Plant Physiol. (1975) 55, 114–119

Multiple Forms of Invertase in Developing Oat Internodes[1]

Received for publication March 22, 1974 and in revised form September 23, 1974

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ABSTRACT

Three different invertases are found in the developing internodes of oat (*Avena sativa* cv. Victory). Two soluble invertases (I and II) are separable on diethylaminoethylcellulose and Sephadex columns. They are further distinguished by their kinetic constants, heat stability, and differences in stability and apparent activity optima in response to pH treatments. Relative activities of the two soluble isozymes change considerably during the developmental stages examined. Invertase I activity rises early and begins to fall after maximal activity is reached at 6 hours of incubation. This early increase in activity accompanies the period of most rapid growth rate of the internode. Invertase II activity does not increase significantly during the first 6 hours of internode extension, but rapidly rises to a maximum activity at 16 hours, then declines. The third form of invertase, bound invertase (III), is present in both immature and mature stem tissue. Its activity increases (by 6 hours) during immature growth stages, decreases considerably with maturation, and remains relatively constant in mature tissue.

In oat internodes, a close relationship exists between growth rate and levels of invertase activity (14, 15). The higher levels of invertase activity observed during growth may reflect the increased energy and carbon requirements to sustain the biosynthetic reactions occurring in the growing parts. Enhancements in respiration (1), and protein (13), and cell wall biosynthesis (1) are observed to occur during the growth period. This suggests that carbohydrate in the tissue may be degraded in amounts proportional to growth requirements.

Although the existence of acid and alkaline forms of invertase is documented in plants, a considerable amount of work has been confined to lower plant forms (2, 7, 9), or higher plant storage, or nongrowing tissues (19, 21, 22, 24). These forms differ in properties and cellular location, but how they relate to metabolism is not known. Of particular interest is the identification of significant changes in invertase isozyme patterns during the periods represented by transition from meristematic to mature tissue in sugarcane internodes (25), and from early corn endosperm development to maturation (10), in which early stages in both systems have a preponderance of acid invertase. High acid invertase seems to be characteristic of growing cells (14, 18).

During growth in *Avena* internodes, activity of an acidic invertase is observed (13, 14), and its levels are regulated by hormone, substrate, and end products (15). The present investigation concerns further efforts to clarify the relationship of invertase to growth through identification of three invertases in oat internodes and examination of their respective activities during internode growth.

MATERIALS AND METHODS

Plant Material. Oat plants (*Avena sativa* cv. Victory) were grown in the greenhouse for 40 to 45 days as in previous studies (14). Shoots of uniform height were selected from next-to-last internodes, located immediately below the peduncular node, with lengths of 1 cm. One-centimeter stem segments, which included the next-to-last internodes, sheath base portions, and basal nodes, were then isolated from these shoots with a razor blade cutting device. Excised segments were placed upright in perforated Plexiglas frames on filter paper in 6-cm Petri dishes. Each dish contained 5 to 10 segments with their node ends in contact with 2 ml of solution.

Segments were routinely allowed to grow at 28 C in the dark. For growth experiments, length of the segments were measured with a mm ruler. Experiments were repeated at least twice.

Extraction and Fractionation of Soluble Invertase. Invertase was extracted from 100 oat stem segments with 0.1 M phosphate-citrate buffer (pH 5.0) containing 1 mM disodium EDTA. The segments were finely ground in 30 ml of cold buffer in a cold mortar and pestle. The homogenate was centrifuged at 30,000*g* for 20 min at 0 C. The pellet was re-extracted a second time with 10 ml of cold buffer, and following centrifugation, the pellet was stored at −60 C for later analysis of the bound invertase. Solid ammonium sulfate was added slowly to the supernatant fraction at 2 C with gentle stirring. The resultant mixture was allowed to stir gently for 20 min in the cold, after dissolution of the solid $(NH_4)_2SO_4$, before centrifugation at 20,000*g* for 20 min. The protein fraction, precipitating between 35 and 60% ammonium sulfate saturation, was retained, as it contains approximately 90% of the soluble invertase activity. This protein precipitate was suspended in 4 ml of cold 0.1 M phosphate-citrate buffer (pH 5.0). After the mixture was clarified by centrifugation, the enzyme solution was placed immediately on a column (2.5 cm × 30 cm) of Sephadex G-200 buffered with 0.1 M phosphate-citrate buffer (pH 5.0) at 2 C. The column was washed with the same buffer solution, and 5-ml fractions were collected at 20-min intervals.

Active invertase fractions from the G-200 column were pooled and treated with solid ammonium sulfate at 0 C. The precipitated protein from 0 to 60% saturation was collected by centrifugation and dissolved in 2 ml of 0.05 M phosphate-citrate buffer (pH 6.0). After the mixture was clarified by centrifugation, the solution was desalted on Sephadex G-25 at 2 C. The protein eluent was added to a DEAE-cellulose (Calbiochem

[1] This work was supported by a grant from The American Cancer Society.

