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Biophysical Chemistry 65 (1997) 211–220

Biophysical Chemistry

Kinetic model for carbon partitioning in *Solanum tuberosum* tubers stored at 2°C and the mechanism for low temperature stress-induced accumulation of reducing sugars

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Received 2 October 1996; accepted 2 November 1996

Abstract

Exposure to low but nonfreezing temperatures induces the breakdown of starch and the accumulation of sucrose, glucose and fructose in potato tubers, a complex phenomenon known as low-temperature sweetening (LTS). A kinetic model for the degradation of starch to sucrose, fructose, glucose, hexose phosphates and carbon dioxide in 2°C-stored mature *Solanum tuberosum* cv. Norchip (LTS-sensitive) and *Solanum tuberosum* seedling ND860-2 (LTS-tolerant) tubers is presented in this work. Analysis of sugar accumulation data in tubers grown in 1993 and 1994 showed no significant differences in the rates of conversion of starch to hexose phosphates and hexose phosphates to sucrose for both cultivars ($P > 0.05$). The rate constant corresponding to invertase activity was 2.3 day^{-1} for Norchip tubers and 1.1 day^{-1} for ND860-2 tubers grown in 1993 ($P \leq 0.05$); however, no significant differences were observed in invertase activity for 1994-grown tubers ($P > 0.05$). The accumulation of the reducing sugars fructose and glucose was found to be dependent on the relative difference in rate constants corresponding to invertase activity and glycolytic/respiratory capacity. This difference was 3–4 fold greater for Norchip in 1993, and 4–6 fold greater for Norchip in 1994, than for ND860-2 ($P \leq 0.05$). Results from the analysis also suggest that the amount of available starch for degradation was greater in Norchip tubers than ND860-2 tubers ($P \leq 0.05$). Our analysis suggests that tubers with decreased invertase activity coupled to increased glycolytic/respiratory capacity should be more tolerant to low-temperature stress.

Keywords: Sucrose; Glucose; Fructose; Invertase; Respiration; Glycolysis; Starch degradation; Potatoes

1. Introduction

Low temperature sweetening (LTS) is a phenomenon that occurs when potato tubers are exposed to cold temperatures, usually below 8–10°C, and results in the accumulation of starch breakdown products, primarily sucrose and the reducing sugars glucose and fructose [1–5]. Although this phenomenon has been recognized for centuries, our understanding of the mechanisms responsible for LTS is relatively incomplete.

The cold stress-induced production of sucrose, fructose and glucose in potato tubers is not believed to be controlled by a single factor, but by the interaction of several pathways of carbohydrate metabolism including starch synthesis, glycolysis, mitochondrial

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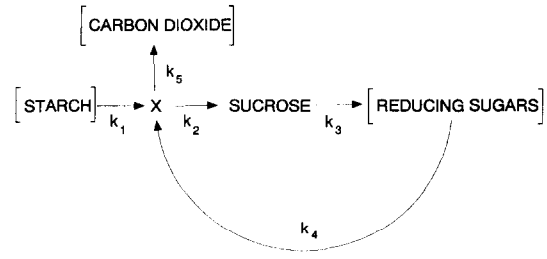
PII S0301-4622(96)02257-0

respiration and gluconeogenesis [1–5]. The interactions among pathways are complex and transient oscillatory behavior in sucrose tissue levels has been observed at 4°C [6]. The exact mechanism responsible for the stress-induced sweetening of potato tissue at the molecular level is still unknown; however, the levels of cellular regulation include: 1) hormones; 2) membrane structure and function; 3) compartmentation and concentration of key ions, substrates, enzymes and other effectors; and 4) enzyme synthesis and activity [1–5].

The purpose of this research was to develop a general mathematical model that describes starch degradation to sucrose, reducing sugars and carbon dioxide in stored mature potato tubers, based on what is currently known about low temperature sweetening. The dynamics of sugar accumulation at 2°C in the LTS-sensitive cultivar Norchip and the LTS-tolerant seedling ND860-2 were determined for tubers grown in 1993 and 1994. A metabolic kinetic model was built to describe the degradation of starch to reducing sugars in order to determine the key regulatory steps in the sequence of events leading to low-temperature sweetening.

