Timing of the Auxin Response in Etiolated Pea Stem Sections

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ABSTRACT

The short term growth response of etiolated pea stem segments (Pisum sativum L., var. Alaska) was investigated with the use of a high resolution growth-recording device. The immediate effect of treatment with indole-3-acetic acid is an inhibition of growth. This inhibition lasts about 10 minutes, and then the rate of elongation rises abruptly to a new steady rate about 4 times the rate of elongation before auxin treatment. This rapid steady rate of elongation, however, continues for only about 25 minutes before declining suddenly to a lower steady rate of growth about 2 times the rate of elongation before the addition of auxin. Pretreatment of the segments with cycloheximide or actinomycin strongly inhibits both phases of auxin-promoted elongation without altering the length of the latent period in response to the hormone.

Recently a number of reports have appeared describing in detail the timing of the growth response of coleoptile segments to indole-3-acetic acid and other auxins (6, 7, 19). It has been shown that, in oat and corn coleoptile segments, there is a latent period of about 10 min following the application of IAA before the increase in rate of elongation begins. The increase in elongation rate, once it begins, occurs rapidly so that a new rapid steady rate of elongation is established within an additional 3 to 5 min.

Such detailed analyses of the timing of the growth response to auxin in dicotyledonous tissues are noticeably lacking. The most frequently employed method of examining elongation responses in stem tissues is to float excised segments on specific growth media and to measure increase in length at intervals of about 1 hr (5, 13, 14, 16, 17). When data obtained in this manner are plotted, they seem to extraplate through zero on the time axis (5, 13, 14, 17). This has led to the claim that there is no latent period at all in the growth response of these tissues to auxin (14). However, information gained by extrapolation to zero time of data obtained in long term experiments may be very unreliable. Since pea stem tissue is used very frequently in plant growth experimentation, it was decided to investigate in more detail the timing of the auxin response in etiolated pea stem segments and to examine the influence of various factors on that timing.

MATERIALS AND METHODS

Plant Material. Alaska peas (Pisum sativum L., var. Alaska), purchased from Asgrow Seed Company, New Haven, Conn., were used in most experiments and grown in trays of vermiculite according to Galston and Baker (8). The trays were placed in a dark room in a light-tight cabinet at 23 to 25°C. Occasional weak red light was employed for watering and other manipulation. With a double-bladed cutter, sections 5 mm in length were cut beginning 1 to 2 mm below the hook in the third internode of 7- to 8-day old plants. The segments were floated on 10⁻³ M phosphate buffer (pH 6.2) for at least 30 min prior to use.

In a few experiments, cucumber hypocotyl segments or oat coleoptile segments were used. Cucumber seeds (Cucumis sativus L., var. Straight Eight) were planted directly on wet filter paper in covered plastic dishes and placed in complete darkness at 23 to 25°C. When the seedlings were 4 to 5 days old, segments were cut as described above for peas. Oats (Avena sativa L., var. Victory) were grown, and 8-mm segments were harvested as described in an earlier paper (7).

Chemicals. Actinomycin D was purchased from Calbiochem, Los Angeles, Calif., and cycloheximide was purchased from Sigma Chemical Co., St. Louis, Mo. Indole-3-acetic acid was obtained from Fisher Scientific Co., Chicago, Ill.

Segment Holder. The high resolution growth-recording device used in these experiments is described in detail in an earlier paper (7). For experiments with pea stem or cucumber hypocotyl segments, modifications of the apparatus were necessary since these tissues are not hollow and do not lend themselves to "stringing" without tissue damage. To hold the stem segments in place, a device was constructed consisting of two parts, a tube-shaped holder (Fig. 1B) and an outer restraining sleeve (Fig. 1A). The pea segment holder is a thin-walled plastic tube 13.5 cm long and 2.5 mm in inside diameter. Toward the top of the tube there is an optical slit (S₁) 2 mm wide and 22 mm long. In addition, there are two opposite helical slits (S₂) beginning 4 mm below the optical slit and running the length of the tube terminating at the sealed end (E). The outer sleeve is a thin-walled plastic tube 10.2 cm long with an inside diameter of 3 mm (Fig. 1A). It is provided with many large openings (O) to allow easy penetration of growth media.

