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Regulation of Invertase Levels in *Avena* Stem Segments by Gibberellic Acid, Sucrose, Glucose, and Fructose[1]

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ABSTRACT

Gibberellic acid and sucrose play significant roles in the increases in invertase and growth in *Avena* stem segments. About 80% of invertase is readily solubilized, whereas the rest is in the cell wall fraction. The levels of both types of invertase change in a similar manner in the response to gibberellic acid and sucrose treatment. The work described here was carried out with only the soluble enzyme. In response to a treatment, the level of invertase activity typically follows a pattern of increase followed by decrease; the increase in activity is approximately correlated with the active growth phase, whereas the decrease in activity is initiated when growth of the segments slows. A continuous supply of gibberellic acid retards the decline of enzyme activity. When gibberellic acid was pulsed to the segments treated with or without sucrose, the level of invertase activity increased at least twice as high in the presence of sucrose as in its absence, but the lag period is longer with sucrose present. Cycloheximide treatments effectively abolish the gibberellic acid-promoted growth, and the level of enzyme activity drops rapidly. Decay of invertase activity in response to cycloheximide treatment occurs regardless of gibberellic acid or sucrose treatment or both, and it is generally faster when the inhibitor is administered at the peak of enzyme induction than when given at its rising phase. Pulses with sucrose, glucose, fructose, or glucose + fructose elevate the level of invertase significantly with a lag of about 5 to 10 hours. The increase in invertase activity elicited by a sucrose pulse is about one-third that caused by a gibberellic acid pulse given at a comparable time during mid-phase of enzyme induction, and the lag before the enzyme activity increases is nearly twice as long for sucrose as for gibberellic acid. Moreover, the gibberellic acid pulse results in about three times more growth than the sucrose pulse. Our studies support the view that gibberellic acid, as well as substrate (sucrose) and end products (glucose and fructose), play a significant role in regulating invertase levels in *Avena* stem tissue, and that such regulation provides a mechanism for increasing the level of soluble saccharides needed for gibberellic acid-promoted growth.

The growth of isolated *Avena* stem segments is markedly stimulated by the application of GA_3, but not significantly by IAA or by kinetin (13, 14, 17, 19, 20). It has also been shown that the concentration of GA_3, which promotes growth, closely parallels that which increases invertase activity in the stem segments (19). The invertase activity is continuously increased in GA_3-treated *Avena* segments for 48 hr, but the activity in water control segments shows an initial increase, followed by a subsequent decrease (19). The maximum enzyme activity in control segments, reached at about 12 hr of incubation, is 50 to 60% of the maximum activity in GA_3-incubated segments (19). GA_3 stimulates the growth of these segments 7-fold over that of the water control segments in the absence of an exogenous supply of sugar (13, 14, 19) and up to 20-fold in the presence of sugar (1, 2, 13, 14). Thus, GA_3 acts both to increase invertase activity and to promote growth. Increased enzyme activity elicited by GA_3 application, however, appears not to be the direct cause of GA_3-induced growth, while the possibility that a GA_3 application causes an increased pool of reducing sugars for sustained growth remains (19).

Gibberellin promotion of invertase activity has also been reported in the literature with other plant materials; *e.g.* lentil epicotyls (32), sugarcane stem tissue (9–11, 29), Jerusalem artichoke tuber (7), corn staminal filaments (30, 31), and beet root slices (28). The present paper concerns the roles played by the substrate, the products, and GA_3 on the level of invertase activity and growth.

MATERIALS AND METHODS

Preparation and Incubation of Segments. One-centimeter stem segments containing intercalary meristems were isolated as reported earlier (1, 16–19) from 45-day-old *Avena* shoots grown in the greenhouse. The segments were surface-sterilized with 10% Clorox (0.5% sodium hypochlorite) for 2 min, then thoroughly washed with 8 to 10 changes of sterile distilled water in 2 min. This treatment was found not to affect the growth response of the segments and effectively prevented any microbial contamination in the incubation medium. Twenty-five segments were then placed horizontally on a disk of filter paper in a 10-cm Petri dish containing 6 ml of treatment solution. Freshly prepared GA_3 was used at 30 μM, and sucrose, where used, at 0.1 M. These concentrations of GA_3 and sucrose, respectively, give maximum growth responses (1, 15–19). Pulse treatments with GA_3 involved incubating the segments in GA_3 for 60 min in the dark, washing the segments with running distilled water for 5 min, followed by 10 changes of sterile distilled water to remove residual GA_3 from the tissue, and transferring the segments to distilled water or sucrose for the rest of the incubation period. The washing of segments was done under diffuse light in the laboratory. The segments were incubated in the dark at 23 C, and necessary manipulations were carried out under a dim green safelight. Growth was measured with a mm ruler to the nearest 0.5 mm.

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In the inhibitor experiments, cycloheximide was used at 10 μg/ml. All experiments were repeated at least three times.