2. Theory

Starch is the energy and carbon source for the production of sugars induced by low-temperature stress [7]. Under low temperature conditions, starch degradation results in an accumulation of reducing sugars (glucose and fructose) via sucrose due to invertase activity [2–4]. Zrenner et al. [8] have recently shown that it is indeed soluble acid invertase that controls the hexose to sucrose ratio in cold-stored potato tubers. These authors also reported that the total amount of reducing sugars accumulated was not, however, correlated with invertase activity. In addition to the changes in sugar accumulation patterns observed at storage temperatures below 8–10°C, respiration rate changes have also been observed [9]. Respiration rate decreases as storage temperature is decreased below 10°C, but at storage temperatures below 5°C respiration is transiently stimulated [10]. Several studies have reported a respiratory burst followed by a subsequent decrease in respiration rate to a new steady state [7,11,12]. The initial respiratory burst has been attributed to the combination of cytochrome-mediated and cyanide-resistant respiration [9]. This respiratory burst is accompanied by a burst in sucrose concentration and subsequent decreases in ATP levels [11]. The interactions between cyanide-resistant respiration, ATP levels and sucrose accumulation are complex and their relationship to LTS unclear at the moment [13]. One aspect to consider, however, is that activation of this cyanide resistant respiration and increases in sucrose levels may be partially independent from each other [9]. Aside from this transient burst in cyanide resistant respiration, cytochrome-mediated respiration has been shown to decrease slightly or remain constant in time for 2°C-stored tubers [7].



Scheme 1.

Scheme 1 depicts the movement of carbon from starch to sucrose, glucose, fructose and carbon dioxide via the metabolic pool of phosphorylated hexose intermediate products represented by X. The k terms represent the rate constants for each degradation step.

The rate constants in the proposed mechanism can be linked to certain enzyme activities based on the current knowledge of the factors involved in the conversion of starch to reducing sugars [1–5]:

k_1 – starch phosphorylase, amylase and/or glucosidase activities;
k_2 – sucrose phosphate synthase activity;
k_3 – invertase activity and/or vacuolar permeability to sucrose;
k_4 – vacuolar permeability to reducing sugars and/or hexokinase activity;
k_5 – glycolytic and/or respiratory activities.

The constants k_1 and k_2 are first order rate constants while k_3, k_4 and k_5 are zero order rate constants. The reasons for assuming zero order behaviour in k_3–k_5 are discussed in Section 2.1.

The degradation of starch to sucrose, reducing sugars (glucose and fructose) and carbon dioxide

depicted in Scheme 1 can then be represented by the following set of differential equations:

$$\frac{d(St)}{dt} = -k_1(St - St_\infty) \quad (1)$$

$$\frac{dX}{dt} = k_1(St - St_\infty) - k_2 X - k_5 + k_4 \quad (2)$$

$$\frac{d(Suc)}{dt} = k_2 X - k_3 \quad (3)$$

$$\frac{d(Red)}{dt} = k_3 - k_4 \quad (4)$$

A mass balance on all species is given by

$$St_0 + Suc_0 + Red_0 + CO_{2_0} + X_0 = St(t) + Suc(t) + Red(t) + C0_2(t) + X(t) \quad (5)$$

where St_0 is the initial starch concentration, St_∞ is the limiting starch concentration as $t \to \infty$, Suc_0 is the initial sucrose concentration, Red_0 is the initial reducing sugars' concentration, CO_{2_0} is the initial carbon dioxide concentration and X_0 is the initial phosphorylated hexose intermediates' concentration. Initial concentrations of all intermediates were assumed to be zero at $t = 0$.

