Inhibition of Gibberellic Acid-induced Elongation in *Avena* Stem Segments by a Substituted Pyrimidine

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ABSTRACT

*Avena* stem segments, which respond with high amplitude, specificity, and sensitivity to gibberellic acid, were used to study the inhibition of gibberellin-induced elongation by the growth retardant α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol (EL-531). It was found that EL-531 strongly inhibits gibberellic acid-induced elongation in this system at a concentration of 1 mM. From a double-reciprocal plot of elongation and gibberellic acid concentration, it seems that EL-531 and gibberellic acid do not compete reversibly for the same site of action. Also, because EL-531 effectively inhibits elongation in internodal tissue dissected away from the node and leaf sheath, it cannot be acting primarily by inhibiting the synthesis or transport of the leaf sheath factor(s). Because EL-531 causes lateral expansion of the stem segments as well as increased diameters of epidermal cells, in a manner very similar to the effects of colchicine, it is suggested that EL-531 inhibits gibberellic acid-induced elongation by somehow interfering with the orientation of the products of cell wall synthesis.

Of growth in an isolated plant part is found in *Avena* stem segments (7). Application of 1 μM GA₃ to these segments in the presence of 0.1 mM glucose can result in a 3- to 10-fold increase over the initial length in 72 hr (2, 13). Net elongation in GA₃-treated segments amounts to 10- to 20-fold over the control. Furthermore, this growth response is highly specific for gibberellin, insofar as it is actually inhibited by auxin (7), cytokinin (4), and ABA (9).

Because the *Avena* stem segment responds with such high amplitude, specificity, and sensitivity to GA₃, it is one of the best systems for the study of GA₃-induced elongation, as well as for the study of substances which inhibit this elongation. The aims of the present investigation are: (a) to document more fully the inhibition of gibberellin action by EL-531 using *Avena* stem segments as the experimental material, and (b) to attempt to gain some knowledge of the physiological processes involved in inhibition by EL-531.

MATERIALS AND METHODS

About 150 oat (*Avena sativa* cv. Victory) plants were routinely grown in flats (41 × 28 × 8 cm) in the greenhouse or, in some cases, in a growth chamber (18 hr light/6 hr dark; 22 C/16 C), for 40 to 45 days. Shoots containing the internodes immediately below the peduncular node (p-1 internodes) with a length of 1 to 3.5 cm were carefully selected. One-centimeter segments were prepared from the shoots with a razor blade cutting device. In some cases, the node and encircling leaf sheath were included in the segment and these are designated “whole segments.” In other experiments, the segments were cut in such a way as to separate the p-1 internodal tissue from the node and leaf sheath; these are designated “isolated internodes” (13). The intercalary meristem was included both in whole segments and in isolated internodes. The segments were placed in Plexiglas frames on filter paper in 6-cm Petri dishes containing 2 ml of treatment solution. The segments were allowed to grow in an upright position, because segments placed on their sides in the liquid medium did not elongate as much, possibly because of the effects of anaerobiosis, and they became curled and difficult to measure. They were routinely allowed to grow at 30 C in the dark in order to avoid the effects of photosynthesis (1) and all manipulations feasible were carried out under a dim green light (13). The lengths of the segments were measured with a millimeter ruler. Widths were measured about 0.5 cm from the base, also with a millimeter ruler. Fresh weights were determined after forcing air through the central lacuna of each segment to remove any residual incubation medium.

EL-531 was applied to the segments by first dissolving it in a small amount of ethanol. The final concentration of ethanol in the treatment solutions never exceeded 0.1 M; this concentration had no effect on the elongation of the segments.

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3 Abbreviations: EL-531, α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol; CBBP, 2,4-dichlorobenzyltributylphosphonium chloride.
Epidermal peels were prepared by nicking the internodes with a razor blade about 5 to 10 mm above the base and the raised tissue was pulled back and stripped off with a pair of jewelers’ forceps. These strips of epidermis were placed in a drop of 1% acetocarmine for 2 min, washed, and stored at 4°C except during periods of microscopic examination or photography.

Gibberellic acid was generously supplied by Mr. Douglas Broadbent of Imperial Chemical Industries, Ltd., Cheshire, England.

