Plant Physiol. (1974) 54, 60–66

Change in Invertase Activity of Sweet Potato in Response to Wounding and Purification and Properties of Its Invertases[1]

Received for publication January 24, 1974 and in revised form March 8, 1974

KAZUNOBU MATSUSHITA AND IKUZO URITANI
Laboratory of Biochemistry, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan

ABSTRACT

When root tissue of sweet potato (*Ipomoea batatas* Lam.) was sliced, acid invertase activity, initially absent in freshly sliced tissue, appeared after a 3- to 6-hour lag phase, rapidly reached a maximum in 18 hours, and thereafter decreased. The increase in invertase activity was accompanied by a decrease in sucrose content of the root tissue. Alkaline invertase activity was present in fresh root tissue, but changed little after wounding. Acid invertase in wounded tissue and alkaline invertase in fresh tissue were purified and their properties were investigated. The acid invertase was a ß-fructofuranosidase and was unaffected by substrate or by any of the cations and several metabolites. The alkaline invertase was more specific for sucrose, was inhibited by glucose and glucose 6-phosphate, and displayed non-Michaelis-Menten kinetics.

Respiratory activity is known to increase markedly in response to wounding or infection in various plant storage tissues. In root tissue of sweet potato, respiratory activity doubles within 20 hr after wounding (11). This respiratory increase may be due to the activity of newly formed mitochondria (3) and the activation of ATP-utilizing systems which involve the production of polyphenols (25). The increased respiratory activity is paralleled with an increase in RNA content (30), and the *de novo* syntheses of peroxidase (36), phenylalanine ammonia-lyase (24), and other enzymes. Thus, storage sugars in the tissue may be used in amounts proportional to the amounts of respiration increased and of polyphenols and other metabolites that are produced in response to wounding. It is also possible that activities of some enzymes participating in sugar decomposition increase at that time. Decomposition of starch (38) and activation of the pentose phosphate pathway (1) occur in sweet potato root tissue in response to wounding or to infection by *Ceratocystis fimbriata* ELL. et HALST. Further, activities of glucose-6-P dehydrogenase and 6-phosphogluconate dehydrogenase increase after wounding (27).

Invertase is found in many plants, and exists in two isozymic forms, acid and alkaline, which differ in properties and cellular localization. Acid invertase has been shown to occur in germinating seeds (5), developing stems (12), and aged tuber discs (8, 32, 34).

This paper deals initially with the fluctuation of invertase activity in sweet potato root tissue in response to wounding. Further, we have purified acid and alkaline invertases and examined their properties in view of assigning their physiological functions in connection with wound response.

MATERIALS AND METHODS

Plant Material. Roots of sweet potato (*Ipomoea batatas* Lam. cv. Norin No. 1) were harvested in the autumn and stored at 10 to 14 C until used. Roots were washed with tap water and sterilized with 0.1% sodium hypochlorite solution followed by thorough washing with tap water. The roots were sliced to 3-mm thickness with a meat slicer, then discs (15 or 19 mm diameter) were prepared from the parenchymatous tissue with a cork borer. The discs were placed on a polyethylene net in a styrol box containing distilled water, and were incubated at 29 ± 1 C.

Extraction of the Enzyme. Seven discs (3 × 19 mm, total wet weight about 7 g) were mixed with 10 ml of 10 mM potassium phosphate buffer, pH 7.0, containing 1% (w/v) sodium isoascorbate, and 1 g of Polyclar AT (poly-(vinylpolypyrrolidone), GAF Corp. New York), and were given two 30-sec homogenizations in a blender (Nihon Seiki Seisakusho, Tokyo) at the maximum speed. The homogenate was squeezed through four layers of cotton gauze and centrifuged at 15,000*g* for 20 min. The supernatant solution was passed through a Sephadex G-25 (coarse) column (1.9 × 20 cm), previously equilibrated with 10 mM potassium phosphate buffer, pH 7.0, to remove low mol wt compounds such as polyphenols, isoascorbate, and sugars. The protein fraction thus obtained was used as crude enzyme preparation. All procedures were carried out at 0 to 4 C.

CELLULAR FRACTIONATION OF THE ENZYMES

Method 1. Seven discs (3 × 19 mm, total fresh weight about 7 g) were mixed with 10 ml of 10 mM potassium phosphate buffer, pH 7.0, containing 0.7 M mannitol, and 1% (w/v) sodium isoascorbate, and 1 g of Polyclar AT and given two 30-sec homogenizations in a blender. The homogenate was squeezed through four layers of cotton gauze, and the residue in the gauze was saved for preparation of the cell wall fraction. The filtrate was centrifuged at 900*g* for 5 min to remove mainly starch granules, and the supernatant fraction was passed through a Sephadex G-25 column (1.9 × 20 cm) pre-equilibrated with 10 mM potassium phosphate buffer, pH 7.0, containing 0.7 M mannitol (washing medium). The protein fraction thus obtained was centrifuged at 14,000*g* for 15 min. The pellet was suspended in 5 ml of washing medium and centrifuged at 14,000*g* for 15 min to sediment the mitochon-

[1] This paper constitutes Part 111 of the Phytopathological Chemistry of Sweet Potato with Black Rot and Injury.

drial fraction. The supernatant material was centrifuged at 100,000*g* for 1 hr and the resulting supernatant (the supernatant fraction) was saved. The pellet was suspended in 5 ml of washing medium and the suspension was centrifuged at 100,000*g* for 1 hr to sediment the microsomal fraction.

The residue which remained in the cotton gauze was suspended in 20 ml of washing medium and homogenized in a glass homogenizer. The suspension was passed through four layers of cotton gauze to collect the cell wall fraction.

The mitochondrial, microsomal, and cell wall fractions were suspended in 10 mM potassium phosphate buffer, pH 7.0, to measure the enzyme activity.

