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Rapid Growth Inhibition of *Avena* Coleoptile Segments by Abscisic Acid[1]

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ABSTRACT

An angular position sensing transducer was used to make continuous measurements of elongation of a column of *Avena sativa* coleoptile segments. Elongation stimulated by 2 μM indoleacetic acid was inhibited by 0.1 mM abscisic acid with a latent period of about 4 or 5 minutes at pH 6.0, 30 C. Full growth inhibition was not established until about 1 hour after the addition of the abscisic acid. The same degree of final growth inhibition could be obtained under the above conditions using 10 μM and 1 μM abscisic acid, but the latent period was longer. Pretreatments with abscisic acid affected the growth rate but did not extend the latent period of a subsequent response to auxin. The short term kinetics of inhibition by abscisic acid were not similar to those of any of the inhibitors of RNA and protein synthesis tested in this system.

In evaluating hypotheses for the mode of action of abscisic acid in any given response, it is important to define precisely the kinetics of that response and to determine the latent period between the hormone application and the beginning of the response to the hormone. There have been several recent reports of ABA responses with latent periods as short as 5 or 10 min. Cummins *et al.* (3) report that ABA may initiate stomatal closure in barley leaves within 5 min. Warner and Leopold (8) used a position-sensing transducer to find a 5.1 min latent period for inhibition of elongation of intact Alaska pea shoots by 10 μM ABA. Zenk (11) reports a latent period of about 5 min for 0.1 mM ABA inhibition of 0.1 μM IAA-promoted elongation in *Avena* coleoptiles as determined by optical measurements. In the present study we have used an angular position-sensing transducer to determine the latent period in the reversal of auxin-promoted elongation of *Avena* coleoptiles by ABA. The continuous measurements of the kinetics of ABA inhibition of elongation will serve as a time course basis for future biochemical studies in the *Avena* coleoptile system. We have also compared the kinetics of inhibition by ABA with those of several known growth inhibitors.

MATERIALS AND METHODS

In order to make precise, continuous measurements of elongation, the methods of Evans and Ray (4) were used with modifications suggested by dela Fuente and Leopold (5). *Avena sativa* L., var. Victory seedlings were grown for 3 days on moist paper towels in the dark and were given a 15-min red light treatment on the 3rd day to suppress mesocotyl growth. All subsequent manipulations were made in dim green light. On the 4th day a double-edged cutting device was used to cut a 1-cm segment from coleoptiles about 3 cm long beginning about 3 mm below the tip. The primary leaves were removed, and 10 of these segments were strung on a wire inserted in the measuring chamber (Fig. 1). A capillary tube was used to keep the coleoptile segments submerged, and a groove in the upper end of the capillary tube held the transducer needle. The angular position-sensing transducer had a maximum displacement of 50° which represented full scale on the recorder. A linear relationship existed between the angle of rotation of the transducer needle and the position of the recorder pen. The growth rate of the coleoptiles could then be computed as a function of the tangent of the angle of needle displacement, since there was a fixed distance from the column of coleoptiles to the center of the transducer turning shaft. The coleoptiles were continuously aerated, and the experiments were carried out at a temperature of 30 C. Bathing solutions in the chamber were rapidly changed by draining the chamber and refilling it from the fluid reservoir. All solutions contained 5 mM succinate buffer, pH 6.0.

Coordinates of data points taken at 1-min intervals from the original recorder graphs were analyzed by an IBM 370 computer and graphed in a normalized form by an IBM 1130 line plotter. The graphs which appear in this paper are tracings of the computer output.

RESULTS

In buffer alone, the coleoptile segments were found to elongate at a rate of about 0.05 mm/hr per 1-cm segment. ABA was not tested directly on this low endogenous growth rate; rather, in order to establish a growth rate of sufficient magnitude so that the subsequent inhibition could be easily demonstrated, auxin was added to the bathing solution. IAA at a concentration of 2 μM was selected for these experiments, since it consistently produced a substantial growth response (Fig. 2). Following a latent period of about 12 min after the addition of the auxin, a new elongation rate of about 0.7 mm/hr per segment was established.