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Corp.) column (1.5 cm × 15 cm) equilibrated at 2 C with 0.05 M phosphate-citrate buffer (pH 6.0). The enzymes were eluted with a linear 0 to 0.5 M NaCl gradient, buffered with 0.05 M phosphate-citrate buffer (pH 6.0). Five-milliliter fractions were collected.

Fractions with invertase I activity were pooled and treated with solid ammonium sulfate at 0 C. Similarly, the 5-ml fractions with invertase II activity were pooled. The precipitated protein (0 to 50% saturation) was collected by centrifugation at 20,000*g*, dissolved in 4 ml of phosphate-citrate buffer (pH 5.0), and dialyzed for 2.5 hr against 200 volumes (two changes) of the same buffer mixture. The final clear solution was distributed among several tubes and stored in a deepfreeze at −60 C. Although the stability of the enzyme preparation is somewhat variable, good activity is retained for several weeks. This enzyme fraction was utilized for determination of the chemical and kinetic properties of invertases I and II.

The amount of invertases I and II in the stem segments during development was measured after ammonium sulfate fractionation (0 to 60%), desalting, and finally fractionating the protein extract on DEAE-cellulose. Each fraction was analyzed for invertase activity and soluble protein. The separate activities were added to give a total activity for the two isozymes.

Measurement of Enzymatic Activity. The reaction mixture contained 60 μmoles of sucrose and 100 μmoles of phosphate-citrate buffer in a total reaction volume of 1.0 ml. The formation of reducing sugar was measured by the method of Nelson (20). The quantity of the enzyme preparation added and the incubation time at 30 C were normally adjusted so that not more than 10% of the substrate was reacted. Absorbance was read with a Klett-Summerson colorimeter (No. 54 filter). For all the invertase preparations reported in this paper, enzyme activity was linearly proportional to time and the amount of protein assayed at all pH values.

Soluble protein was determined according to the method of Lowry *et al.* (17). Bovine serum albumin was used as a standard. Carbohydrate was determined by using the anthrone method of Roe (23).

Extraction and Assay of Bound Invertase. The cellular debris saved from the initial centrifugation was resuspended in 20 volumes of cold acetone and homogenized again with a cold mortar and pestle. The mixture was suspended in fresh acetone, filtered on a Büchner funnel, and the residue was washed twice with fresh cold acetone. A gentle suction was used, and at all times, the material was covered with acetone. After the second wash the preparation was washed with cold peroxide-free diethyl ether while the precipitate was continuously stirred. The ether was filtered off completely. The final powder was scraped from the filter paper into a Petri dish and dried *in vacuo* over P_2O_5 in a desiccator. After thorough drying the powder was stored at −15 C. Enzyme activity was stable for at least several weeks.

For assay of bound invertase III, 10 mg of the cell wall powder was suspended in a reaction mixture consisting of 60 μmoles of sucrose and 120 μmoles of phosphate-citrate buffer (pH 5.0) in a total volume of 0.5 ml. The mixture was incubated at 30 C, and the reaction was stopped by the addition of 0.5 ml of 0.1 N NaOH. Ten minutes after the addition of NaOH, 2 ml of water were added; the reaction mixture was stirred and then centrifuged at 1,500*g*. One ml of the supernatant fraction was removed and assayed for reducing sugar content by the method of Nelson (20).

RESULTS

Chromatographic Separation of the Invertase Isozymes. Initial studies indicated that two peaks of invertase activity could be resolved by column chromatography of cell homogenates from developing oat internodes on DEAE-cellulose (Fig. 1). The invertase activities from fractions 5 to 7 and 11 to 15 are designated, respectively, as I and II.

The existence of two soluble invertase isozymes in the internodes during both cell elongation and maturation can also be demonstrated after fractionation of cell homogenates by Sephadex G-200 chromatography. The ratio of total activities in both peaks is similar to that found when identical cell homogenates are separated on DEAE-cellulose, suggesting that the isozymic forms are not artifacts of the DEAE-cellulose separation procedure. A typical elution profile from a tissue homogenate of developing stem segments is depicted in Figure 2. Void volume (V_o) was determined with Dextran 2000, assayed with the anthrone reagent. Invertase I eluted with the exclusion volume (V_o) of the column bed. Sephadex G-200 has an exclusion limit of approximately 300,000; therefore, the mol wt of invertase I is probably larger than this value. In-

