Avena Coleoptile Elongation: Stimulation by Fluorophenylalanine

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Abstract. A 100 to 150% stimulation of Avena coleoptile segment elongation by the amino acid analogue p-fluorophenylalanine (FPA) has been observed. The effect is reversed by phenylalanine and is not seen with comparable concentrations of sodium fluoride. FPA does not alter elongation of red-irradiated segments. Stimulation by FPA occurs only when the apex is intact and the segments are incubated in the absence of exogenous auxin. In the presence of FPA, [3H]leucine uptake by coleoptile segments is reduced by 34% and protein synthesis by 42%. When pre-incubated on labeled media and subsequently transferred to unlabeled media, segments fail to incorporate into the protein fraction any of the previously absorbed label. It is therefore difficult to ascertain whether FPA results in a genuine inhibition of protein synthesis in apical coleoptile segments. Possible mechanisms for the action of FPA and its relationship to light dependent elongation are considered.

The amino acid analogue p-fluorophenylalanine (FPA) is one of several anti-metabolites known to inhibit growth and/or protein synthesis in bacteria and higher plants (6,9,11). Inhibition of growth by FPA, in conjunction with the action of related antimetabolites, is normally accepted as an indication that protein synthesis is essential to the growth process. Thus, the conclusion that protein synthesis is required for auxin induced growth processes was based, in part, on the observed inhibition of Avena coleoptile elongation by FPA (9). We have been studying the light dependent elongation of Avena coleoptile tissue, which is known to be mediated by the photomorphogenic pigment, phytochrome (3). In the course of these investigations, we observed that FPA stimulated, rather than inhibited, coleoptile elongation. In this paper the stimulatory action of FPA is described along with experiments bearing on the mechanism of this response and its relationship to both auxin dependent and light dependent elongation of Avena coleoptile segments.

Materials and Methods

Plant Materials. Seeds of Avena sativa L. var. Clintland 60 were obtained from Southern States Cooperative, Baltimore, Maryland. Approximately 50 ml of unhusked seeds were soaked for 30 min in distilled water containing 0.05% Tween-20 (v/v) and then scattered uniformly over the surface of moist vermiculite in closed polyethylene basins (Polly Flex No. 137, Republic Molding Company, Chicago, Illinois). Each basin (12 × 8 × 4 in.) contained approximately 150 g of vermiculite moistened with 600 ml of distilled water. In order to achieve the most uniform growth of the seedlings, the moist vermiculite was thoroughly mixed by hand and carefully leveled. If the vermiculite is excessively wet or tamped down, poor seedling germination and growth results. The basins were then placed in a darkroom at 26 ± 1° until the third day after sowing. Seedlings designated as dark-grown were allowed to develop in total darkness for the entire 3 days. Seedlings designated as light-grown were irradiated with red light for 1 hr approximately 36 hr after sowing and then returned to darkness until the third day. All seedlings were used approximately 72 to 75 hr after sowing.

Segment Growth. Apical segments were prepared by excising the apical 5 mm from coleoptiles ranging in length from 10 to 15 mm. For use in growth tests uniform segments were cut with a razor blade attached to a plexiglas arm, with the length of the segments determined by a pre-set mechanical stop. The primary leaf was not removed, as its presence does not alter the growth of the coleoptile segment (3). For those experiments involving tissue analysis, leafless segments were cut with a hand-held razor blade. Subapical segments were 10 mm in length and were taken approximately 2 to 3 mm below the apex of coleoptiles at least 15 mm long. All segments were collected and randomized on 0.02 M phosphate buffer, pH 6.2. When a sufficient number of segments had been collected, they were distributed in lots of 5 to 10 segments in 50 ml beakers. Each beaker contained 3 or 5 ml of a buffered sucrose medium (BSM) consisting of the above phosphate buffer plus 1.5% sucrose and...
the beaker was covered with aluminum foil to prevent evaporation. The length of the segments was measured to the nearest 0.5 mm after 18 to 20 hr incubation in darkness at 26 ± 1°C. Within each experiment, all treatments were conducted in duplicate and usually in triplicate lots. All experiments were conducted a minimum of 3 times.

