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Acid and Alkaline Invertases in Suspension Cultures of Sugar Beet Cells

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ABSTRACT

Alkaline invertase was induced during the initiation of suspension cultures of single cells from leaf explants of sugar beets in Murashige-Skoog liquid medium which contained benzyladenine. This activity was barely detectable in the leaves themselves. In suspension cultures, the presence of both acid and alkaline invertases was detected; alkaline invertase was only present in the cytoplasm of the cultured cells, whereas acid invertase was present in the cytoplasm and cell walls, and was also detected in the culture medium. The cell wall contained at least three types of acid invertase; two of these activities were solubilized by saline (saline-released) and EDTA (EDTA-released), respectively, and the third remained tightly associated with the cell wall. Saline-released and EDTA-released invertases from the cell wall showed the significant differences in their properties: the saline-released enzyme had the highest affinity for sucrose among the invertases tested, and was easily bound to cell walls, to DNA, and to a cation exchanger, unlike the EDTA-released enzyme. Sucrose is the source of carbon for plant cells in suspension culture and is probably degraded in the cell wall by the saline-released invertase, which had the highest activity and the highest affinity for sucrose. Hexose products of this degradation would be transported to cytoplasm. Soluble invertase, EDTA-released invertase from the cell wall, and one of two extracellular invertases behaved similarly upon chromatography on DEAE-cellulose. They had similar activity profiles with changing pH, and similar K_m values for sucrose. Thus it appears that they are identical. Two extracellular invertases found in the growth medium of the suspension cultures were probably identical with those in the soluble fraction of callus and seedlings of sugar beets, because they showed similar behaviors during chromatography on DEAE-cellulose, and had similar activity profiles with changing pH and K_m values for sucrose.

Plant invertases have been extensively studied and are well known to be involved in the degradation of sucrose, which is an important storage compound in higher plants and is the usual form of carbon involved in transportation of nutrients to developing organs. There are acid and alkaline (or neutral) invertases which are defined on the basis of the pH required for maximum activity. Both types of enzyme often occur in the same tissue (2, 9, 11), but acid invertase is generally situated in the cytoplasm and in the cell wall in the intact tissues (1, 3), and, in the case of cultured cells, the enzyme is secreted into the medium (16–18). Alkaline invertase is located exclusively in the cytoplasm (11, 17). Enzymes bound to the cell wall are generally released by high concentrations of NaCl, but often considerable activity remains in the residual cell wall after treatment with saline (5, 8, 19). It is of interest to determine whether the saline-released, wall-bound enzyme and the enzyme that remains bound are identical, and whether, if different, they have different physiological functions.

Suspension cultures of plant cells are suitable systems for a variety of biochemical investigation, and have used such a system to investigate the relationship between utilization of external sucrose and the various types of invertase in suspension cultures of cells derived from sugar beets.

We report here the existence of various invertases in the soluble, cell wall, and extracellular fractions, of sugar beet cells in suspension culture with emphasis on the wall-bound invertases which are present as three different forms. Invertases in cells in liquid culture are compared with those in callus and intact tissue of seedlings, and the relationship between the uptake of sucrose from the culture medium by cells and the various types of invertase is discussed.

MATERIALS AND METHODS

Plant Material. Sugar beet (*Beta vulgaris* L.) plants were grown on vermiculite for 4 weeks in a growth chamber, with a 16h:8h light:dark cycle at 24°C.

Induction of Callus and Suspension Cultures of Cells from Leaf Explants. Callus from leaf explants of sugar beets was initiated by the methods of Saunders (13) as follows. The harvested 4-week-old leaves were surface-sterilized in 70% ethanol for 30 s, then in a solution of sodium hypochlorite (approximately 0.05%) for 10 min, and washed three times with sterile water, and cut into 4 × 4 mm^2 squares. The basal medium was solid MS[1] medium (10) supplemented with 3% sucrose, and 0.8% agar (Wako Pure Chemical Industries. Ltd.). The pH was adjusted to 5.8 with 1 N KOH before autoclaving (15 min at 120°C). Four leaf explants were placed on solid medium in Petri dishes (90 × 20 mm) with 35 to 40 ml of medium per plate and cultured in dim light at 32°C. Callus cultures were initiated about 3 weeks after inoculation of leaf explants and were subcultured every 3 weeks on MS medium without plant hormones. Suspension cultures of cells were prepared by the transfer of samples of callus into liquid MS medium without plant hormones and the cells then were sieved through nylon mesh of pore size 84 to 295 μm. Cells were subcultured every 7 d.