Stem sections are loaded into the holder one at a time and are pushed gently down the tube until a total of 20 segments have been inserted. A small metal cylinder (M) weighing 150 mg is then inserted into the holder so that it rests upon the uppermost segment of the column and serves to keep the segments in contact. The sleeve described above is then slipped over the lower (segment-containing) portion of the holder, and the entire assembly is suspended within a glass growth measurement chamber (see Reference 7) which is then filled with the desired growth medium. The purpose of the outer sleeve in the assembled holder is to prevent the holder from spreading enough to allow the segments to fall out. At the same time the holder is flexible enough to allow the segments within to slide freely so there is no mechanical inhibition of elongation.

In order to follow elongation of the entire column of segments in the assembled device, it is necessary only to measure the vertical displacement of the metal weight as the growing seg-

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and refilling the chamber through the appropriate outlet and connecting funnels. These manipulations take about 30 sec. All growth media used in these experiments contained $10^{-3}$ M phosphate buffer, pH 6.2.

RESULTS

Timing of the Auxin Response. Figure 2, curve C, illustrates the response of pea stem segments to $10^{-5}$ M IAA. The immediate effect of IAA is to inhibit growth. This reduced rate of elongation continues for about 10 min before the growth rate rises suddenly to a steady value about 4-fold greater than that prevailing before auxin treatment. In this respect, the effect of IAA on elongation during the first 30 min after its addition is identical in pea stem segments and in corn or oat coleoptile segments. That is, in many cases IAA also inhibits growth during the 10-min period following its addition to coleoptile segments. This is shown but not described in earlier papers by one of us (see Fig. 1, curve B, in Reference 6 and Fig. 7, curve E, in Reference 7). There is, however, an important difference in the timing of the growth response to auxin in pea stem vs. coleoptile segments. In pea stem segments the initial rapid rate of elongation following the 10-min latent period persists only about 25 min. There is then a rather sudden decline in the rate of elongation to a value about 2-fold greater than the rate of elongation before the addition of auxin. This

![Diagram of growth response](https://plantphysiol.org)

**Fig. 1.** A: Restraining sleeve for tissue segment holder. Made from plastic tubing 10.2 cm long and 3 mm internal diameter. O: Openings to allow penetration of growth media. B: Segment holder. Made from plastic tubing 13.5 cm long and 2.5 mm internal diameter. S: Optical slit for light passage; M: weight; P: row of pea stem segments; S: helical slits in holder to allow penetration of growth media; E: sealed end of holding tube. Assembly of components described in text.

**Fig. 2.** Timing of the growth response of pea stem segments and cucumber hypocotyl segments to IAA. Growth medium changed from phosphate buffer to buffered IAA at the arrow. IAA concentration $10^{-3}$ M in experiments of curves A and C, $10^{-4}$ M in B, and $10^{-5}$ M in D. B, C, and D done with pea stem segments; A, cucumber hypocotyl segments. The vertical bar at the end of each curve in this and subsequent figures represents 1 mm of elongation, for that particular curve, for the entire column of segments.
lowered rate of auxin-promoted elongation then persists for as long as we have followed it (up to 90 min). Curve A in Figure 2 shows that the response of segments of etiolated cucumber hypocotyl tissue to auxin exhibits the same timing, i.e., initial inhibition followed by a biphasic positive response.