Enzyme Extraction and Assay. For the preparation of the crude invertase fraction, 12 segments were removed at various times of incubation and frozen by placing them in a deep freeze refrigerator (about 20 C) until the segments in a set of experiments were accumulated. Freezing was not found to affect invertase activity upon subsequent extraction. The 12 segments were thoroughly hand-ground (until no fiber material could be seen) in a 70-ml porcelain mortar with 4 ml of cold 5 mM phosphate-citrate buffer (pH 5.0) and about 1.5 g of sand. This and all subsequent steps were carried out at 0 to 4 C. The brei was strained through four layers of cheesecloth and centrifuged at 1700*g* for 5 min with an International clinical centrifuge. Invertase activity was examined for both pellet and supernatant fractions; the pellet enzyme represents enzyme bound to particulate fractions, presumably the cell wall (7, 33), and the supernatant contains soluble enzyme. In order to remove endogenous substrate and possible inhibitors, the supernatant was dialyzed against 1 liter of distilled water at 4 C with three changes of water in 60 min. The enzyme activity is not significantly different when dialysis is done against water as compared with buffer. The pellet fraction was suspended in 2.0 ml of extraction buffer with gentle shaking, followed by dialysis as for the supernatant fraction. The sand was removed by very careful decanting of the pellet liquid from the centrifuge tube after the sand had settled.

The enzyme reaction mixture consisted of 0.1 ml extract, 60 μmoles sucrose, and 30 μmoles of phosphate-citrate buffer (pH 5.0) in a total volume of 1.0 ml. The enzyme reaction mixture at 30 C was stopped by adding 1 ml of alkaline reducing sugar reagent (27). The reducing sugar content was then determined by the standard method (27) with arsenomolybdate. The resulting color was read with a Klett-Summerson colorimeter with a green (No. 54) filter. Protein was determined according to the method of Lowry *et al.* (24). The invertase specific activity is expressed as μmoles glucose produced/mg protein·hr at 30 C.

Gibberellic acid was obtained from Imperial Chemical Industries, Ltd., England; Cycloheximide from Sigma Chemical Co., St. Louis, Mo.; and Victory oat seed from the Swedish Seed Assoc., Svalöf, Sweden.

RESULTS

Time Course Changes in Invertase Activity in "Wall" and Soluble Fractions and in the Incubation Medium. Invertase has been shown to be associated with the wall fraction in some tissues (7, 23, 33, 34), while the invertase thus far studied with *Avena* stem segments is primarily in the soluble fraction. In order to test whether or not the changes of invertase activity observed in the tissue extract may result from a release of enzyme from the "wall" to the soluble fraction, stem segments were treated with water, sucrose, or a GA_3 pulse for varying periods and then removed for the analysis of enzyme activity in the wall and soluble fractions at various times during incubation. The results (Table I) indicate that the enzyme activity is increased in the wall fraction in a manner similar to the soluble fraction, but a predominant portion of the total activity (72.8–88.1%) is in the soluble fraction at any time of tissue incubation. In view of a remarkable over-all consistency in the ratio of soluble to wall fractions, the range of 12 to 27% ascribable to the wall fraction may be taken as experimental variation mainly due to preparation and assays of soluble and wall fractions.

Since the bulk of invertase is associated with the cell walls in other tissues (7, 23, 33, 34), the above results can be interpreted in terms of an invertase being loosely associated with the wall. If this is the case, the enzyme would be released into the surrounding medium during the incubation of the segments, or it is solubilized into the soluble fraction during enzyme preparation. In order to test for a possible leakage of the enzyme into the incubation medium, enzyme activity in this medium was carefully assayed at the various times of incubation in water, GA_3, and sucrose. In none of the treatments could we detect invertase activity in the medium during the 48 hr incubation period. These experiments were repeated several times with different amounts of the medium and incubation times; the results were consistently negative.

Table I. *Changes in Wall and Soluble Invertase in Avena Stem Segments Incubated in Water, Sucrose, and GA_3*

The preparations of supernatant and wall fractions were made as described under "Materials and Methods." GA_3 was given in a 60-min pulse at the beginning of incubation; sucrose was given continuously throughout the incubation period. The experiment was carried out in the dark at 23 C.

Treatment	Invertase Activity		Invertase Activity	
	Supernatant	Pellet (wall fraction)	Supernatant	Pellet (wall fraction)
hr	*μmoles glucose liberated/12 segments·min*		*% total for each treatment*	
Water				
0	603	100	85.5	14.1
14	1642	552	75.5	24.5
24	1419	354	80.5	19.5
48	993	220	82.1	17.9
Sucrose (0.1 M)				
0	603	100	85.5	14.5
14	1619	444	78.4	21.6
24	1809	678	72.8	27.2
48	1394	288	82.9	17.1
GA_3 (30 μM)				
0	603	100	85.5	14.5
14	3665	750	87.1	12.9
24	6675	900	88.1	11.9
48	4856	888	84.6	15.4

From these results, it can be concluded that (*a*) the enzyme resides mostly in the soluble fraction and about 20% of the total activity occurs in the wall fraction; (*b*) the pattern for increase in level of enzyme activity is the same for both wall and soluble fractions; and (*c*) there is no detectable enzyme activity in the medium at any time of tissue incubation. Since about 80% of invertase in *Avena* stem segments can readily be solubilized (Table I), and there is no detectable invertase secreted in the medium, it is possible that the majority of enzyme is either present in the cytosol or associated with the cell wall very loosely. These solubility characteristics are different from the invertases from other plant tissues (7, 23, 33, 34) and may perhaps reflect certain differences in the enzyme properties.

Time Course Changes in GA_3-induced Invertase Activity and Growth. In order to ascertain whether the continous presence of GA_3 is required to maintain the increased level of invertase in *Avena* stem segments, GA_3 pulses of 60-min duration were started at 0, 14, and 25 hr. These times correspond to the period of increase, peak, and decline, respectively, of soluble invertase activity in water control segments. The segments which received either water or GA_3 continuously throughout the experimental period served as controls. A typical set of results is shown in Figure 1 for soluble enzyme

activity (Fig. 1A) and growth (Fig. 1B) for water-incubated segments.