In the inhibitor studies, the following procedure was used. The coleoptile segments were first placed in the measuring chamber in buffer for 1 hr in order to allow depletion of endogenous IAA. The segments elongated at a minimal rate until the 2 μM IAA was added, and the new higher elongation rate was established. One hr later, IAA at the same concentration plus the inhibitor to be tested were added simultane-

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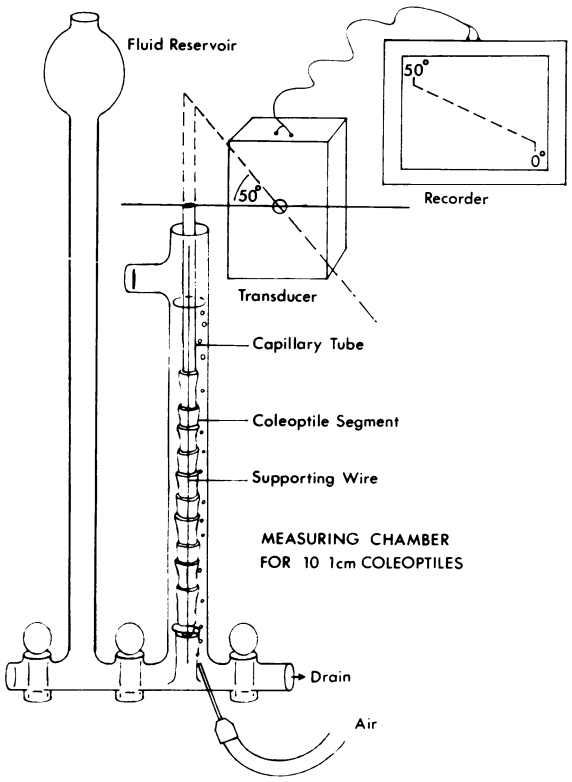

FIG. 1. Apparatus for continuous measurement of elongation of a column of 10 1-cm coleoptile segments.

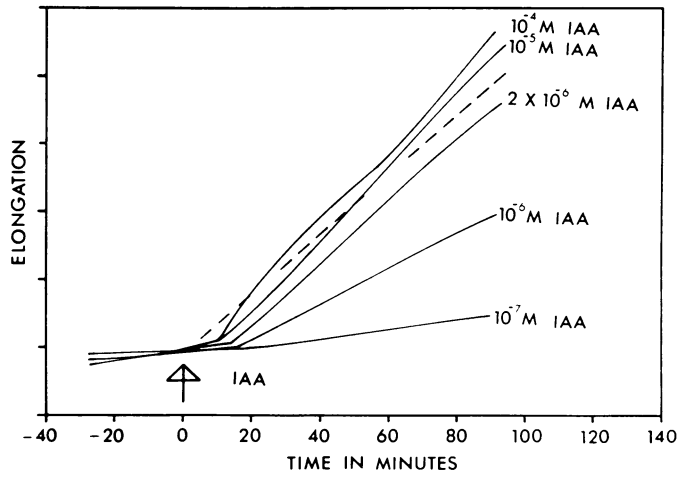

FIG. 2. Time course of growth promotion by various concentrations of IAA. The slope of the dashed line represents a growth rate of 0.7 mm/hr per segment. Coleoptiles were placed in the chamber with succinate buffer at −60 min.

ously, and the elongation of the coleoptile segments was measured for the desired period of time.

If inhibitor studies are to be performed in this manner, it is necessary to ascertain both how soon the auxin-induced rate becomes constant and how long this maximum rate continues. A steady maximum growth rate was well established 20 to 25 min after the first addition of auxin; draining and replacing the IAA does not alter the growth rate which subsequently remained constant for longer than 1 hr. The computer was programmed to use a least squares analysis to find the best fitting straight line through the 35 data points directly preceding time zero in any given trial. The slope of this best fit line is utilized in finding the average elongation rate of the coleoptiles when treated with IAA only. After the addition of an inhibitor, the growth rate is checked at 1-min intervals to find the point of significant deviation from the initial IAA-induced elongation rate. The term "latent period," as it is used, here refers to the time in minutes from the addition of the growth substance to the first indication of growth acceleration or inhibition as detected by a deviation in the elongation rate.