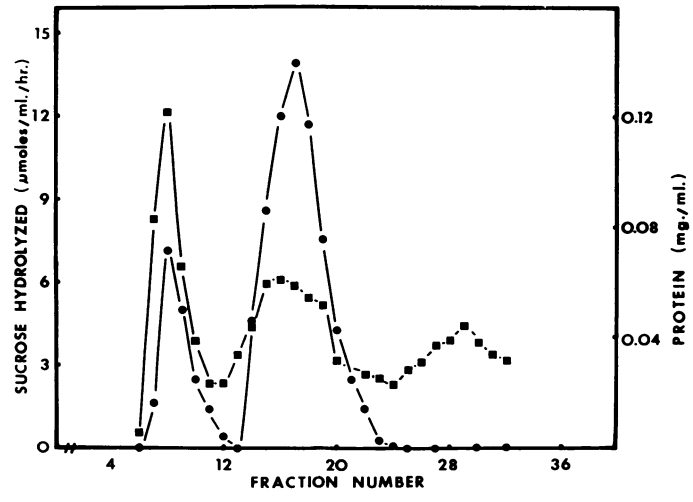

FIG. 1. Elution profile with DEAE-cellulose column fractionation of an extract made from excised stem segments as described under "Materials and Methods." After elution of 5-ml fractions from this homogenate, protein (■) and invertase activity (●) were assayed as described under "Materials and Methods."

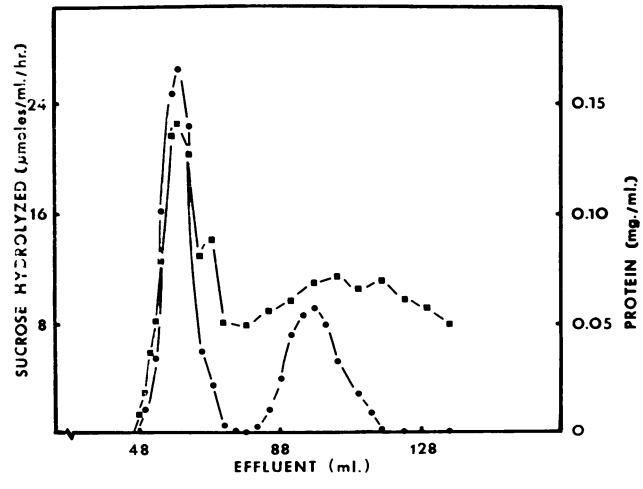

FIG. 2. Elution profile with Sephadex G-200 fractionation of a homogenate from developing stem segments as described under "Materials and Methods." Protein (■) and invertase activity (●) were assayed in the eluate as described under "Materials and Methods."

vertase II eluted at a later position than invertase I, indicating a lower mol wt. An approximate mol wt could not be obtained because of the lack of appropriate mol wt protein standards.

pH Relationship of Oat Invertase Activity. The pH optima for invertases from a number of higher plants have been reported to be around 4.5 to 5.0 or 7.0 to 8.0. The effects of pH on enzyme instability and on ionization of groups in the active site of the enzyme, however, have not been separated. Also, reactions have been performed at a single substrate concentration without reporting its relationship to the K*m* (5). Therefore, it seemed desirable to study the pH-activity profile in greater detail.

As a first step in evaluation of the effect of pH on enzymic sucrose hydrolysis by the soluble and bound enzymes, reactions were conducted by using a range of buffers which maintained good buffering capacity over the pH range examined (Fig. 3). Optimal activity for the bound invertase II and invertase III forms was obtained at pH 5.3; that for invertase I occurred at pH 4.9. The shapes of the curves differ slightly in detail.

Stability of the soluble isozymes as a function of pH was measured using the standard conditions, except for adjustment to a series of pH values. In one series of experiments, the enzyme was preincubated at 30 C for 20 min at various pH values. Standard activity assays were then performed after adjustment of the pH to 5.0. Invertase I appeared to be stable under the test conditions from pH 4.1 to 7.0; inactivation was very significant below pH 3.3 (Fig. 4). In contrast, invertase II

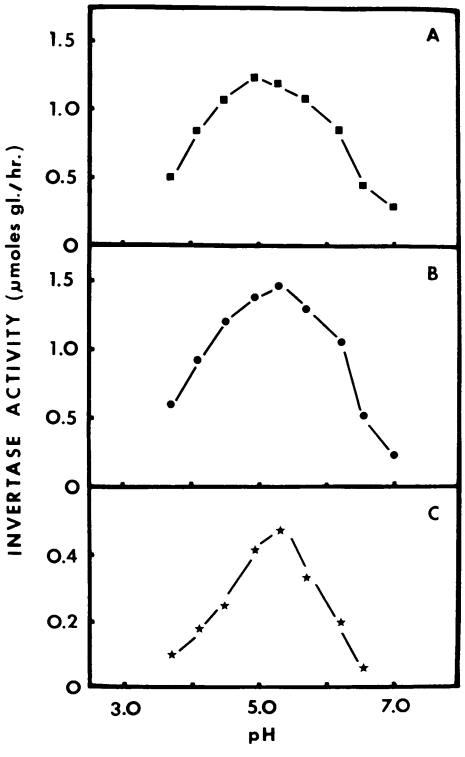

FIG. 3. Effect of pH on the activity of invertases I, II, and III. Reaction mixtures of 1.0 ml contained 100 μmoles of buffer; 60 μmoles of sucrose; and 24 μg of invertase I, 17 μg of invertase II, or 10 mg of cell wall-bound III (A, B, and C, respectively). Buffers used were citrate (pH 3.3, 3.7, and 4.1), acetate (pH 4.1, 4.5, 4.9, and 5.3) and potassium phosphate-citrate (pH 5.3, 5.7, 6.2, 6.6, and 7.0). The values obtained at the same pH values have been used to normalize the data on the basis of phosphate-citrate buffer values. Each value presented is the mean of three replicates.