The two control treatments (curves 1 and 2 in Fig. 1A) show essentially the same pattern as reported earlier (19), although the total treatment period is about twice as long in these experiments as in the previous report. It should be noted that because of the prolonged incubation period, the enzyme activity at 48 hr in water-control segments is significantly lower than the level at 0 hr, and that a slight decline from the highest level of activity is detectable in continuous GA_3 control after 50 hr of treatment (Fig. 1A). The maximum growth attained by these control segments (curves 1 and 2, Fig. 1B) was 0.2 cm for water control and 1.0 cm for continuous GA_3 control during the first 24 hr of incubation, but the activity declines thereafter to that of the water control. GA_3 pulses given a 14 and 25 hr of incubation show lag periods of about 10 and 20 hr, respectively, prior to an increase in the enzyme activity above water control. The level of maximum enzyme activity reached decreases with increased delay of GA_3 pulse treatments: 72 μmoles glucose/mg protein·hr for 0 time pulse (curve 3), 50 for 14 hr pulse (curve 4), and 38 for 25-hr pulse (curve 5). After the peaks are reached for the 14- and 25-hr treatments, both enzyme activity curves follow a similar pattern of decrease in enzyme activity.

The growth curves (Fig. 1B) indicate that the growth induction by GA_3 pulses takes place with much shorter lag periods, but the maximum growth achieved decreases with increasing delay of GA_3 pulse treatments. The maximum growth attained is 1.0 cm for the pulse at 0 hr, 0.4 for the pulse at 14 hr, and 0.3 for the pulse at 25 hr.

Figure 2 indicates the time course changes in invertase activity and growth for sucrose-treated segments. The results indicate that (*a*) the enzyme activity for sucrose control (curve 1) rises after a short lag period to a peak of maximum activity (74 μmoles glucose/mg protein·hr) in 24 hr, then decreases slowly during the next 140 hr; (*b*) in the presence of a continuous supply of sucrose and GA_3 (curve 2), the invertase activity parallels the sucrose control for the first 24 hr and continues to rise to a maximal activity of 155 μmoles glucose/mg protein·hr by 72 hr, and then declines slightly after 100 hr of incubation; (*c*) with a GA_3 pulse at 0 hr (curve 3), the enzyme activity follows that of the sucrose + GA_3 control during the first 48 hr, where the activity reaches a peak of 135 μmoles glucose/mg protein·hr at 48 hr, then drops slowly during the next 120 hr; (*d*) a pulse with GA_3 at 24 hr (curve 4) causes an increase of invertase above the sucrose control after a lag of about 12 hr, reaching a peak activity of 125 μmoles glucose/mg protein·hr at 60 hr, after which time the enzyme activity declines; (*e*) a pulse with GA_3 at 48 hr also causes a striking increase in invertase activity after a 24-hr lag period; upon reaching a peak of 115 μmoles glucose/mg protein·hr at about 100 hr, the enzyme activity decreases rapidly thereafter; and (*f*) a GA_3 pulse at 72 hr (curve 6) causes only a slight increase in invertase activity after a lag period of about 30 hr.

When Figures 1A and 2A are compared, it can be seen that (*a*) the presence of sucrose in the medium suppresses the in-