Method 2. Seven discs were mixed with 10 ml of 10 mM potassium phosphate buffer, pH 5.5, 6.4, or 7.5, containing 1% (w/v) sodium isoascorbate and 1 g of Polyclar AT and given two 30-sec homogenizations in a blender. The homogenate was squeezed through three layers of cotton gauze. The filtrate was centrifuged at 15,000*g* for 20 min and the supernatant solution was passed through a Sephadex G-25 column (1.9 × 20 cm) pre-equilibrated with 10 mM potassium phosphate buffer, pH 7.0. The column effluent that contained proteins (supernatant fraction) was saved. The debris collected in the cotton gauze was suspended in 30 ml of 10 mM potassium phosphate buffer, pH 5.5, 6.4, or 7.5, containing 1% (w/v) sodium isoascorbate (washing medium), and the suspension was passed through three layers of cotton gauze. The debris was suspended in 50 ml of washing medium and the suspension centrifuged at 1,000*g* for 10 min. The resulting pellet was suspended with 10 mM potassium phosphate buffer, pH 7.0, to a final volume of 30 ml, of which 5 ml was dialyzed overnight against 500 ml of the same buffer; the buffer was changed twice during dialysis (29). The dialyzed suspension was designated the cell wall fraction.

Enzyme Assays. The reaction mixture for assay of acid or alkaline invertase in the soluble, mitochondrial, and microsomal fractions consisted of 0.1 M sucrose, enzyme, and 0.05 M potassium acetate buffer, pH 4.6, or potassium phosphate buffer, pH 7.6, in a final volume of 1 ml. The mixture was incubated at 30 C for 1 hr, and then 1 ml of Nelson-Somogyi copper reagent (31) was added to stop the reaction. The mixture was boiled for 10 min, cooled, and mixed with 1 ml of arsenomolybdate reagent (31) and 20 ml of water. The absorbance of blue color was measured at 660 nm. The enzyme activity was expressed as 1 unit when 1 μg of reducing sugar was produced during incubation using glucose as standard.

Invertases of the cell wall fraction were assayed in a reaction mixture (2 ml) containing 0.1 M sucrose, 0.05 M potassium acetate buffer, pH 4.6, or sodium pyrophosphate buffer, pH 8.0, and 1 ml of the cell wall fraction. After shaking at 30 C for 1 hr, the reaction mixture was cooled and centrifuged at 3000 rpm. Enzyme activity was measured as described above using 1 ml of the supernatant liquid.

Assay of Protein. The trichloroacetic acid-insoluble fraction was digested with 10 N sulfuric acid containing 2 mg/100 ml $CuSeO_3$. Protein content of each enzyme preparation was calculated from the nitrogen content of the digested sample determined by the Nessler's reagent (16).

Extraction and Assay of Sugars. Four discs (3 × 15 mm, total wet weight about 2 g) were heated at 90 C for 5 min in 10 ml of 80% (v/v) ethanol, cooled, and homogenized for 1 min in a blender. The homogenate was centrifuged at 3,000 rpm for 10 min. The residue was extracted by heating at 90 C for 5 min in 10 ml of 80% (v/v) ethanol, and by centrifugation as before. The extraction procedure was repeated once more. All the supernatant fractions were combined and evaporated to dryness at 50 C. The residue was dissolved in water, and the solution was made up to 100 ml. Aliquots were taken for determination of total sugar and reducing sugar by the phenol-sulfuric acid (7) and Nelson-Somogyi methods, respectively. Sucrose was determined by the phenol-sulfuric acid method using the fraction eluted from a Dowex-1 column (0.9 × 6 cm in borate form) (17).

Purification of Acid Invertase. Sliced sweet potato root tissue (200 g), incubated for 18 hr, was mixed with 200 ml of 10 mM potassium phosphate buffer, pH 7.0, containing 1% (w/v) sodium isoascorbate and 10 *g* of Polyclar AT and subjected to two 30-sec homogenizations in a blender. The homogenate was squeezed with three layers of cotton gauze and the filtrate centrifuged at 12,000*g* for 20 min. The supernatant liquid was passed through a Sephadex G-25 (coarse) column pre-equilibrated with 10 mM potassium phosphate buffer, pH 7.0. The crude extract thus obtained was placed on a DEAE[2]-cellulose column (3.6 × 24 cm) pre-equilibrated with 10 mM potassium phosphate buffer, pH 7.0, and the enzyme was eluted with the same buffer containing 0.05 M NaCl. The fraction showing acid invertase activity was made 80% saturated with solid ammonium sulfate. The resultant precipitate was suspended in 10 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM DTT and dialyzed against the same buffer for 3 hr. After centrifuging the dialyzed enzyme preparation at 13,000*g* for 20 min, the supernatant liquid was applied to a Sephadex G-200 (5 × 82 cm) preequilibrated with DKP-buffer. The enzyme was eluted with the same buffer at a flow rate of 10 to 15 ml/hr. To the fraction with invertase activity, solid ammonium sulfate was added to give 80% saturation. The precipitate was suspended in DKP-buffer and the suspension was dialyzed against the same buffer for 3 hr. After centrifuging the dialysate at 12,000*g* for 20 min, the supernatant was applied to a hydroxyl-apatite column (1.8 × 10.5 cm) previously equilibrated with DKP-buffer. The column was washed with 50 ml of the same buffer, and then the enzyme was eluted with a linear gradient using 125 ml of DKP-buffer and 125 ml of 0.3 M potassium phosphate buffer, pH 7.0, containing 0.5 mM DTT. The flow rate was 12 ml/hr. The fractions with acid invertase activity were pooled and taken to 80% saturation with solid ammonium sulfate. The resultant precipitate was collected by centrifugation and the precipitate suspended in 10 mM potassium phosphate buffer, pH 6.0, containing 0.5 mM DTT. The suspension was dialyzed against the same buffer overnight and then centrifuged at 12,000*g* for 20 min to remove the precipitate. The supernatant liquid was placed on a CM-Sephadex column (1.8 × 7 cm) and the above buffer was passed through the column. Invertase activity was not retained on the column. Fractions with invertase activity were pooled, dialyzed against 0.05 M potassium phosphate buffer, pH 8.0, containing 0.5 mM DTT for 2 hr, and applied to a DEAE-Sephadex column (1.3 × 5 cm) preequilibrated with the same buffer. The column was washed with 50 ml of the same buffer, and the enzyme was eluted with a linear gradient using 200 ml of 0.05 M potassium phosphate buffer, pH 8.0, containing 0.5 mM DTT and 200 ml of the same buffer containing 0.5 mM DTT and 0.5 M sodium chloride.