Curves B and D in Figure 2 show that the timing of the response to auxin is essentially the same at various auxin concentrations. The initial inhibition still appears, followed after 10 min by the biphasic response curve as described above. This is in spite of the fact that auxin uptake during the first 30 min has been shown to be directly related to external auxin concentration (4, 9, 15). This suggests that the time required for auxin uptake from concentrations ranging from $10^{-4}$ to $10^{-4}$ M represents a very minor fraction of the total latent period. The biphasic nature of the growth-response curve, however, is somewhat more pronounced at higher concentrations of auxin since the initial growth promotion is greater. Also, the initial positive phase of the elongation response to auxin seems to be somewhat shorter at higher auxin concentrations (13 min in the response to $10^{-4}$ M IAA shown in curve B vs. 23 min in the response to $10^{-3}$ M IAA shown in curve C of Fig. 2).

Cycloheximide. Cycloheximide is known to be a potent inhibitor of translation in both plants (10) and animals (11). Its ability to inhibit elongation in plants is well documented (7). Figure 3 shows the effect of cycloheximide pretreatment on the timing of the auxin response in pea stem segments. Segments were pretreated in the chamber with 2 $\mu$g/ml cycloheximide for 35 min, at which time the growth medium was changed to the same concentration of cycloheximide plus $10^{-3}$ M IAA. Cycloheximide pretreatment appears to have no significant effect on the length of the latent period in response to auxin even though there is a marked reduction in elongation rate during both phases of auxin-promoted growth. In some cases, pretreatment with cycloheximide or actinomycin D (see below) seemed to eliminate the initial inhibitory effect of auxin on elongation (Fig. 3, curve B, D).

**Fig. 3.** Effect of cycloheximide pretreatment on response of etiolated pea stem segments to IAA. A: Growth medium changed from buffer to $10^{-3}$ M IAA at the arrow; B: segments treated in the chamber with 2 $\mu$g/ml cycloheximide for 35 min just prior to changing growth medium to 2 $\mu$g/ml cycloheximide plus $10^{-3}$ M IAA at arrow.

**Fig. 4.** Effect of varying length of pretreatment period with actinomycin D on response of pea stem segments to IAA. A: Growth medium changed from buffer to $10^{-4}$ M IAA at arrow; B, C, and D: growth medium changed from 10 $\mu$g/ml actinomycin D to 10 $\mu$g/ml actinomycin D plus $10^{-3}$ M IAA at arrow. Pretreatment time with actinomycin D was 1 hr in B, 3 hr in C, and 6 hr in D.
and Fig. 4, curve C). This may not be a specific effect of the inhibitors, however, since the initial inhibitory phase of auxin action was sometimes absent in control experiments as well.

**Actinomycin D.** Actinomycin D has been shown to inhibit protein synthesis by interfering with DNA-dependent RNA synthesis (20). Low concentrations of actinomycin D strongly inhibit growth in a variety of plant tissues (7, 12, 14). Figure 4 shows the effect of varying lengths of pretreatment with actinomycin D (10 µg/ml) on the growth response of pea stem segments to auxin. A 1-hr pretreatment with actinomycin D has practically no effect on the growth response of etiolated pea stem segments to auxin during the first 60 min, even though a 1-hr pretreatment with the same solution of actinomycin D almost entirely eliminates the growth response of oat coleoptile segments to auxin (see Fig. 5).

That the difference in sensitivity of etiolated coleoptile and pea stem tissue to actinomycin D might be due to a difference in permeability to the antibiotic is suggested by the results shown in curves C and D of Figure 4. After a 3-hr pretreatment with 10 µg/ml actinomycin D, the inhibition of the elongation of pea stem segments is evident from the beginning of growth promotion by auxin (curve C). A 6-hr pretreatment with actinomycin D results in even more severe inhibition of the initial auxin response (curve D). Notice, however, that neither of the actinomycin-inhibited responses to auxin is accompanied by an extended latent period.