Since growth inhibition was most rapid with 0.1 mM ABA (Fig. 3), this concentration was most thoroughly investigated. In 10 trials of this experiments, the longest latent time recorded was 12 min, with the mode of the series being 4 min. The shortest latent period recorded was 1 min; both temperature and pH were monitored during this trial, and neither showed fluctuations near the time of ABA addition which could account for the recorded decrease in elongation rate. Although inhibition begins rapidly, a constant lowered elongation rate was not established until about 1 hr after the addition of the ABA.

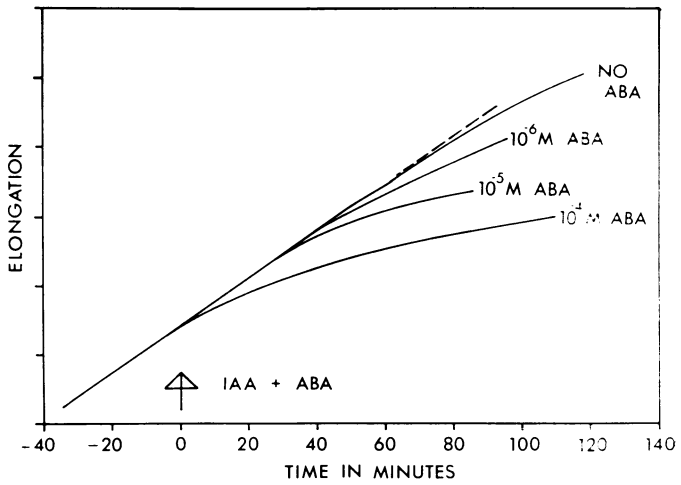

FIG. 3. Time course of inhibition of auxin-promoted elongation by various concentrations of ABA. Coleoptiles were first treated with 2 × 10^{-6} M IAA at −60 min. At time zero, this same concentration of IAA plus the concentration of ABA indicated were added simultaneously.

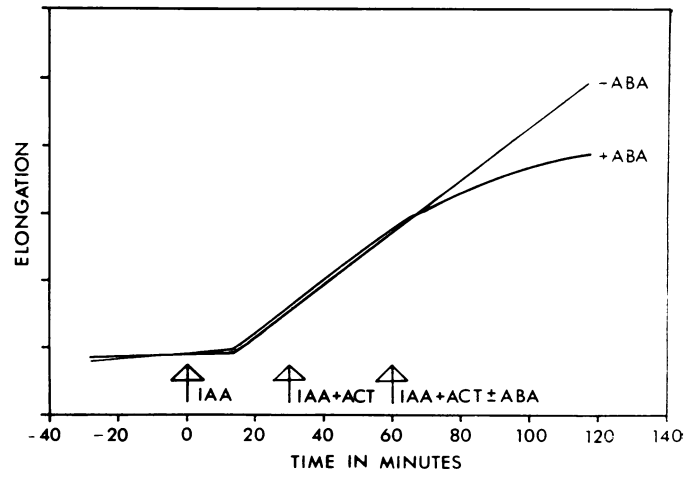

FIG. 4. Thirty-min pretreatment with actinomycin D before addition of ABA. The actinomycin D at 20 mg/ml does not decrease the IAA-induced growth rate (upper curve) nor does it alter the kinetics of the inhibition by ABA (lower curve).

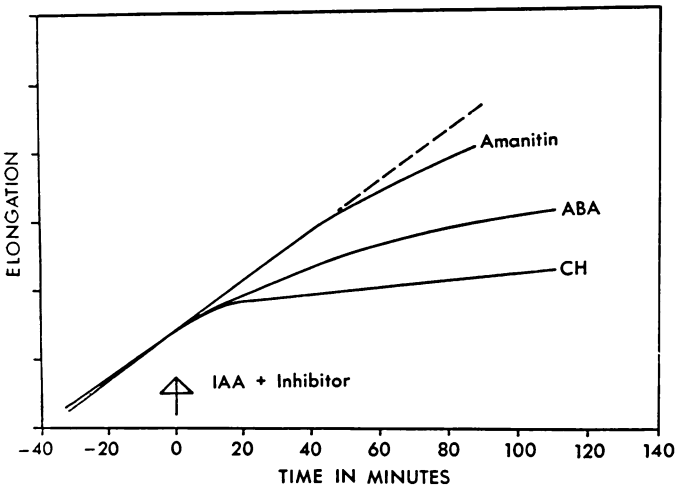

FIG. 5. Time course of inhibition of IAA-induced elongation by cycloheximide and α-amanitin. Coleoptiles were first treated with 2×10^{-6} M IAA at −60 min. At time zero, this same concentration of IAA plus 10 μg/ml α-amanitin or 10 μg/ml cycloheximide were added simultaneously. Time course for 10^{-4} M ABA is shown for comparison.

