Activity and Stability of a Soluble Acid Invertase from Sugarbeet Roots

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ABSTRACT

Soluble acid invertase catalyzes the hydrolysis of sucrose to the invert sugars, glucose and fructose, and has been implicated in sucrose loss during sugarbeet root development, postharvest storage and processing. To better understand the ability of this enzyme to degrade sucrose during sugarbeet root production and processing, the activity and stability of the major sugarbeet root soluble acid invertase were determined under different physical conditions. Maximum enzyme velocity was observed at sucrose concentrations above 20 mM, with a K_m for sucrose of 8.9 mM. The enzyme exhibited a plateau of activity at pH 5.0 to 5.5, a pH range in which most plant soluble acid invertases exhibit high activity. Enzyme activity increased at pH values less than 5.0, possibly due to the loss of an inhibitor. Irreversible inactivation of the enzyme occurred at pH values of 7.5 or greater. Enzyme activity was greatest at 35°C and declined rapidly at temperatures above or below this optimum. At 5°C, the enzyme retained 16% of its maximal activity. Partial inactivation of the enzyme occurred at 40° to 50°C; complete inactivation was observed at 55°C and above. Inactivation at elevated temperatures was rapid and irreversible. This study indicates that the major soluble acid invertase is likely to operate at or near its maximum velocity in sugarbeet roots, is capable of hydrolyzing sucrose at the temperatures

typical of sugarbeet root storage, and is active at the pH conditions typical of sugarbeet root processing.

Key words: acid invertase, *Beta vulgaris*, sucrose, sugarbeet

Sucrose catabolism is a fundamental metabolic process that is essential for the growth, development and maintenance of the sugarbeet crop. Conversion of sucrose to its constituent monosaccharides allows plants to utilize sucrose and provides the metabolic energy and the biosynthetic substrates to support growth during production, heal wounds incurred during harvest, and maintain healthy tissue during production and postharvest storage (Dennis *et al.*, 1997). Sugarbeet root sucrose catabolism, however, is costly to the sugarbeet industry. Sucrose is lost during sugarbeet root production, postharvest storage and processing due to the activity of sucrose catabolizing enzymes. These enzymes reduce the yield of extractable sucrose from the sugarbeet crop directly by their ability to degrade sucrose and indirectly by producing invert sugars which increase the loss of sugar to molasses during processing (Oldfield *et al.*, 1971, Walker *et al.*, 1960). Four enzyme activities, soluble acid invertase, insoluble acid invertase, alkaline invertase and sucrose synthase, are responsible for nearly all sucrose catabolism in sugarbeet root. Although the presence of these sucrolytic enzymes is well documented, their *in planta* functions are largely unknown. Several studies, however, have shown a correlative relationship between soluble acid invertase activity and sugarbeet carbohydrate content. It has been suggested from these studies that soluble acid invertase causes sucrose loss during sugarbeet root development, postharvest storage and processing (Berghall *et al.*, 1997; Giaquinta, 1979; Harvey and Dutton, 1993; Wyse, 1974).

Soluble acid invertase is a vacuolar enzyme that catalyzes the irreversible hydrolysis of sucrose to glucose and fructose. The enzyme is the major sucrolytic enzyme activity in young sugarbeet roots (Giaquinta, 1979). Its activity declines rapidly with root development and has been shown to be minimal during most stages of root development, in mature roots and roots in postharvest storage (Giaquinta, 1979; Wyse, 1974). Several studies have compared soluble acid invertase activity with sucrose and invert sugar concentrations in sugarbeet roots. Berghall *et al.* (1997) and Giaquinta (1979) demonstrated an inverse correlation between sucrose accumulation and soluble acid invertase activity in developing sugarbeet roots. They observed high acid invertase activity and a low sucrose concentration in young sugarbeet roots. With root maturation, soluble acid invertase activity declined in parallel with an increase in root sucrose concentration. Correlations between soluble acid invertase activity, sucrose loss and invert sugar formation have also been observed during sugarbeet root postharvest storage and the initial stages of sugarbeet root

processing. Wyse (1974) and Berghall *et al.* (1997) observed an increase in soluble acid invertase activity during long term storage of sugarbeet roots that correlated highly with an increase in glucose and fructose concentrations. Sucrose loss in sugarbeet roots which had deteriorated during storage due to microbial activity and sucrose loss during the initial extraction of sugarbeet cossettes have also been attributed to soluble acid invertase activity (Berghall *et al.*, 1997; Oldfield *et al.*, 1979).

