

# Rapid Inhibition of Auxin-induced Elongation of *Avena* Coleoptile Segments by Cordycepin<sup>1</sup>

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## ABSTRACT

The effects of cordycepin (3'-deoxyadenosine), an RNA synthesis inhibitor, on auxin-induced elongation in *Avena* coleoptile segments were studied with a position-sensing transducer. Cordycepin rapidly inhibited auxin-stimulated growth in the coleoptile segments whether added before, at the same time as, or after, the 2  $\mu$ M auxin treatment. Midcourse additions of 100, 50, and 25  $\mu$ g/ml cordycepin inhibited auxin-promoted elongation in an average of 18, 22, and 35 minutes, respectively. Additions of cordycepin before or at the same time as the auxin treatment partially inhibited the magnitude of the subsequent auxin-promoted growth but did not appreciably alter the latent period of the auxin response. It was concluded that if cordycepin is inhibiting the synthesis of RNA required for growth, the decay time for this RNA may be considerably shorter than that suggested in the literature from actinomycin D experiments. Preliminary kinetic evidence indicated that cordycepin does not inhibit auxin-induced elongation by acting as a respiratory inhibitor. Studies in mung bean shoot mitochondria demonstrated that cordycepin has no effect on respiration, respiratory control, or ADP/oxygen ratios.

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Cunningham *et al.* (3) reported the isolation of cordycepin from a culture broth of the fungus, *Cordyceps militaris* (Linn) Link. They found cordycepin to inhibit the growth of many strains of *Bacillus subtilis*, but they further observed that its toxicity was of low order. Subsequently, cordycepin was also isolated from cultures of *Aspergillus nidulans* (Eidam) Wint (10). Besides inhibiting growth in some bacteria, cordycepin has also been found to inhibit growth in various mammalian systems such as Ehrlich ascites tumor cells (17), human tumor cell (H. Ep. No. 1) cultures (16), and chick fibroblast cultures (5).

Cordycepin has been identified to be 3'-deoxyadenosine, although the compound is probably phosphorylated before it acts as an inhibitor. Most studies point towards nucleic acid synthesis as the most logical site of inhibition (6). If cordycepin is incorporated into a growing RNA chain, the absence of oxygen

at the 3' position inhibits subsequent phosphodiester bond linkage. Hence, cordycepin terminates RNA chain elongation.

In mammalian cells, cordycepin has a much smaller inhibitory effect on the synthesis of heterogenous nuclear RNA than has actinomycin D (13), and it has been shown that the post-transcriptional addition of poly A (150-200 residues of adenylic acid) to heterogeneous nuclear RNA is much more sensitive to cordycepin than to actinomycin D (9). Experimentally, it has been found that cordycepin inhibits the arrival of mRNA to the polyribosomes in the cytoplasm. Cordycepin has also been reported (18) to inhibit the synthesis of ribosomal and transfer RNA in HeLa cells.

Little use has been made of cordycepin with plant systems (2, 7, 12, 19). Nooden (12) reported that 5 mM cordycepin caused a 50% decrease in growth of corn coleoptiles within 1 hr. Stockert *et al.* (19) found that 0.1 mM cordycepin caused nearly 80% of nucleoli to exhibit segregation within 2 hr in meristematic cells (22nd hr, 0.1% caffeine-induced binucleate cells) of *Allium cepa* root tips. They concluded that this nucleolar aberration was probably related to the effect of cordycepin on RNA synthesis. In unpublished studies (M. Cline) there is evidence that cordycepin inhibition of RNA synthesis in plant systems is general for all species of RNA.

The present experiments were carried out with a high resolution continuous growth measuring apparatus to determine the effects of cordycepin, an RNA synthesis inhibitor, on auxin-promoted elongation in *Avena* coleoptile segments.

## MATERIALS AND METHODS

An angular position-sensing transducer was used to measure continuous elongation in 4- to 5-day-old coleoptile segments of *Avena sativa* L. cv. Victory as described previously (15). Ten 1-cm segments, with primary leaves removed, were strung vertically on a wire and immersed in a measuring chamber containing 5 mM succinate buffer, pH 6.0. After a 1-hr equilibration period in the buffer solution, the coleoptile segments were treated with 2  $\mu$ M auxin and/or cordycepin in a buffered solution, as indicated under "Results." Cordycepin was purchased from Sigma Chemical Co.