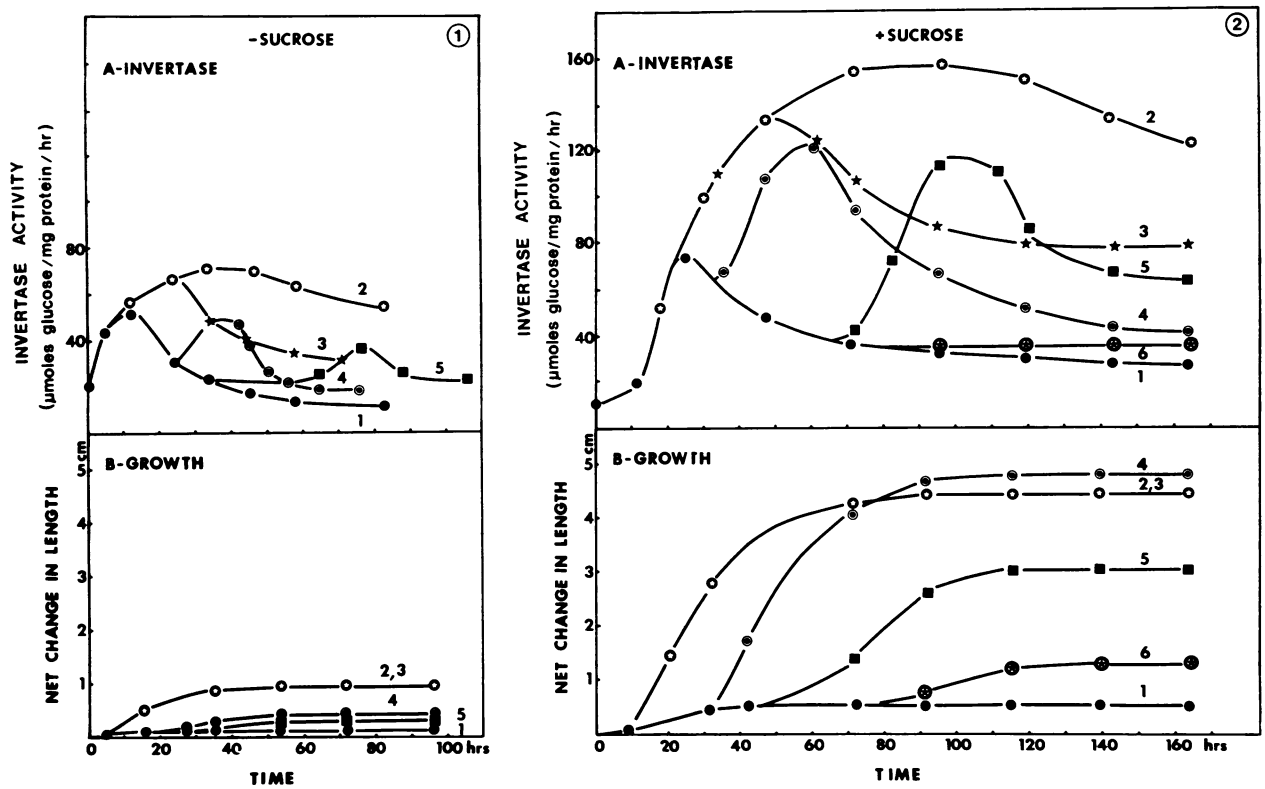

FIG. 1. Changes in invertase activity (A) and growth (B) for *Avena* (stem) segments incubated in absence of substrate. Treatments include: (1) water control, (2) continuous GA_3, (3) GA_3 pulse at 0 hr, (4) GA_3 pulse at 14 hr, (5) GA_3 pulse at 25 hr. GA_3 pulse treatments were 60 min; GA_3 was used at 30 μM. For growth curves in B, the standard error of the mean for treatments 2 and 3 is $\pm$0.05 cm at 96 hr. The growth differences in treatments 1, 4, and 5 are small and not significantly different (standard errors = $\pm$0.02 cm).

FIG. 2. Changes in invertase activity (A) and growth (B) for *Avena* stem segments incubated in presence of sucrose (0.1 M). Treatments include (1) sucrose control, (2) continuous GA_3, (3) GA_3 pulse at 0 hr, (4) GA_3 pulse at 24 hr, (5) GA_3 pulse at 48 hr, (6) GA_3 pulse at 72 hr. GA_3 pulse treatments were 60 min; GA_3 was used at 30 μM. For growth curves in B, standard errors of means for above treatments are: (1) $\pm$0.03 cm, (2, 3) $\pm$0.15 cm, (4) $\pm$0.13 cm, (5) $\pm$0.11 cm, (6) $\pm$0.07 cm. Treatments 2, 3, and 4 show no significant difference in growth at 160 hr; significant differences do occur at 160 hr for growth in treatments 1, 5, 6, and 4 (2, 3).

crease in enzyme level during the first 12 hr; (*b*) greater increases in level of invertase activity are produced by GA_3 in the presence of sucrose; (*c*) the peaks of enzyme activity brought about by delayed GA_3 pulses decrease with time of delay in the water series, whereas delayed GA_3 pulses gave practically the same enzyme activity as the GA_3 pulse at 0 hr in the sucrose series; (*d*) the lag periods prior to the enzyme level increases elicited by GA_3 pulses, tend to be shorter in the presence of sucrose than in the absence of sucrose; and (*e*) the decrease in enzyme activity after reaching the peak is usually slower in the sucrose series than in the water series.

Figure 2B shows the growth response of *Avena* stem segments to GA_3 in the presence of sucrose. During the period when *Avena* stem segments are growing most rapidly in the sucrose control, *i.e.* between 0 and 30 hr, GA_3 pulse treatments are most effective, causing a 9-fold increase in growth over that of control segments. After the control growth reaches a plateau, *i.e.* after 48 hr, the growth induction by GA_3 pulse treatments becomes increasingly less with time.

When Figure 2B is compared with Figure 1B, it is seen that sucrose and GA_3 together cause much more than an additive growth response (see also refs. 1 and 2). Furthermore, the presence of sucrose in the incubation medium makes the segments more responsive to delayed GA_3 pulses than in the absence of sucrose. It is noteworthy that there is a much longer lag period for growth with continuous GA_3 and for a GA_3 pulse at 0 hr in the sucrose series than in the water series. The longer lag for growth parallels closely the lag period noted in the invertase activity.

Inspection of Figures 1 and 2 points to an approximate correlation between the increase in invertase activity and the growth stimulated by GA_3 pulse treatment: invertase activity increases during the phase of active growth, whereas invertase activity decreases, when the growth of the segments begins to cease. Furthermore, a continuous supply of GA_3 in either set of experiments retards the decline of enzyme activity. It should also be noted, in particular for delayed pulse treatments, that GA_3 increases growth with a much shorter lag than it does in the case of invertase activity. It thus appears that the GA_3 first stimulates the growth and then causes an increase in level of enzyme activity during the active growth phase. In view of the far larger increment of growth elicited by GA_3 in the presence of sugar than in its absence, this later increase in invertase activity may be significant in sustaining growth with an increased pool of reducing sugar in the segments.