The eluates with invertase activity were concentrated in a collodion bag and dialyzed against DKP-buffer. The dialysate was stored at −20 C as the purified enzyme preparation.

---

[2] Abbreviations: DEAE: diethylaminoethyl; DTT: dithiothreitol; CM: carboxymethyl; DKP-buffer: 10 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM DTT.

The procedure resulted in a purification of 122-fold (Table I).

Purification of Alkaline Invertase. Two hundred g of parenchymatous tissue of fresh sweet potato roots were mixed with 200 ml of DKP-buffer containing 1% (w/v) sodium isoascorbate and given two 30-sec homogenizations in a blender. The homogenate was squeezed with three layers of cotton gauze and the filtrate centrifuged at 15,000*g* for 20 min. The supernatant liquid was passed through a Sephadex G-25 (coarse) column. To the crude extract obtained, solid ammonium sulfate was added and the fraction, precipitated between 20 and 40% saturation, was collected by centrifugation at 12,000*g* for 20 min. The precipitate was suspended in DKP-buffer, and the suspension was dialyzed for 4 hr against the same buffer and then dialyzed for 1.5 hr against 0.05 M potassium phosphate buffer, pH 8.0, containing 0.5 mM DTT. The dialyzed sample was centrifuged at 15,000*g* for 20 min, and the supernatant liquid layered on a DEAE-cellulose (Whatman DE-52) column (2.5 × 17 cm), previously equilibrated with the above buffer. The column was washed at a flow rate of 1 ml/min with a concave gradient of NaCl using 400 ml of the above buffer and 200 ml of the same buffer containing 1 M NaCl. The fractions with alkaline invertase activity were pooled and the pooled sample was saturated with ammonium sulfate to 80%. The precipitate was collected by centrifugation at 12,000*g* for 20 min and dissolved in DKP-buffer. The enzyme preparation then was pumped into Sephadex G-200 column (5 × 66 cm), previously equilibrated with DKP-buffer, and the enzyme was eluted with the same buffer at a flow rate of 20 to 30 ml/hr.

The fractions containing enzyme activity were pooled, concentrated in a collodion bag, and stored at −70 C until used.

Results of this purification procedure are shown in Table II; a 133-fold purification was achieved.

RESULTS

Change in Invertase Activity in Response to Wounding. The pH dependency of invertase activity in enzyme extracts of fresh and sliced tissues was examined (Fig. 1). Whereas invertase activity in fresh tissue exhibited maximal activity at about pH 8, the activity in preparations from sliced tissue had a maximum at pH 8 but remained significantly high over a broad range of acidic pH. After slicing, acid invertase activity increased markedly but alkaline invertase activity changed little.

Table I. *Summary of Results of Purification of Acid Invertase from Sliced Sweet Potato Root Tissue*

Step	Activity	Protein	Specific Activity	Recovery
	units	*mg*	*units/mg*	%
Crude extract	174900	450	389	100
DEAE-cellulose	216000	129.5	1670	123
Sephadex G-200	180000	21.08	8570	103
Hydroxyl apatite	123350	8.69	14200	71
CM-Sephadex	123000	3.69	33300	70
DEAE-Sephadex	56000	1.15	48700	32

Table II. *Summary of Results of Purification of Alkaline Invertase from Fresh Sweet Potato Root Tissue*

Step	Activity	Protein	Specific Activity	Recovery
	units	*mg*	*units/mg*	%
Crude extract	117000	480	244	100
$(NH_4)_2SO_4$ 0.2-0.4	109340	325	336	93.5
DE-52 (DEAE-cellulose)	73900	149	496	63.2
Sephadex G-200	46000	1.67	27500	39.3

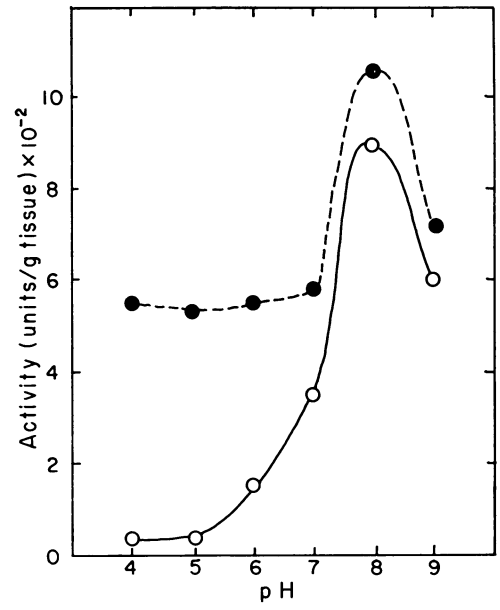

FIG. 1. pH dependency of invertase activity in crude extracts from fresh and sliced sweet potato root tissues. Tissues were incubated for 24 hr after slicing. Reactions were performed in 0.1 M citrate-phosphate buffer (pH 4–9). ○: Invertase activity of fresh tissue ; ●: invertase activity of sliced tissue.