Mitochondria were isolated from 4-day-old etiolated mung bean shoots (*Phaseolus aureus* L.) by standard isolation procedures. Approximately 100 g of shoots were placed in 200 ml of grinding media containing: 0.4 M mannitol, 50 mM tris-Tricine (pH 7.4), 1 mg/ml BSA, 5 mM EDTA, and 4 mM cysteine. The shoots were disrupted with Moulinex mixer for 15 sec, and the solution was passed through a nylon mesh cloth. The suspension was centrifuged at 600g for 10 min, and

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the supernatant was decanted and recentrifuged at 14,500g for 10 min. The pellet was resuspended in approximately 5 ml of the grinding medium and layered on a discontinuous gradient composed of 10 ml of 1.1 M sucrose and 5 ml of 1.5 M sucrose. The gradient was centrifuged in a swinging bucket head for 20 min at 20,000g, and the mitochondria were collected between the 1.1 M and 1.5 M fractions. The mitochondrial suspension was diluted slowly with 0.3 M sucrose to a final concentration of approximately 0.8 M sucrose, and the suspension was centrifuged at 14,500g for 5 min. The mitochondrial pellet was resuspended in a small volume of the grinding medium at a concentration of approximately 10 mg/ml. Oxygen was determined on a Beckman Model 160 gas analyzer.

## RESULTS

**Pretreatments with Cordycepin.** Figure 1 shows the results of a 30-min pretreatment with either 50 or 100  $\mu\text{g/ml}$  of cordycepin. At the end of the 30-min pretreatment period, the cordycepin solution was removed and a 2  $\mu\text{M}$  auxin solution was added. Accelerated elongation of the coleoptile segments occurred in response to the auxin treatment, although at a considerably reduced magnitude compared with the control which did not have a cordycepin pretreatment. The pretreatment with 50  $\mu\text{g/ml}$  of cordycepin had a weaker inhibiting effect on the auxin-stimulated growth than did the pretreatment with 100  $\mu\text{g/ml}$  of cordycepin. It is important to note that the length of the latent period which is the time between the beginning of the auxin treatment and the beginning of the accelerated elongation response (typically about 15 min in our system) was not appreciably altered by cordycepin pretreatments.

If cordycepin was re-added with the auxin at the end of the 30-min cordycepin pretreatment (Fig. 2), the auxin-induced growth of coleoptile segments was reduced compared with that of coleoptiles to which cordycepin had not been re-added.

**Simultaneous Treatments with Cordycepin and Auxin.** When cordycepin (50 or 100  $\mu\text{g/ml}$ ) was added at the same time as 2 mM auxin without a cordycepin pretreatment, the subsequent auxin-accelerated growth still occurred, although at a much reduced rate (Fig. 3). The auxin-stimulated growth rate within the 1st hr was substantially greater than when a cordycepin pretreatment (Fig. 2) also was given. When cordy-

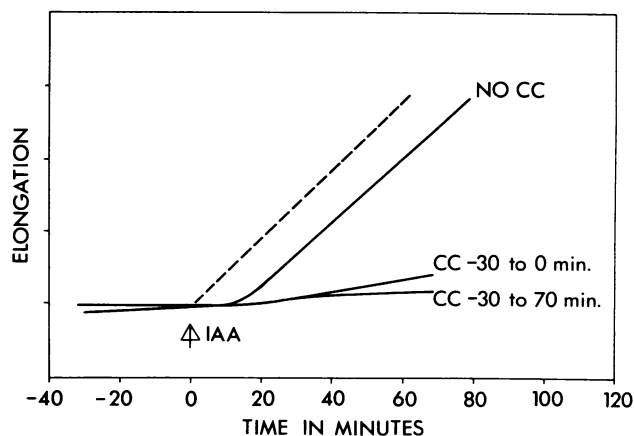


FIG. 2. Time course of 2  $\mu\text{M}$  auxin-induced elongation with 30-min pretreatment of 100  $\mu\text{g/ml}$  of cordycepin (CC). At time zero, either cordycepin was removed and auxin added, or cordycepin was removed and both cordycepin and auxin were added. No CC: auxin-stimulated growth with no cordycepin treatment. The slope of the dashed line represents a growth rate of 0.7 mm/hr·segment.