The studies described above have led their authors to suggest that soluble acid invertase activity is incompatible with sucrose storage (Giaquinta, 1979), and responsible for sucrose loss during postharvest storage (Berghall *et al.*, 1997; Wyse, 1974) and processing (Oldfield *et al.*, 1979). In these studies, enzyme activity assays were used to determine soluble acid invertase activity in relation to root carbohydrate content. Enzyme activity assays, however, measure activity at optimum reaction conditions. Although useful in determining the quantity of active enzyme present, these assays do not measure activity under the different environmental conditions likely to occur in the sugarbeet root during development, postharvest storage and processing. To better assess the capacity of soluble acid invertase to degrade sucrose at the physical conditions encountered during root development, postharvest storage and processing, a soluble acid invertase was isolated from sugarbeet roots, and its activity and stability under different environmental conditions were determined. Sugarbeet roots contain at least two soluble acid invertase isoenzymes (Klotz and Finger, unpublished results). Nearly all soluble acid invertase activity, however, is due to a single isoenzyme. This major isoenzyme of soluble acid invertase is found in sugarbeet roots throughout all stages of development and is the subject of this study. A second minor soluble acid invertase isoenzyme is found only in the roots of young seedlings.

MATERIALS AND METHODS

Plant material: Sugarbeet hybrid, VDH66156 (Van der Have, Netherlands) was greenhouse grown in Sunshine mix #1 (Sun Gro Horticultural Products, Canada) with supplemental light and 16 h days. Roots were harvested 5 to 6 weeks after planting, rapidly frozen with liquid N_2 and lyophilized. Harvest date was chosen to maximize yield of soluble acid invertase for enzymatic studies.

Enzyme extraction and partial purification: Lyophilized roots were ground to a powder and homogenized in extraction buffer containing 50 mM HEPES-NaOH, pH 7.5, 5 mM β-mercaptoethanol, 10 mM Na_2SO_3, 1 mM EDTA, 1 mM $MgCl_2$, 1 mM benzamidine and 100 μM PMSF. The homogenate was filtered through four layers of cheesecloth and centrifuged for 30 min at 17,000*g* to remove insoluble material. Proteins were

fractionated by sequential precipitation with increasing concentration of $(NH_4)_2SO_4$. Proteins were collected by centrifugation at 17,000*g* after precipitation with $(NH_4)_2SO_4$ at 0 to 20%, 21 to 40%, 41 to 60% and 61 to 80% saturation. Proteins were solubilized in resuspension buffer (25 mM HEPES-NaOH, pH 7.5; 5 mM β-mercaptoethanol). Protein solutions were dialyzed overnight against dialysis buffer (10 mM HEPES-NaOH, pH 7.5; 1 mM β-mercaptoethanol). All steps were performed at 4°C.

Isoelectric focusing gel electrophoresis and activity staining: Dialyzed fractions from $(NH_4)_2SO_4$ fractionation were subjected to flat bed isoelectric focusing on polyacrylamide gels with ampholines in the pH range of 3.5 to 9.5 (Amersham Pharmacia Biotech, Sweden). Electrophoresis was conducted for 1.5 h at 0.10 W/cm^2 at 10°C. Acid and alkaline invertase activities were localized in gels by activity staining. Gels were incubated for 30 min at 35°C in 100 mM NaOAc, pH 4.7 or 100 mM HEPES-NaOH, pH 7.7 with 100 mM sucrose for acid or alkaline invertase activity, respectively. Gels were rinsed with distilled H_2O and stained with 0.1% 2,3,5-triphenyltetrazolium chloride and 0.5 N NaOH warmed to 100°C (Gabriel and Wang, 1969). Control gels were run as described above except sucrose was omitted from the incubation solution. Isoelectric points were determined by comparison of the mobility of the enzymes in an isoelectric focusing gel with standards of known pI (BioRad, Hercules, CA, USA)

Invertase and total protein assays: All assays were conducted with the dialyzed 61 to 80% $(NH_4)_2SO_4$ protein fraction described above. The invertase assay was a modification of the two step protocol of Goldstein and Lampen (1975). The protein fraction (20 to 50 µl) was incubated for 30 min at 37°C with 100 mM NaOAc, pH 4.7 and 100 mM sucrose or 100 mM HEPES-NaOH, pH 8.0 and 100 mM sucrose in a 100 µl total volume for acid or alkaline invertase activity assays, respectively. Reaction was stopped by addition of an equal volume of 0.5 M potassium phosphate, pH 7.0 and boiled for 3 min. Glucose concentration was determined by addition of 1 ml of a solution containing 14 U glucose oxidase, 1 U peroxidase, 24 µM *o*-dianisidine and 38% glycerol and incubation for 30 min at 30°C. Reaction was stopped by addition of 1.5 ml 6N HCl, and the absorbance at 540 nm was measured. Total protein was measured by the method of Bradford (1976) using bovine serum albumin as standard.