Therefore, a time course study was done in acid (pH 4.6) and alkaline (pH 8.0) invertase activities of the root tissue after slicing. After a lag phase of 3 to 6 hr following slicing of the tissue, acid invertase activity increased markedly and reached a maximal value in 18 hr (Fig. 2). After reaching the maximal activity, acid invertase activity decreased rapidly for the next 12 hr, and reached a constant value thereafter. On the other hand, alkaline invertase activity remained essentially unaltered during the 48-hr period after slicing, although a few samples showed slight increases in activity.

DEAE-Cellulose Elution Pattern of Invertase Activity. When chromatography of the invertases from sliced tissue was done on DEAE-cellulose, acid invertase was eluted before alkaline invertase. In addition, the latter activity showed a multicomponent elution pattern (Fig. 3B). Since alkaline invertase from fresh tissue showed a single component elution pattern (Fig. 3A), it was considered likely that the apparent multiple forms of alkaline invertase in the sliced tissue preparation were produced by interaction with polyphenols which occurred in high concentration. However, the DEAE-cellulose elution pattern of mixed enzyme preparation from fresh and sliced tissues agreed closely with the pattern that was prepared by overlapping two individual enzyme elution patterns from fresh and sliced tissues (Fig. 3C). Therefore, the multiple forms of alkaline invertase from sliced tissue were believed not to be artifacts, but products associated with the wound response.

Change in Sucrose Content in Response to Wounding. It was shown that acid invertase activity changed markedly with wounding. Therefore, it was of interest to determine if changes in sucrose content paralleled changes in acid invertase activity. Sucrose increased slightly during the first 12 hr after slicing,

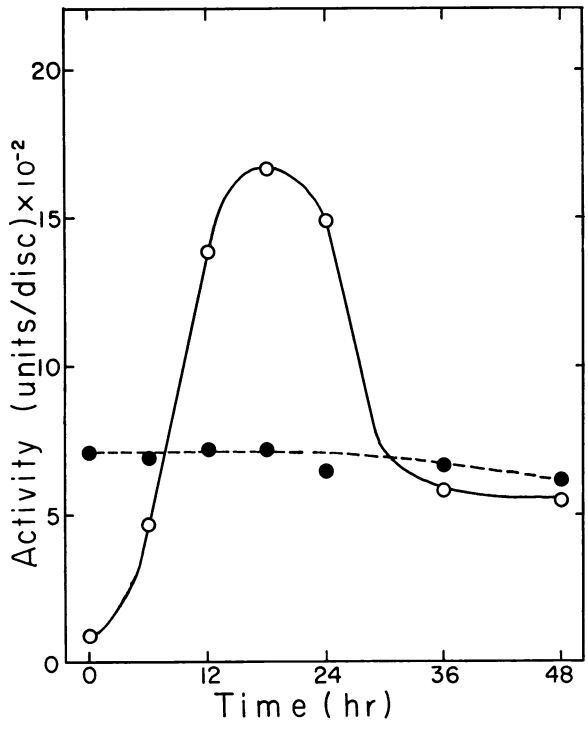

FIG. 2. Time course of invertase activity of sweet potato discs during incubation. The reaction mixture (1.0 ml) for acid invertase activity consisted of 0.05 M potassium acetate buffer, pH 4.6, 0.1 M sucrose, and 0.1 ml of the crude extract. The reaction mixture (1.0 ml) of alkaline invertase activity consisted of 0.05 M sodium pyrophosphate buffer, pH 8.0, 0.1 M sucrose, and 0.1 ml of the crude extract. ○: Acid invertase activity; ●: alkaline invertase activity.

then decreased during the next 12 hr, and reached a plateau thereafter (Fig. 4). Sucrose decreased approximately 10 mg/g tissue during the 12 to 24 hr interval after slicing. On the other hand, reducing sugar increased particularly during the period between 12 and 24 hr after slicing, concomitantly with the decrease in sucrose content.

Cellular Distribution of Invertase Activity. The results in Table III show that acid invertase activity existed in both the supernatant and cell wall fractions and that alkaline invertase activity was solely located in the supernatant fraction. The effect of pH on the extraction of the cell wall-bound enzyme was also investigated. Table IV shows that 20% of the acid invertase activity was located in the cell wall fraction, and that the content of cell wall-bound enzyme was not greatly influenced by pH of extraction buffer.

PROPERTIES OF PURIFIED ACID AND ALKALINE INVERTASES

Optimum pH. Acid and alkaline invertases showed optimum pH values at 4.6 and 7.6, respectively.

Km for Sucrose. As shown in Figure 5, Km value of acid invertase, obtained with the Lineweaver-Burk double reciprocal plot, which showed a straight line, was 4.5 mM for sucrose, in either potassium or sodium acetate buffer.

On the other hand, the double reciprocal plot of alkaline invertase in potassium phosphate buffer was biphasic, and Km values were 8.3 and 32 mM in low and high sucrose concentrations, respectively. Hill coefficient values obtained from the above data were 1.04 and 0.87 for acid and alkaline invertases, respectively. However, in sodium phosphate buffer the plot indicated a straight line, from which the Km value was determined as 16 mM.

Substrate Specificity. Acid invertase of sweet potato was a β-fructofuranosidase which was reactive with raffinose, while sweet potato alkaline invertase was more specific for sucrose with less activity for raffinose (Table V).

Effects of Metabolites on the Invertase Activity. Effects of metabolites related to sucrose metabolism on invertase activity were examined to determine if acid or alkaline invertase activity was regulated by those metabolites.

