fig. 3) militates against any overriding influence by changes in permeability or pool size or both.

Our data thus affirm that the ethylene-stimulated RNA synthesis of ribosomes in period II when slices (1.5 g, 2 to 3 mm thick) were incubated for 6 hr at 25°C in 5 ml of an incubation medium (10 mM potassium phosphate buffer, pH 6.8; 0.1 M sucrose; 6 mM 2-mercaptoethanol; 50 μg/ml chloramphenicol; and 25 μg/ml uridine-5'-H). Ribosomes were fractionated by SDG (10 to 34%) centrifugation for 5 hr (0-4°C) at 25,000 rpm using a Spinco SW 25.1 rotor. Sedimentation coefficients (S) are indicated.

Table I. Effect of in Vitro Application of Ethylene and Ethephon on Synthesis of Ribosomes, rRNA, and sRNA in Slices of Fig Fruits Incubated with Uridine-5'-H

Fruits were in the second half of period II. The methods of incubation were those described in Fig. 1.

<table>
<thead>
<tr>
<th>Date of Treatment</th>
<th>Treatment</th>
<th>Percentage of Increase in Specific Radioactivity* over Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ribo-</td>
<td>rRNA</td>
</tr>
<tr>
<td></td>
<td>somes</td>
<td></td>
</tr>
<tr>
<td>7/15</td>
<td>Ethylene, 5 μl/liter</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>Ethephon, 10 μl/liter</td>
<td>13.9</td>
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<tr>
<td>7/20</td>
<td>Ethylene, 5 μl/liter</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>Ethephon, 10 μl/liter</td>
<td>24.1</td>
</tr>
</tbody>
</table>

1 Specific radioactivity = cpm × 10^-3/A254.

2 Calculated from SDG sedimentation profiles.

3 Calculated from MAK column elution profiles.

Fig. 1. Effect of ethylene and Ethephon (in vitro) on the synthesis of ribosomes in fig fruits. Fruits were in the second half of growth period II when slices (1.5 g, 2 to 3 mm thick) were incubated for 6 hr at 25°C in 5 ml of an incubation medium (10 mM potassium phosphate buffer, pH 6.8; 0.1 M sucrose; 6 mM 2-mercaptoethanol; 50 μg/ml chloramphenicol; and 25 μg/ml uridine-5'-H). Ribosomes were fractionated by SDG (10 to 34%) centrifugation for 5 hr (0-4°C) at 25,000 rpm using a Spinco SW 25.1 rotor. Sedimentation coefficients (S) are indicated.

Fig. 2. Effect of ethylene and Ethephon in vitro on the synthesis of RNA from fig fruits. Fruit slices (5 g) were prepared and incubated with 50 μg uridine-5'-H in 10 ml of medium as described in Figure 1. RNA fractions were eluted from a MAK column with a NaCl gradient (0.2 M to 1.2 M).

It may be argued that ethylene treatment, in vitro or in vivo, increased the tissue permeability (11), resulting in precursor pools of higher specific radioactivity and increased rates of synthesis that are more apparent than real. However, total tissue content of labeled precursors was not increased by ethylene treatment (N. Marei and R. Romani, in preparation). This observation is in accord with the lack of an ethylene effect on permeability reported for several plant tissues (2, 3, 12). Moreover, a decline in RNA and ribosomal synthesis coincident
thesis occurs whether the gas is applied to unharvested fruit or to tissue slices. The RNA synthesis is of a rather general nature, including the synthesis of new ribosomes, rRNA, and sRNA. Whether mRNA or DNA-like RNA were also synthesized could not be determined from our study. Holm and Abeles (3) reported that ethylene enhanced synthesis of all RNA fractions, but particularly mRNA and rRNA, in the abscission zone cells of aged bean explants.

Of particular interest was the transient nature of the response when ethylene was applied in vivo (Fig. 3). The dramatic ethylene-triggered transition of the fig fruit from a quiescent phase (period II) to one of rapid growth and maturation is either mediated or accompanied by equally rapid but short-lived increases in ribosomal synthesis. The burst in ribosomal synthesis is not an isolated event, for it is accompanied by sustained and elevated levels of protein synthesis. These findings are in accord with the reported effects of postharvest applications of ethylene to apple slices (4), where ethylene-stimulated protein synthesis was preceded by an increase in the synthesis of RNA.

A sequential order of events was clearly discernible in the rapid response of fig fruits to ethylene. A sharp rise in respiratory activity was noted after 24 hr of exposure to ethylene in vivo (7). Changes in color, texture, and an increase in fruit (7) and cell size (N. MAREI and M. Bradley, in preparation) were first detected after 48 hr of continuous treatment. It would appear, therefore, that the sudden rise in RNA and ribosomal synthesis reported here is closely linked with the initiation of ripening and the final phases in fruit development.

**LITERATURE CITED**