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Promotion of Growth and Invertase Activity by Gibberellic Acid in Developing Avena Internodes[1]

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Abstract. **Gibberellic acid (GA_3) induces invertase activity within 6 hours in *Avena* stem segments that are incubated in the dark at 23°. The maximum amount of promotion is about 5 times that of invertase activity in untreated segments. GA_3 causes significant promotion of invertase activity at concentrations as low as 3×10^{-5} μM GA_3. The increase in invertase activity elicited by GA_3 between 3×10^{-5} μM and 300 μM closely parallels the growth promotion that is caused by GA_3 over this concentration range. In control segments, invertase activity rises steeply during the first 6 hours of incubation, then decays slowly between 12 and 48 hours. In GA_3-treated segments, the invertase activity also rises during the first 6 hours, parallel to that in control segments and continues to rise during the next 42 hours. These changes in invertase activity during 48-hour incubation periods do not parallel the changes in growth that occur in control and GA_3-treated segments. Cycloheximide at 10 μg/ml abolishes all GA_3-promoted growth and invertase activity in these segments. Actinomycin D at 40 and 80 μg/ml decreases GA_3-promoted growth by 20 % and invertase activity by 38 and 44 %, respectively. The data clearly support the idea that protein synthesis is necessary for GA_3-promoted growth and invertase activity in *Avena* stem segments.**

We have previously found that gibberellic acid (GA_3) accelerates in both light and dark the rate of lengthening of stem segments isolated from intercalary meristem portions of next-to-last internodes of *Avena sativa* (7). This response is primarily caused by an increase in the rate of cell lengthening in these segments. One of the possible mechanisms by which GA_3 accelerates this growth is through the promotion of invertase synthesis as it does with α-amylase (5, 10, 17, 18). This would result in release of reducing sugars that could be used in polysaccharide biosynthesis in elongating cells of these internodal segments. This idea seems attractive in view of the fact that GA_3 has been reported to augment invertase synthesis in other systems; namely, isolated sugar cane stem segments (12), staminal filaments of *Zea mays* (13, 14), beet root slices (11), and in Jerusalem artichoke tuber tissue (2). In sugar cane tissue, Hatch and Glasziou (4) found a strikingly close correlation between invertase activity and growth rate in internodes of different ages.

In preliminary experiments, we found marked promotion by GA_3 of growth and invertase activity in dark-incubated *Avena* internodal segments. This paper therefore aims at determining how close the relationship is between GA_3-promoted growth and GA_3-augmented invertase activity. It also sheds light on the mode of action of GA_3 in control of the growth process in this system.

Materials and Methods

One cm segments were isolated from next-to-last internodes (fig 1A) of *Avena sativa* cv. "Victory" (45 days old) with a razor blade cutting device. The segments (fig 1B) included the basal node, the basally located intercalary meristem portion of the internode, and an enclosing sheath base portion. The sheath base, which no longer grows, was included to provide support for the extending internode. The segments will be referred to as I.M. segments in this paper.

Twenty-five I.M. segments were placed horizontally on a disk of filter paper in a 5-cm petri dish (fig 1C) containing 2.5 ml of solution to be tested. Control solution was distilled water. GA_3 was used at 30 μM unless otherwise stated. This concentration evokes a maximal response in these segments (7). Concentrations of cycloheximide and actinomycin D are included in the text. The segments were incubated in the dark at 23° for periods up to 48 hours. This was sufficient to obtain a saturation growth response to gibberellic acid in *Avena* I.M. segments.

Crude extracts were then obtained from 20 I.M. segments for the determination of invertase activity. Methods essentially follow those of Glasziou *et al.* (3) and Sacher *et al.* (12). Extraction and invertase activity were run at pH 5.0 (using phosphate buffer, 5 mM for extraction, 50 mM for invertase