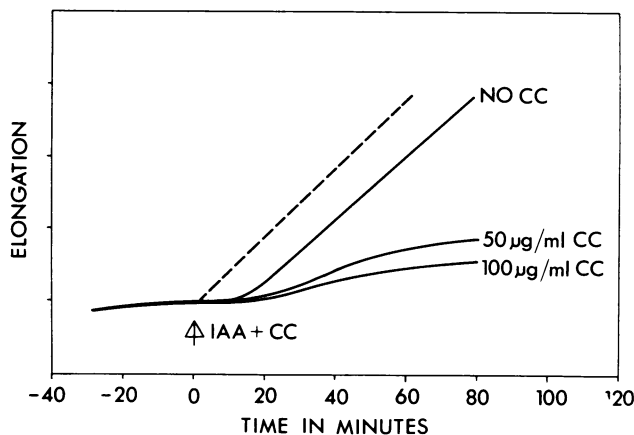


FIG. 3. Time course of 2  $\mu\text{M}$  auxin-induced elongation with simultaneous additions of cordycepin (CC). The slope of the dashed line represents a growth rate of 0.7 mm/hr·segment.

cepin was present with auxin, the growth curve leveled off to some extent after 1 hr. The reduced growth rates which occurred at both concentrations (50 or 100  $\mu\text{g/ml}$ ) of cordycepin did not differ greatly. As with cordycepin pretreatments, the typical 15-min latent period for the coleoptile segment response to auxin was not appreciably altered by the presence of cordycepin.

**Midcourse Treatments with Cordycepin.** After auxin-promoted elongation was underway, the 2  $\mu\text{M}$  auxin solution was removed, and a new solution containing auxin plus cordycepin (25, 50, or 100  $\mu\text{g/ml}$ ) was added immediately. The inhibition of the auxin-stimulated elongation by cordycepin was very rapid (Fig. 4). There was considerable overlap in the range of lengths of the latent periods for individual trials with the three concentrations of cordycepin tested. This overlap may be indicative of the fact that the concentrations tested did not differ greatly (e.g., by orders of magnitude). Also, it is possible that this overlap was due to some differences in developmental age of coleoptiles and, perhaps, to small concentration differences in solutions because of incomplete rinsing of the measuring chamber in some of the trials. The average latent periods based on a minimum of 10 trials for each concentration of cordy-

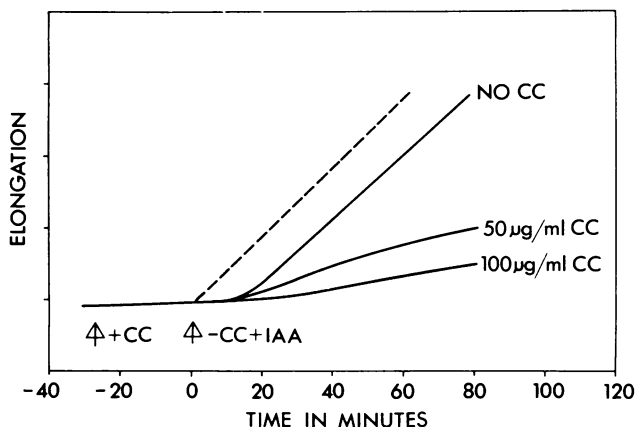


FIG. 1. Time course of 2  $\mu\text{M}$  auxin-induced elongation following 30-min pretreatment with 50 or 100  $\mu\text{g/ml}$  of cordycepin (CC). At time zero, cordycepin was removed and 2  $\mu\text{M}$  auxin was added. No CC: auxin-stimulated growth with no cordycepin pretreatment. The slope of the dashed line represents a growth rate of 0.7 mm/hr·segment.

cepin were  $17.5 \pm 5.3$ ,  $22.1 \pm 9.9$ , and  $34.6 \pm 9.8$  min for the 100, 50, and 25,  $\mu\text{g/ml}$  cordycepin concentrations, respectively ( $P = 0.05$ ).