Acid invertase activity was not influenced by glucose-1-P, glucose-6-P, fructose-6-P, UDP-glucose, ADP-glucose, ATP, ADP, D-glucose, or fructose, when 1 to 2 mM concentration was used. On the other hand, alkaline invertase was inhibited slightly by 2 mM glucose and glucose-6-P (12 and 20% inhibition, respectively), but was not influenced by the other metabo-

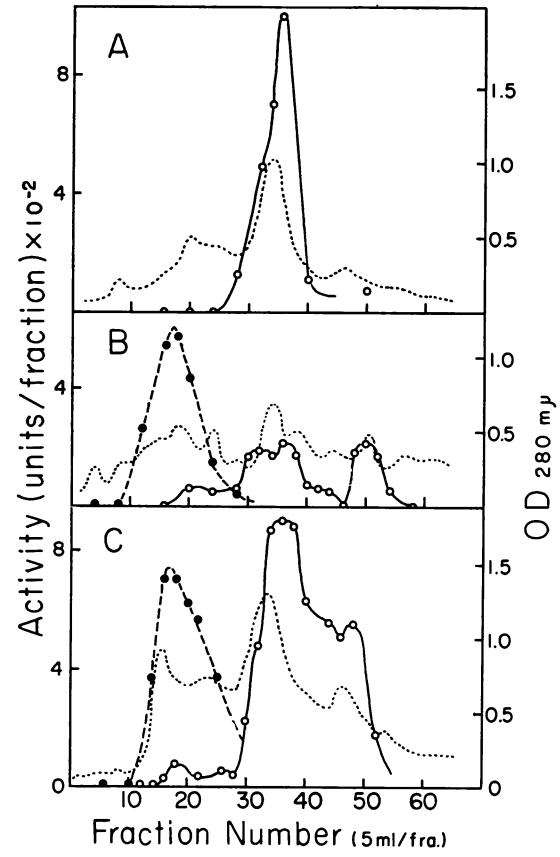

FIG. 3. DEAE-cellulose elution patterns of invertase preparations from fresh tissue, sliced tissue, and mixture of fresh and sliced tissues. A: Fresh tissue; B: 18-hr-incubated sliced tissue; C: a mixture of fresh tissue and sliced tissue. Twenty g fresh weight of tissue were used for extracts of fresh and sliced tissue; the mixture was prepared from 20 g (fresh weight) each of fresh and sliced tissue. Crude extracts were fractionated with ammonium sulfate (80% saturation). The resultant precipitate was suspended in 10 mM potassium phosphate buffer, pH 7.0, and the suspension dialyzed against 2 l of the same buffer. Each dialysate was applied to a DEAE-cellulose column (2.0 × 17 cm) pre-equilibrated with 10 mM potassium phosphate buffer, pH 7.0, and eluted with 100 ml of 0.05 M potassium phosphate buffer, pH 7.0, followed by a concave gradient using 200 ml of the starting buffer and 100 ml as the limiting buffer, additionally containing 0.5 M sodium chloride. Enzyme activity was assayed in 0.1 M sodium phosphate buffer, pH 8.0, and 0.1 M citrate phosphate buffer, pH 4.0. ●: acid invertase activity; ○: alkaline invertase activity; ----: *A*, 280 nm.

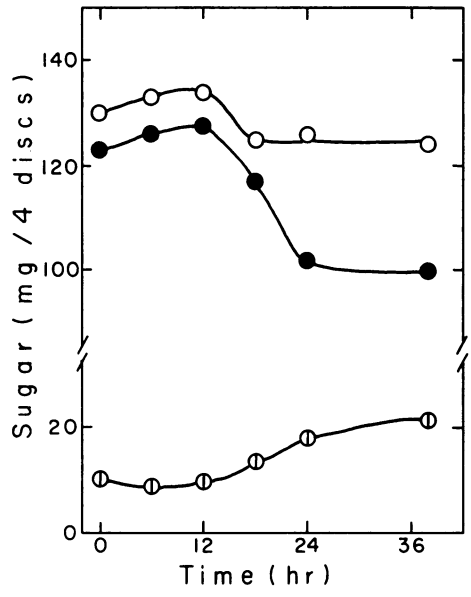

FIG. 4. Changes in sugar contents of sweet potato discs during incubation. Levels of total and reducing sugars were determined using phenol-sulfuric acid and Nelson-Somogyi methods, respectively, in extracts obtained as described in the text. Sucrose was determined with phenol-sulfuric acid method, on the fraction eluted from Dowex-1 column (0.9 × 6 cm in borate form) with 100 ml of 5 mM borate, pH 8.9. ○: Total sugar; ●: sucrose; ⓘ: reducing sugar.

Table III. *Cellular Distribution of Invertase Activity in Fresh and Sliced Sweet Potato Root Tissues*

The method of fractionation is described as Method 1 under "Materials and Methods." Enzyme reaction was conducted in 0.05 M potassium acetate buffer, pH 4.6, and 0.05 M sodium pyrophosphate buffer, pH 8.0.

Fractions		Acid Invertase Activity (pH 4.6)	Alkaline Invertase Activity (pH 8.0)
		units/disc	
Cell wall	F[1]	trace	trace
	S[2]	570	trace
Mitochondria	F	16	trace
	S	32	11
Microsomes	F	13	trace
	S	132	18
Supernatant	F	112	235
	S	2350	403

[1] Fresh tissue disc.
[2] Sliced tissue disc (18 hr).

lites, when the reaction was performed in a 0.1 M concentration of sucrose (data not shown).

Effects of Ions on the Invertase Activity. Sweet potato acid invertase activity was inhibited only by I^- and NH_4^+, while sweet potato alkaline invertase activity was affected by various ions (Table VI). Alkaline metal ions and related cations affected alkaline invertase activity in order of $Li^+ > Na^+ > K^+ > Rb^+ > NH_4^+ > tris^+$, and anions affected the activity in order of $F^- > NO_3^- > Br^- > I^-$ and Cl^-. The interrelated effect of cations and anions on invertase activity prevented distinction between activator and inhibitor. However, information presented in Figure 6 suggested that Na^+ and K^+ were activators and that Cl^- was an inhibitor.