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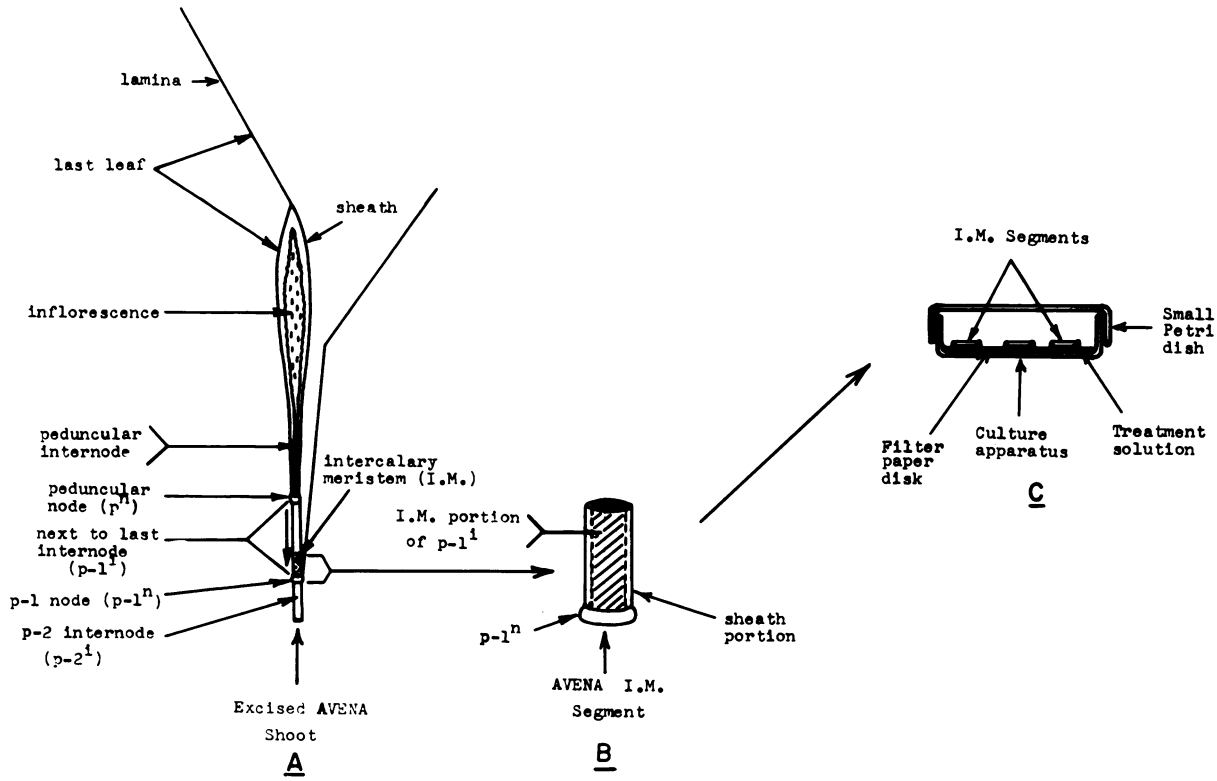

FIG. 1. Illustration of next-to-last internode ($p\text{-}1^i$) in the *Avena* shoot (A) from which basally located intercalary meristem (I.M.) segments (B) were excised. I.M. segments were floated on 2.5 ml incubation medium as shown in (C).

reaction), which we have found to be the optimum pH for invertase extracted from *Avena* internodal segment tissue. Reducing sugars were determined according to methods of Somogyi (16). Invertase activity is expressed as glucose μg equivalents per mg dry weight of tissue per hour at 30°.

Cycloheximide was obtained from Sigma Chemical Company, St. Louis, Missouri and actinomycin D from Merck and Company, Rahway, New Jersey. Gibberellic acid was supplied by Plant Protection Ltd., England. "Victory" oats were obtained from Allmanna Svenska Utsades A.B., Svalof, Sweden.