Fructose inhibition: Enzyme activity with 10 and 100 mM sucrose was measured with and without added D-fructose to determine the extent of fructose inhibition on the reaction. Concentrations of added fructose were 5, 10, 15, 20, 25 and 30 mM.

pH and temperature optima: The pH optimum was determined by assaying enzyme activity as described above using 100 mM buffers ranging from pH 2.5 to 9.0 at 0.5 pH unit intervals in place of NaOAc, pH

4.7. Buffers used were citric acid-NaOH (pH 2.5 to 6.0), HEPES-NaOH (pH 6.5 to 8.0) and bicine-NaOH (pH 8.5 to 9.0). A temperature optimum was determined by assaying enzyme activity at 5 to 60°C for 30 min. The protein extract was preincubated for 10 min at the reaction temperature before initiation of the reaction with sucrose.

pH and temperature inactivation: The ability of varying pH conditions to irreversibly inactivate soluble acid invertase was determined by preincubation of the enzyme with 100 mM of the appropriate buffer for 15 to 180 min at 4°C. The pH ranged from 2.5 to 9.0 at 0.5 pH unit intervals using the buffers described above. Activity assay was conducted as described above with 100 mM NaOAc, pH 4.7. Thermal inactivation was measured by preincubation of the enzyme in dialysis buffer at 40, 45, 50 and 55°C for 5 to 30 min. After temperature treatment, the enzyme was incubated at 4°C for 30 min to allow the protein to refold. Activity assay was conducted as above.

RESULTS AND DISCUSSION

Isolation of soluble acid invertase activity: The major soluble acid invertase isoenzyme of sugarbeet (*Beta vulgaris* L.) root was isolated and its activity and stability under different physical conditions were determined. The soluble acid invertase isoenzyme was isolated from other invertase activities by centrifugation and ammonium sulfate fractionation. Insoluble acid invertase activity remained bound to the cell wall during extraction and was removed by centrifugation. Alkaline invertase isoenzymes were removed by ammonium sulfate fractionation (Table 1). Ammonium sulfate precipitation at 21 to 60% saturation removed nearly all alkaline invertase activity from crude extracts with most alkaline invertase activity precipitating at 21 to 40% ammonium sulfate saturation. After removal of alkaline invertase activity, soluble acid invertase was precipitated by increasing the ammonium sulfate saturation to 80%. The ammonium sulfate fractionation resulted in a 3.5-fold enrichment of acid invertase activity with only a trace of contaminating alkaline invertase activity. The 61 to 80% ammonium sulfate fraction was used in all subsequent experiments.

Isoelectric focusing confirmed the presence of a single soluble acid invertase isoenzyme in sugarbeet root extracts. Fractions from ammonium sulfate precipitation were subjected to isoelectric focusing polyacrylamide gel electrophoresis and stained for acid and alkaline invertase activities (Fig 1). The 61 to 80% ammonium sulfate fraction contained a single acidic isoenzyme with an isoelectric point of 4.7 (Fig 1A). Isoelectric focusing revealed the presence of at least two alkaline invertase isoenzymes in sugarbeet roots with isoelectric points of 5.3 and 5.9 (Fig 1B). Some separation of alkaline invertase isoenzymes by

Table 1. Separation of acid and alkaline invertase activities by ammonium sulfate fractionation. Specific and relative activities for soluble acid invertase and alkaline invertase of protein solutions obtained by ammonium sulfate fractionation after dialysis. Relative activity is the activity of the fraction to the crude extract.

Ammonium Sulfate Concentration (% saturation)	Alkaline Invertase		Acid Invertase	
	Specific Activity (μmol glucose h^{-1} mg $protein^{-1}$)	Relative Activity	Specific Activity (μmol glucose h^{-1} mg $protein^{-1}$)	Relative Activity
Crude Extract	0.681	1.00	0.130	1.00
0 – 20% $(NH_4)_2SO_4$	0.237	0.34	0.003	0.02
21 – 40% $(NH_4)_2SO_4$	1.550	2.27	0.003	0.02
41 – 60% $(NH_4)_2SO_4$	0.864	1.26	0.210	1.61
61 – 80% $(NH_4)_2SO_4$	0.060	0.08	0.450	3.46