Direct induction of a suspension culture of cells from leaf explants was performed as follows. Fifty leaf explants were inoculated into 10 ml of MS liquid medium which contained 0.25 mg/L BA in a 50 ml Erlenmeyer flask and then agitated on a reciprocal shaker in dim light at 27°C for 10 d. The single cells released from the edges of the explants during cultures were sieved through a nylon with pore size 295 μm, then collected on nylon with pore size 84 μm, and transferred into MS liquid

[1] Abbreviations: MS, Murashige-Skoog; BA, benzyladenine.

medium that contained 0.25 mg/L BA. After 2 to 3 weeks, clusters of cells began to form and then developed rapidly. The suspension cultures thus obtained were subcultured every 7 d. Cells from callus and cells in suspension culture were autonomous nature with respect to their hormal requirements.

Preparation of Invertases. *Preparation of Soluble Invertase.* Cells, cultured for 7 d, were separated from the medium by vacuum filtration, washed with 10 mM phosphate buffer (pH 7.4) and weighed. The cells (100 g fresh weight) were washed with 5 mM Na-phosphate buffer (pH 7.4), then homogenized with three volumes of the same buffer in a Polytron homogenizer (Kinematica) for 3 min. The homogenate was filtered through nylon screen with 42 μm pore size. Solid ammonium sulfate was added to the filtrate to a final concentration equivalent of 70% saturation. After centrifugation, the precipitate was dissolved in a small amount of 5 mM Na-phosphate buffer (pH 7.4) and dialyzed overnight at 4°C against the same buffer. After centrifugation of the dialyzed solution, the supernatant was applied to a DEAE-cellulose column (2.5 × 30 cm), preequilibrated with 5 mM phosphate buffer (pH 7.4). The column was first washed with the phosphate buffer to remove the unadsorbed proteins, and then eluted with a linear gradient from 0 to 0.3 M NaCl in 500 ml of the buffer. Fractions containing active invertase were pooled and designated as soluble invertase.

Preparation of Wall-Bound Invertase. The cell wall fraction, obtained by filtration of the homogenate as described above, was washed thoroughly with distilled water and then suspended in 0.1% (w/v) sodium deoxycholate. After the suspension had been allowed to stand at room temperature for 2 h, the residue was washed thoroughly with distilled water to remove deoxycholate. No invertase activity was removed by washing the cell walls with the deoxycholate. The purified cell walls thus obtained were used for the solubilization of wall-bound invertase.

Preparation of Extracellular Invertase. The medium obtained by filtration of suspension cultures was brought to 70% saturation by the addition of solid ammonium sulfate. The precipitate was collected by centrifugation, dissolved in a small amount of 5 mM phosphate buffer (pH 7.4), and dialyzed against the same buffer. The dialyzed solution was applied to a DEAE-cellulose column (2.5 × 30 cm), preequilibrated with 5 mM phosphate buffer (pH 7.4). The column was washed with the same buffer and then eluted with a linear gradient 0 to 0.3 M NaCl in 500 ml of the buffer. The fractions containing active invertase were pooled and designated as extracellular invertase.

Readsorption of Invertase to Cell Wall. To test the adsorption of the enzyme to the cell wall, the purified cell walls were further treated as follows. The cell walls were suspended in distilled water, heated at 100°C for 15 min to inactivate the enzyme, and then washed with a large quantity of distilled water. The resulting cell walls were free of invertase activity. To test for readsorption, the amount of activity which was adsorbed to the wall was estimated, after enzyme and walls were allowed to interact, by subtracting the activity in the filtrate after reaction from the original activity added. Thus, a suspension of 0.5 g of cell walls (wet weight) in 10 ml of a solution of enzyme at various concentrations of NaCl was allowed to stand for 30 min at room temperature and then passed through filter paper. The activity remaining in the filtrate was estimated as described below.