RESULTS

Dose Response of Inhibition by EL-531. In order to establish the concentration range over which EL-531 effectively inhibits GA₃-induced elongation, whole stem segments were treated with concentrations of EL-531 ranging from 10⁻⁷ to 10⁻⁴ M, together with 0.1 M glucose, and 100 μM GA₃. The results (Fig. 1) show that EL-531 produced no inhibition at 10⁻⁷ M and only slight inhibition of elongation at 10⁻⁶ M, whereas at 10⁻⁵, 10⁻⁴, and 10⁻³ M EL-531 caused significant inhibition. At 10⁻⁴ M EL-531, the amount of elongation was no greater than the control without exogenously supplied GA₃. Clearly, EL-531 is highly effective in inhibiting the elongation of Avena stem segments, even in the presence of a concentration of GA₃ 100-fold in excess of the level needed for maximal elongation (2, 13). The effective concentration range here for EL-531 is similar to the range Leopold found for lettuce hypocotyl (11).

Nature of Inhibition of GA₃-promoted Elongation by EL-531. In further experiments, the inhibition of elongation by EL-531 was studied using a modified double-reciprocal or Lineweaver-Burk plot. Whole stem segments were incubated with 0.1 M glucose and with concentrations of GA₃ ranging from 0.1 to 1 μM, with or without 100 μM EL-531. In Figure 2, the inverse of the final elongation is presented on the ordinate and the inverse of the GA₃ concentration is presented on the abscissa. Over the range of GA₃ concentrations tested, such a plot gave straight lines for both control and EL-531 treatments. The two straight lines intersect at a point on the abscissa, indicating noncompetitive inhibition; the inhibition of elongation by EL-531 cannot be overcome entirely by an increased supply of GA₃. This result suggests that GA₃ and EL-531 are not competing reversibly for a common site of action.

Effects of EL-531 in Absence of Node and Sheath. In a previous study (13), it was shown that maximal elongation of the internodal part of Avena stem segments is dependent upon some factor(s) produced in the leaf sheath and transported to the internode via the vascular plexus in the node. In order to test whether EL-531 was somehow acting on the synthesis and/or transport of the leaf sheath factor(s), the

Fig. 1. Effect of various concentrations of EL-531 on GA₃-induced elongation of Avena stem segments. Samples of five to six whole stem segments were treated with solutions containing various concentrations of EL-531, along with 0.1 M glucose and 100 μM GA₃. Mean final (maximal) elongation is plotted with standard errors.

Fig. 2. Effect of EL-531 on final elongation of Avena stem segments supplied with various concentrations of GA₃. Samples of five to six whole segments were treated with solutions containing various concentrations of GA₃, along with 0.1 M glucose with or without 100 μM EL-531.

Fig. 3. Time course of elongation of Avena stem segments as affected by EL-531. Samples of five isolated internodes were treated with solutions containing 0.1 M glucose with or without 100 μM GA₃ and with or without 1 mM EL-531. Mean elongation is plotted with standard errors.
inhibitor was supplied to isolated internodal tissue in the presence of 0.1 M glucose with or without 100 μM GA₃. The results (Fig. 3) are given as the time course of elongation of the isolated internodes. As described previously (12), GA₃ caused substantial promotion of elongation in these isolated internodes. Furthermore, EL-531, here given at a concentration of 1 mM, caused a rapid inhibition of GA₃-induced elongation; this could be observed as early as 6 hr after simultaneous application of GA₃. At 72 hr, when final elongation had been achieved, internodes treated with GA₃ plus EL-531 showed only 19% of the elongation achieved when GA₃ was supplied without inhibitor, whereas internodes treated with EL-531 but not supplied with exogenous GA₃ showed 60% of the elongation achieved without inhibitor and without GA₃. The effect of EL-531 is considerably more pronounced in the case of GA₃-treated internodes. The remarkable inhibition of elongation in isolated internodes caused by EL-531 shows that the inhibitor does not act, at least not primarily, by interfering with the synthesis or transport of the leaf sheath factor(s) but rather that it directly affects the elongating internodal tissue.

**Cytological Effects of EL-531.** In addition to an inhibition of elongation, EL-531 also caused swelling of the internodal tissue, especially near the base, whether or not the node and sheath were present. In order to investigate this phenomenon on a cytological level, epidermal peels were prepared from internodes incubated with GA₃ and 0.1 M glucose, with or without 1 mM EL-531. Epidermal peels from the internodes treated with GA₃ alone (Fig. 4A) show rows of long and short cells, extensively described previously (8). In peels from internodes treated with GA₃ plus EL-531 (Fig. 4B), the cells show generally increased diameters, in a manner remarkably similar to colchicine-treated *Avena* internode cells (see Fig. 8 in ref. 5). When cells in cross sections from the internodes were counted, no evidence could be found for an increased number of cells in EL-531-treated tissue. It seems that treatment with EL-531 causes lateral expansion of the *Avena* internodes by promoting increased cellular diameter, in a manner similar to the effect of colchicine.