Radio-carbon Incorporation. Incubations with radioactive carbon were conducted in 60 mm Stender dishes with a center well 12 mm in diameter and 18 mm deep. During incubation, the dish was sealed with petroleum jelly (Vaseline) and the center well contained 0.5 ml of 5% KOH (w/v) to trap evolved CO₂. Preliminary experiments indicated that incubation under these conditions did not alter the growth response.

Tissue Analysis. Following incubation, the segments were chilled on ice to prevent further incorporation of label. The CO₂ collected in the centerwell was analyzed by transferring an aliquot directly to a scintillation vial. Where media activity are indicated, an aliquot of the medium was similarly transferred directly to a scintillation vial. Each lot of segments (25-40 segments) was then collected in a perforated crucible, washed with several volumes of cold buffered sucrose and blotted on filter pads. The tissue was homogenized in 1.0 ml of cold 5% perchloric acid (PCA) (v/v) with a motor-driven glass tissue grinder and quantitatively transferred to a 15 ml Corex glass high speed centrifuge tube. The homogenate was allowed to stand on ice for 15 min before centrifugation at 30,000 g for 15 min at 4°C. The precipitate was resuspended in 1.0 ml PCA, heated at 90°C for 10 min in order to extract nucleic acids, and washed once with PCA at room temperature. Both supernatants were combined and brought to a total volume of 4.0 ml. The pooled supernatants were considered the acid soluble fraction. Protein in the remaining precipitate was solubilized in 1.0 ml of 5% KOH (w/v) at 30°C overnight.

Radioactive Counting Techniques. Aliquots, usually 0.1 ml, of all samples were added to 10 ml of scintillation fluid in polyethylene vials and counted in a Packard Tri-carb Model 3310 liquid scintillation counter. The scintillation fluid consisted of 100 grams naphthalene, 10 grams POP, 250 mg POPOP, 200 ml methanol, and 1 liter dioxane. Unquenched fluid yielded 80% efficiency with a ³⁴Cl-toluene standard. All samples were counted for a minimum of 10,000 counts above background and corrected to 100% efficiency.

Light Sources. All operations were conducted under a dim green safelight which consisted of two 15-W green fluorescent tubes behind one-eighth inches of Rohm and Haas Plexiglas, Amber 2451 and Blue 2045. The red light source consisted of four 15-W red fluorescent tubes behind one-eighth inches of red Plexiglas (Rohm and Haas 2444). This source provided approximately 1.4 μw cm⁻² nm⁻¹ at 650 nm, as measured with an ISCO model SR spectroradiometer (Instrumentation Specialties Company, Lincoln, Nebraska).

Results

Effect of FPA on Growth. The effect of FPA on elongation of apical coleoptile segments is shown in figure 1. For these experiments, segments were cut from dark-grown seedlings and incubated on buffered sucrose medium (controls) or on buffered sucrose containing the indicated concentration of FPA. One-half the lots of segments at each concentration of FPA were exposed only to the dim green safe light prior to incubation in darkness. The remaining lots received 5 min of red light at the beginning of the incubation. Over the range of concentrations tested, FPA clearly stimulates the elongation of non-irradiated segments. In the 2 experiments summarized in figure 1, elongation at 10⁻² M FPA is stimulated by approximately 100%. Stimulations as high as 150% have been observed in other experiments. In all cases observed thus far, elongation on 10⁻² M FPA is approximately equivalent to red-irradiated controls. Total elongation of red-irradiated segments, however, is not significantly influenced by FPA over the same range of concentrations, while the increment due to red light decreases to zero. Concentrations greater than 10⁻² M were not tested in order to avoid the possibility of complications introduced by what are normally considered non-physiological levels. At 10⁻² M, however, there is no visible evidence of tissue deterioration or other signs of toxicity. In addition, the effect of FPA on respiration was tested by following oxygen consumption in a Gilson model GP-14 differential respirometer. Twenty-five segments were floated on 3.0 ml of buffered sucrose containing 5 × 10⁻³ M FPA. No measurable change in rate of

![Figure 1](http://www.plantphysiol.org/figure/1.png)

Fig. 1. The effect of FPA on elongation of apical coleoptile segments in darkness (●) and following 5 min irradiation with red light (○). Controls shown are elongation in the absence of FPA.
Table I. Reversal of FPA-induced Coleoptile Elongation by PA