Effects of Cycloheximide on Increases and Decreases in Invertase. It was previously reported that 10 μg/ml cycloheximide was a potent inhibitor of GA_3-augmented growth and invertase activity (19). The time course patterns of cycloheximide inhibition of invertase activity were thus examined by treating segments in the presence and absence of GA_3 with cycloheximide at three distinct phases of enzyme activity: during the initial phase of activity increase, at the peak of enzyme activity, and during the phase of declining activity.

Results for water-treated segments (Fig. 3A) indicate that cycloheximide causes an earlier and faster decrease in invertase activity than the activity pattern for the control without cycloheximide. It is of interest to note that the decay curve shows a lag when cycloheximide is added during the phase of activity increase. Results for GA_3-treated segments (GA_3 supplied continuously) are shown in Figure 3B. The curves here indicate that the decay of invertase is delayed by at least 3 hr, regardless of the time of cycloheximide addition.

To segments which were incubated continuously with

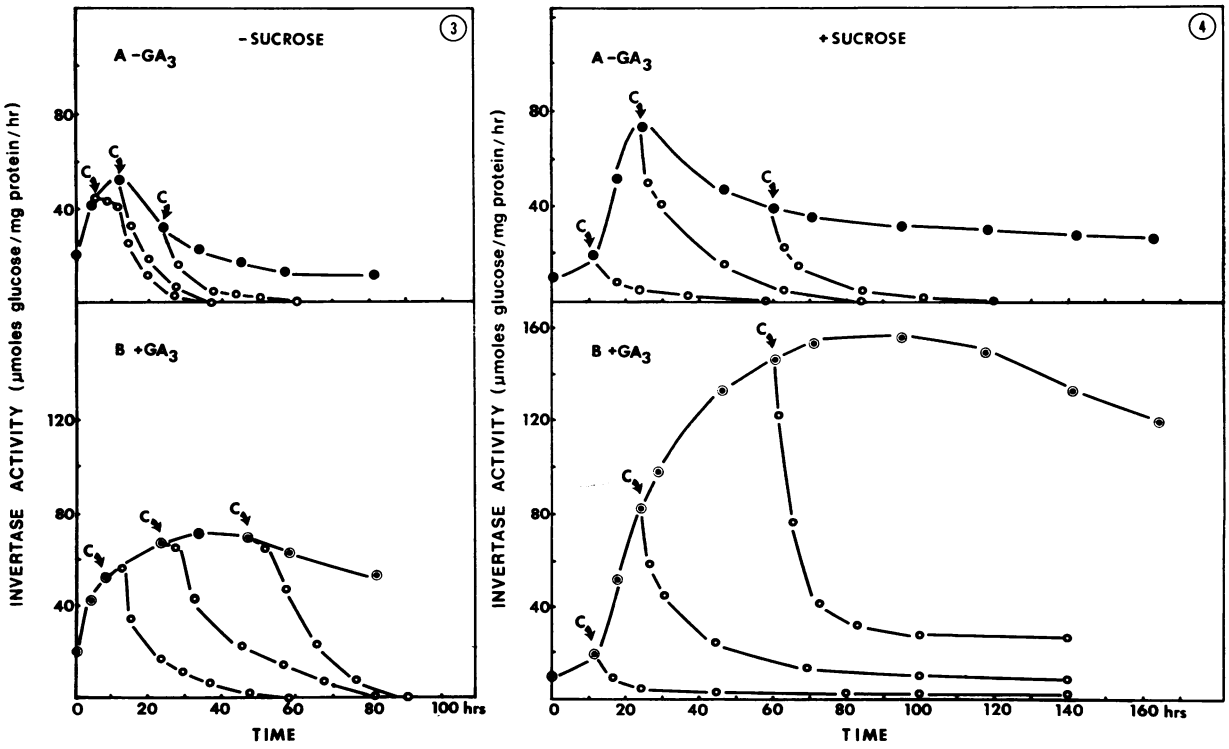

FIG. 3. Effect of cycloheximide (10 μg/ml) on the decay of invertase in water-treated (A) and GA_3-treated (B) *Avena* stem segments. For treatments in A, segments were incubated in distilled water and transferred to cycloheximide at 6, 12, and 24 hr (arrows). For GA_3-treated segments (B), the segments were first incubated in 30 μM GA_3, then transferred to GA_3 + cycloheximide at 9, 24, and 48 hr for the balance of the incubation period.

FIG. 4. Effect of cycloheximide (10 μg/ml) on the decay of invertase in sucrose-treated (A) and GA_3 + sucrose-treated (B) *Avena* stem segments. Segments were transferred to cycloheximide + sucrose (graph A) or cycloheximide + sucrose + GA_3 (graph B) at 12, 24, and 60 hr (indicated by arrows on curves).

sucrose alone and sucrose + GA_3, cycloheximide was given at 12, 24, and 60 hr of incubation. The results (Fig. 4, A and B) show that the invertase activity decreases markedly after administration of cycloheximide without any lag. The most striking decay pattern is seen where cycloheximide is added at 60 hr in the GA_3 + sucrose series (Fig. 4B): the activity drops 75% within 12 hr after adding cycloheximide. These observations clearly vary from the response seen in treatments made in the absence of sucrose, and they differ from other systems (21, 34, 35) where the enzymes studied are stabilized after the addition of cycloheximide.

To test for possible leakage of invertase from the tissue, the medium in which segments were incubated following cycloheximide treatment was assayed for invertase activity for each of the above treatments at different times throughout the respective incubation periods. In no case could any invertase activity be detected in the medium for any of the treatments.