Results

The Effect of GA_3 Concentration on Growth and Invertase Activity. The first set of experiments was aimed at determining (a) the lowest level of GA_3 which promotes invertase activity and (b) the closeness of the relation between GA_3-promoted growth and GA_3-augmented invertase activity. The effects of different concentrations of GA_3 on linear growth and invertase activity in *Avena* I.M. segments cultured for 48 hours in the dark at 25° are shown in figure 2. The curve for growth indicates that GA_3 causes a significant acceleration of linear extension in these segments at concentrations varying between 3×10^{-5} μM and 300 μM GA_3. This increase in growth elicited by GA_3 is also accompanied by a significant promotion of invertase activity. The amount of promotion in invertase activity is as great as 4 times the control level for GA_3 at 3×10^{-3} μM; growth is augmented as much as 6 times the control level for GA_3 at 3×10^{-2} μM. Comparison of the 2 curves in figure 2 shows further that GA_3-promoted growth and GA_3-promoted invertase activity are closely parallel over the entire concentration range of GA_3

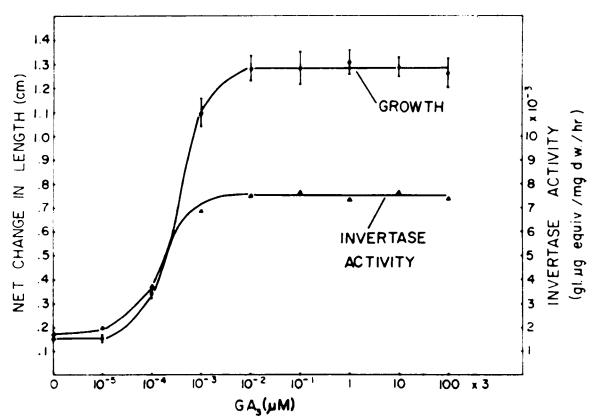

FIG. 2. Comparison of net linear growth and invertase activity in *Avena* I.M. segments incubated in GA_3 varying concentration from 3×10^{-5} to 300 μM for 25 hours in the dark at 23°.

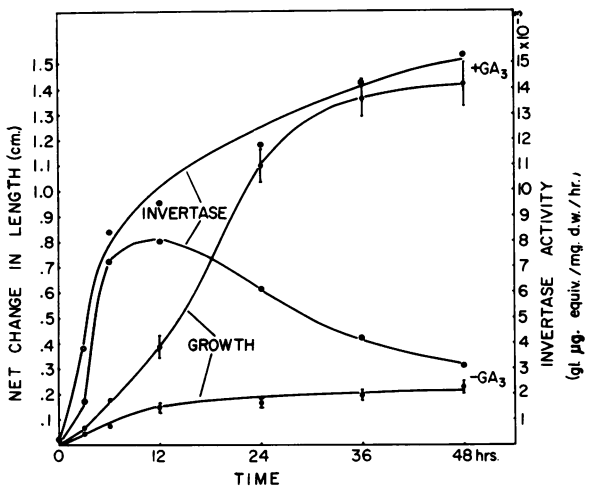

FIG. 3. Time-course changes in effects of GA_3 on growth and invertase activity in *Avena* I.M. segments incubated in the dark at 23° for 48 hours.

employed. Saturation occurs for both growth and invertase activity promotion above 0.03 µM GA_3.

Time-course Changes of GA_3-promoted Invertase Activity. The next part of this study was concerned with the question of the amount of time necessary for GA_3 to cause induction of enzyme activity in *Avena* I.M. segments. Figure 3 illustrates the effect of GA_3 at 30 µM on growth and invertase activity in segments incubated in the dark for 48 hours. The curves indicate that (a) GA_3 stimulates growth by 7-fold over control segments; (b) growth reaches a maximum level at about 36 hours; (c) invertase activity in control segments increases with treatment period, reaches a maximum at about 12 hours, and declines thereafter; (d) the invertase activity in GA_3-treated segments rises parallel to that in control segments during the first 6 hours of growth, starting within 3 hours after time zero; it then increases gradually during the remainder of the growth period (6 to 24 hrs); (e) this contrasts with invertase activity in control segments, which drops steadily between 12 and 48 hours after time zero.

The Effects of Inhibitors of Protein and RNA Synthesis on GA_3-promoted Growth and Invertase Activity. The question of whether GA_3 causes synthesis of invertase or merely activates preexisting enzyme was next tested using inhibitors of protein and RNA synthesis.

In *Avena* I.M. segments (table I), cycloheximide at 10 and 20 µg/ml completely abolished GA_3-promoted growth; at 5 µg/ml, it suppressed growth about 30 %. Cycloheximide was also equally effective in suppressing GA_3-promoted invertase activity (table I). At 5, 10, and 20 µg/ml, it completely nullified all GA_3-promoted activity.