Five mm apical segments were cut from totally dark-grown seedlings and incubated in darkness for 18 hr at 25 ± 7°C. FPA and PA were provided at 10-2 M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Elongation (mm ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td>FPA</td>
<td>2.47 ± 0.16</td>
</tr>
<tr>
<td>PA</td>
<td>1.27 ± 0.15</td>
</tr>
<tr>
<td>FPA + PA</td>
<td>1.07 ± 0.08</td>
</tr>
</tbody>
</table>

A series of experiments was conducted in order to clarify some of the conditions under which FPA is able to stimulate or inhibit elongation of coleoptile tissue. In these experiments, the following 3 factors were tested in all possible combinations for their influence on the action of FPA: (1) source of the segments within the coleoptile, i.e., apical vs. subapical; (2) pretreatment of the seedlings with red light; and (3) availability of exogenous indoleacetic acid (IAA). These growth tests were all conducted with the incubation of segments in total darkness. The results, summarized in table III, indicate that the direction and magnitude of FPA is indeed dependent upon the source of tissue and conditions of the experiment. It is evident from these data that FPA does inhibit elongation of coleoptile segments. Both apical and subapical, when they are excised from seedlings previously irradiated with red light and if they are incubated in the presence of indoleacetic acid (IAA). These results therefore both confirm and extend the previously published report of an inhibitory action by FPA on IAA-induced coleoptile elongation (9). On the other hand, FPA clearly stimulates elongation of apical segments when incubated in the absence of IAA, regardless of whether the seedlings were dark-grown or pretreated with red light. Under all other conditions, FPA is essentially without effect. Thus, in order to observe the stimulatory action of FPA on coleoptile segments the apex must be present and there must be no exogenous auxin in the medium.

Effect of FPA on Protein Synthesis. Before testing for the ability of FPA to interact with protein synthesis, the kinetics of amino acid incorporation by apical segments were examined. Lots of 40 leafless segments were incubated on 5 ml of buffered sucrose medium containing 1 µc of 1-leucine-1-14C (Calbiochem) as described in the methods. At hourly intervals, random lots were harvested, the segments fractionated, and the fractions assayed for activity. The results (fig 2) show that there is a continuous and near-linear uptake and incorporation of label into both the acid soluble and KOH-soluble fractions over a period of 4 hr. Evolution of CO2 occurs more slowly over the first 2 hr, but by the

Table II. Action of Fluoride on Elongation of Apical Coleoptile Segments

Five mm segments were cut from totally dark-grown seedlings. Red light was given for 5 min at beginning of incubation. Total incubation time: 18 hr at 25 ± 1°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Elongation (mm ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.73 ± 0.09</td>
</tr>
<tr>
<td>FPA (5×10-3 M)</td>
<td>2.55 ± 0.09</td>
</tr>
<tr>
<td>NaF (5×10-5 M)</td>
<td>1.75 ± 0.11</td>
</tr>
<tr>
<td>NaF (5×10-3 M)</td>
<td>0.98 ± 0.02</td>
</tr>
</tbody>
</table>

Table III. Effect of FPA on Segment Growth

See text for details.

<table>
<thead>
<tr>
<th>Seedlings</th>
<th>Segment</th>
<th>Control</th>
<th>FPA</th>
<th>IAA</th>
<th>FPA+IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark-grown</td>
<td>Apical</td>
<td>1.43 ± 0.13</td>
<td>2.47 ± 0.16</td>
<td>...</td>
<td>2.33 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Subapical</td>
<td>2.26 ± 0.13</td>
<td>...</td>
<td>2.77 ± 0.22</td>
<td>...</td>
</tr>
<tr>
<td>Light-grown</td>
<td>Apical</td>
<td>1.13 ± 0.09</td>
<td>1.37 ± 0.08</td>
<td>5.72 ± 0.22</td>
<td>5.69 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Subapical</td>
<td>0.63 ± 0.06</td>
<td>...</td>
<td>3.03 ± 0.17</td>
<td>1.67 ± 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.93 ± 0.27</td>
<td>2.66 ± 0.14</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.63 ± 0.19</td>
<td>9.37 ± 0.30</td>
<td>7.23 ± 0.24</td>
<td></td>
</tr>
</tbody>
</table>
an apparent inhibition of protein synthesis. However, activity evolved as carbon dioxide was reduced by 46% and that of the acid soluble fraction by 19%. The addition of FPA to the medium thus resulted in an overall reduction of uptake and incorporation by approximately 34%. On the basis of these results, it is not clear whether FPA specifically inhibits protein synthesis. The reduced level of incorporation into the KOH soluble fraction could merely reflect a general reduction in the total uptake of the label into the tissue.