These equations were simultaneously solved by integration, rearrangement and substitution to obtain the following set of solutions:

$$St(t) = (St_0 - St_\infty)e^{-k_1 t} + St_\infty \quad (6)$$

$$X(t) = \frac{k_1(St_0 - St_\infty)}{k_2 - k_1}[e^{-k_1 t} - e^{-k_2 t}] + \frac{k_4 - k_5}{k_2}[1 - e^{-k_2 t}] \quad (7)$$

$$Suc(t) = \frac{k_2(St_0 - St_\infty)}{k_2 - k_1}[1 - e^{-k_1 t}] + \frac{k_1(St_0 - St_\infty)}{k_2 - k_1}[e^{-k_2 t} - 1] + \frac{k_4 - k_5}{k_2^2}[e^{-k_2 t} - 1] + \left(\frac{k_4 - k_5}{k_2} - k_3\right)t \quad (8)$$

$$Red(t) = (k_3 - k_4)t \quad (9)$$

By difference, from the mass balance, the concentration of CO_2 could be estimated:

$$CO_2(t) = St_0 + Suc_0 + Red_0 + CO_{2_0} + X_0 - St(t) - Suc(t) - Red(t) - X(t)$$

This model represents the net degradation of starch to sucrose, reducing sugars and CO_2 via hexose phosphate intermediates. In Scheme 1 this net degradation is represented by single arrows indicating the direction of net carbon flow.

In the development and application of the model several assumptions were made. These are listed in the following subsection.

2.1. Assumptions of the model

1. *All effects are purely kinetic in nature. No enzyme induction is occurring.* LTS involves fine metabolic control rather than coarse metabolic control [1,4]. No substantial changes in the V_{max} of several enzymes involved in carbohydrate metabolism have been observed upon cold storage of potato tubers [14,15]. In contrast, the Q_{10} (change in reaction rate for every 10°C change in temperature) of glycolytic enzymes such as ATP-dependent phosphofructokinase and pyruvate kinase increase significantly (enzyme activity is decreased upon cooling) in the range 2–10°C [14].

2. *Enzymes are working at V_{max} and/or the system is mass transfer limited* for the hydrolysis of sucrose to reducing sugars and transfer of sucrose into the vacuole (k_3 is a zero order rate constant), for the transfer of reducing sugars out of the vacuole and phosphorylation to hexose phosphates, and for the respiration of reducing sugars to carbon dioxide (k_4 and k_5 are zero order rate constants). The K_m of soluble acid invertase in potatoes (sucrose concentration at half maximal velocity) has been reported as 3.33 mM [16]. Taking into consideration the molecular weight of sucrose (342 g/mol) and the density of a potato tuber (1.1 g/cm^3), this K_m value can be transformed to units of mg of sucrose per g of dry potato tissue. The K_m of soluble acid invertase was then calculated to be 1.04 mg/g d.w. Potato tuber sucrose concentrations reach levels of 80–100 mg/g d.w. in 1993 and 40 mg/g d.w. in 1994. We believe that our assumption of zero order behavior in the

rates of sucrose breakdown by acid invertase is therefore justified. Sucrose hydrolysis by acid invertase will lead to high amounts of fructose and glucose being produced in the vacuole. Removal of these reducing sugars from the vacuole will most probably be mass transfer limited by the capacity of glucose and fructose transporters in the vacuolar membrane (tonoplast). The rate of removal of glucose and fructose will also depend on how quickly they are phosphorylated into hexose phosphates and utilized in glycolysis and respiration. We have also assumed that there is no significant starch synthesis occurring in LTS-stressed potato tubers at 2°C. Mohabir and John [17] have shown that starch synthesis in potato tuber discs displayed an optimum at about 21.5°C, with starch synthesis decreasing rapidly as temperature is decreased or increased. ADP-glucose pyrophosphorylase, the main regulatory enzyme in starch biosynthesis [18], has been reported to be inactivated at low temperatures with modification of its regulatory properties [19]. As well, ADP-glucose pyrophosphorylase is strongly inactivated by inorganic phosphate [20]. Inorganic phosphate has been postulated to leak from the vacuole into the cytosol during LTS from where it could be translocated into the amyloplast [3]. In the amyloplast, inorganic phosphorous would inhibit ADP-pyrophosphorylase activity and stimulate starch breakdown via starch phosphorylase. Shekhar and Iritani [21] have reported increases in inorganic phosphorous content in cold-stressed potato tubers. Considering we are dealing with postharvest storage of a mature tuber, i.e., beyond the growth and development stage, under conditions which would inhibit ADP-glucose pyrophosphorylase, the main enzyme responsible for starch synthesis, we believe that our assumption that starch synthesis during LTS is not significant, is warranted.

