Nonphysiological Binding of Ethylene by Plants

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ABSTRACT

Ethylene binding to seedling tissue of Vicia faba, Phaseolus vulgaris, Glycine max, and Triticum aestivum was demonstrated by determining transit time required for ethylene to move through a glass tube filled with seedling tissue. Transit time for ethylene was greater than that for methane indicating that these tissues had an affinity for ethylene. However, the following observations suggest that the binding was not physiological. Inhibitors of ethylene action such as Ag⁺ ions and CO₂ did not decrease binding. Mushrooms which have no known sites of ethylene action also demonstrated ethylene binding. The binding of acetylene, propylene, ethylene, propane, and ethane more closely followed their solubility in water than any known physiological activity.

Previous investigators have shown that ethylene can bind to plants (2, 3, 5–7, 10, 11). The purpose of the work reported here was to take advantage of that fact and test the concept of using an affinity column of living tissue to measure ethylene binding sites. The affinity column approach was designed to exploit the fact that ethylene, as a gaseous hormone, could be readily measured in the column effluent, and its movement through the tube (transit time) could be compared to a physiologically inert standard such as methane. An increase in transit time of ethylene as compared to methane would indicate binding, and it should be possible to design experiments that would relate binding to physiological properties of the gas. However, as discussed below, while ethylene-binding was demonstrated with this ‘chromatographic’ technique, it did not correlate with the physiology of the gas.

MATERIALS AND METHODS

Plant Material. Seeds of Vicia faba ‘Herz freya’ (fababean), Phaseolus vulgaris ‘Black Valentine’ (bean), Glycine max (soybean), and Triticum aestivum ‘Hard red winter’ (wheat) were germinated in moist vermiculite after sterilization with Clorox:water (1:5). Mushrooms (Agaricus bisporus) were purchased in a local market.

Equipment. The affinity of plant material to ethylene and other hydrocarbon gases was measured by determining the transit time required for gases to move through a glass column (100 cm long, 1.5 cm inside diameter, volume 265 cm³) filled with plant or other material. The column was filled with approximately 150 ± 10 g, fresh weight, of seeds, cotyledons, 1-cm sections of seedling axes, or 0.13 cm² pieces of mushroom tissue. The ends of the column were sealed with rubber vaccine stoppers. Plastic tees were inserted in both stoppers. One arm of each tee was covered with a vaccine stopper through which either gases were added or removed with a 1-ml plastic syringe. At the entrance of the column, the other arm of the tee was attached to a compressed air cylinder by rubber tubing. The flow rate of air, 10% CO₂ in air or N₂, was 13 to 14 ml per min. Rubber tubing at the exit tee was held 1 cm below water in a beaker. This insured that gas samples from the column were not diluted with ambient air. The column effluent was measured once a minute for 6 to 20 min after a mixture of methane and other hydrocarbon gases was injected into the entrance tee of the column. These experiments were run at room temperature 23°C. Ethylene and other hydrocarbon gases were measured with a gas chromatograph.

Mixtures of gases used in these experiments were prepared by mixing known volumes of gases in gas sample bags. Ethylene concentration was checked against a commercial standard.

Seeds were treated with AgNO₃ by immersing them in a 100-ppm solution for 10 min and then blotting to excess with paper towels. The CS₂ treatment was a 2-min exposure of 1000 ppm in a glass desiccator.

Calculations. The following method was used to measure hydrocarbon gas transit times (T-hydrocarbon) and the per cent increase in transit time (%ΔT). The peak heights of gases from similar time collection periods were averaged and plotted on a graph as shown in Figure 1. Column runs were replicated two to five times. A midpeak line was drawn horizontally through the peak. A second line, drawn from the center of the midpeak line and perpendicular to the abscissa, indicated that peak’s transit time. The per cent increase in transit time of a hydrocarbon was calculated by the following equation: %ΔT = ((T_h - T_m)/(T_m)) × 100. (T_h and T_m are the transit times of the hydrocarbon gas and methane, respectively).

RESULTS

Figure 1 shows that ethylene and methane have similar transit times through a column filled with dry bean seeds. Table I summarizes the results of similar experiments with the column filled with glass beads, imbibed seeds with intact seed coats, or germinating seeds. A representative experiment showing ethylene binding with apical tissue from soybeans is shown in Figure 2. In this experiment, the appearance of ethylene in the effluent was delayed by 0.5 min compared to the methane standard.

Results presented in Table I show that ethylene has a longer transit time than methane when the column was filled with cotyledons, germinating wheat, or pieces of mushroom tissue. It was assumed that fababean cotyledons have an ethylene attachment site since they contain ethylene monooxygenase which converts ethylene to ethylene oxide (4). The contribution of this site to ethylene binding could be evaluated by treating the seeds with 100 ppm CS₂ for 10 min before placing them in the glass tube. CS₂ has been shown to be an inhibitor of ethylene monooxygenase (4). As shown in Figures 3 and 4, CS₂ treatment of fababean cotyledons increased the amount of ethylene in the column effluent but it did not affect ethylene transit time.

A range of ethylene concentrations were used to see if it was possible to saturate the binding site with excessive concentrations of ethylene. Table II indicates that the transit time of ethylene
was not influenced by the initial application of 100 to 10,000 ppm ethylene. Because of dilution (approximately 100-fold) as ethylene moved down the tube, it was not possible to evaluate the effect of lower concentrations of ethylene.