In the same experiment, the influence of a 5 min red irradiation on incorporation of label was also tested. Red light does not significantly alter the incorporation of label into the KOH soluble fraction, but activity in the acid soluble pool is increased by 18% and evolution as carbon dioxide by 36% (table IV). Red light thus resulted in a stimulation of uptake and incorporation by 16%, although none of this increase is reflected in the KOH (protein) fraction.

Table V. Failure of Apical Coleoptile Segments to Incorporate Previously Absorbed Leucine Into Protein

Values are means of duplicate samples. Segments were incubated for 2 hr on 10.0 \( \mu \)C L-leucine-1-\( ^{14} \)C, followed by 2 hr on medium without label. Control analyses were made after the first 2 hr incubation. BSM and FPA analyses were made after the second 2 hr incubation on buffered sucrose medium and 10\( ^{-2} \) M FPA respectively.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Control</th>
<th>BSM</th>
<th>FPA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radioactivity in segments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO(_{2})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOH soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td>2,130</td>
<td>4,300</td>
<td></td>
</tr>
<tr>
<td><strong>Total activity</strong></td>
<td>42,689</td>
<td>46,506</td>
<td>46,499</td>
</tr>
</tbody>
</table>

It is apparent from these data that the simultaneous application of label and FPA does not provide a satisfactory answer to the question of whether FPA inhibits protein synthesis. In an attempt to provide a more definitive answer, segments were first incubated for 2 hr on a buffered sucrose medium containing \( ^{14} \)C-leucine. They were then washed and transferred to unlabeled media, both with and without FPA, for a second 2 hr incubation. The segments were fractionated and assayed for activity after the second 2 hr incubation. An additional sample was assayed after the first 2 hr incubation in order to provide an estimate of activity in each fraction at the time of transfer to the unlabeled media. In this manner, the effect of FPA on incorporation of label could be separated from its effects on uptake. These results are presented in table V. There is no difference between the levels of incorporation of label into

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Table IV. Effect of Red Light and FPA Upon Incorporation of \( ^{14} \)C-Leucine into Protein of Apical Coleoptile Segments

Values are means of duplicate samples. Twenty-five segments were incubated in dishes containing 5 ml buffered sucrose and 1 \( \mu \)C L-leucine-1-\( ^{14} \)C. Red light was given for 10 min at the beginning of the incubation. Analyses were performed after 4 hr incubation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO(_{2})</th>
<th>Acid soluble</th>
<th>KOH soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16,704</td>
<td>34,065</td>
<td>34,178</td>
</tr>
<tr>
<td>Red light</td>
<td>22,675 (+36)</td>
<td>40,253 (+18)</td>
<td>35,336 (+3)</td>
</tr>
<tr>
<td>10(^{-2}) M FPA</td>
<td>9,032 (-46)</td>
<td>27,724 (-19)</td>
<td>19,793 (-42)</td>
</tr>
</tbody>
</table>

\(^1\) Values in parentheses are percentage change over controls.
protein in the presence or absence of FPA (KOH soluble fraction, FPA vs. BSM). More significant however, is the apparent failure of the segments to incorporate any label into protein once transferred to the unlabeled medium (KOH soluble fraction, BSM vs. Control), in spite of the presence of a large soluble pool at the time of transfer (Acid soluble fraction, Control). Activity is lost from the soluble pool but is accounted for as evolved carbon dioxide and soluble components released to the medium.