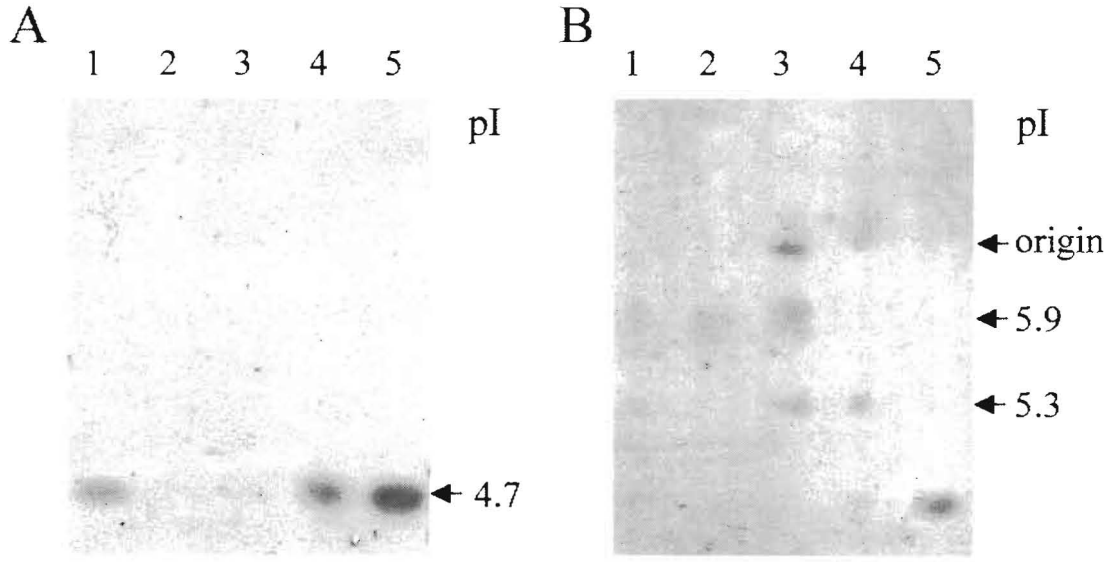

Fig 1. Activity stained isoelectric focusing gel of crude extract and fractions from ammonium sulfate precipitations. Lane 1: crude extract, lane 2: 0-20% ammonium sulfate fraction, lane 3: 21-40% ammonium sulfate fraction, lane 4: 41-60% ammonium sulfate fraction, lane 5: 61-80% ammonium sulfate fraction. (A) Stained for acid invertase activity at pH 4.7. (B) Stained for alkaline invertase activity at pH 7.7. Bands occurring at origin in (B), lanes 3 and 4 are due to protein precipitation at the site of loading. Some acid invertase is evident in (B), lane 5, due to a localized pH effect caused by ampholines.

ammonium sulfate fractionation was evident. Although two alkaline invertase isoenzymes were evident in the 21 to 40% ammonium sulfate fraction, only the more anionic isoenzyme was present in the 41 to 60% fraction. No staining was observed in control gels in which the isoelectric focused proteins were incubated without sucrose (data not shown). Masuda *et al.* (1987) have previously reported the presence of two alkaline invertases in sugarbeet root. Alkaline invertases are cytoplasmic enzymes that catalyze the same sucrose hydrolysis reaction as acid invertases (Tymowska-Lalanne and Kreis, 1998). Alkaline invertase activity is present in sugarbeet root at all but the earliest stages of development (Masuda *et al.*, 1987), at harvest and during postharvest storage (Wyse, 1974). To date, no function has been definitively established for alkaline invertases in plants and the significance of their presence in sugarbeet root is not known.

Enzyme kinetics: The major soluble acid invertase isoenzyme of sugarbeet roots follows Michaelis-Menton kinetics (Fig 2). At concentrations above 20 mM sucrose, the reaction rate was essentially zero order and was independent of sucrose concentration (Fig 2A). The

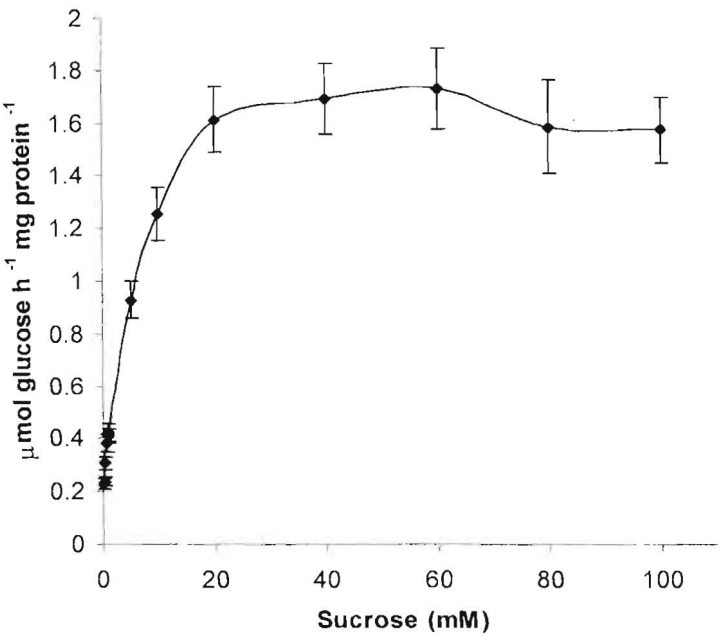

Fig 2A. Effect of sucrose concentration on soluble acid invertase activity at pH 4.7 and 37°C. Error bars = one standard deviation.