Treatments which potentially regulate or influence hormonal action of ethylene such as pretreating seeds with AgNO₃, including 10% CO₂ in the airstream, or using N₂ as a carrier gas, did not have an effect on ethylene transit time (Table III).

The relative affinities of saturated and unsaturated hydrocarbon gases to soybean tissues compared to a methane standard is shown in Table IV. For a comparison, a listing of the relative solubility of these gases in water with respect to a methane standard is presented.

**Table I. The Affinity of Hydrocarbon Gases to Seeds, Glass Beads, and Mushrooms**

<table>
<thead>
<tr>
<th>Material</th>
<th>T-Methane</th>
<th>T-Ethylene</th>
<th>%ΔT^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass beads</td>
<td>10.56</td>
<td>10.56</td>
<td>0</td>
</tr>
<tr>
<td>V. faba, dry seeds</td>
<td>10.25</td>
<td>10.25</td>
<td>0</td>
</tr>
<tr>
<td>V. faba, imbued with seed coats</td>
<td>11.32</td>
<td>11.32</td>
<td>0</td>
</tr>
<tr>
<td>V. faba, 3-d-old seedlings</td>
<td>11.63</td>
<td>12.00</td>
<td>3.2</td>
</tr>
<tr>
<td>P. vulgaris, dry seeds</td>
<td>9.76</td>
<td>9.76</td>
<td>0</td>
</tr>
<tr>
<td>P. vulgaris, 2-d-old seedlings</td>
<td>10.73</td>
<td>11.24</td>
<td>4.8</td>
</tr>
<tr>
<td>G. max, 3-d-old seedlings</td>
<td>11.39</td>
<td>12.46</td>
<td>9.4</td>
</tr>
<tr>
<td>T. aestivum, 3-d-old seedlings</td>
<td>14.76</td>
<td>14.88</td>
<td>0.8</td>
</tr>
<tr>
<td>Mushrooms</td>
<td>12.34</td>
<td>12.90</td>
<td>4.5</td>
</tr>
</tbody>
</table>

^* Per cent increase in transit time.

^b Transit time of methane and ethylene through the column.

**DISCUSSION**

Earlier studies by others on ethylene binding (2, 3, 5–7, 10, 11) attempt to elucidate the site and mode of ethylene action. Kende and Gardner (8) and Rubery (9) have presented excellent reviews on the nature and significance of hormone binding studies. The following problems confront the investigator of plant hormone binding sites. The number of sites are small. Estimates of their number range from 500 (1) to 6000 per cell (9). Affinity for ethylene is low. For example, ethylene effects on seedling growth are rapid and reversible (12). Unspecific binding sites and ethylene metabolism (4) have to be accounted for.
Table IV. Affinity of G. max to Various Hydrocarbon Gases

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Hydrocarbon Gas</th>
<th>T-Methane (min)</th>
<th>T-Hydrocarbon (min)</th>
<th>%ΔT</th>
<th>Solubility of Gas in Water Compared to Methane (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledons</td>
<td>Ethane</td>
<td>11.48</td>
<td>11.13</td>
<td>-3.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>Ethylene</td>
<td>11.39</td>
<td>12.46</td>
<td>9.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>Propane</td>
<td>9.09</td>
<td>9.41</td>
<td>3.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>Propylene</td>
<td>10.01</td>
<td>11.08</td>
<td>10.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Seedling axis</td>
<td>Ethylene</td>
<td>12.39</td>
<td>12.90</td>
<td>4.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Seedling axis</td>
<td>Acetylene</td>
<td>11.48</td>
<td>17.34</td>
<td>51.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

- **a** Per cent increase in transit time.
- **b** Solubility of methane in water = 9.0 ml gas/100 ml H₂O.
- **c** Transit time of methane and hydrocarbon through the column.

The purpose of the work described here was to develop a technique which would demonstrate ethylene binding in a way that the findings could be correlated with known features of the mode of action of the gas. The data presented here demonstrated that a glass tube filled with cotyledons or other parts of germinating seeds absorbed ethylene with a greater affinity than a methane standard. The advantage of this 'plant chromatograph' is that it is fast, simple, and demonstrates binding without the use of radioisotopes. However, the ethylene binding observed with this system appears to be of a physical nature and not due to the presence of a hormonal site of action.

This conclusion is based on the following observations. The binding was nonspecific; retention of ethylene by mushroom tissue indicates that binding can occur in tissue that is not known to respond to the gas. Binding was not saturated at concentrations of the gas known to physiologically saturate ethylene action. Competitive inhibitors of ethylene action such as 10% CO₂, 100 ppm AgNO₃, and a N₂ gas phase did not decrease binding. Finally, the affinity of ethylene action analogs to plant tissue was more closely associated with their solubility in water than their physiological activity. The physiological activity of hydrocarbon

**Fig. 3.** Transit times of methane and ethylene from a column filled with fababean cotyledons.

**Fig. 4.** Transit times of methane and ethylene from a column filled with fababean cotyledons pretreated with 1000 ppm CS₂ for 10 min. Note the increase in peak height of ethylene compared to that shown in Figure 3.

is in the order of ethylene > propylene > acetylene > ethane, while the binding affinity was acetylene > propylene > ethylene > propane > ethane. The relative solubility of these gases in water is acetylene > propylene > ethylene > propane > ethane.

Acknowledgments—I wish to acknowledge the excellent technical assistance of L. Dunn and G. Lightner for the computer-generated figures.

LITERATURE CITED

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