Effects of Pulse Treatments with Substrate (Sucrose) and End Products (Glucose and Fructose) on Level of Invertase Activity. In order to determine how GA_3 and substrate and end products might regulate *in vivo* levels of invertase (see also, 12, 25, 26), segments were incubated in 30 μM GA_3 for 15 hr, then given a 60-min pulse with either 0.1 M sucrose + GA_3 or 0.1 M glucose and 0.1 M fructose + GA_3, followed by vigorous washing for 5 min in continuously flowing distilled water and transfer of the segments back to fresh GA_3. The results of this experiment are shown in Figure 5A.

Following a 60-min pulse with sucrose, there is a significant decrease in invertase activity for about 10 hr compared with the GA_3 control. This is followed by a significant rise in enzyme activity during the next 15 hr; after this, the activity decreases precipitously within 8 to 10 hr, then rises slightly, with the final activity almost the same as that of the GA_3 control at 70 hr. Following a 60-min pulse with glucose + fructose, the invertase activity drops very slightly relative to the GA_3 control activity for about 5 hr, then rises sharply, almost parallel to the sucrose-pulsed segments. The total amount of increase in level of invertase activity elicited by the glucose + fructose pulse treatment is essentially the same as obtained with the sucrose pulse treatment. After the peak, the invertase activity in the glucose + fructose pulse treatment decreases over a 10-hr period, then rises slightly and falls again. The final activity of invertase at 70 hr is slightly less than that for the GA_3 control and the sucrose pulse treatment. These results indicate that both substrate and end products may cause an increase in invertase activity after distinct lag periods. The initial decrease persists twice as long for the sucrose pulse as for the glucose + fructose pulse treatment, but the general patterns after the pulse are essentially the same for the two treatments.

To examine the glucose + fructose results more closely, each hexose was used separately at a concentration of 0.2 M (the same mole equivalent as 0.1 M sucrose) in the presence of GA_3, starting the 60-min pulse at 15 hr, as above. The results in Figure 5B indicate a distinct decrease in invertase activity, compared with the GA_3 control, immediately following the pulse with glucose or fructose. The decrease in enzyme activity following the fructose pulse is greater and longer than that for the glucose pulse. Following this period of decreased enzyme activity, the level of invertase activity rises sharply for about 15 hr for both sugar pulse treatments. The amount of elevation of invertase is greater for the glucose pulse than for the fructose pulse. After the peaks are reached, the levels of invertase activity decrease steeply below the GA_3 control levels at 40 to 50 hr of incubation, and then the activity gradually rises to the level of the GA_3 control by 70 hr of incubation.

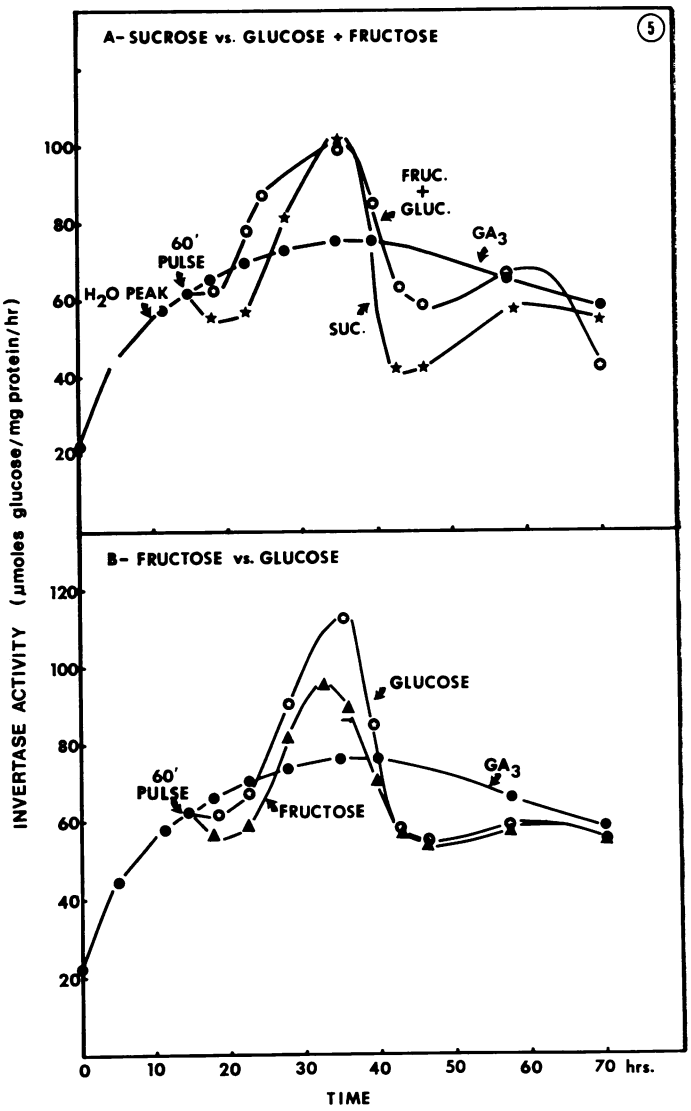

FIG. 5. Effect of a pulse treatment with sucrose (Suc) or glucose + fructose (A) and glucose or fructose (B) on the invertase level. A 60 min pulse treatment was given with 0.1 M sucrose, 0.1 M glucose + 0.1 M fructose (Gluc + Fruc), 0.5 M glucose, or 0.2 M fructose at 15 hr of incubation of segments in 30 μM GA_3. All pulse treatments with sugars were made in the presence of 30 μM GA_3.