Estimation of Neutral Sugars. *Internal Sugars.* Five g (fresh weight) of cultured cells obtained by vaccum filtration and then washing with deionized water were treated by boiling in 30 ml of 80% ethanol for 1 h to inactivate the enzyme. After being cooled, the cells were homogenized in a Polytron homogenizer. Extraction with ethanol was repeated once and then the sample was filtered through a glass filter. The filtrate was concentrated *in vacuo* and then dissolved in distilled water.

External Sugars. The growth medium, separated from cells by vacuum filtration, was used for determination of external sugar.

Sucrose were estimated by the resorcin-perchloric acid method (12). Reducing sugars were determined by the Nelson-Somogyi method (14) with glucose as standard. Starch was estimated by the Somogyi-Nelson method after digestion with α-amylase (Sigma) at 30°C for 24 h.

Assays of Enzymatic Activity. Invertase activity was assayed as in a reaction mixture that consisted of 60 mM sucrose, 20 mM citrate buffer (pH 5.0 for acid invertase and pH 8.0 for alkaline invertase) and 300 μl of a solution of enzyme, in a final volume of 1 ml. The mixture was incubated at 37°C for 30 min. The amount of reducing sugar liberated was determined by the method of Somogyi-Nelson (14). One unit of invertase activity was defined as the amount of enzyme that catalyzed the production of 1 μmol of reducing sugar as glucose per min at 37°C.

RESULTS

Acid and Alkaline Invertases in Leaf Explants, BA-Treated Explants, and Suspension Cultures. When the leaf explants were cultured in liquid MS medium that contained 0.25 mg/L BA as sole plant hormone, they expanded significantly, and their wet weight increased about 20-fold after 10 d in culture. There was a simultaneous and spontaneous release of single cells from the edges of the expanded explants. When the single cells sieved through nylon screen were transferred to fresh liquid MS medium plus 0.25 mg/L BA and cultured for about 2 weeks, the cells were observed to form clusters and grew actively at the same rate whether or not auxin was present. These results suggest that cultured cells directly induced from leaf explants are able to produce their own growth hormone. It should be noted that hormone-independent growth was also observed in the case of callus initiated from leaf explants. We examined the changes in the activities of acid and alkaline invertases in the fresh and BA-treated leaf explants, and in cells in suspension culture (Table I). When the leaf explants were cultured in the presence of BA for 10 d, acid invertase activity was increased 2.8-fold expressed on the basis of fresh weight of cells, while the formation of clusters in suspension culture caused a decrease in acid invertase activity. Alkaline invertase activity was hardly detectable in fresh and BA-treated leaf explants or single cells, but was found in cultured cells. These results suggest that the treatment of explants with BA was accompanied by an increase in acid invertase activity, and the formation of clusters of cells in suspension culture was accompanied by a slight decrease of acid invertase activity and the induction of alkaline invertase.

Changes in Neutral Sugars and Activities of Acid and Alkaline Invertase in Suspension. Figure 1 shows the changes in the rate of cell growth, and levels of internal and external neutral sugar in suspension cultures of sugar beet cells. In this research, the liquid medium contained 3% sucrose as the carbon source. A logarithmic rate of growth was observed between 3 and 9 d after subculture. Levels of sucrose, reducing sugar, and starch were measured at 3-d intervals in cells and media from suspension cultures. Intracellular levels of sucrose and reducing sugar showed a steady increase until d 9, or until the late logrithmic phase of growth, followed by a rapid decrease. The maximum concentration of sucrose was about 32 mg/g fresh weight at the

Table I. *Changes in Activities of Acid and Alkaline Invertase during Initiation of Cultured Cells*

One unit 1 μmol reducing sugar per min at 32°C.

	Acid Invertase	Alkaline Invertase
	units/g fresh weight	
Leaves	0.30	
BA-treated leaves	0.83	
Cultured cells	0.71	1.3