$K_{m[sucrose]}$ for acid invertase was 8.9 mM as calculated from a Lineweaver-Burk plot (Fig 2B). Vacuolar sucrose concentrations in sugarbeet roots

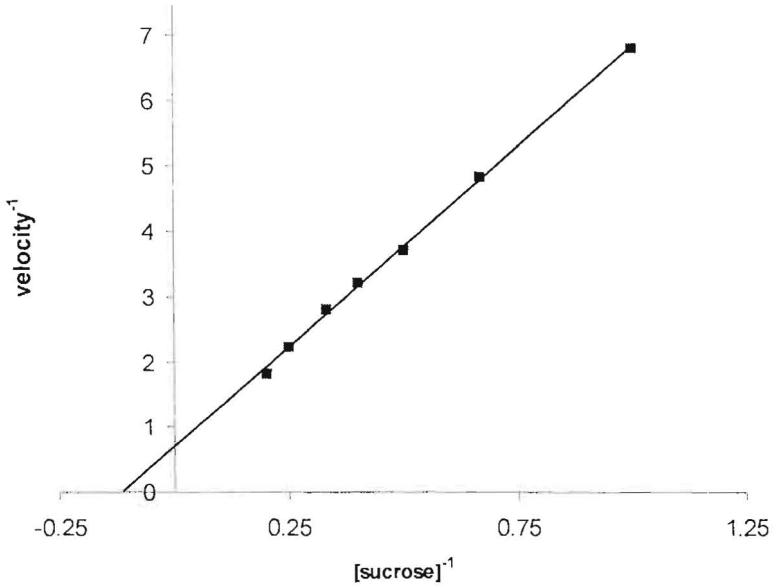

Fig 2B. Lineweaver-Burk plot of soluble acid invertase activity for varying concentrations of sucrose. Sucrose concentration is expressed in mM, velocity in µmole glucose h^{-1} mg $protein^{-1}$. Error bars = one standard deviation.

are in large excess of this K_m value and can reach 600 mM in mature sugarbeet roots (Semenov, 1986). The enzyme, therefore, is likely to function at its maximum velocity *in planta*. K_m values in the range of 2 to 13 mM are typical for soluble acid invertases isolated from plants (Hawker, 1985). Masuda *et al.* (1988) reported a K_m of 3.8 mM for a soluble acid invertase isoenzyme found in sugarbeet suspension cultured cells, but whether this is the same acid invertase isoenzyme found in roots is not known.

Many plant soluble acid invertase isoenzymes exhibit inhibition by fructose (Sturm, 1999; Kingston-Smith *et al.*, 1999). No enzyme inhibition by fructose was observed at either high or low sucrose concentrations for the major soluble acid invertase isoenzyme of sugarbeet root. Fructose concentrations of 5 to 30 mM had no effect on enzyme reaction rate with 10 or 100 mM sucrose (data not shown).

pH optimum and pH stability: Soluble acid invertase exhibited a plateau of activity at pH 5.0 to 5.5 (Fig 3). Plant soluble acid invertases