**Comparison with Effects of Colchicine.** To estimate quantitatively the similarity between the effects of EL-531 and colchicine, the widths, lengths, and fresh weights of samples of isolated internodes were determined, after they had been incubated with 1 mM EL-531 or 1 mM colchicine, either in the presence or absence of 100 μM GA₃ (Table I). As before, application of GA₃ (treatment 2) clearly promoted elongation over the control (treatment 1) as shown by the total length at the end of 24 hr. As expected, the hormone caused an increase in the fresh weight per internode, as compared with the control, although the fresh weight/cm of length actually decreased slightly with GA₃ treatment. As reported above, EL-531, applied alone (treatment 3) or with GA₃ (treatment 4), inhibited elongation. Treatment with EL-531 also resulted in

Fig. 4. A: Photomicrograph of epidermal peel from isolated internode treated with 100 μM GA₃ plus 0.1 M glucose (× 2300); B: photomicrograph of epidermal peel from isolated internode treated with 100 μM GA₃, 1 mM EL-531, and 0.1 M glucose (× 3450).
greater width of the internodes compared with the control, especially if GA₃ was supplied concomitantly (treatment 4). This lateral enlargement is reflected by an increased fresh weight/cm of length (treatment 3 compared with treatment 1, and treatment 4 compared with treatment 2). Colchicine applied alone (treatment 5) or with GA₃ (treatment 6) also inhibited elongation. At this concentration, it was more effective in the inhibition of GA₃-induced elongation than was EL-531 (treatment 6 compared with treatment 4). Application of colchicine also resulted in increased diameters of the internodes, which was reflected by an increased fresh weight/cm of length (treatment 5 compared with treatment 1, and treatment 6 compared with treatment 2). Although the GA₃-induced increase in fresh weight/cm in the presence of either inhibitor varied considerably from experiment to experiment, EL-531 and colchicine always inhibited elongation and produced swelling of the internodes. From this standpoint, the two inhibitors have similar effects.

**DISCUSSION**

From the results presented in this study (Fig. 1), it is clear that EL-531 is a potent inhibitor of GA₃-induced elongation of *Avena* stem segments and is effective over a range of concentrations similar to those effective in the inhibition of GA₃-induced elongation of lettuce hypocotyl (11). The effect of EL-531 in *Avena* stem segments cannot be overcome entirely by even high concentrations of exogenous GA₃ (Figs. 1 and 2). It appears that EL-531 does not interfere with GA₃ action in *Avena* stem segments by competing reversibly with GA₃ for a common site of action (Fig. 2). Clearly, EL-531 does not act primarily by interfering with the synthesis or transport of the sheath factor(s) that is necessary for maximal elongation (Fig. 3).

Colchicine and EL-531 show remarkable similarities in their actions toward GA₃-induced elongation in *Avena* stem segments. Both inhibit elongation dramatically, while at the same time inducing lateral growth (swelling), accompanied by increased fresh weight/cm of length of the internodes (Table I). Cytologically, application of colchicine or EL-531 results in increased diameters of epidermal cells (Fig. 4). This swelling suggests a possible mechanism for the action of EL-531 in inhibiting elongation. In at least some plant cells, colchicine is known to disrupt microtubule formation (14), resulting in increased cellular diameter as a result of reorientation of cellulose microfibrils (3). It seems reasonable to suggest that EL-531 interferes (in some unknown way) with the orientation of the products of cell wall synthesis necessary for GA₃-induced elongation. A recent study has shown that increased wall synthesis is involved in GA₃-induced elongation of *Avena* stem segments (12). A possible weakness of this proposed mechanism is its inability to explain readily the promotion by EL-531 of rooting in shoot cuttings (10), which, incidentally, occurs optimally at a lower concentration of EL-531 than that found most effective in this study.

Shibaoka (15) has reported an inhibition of GA₃-induced elongation in azuki bean epicotyl sections by colchicine. As with *Avena* stem segments (6), GA₃ seems to stimulate elongation in azuki epicotyl sections by some process that does not include cell division; therefore, colchicine could not inhibit the effect of GA₃ simply by the inhibition of cell division in either of these systems. Because colchicine had little effect on auxin-induced elongation of azuki bean epicotyls, Shibaoko (15) suggested that wall microtubules participate in the response to gibberellins in a special way. Certainly, the present results with *Avena* stem segments lend some support to the idea that GA₃-induced elongation requires appropriate microtubule orientation.

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**LITERATURE CITED**