The rate constants in this model represent both the activity of enzymes and / or whole enzyme pathways, and / or the transfer of materials in and out of subcellular organelles. In such a phenomenological / semi-mechanistic model, it is not possible to unambiguously differentiate between mass transfer and catalytic kinetic effects. However, we assume that the rate constants obtained correspond to enzyme catalytic constants such as k_{cat} since mass transfer limitations should be similar for the two tubers. Differences would, therefore, be attributed to enzyme activities.

3. Materials and methods

Plant material. Mature potato tubers (*Solanum tuberosum* L.) were harvested on September 27 in 1993 and on September 19 in 1994 from fully senesced potato plants of the seedling 'North Dakota 860-2' (ND860-2) and the cultivar Norchip. Tubers were grown at the Cambridge Agricultural Research Station (Ontario Ministry of Agriculture, Food and Rural Affairs, Cambridge, Ont.) using standard agronomic practices. Tubers were cured for 2 weeks at 15°C and then placed at 2°C with 95% relative humidity for the remainder of the study.

Sugar extraction and analysis. The sugars sucrose, glucose and fructose were extracted from randomly selected potato tubers by blending a ratio of 10 g of potato tuber with 8 ml of HPLC grade methanol in a Waring Commercial Blender for 90 seconds. 0.5 g of charcoal were added for each 10 g of tuber and the samples were shaken for 20 minutes and then refrigerated for a minimum of 1 hour. Samples were vacuum filtered and supernatants collected and stored at 4°C until analysis. The sampling period varied from 55 days in 1993 to 73 days in 1994.

Prior to HPLC analysis, supernatants were cleaned by first passing them through a SepPak Alumina A cartridge followed by a 0.45 micron nylon filter. Sucrose, glucose and fructose concentrations (mg/g dry weight) were determined by high pressure liquid chromatography (HPLC) as described by Wilson et al. [22]. HPLC analysis was performed on three individual samples and the values averaged.

Data analysis. The model equations were fitted to the experimental data using nonlinear least squares methods using the software package Grafit [23], which uses the method of Marquart [24] using a numerical second order method to calculate partial differentials. Simple weighting was used for the sucrose data set, while robust (simple) data weighting was used for the fructose and glucose data set in both years [25]. For the 1993 glucose and fructose data, the model was fitted to data in the range 20–48

Table 1
Parameters obtained from non-linear regression fits of sucrose, glucose and fructose whole tissue accumulation data at 2°C to the model developed in this study

Parameter	ND860-2 ('93)	ND860-2 ('94)	Norchip ('93)	Norchip ('94)
k_1 (day^{-1})	0.100^{1} [a]	0.073^{1}	0.109^{1}	0.114^{1}
k_2 (day^{-1})	0.098^{1}	0.077^{1}	0.103^{1}	0.108^{1}
k_3 (day^{-1})	1.095^{1}	0.662^{1}	2.282^{2}	1.227^{1}
$k_3 - k_4$ (day^{-1})				
Fructose	$0.141^{1,5}$	0.0217^{2}	0.544^{3}	$0.134^{4,5}$
Glucose	0.211^{1}	0.0314^{2}	0.635^{3}	$0.136^{4,5}$
k_4/k_5	1.007^{1}	1.013^{1}	1.056^{1}	1.095^{1}
$St_0 - St_\infty$ (mg/g d.w.)	126^{1}	65^{2}	170^{3}	77^{2}

[a] Values with the same superscript within a row are not significantly different from each other ($P > 0.05$).

days, while for the 1994 glucose and fructose data, the model was fitted to data in the range 10–49 days. The criterion for convergence was a less than 0.01% change in the reduced chi-square (χ^2) value upon variation of parameters and lack of sensitivity to initial conditions (sensitivity analysis).

Simulations. A fourth order, error-controlled Runge–Kutta routine (1×10^{-6} maximum relative error and a 0.01 step size) was used to numerically simulate the whole proposed kinetic model. For this purpose, the software package Scientist 2.0 (Micromath Scientific Software, Salt Lake City, UT) was used. The values for rate constants and initial starch concentrations used were those derived from the nonlinear fits to our experimental data (see Table 1).