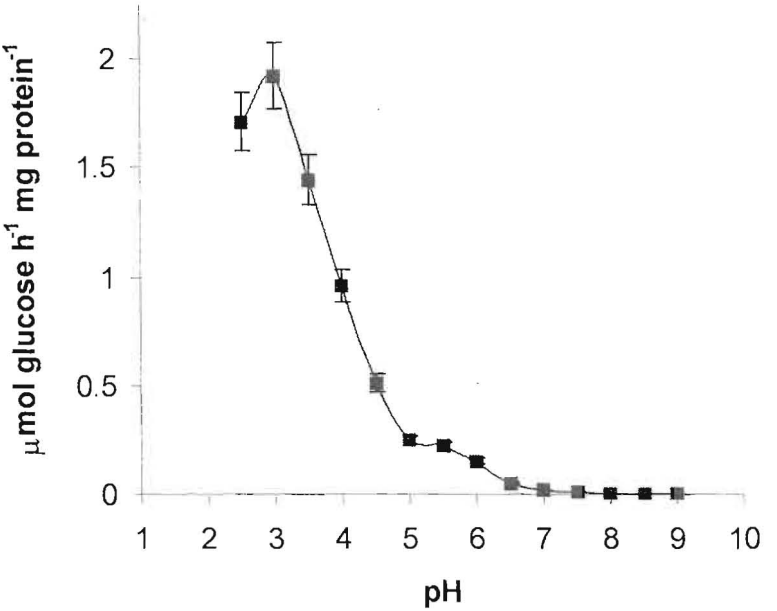

Fig 3. Effect of pH on soluble acid invertase activity. Activity was measured at 37°C in 100 mM buffer at the given pH. Buffers used: citric acid-NaOH (pH 2.5 to 6.0), HEPES-NaOH (pH 6.5 to 8.0), bicine-NaOH (pH 8.5 to 9.0). Error bars = one standard deviation.

typically exhibit optimum activity at pH 4.5 to 5.0 (Tymowska-Lalanne and Kreis, 1998), and a pH optimum of 5.0 has previously been reported for a soluble acid invertase from sugarbeet suspension cultured cells and roots of red beet (Masuda *et al.*, 1988; Milling *et al.*, 1993). With increasing pH, activity gradually declined until all activity was lost at pH 8.0. Surprisingly, invertase activity increased 7.5-fold with a decrease in pH from 5.0 to 3.0. This activity increase could not be attributed to buffer effects since all assays between pH 2.5 and 6.0 used the same concentration of sodium citrate buffer. Controls also established that the activity increase was not due to acid hydrolysis of sucrose. Although the cause of this increase in activity is unknown, a similar pH response has been observed with acid invertases from other plant species. Straus (1962) examined the pH profile for cell wall acid invertases isolated from several plant species. Four of the six invertases examined exhibited an increase in

enzyme activity at pH 3.8 or less. In potato (*Solanum tuberosum* L.), a similar increase in acid invertase activity was observed at low pH values and was caused by a decrease in effectiveness of a specific proteinaceous acid invertase inhibitor (Pressey, 1967). Like potato, sugarbeet contains a proteinaceous acid invertase inhibitor (Pressey, 1968). The increase in sugarbeet acid invertase activity observed in these studies may be due to the loss or decreased effectiveness of this inhibitor. It is noteworthy that the major soluble acid invertase isoenzyme was active at pH values typical of sugarbeet processing. Solution pH during sugarbeet root extraction is typically in the range of 5.0 to 6.6 (Vetter, 1998), a pH range in which the enzyme exhibited activity. Lower pH values occur during the processing of diseased roots and pH values as low as 4.5 have been reported (Vetter, 1998). At these lower pH values, the enzyme's ability to hydrolyze sucrose was enhanced.

Stability of the soluble acid invertase isoenzyme was also dependent on pH (Fig 4). Enzyme stability was measured over three

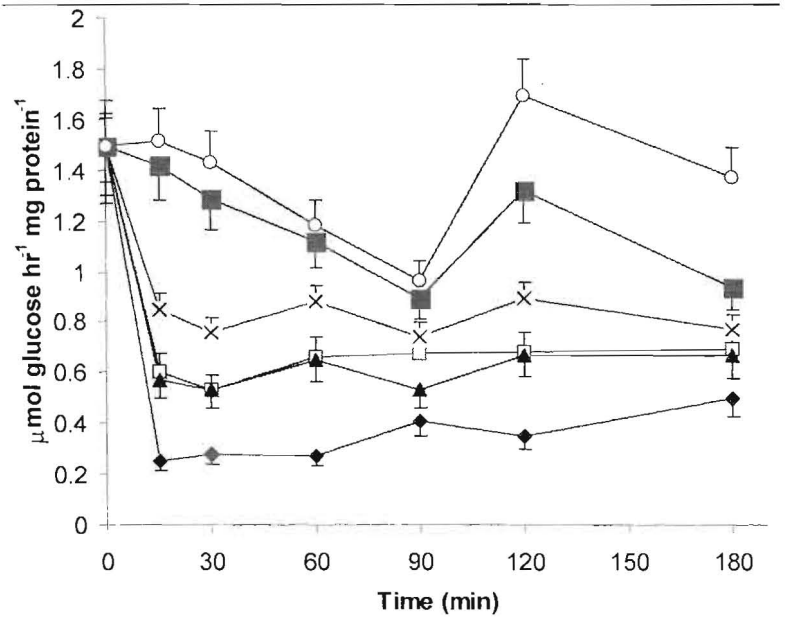

Fig 4. Stability of soluble acid invertase activity at different pH values. Enzyme was incubated at 4°C with 100 mM buffer of the given pH for the indicated length of time, and assayed for activity at pH 4.7. Buffers used were citric acid-NaOH (pH 3, 4.7), HEPES-NaOH (pH 7.5, 8.0), bicine-NaOH (pH 8.5, 9.0). pH 3.0 (O), pH 4.7 (■), pH 7.5 (x), pH 8.0 (▲), pH 8.5 (□), and pH 9.0 (♦). Error bars = one standard deviation.

