Biochemical Properties of Three Sugarbeet Root Sucrolytic Enzymes and their Implications for Postharvest Sucrose Loss

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Introduction

Sucrose is lost during sugarbeet root postharvest storage and processing due to the presence of endogenous enzymes capable of degrading sucrose. Sugarbeet roots contain a number of enzymes that convert sucrose to invert sugars. These enzymes are present at harvest and allow roots to remain metabolically active until frozen or processed. Sucrose loss due to continued metabolism is costly to the sugarbeet industry. It has been estimated that 100 to 250 g of sucrose is lost per day per ton of roots during storage (Bugbee, 1993, van der Poel *et al.*, 1998). Sucrolytic enzymes also contribute to the sucrose loss that occurs when stored roots thaw and during the initial stages of processing. In both cases, cell rupture caused by a freeze-thaw cycle or slicing during the first steps of processing, eliminates the cellular compartmentalization that separates sucrose from the enzymes that degrade it. Sucrose loss by the action of these enzymes occurs directly by the degradation of sucrose and indirectly by the formation of invert sugars that increase the loss of sugar to molasses.

The role of individual sucrolytic enzymes in postharvest sucrose loss is unknown. Several studies, however, have suggested the involvement of soluble acid invertase and/or sucrose synthase activities (Berghall *et al.*, 1997; Sakalo & Tyltu, 1997; Wyse, 1974). Soluble acid invertase is a vacuolar enzyme that catalyzes the hydrolysis of sucrose to glucose and fructose. Soluble acid invertase activity is found at low levels in sugarbeet roots at harvest and has been observed to increase during storage in parallel with an increase in invert sugar concentrations (Berghall *et al.*, 1997; Wyse, 1974). Sucrose synthase is a cytoplasmic enzyme that catalyzes the reversible reaction of sucrose with uridine 5'-diphosphate (UDP) to form UDP-glucose and fructose. Sucrose synthase is the predominant sucrolytic activity in postharvest sugarbeet roots and occurs at levels far in excess of any other sucrolytic enzyme activity. To better assess the ability of these enzymes to degrade sucrose under typical postharvest storage and processing conditions, the major soluble acid invertase isoenzyme and two sucrose synthase isoforms of sugarbeet roots were isolated and their activities under different environmental conditions were determined.

Materials and Methods

Protein purification

Enzymes were isolated from greenhouse grown roots of sugarbeet hybrid VDH66156. Soluble acid invertase was partially purified from lyophilized 5 to 6 week old root tissue by homogenization in 50 mM HEPES, pH 7.5, 10 mM Na_2SO_3, 5 mM β-mercaptoethanol, 1 mM EDTA, 1 mM $MgCl_2$, 1 mM benzamidine and 100 μM PMSF. Homogenate was filtered through

cheesecloth and centrifuged to remove cell debris. Acid invertase was precipitated with $(NH_4)_2SO_4$ at 61 to 80% saturation and dialyzed against 10 mM HEPES, pH 7.5 and 1 mM β-mercaptoethanol before use. Sucrose synthase isoforms were partially purified from 6 and 16 week old roots for sucrose synthase I and sucrose synthase II, respectively. Lyophilized tissue was homogenized with 50 mM HEPES, pH 7.2, 10 mM Na_2SO_3, 5 mM β-mercaptoethanol and 1 mM $MgCl_2$. Homogenate was filtered through cheesecloth and centrifuged. Sucrose synthase isoforms were precipitated by $(NH_4)_2SO_4$ at 20 to 45% saturation and dialyzed against 10 mM HEPES, pH 7.2 and 1 mM β-mercaptoethanol. Dialyzed fractions were purified over a cibachron blue column eluted with 0.5 M NaCl. Sucrose synthase II was further purified by passage over a Q-sepharose column eluted with a 0.2 to 0.6 M NaCl gradient. Sucrose synthase isoforms were dialyzed as described above after passage over each column.

Enzyme activity assays

Acid invertase activity was determined by the method of Goldstein and Lampen (1975). Sucrose synthase activity was determined by the method of Somogyi (1952). Total protein was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Results and Discussion

The effect of environmental conditions on the sucrose degrading ability of the major soluble acid invertase and two sucrose synthase isoforms of sugarbeet roots was determined. The major soluble acid invertase isoenzyme is responsible for nearly all soluble acid invertase activity in sugarbeet roots. A second soluble acid invertase isoenzyme is also found in sugarbeet roots, although its activity has only been detected in seedlings. Sugarbeet root sucrose synthase activity is due to two sucrose synthase isoforms (sucrose synthase I and sucrose synthase II). Both sucrose synthase isoforms are present in sugarbeet roots at harvest and occur in nearly equivalent amounts. The effect of environmental conditions on the activities of these two sucrose synthase isoforms was determined separately.