Statistical analysis. Differences in the rate constants were analyzed statistically using the software package Prism (GraphPad Software, San Diego, CA). A one-way analysis of variance was used to establish significant effects in the four treatments (two cultivars, two growing years). Unpaired two-way *t*-tests were then subsequently used to establish significant ($P \leq 0.05$) differences among treatments.

4. Results and discussion

Fig. 1 shows the patterns of sucrose accumulation for 1993 and 1994-grown ND860-2 and Norchip tubers stored at 2°C. In both years a characteristic sigmoidal accumulation pattern was observed. The solid lines represent the best fit line of our model to the data. Differences in the accumulation patterns between 1993 and 1994 reflect differences in growing conditions between the two years. 1994 was a close to ideal growing year, hence the sucrose accumulation patterns of ND860-2 and Norchip are similar. The amount of sucrose produced at 2°C in 1994 was about half of that produced in 1993.

In 1993, Norchip, an LTS-sensitive cultivar, accumulated more sucrose than ND860-2, an LTS-tolerant seedling. This growing year was particularly wet and cold. The potato tubers were subjected to much greater environmental stresses in the 1993 growing season than in the 1994 growing season.

Figs. 2 and 3 show the patterns of fructose and glucose accumulation for ND860-2 and Norchip tubers stored at 2°C in 1994 and 1993. Our results suggest that the rate and amount of reducing sugar accumulation depends on the difference in rate constants for the sucrose hydrolysis step (invertase activity) and the rate constant for the removal of reducing sugars from the vacuole and/or hexokinase activity. The solid lines represent the best fit lines of our model to the data. The rates of accumulation of fructose and glucose are greater for Norchip (LTS-sensitive) than for ND860-2 (LTS-tolerant) in both years ($P \leq 0.05$). The rates of glucose accumulation and fructose accumulation are not significantly different from each other in 1993 or 1994 ($P > 0.05$). This suggests that these sugars are indeed the product of sucrose hydrolysis by invertase, and that there is no preferential removal of one sugar over the other due to specific transport systems in the vacuole, and/or specific glucokinase/fructokinase activities.

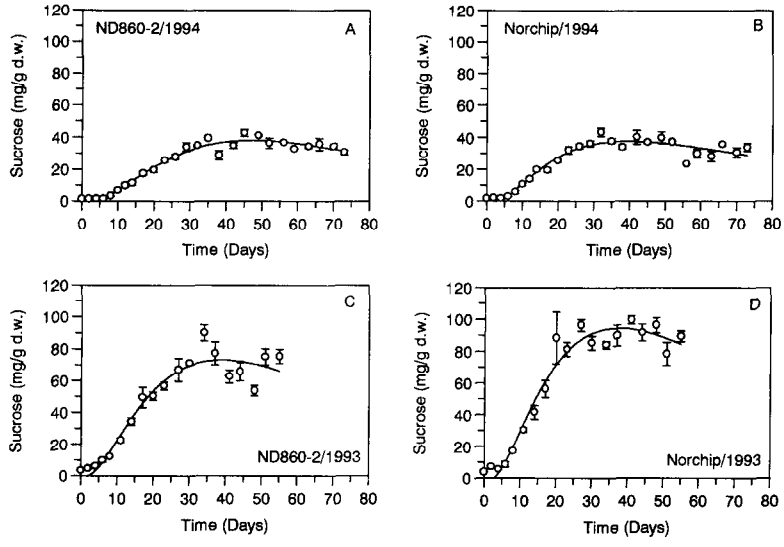


Fig. 1. Dynamic patterns of sucrose accumulation in 2°C-stored whole potato tubers grown in 1993 and 1994. (A) LTS-tolerant ND860-2, 1994; (B) LTS-sensitive Norchip, 1994; (C) LTS-tolerant ND860-2, 1993; (D) LTS-sensitive Norchip, 1993. Symbols and error bars represent the average and standard error of three replicates. Solid lines correspond to the best fit line of the data to the kinetic model developed in this study obtained by nonlinear regression.

The rate of removal will probably depend on relative fluxes through glycolysis and respiration.