hours at six pH values ranging from 3.0 to 9.0. The enzyme was unstable at alkaline pH. An irreversible loss of activity occurred within fifteen minutes at pH 7.5, 8.0, 8.5 and 9.0. The extent of inactivation increased with each incremental increase in pH. At pH 7.5 and 9.0, activity declined by 43% and 83%, respectively. Short exposures to acidic pH values also caused a loss of enzyme activity. Invertase activity decreased by 36 to 40% after 90 minutes at pH 3.0 and 4.7. Further incubation (90 to 120 min) at these pH values resulted in a recovery of activity, followed by a decline in activity (120 to 180 min) at a rate comparable to the decline observed in the first 90 minutes. The cause for this return and subsequent loss of activity is unknown, but reproducible. Clearly, pH conditions affected the stability of the soluble acid invertase isoenzyme. Alkaline conditions were more effective at denaturing the enzyme and irreversibly reducing its activity. No pH value examined, however, completely eliminated acid invertase activity.

Temperature optimum and thermal stability. The major soluble acid invertase isoenzyme exhibited greatest activity at 35°C (Fig 5). Its

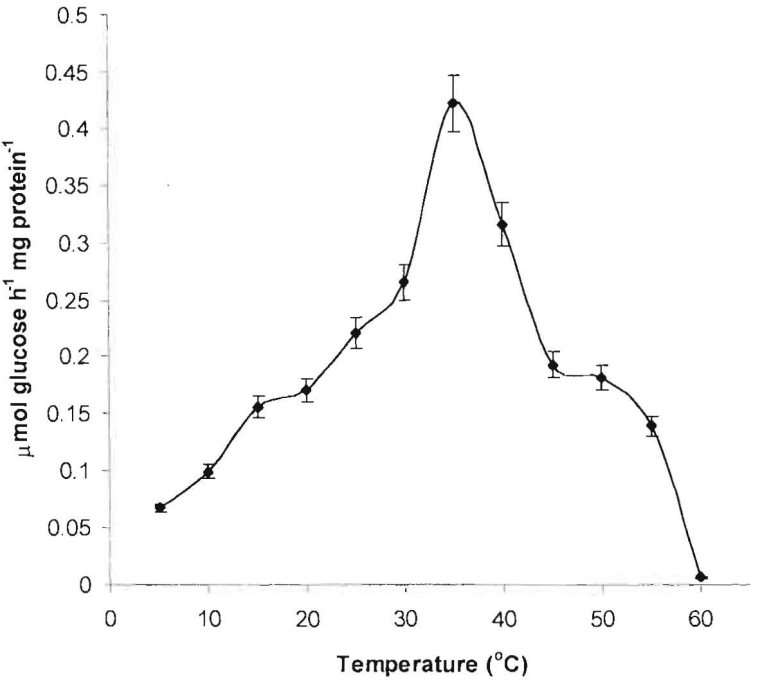

Fig 5. Effect of temperature on soluble acid invertase activity at pH 4.7. Error bars = one standard deviation.

activity, however, decreased sharply with a 5°C temperature change in either direction. At 30° and 40°C, activity was 63 and 75% of the activity at 35°C, respectively. The enzyme was completely inactive at 60°C, but maintained 16% of its maximum activity at 5°C. The maintenance of activity at relatively low temperatures suggests the ability of this enzyme to degrade sucrose under typical postharvest storage conditions. Although storage temperatures are variable, the optimal temperature for sugarbeet root storage is 4 to 6°C (Bugbee, 1993).

The enzyme's thermal stability was also determined since irreversible thermal inactivation of sucrose degrading enzymes is critical to limit sucrose loss during sugarbeet root processing. Enzyme activity was measured at optimum pH and temperature conditions after incubation at elevated temperatures for different time durations. In this way, the enzyme's ability to renature and regain activity after thermal inactivation could be assessed. The soluble acid invertase isoenzyme was unstable to short incubations at elevated temperatures (Fig 6). Irreversible loss of