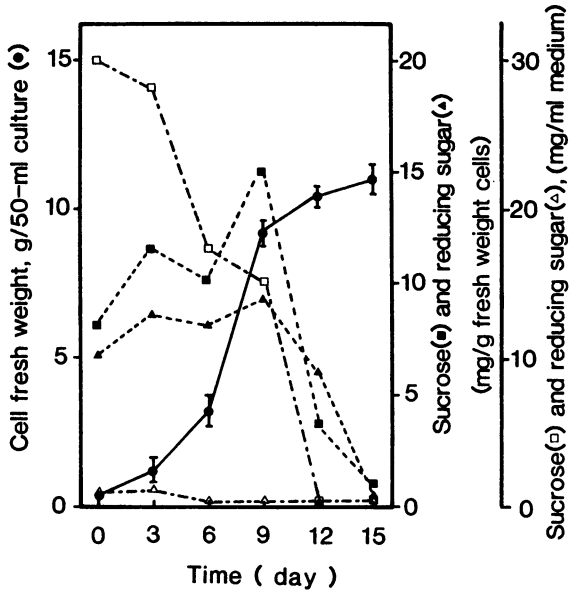

FIG. 1. Growth of and utilization of sugar by sugar beet cells in suspension culture. Internal and external sugars were determined as described in "Materials and Methods." Values for growth of cells represent the means of results of triplicate determinations, and the vertical bars indicate the SD.

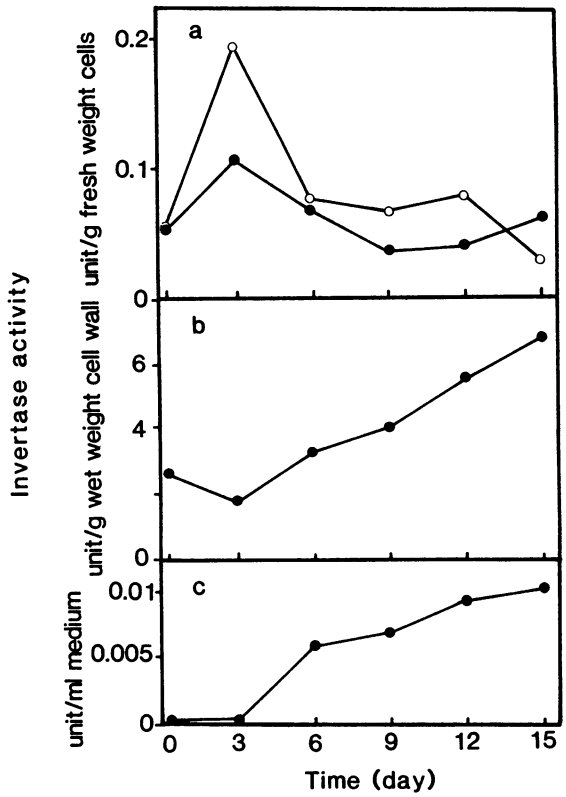

FIG. 2. Changes in the activities of acid and alkaline invertase in soluble, cell wall, and extracellular fractions. (○), Alkaline invertase; (●), acid invertase. a, Soluble invertase; b, cell wall invertase; c, extracellular invertase.

end of the period of logarithmic growth. In the medium, the amount of sucrose decreased rapidly during the logarithmic growth of cells, and all sucrose had disappeared by d 12, at the plateau phase, but no increase in the level of reducing sugar with the decrease in level of sucrose was observed throughout growth. Starch was barely detectable at any stage of growth. Figure 2 shows the changes in the activities of acid and alkaline invertases in the cells, in the soluble, cell wall, and extracellular fractions. Alkaline invertase activity was found to be present only in the soluble fraction, reached a maximum level during the first 3 d, and then decreased. Alkaline invertase activity was relatively higher than the acidic activity. Acid invertase was present in the soluble, cell wall, and extracellular fractions. Soluble acid invertase activity remained approximately constant during all phase of growth of cultures. Extracellular invertase activity in the medium appeared on d 3 when the cells had begun logarithmic growth and thereafter increased steadily, although its activity was low. The activity of wall-bound invertase was relatively very much higher than that of the soluble and extracellular invertases, and increased steadily during growth of cultures. The amounts of invertase in each fraction (Fig. 2), were all different, which suggests differences in the contribution of each enzyme, to the degradation of sucrose. The total amounts of invertase in each fraction, however, may be a measure of the relative importance of each fraction in the degradation of sucrose. Thus, total amounts were calculated for a 9-d old culture, in which sucrose was still present in the medium. For soluble invertase there were 0.61 unit of the alkaline and 0.47 unit of acid invertase, 24.5 units of cell wall invertase, 1.1 units of extracellular invertase. Total amounts of wall-bound invertase was also much higher than those of soluble and extracellular invertases.