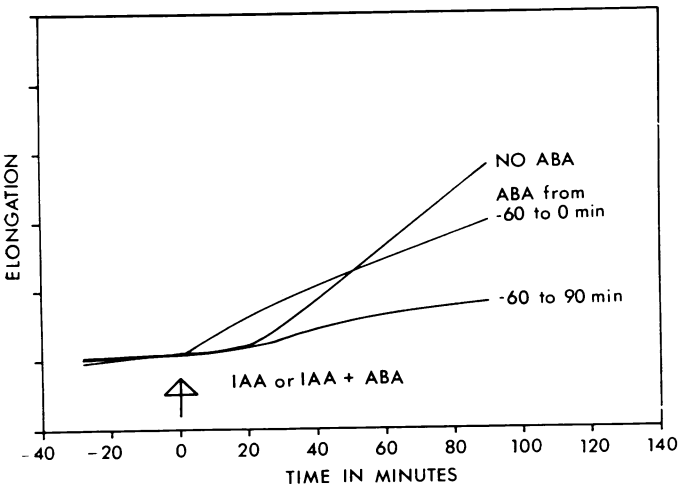

FIG. 6. Effects of 1-hr pretreatment with ABA. In these trials the ABA concentration is 10^{-4} M and the IAA concentration is 2×10^{-6} M. When ABA is removed at time zero and IAA added, an immediate stimulation of elongation as shown above was observed in about half the trials made; in the other trials the normal 10- to 12-min latent period for auxin action was observed, although the growth rate following ABA treatment was lower.

Several inhibitors with known modes of action were tested in the same manner as ABA. The RNA transcription inhibitors 5-fluorouracil and actinomycin D in concentrations up to 100 μg/ml did not significantly lower the elongation rate during the 1st hr after their addition.

Since midcourse addition of actinomycin D does not inhibit IAA-promoted elongation, it is possible to test the effect of actinomycin D pretreatment on the ABA response. The coleoptile segments were first placed in buffer for 60 min, then 2 μM IAA for 30 min, next 2 μM IAA plus 20 μg/ml actinomycin D for 30 min, and finally 2 μM IAA plus 20 μg/ml actinomycin D plus 0.1 mM ABA for 60 min longer (Fig. 4). The inhibition response was typical for ABA, indicating that the 30-min pretreatment with actinomycin D had no effect on the ABA response.

Preliminary studies with α-amanitin, an inhibitor of eucaryotic RNA polymerase, indicate that 10 μg/ml may cause a moderately rapid inhibition of elongation (Fig. 5), but higher concentrations have not yet been examined in this system. The protein synthesis inhibitor cycloheximide caused rapid inhibition of elongation with maximum inhibiton reached about 15 min after the addition of cycloheximide (Fig. 5).

The effect of ABA pretreatment on the kinetics of the IAA response was also studied. Thirty-minute pretreatments with ABA had no appreciable effect on the latent period before auxin-induced stimulation, although increasing concentrations of ABA caused proportionate decreases in the magnitude of the auxin response. The same general results were found for 60-min pretreatments with ABA with one interesting exception. When coleoptiles were treated for 1 hr with 0.1 mM ABA and then transferred to 2 μM IAA alone (Fig. 6), an immediate promotion of elongation was found in three out of six trials. Hence, in these cases the latent period for the IAA response was eliminated. This rapid growth promotion never occurred if ABA was added along with the IAA, nor was an immediate response found if buffer alone or a fresh solution of ABA was added after the 1-hr pretreatment with ABA.

DISCUSSION

Continuous elongation curves obtained by means of a position-sensing transducer demonstrate that ABA inhibits IAA-promoted elongation in *Avena* coleoptiles following a latent period of about 4 or 5 min. This essentially confirms reports of other workers (8, 11) concerning rapid inhibition of tissue elongation by ABA. However, full growth inhibition was not established until about 1 hr after the addition of 0.1 mM ABA.