In order to determine whether GA_3 was capable

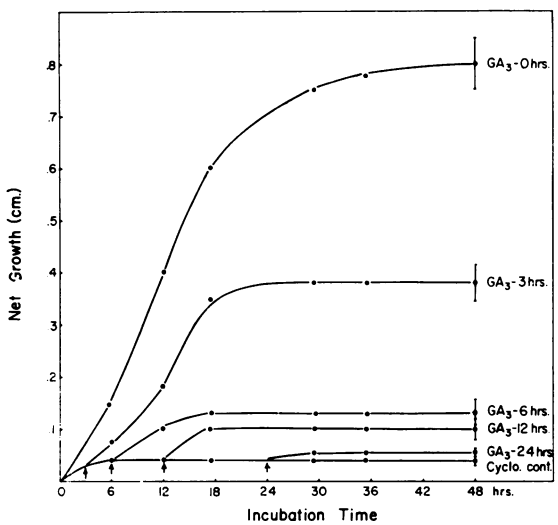

FIG. 4. Time-course growth responses of *Avena* I.M. segments pretreated with cycloheximide (10 µg/ml) for various lengths of time (as indicated by arrows on graph), then transferred to GA_3 (30 µM) at the times indicated at far right side of graph. I.M. segments (25 per dish) were incubated in the dark at 23° for 48 hours.

Table I. *Effect of Different Concentrations of Cycloheximide on the Growth of* Avena *I.M. Segments in Presence and Absence of GA_3*

25 I.M. segments per culture dish were incubated 48 hours in the dark at 23°. Experiments were repeated twice.

Measurement	H_2O control	Cycloheximide 0.5	5.0	10.0	20.0	GA_3 control	Cycloheximide + 30 µM GA_3 0.5	5.0	10.0	20.0
			µg/ml			30 µM		µg/ml		
Net growth (mm) After 48 hrs	0.24 ±0.01	0.28 ±0.02	0.17 ±0.02	0.11 ±0.02	0.07 ±0.01	1.38 ±0.06	0.95 ±0.09	0.49 ±0.06	0.14 ±0.02	0.05 ±0.01
% of control	100	116	71	46	29	100	69	35	10	4
Invertase activity (glucose µµg equiv. per mg dry wt per hr)	1.90	2.05	1.80	1.10	0.83	4.70	4.30	1.80	1.30	0.67

Table II. *Invertase Activity in* Avena *I. M. Segments Pretreated with Cycloheximide for Various Lengths of Time Followed By Incubation in GA_3 and the Reverse Treatment*

25 I.M. segments per culture dish, incubated for 48 hrs in the dark at 23°. Experiments were repeated twice. The concentration of GA_3 was 30 μM. Invertase activity was determined at the end of 48 hrs.

H_2O control	48 hrs Cycloheximide		Hours of pretreatment with cycloheximide 0	3	6	12	24
	10 μg/ml		*Glucose μμg equivalents per mg dry wt per hr*				
1.25	0.75		1.84	1.70	0.83	0.63	0.54
H_2O control	48 hrs GA_3	48 hrs Cycloheximide	Hours of pretreatment with GA_3 0	3	6	12	24
		10 μg/ml	*Glucose μμg equivalents per mg dry wt per hr*				
1.90	4.00	0.21	0.30	0.20	0.23	0.27	0.29

of reversing the inhibition of growth and invertase activity caused by cycloheximide, GA_3 was next added to segments pretreated with cycloheximide for varying lengths of time. The results indicate that GA_3 does partially restore growth and invertase activity in I.M. segments (fig 4, table II). The reversal of cycloheximide inhibition by GA_3 depends on the period of cycloheximide pretreatment: the longer the pretreatment, the less the recovery (fig 4). The growth rate and net growth of I.M. segments given GA_3 after 3 hours pretreatment with cycloheximide is about one-half that of non-pretreated GA_3 control segments. The segments pretreated for 6 and 12 hours with cycloheximide grow only slightly; this increase in growth rate lasts for about 6 hours, then decays quickly to zero. Thus, after 48 hours, the total growth with these treatments is only 10 % of that of segments given continuous GA_3 treatment.