Release of Acid Invertase from Cell Wall by Saline and Chelating Agent. Figure 3a shows that NaCl was effective in releasing invertase from the cell wall fraction, and the release of the enzyme began at 0.3 M NaCl and reached a maximum rate at about 0.5 M. The extracts obtained by treating the cell wall with 2.0 M NaCl for 17 h at 4°C contained 45% of the total invertase activity in the cell wall.

Next we attempted to determine whether residual invertase,

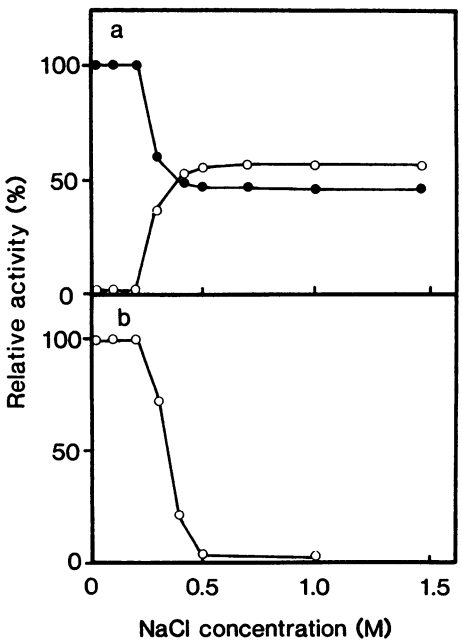

FIG. 3. Effect of increasing concentrations of NaCl on release of acid invertase from cell walls and readsorption to cell walls. a, Release; (○), released activity; (●), residual activity. b, Readsorption. Release of acid invertase from cell walls and readsorption to cell walls were performed as described in "Materials and Methods."

which was not released from the cell wall by treatment with saline could be solubilized by treatment with EDTA, a chelating agent often used for extraction of pectic substance from cell walls. Figure 4b shows that the release of invertase by EDTA is dependent on temperature, with a maximum rate of release between 27 and 32°C. The decrease in enzymic activity at 40°C and above is probably due to thermal inactivation. The time course shows that 36 h is required for complete release of the enzyme (Fig. 4a). The amount of acid invertase released by the chelating agent, under optimal condition at 27°C for 36 h, was 20 to 25% of amount of invertase left in the saline-treated wall, and the remaining activity was still high. These results suggested that the cell wall may contain at least three types of acid invertase—saline-released, EDTA-released, and a third invertase resistant to release by these methods.

Readsorption of Saline-Released Acid Invertase to Cell Wall. Dialysis against deionized water of the extracts obtained by treating the cell walls with 2 M NaCl resulted in a precipitate in the dialysis bag. The invertase activity was found in this precipitated fraction after centrifugation, a result which suggests that the enzyme came out of solution at low concentrations of salt. However, when the precipitate was dissolved in a small volume of 1.0 M NaCl and then was chromatographed on a column of Sephacryl S-300, preequilibrated with 1.0 M NaCl, the enzyme did not come out of solution. Furthermore, the enzyme was precipitated by the addition of calf thymus DNA. It appears, therefore, that the saline-released invertase from the cell walls forms an insoluble complex with DNA. This result is in good agreement with results for saline-released acid invertase from cell walls of sugar beet seedlings and aged slices of mature roots (7, 8). However, it is not evident whether the DNA that binds to acid invertase is actually situated on the cell wall *in vivo* or is an artifact from contaminant by nuclear DNA during preparation of cell walls. Saline-released acid invertase was easily readsorbed to the cell wall at concentrations of NaCl below 0.25 M (Fig. 3b). No adsorption was observed with soluble invertase, with EDTA-released invertase from the cell wall, and with two extracellular invertases.

Behavior of Soluble, Wall-Bound, and Extracellular Acid Invertases during Chromatography on DEAE-Cellulose. Figure 5 shows the elution profiles of acid invertases from soluble, cell wall, and extracellular fractions, after chromatography on DEAE-cellulose. Both soluble enzymes and the EDTA-released enzyme from the cell wall had one active peak, which, in each case, was eluted by the same concentration of NaCl of about 0.12 M, while the extracellular enzyme was separated into two active peaks (designated I and II). Peak II emerged at the same concentration of NaCl as the peak of soluble and EDTA-released enzymes. The saline-released enzyme from cell wall was not adsorbed to the anion exchanger.