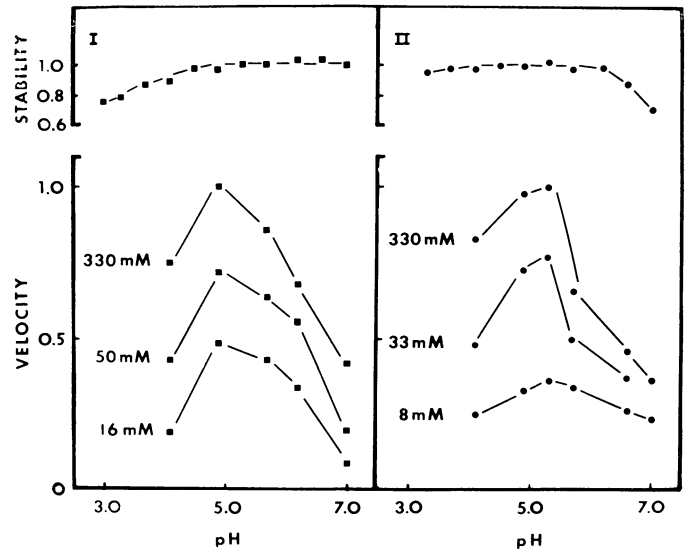

FIG. 4. Effect of pH on oat invertase isozymes I and II. Enzyme stability (■) after preincubation at various pH values is expressed as a fraction (1.0 = complete stability). Initial velocities (●) for the enzymic hydrolysis of sucrose are normalized about the value at pH 4.9 (invertase I) or pH 5.3 (invertase II) at 0.33 M sucrose. Sucrose concentrations are indicated on the appropriate curves.

activity was greater than invertase I at lower pH values, and it was diminished near a neutral pH. It is apparent from these results that the isozymes can be further characterized by differences in their stability and activity optima in response to pH treatments.

The shape of initial reaction velocity *versus* pH curves (Fig. 4) is strongly dependent on sucrose concentrations. Initial velocities were determined at 2-min intervals for approximately 10 min without preincubation. Controls (boiled enzyme, included for every treatment) revealed that nonenzymatic hydrolysis was not significant in the time intervals employed.

Effect of Temperature on Invertases I and II. Invertase I is clearly more thermolabile to heat treatment (45 C) than invertase II (Fig. 5). Preincubation at 45 C for 6 min results in a 75% loss in invertase I activity, but correspondingly, only a 25% loss in invertase II activity. After 20 min of preincubation at 45 C, invertase I retains about 15%, and invertase II about 60%, of their respective original activities.

Substrate Specificity. The three forms of oat invertase were characterized by their ability to act upon certain saccharides. The nonreducing saccharides were examined at pH 5.0 using the standard assay conditions, except that two different final concentrations of each test sugar were employed (50 and 100 mM). These same concentrations were used for reducing saccharides which were tested at pH 5.0 and 6.0 and were assayed for glucose liberation as measured by the glucose oxidase method (Worthington).

The results of this study are summarized here. Negative results represent activity less than 1% of that for sucrose under identical assay conditions. Each form of invertase exhibited identical substrate specificities: invertases I, II, and III will hydrolyze the three unsubstituted B-fructofuranosides tested (sucrose, raffinose, and methyl-*p*-D-fructofuranoside), and cleave a substituted β-fructofuranoside (melezitose). The invertases (I, II, and III) also fail to cleave nonreducing O-glucosides or β-galactosides (maltose, trehalose, and lactose). The substrate specificities are thus consistent with that for a