Table IV. *Effect of Extraction pH on Cellular Distribution of Invertases in Sliced Sweet Potato Root Tissue*

Seven discs (3 × 19 mm, about 7 g) of sliced sweet potato root tissue incubated for 18 hr were homogenized in 10 ml of 0.01 M potassium phosphate buffer (pH 5.5, 6.4, or 7.5). The detailed procedures are described as Method 2 under "Materials and Methods."

Fractions	pH	Acid Invertase Activity	Alkaline Invertase Activity
		units/disc	
Supernatant	5.5	1490	543
	6.4	1660	643
	7.5	1460	529
Cell wall	5.5	409	19
	6.4	332	13
	7.5	321	4

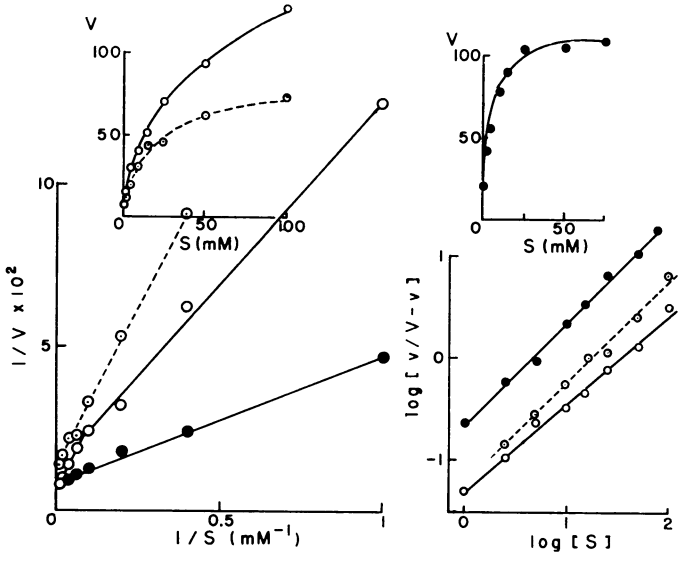

FIG. 5. Effects of sucrose concentration on the activities of acid and alkaline invertases. The assay mixture (1.0 ml) contained various concentrations of sucrose, enzyme preparation, and 0.05 M potassium acetate buffer, pH 4.6, potassium phosphate buffer, pH 7.6, or sodium phosphate buffer, pH 7.6. ●: Acid invertase (115 units); ○: alkaline invertase (128 units) in potassium phosphate buffer; ⊙: alkaline invertase (69 units) in sodium phosphate buffer. The double reciprocal plot and Hill plot were shown by calculation by method of least squares.

Table V. *Substrate Specificity of Acid and Alkaline Invertases*

The assay mixture contained 0.05 M potassium acetate buffer (pH 4.6) or potassium phosphate buffer (pH 7.6), 0.05 M concentrations of each substrate, and enzyme solution in a total volume of 100 μl. Enzyme activity was expressed as percentage of the activity obtained when sucrose was used (97 and 44 units in acid and alkaline invertases, respectively).

Substrates	Acid Invertase Activity	Alkaline Invertase Activity
	%	
Sucrose	100	100
Raffinose	23	5.8
Melezitose	3	0

Table VI. *Effects of Ions on the Activities of Acid and Alkaline Invertases*

The assay mixture consisted of 0.005 M potassium acetate buffer, pH 4.6, or potassium phosphate buffer, pH 7.6, 0.1 M sucrose, 0.05 M concentrations of each salt and enzyme solution in a total volume of 1 ml. Enzyme activity was expressed as percentage of the activity obtained without salt (control), which was 37 or 63 units in acid or alkaline invertase, respectively.

Salts	Acid Invertase Activity	Alkaline Invertase Activity
	%	
Control	100	100
KF	97	163
KCl	97	84
KBr	103	94
KI	52	88
KNO_3	96	118
LiCl	100	142
NaCl	101	87
RbCl	95	78
NH_4Cl	7	53
Tris-Cl[1]	95	25

[1] Tris-Cl was adjusted to pH 4.5 or 7.5 for acid or alkaline invertase.

DISCUSSION

Alteration of Sucrose Contents and Invertase Activity in Response to Wounding. In sweet potato root tissue, the increase in respiratory activity in response to wounding is accompanied by a decrease in sucrose content. In addition, starch was also shown to be degraded after a lag phase of 18 hr (38). Therefore, the respiratory increase in response to wounding seems to be dependent upon the oxidation of products released by sucrose and starch breakdown. Sucrose seems to be used mostly during the 12- to 24-hr interval after wounding and starch seems to be the main substrate after 24 hr.

Acid invertase increased rapidly after a lag phase of 3 to 6 hr, reached the maximum in 18 hr, and decreased rapidly thereafter. The increase in acid invertase activity parallels the decrease in sucrose content, and invertase activity is sufficient to account for the rate of sucrose disappearance that is detected in cut tissue. On the other hand, the activity of alkaline invertase for the most part scarcely changed in response to wounding. Therefore, it seems that alkaline invertase is not directly involved in the decrease in sucrose content after wounding. However, no role is apparent for the multiple forms of alkaline invertase that appear in response to wounding.

Properties and Physiological Function of Invertases. Both acid and alkaline invertases have been found in many higher plants (5, 6, 12, 22, 34), and the existence of both enzymes in higher plants is thought to be universal. Acid invertases in higher plants are generally β-fructofuranosidases, having pH optima of 4.0 to 5.5 and K*m* values of 2 to 13 mM (5, 28), whereas alkaline invertases have pH optima of 7.0 to 7.8 and K*m* values of 9 to 25 mM and, at least in the case of the bean enzyme (5), seem to be specific for sucrose. Further, the activity is known to be inhibited by $tris^+$ in some higher plants (5, 12). On the other hand, the invertases of yeast and *Neurospora* are known to be β-fructofuranosidases, having pH optima in the acidic region (21). The yeast enzyme is inhibited by I^- (21). Animal intestinal invertase was reported to be an α-glucosidase with a pH optimum of 6.7, and to be activated by alkaline metal ions and inhibited by $tris^+$ and NH_4^+ (19, 37).