The order of treatment with cycloheximide and GA_3 is reversed in the next experiments; the segments were pretreated for varying lengths of time with GA_3 followed by transfer of the segments to an inhibitory concentration of cycloheximide (10 μg/ml).

The results in figure 5 indicate that irrespective of the length of time of pretreatment of segments in GA_3, the subsequent treatment with cycloheximide brings about a complete cessation in growth, usually within 6 hours after segments are treated in cycloheximide. The effect of this inhibitor on invertase activity is equally drastic (table II). The cycloheximide abolishes all GA_3-promoted invertase activity; in fact, the inhibitor depresses it to levels one-seventh to one-eighth that of invertase activity in water control segments. It is obvious that after this 48-hour incubation, the effect of GA_3 in promoting invertase activity is completely lost following treatment with inhibitor.

We next considered the possibility that GA_3 was operating at some step more closely linked to RNA synthesis. Actinomycin D, which blocks DNA-dependent RNA synthesis, was tested. Actinomycin D has little or no effect on GA_3-promoted growth except at 40 and 80 μg/ml, where it depresses net growth only by 20 %. This is not a large decrease considering the amount of inhibitor employed. The amount of depression in invertase activity was not significant except at 40 and 80 μg/ml; here, the amount of inhibition of enzyme activity was 38 % at 40 μg/ml and 44 % at 80 μg/ml. Thus, while actinomycin D at high concentrations has only a slight depressing effect on GA_3-promoted growth, it is capable of eliciting considerable repression of invertase activity. This observation tends to give additional support to the idea that not all GA_3-

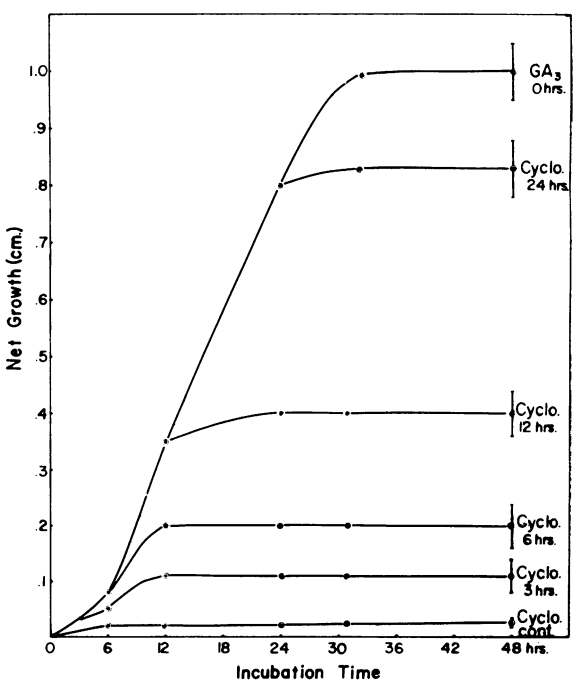

FIG. 5. Time-course growth responses of *Avena* I.M. segments pretreated with GA_3 (30 μM) for various lengths of time (as indicated in table II), then transferred to cycloheximide (10 μg/ml) at times indicated at far right side of graph. I.M. segments (25 per dish) were incubated in the dark at 23° for 48 hours.

promoted growth in *Avena* I.M. segments can be attributed to an increase in invertase activity alone. It is also likely that very little actinomycin D was actually taken up by the I.M. segments. This would explain the absence of an effect of the inhibitor on growth and invertase activity except at extremely high concentrations of the inhibitor. Thus, it is not clear from these results whether DNA-dependent RNA synthesis is really necessary for GA_3-promoted growth and invertase activity in *Avena* I.M. segments.