**Effects of Cordycepin on Respiratory Activity in Mitochondria.** Concentrations of 25 and 100  $\mu\text{g/ml}$ , as used in coleoptile segment studies, showed no effect on respiration, respiratory control or ADP/O ratios in mung bean shoot mitochondria

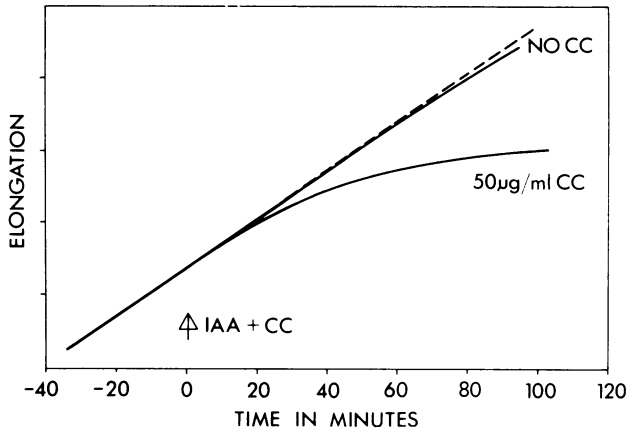


FIG. 4. Time course of inhibition of 2  $\mu\text{M}$  auxin-induced elongation with 50  $\mu\text{g/ml}$  of cordycepin (CC). Coleoptile segments were first treated with auxin for at least 45 min. At time zero, auxin and cordycepin were added simultaneously. The slope of the dashed line represents a growth rate of 0.7 mm/hr·segment.

(Fig. 5). Intermediate concentrations produced similar results. No evidence of a reduction of ATP synthesis or uncoupling effects was observed, which demonstrates that cordycepin is not inhibiting energy production in mitochondria.

**DISCUSSION**

The data presented here show that cordycepin inhibits auxin-induced elongation very rapidly, and preliminary data of the authors (2) indicate that cordycepin strongly inhibits  $^{32}\text{P}$  incorporation into polydisperse RNA of *Avena* coleoptile segments within 30 min. These findings are at variance with results of similar experiments using actinomycin D as an inhibitor. Actinomycin D inhibits RNA synthesis in *Avena* coleoptile segments within 30 min (4) but has no significant effect on auxin-induced elongation (in midcourse additions) during the 1st hr (15). This appears to be a common response of many kinds of hormone-treated tissues to actinomycin D; if the antibiotic is added after the hormone-stimulated growth response is underway, RNA synthesis is inhibited much more rapidly than the hormone-induced growth response (1, 4, 11, 14). Assuming that continued RNA synthesis is necessary for continued growth, this lag between the inhibition of RNA synthesis and the inhibition of growth response has been attributed to the decay time of the residual mRNA which was produced before the addition of actinomycin D (8). If one assumes that cordycepin has no deleterious effects on growth (other than on RNA synthesis) a central question is, why does a midcourse addition of cordycepin inhibit the hormone-induced growth response