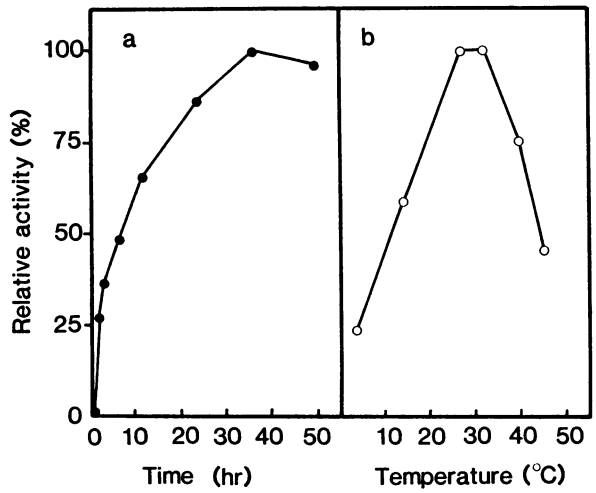

FIG. 4. Time course of release of acid invertase from cell walls by EDTA, and effect of temperature on the release. a, Time course of release of acid invertase release: the cell walls (0.5 g weight) were suspended in 5 ml of 0.5% (w/v) EDTA (0.05 M K-phosphate, pH 6.8) and incubated at 27°C. b, Effect of temperature: suspensions of cell walls in a solution of EDTA were incubated at temperatures between 4 and 45°C for 36 h.

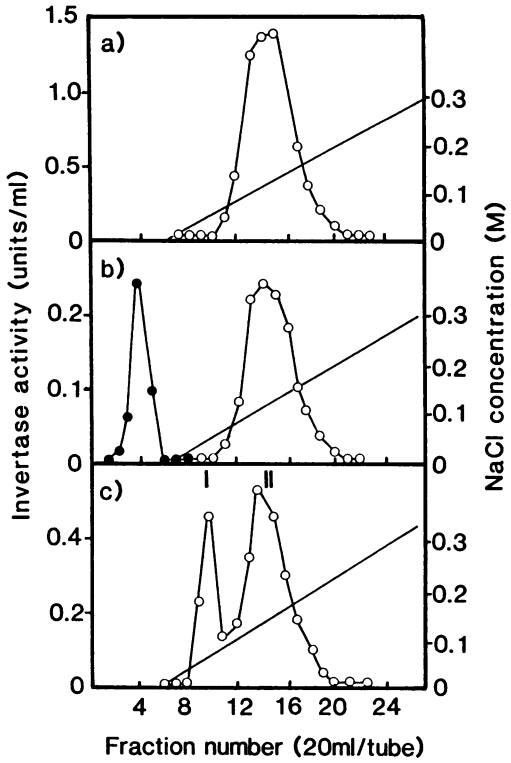

FIG. 5. Chromatography on DEAE-cellulose of soluble, wall-bound (saline-released and EDTA-released), and extracellular invertase. In the case of soluble and extracellular invertases, each crude extract was brought to 70% saturation by addition of solid $(NH_4)_2SO_4$. The precipitate was collected by centrifugation, dissolved in a small amount of 5 mM phosphate buffer (pH 7.4), and then dialyzed against the same buffer. The dialyzed solution was applied to a column of DEAE-cellulose (1.5 × 20 cm). In the case of the two wall-bound invertases (wall saline-released and EDTA-released), two samples were mixed, dialyzed against 5 mM phosphate buffer (pH 7.4), and then applied to a DEAE-cellulose column (1.5 × 20 cm). a, Soluble invertase; b, wall saline-released (●) and wall EDTA-released (○) invertases; c, extracellular invertase.

Properties of Various Types of Acid Invertase in Cells in Suspension Culture. Dependence on pH and the K_m values for sucrose of the soluble, the EDTA-released acid invertase, and extracellular enzyme II, which showed the same behavior during chromatography on DEAE-cellulose were compared. These acid invertases showed similar profiles of activity in the changes in pH (optimum pH was 5.0) and K_m values of 3.8 to 4.4 mM for sucrose (Table II), indicating that they are probably identical. Both extracellular invertase I and II had an optimum pH of 5.0, but showed different profiles of activity with changes in pH; that is, the activities of enzymes I and II at pH 3.0 were 28 and 55%, respectively, of maximum activity. Extracellular invertase I had a K_m value of 12.3 mM for sucrose, while invertase II had a K_m value of 4.4 mM (Table II). The saline-released acid invertase from the cell wall had the highest affinity for sucrose, with a K_m value of 0.56 mM, of all the invertases examined.