**Discussion**

The experiments reported here demonstrate that the amino acid analog, p-fluorophenylalanine, stimulates the growth of excised apical coleoptile tissue by 100% or more. Walton has demonstrated a similar, although smaller, positive effect of FPA on excised *Phaseolus* embryos (12). These data would appear to contradict previous reports that FPA inhibits the growth of plant tissues, including excised coleoptile segments (9). However, the data summarized in table III confirm the previous report of inhibition and illustrate that both the direction and magnitude of FPA action is dependent upon the conditions under which the test is conducted. The requisites for stimulation by FPA are that the apex remain intact and that there be no IAA in the medium. Pretreatment of the seedlings with red light does not influence the stimulatory action of FPA on subsequently excised apical segments. The basis for such a differential response of coleoptile tissue to FPA is not clear, although it may be suggested that removal of the endogenous hormone supply imposes new limitations on the growth process.

The normally observed inhibitory action of FPA is believed to result from an inhibition of protein synthesis or the formation of non-functional protein (11). It is not clear whether FPA inhibits protein synthesis in the system reported here. Attempts to test for the ability of FPA to inhibit incorporation of previously absorbed C-leucine were unsuccessful, since even in the absence of FPA, apical segments fail to incorporate into the protein fraction a significant amount of previously absorbed label. Since the kinetic experiment shows a continuous uptake and incorporation of label over the 4 hr period used for all uptake experiments, the results presented in table V cannot be attributed to an inability of the segments to synthesis protein during the second 2 hr incubation. A more likely interpretation is that the amino acid present in the soluble pool is not readily available to the protein synthetic apparatus. Although the data presented in table IV indicate a strong inhibitory effect of FPA on uptake of the label, it should be noted that inhibition of incorporation into the protein fraction is twice that observed for the acid soluble fraction. This suggests the possibility of a genuine inhibition of protein synthesis by FPA.

The observed stimulation of elongation by FPA is interesting for 2 reasons. First, it would be of interest to know the mechanism by which an inhibitor of protein synthesis is able to stimulate a growth response. Despite the dangers inherent in interpretation of inhibitor action, a study of this process may add measurably to our knowledge of growth regulation in this tissue. The simplest hypothesis consistent with these facts is that FPA reduces the activity of a protein whose presence normally limits growth. Also to be considered, however, is the role of phenylalanine in the synthesis of phenolic derivatives, some of which (*e.g.*, coumarin) are known to inhibit plant growth (5, 8). It is conceivable that FPA could influence the synthesis of such compounds through depressed enzyme synthesis and/or activity, thereby relieving the tissue of a natural inhibition. One such enzyme could be phenylalanine ammonia lyase (PAL). This enzyme is responsible for the conversion of phenylalanine to trans-cinnamic acid, which is known to have anti-auxin activity (4, 10). Preliminary experiments in this laboratory have demonstrated that PAL is present in acetone powders of *Avena* seedlings, that the enzyme is competitively inhibited by FPA in vitro and that trans-cinnamic acid inhibits the elongation of apical coleoptile segments in the presence of FPA (Hopkins, unpublished observations). Experiments are currently in progress to determine the effect of FPA on PAL activity in vivo.

Of potentially greater interest than the mechanism of action of FPA itself in this system is the relationship between FPA and the red light effects. The apparent inability of FPA to inhibit red light-induced elongation suggests that synthesis of new protein may not be necessary for the expression of this phytochrome-dependent process. Furthermore, red light does not influence incorporation of label into the protein fraction although a significant increase in total uptake was observed. Mohr was unable to detect a similar light-stimulated uptake in mustard seedlings (7), but this would not be the first case of difference in phytochrome-related phenomena attributable to monocot-dicot distinctions (1, 2). Indeed, the growth responses of *Sinapis* hypocotyls and *Avena* coleoptiles are themselves opposite in nature (3, 7). The increased uptake observed in coleoptile segments may be simply a reflection of increased growth, while the growth rate of hypocotyls is reduced by red light and increased uptake of label would not necessarily be expected.

The apparent substitution of FPA for red light in promoting elongation of coleoptile segments would be consistent with the idea of a common or closely related mechanism of action. A study of the mechanism by which FPA stimulates elongation could conceivably lead to some insight as to the mechanism of action of the photomorphogenetic pigments. That FPA and red light do not exert the same apparent effect on protein synthesis is not necessarily inconsistent with this hypothesis. FPA would be expected to reduce protein synthesis in general, while red light might be expected to act more specifically,
perhaps regulating synthesis of certain critical proteins. An evaluation of this hypothesis cannot be made until a more critical examination of specific protein components of the coleoptile segments is completed.

**Literature Cited**