Rate constants derived from the model fit to our data are presented in Table 1. Interestingly, no significant differences ($P > 0.05$) were detected in k_1 or k_2 between Norchip and ND860-2 in 1993 or

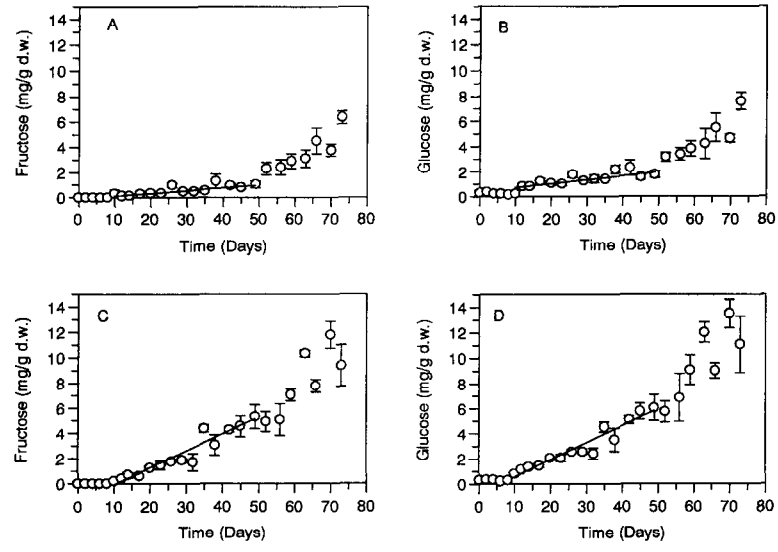


Fig. 2. Dynamic patterns of fructose and glucose accumulation in 2°C-stored whole potato tubers grown in 1994. (A) LTS-tolerant ND860-2, fructose; (B) LTS-tolerant ND860-2, glucose; (C) LTS-sensitive Norchip, fructose; (D) LTS-sensitive Norchip, glucose. Symbols and error bars represent the average and standard error of three replicates. Solid lines correspond to the best fit line of the data to the kinetic model developed in this study obtained by linear regression.

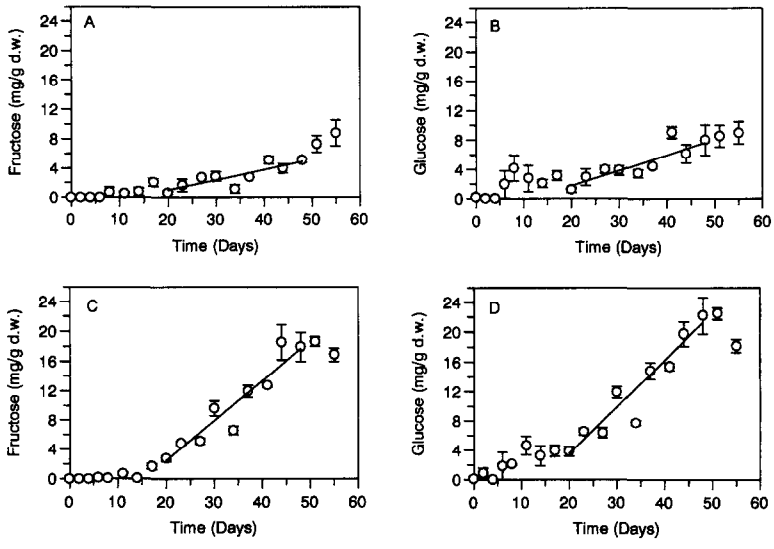


Fig. 3. Dynamic patterns of fructose and glucose accumulation in 2°C-stored whole potato tubers grown in 1993. (A) LTS-tolerant ND860-2, fructose; (B) LTS-tolerant ND860-2, glucose; (C) LTS-sensitive Norchip, fructose; (D) LTS-sensitive Norchip, glucose. Symbols and error bars represent the average and standard error of three replicates. Solid lines correspond to the best fit line of the data to the kinetic model developed in this study obtained by linear regression.

1994. In fact, these rate constant were not significantly different from each other ($P > 0.05$). As stated before, k_1 represents starch degradative enzyme systems such as starch phosphorylase, amylases, glucosidases, while k_2 represents the activity of sucrose phosphate synthase (SPS). Our results suggest that these enzyme systems do not play a key role in the induction of LTS.