Discussion

The present studies have shown that GA_3 induces invertase activity within the first 6 hours after *Avena* I.M. segments are placed in hormone solution. This compares favorably with 6 hours cited by Varner (17) for induction of α-amylase in barley aleurone by GA_3 and 24 hours for promotion of invertase activity in chick embryos by hydrocortisone (6). GA_3 has been shown to accelerate growth in I.M. segments within 40 minutes after placing them in hormone solution (Paul Adams, unpublished results). This means that a sizeable component of GA_3-promoted growth actually takes place before the hormone increases invertase activity. One cannot escape the conclusion that the enhanced invertase activity elicited by GA_3 does not account for all of the GA_3-promoted growth in these segments.

The maximum amount of enhancement in invertase activity caused by GA_3 in *Avena* I.M. segments is as much as 5 times that of invertase activity in control segments after incubation periods of 48 hours (fig 2). This compares with a maximal increase in invertase activity caused by GA_3 of about 3 times in staminal filaments of *Zea mays* (13, 14) and 1.3 times in beet root slices (11). Crispeels and Varner (1) have reported an increase in α-amylase activity in barley aleurone layers of about 5 times. The maximal amount of enhancement in invertase activity caused by GA_3 in these different systems, including *Avena* I.M. segments, is thus within the same order of magnitude.

In dosage-response experiments, we have shown that both growth and invertase activity in *Avena* I.M. segments starts to increase at concentrations of GA_3 between 3×10^{-5} μM and 3×10^{-4} μM (fig 2); the lower limit is actually closer to 3×10^{-5} μM GA_3. Schaeverbeke (15) reports that GA_3 starts to augment invertase activity in *Zea mays* staminal filaments at a concentration of 3×10^{-3} μM with the greatest amount of promotion occurring at 30 μM GA_3. Comparing these data with those for α-amylase, the promotion in synthesis of this enzyme by GA_3 starts at 10^{-5} μM in barley aleurone layers (17, 18). Therefore, GA_3-induced invertase activity in *Avena* I.M. segments has a much lower limit of sensitivity than in *Zea mays* filaments and is nearly the same as in the barley α-amylase system.

One of the surprising results of the present study is the occurrence of a sharp rise in invertase activity in control I.M. segments during the first 6 hours of incubation in the dark, followed by decrease in invertase activity during the next 42 hours. The rise in control segments parallels closely the increase in invertase activity that occurs in GA_3-treated segments; however, with the latter, the invertase activity rises continually for 42 hours. The rise and decay in invertase activity in the control segments could be interpreted as being due to the turnover of invertase in this system.

When growth and invertase activity are compared in control and GA_3-treated segments, a significant discrepancy is observed. In the control system, while invertase activity increases, there is only a slight increase in dark growth. In the GA_3 system, where the increase in invertase activity parallels that in control segments (0–6 hrs), growth is accelerating at a marked rate. What this apparently means is that the burst in invertase activity during the first 6 hours of incubation, in either system, is probably not the direct cause of the growth by cell lengthening that occurs during this period.

GA_3 induces invertase activity after the first 6 hours of incubation. This would bring about an increase in the pool of reducing sugars, which in turn would be available for sustaining high growth rates that occur in gibberellin-treated segments over 6 to 36 hours in the dark (cf. fig 3). Such a system suggests a regulatory mechanism by which elongating cells in the intact internode are able to release substrate(s) for growth. This is based on the assumption that the developing internode has some mechanism for producing gibberellin(s) during the log phase of intercalary growth.

The results from the experiments with inhibitors clearly support the idea that protein synthesis is necessary for GA_3-promoted growth and GA_3-promoted invertase activity in *Avena* I.M. segments. This has also been shown for other systems (8, 14, 17, 18). In I.M. segments, cycloheximide blocks both processes. It is probable that cycloheximide acts at a different site from the site of GA_3 action. The results with actinomycin D are not nearly as clear-cut. We do obtain considerable decrease in invertase activity but very little effect of this inhibitor on GA_3-promoted growth. It is possible that very little actinomycin D is taken up by the segments. The point that can be made here is that GA_3 is probably causing the synthesis of invertase, but at what site(s) it is acting is not yet clear. Unequivocal proof that GA_3 is inducing the synthesis of invertase must await further studies on inhibitors and incorporation of labeled amino acids into protein, similar to the work of Nooden and Thimann (9), Varner (17), and Varner and Chandra (18).

Acknowledgments

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