**DISCUSSION**

These data show that IAA does not immediately promote elongation in pea stem segments as has been claimed. On the contrary, in most cases the immediate effect of IAA is to inhibit elongation. This phenomenon has also been observed in a variety of other tissues and is presently under further investigation. The data also offer an explanation for the apparent lack of a latent period in the response of pea stem segments to IAA during long term experiments. Clearly, elongation measurements made every 30 min or every hour after the addition of IAA would fall along a straight line representing the steady rate of elongation during the second phase of auxin-promoted elongation. The initial biphasic nature of the elongation response would go undetected. Hence, extrapolation of a line drawn through points obtained in long term experiments would be expected to pass almost exactly through zero on the time axis (Fig. 2, curve C), and the 10-min latent period would not be seen. This example only serves to emphasize the warnings of a number of authors against the practice of extrapolation to zero time of curves obtained from long term measurements (1, 2).

The biphasic growth response to auxin also occurs in segments of etiolated cucumber hypocotyl tissues (Fig. 2, curve A) and, at low auxin concentration, also in coleoptiles of corn and oats (Evans, unpublished). We do not at present have an unequivocal explanation for the biphasic nature of the growth response of these tissues. Perhaps it is related to the initial inhibition of elongation by auxin. It is known that in the presence of a number of inhibitors of elongation (cyandine, mannitol, etc.) some sort of growth potential is accumulated so that, after the removal of the inhibitor, a burst of growth occurs. This phenomenon has been demonstrated in coleoptile segments and is referred to as stored growth (18). Perhaps the initial burst of growth following the latent period in the response of pea stem tissue to auxin is a result of growth potential accumulated during the period of inhibition that follows the addition of auxin. That higher concentrations of auxin do not elicit a biphasic growth response in coleoptile segments may be because optimal concentrations of auxin saturate the elongation process in this tissue. If this were the case, the addition or subtraction of a small amount of growth potential would affect the steady rate of elongation only at lower concentrations of auxin where the growth process is submaximal. This interpretation is supported by the observation of Ray that the stored growth phenomenon in coleoptile segments does not occur in the presence of optimal levels of IAA (18). The possibility that the reduction in elongation rate after 25 to 35 min in auxin might be due to a stimulation of ethylene production has also been considered. However, this seems unlikely since the elongation response of pea stem segments to 10^{-5} M IAA is strongly biphasic even though this concentration of auxin causes no significant increase in ethylene production in pea stems (3). Ethylene production cannot account for the biphasic response seen in coleoptile segments at low auxin concentration either, since coleoptile straight growth has been shown to be rather insensitive to ethylene (3) and since higher concentrations of auxin that would be expected to stimulate the production of even more ethylene (3) do not cause a biphasic growth response.

**Effect of Antibiotics.** Elongation of etiolated pea stem segments in response to IAA was strongly reduced by brief pretreatment with cycloheximide. This high degree of sensitivity to cycloheximide has also been demonstrated in coleoptiles in earlier work (7). However, cycloheximide pretreatment of coleoptile segments has been reported to shorten somewhat the latent period in response to auxin (7). This does not seem to be the case with pea stem tissue.

Both phases of the positive growth response of pea stem segments to auxin are very sensitive to cycloheximide pretreatment. Each is strongly reduced after 35 min in 2 µg/ml of the antibiotic.

The data also show that actinomycin D pretreatment can strongly inhibit the initial growth response of pea stem segments to auxin. However, this requires rather long pretreatment (3 hr or more), suggesting that the uptake of actinomycin D by etiolated pea stem segments may be rather sluggish in comparison with the uptake of cycloheximide. These strong inhibitions of the initial auxin response in etiolated pea stem segments by long actinomycin D pretreatment are in contrast to data obtained by Penny and Galston in similar experiments with green stem tissue of the same species of pea (14). These workers found that, regardless of the length of the pretreatment, actinomycin D did not begin to inhibit the elongation response to auxin until 2 hr after the addition of the hormone.

It is also clear from these growth curves that, as with cycloheximide, the inhibition of the auxin response by actinomycin D pretreatment is not accompanied by an extension of the latent period in response to the hormone. Similar data for coleoptile
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