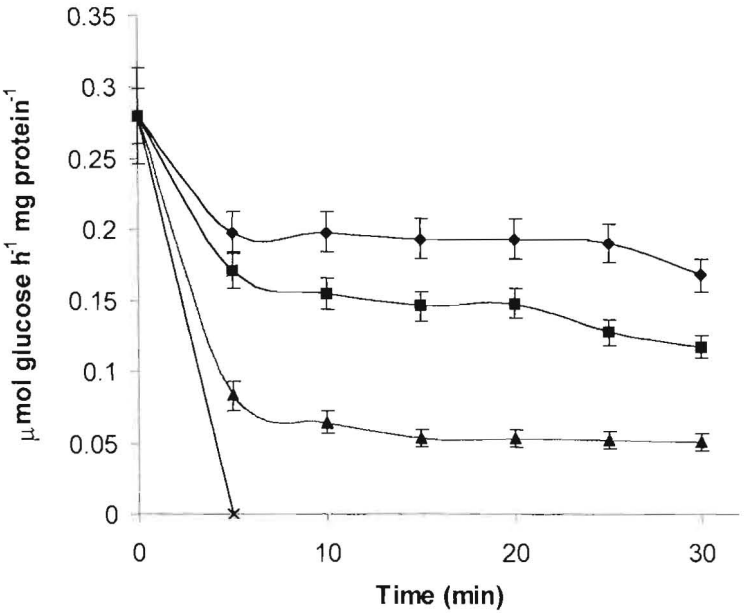

Fig 6. Stability of soluble acid invertase activity at different temperatures. Enzyme was incubated at given temperature for the indicated time period, allowed to refold by incubation at 4°C for 30 minutes, and assayed at pH 4.7 and 37°C. 40°C (♦), 45°C (■), 50°C (▲), and 55°C (x). Error bars = one standard deviation.

activity was observed at 40°C or above, and was rapid and temperature dependent. Within five minutes, activity was reduced by 29, 39, 70 and 100% at 40°, 45°, 50°, and 55°C, respectively. Extended incubation at elevated temperatures caused little or no additional loss in activity beyond what occurred after 5 minutes.

Sugarbeet roots are typically extracted at 68 to 75°C (Vetter, 1998). At these temperatures, the major soluble acid invertase isoenzyme was completely and irreversibly inactivated. The extraction temperatures used in sugarbeet root processing, however, do not preclude sucrose degradation by the action of this enzyme. Because sugarbeet roots are sliced at cold or freezing temperatures and warmed to optimum extraction temperatures, a time period exists during processing in which temperatures are not sufficient to inactivate this enzyme. During this warming period, sucrose degradation by soluble acid invertase is possible.

A study of the activity and stability of the major soluble acid invertase isoenzyme of sugarbeet root suggests that the enzyme is capable of degrading sucrose under the conditions found during root development, postharvest storage and processing. Kinetic data indicate that the enzyme is likely to function at or near its maximum velocity in sugarbeet roots. Sucrose accumulation, therefore, is unlikely in cells containing this enzyme activity, since sucrose storage and soluble acid invertase are both localized in the vacuole (Leigh *et al.*, 1979). Sucrose degradation by soluble acid invertase may be controlled in sugarbeet roots by limiting the quantity of active enzyme and restricting its access to sucrose by compartmentalization. Soluble acid invertase activity is low during the stages of development when sucrose accumulates in the sugarbeet root (Giaquinta, 1979), and during postharvest storage (Wyse, 1974). While soluble acid invertase activity is limited during periods of sucrose storage, whether soluble acid invertase activity is controlled by limiting biosynthesis of the enzyme or by inhibiting enzyme activity is not known. Sucrose degradation is also likely to be controlled by maintaining soluble acid invertase and sucrose storage in different cells or tissues. In other plant species, soluble acid invertase has been shown to be localized to specific tissues and cells (Kingston-Smith *et al.*, 1999). Cellular compartmentalization, however, can be lost due to harvest damage, freeze-thaw cycles during postharvest storage, and slicing of roots for processing, and may be a cause of sucrose loss during harvest, postharvest storage and processing of sugarbeet roots.

The extent of sucrose loss to soluble acid invertase has not been quantified. This study, however, reveals the potential of this enzyme to degrade sucrose during sugarbeet root postharvest storage and processing. The major soluble acid invertase isoenzyme exhibited activity at the

environmental conditions typical of postharvest storage and the initial stages of processing. The enzyme retained activity at the cold temperatures typical of sugarbeet root storage. The enzyme was also active at the pH and temperature conditions that occur in the initial stages of processing in which cold or frozen roots are sliced and warmed to optimum extraction temperatures. At optimum extraction temperatures, the major soluble acid invertase isoenzyme was completely and irreversibly inactivated. Rapid warming of cossettes to optimum extraction temperature, therefore, would limit sucrose hydrolysis by soluble acid invertase during sugarbeet root processing. Sucrose loss by soluble acid invertase during processing can also be minimized by pH control of the extraction medium. Soluble acid invertase activity is reduced at pH values of 6.0 or greater and enhanced at pH values less than 5.0.

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