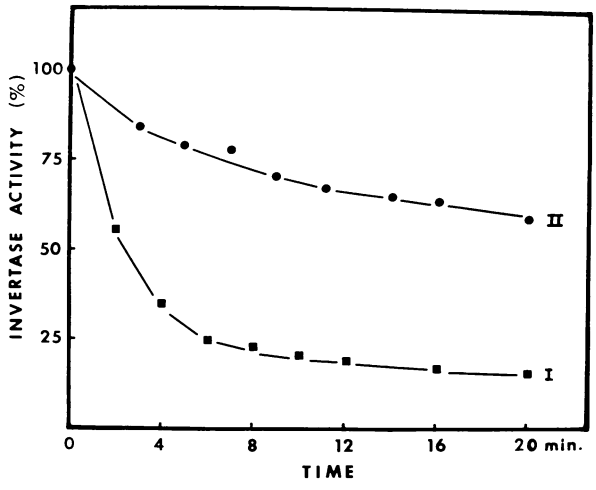

FIG. 5. Effect of preincubation at 45 C on inactivation of invertases I and II. Invertase I (45 μg) or invertase II (32 μg) was preincubated at 45 C with 100 μmoles of phosphate-citrate buffer (pH 5.0) for the times indicated. After preincubation, the enzyme was cooled, and sucrose (60 μmoles) was added to start the reaction. The total reaction volume was 1.0 ml, and the reaction mixture was incubated for 10 min at 30 C. Each point represents the mean of two replicates.

β-fructofuranosidase (3.2.1.26) for each of these invertase isozymes.

Differences in Kinetic Properties of Soluble Invertases. The effect of substrate concentration on the splitting of sucrose and raffinose was examined next. Incubations were performed according to the standard assay procedure for invertase, with the exception that for each fructoside, a minimum of six different substrate concentrations were employed (16–333 mM). Incubations were performed in duplicate. The Michaelis constants (K*m*) and maximum velocities (V_{max}) were calculated from $(S)/V$ *versus* (S), and $1/(S)$ *versus* $1/V$ plots of the data which were linear. Initial velocities at each substrate concentration were computed, and regression analysis of the points was performed with the aid of an IBM 360 computer which determined the slope and intercept for each series of replicates. From the results presented in Table I it is evident that the two soluble invertases can be distinguished by their reaction kinetics. Sucrose exhibits the smallest K*m* and the largest V_{max} for both invertase I and II, in comparison with those for raffinose. However, invertase I has a larger K*m* for sucrose (19 mM) and a higher relative V_{max} (100) than invertase II which has a much lower K*m* (5 mM) for sucrose and a lower relative V_{max} (79) under identical conditions. Thus, between invertase I and invertase II, sucrose is a better substrate at high concentrations for the former, whereas the latter splits sucrose more rapidly at low substrate concentrations. Raffinose, as a substrate for the two invertases, shows the same trend.

When the K*m* and V_{max} of invertase I for sucrose were plotted as a function of pH (Fig. 6B), little change in K*m* was noted between pH 4.1 and 6.2. Above pH 6.2 the K*m* rose rapidly, while the V_{max} for invertase I gradually decreases at a pH above 4.9. In contrast the K*m* of invertase II is relatively constant over the pH range of 5.3 to 6.6, and it increases on either side of this plateau (Fig. 6A). At a pH greater than 5.7 the V_{max} decreases for invertase II. Both soluble forms show a large decrease in V_{max} occurring toward the neutral pH region. Because the substrate has no ionizable groups, this decrease seems to be the result of protonation of a group on the enzyme with a pK near 6.8.

Bound Invertase. A third form of invertase (invertase III) present in developing oat internodes was bound to cellular debris after extraction. Starch granules, selectively separated from the cell walls by passage through Miracloth, had little invertase activity. When the cell wall pellets were extracted a second time with the same buffer, no activity was recovered in the soluble fraction. Extractions at higher or lower pH values had the same negative effect. The bound invertase was not solubilized by salt (1 M), organic solvents, 8 M urea, dimethylformamide, Tween 80, or Triton X-100. These results suggest that the enzyme is indeed cell-wall bound.

Effect of Various Treatments on Invertase I and II Relationships. In order to correlate the different levels of invertase I and II activity with different developmental states of the tissue, the role of possible interconversion or selective inactivation had to be clarified. The effect of β-mercaptoethanol or dithio-

Table I. *Kinetic Constants for Soluble Oat Invertases I and II*

Substrate	Invertase I		Invertase II	
	K*m*	V_{max} (relative)	K*m*	V_{max} (relative)
	mM		mM	
Sucrose	19.0	100[1]	5.0	79
Raffinose	124.0	63	42.0	24

[1] Measured at 30 C with 100 mM potassium phosphate-citrate buffer at pH 5.0.