Chrispeels and Varner (1, 2) have found that midcourse additions of ABA and the transcriptional inhibitors 6-methyl purine and 8-azaguanine (but not actinomycin D) inhibit gibberellin-promoted α-amylase production in barley seed aleurone layers. The latent period for the ABA inhibition is 2.5 to 3 hr, whereas that for 6-methyl purine and 8-azaguanine is 2.5 to 4 hr depending on the concentration used. Because the kinetics of inhibition of these three responses were found to be quite similar, they tentatively suggested the possibility of ABA acting as an inhibitor of a specific RNA fraction.

The failure of the transcriptional inhibitor actinomycin D to inhibit IAA-promoted elongation of *Avena* coleoptiles when added midcourse agrees with numerous reports that actinomycin D is slow to inhibit hormone-mediated responses once they have been initiated. In order to cause significant inhibition in hormone-mediated responses, actinomycin D must usually be applied either before or at the beginning of the hormone treatment, even though it has been demonstrated that the uptake of actinomycin D by oat coleoptile tissue is rapid (4). This inability of midcourse additions of actinomycin D to inhibit many hormone-induced responses has caused investigators to question the role of RNA synthesis in hormone activity. Some workers have found that actinomycin D inhibits precursor incorporation into RNA without inhibiting the hormone-induced response. They have suggested the possibility that actinomycin D in these cases does not inhibit the specific fraction of RNA which is responsible for the given response.

The experiments involving actinomycin D pretreatment of the ABA response (Fig. 4) were carried out to test whether the translation inhibition hypothesis of Ihle and Dure (6) for ABA control of cotton seed germination might have applicability in the present system. In a series of elegant experiments, these workers found that if actinomycin D were added with ABA during precocious germination, the ABA-induced inhibition of carboxypeptidase and isocitritase was negated. They suggest

that ABA either inhibits RNA synthesis which is required for the production of a translational inhibitor or acts as a corepressor with a product of RNA synthesis to effect translational inhibition. In the present experiments actinomycin D treatments did not alter the kinetics of ABA inhibition, thus suggesting the nonapplicability of the translation inhibition hypothesis under the conditions of the present system.

Midcourse additions of 5-fluorouracil, an inhibitor of ribosomal RNA synthesis, also had no effect on coleoptile elongation. This substantiates electron microscope observations that little or no ribosome synthesis occurs in *Avena* coleoptiles during the period of cell elongation. The preliminary evidence that α-amanitin inhibits elongation within 1 hr warrants further investigation since the inhibition of RNA polymerase could be a tool necessary to assess adequately the role of RNA synthesis in this system. α-Amanitin has been studied in purified animal enzyme systems (7), but little is known about other possible metabolic effects of the toxin.

Although the protein synthesis inhibitor cycloheximide causes inhibition of elongation with latent periods which fall within the range found for inhibition by ABA, the kinetics of inhibition for the two substances are different. Inhibition is virtually complete 15 min after the addition of cycloheximide in contrast to the hour or more required in the case of ABA. The similarity of the latent periods for inhibition of IAA-promoted elongation by ABA and cycloheximide may be coincidental, since ABA also inhibits low pH-induced elongation which is insensitive to cycloheximide (Rehm, unpublished data). ABA could not be shown to have kinetics of inhibition similar to the transcriptional and translational inhibitors tested. Now that a time course has been worked out for ABA action in *Avena* coleoptile elongation, rigorous biochemical studies may be performed in this system where both cell division and ribosomal RNA synthesis are negligible.

ABA pretreatment studies were made to further investigate the nature of the ABA interaction with IAA. ABA pretreatment for 30 or 60 min prior to the addition of IAA does not increase the latent period for the promotion of growth, although the magnitude of the IAA response is decreased. However, in a limited number of trials, simultaneous removal of ABA and the addition of IAA caused immediate stimulation of growth; neither temperature nor low pH caused this increase in growth rate, and at present we have no adequate explanation for this anomalous result. Van Overbeek *et al.* (9, 10) have found that in long term studies with *Lemna* cultures incubated for 7 days with ABA and then transferred to medium without ABA, growth resumed with no apparent lag at a rate greater than nontreated cultures. In the present study, however, the immediate response was IAA-dependent, with no growth response if the coleoptiles were transferred to buffer only.

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