Ethylene Enhanced Release of \( \alpha \)-Amylase from Barley Aleurone Cells

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Ethylene induces various physiological effects in higher plants (3) and recent evidence suggests that at least some of these events are mediated by influencing protein synthesis (1, 5). This paper describes the effects of ethylene on the synthesis and release of \( \alpha \)-amylase from barley aleurone layers. Varner and co-workers (10, 11), have shown that \( \alpha \)-amylase is produced de novo in aleurone layers following treatment with gibberellic acid (GA\(_3\)) and that amylase is secreted from the aleurone layers by a specific secretory mechanism.

The methods used for preparation of barley aleurone layers and determination of \( \alpha \)-amylase are similar to those described by Chrispeels and Varner (4) and Jones and Varner (6). The amount of \( \alpha \)-amylase obtained from the incubating aleurone layers was expressed in terms of units of \( \alpha \)-amylase

\[
\Delta \text{OD} \times T, \quad \text{volume of incubate; } t = \text{time of incubation with sample; } v = \text{sample volume (6).}
\]

Incubation of aleurone layers was accomplished in vaccine-capped flasks and ethylene was introduced through the caps using gas-tight syringes.

The secretion of \( \alpha \)-amylase into the medium surrounding aleurone cells follows a characteristic pattern. During the first 5 to 8 hours following GA\(_3\) application no \( \alpha \)-amylase is secreted, this is referred to as the lag period. Following this lag period, \( \alpha \)-amylase secretion is linear with time for approximately 12 to 18 hours after which secretion declines. Consequently, in most experiments the flasks were sampled after 18 hours of incubation.

Simultaneous addition of ethylene and GA\(_3\) to aleurone layers resulted in an increase in the amount of \( \alpha \)-amylase present in the medium (fig 1). However, when aleurone layers were treated with ethylene alone, a marked reduction in the amount of \( \alpha \)-amylase was noted (fig 1). In an attempt to separate the effects of ethylene from those of GA\(_3\), the growth regulators were added separately during the incubation period.

Following incubation of aleurone layers with GA\(_3\) or ethylene for 5 hours, the aleurone layers were removed, washed in buffer for 30 minutes and incubated for the remaining 12 hour period with the appropriate regulator.

Addition of GA\(_3\) for a time period corresponding to the length of the lag period (5 hrs) followed

Fig. 1. Effect of gibberellic acid and ethylene on \( \alpha \)-amylase release from aleurone layers. (●—●) \( \alpha \)-amylase released in the presence of GA\(_3\) at 0.05 \( \mu \text{g/ml}\) and ethylene; (○—○) \( \alpha \)-amylase released in the presence of ethylene alone.

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by addition of ethylene for the remaining incubation period (12 hrs) resulted again in an increase in the amount of α-amylase present in the medium (table I). However, addition of ethylene to aleurone layers for a time period corresponding to the lag period and of GA during the next 12 hours resulted in a reduction in the amount of α-amylase present in the medium (table II). This effect of ethylene pretreatment, although not marked, was consistent in all experiments.

There are 2 ways in which ethylene could increase or decrease the amount of α-amylase found in the medium surrounding aleurone cells. Firstly, it could affect enzyme synthesis, or secondly, it could affect the rate of enzyme secretion. To distinguish between an effect on synthesis and one on secretion an examination was made of the total amount of α-amylase induced by GA$_3$ and ethylene treatments, namely, the amount of α-amylase secreted by the cells together with that remaining within the aleurone cells. Aleurone cells were extracted by grinding with sodium acetate buffer (pH 4.8) and following centrifugation of the homogenate, α-amylase was determined according to the previously described method. Determinations of medium and extracted α-amylase following treatment of aleurone layers with GA$_3$ during the lag period and ethylene during the secretory period are shown in table I. Since the totals are identical for each treatment, it is concluded that ethylene does not affect α-amylase synthesis but rather that its effect is on secretion. Similarly, an examination was made of medium and extracted α-amylase following treatment of aleurone layers with ethylene during the lag phase and GA$_3$ during the secretory phase. Again, as the total amount of enzyme produced following ethylene treatment is the same as that of the control (table II), it is concluded that the effect of ethylene is indeed on the secretion of α-amylase as opposed to synthesis.

Several workers have reported effects of ethylene on membrane permeability (2, 7, 9) and it is possible that the effects of ethylene in this system are being mediated via effects on cell membranes. Although the mechanism of α-amylase secretion is unknown, histological observations by Paleg and Hyde (8) indicate that membrane bound vesicles are involved. Ethylene could affect the formation of these vesicles or the eventual discharge of their contents. It is difficult, however, to envisage that ethylene could both inhibit or accelerate the release of α-amylase from aleurone cells via the same mechanism. Further evaluation of this data must therefore await the elucidation of the mechanism of α-amylase secretion in barley aleurone cells.

Although ethylene exhibits a significant effect on the secretion of α-amylase from barley aleurone cells, the magnitude of this effect makes this system less attractive for the study of ethylene effects on secretion. To establish conclusively a role of ethylene in secretion, other systems should be investigated.

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**Table I. Effect of Ethylene Added for 5 Hours and Gibberellic Acid (0.05 μg/ml) Added for 12 Hours on Secreted and Extractable α-Amylase**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GA$_3$ 5 hrs</th>
<th>C$_2$H$_4$ 12 hrs</th>
<th>Amylase units $\Delta OD \times T \times V$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>Extracted</td>
<td>Total</td>
</tr>
<tr>
<td>Control GA$_3$ 0.05 μg/ml</td>
<td>69</td>
<td>27</td>
<td>96</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + 0.1 ppm C$_2$H$_4$</td>
<td>69</td>
<td>25</td>
<td>94</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + 10 ppm C$_2$H$_4$</td>
<td>85</td>
<td>13</td>
<td>98</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + 10 ppm C$_2$H$_4$</td>
<td>81</td>
<td>16</td>
<td>97</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + 100 ppm C$_2$H$_4$</td>
<td>75</td>
<td>18</td>
<td>93</td>
</tr>
</tbody>
</table>

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**Table II. Effect of Gibberellic Acid Added for 5 Hours and Ethylene Added for 12 Hours on Secreted and Extractable α-Amylase**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C$_2$H$_4$ 5 hr</th>
<th>GA$_3$ 12 hrs</th>
<th>Amylase units $\Delta OD \times T \times V$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>Extracted</td>
<td>Total</td>
</tr>
<tr>
<td>Control GA$_3$ 0.05 μg/ml</td>
<td>45</td>
<td>16</td>
<td>61</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + C$_2$H$_4$ 0.1 ppm</td>
<td>40</td>
<td>17</td>
<td>57</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + C$_2$H$_4$ 10 ppm</td>
<td>40</td>
<td>19</td>
<td>59</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + C$_2$H$_4$ 10 ppm</td>
<td>38</td>
<td>19</td>
<td>57</td>
</tr>
<tr>
<td>GA$_3$ 0.05 μg/ml + C$_2$H$_4$ 100 ppm</td>
<td>40</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

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Literature Cited