Sweet potato acid invertase is a β-fructofuranosidase which has a pH optimum of 4.6, a K*m* of 4.5 mM, and is inhibited by I^- and NH_4^+. Sweet potato alkaline invertase is more specific for sucrose than the acid invertase, and has pH optimum of 7.6 and a K*m* of 32 mM in high concentrations of sucrose. Further it is activated by Na^+ and K^+, and inhibited by Cl^- and possibly by $tris^+$ and NH_4^+.

In many plant tissues an appreciable proportion of acid invertase exists in cell wall (2, 8, 12, 14, 18, 29, 32, 34), and a part of acid invertase may be associated with the tonoplast (6, 22, 35). In our studies, we found that a significant amount of acid invertase was associated with the cell wall fraction of sweet potato roots.

In sweet potato alkaline invertase, the Lineweaver-Burk double reciprocal plot yielded a biphasic curve, and its Hill coefficient was less than 1, when enzyme activity was tested in the presence of K^+. However, when enzyme assays were done with the presence of Na^+, the reciprocal plot gave a straight line and its Hill coefficient was 1. These results suggest that sucrose binds to alkaline invertase with negative cooperativity (20) in the presence of K^+, which is the main cation in sweet potato (26).

As described above, the activity of sweet potato alkaline invertase seems to be regulated by the substrate, and by certain metabolites and ions. Since in intact tissue nearly all the invertase activity can be attributed to alkaline invertase, it seems that the enzyme is responsible for sucrose decomposition in the absence of injury. In the case of the acid invertase, which is rapidly formed in response to wounding, regulation by substrate and small effector molecules apparently does not occur. Therefore, the control of sucrose decomposition by acid invertase may occur by induction and turnover of the enzyme in the injured tissue.

Development and Decrease of Acid Invertase in Response to Wounding. Since acid invertase was not localized in any cellular fractions of fresh tissue, the increase in soluble enzyme

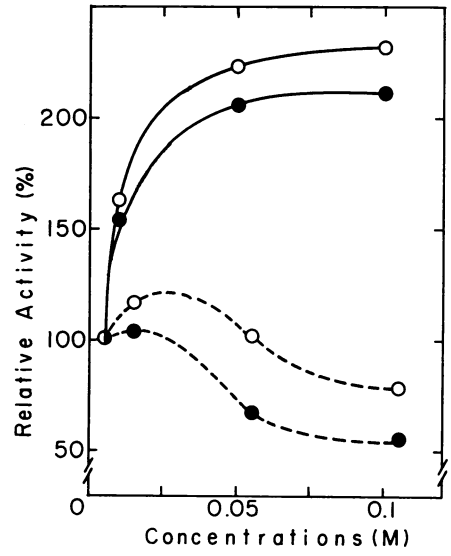

FIG. 6. Effects of ion concentration on the activity of alkaline invertase. Enzyme assays were done in potassium and sodium phosphate buffers, pH 7.6, in 0.005, 0.01, 0.05, and 0.1 M concentrations. Enzyme activity was, likewise, assayed in 0.005 M potassium phosphate buffer containing 0.01, 0.05, or 0.1 M KCl and in 0.005 M sodium phosphate buffer containing 0.01, 0.05, or 0.1 M NaCl. Enzyme activity is expressed as percentage of the activity in 0.005 M buffer. ○——○: Sodium phosphate buffer; ●——●: potassium phosphate buffer; ○- - -○: NaCl; ●- - -●: KCl.

activity with wounding is not due to its release from a particulate form. Therefore, the development of the enzyme activity with wounding may be based on one or more of the following mechanisms: (*a*) the enzyme precursor is activated to the enzyme, (*b*) the inactivating system of the enzyme disappears, or (*c*) the enzyme is newly synthesized *de novo*.

Acid invertase decreases markedly after attaining a maximum following wounding. Such a disappearance of enzyme activity was also observed in phenylalanine ammonia-lyase of Gherkin seedling (9) and Alaska pea (13), which in both cases was preceded by the appearance of an inactivating system dependent upon *de novo* protein synthesis. Protein inhibitors of invertase were also found in potato (33) and in maize (15). Further, a proteinous inhibitor of invertase synthesis was shown to be secreted from Jerusalem artichoke tuber discs during aging (4). It has been suggested by Filner *et al.* (10) and by Marcus (23) that enzyme disappearance requiring new protein synthesis may be an important, specific regulatory mechanism in higher plants. Therefore, the mechanisms behind the increase and decrease in acid invertase activity in response to wounding in sweet potato roots may represent such a regulatory function and therefore should be elucidated.

Acknowledgment—The authors are very grateful to Dr. T. Kosuge, Department of Plant Pathology, University of California at Davis for kindly reading the manuscript and making helpful suggestions.