Thus under our experimental conditions, fructose is more effective than glucose in lowering invertase activity immediately after a 60-min pulse; such a result is similar to that observed for the sucrose pulse. This lag period is relatively short compared to the relatively long ensuing period when the invertase level rises sharply and then decreases. Of interest in this connection is the observation of Glasziou *et al.* (11) that D-fructose is more effective in lowering the level of invertase activity than D-glucose in mature sugarcane stem tissue, paralleling our results with *Avena* stem segments, except for the fact that they obtain greater decrease in enzyme activity after hexose addition than we do in *Avena*.

In order to test whether GA_3 or sucrose is more effective in elevating invertase activity in *Avena* segments, we next conducted a "reciprocal pulse" experiment. Here either a GA_3 60-min pulse (in the presence of sucrose) was administered at 15 hr or a 60-min pulse with sucrose (in the presence of GA_3) was given at 15 hr. The respective controls were continuous sucrose and continuous GA_3.

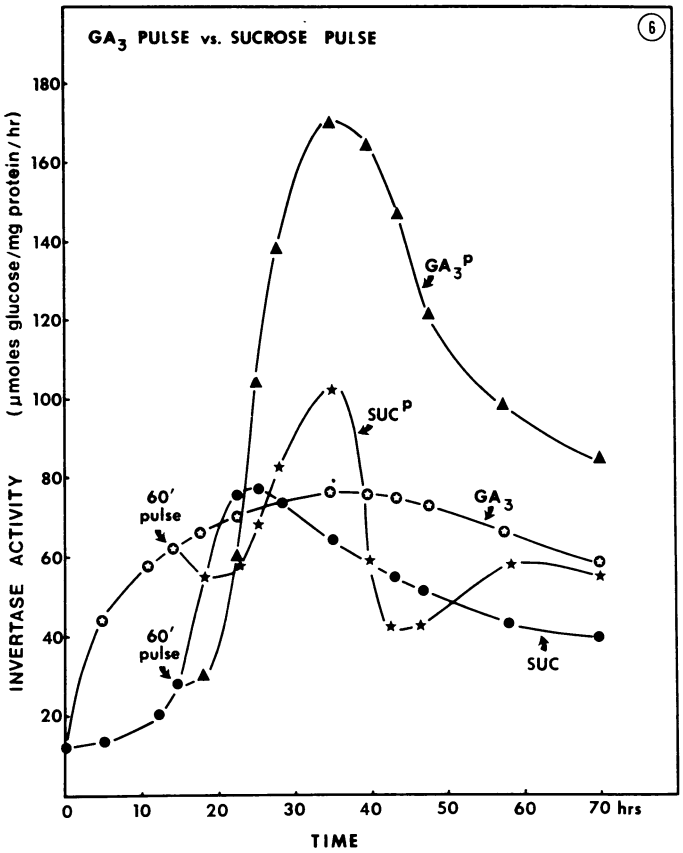

FIG. 6. Time course changes in level of invertase activity in a reciprocal pulse experiment. Segments were either pulsed with 0.1 M sucrose for 60 min at 15 hr of incubation in the presence of 30 μM GA_3 (suc^P) or with 30 μM GA_3 for 60 min at 15 hr of incubation in presence of 0.1 M sucrose (GA_3^P curve). Respective control treatments were continuous 30 μM GA_3 and continuous 0.1 M sucrose.

The results in Figure 6 indicate that both GA_3 and sucrose pulse treatments result in a lag before the level of invertase starts to increase; the lag for the sucrose pulse is about two times longer than for the GA_3 pulse (10 *versus* 5 hr). The peaks for both treatments occur at about 35 hr, 20 hr after initiation of the pulse treatments. The amount of net increase in invertase activity after the GA_3 pulse is about three times greater than that for the sucrose pulse. After the peak for the GA_3 pulse treatment, the enzyme activity follows a slow decline similar to earlier experiments (Fig. 2A). The pattern for invertase changes for the sucrose pulse is essentially the same as that described in Figure 5A. These observations clearly suggest that a GA_3 pulse treatment is far more effective in elevating invertase level than a sucrose pulse under these experimental conditions. In view of the observation that treatment with GA_3 alone causes a marked decrease in levels of endogenous glucose, fructose, and sucrose, while sucrose treatment raises the endogenous levels of these sugars (1), the presence of high levels of endogenous carbohydrates in the tissue appears to make a more drastic increase in the enzyme level in response to the hormone pulse treatment.

The net growth of the segments in these treatments at 48 hr of incubation was as follows: GA_3 control = 0.54 + 0.02 cm; sucrose control = 0.33 + 0.03 cm; GA_3 pulse = 2.79 + 0.12 cm; sucrose pulse = 0.75 + 0.02 cm. The growth of segments receiving the GA_3 pulse is about three times greater than that for the sucrose pulse, paralleling closely the results obtained for differences in amount of increase in invertase activity. Our results, therefore, support the view that the greater growth seen with the GA_3 pulse, as compared with the sucrose pulse, could have resulted from the much greater amount of increase in invertase activity elicited by this same treatment.