Significant differences in the value of k_3 (invertase activity) were, however, evident. The value of k_3 for Norchip in 1993 was twice that of ND860-2 ($P \leq 0.05$). In 1994, however, no significant differences in k_3 between the cultivars were evident ($P > 0.05$). Interestingly, $k_3 - k_4$, the difference in rate constant between invertase activity and the rate of removal of glucose and fructose from the vacuole, and/or hexokinase activity, seems to be the controlling factor responsible for reducing sugar accumulation in our system. This difference in rate constants was 3–4-fold higher for Norchip (LTS-sensitive) in 1993, and 4–6-fold higher in 1994 than for ND860-2 (LTS-tolerant). It would seem, therefore, that increased invertase activity coupled with a decreased ability to remove reducing sugars from the vacuole and return them to the hexose phosphate pool is controlling the amount of reducing sugars accumulated in the tissue.

Zrenner et al. [8] found a strong correlation between the hexose/sucrose ratio and the extractable soluble acid invertase activity in 24 potato cultivars. They also isolated a cold-inducible acid invertase cDNA from the potato cultivar Desirée and developed clones expressing the cDNA in an antisense orientation. Analysis showed that inhibition of the soluble acid invertase activity lead to decreased hexose and increased sucrose contents when compared to controls. The hexose/sucrose ratio was again found to decrease with decreasing invertase activities. The total amount of soluble sugars did not significantly change in either study and it was concluded that invertases do not control the total combined amount of glucose, fructose and sucrose in cold stored potato tubers, but are involved in the regulation of the ratio of hexose to sucrose.

The ratio k_4/k_5 is close to unity in both years and does not vary among cultivars or years. This means that the value of k_4 and k_5 are linked to each other, and k_5 represents glycolysis and respiration.

Hence, the flux of material through these pathways controls the value of k_4. It would therefore seem that increased basal glycolytic/respiratory capacity and decreased invertase activity at low temperatures should minimize reducing sugar accumulation in potato tubers. The results from our analysis agree closely with recent work by Viola and Davies [26] who suggest that glycolytic metabolism is severely impaired in low-temperature stressed potato tubers. ATP-dependent 6-phosphofructokinase (ATP-PFK) has been described in the literature as the main cold-labile regulatory enzyme responsible for controlling carbon flux through glycolysis [1]. However, recent work by Burrell et al. [27] with transgenic potatoes has shown that changing the amount of ATP-PFK present does not lead to changes in respiratory activity. These results suggest that PFK is not controlling the flux of metabolites through glycolysis, resulting in increased respiratory activity. Plants also have a pyrophosphate-dependent PFK, which may play a role in controlling glycolytic fluxes [2,3]. This issue is, however, far from being solved and much work remains to be done.

Previous work by our group has shown that ATP-dependent phosphofructokinase (ATP-PFK) activity and respiration rates in Norchip (LTS-sensitive) tubers are significantly lower than ATP-PFK activity and respiration rates in ND860-2 tubers (LTS-tolerant) at both 4°C and 12°C [28]. In addition, it was clearly shown that respiration was severely impaired in both cultivars upon exposure to low temperatures, probably due to an impaired glycolytic metabolism. Whether decreased ATP-PFK activity is responsible for decreased respiratory activity remains to be proven.