LITERATURE CITED

1. Akazawa, T. and I. Uritani. 1962. Pattern of carbohydrate breakdown in sweet potato roots infected with *Ceratocystis fimbriata*. Plant Physiol. 37: 662–670.
2. Arnold, W. N. 1966. β-Fructofuranosidase from grape berries. II. Solubilization of a bound fraction. Biochim. Biophys. Acta 128: 124–129.
3. Asahi, T., Y. Honda, and I. Uritani. 1966. Increase of mitochondrial fraction in sweet potato root tissue after wounding or infection with *Ceratocystis fimbriata*. Plant Physiol. 41: 1179–1184.
4. Bradshaw, M. J., J. M. Chapman, and J. Edelman. 1970. Enzyme formation in higher plant tissue: a protein inhibitor of invertase synthesis secreted by tissue slices of plant storage organ. Planta 90: 323–332.
5. Cooper, R. A. and R. N. Greenshields. 1964. The partial purification and some properties of two sucrases of *Phaseolus vulgaris*. Biochem. J. 92: 357–364.
6. Copping, L. G. and H. E. Street. 1972. Properties of the invertases of cultured sycamore cells and change in their activity during culture growth. Physiol. Plant. 26: 346–354.
7. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356.
8. Edelman, J. and M. A. Hall. 1964. Effect of growth hormones on the development of invertase associated with cell walls. Nature 201: 296–298.
9. Engelsma, G. 1968. Photoinduction of phenylalanine deaminase in Gherkin seedlings. III. Effects of excision and irradiation of enzyme development in hypocotyl segments. Planta 82: 355–368.
10. Filner, P., J. L. Wray, and J. E. Varner. 1969. Enzyme induction in higher plants. Science 165: 358–367.
11. Greksák, M., T. Asahi, and I. Uritani. 1972. Increase in mitochondrial activity in diseased sweet potato root tissue. Plant Cell Physiol. 13: 1117–1121.
12. Hatch, M. D., J. A. Sacher, and K. T. Glasziou. 1963. Sugar accumulation cycle in sugar cane. I. Studies on enzymes of the cycle. Plant Physiol. 38: 338–343.
13. Hyodo, H. and S. F. Yang. 1971. Ethylene-enhanced synthesis of phenylalanine ammonia-lyase in pea seedlings. Plant Physiol. 47: 765–770.
14. Jaynes, T. A. and O. E. Nelson. 1971. Invertase activity in normal and mutant maize endosperms during development. Plant Physiol. 47: 623–628.
15. Jaynes, T. A. and O. E. Nelson. 1971. An invertase inhibitor in maize endosperm and factors affecting inactivation. Plant Physiol. 47: 629–634.
16. Johnson, M. J. 1941. Isolation and properties of a pure yeast polypeptidase. J. Biol. Chem. 137: 575–592.
17. Khym, J. X. and L. P. Zill. 1952. The separation of sugars by ion exchange. J. Amer. Chem. Soc. 74: 2090–2094.
18. 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.
19. Kolinska, J. and G. Semenza. 1967. Studies on intestinal sucrase and on intestinal sugar transport. V. Isolation and properties of sucrase-isomaltase from rabbit small intestine. Biochim. Biophys. Acta 146: 181–187.
20. Koshland, D. E., Jr. 1971. The molecular basis for enzyme regulation. *In:* P. D. Boyer, ed., The Enzymes, Vol. 1. Academic Press, New York. pp. 341–396.
21. Lampen, J. O. 1971. Yeast and *Neurospora* invertase. *In:* P. D. Boyer, ed., The Enzymes, Vol. 5. Academic Press, New York. pp. 291–305.
22. Lyne, R. L. and T. ap Rees. 1971. Invertase and sugar content during differentiation of roots of *Pisum sativum*. Phytochemistry 10: 2593–2599.
23. Marcus, A. 1971. Enzyme induction in plants. Annu. Rev. Plant Physiol. 22: 313–336.
24. Minamikawa, T. and I. Uritani. 1965. Phenylalanine ammonia-lyase in sliced sweet potato roots. Effect of antibiotics on the enzyme formation and its relation to the polyphenol biosynthesis. Agr. Biol. Chem. 29: 1021–1026.
25. Minamikawa, T. and I. Uritani. 1965. Phenylalanine ammonia-lyase in sliced sweet potato roots. J. Biochem. 57: 678–688.
26. Murata, T. and Akazawa. 1968. Enzymic mechanism of starch synthesis in sweet potato roots. I. Requirement of potassium ions for starch synthetase. Arch. Biochem. Biophys. 126: 873–879.
27. Muto, S., T. Asahi, and I. Uritani. 1969. Increase in the dehydrogenase activities of the pentose phosphate pathway in sweet potato root tissue after slicing. Agr. Biol. Chem. 33: 176–189.
28. Nakagawa, H. 1972. Purification and some properties of two types of β-fructofuranosidase from tomato fruit. Agr. Biol. Chem. 36: 18–26.
29. Nakagawa, H., T. Hashimoto, N. Ogura, and H. Takenhana. 1972. Cell wall-bound enzyme: properties of β-fructofuranosidase bound to tomato cell wall. Agr. Biol. Chem. 36: 697–702.
30. Nawa, Y., T. Asahi, and I. Uritani. 1970. Modification of Ogur-Rosen method for estimation of RNA in fresh and wounded sweet potato root tissues and change in RNA contents after the cutting. Plant Cell Physiol. 11: 531–539.
31. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375–380.
32. Palmer, J. M. 1966. The influence of growth regulating substances on the development of enhanced metabolic rates in time slices of beet root storage tissue. Plant Physiol. 41: 1173–1178.
33. Pressey, R. 1966. Separation and properties of potato invertase and invertase inhibitor. Arch. Biochem. Biophys. 113: 667–674.
34. Ricardo, C. P. P. and T. ap Rees. 1970. Invertase activity during the development of carrot roots. Phytochemistry 9: 239–247.
35. Sacher, J. A., M. D. Hatch, and K. T. Glasziou. 1963. Sugar accumulation cycle in sugar cane. III. Physical and metabolic aspects of cycle in immature storage tissue. Plant Physiol. 38: 348–354.
36. Shannon, L. M., I. Uritani, and H. Imaseki. 1971. *De novo* synthesis of peroxidase isozymes in sweet potato slices. Plant Physiol. 47: 493–498.
37. Takesue, Y. 1969. Purification and properties of rabbit intestinal sucrase. J. Biochem. 65: 545–552.
38. Uritani, I. and Kato, C. 1973. Change of carbohydrate in diseased and cut-injured sweet potato root tissue. Abstract of the Annual Meeting of Agr. Chem. Soc. Japan, Tokyo, April. p. 138.