Short Communication

Ethylene Production and Enzyme Induction in Excised Plant Tissues

G. Engelsma and J. M. H. van Bruggen
Philips Research Laboratories, N. V. Philips' Gloeilampenfabrieken, Eindhoven, Netherlands

Excising plant tissues has been found to increase the activity of phenylalanine ammonia-lyase (EC 4.1.3.5) (1, 4, 11, 12). This increase is probably due to de novo synthesis of this enzyme, since inhibitors of protein synthesis block the rise of PAL activity (4, 11, 12). We suggested earlier (4) that the increase of PAL in gherkin hypocotyl segments was due to the release into the medium of reaction products (hydroxy-cinnamic acids) which by a feedback mechanism caused repression of PAL synthesis. Other hypotheses that have been advanced include the release of proteinaceous inhibitors of enzyme synthesis (2) and ethylene, which is produced in response to tissue wounding (7, 10).

Support for the latter hypothesis stems from the work by Imaeki et al. (7) with sliced sweet potato roots and by Riev et al. (10) who worked with flavedo tissue excised from mature grapefruits. This paper presents data which indicate that there was no correlation between ethylene production and PAL development in gherkin hypocotyl segments, and that ethylene did not increase PAL activity in intact hypocotyl tissue but did so in excised tissue.

Materials and Methods

Intact 3-day-old gherkin seedlings (Cucumis sativus L., cv. "Venlose niet plekkers", strain Tercken VI) were grown in the dark at 25°C, and 2- and 10-mm segments were excised from the hypocotyl as described earlier (4). In experiments where 2-mm segments were used, five adjacent segments were excised immediately below the plumular hook. Plant material was exposed to various concentrations of ethylene in 35-liter glass jars equipped with a built-in electric fan to stir the air. Intact seedlings were placed in the jars in the boxes in which they had been grown. Segments were treated with ethylene by placing them in Petri dishes containing a 1-mm layer of water. Controls were placed in jars without ethylene. Where indicated, seedlings were irradiated with blue light (150 µW/cm²) in accordance with the method of Meijer (8).

Extraction and in vitro assay of PAL activity was performed as described before (3).

Ethylene production from plant tissue was measured by removal of 1-ml samples from 85-ml containers which were connected directly to a gas chromatograph. Ethylene was measured with a hydrogen flame ionization gas chromatograph fitted with a 200 × 0.22 cm Porapak Q column, 100 to 200 mesh, operated at 60°C.

Ethylene Production and PAL Activity of Gherkin Hypocotyl Segments. Figure 1 shows rates of ethylene production and increase of PAL activity in gherkin hypocotyl segments as a function of time after excision. During the first 4 hr following excision, ethylene was produced at a rate of 3.7 µmol per g fr wt per hr, which thereafter increased rapidly by a factor of 10. However, the PAL activity increased at a maximum rate immediately after excision and then leveled off after 16 hr, as shown previously (4). The data in Figure 1 indicate the lack of a correlation between ethylene production and the increase in PAL activity.

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1 Abbreviation: PAL: phenylalanine ammonia-lyase.
The next question was whether the slow rate of ethylene production during the initial phase following excision was equal to that of the corresponding tissue in the intact plant or whether it was already affected by excision. For this reason we compared ethylene production of intact hypocotyls with segments excised from the top and the bottom of similar hypocotyls. The results (Table 1) indicate that excision did increase the rate of ethylene evolution. However, a correlation between ethylene production and PAL development was not observed, since 2-mm and 10-mm segments produced equal amounts of ethylene, even though 2-mm segments produced three times as much PAL as 10-mm segments (4).

Effect of Ethylene on the PAL Activity in Intact and Excised Gherkin Hypocotyl Tissue. The PAL level in the gherkin hypocotyl remained more or less constant in the dark at room temperature when calculated on a fresh weight basis (5). Evidence was presented (6) that this constant level represented a balance between PAL synthesis and inactivation. Figure 2 shows that 24 hr of incubation in the dark with 0.1 to 800 μl/l ethylene lowers the PAL level. A time course experiment with 2.5 μl/l ethylene indicated that there was no temporary stimulation (Fig. 3). Similar data were obtained with irradiated hypocotyls. As shown earlier (3), the PAL activity reaches a maximum 3 hr after onset of the irradiation. In the presence of 0.1 to 800 μl/l we found that 3 hr of irradiation led to a lower level of PAL activity. A time course experiment with 2.5 μl/l ethylene indicated no change in the position of the PAL activity peak (Fig. 3).

In contrast to these results, we found that levels of ethylene which caused maximum inhibition of PAL synthesis in intact seedlings (both dark and light treated) increased the PAL activity in 2-mm hypocotyl segments (Fig. 2). The stimulation became measurable 2 to 3 hr after application of ethylene (Fig. 1).

CONCLUSIONS

The results indicate that if ethylene plays a role in PAL synthesis in excised gherkin hypocotyl tissue, it does so only in combination with the formation or disappearance of another factor. It has been shown before (4) that hydroxycinnamic acids, which inhibit PAL synthesis, are released into the medium. In more recent experiments, we have found that in addition to these compounds a heat-labile and nondialyzable inhibitor of PAL synthesis is released after excision. This compound, which is possibly of a similar nature to the proteinaceous inhibitors of invertase and peroxidase synthesis recently discovered in various plant tissues (2, 9), may be crucial for the ethylene effect on PAL synthesis in gherkin hypocotyl tissue.

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LITERATURE CITED

4. Edelmann, G. 1968. Photoinduction of phenylalanine deaminase in gherkin

Table 1. Rate of Ethylene Production of Excised Gherkin Hypocotyls and of Smaller Segments from Different Parts of the Hypocotyl

<table>
<thead>
<tr>
<th>Material</th>
<th>Ethylene (μmoles per g fresh wt per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire hypocotyl (30 mm)</td>
<td>1.1</td>
</tr>
<tr>
<td>10-mm Segments top</td>
<td>3.6</td>
</tr>
<tr>
<td>2-mm Segments top</td>
<td>3.7</td>
</tr>
<tr>
<td>10-mm Segments bottom</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of ethylene concentration on the development of PAL activity in the hypocotyl of intact gherkin seedlings in darkness (measured after 24-hr incubation); in the light (measured after simultaneous incubation and irradiation of 3 hr); and in 2-mm hypocotyl segments in darkness (measured after 24-hr incubation). All experiments started from 3-day-old dark-grown seedlings and were carried out at 25 C.

Fig. 3. Time course of the development of PAL activity in the hypocotyl of gherkin seedlings which had from time zero been exposed to 2.5 μl/l ethylene and either irradiated simultaneously or kept in darkness.