Effect of Temperature

The effect of temperature on the activity of the major soluble acid invertase isoenzyme and two sucrose synthase isoforms was determined (Fig. 1). The optimum temperatures for acid invertase, sucrose synthase I and sucrose synthase II were 35°, 50° and 40-45°C, respectively. The acid invertase isoenzyme and sucrose synthase II were completely and irreversibly inactivated at temperatures of 60°C or greater. Inactivation of sucrose synthase I required temperatures of 65°C or greater. A temperature of at least 65°C, therefore, is required to completely inactivate all three enzymes. Sugarbeet roots are typically extracted at 68 to 75°C (van der Poel *et al.*, 1998). Although these extraction temperatures are sufficient to completely inactivate all three enzyme activities, sucrose degradation by these enzymes during processing is not precluded. Sugarbeet roots are sliced at cold or freezing temperatures and warmed to optimum extraction temperatures. A time period, therefore, exists during processing in which temperatures are not sufficient to inactivate these enzymes. Of particular note, is the heat stability of the two sucrose synthase isoforms which exhibited increasing activity with

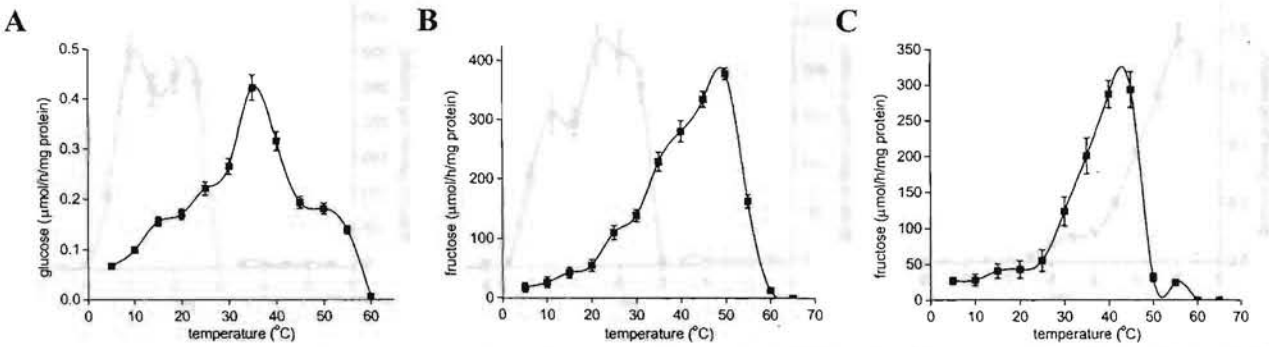

Figure 1: Temperature effect on activity of (**A**) the major soluble acid invertase isoenzyme, (**B**) sucrose synthase I, and (**C**) sucrose synthase II. Error bars = one standard deviation.

temperature increases up to 50° and 45°C for sucrose synthase I and sucrose synthase II, respectively. All three enzymes retained a portion of their activity at temperatures typical of storage. At 5°C, the acid invertase isoenzyme, sucrose synthase I and sucrose synthase II retained, respectively, 16, 8 and 14% of their activity relative to their activity at 35°C. This suggests that the major soluble acid invertase isoenzyme and both sucrose synthase isoforms are capable of degrading sucrose during postharvest storage.

Effect of pH

The activities of the major soluble acid invertase isoenzyme and two sucrose synthase isoforms were also dependent on solution pH (Fig. 2). Acid invertase exhibited a plateau of activity at pH 5.0 to 5.5, and its activity increased 7.5 fold with a decrease in pH from 5.0 to 3.0. Although the cause of the activity increase between pH 3.0 and 5.0 has not been determined, a similar pH response has been observed for an acid invertase in potato and is due to a decreased effectiveness of a specific acid invertase inhibitor (Pressey, 1967). Sucrose synthase I was active in the pH range of 5.5 to 8.0; sucrose synthase II was active in the pH range of 5.5 to 7.5. Solution pH during sugarbeet root extraction is typically in the range of 5.0 to 6.6 (van der Poel *et al.*, 1998). At these pH values, sucrose degradation can occur by the action of acid invertase and/or sucrose synthase isoenzymes. Lower pH values have been observed during the processing of diseased roots and pH values as low as 4.5 have been reported (van der Poel *et al.*, 1998). Sucrose loss due to acid invertase activity would be expected to be exacerbated by these conditions.

Acknowledgments

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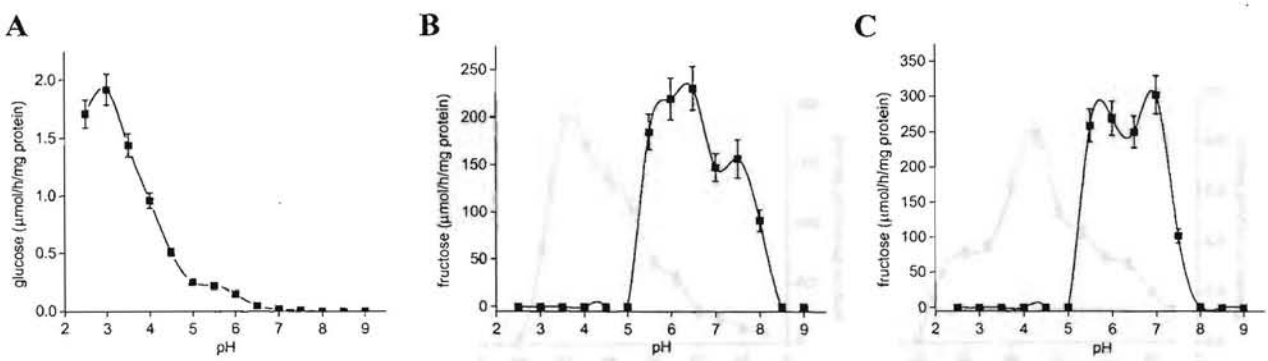

Figure 2: pH effect on activity of (**A**) the major soluble acid invertase isoenzyme, (**B**) sucrose synthase I, and (**C**) sucrose synthase II. Error bars = one standard deviation.

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