An interesting result from the fits to our data was the difference observed in available starch for degradation ($St_0 - St_\infty$). The amount of starch degraded was always greater for Norchip than for ND860-2 ($P \leq 0.05$) in both years. This result suggests that there may be differences in the availability of starch for LTS-induced degradation. It is possible that the starch from the LTS-sensitive cultivar is more susceptible, or more available, to degradation than starch from LTS-tolerant cultivars. This conclusion would tend to support the proposal that starch structure plays a role in LTS-sensitivity or tolerance [29]. A point to consider is that we are not suggesting that the starch substrate becomes depleted, but rather that the amount of "initial available starch" becomes depleted. Even though the sole source of carbon for LTS has been shown to be starch [7], microscopic studies on the morphology of starch granules derived from potatoes stored at 4°C (under LTS conditions) have shown that starch granule morphology was not significantly different from that of starch granules derived from potatoes stored at 10°C [30]. This is surprising, since amylase treatment of isolated starch granules clearly demonstrates surface pitting [29]. This would agree with our proposal that a "pool" of available starch exists which is more susceptible to enzymatic degradation than the main bulk of crystalline starch (about 6–17% w/w of total starch). Moreover, results from our laboratory [29,31] have shown that small starch granules are preferentially degraded over large starch granules. Work by Nielsen et al. [32] has shown that small starch granules are more extensively phosphorylated than large starch granules during cold-storage of potato tubers. Increased phosphorylation would lead to a more open, hydrated substrate structure (starch), more prone or available to enzymatic degradation. Maybe starch with a greater amount of phosphorylation is more labile than starch with a lesser degree of phosphorylation. These results would imply that greater LTS tolerance could be correlated with decreased levels of starch phosphorylation in potato tubers.

Fig. 4 shows a simulation of the complete model using rate constants derived from the nonlinear regression fits to our experimental data. The simulated patterns agree closely with the experimentally obtained patterns for sucrose (Fig. 1) and glucose/fructose (Figs. 2 and 3) accumulation. The only difference is the observed "lag time" in the accumulation of reducing sugars observed in the experimental data (Figs. 2 and 3). We have no mechanistic explanation for this lag time. A transient increase in hexose-phosphate intermediates was reported by Isherwood [12] in LTS-tubers, while a gradual increase in the amount of CO_2 produced was reported by Isherwood [7]. These observations agree with our simulations.

In conclusion, analysis of sucrose, glucose and fructose accumulation data at 2°C suggests that low-temperature induced reducing sugar accumulation is due mainly to relative differences between invertase

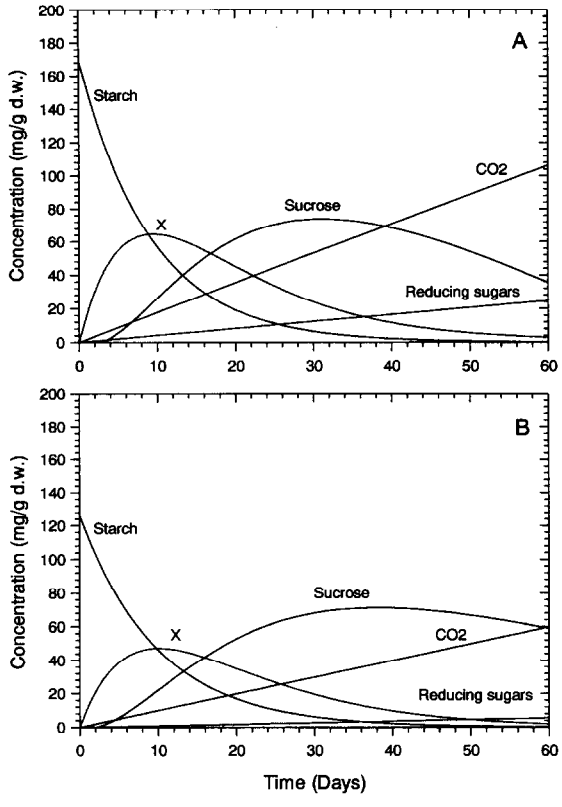


Fig. 4. Numerical simulation of the whole kinetic model developed in this study shown here for 1993-grown Norchip (A) and ND860-2 (B). Values for the parameters used are those listed in Table 1.

activity and glycolytic/respiratory capacity. A high invertase activity coupled with decreased glycolytic/respiratory capacity results in an increased accumulation of reducing sugars in potato tuber tissue at low temperatures. LTS-sensitive potato cultivars may contain greater amounts of labile starch than tolerant ones.

Acknowledgements

The authors acknowledge the financial assistance of the Ontario Ministry of Agriculture, Food and Rural Affairs (Ontario Food Processing Research Fund), The Ontario Potato Growers' Marketing Board, the Ontario Potato Cultivar Evaluation Association, and the Canadian Potato Chip and Snack Food Association. The authors acknowledge the valuable suggestions from Dr. Robert Lencki.

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