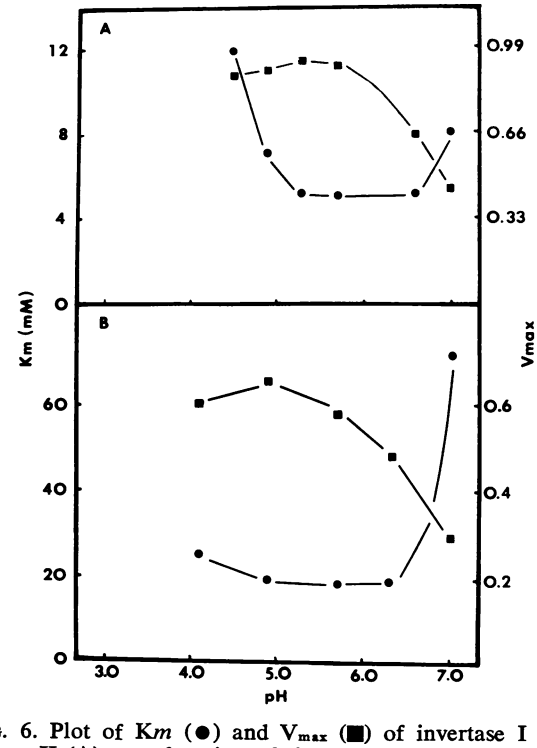

FIG. 6. Plot of K*m* (●) and V_{max} (■) of invertase I (B) and invertase II (A) as a function of the pH of the reaction mixture. Duplicates of invertase I and II were reacted for 15 min with six sucrose concentrations at each pH. Volume of the reaction mixture was 1 ml. It contained: 30 μg of invertase I or 20 μg invertase II (A or B, respectively); acetate buffer (pH 4.1, 4.5, 4.9, 5.3, and 5.7) or potassium phosphate buffer (pH 5.3, 5.7, 6.2, 6.6, and 7.0) at 50 mM; and sucrose from 33 mM to 333 mM. Invertase activity in acetate buffer was normalized at pH 5.3/5.7 to correspond to its activity in phosphate buffer.

threitol, during preparation on the relative proportions of invertases I and II, was therefore assayed. The enzymes were prepared as described under "Materials and Methods" except that 10 mM β-mercaptoethanol or 5 mM dithiothreitol was included or excluded in the buffer solution used, as appropriate. The results indicated that the relative proportions of invertase I to II remained relatively constant in preparations made with or without β-mercaptoethanol or dithiothreitol.

Inclusion of 5% glycerol in the extraction medium slightly enhanced the total level of extractable invertase, presumably by stabilization of the enzymes. However, preferential stabilization of either invertase I or II was not observed. Brief exposure of invertase I or II to dilute HCl acid leads to loss in enzyme activity, but no interconversion was observed. Likewise, storage of crude extracts or purified enzyme at −5 C to −60 C was not found to alter the relative ratios of the two isozymes.

The possibility that invertase II could arise from invertase I via limited proteolysis may also be excluded. The existence of a protease (or carbohydrase) present in the crude extract resulting in the interconversion of I to II was not observed when invertase I was incubated with crude extract at 30 C for 1 to 2 hr, followed by DEAE-cellulose column chromatography.

Changes in Invertase I and II Activity during Internodal Development. Analysis of the relative activities of invertase I and II during excised internode development indicates that the two isozymes follow a similar pattern of an increase, and then later, a decrease in activity (Fig. 7). Invertase I activity rises early and begins to fall by 6 hr of incubation. This early increase in activity accompanies the period of most rapid growth rate of the internode (Table II, and ref. 14). In contrast, invertase II activity does not increase significantly during the first 6 hr of internodal growth; instead, it rapidly rises to a maximum at 16 hr. Its activity then declines. It is important to emphasize that even though the relative amounts of invertase I and II activities change during development, both activities are present throughout the periods examined.

In several storage plant systems, including potatoes (21), corn endosperm (11), and red beets and sugar beets (22), an invertase-inactivating protein has been found which is present only at certain stages of development or under certain storage temperature conditions. The presence of such factors as this,

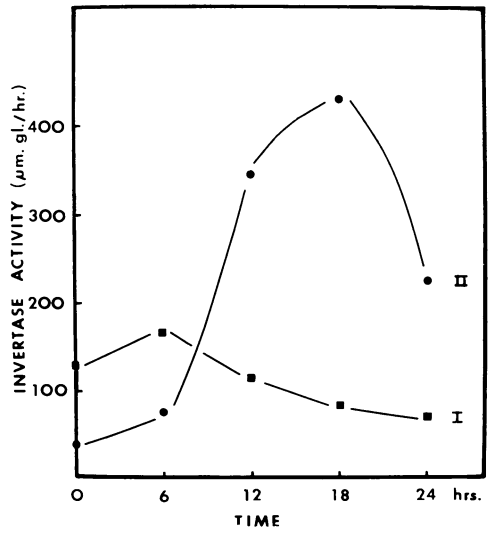

FIG. 7. Changes in invertase I (■) and II (●) activity during internodal development. Excised stem segments were incubated on H_2O at 23 C and harvested at various times.