DISCUSSION

Our data and those of Chrispeels and Varner (6) and Filner *et al.* (8) demonstrate that GA_3 must be present continuously to maintain high levels of enzymes (*e.g. i*nvertase, α-amylase, ribonuclease) in the tissues. Bradshaw and Edelman (3) have reported that a rise in endogenous gibberellin precedes the increase in the level of invertase activity in aging Jerusalem artichoke tuber tissue. Our results (Figs. 1A, 2A, and 6) indicate that the presence of sucrose greatly enhances the GA_3 effect on the elevation of invertase activity as compared to the absence of sucrose. Substrate appears to stabilize the enzyme (19) or help the induction of enzyme (22, 26). In the presence of sucrose, increased levels of invertase are correlated with the sustained growth of *Avena* stem segments (Fig. 2). Copping and Street (5) find a close correlation between a rise in invertase activity and growth in cultured sycamore cells, which parallels the results reported herein.

Sucrose added from the beginning of incubation (Fig. 2A), or in pulses (Figs. 5A and 6), shows a lag period of 10 to 12 hr before the enzyme activity increases in the segments. Fructose, but not glucose, can cause a lag similar to sucrose in invertase induction (Fig. 5B), but the pulse treatment with glucose + fructose causes essentially the same lag period as the glucose pulse. The length of the lag period cannot be explained solely by the substrate or end product effect. Increase in the level of invertase activity by sugars alone (Figs. 5 and 6) is relatively small when compared with the effect of GA_3 (Fig. 6). The exact nature of how both the substrate and the products can cause an increased level of invertase is difficult to understand, particularly in view of the fact that glucose or fructose can be utilized without forming large amounts of sucrose endogenously (unpublished observation of M. Montague). Furthermore, the *Avena* stem segment does not contain much sucrose immediately after harvest, and the endogenous levels of glucose and fructose are higher in sucrose-incubated tissue than sucrose (1). The absence of significant amounts of endogenous sucrose could be explained by the presence of a very active invertase in the tissue as shown here, previously (19), and by Adams (1).

The rise and subsequent fall in the level of invertase observed repeatedly in this study (Figs. 1A, 2A, 5, and 6) poses a question as to whether the phenomenon is due to either turnover of the enzyme or to the activation-deactivation of the enzyme action. In the *in vitro* enzyme assays, it has been repeatedly shown that the enzyme activity is not stimulated by the addition of GA_3 (19). The apparent *Km* for sucrose of our invertase averages 20 mM and ranges from 12 to 24 mM with relatively small changes throughout the experimental periods tested (Table II). These results are taken to indicate that the conformational properties of the enzyme are not altered greatly during growth of the segments. The cycloheximide effects (Figs. 3 and 4) are somewhat unexpected, especially if the inhibitor simply turns off synthesis of the enzyme. Since the enzyme activity decreased markedly in the segments following addition of cycloheximide, and no activity was detected in the medium, the most likely explanation of the cycloheximide experiments may be that the enzyme, which is constantly synthesized and degraded, follows the pathway of degradation. This further suggests that in the presence of a

Table II. *Changes in the K_m of Avena Soluble Invertase for Sucrose following Incubation of Stem Segments in Water and GA_3*

Segments were incubated in the dark at 23 C. GA_3 (30 μM) was used continuously for the 48-hr incubation period. Experiment was repeated three times; this is a typical set of results.

Incubation Time	Km Values for Sucrose	
	$-GA_3$	$+GA_3$
hr	*mM*	
0	24	
12	22	12
24	22	16
48	20	24

constant supply of GA_3 the synthetic activity overrides the degradation pathway. The idea of enzyme turnover is attractive in view of certain similarities of our observations to other cases of GA_3-stimulated enzyme synthesis (3, 4, 6, 8, 31, 32). This possibility should include in our case the *de novo* synthesis of invertase to be stimulated mostly by GA_3, and somewhat by sugars, and the degradation of the enzyme to appear after the synthetic processes slow down, presumably due to decreased level of GA_3 or sugars. The nature of the degradative processes in unclear, but it may be speculated to involve a protease.

We have concluded earlier that the elevation of invertase activity is not the direct cause of GA_3-induced growth in *Avena* stem segments. The present work not only confirms this conclusion, but also extends it to explain the sustained growth of the segments treated with both GA_3 and sucrose (Fig. 2). The sustained growth is considered to be the result of the increased pool size of reducing sugars in the cytosol (1).

In the final analysis, what can we interpret from the data presented herein regarding hormone, substrate, and end product regulation of invertase in *Avena* stem segments? Our view is as follows. (*a*) GA_3 enhances growth before invertase activity increases; (*b*) this gibberellin-promoted growth creates a "mass action" effect, exerting a significant drain on the soluble saccharide pool; (*c*) as a result of this, invertase is derepressed, and the activity increases; (*d*) meanwhile, growth continues, such that all sugars or essential co-factors or both are used up; (*e*) as a consequence, invertase now disappears because it constantly turns over, and there is not enough substrate (amino acids) or co-factors or both to continue its synthesis. In this same context, high levels of sugars would repress invertase, as seen after 0 hr or after a sugar pulse, whereas intermediate levels derepress the enzyme. Similar results have been obtained for yeast cultures grown in different levels of glucose (J. E. Varner, personal communication). We wish to emphasize that such an interpretation, as given above, is speculative in nature. However, it is useful as a working hypothesis to explain the consistently observed rise and fall in level of invertase activity after sugar and GA_3 pulse treatments and will serve to stimulate further studies on the nature of hormone and sugar regulation of invertase in *Avena* stem segments.

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