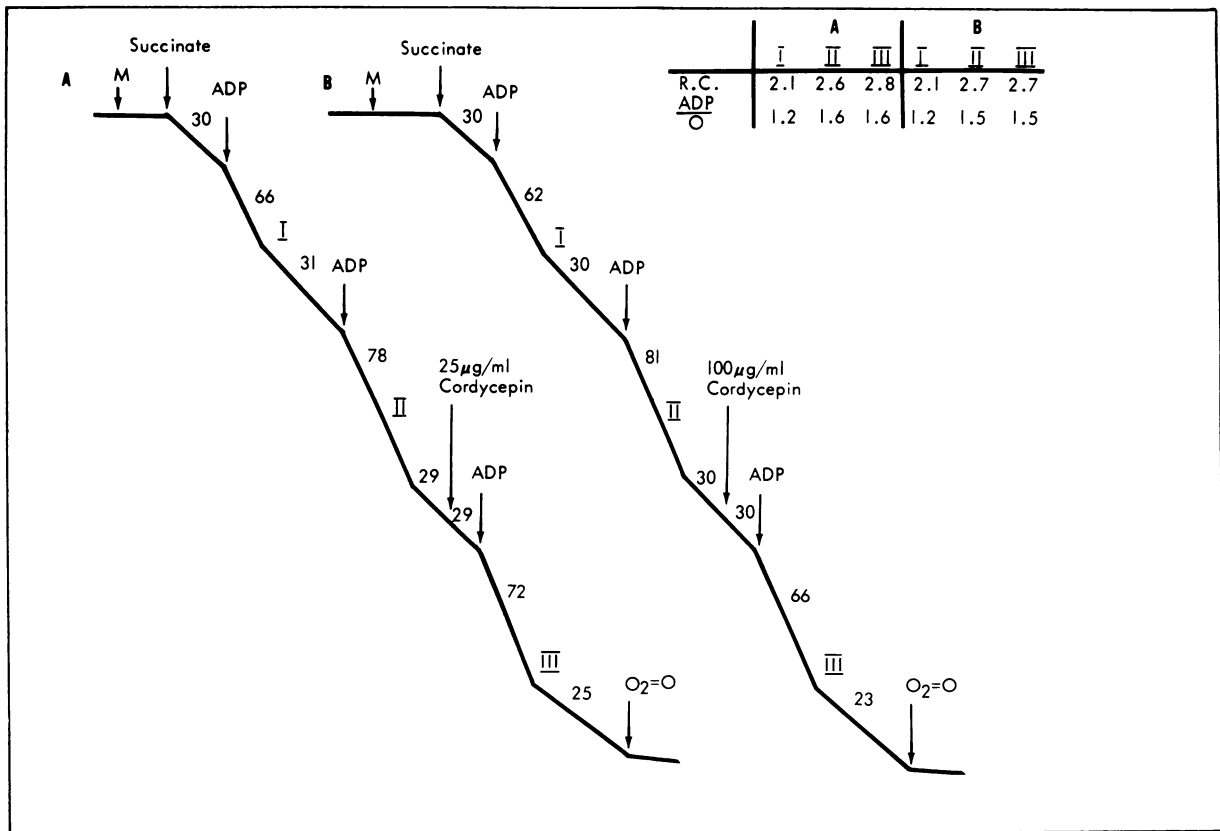


FIG. 5. Oxygraph trace of respiration and coupling parameters of mung bean mitochondria. The complete reaction media of 2.4 ml contained: 0.4 M mannitol, 50 mM tris-Tricine (pH 7.0), 4 mM MgCl<sub>2</sub>, 2 mM K-PO<sub>4</sub>, 1 mg/ml of BSA, and 8 mM succinate. The first addition of ADP was 120 nmoles, subsequent additions were 240 nmoles. Numbers on traces show nmoles O<sub>2</sub>/min. A: Addition of 25  $\mu\text{g/ml}$  of cordycepin; B: addition of 100  $\mu\text{g/ml}$  of cordycepin. Protein was 0.5 mg; M: mitochondria.

so much more rapidly than does actinomycin D? If growth-specific RNA synthesis is inhibited by cordycepin, it would suggest that the half-life of the mRNA involved is not as long as previous actinomycin D experiments have indicated.

We have already mentioned the conclusion of the original discoverers (3) of cordycepin; *i.e.*, that its toxicity as an antibiotic is of a "low order." Stockert *et al.* (19) have reported cordycepin-induced aberrations in nucleoli to be completely reversible within 24 to 36 hr after removal of the antibiotic from onion root tip cells. The fact that both pretreatments and simultaneous additions of cordycepin caused only partial decreases in auxin-promoted growth, indicated that the tissue was not strongly poisoned.

Siev *et al.* (18) found DNA and protein synthesis to be unaffected by 50  $\mu\text{g}/\text{ml}$  of cordycepin in HeLa cells. However, 0.5 mM cordycepin has been reported (J. Key, personal communication) to inhibit incorporation of labeled amino acids into protein of soybean hypocotyls pretreated with auxin by about 35% after 2 hr incubation. Hypocotyl elongation is inhibited by about 48%. Hence, the possibility that cordycepin inhibits growth in coleoptiles via translational processes still remains.

Because cordycepin is an analog of adenosine, it might be reasonable to suppose that there could be interference with oxidative phosphorylation. However, we could find no evidence that cordycepin affected the ADP/oxygen ratios in mung bean shoot mitochondria. Furthermore, the fact that the 15-min latent period for auxin-stimulated growth of the coleoptile segments was not appreciably altered would suggest that cordycepin was not acting as a respiratory inhibitor like KCN which is known to cause a 2- to 3-fold extension of the latent period (4).

6-Methyl purine is a transcriptional inhibitor which is known to rapidly inhibit certain hormone-induced responses in some ways similar to those of cordycepin (7; J. Key, personal communication). The precise mode of action of 6-methyl purine is unknown but there is evidence (J. Varner, personal communication) to indicate that it terminates a growing RNA chain at the point of its incorporation.

In summary, we have found that cordycepin, an RNA synthesis inhibitor, inhibits auxin-stimulated growth very rapidly. As such, cordycepin may prove to be a useful tool in the elucidation of relationships between growth and genetic control mechanisms in plant cells. Since possible side effects of cordycepin have not been completely explored, it may be advisable to exercise discretion in its use.

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