Comparison of Chromatographic Behavior and Properties of Extracellular Invertase with Those of Soluble Invertase of Callus

Table II. *K_m Values for Sucrose of Invertases from Cells in Suspension Culture from Callus, from Seedlings, and from Mature Roots*

	Acid Invertase	Alkaline Invertase
Cells in suspension culture		
Soluble invertase	3.8	33.3
Cell wall invertase		
Saline-released	0.56	
EDTA-released	4.2	
Extracellular invertase		
Invertase I	12.3	
Invertase II	4.4	
Callus		33.3
Invertase I	11.6	
Invertase II	3.6	
Seedlings		
Invertase I	12.6	
Invertase II	4.2	
Mature roots		33.3

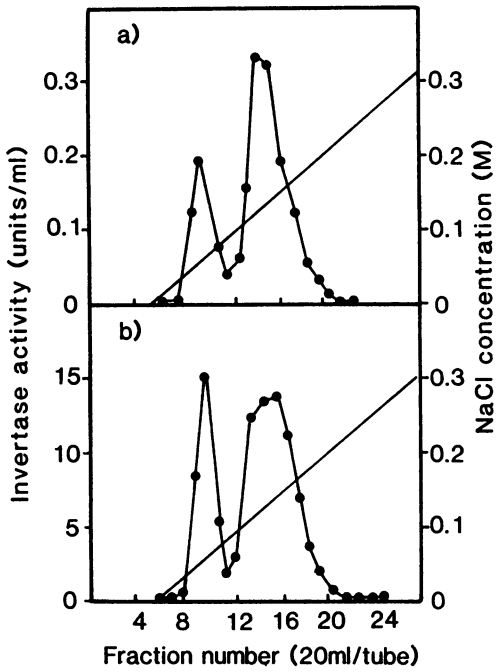

FIG. 6. Chromatography on DEAE-cellulose of acid invertase from callus and seedlings of sugar beets. Preparation of acid invertase from callus and seedlings was the same as that used for the soluble invertase from cells in suspension culture and described in the legend to Figure 5.

and Sugar Beet Seedlings. Soluble acid invertase from both callus and 4-d-old seedlings of sugar beets were separated into two active peaks (enzyme I and II of callus and seedlings), which were eluted at concentrations of 0.08 and 0.12 M NaCl, respectively, after chromatography on DEAE-cellulose (Fig. 6). This result was in good agreement with that for extracellular acid invertases I and II of cells in suspension culture. In addition, extracellular enzyme I was probably identical to soluble invertase I from callus and seedlings because these enzymes showed the similar profiles of activity with changes in pH (data not shown) and K_m values for sucrose (Table II). Similarly, extracellular acid invertase II appears to correspond to the soluble invertase II from callus and seedlings.

DISCUSSION

Cells in suspension cultures derived from leaf explants of sugar beets contained both acid and alkaline invertases. Alkaline invertase was barely detectable in fresh and BA-treated leaf explants from which the cells in suspension cultures were derived, and appeared in cells in suspension cultures induced from single cells that were released from leaf explants in medium that contained BA. Thus, the division of cells and formation of clusters from single cells were apparently accompanied by induction of alkaline invertase, an important regulatory enzyme in the accumulation of sucrose. It is of interest to ascertain how, in the metabolism of sucrose, the appearance of alkaline invertase during initiation of clusters of cultured cells is relative to the parallel accumulation of alkaline invertase during the accumulation of sucrose, during growth of sugar beet roots, as described below.