Table II. *Activity of Bound Invertase III during Excised Internode Development*

Time	Growing Tissue		Mature Tissue	
	Net growth	Invertase activity[1]	Net growth	Invertase activity
hr	*cm*	*nmoles glucose/mg·hr*	*cm*	*nmoles glucose/mg·hr*
0	0.0	29.8	0.0	19.4
6	0.10	41.0	0.0	18.8
12	0.20	35.3	0.0	17.8
18	0.24	28.8	0.0	15.9
24	0.27	29.4	0.0	12.7

[1] Invertase activity is given as the mean of three to four replicates.

which might change during development, could have marked effects upon invertase activity during extraction. Two approaches were used to assess such a possibility. Purified invertase preparations were incubated with either (*a*) various concentrations of dialyzed cell homogenates, in particular from later growth stages when invertase activity is observed to decline, or (*b*) protein fractions separated by column chromatography. Subsequent assay for invertase activity indicated the absence of such specific inactivating factor in *Avena* internode tissue.

Activity of Bound Invertase during Development. The activity of bound invertase in immature and mature sections of the stem at several stages of development is given in Table II. Assays for the bound invertase were more variable than those for the soluble invertases. Therefore, activity is presented as the mean of three to four replicates. Two hundred 20-mm stem segments were isolated from the bases of selectively chosen, growing internodes that had extended 20 mm. The segments containing the node, intercalary meristem, and the elongating region of the growing internode, as well as a portion of mature tissue, were then transferred to Petri dishes for incubation as described under "Materials and Methods." As appropriate, 40 stem segments were randomly removed at different times and the basal growing, 7-mm region (immature tissue) was excised. Likewise, a 7-mm portion containing the uppermost mature, nongrowing portion was removed. These two groups of stem sections were then analyzed separately for bound invertase as described under "Materials and Methods."

The results with the immature tissue clearly show that there are high levels of bound invertase during the early phases of stem growth in culture (0–12 hr), associated with pronounced cell wall development (1). As growth of the stem continues (12–24 hr), the relative amount of invertase activity per mg of cell wall material decreases, and is indistinguishable from that for time zero tissue. This suggests that invertase associated with the cell wall may be bound only during periods of rapid cell division or initial cell elongation. Support for this is seen with the incubation of mature stem tissue (Table II), in which levels of bound invertase remain stationary during the incubation times examined. The relative amount of bound invertase is considerably lower (approximately 50%) in mature tissue than that in growing tissue.

DISCUSSION

Two soluble invertases have been identified and characterized in developing oat internodes. Their relative activities change considerably at different stages of development. However, they are distinct and apparently noninterconvertible

enzymes. Both soluble enzymes have acid pH activity optima, in contrast to other plant systems (4, 18, 25) where single acid and neutral invertases exist. This may reflect subtle differences in the regulation of the invertase activities in *Avena* tissue.

In freshly excised stem tissue, invertase I activity is much higher than that of invertase II. At this period, the internode is composed almost entirely of meristematic and elongating cells (Jones, unpublished data). Invertase I activity continues to rise during incubation preceding the period of greatest growth (*i.e.*, cell elongation), and it then declines. In the absence of exogenous substrate and hormone, cell division activity within the excised intercalary meristem rapidly declines (12, and Jones, unpublished data). Decline in division activity of the meristem is accompanied by a decline in invertase I activity. These observations suggest that this isozymic form of invertase may be linked with metabolism of meristematic cells.

There is little invertase II activity present while meristematic activity predominates in oat stem tissue. An increase in invertase II activity accompanies the initial period when cell maturation predominates. Its activity then declines. Copping and Street (5) also find a close correlation between a rise in invertase activity and growth in cultured sycamore cells. Although it is difficult to draw firm conclusions from such observations, it is apparent that the isozymic complements of invertase are altered during cell maturation. This further suggests possible differences in the physiological function(s) of invertase I and II during oat internode development.

The invertase thus far studied in oat stem segments is primarily in the soluble fraction (15), in contrast to other tissues where the bulk invertase activity is associated with the cell wall fraction (6, 16, 28). Invertase activity could not be removed from the cell wall fraction by the procedures tested. Other workers have similarly found invertase tightly associated with the cell wall. These results tend to rule out the possibility that the observed rise in soluble oat invertase activity is caused by exchange of bound invertase with the cytoplasm. The existence of a minor amount of loosely bound invertase cannot be entirely ruled out. Under these conditions, the enzyme could be released into the incubation medium, or it could be solubilized into the soluble fraction during enzyme extraction. The former possibility consistently has not been observed in previously cited studies (13, 15).

Our data show that the invertase III activity, which is associated with the cell wall, increases rapidly during the growth phase of excised immature tissue. A comparable increase in wall-bound invertase activity is not observed upon incubation of mature stem tissue. In fact, the amount (on a per mg of cell wall basis) of bound invertase activity is considerably lower in mature tissue (50%) than that found in growing tissue. Similar distribution patterns have been described for invertases in varying regions of developing roots, *e.g.*, in pea (27) broad bean (3), and corn (8). These observations suggest that the invertase associated with the cell wall may be bound only during periods of initial or rapid cell elongation. The evidence also suggests that wall-bound enzyme is inactivated and not replaced during maturation. Thus a means is provided by which transported sucrose may preferentially be taken up in actively growing oat cells. This assumes that hydrolysis of sucrose at the cell surface is a prerequisite to its uptake (26).

LITERATURE CITED

1. Adams, P. A. 1969. Studies on gibberellic acid-induced growth in *Avena* stem segments. Ph.D. dissertation. University of Michigan, Ann Arbor.
2. Beteta, P. and S. Gascon. 1971. Localization of invertase in yeast vacuoles. FEBS Lett. 13: 297-300.
3. Brown, R., W. S. Reith, and E. Robinson. 1952. The mechanism of plant cell growth. Soc. Exp. Biol. Symp. 6: 329-347.
4. Cooper, R. A. and R. N. Greenshields. 1964. The partial purification and properties of two sucrases of *Phaseolus vulgaris*. Biochem. J. 92: 357-364.
5. Copping, L. D. and H. E. Street. 1972. Properties of the invertases of cultured sycamore cells and changes in their activity during culture growth. Plant Physiol. 26: 346-354.
6. Edelman, J. S. and M. A. Hall. 1964. Effect of growth hormones on development of invertase associated with cell walls. Nature 201: 296-297.
7. Gascon, S., N. P. Newmann, and J. O. Lampen. 1968. Comparative study of the properties of the purified internal and external invertase from yeast. J. Biol. Chem. 243: 1573-1577.
8. Hellebust, J. A. and D. F. Forward. 1962. The invertase of the corn radicle and its activity in successive stages of growth. Can. J. Bot. 40: 625-628.
9. Hoshino, J., T. Kaya, and T. Sato. 1964. Formation and characterization of three types of yeast invertase. Plant Cell Physiol. 5: 495-506.
10. Jaynes, T. A. and O. E. Nelson. 1971. Invertase activity in normal and mutant maize endosperms during development. Plant Physiol. 47: 625-628.
11. Jaynes, T. A. and O. E. Nelson. 1971. An invertase inactivator in maize endosperm and factors affecting inactivation. Plant Physiol. 47: 629-634.
12. Jones, R. A. and P. B. Kaufman. 1971. Regulation of growth in *Avena* stem segments by gibberellic acid and kinetin. Physiol. Plant. 24: 491-497.
13. Jones, R. A. and P. B. Kaufman. 1971. Regulation of growth and invertase activity by kinetin and gibberellic acid in developing *Avena* internodes. Physiol. Plant. 25: 198-203.
14. Kaufman, P. B., N. Ghoseh, and H. Ikuma. 1968. Promotion of growth and invertase activity by gibberellic acid in developing *Avena* internodes. Plant Physiol. 43: 29-34.
15. Kaufman, P. B., N. Ghoseh, J. D. LaCroix, S. L. Soni, and H. Ikuma. 1973. Regulation of invertase levels in *Avena* stem segments by gibberellic acid, sucrose, glucose, and fructose. Plant Physiol. 52: 221-228.
16. Klis, F. M. and A. Hak. 1972. Wall-bound invertase activity in *Convolvulus callus:* increase after subculturing, and paradoxical effects of actinomycin D, cycloheximide, and thienylalanine. Physiol. Plant. 26: 364-368.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
18. Lyne, R. L. and T. Aprees. 1971. Invertase and sugar content during differentiation of roots of *Pisum sativum*. Phytochemistry 10: 2593-2599.
19. Nakagawa, H. 1972. Purification and some properties of two types of β-fructofuranosidase from tomato fruit. Agric. Biol. Chem. 36: 18-26.
20. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380.
21. Pressey, R. 1966. Separation and properties of potato invertase and inhibitor. Arch. Biochem. Biophys. 113: 667-674.
22. Pressey, R. 1968. Invertase inhibitors from red beet, sugar beet, and sweet potato roots. Plant Physiol. 43: 1430-1434.
23. Roe, J. H. 1966. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 212: 335-343.
24. Sacher, J. A. 1966. The regulation of sugar uptake and accumulation in bean pod tissue. Plant Physiol. 41: 181-189.
25. Sacher, J. A., M. D. Hatch, and K. T. Glasziou. 1963. The sugar accumulation cycle in sugar cane I. Studies on the enzymes of the sugar cycle. Plant Physiol. 38: 338-343.
26. Sacher, J. A., M. D. Hatch, and K. T. Glasziou. 1963. Sugar accumulation cycles in sugar cane III. Physical and metabolic aspects of the cycle in immature storage tissues. Plant Physiol. 38: 348-354.
27. Sexton, H. R. and J. F. Sutcliffe. 1969. The distribution of B-glycerophosphatase in young roots of *Pisum sativum* L. Ann. Bot. 33: 407-419.
28. Strauss, J. 1962. Invertase in cell walls of plant tissue cultures. Plant Physiol. 37: 342-348.