The cultured cells contained only a very low level of sucrose (32 mg/g fresh weight at its maximum), compared with mature roots of sugar beets. As reported in another study (H Masuda, T Takahashi, S Sugawara, unpublished data), during growth of sugar beets alkaline invertase activity appeared when roots began to enlarge and simultaneously to store sucrose, and the activity increased in parallel with enlargement of roots and storage of sucrose. By contrast, acid invertase activity was very high in immature tissues, decreased rapidly before roots began to enlarge, and was hardly detectable in mature roots. Apparently, in mature roots alkaline invertase plays an important role in the accumulation of sucrose, but acid invertase probably disappears because it hinders the accumulation of sucrose. However, cultured cells contained alkaline invertase in the cytoplasm, but simultaneously contained acid invertase in the cytoplasm and cell walls, and excreted it into the medium. If mutant cells, in which only alkaline invertase occurs but the acid enzyme is barely detectable, can be selected from cultured cells, they might be as capable of accumulating a large amount of sucrose as the cells of the mature roots of sugar beets.

The cell wall contained three types of acid invertase. Two of the enzymes were solubilized by treatment of the cell wall with saline and then with a chelating agent, while the third one remained tightly associated with the cell wall. The saline-released invertase was solubilized at concentrations of NaCl above 0.3 M and was readsorbed to the cell walls at concentrations below 0.25 M, a result which indicates that the enzyme was ionically bound to the cell wall. This result is in good agreement with the observations of saline-released acid invertase from cell walls of sugar beet seedlings (6). EDTA-released invertase was gradually liberated from cell walls under optimal conditions at 27°C for 36 h, after treatment with saline. It may be bound covalently to the pectic moiety of the cell wall. Wall-bound enzymes have previously been solubilized with detergents such as Tween 20, Triton X-100 (19), and a pectolytic enzyme (15), as well as saline, but no attempt was previously made to solubilize them with the chelating agent, EDTA. Saline-released and EDTA-released invertases were significantly different from each other: (a) saline-released invertase exhibited the highest activity and the highest affinity for sucrose (K_m, 0.56 mM) among the invertases tested, suggesting its probable involvement in degradation of sucrose transported into the cell from the medium, as described below; (b) saline-released invertase bound easily to cell walls, to DNA, and to a cation exchanger, whereas no binding to these materials was observed with the EDTA-released enzyme.

Acid invertase, which emerged at a concentration of NaCl of about 0.12 M after chromatography on DEAE-cellulose was found to be present in all three fractions (cytoplasm, cell walls, and medium). The enzyme in each fraction is probably identical because each of the three enzymes showed approximately similar profiles of activity with changing pH and K_m values for sucrose. These findings suggest that some of the soluble acid invertase passes through the plasma membrane and then some binds to the cell wall, while the rest is secreted into the medium. The saline-released invertase is ionically adsorbed to the cell wall and

remains there. Furthermore, one of the two extracellular invertases (enzyme I), which was found only in medium, might be secreted directly, and not via the cell wall, into the medium.

The level of sucrose in the medium decreased rapidly during the logarithmic phase of growth and was nearly depleted from the medium after 12 d, at the plateau phase of growth, but the decrease in level of sucrose was not accompanied by an increase in reducing sugars in the medium throughout the period of cell growth. This result suggests that external sucrose is preferentially taken to the cell surface before it is hydrolyzed by extracellular invertase, because of the low activity of this enzyme. Thus, the sugar is probably degraded in the cell wall by the saline-released invertase, which has the highest activity and the highest affinity for sucrose, and the products of degradation are finally transported to the cytoplasm as hexoses. A similar result, namely that wall-bound invertase is involved in the uptake of sucrose, was reported for tobacco callus (17). These results differ from those of Kanabus *et al.* (4) who reported that, in carrot cells in liquid culture, hexoses released by the action of extracellular invertase in the medium are the source of most of the carbon in the cell.

It is noteworthy that the two acid invertases released into the medium of suspension cultures were separable by chromatography on DEAE-cellulose, and that these enzymes were also found in the soluble fraction of the callus and the seedlings of sugar beet. The enzyme corresponding to the two acid invertases (enzymes I and II) in cells in suspension culture, in callus, and in seedlings, exhibited similar behavior upon chromatography on DEAE-cellulose, as well as similar profiles of activity with changing pH and K_m values for sucrose. These similarities suggest that the soluble fraction of callus and seedlings, and the medium from suspension cultures, contained identical acid invertases. Thus, the invertase found in the medium may be present in a soluble form, and not bound to the cell wall, in intact plants.

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